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The response of subcutaneous connective tissue to newly developed calcium phosphate-based root canal sealers

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Abstract

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Aim To evaluate the histopathologic biocompatibility of two new calcium phosphate-based sealers (CPS-1 & CPS-2) with a commercially available calcium hydroxide-based sealer (Acroseal).

Methodology Polyethylene tubes were filled with freshly mixed sealers and implanted subcutaneously in the dorsal connective tissue of rats. Empty tubes were used as controls. Histopathological examinations were conducted at 7, 15, 30, 60 and 90 days after the implantation procedure. The presence of inflammation and predominant cell types were analysed statistically with Mann–Whitney *U* and Kruskal–Wallis non-parametric tests. Fibrous connective tissue thickness adjacent to each sample was recorded. Differences were tested for significance using ANOVA and 'Duncan's' multiple comparison test ($P < 0.05$).

Results CPS-1 sealer was associated with severe inflammation and remained an irritation throughout the 90-day implantation period; the tissue reaction pattern was stromal fibrosis. The control, CPS-2 and Acroseal sealers had similar patterns of irritation, which were more severe initially and diminished with time creating a thin fibrous capsule around the implant with a complete absence of inflammatory cells. There was no difference in tissue reaction between the control, CPS-1, CPS-2 and Acroseal groups amongst the first two observation periods ($P > 0.05$). However, there was a highly significant difference between the same groups at the last two observation periods ($P < 0.01$). Also, there were highly significant differences between the observation periods within all four groups at 7, 15, 30, 60 and 90 days ($P < 0.01$).

Conclusion CPS-1 sealer was not biocompatible. CPS-2 sealer and Acroseal had a favourable biocompatibility level based on the histological findings.

Keywords: Acroseal, calcium phosphate-based sealers, connective tissue reactions, histopathologic method.

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Introduction

One of the aims of root canal treatment is to fill the root canal system with an impervious, biocompatible and dimensionally stable filling material (Nguyen 1991). Endodontic filling materials are placed directly on to vital tissues, and the tissue response to these materials is of importance because it may influence the outcome of treatment (Sundqvist & Figdor 2003). One of the requirements of an ideal root canal sealer is that it should be non-irritating to the periapical tissues and compatible with the surrounding living connective tissues (Gutmann & Witherspoon 2002). Although a wide variety of endodontic sealers are available commercially, many create tissue irritation (Ioannis & Nikolaos 1998, Lee *et al.* 2002, Schwarze *et al.* 2002a,b, Hauman & Love 2003).

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Continuing advances in the field of implantable calcium phosphate biomaterials have produced promising results. It is well known that calcium phosphate cement (CPC) has a composition almost identical to that of tooth and bone mineral (Hong *et al.* 1990, Yuan *et al.* 2000). The low degree of toxicity makes this material useful in applications in which the cement is in contact with vital tissues (Fukase *et al.* 1990). Therefore, it has been suggested as a useful material for endodontic therapy (Sugawara *et al.* 1992, Yoshikawa *et al.* 1997, Geurtsen 2001).

Biological properties are amongst the most important aspects of root filling materials because the release of substances from the sealers may generate reactions in the periapical tissue (Bernath & Szabo 2003, Hauman & Love 2003, Kaplan *et al.* 2003, Ho *et al.* 2006). The principal methods used are cytotoxicity tests conducted *ex vivo* on cell or tissue cultures (Saidon *et al.*2003) and subcutaneous connective tissue (Modaresi *et al.* 2005), or bone implantation methods in experimental animals (Zmener *et al.*2005).

Recently, new calcium phosphate-based sealers have been developed and their physicochemical properties tested (Khashaba *et al.* 2006, 2010). These materials harden into cements when mixed with polymeric acids. Their biological properties in terms of cytotoxicity had been previously investigated on cell culture of fibroblasts derived from primary human gingival and L929 cell lines and compared with a commercial calcium hydroxide-based sealer, Acroseal (Specialités-Septodont; Saint Maur-des-Fossés, Cedex, France) (Khashaba *et al.* 2009). When compared with Acroseal, the cytotoxicity of CPS-1 & CPS-2 was found to be less toxic. (Khashaba *et al.* 2009). Consequently, the aim of this study was to further evaluate histopathologically the response of the tissues to the two new calcium phosphate-based root canal sealers (CPS-1, CPS-2) and to compare these sealers with the calcium hydroxide-based sealer (Acroseal) after implanting them in the subcutaneous tissue of rats.

The hypothesis of the current study is that the new calcium phosphate-based sealers (CPS-1 and CPS-2) will be biocompatible with subcutaneous connective tissue to potentially provide a safer alternative to currently available sealers.

Material and methods

The protocol for this experiment was approved by the Institutional Animal Care and Use Committee of the Medical College of Georgia, USA. All procedures and animal care were carried out in accordance with the guidelines laid down by the National Institutes of Health, USA (National Institutes of Health, 2006) through the Public Health Service Policy on Human Care and Use of Laboratory Animals.

Seventy-five white male Sprague–Dawley rats 3–4 months old weighing between 200 and 250 g were obtained commercially and given standard food pellets and water *ad libitum*. The specimens were divided into three groups of five animals each for an experimental period of 7, 15, 30, 60 and 90 days, respectively.

Preparation of cements

Two polymeric calcium phosphate-based sealers and a calcium hydroxide sealer were evaluated (Table 1).

Preparation of calcium phosphate cement powder

The powder of the CPCs was derived from a mixture of monocalcium phosphate (MCP), calcium oxide (CaO) and synthetic hydroxyapatite (SHAP6). Both the MCP and the calcium oxide powder were crushed separately in an agate mortar and then sieved through a mesh to obtain an average particle size of 80 microns. A mixture of MCP and CaO was prepared at a Ca/P ratio of 1.67, the same ratio present in bone and dentine (Jarcho 1986). This mixture constituted 60% of the total weight of the powder. A 40% weight of synthetic hydroxyapatite (SHAP6) molecules sintered at 600 °C (Abd-el Fattah & Selim 1991) were added to increase the strength of the cement powder and to act as seeds for nucleation of more hydroxyapatite (Bermudez *et al.* 1994). The powder was sieved and mechanically mixed in a shaking apparatus (Shaking apparatus Turbula, Pharmaceutical Faculty, Frankfurt, Germany).

Preparation of aqueous solutions of polymeric liquids

Two types of polymeric liquids (Table 1) were each mixed individually with the calcium phosphate powder: (i) modified polyacrylic acid and (ii) a 35% (w/w) aqueous solution of polymethyl vinyl ether maleic acid, prepared by dissolving 35 g of the white powder of polymethyl vinyl ether maleic anhydride (PMVE-MA) copolymer (molecular weight 50 000) in 100 mL of distilled water at 60 °C. This solution was maintained at this temperature for 24 h in a shaker incubator. The 35% w/w aqueous solution of PMVE-MA was mixed

Table 1 Composition of the materials and their manufacturers

Material	Composition	Trade name	Manufacturers
Monocalcium phosphate monobasic (MCPM)		Calcium Phosphate Monobasic	Sigma-Chemical Laboratories, St Louis, MO, USA
Calcium oxide			Adwic Chemical Laboratories, Cairo, Egypt
Synthetic hydroxyapatite (SHAP6)	Chemically precipitated Ca $(OH)_2$ and concentrated phosphoric acid.		Prepared at the Medical College of Georgia, Augusta, GA, USA
Polyacrylic acid	Polyacrylic acid, itaconic acid, and tartaric acid as stabilizer	G.C.R	Advanced Research Inc., Dental division, Leeds, UK
Polymethyl vinyl ether maleic anhydride copolymer (white powder) PMVE-MA			Sigma-chemical Laboratories
Calcium hydroxide based sealer (Acroseal)	Base glycyrrhetinic acid (enoxolone), methenamine, radiopaque excipient (bismute subcarbonate, colophone, paraffin oil, and terbentine) Catalyst calcium hydroxide, diglicidyl ether biphenol A, radiopaque excipient (bismute subcarbonate and 10 yellow pigment)		Saint Maur des Fosses

with CPC powder to form the polymeric CPC cement (CPS-2 cement).

Preparation of calcium phosphate-based sealers

The calcium hydroxide-based root canal sealer (Acroseal) was prepared in the manner advised by the manufacturers for clinical use.

The polymeric calcium phosphate-based sealers were prepared as follows:

CPS-1: CPC powder (MCP + CaO) + SHAP6 (60% w + 40% w). Liquid: aqueous solution of polyacrylic acid

CPS-2: CPC powder: (MCP + CaO) + SHAP6 (60% w + 40% w). Liquid: a 35% (w/w) aqueous solution of polymethyl vinyl ether maleic acid.

The two polymeric liquids previously mentioned were mixed individually with the calcium phosphate powder in a ratio of 4 : 1. This ratio was selected for the preparation of the two types of cements because this ratio demonstrated good handling characteristics and working time.

Handling time was determined as follows: The mixing phase (30 s) was deemed to have started as soon as all constituents had been combined. A timer was started when all of the liquid had been added to the powder. The period between mixing and the point the cement can be used is the waiting phase (dough time). The end of the waiting phase was recorded when the cement mass no longer adhered to a surgically gloved finger.

The working phase immediately followed the waiting phase and ended when upon folding the cement, it no longer adhered to itself. The end of the working time until the moment at which the cement had become completely hardened was recorded as the setting phase.

All materials were loaded into autoclaved polyethylene carriers (Poltetra fluoroethylene, Du Pont, Habia, Knivsta, Sweden) 10 mm long with 1.5 mm inner diameter and 2 mm outer diameter, by means of sterile insulin syringes (Injex Industria Cirurgica Ltda, Ourinhos, SP, Brazil) in such a way that the material filled the tube entirely with no overflow.

The animals were anaesthetized by intramuscular administration of a rodent anaesthesia cocktail, 1.5 mL ketamine HCl (100 mg mL^{-1}), 1.5 mL xylazine HCl (20 mg mL^{-1}) and 0.5 mL acepromazine (10 mg mL^{-1}), 0.2–0.5 mL per 100 g of animal weight and stabilized on a surgical table. The surgical sites on the dorsal skin were shaved, disinfected with 5% iodine solution and an incision approximately 15 mm long made through the full thickness of the skin using a number 15 blade (Aesculap AG and Co. KG, Tutlingen, Germany). Subsequently, lateral to the incisions, four subcutaneous pockets were created by blunt dissection with scissors. Under aseptic conditions, the tubes containing freshly mixed sealers were implanted immediately into the subcutaneous pockets. Each animal received three tubes filled with the sealers and one empty sterilized tube to serve as

control. Care was taken to prevent spilling of the materials into the tissue. Finally, the incisions were closed with 3/0 silk suture.

The animals were maintained in cages on a regular diet and water *ad libitum*. After the experimental periods, all animals from the group were killed by an overdose of anaesthetic. The dorsal skin was shaved, disinfected, and the implants together with their surrounding tissues were removed and fixed in 10% formalin solution for 24 h and processed for paraffin embedding. The tube and the connective tissue adjacent to both open ends of each tube were serially sectioned along the long axis of the tube at a microtome setting of 5 μm thickness. Each step of the histological process (i.e. dehydration, cleaning and paraffin infiltration) was performed following the same protocol for each sample. Five-micrometre-thick sections were stained with haematoxylin and eosin, and histological examination for selected sections was performed using a Leica DM 4000–B light microscope (Leica, Wetzlar, Germany).

Evaluations of inflammatory scores were carried out in 10 separate areas of sections at ×400 magnifications (Zmener *et al.* 1990) by Leica DC-500 camera and QWin 3.3 image analyzer software system (Leica). The observer was blinded to treatment allocation. The mean values for the materials were obtained from the sum of the cells counted in ten separate areas. The type of inflammatory cells was also noted. The inflammatory reactions were scored and evaluated as:

0 – None or few inflammatory cells and no reaction;
1 – $n < 25$ cells and mild reaction;
2 – between 25 and 125 cells and moderate reaction;
3 – $n > 125$ cells and severe reaction (Costa *et al.* 2000). Fibrous capsule thickness was measured using the Leica DC-500 Camera attached to the microscope using Leica QWin 3.3 image analyzer software. Five separate walls from each capsule of every sample were photographed, and the thickest cross-section length of the capsule was measured.

Quantitative data of the fibrous capsule thickness were tested for statistical significance using analysis of variance and multiple comparisons 'Duncan's test'. The results of the inflammatory reactions were tested with the Mann–Whitney *U* and Kruskal–Wallis non-parametric tests. Significance was established at $P < 0.05$.

Results

All animals remained in good health during the implantation periods. No postoperative complications were observed and the surgical sites healed with no infection.

The connective tissue adjacent to the open end of the polyethylene tubes was evaluated, and descriptive analyses of the histologic findings were made. The mean and standard error of inflammatory cell numbers are shown in Table 2.

Seven days

Histological evaluation of tissue response at 7 days revealed a severe inflammatory reaction in the control group (empty tube) and those with CPS-1 and CPS-2 sealers; the tissue was infiltrated predominantly with macrophages and lymphocytes. The presence of polymorphonuclear leukocytes, hyperaemia and dilated blood vessels was also observed (Fig. 1a–c),

In the commercially available Acroseal sealer, areas of liquefactive necrosis were observed with moderate inflammatory cell infiltration mainly macrophages (Fig. 1d).

The tissue responses to the three tested materials and the control group were similar. The median inflammatory reaction scores of the three groups were 3 ($P > 0.05$). Mean values of fibrous tissue thickness were 2.10, 2.80, 1.69 and 2.30 μm in the control group, CPS-1, CPS-2 and Acroseal, respectively ($P > 0.05$).

Table 2 The mean and standard deviation of fibrous capsules and the inflammatory cell numbers

Observation periods (days)	The inflammatory cell numbers				Thickness of fibrous capsules (μm)			
	Control	CPS-1	CPS-2	Acroseal	Control	CPS-1	CPS-2	Acroseal
7	126.33 ± 1.2	138.33 ± 1.2	134.33 ± 1.2	136.33 ± 1.2	2.10 ± 0.211	2.80 ± 0.211	1.69 ± 0.078	2.30 ± 0.198
15	70.33 ± 0.6	78.33 ± 0.8	72.33 ± 0.8	73.66 ± 0.7	3.50 ± 0.120	3.66 ± 0.430	2.80 ± 0.202	3.64 ± 0.244
30	10.33 ± 0.7	68.33 ± 0.8	63.33 ± 0.8	64.33 ± 0.7	4.13 ± 0.511	4.26 ± 0.511	3.82 ± 0.266	4.13 ± 0.511
60	6.32 ± 0.7	79.33 ± 1.2	23.16 ± 0.7	24.33 ± 0.6	3.34 ± 0.504	4.32 ± 0.511	2.53 ± 0.104	2.63 ± 0.109
90	1.22 ± 0.6	80.33 ± 1.2	13.15 ± 0.7	14.33 ± 0.6	2.03 ± 0.214	4.50 ± 0.630	2.43 ± 0.098	2.58 ± 0.107

Fifteen days

Histological evaluation of tissue response to the materials at 15 days revealed that the cellular reaction remained dominated by mononuclear cells, with the presence of macrophages, plasma cells and multinucleated giant cells (Fig. 2a).

The median inflammatory cell score of the control group, CPS-1, CPS-2 and Acroseal was 2 ($P > 0.05$). The thicknesses of the fibrous tissue were 3.50 3.66, 2.80 and 3.64 μm in the control group, CPS-1, CPS-2 and Acroseal, respectively ($P > 0.05$) (Fig. 2b–e).

Thirty days

The histological evaluation of tissue response at 30 days revealed that the degree of inflammation had decreased relative to the 15-day evaluation. However, it was still dominated by lymphocytes in all groups. Although less in number, macrophages and plasma cells were also present in the inflammatory infiltration of each group. It was noted that the decrease was much more evident in the control and CPS-2 groups, respectively (Fig. 3a,b).

At 30 days post-implantation, mononuclear cells appeared qualitatively less common than at 15 days. In the CPS-1 group, the cellular reaction was characterized by the presence of multinucleated giant cells with engulfed particles of the material in their cytoplasm; it was not uncommon to see CPS-1 cement particles extruded into the tissue, evoking a foreign body reaction (Fig. 3c). The median inflammatory cell scores of the CPS-1, CPS-2 and Acroseal groups were 2 ($P > 0.05$). The median inflammatory reaction score of the control group was 1 ($P > 0.05$). Mean values of fibrous tissue thicknesses were 4.11, 4.26, 3.82 4.13 μm in the control group, CPS-1, CPS-2 and Acroseal, respectively ($P > 0.05$) (Fig. 3d).

Sixty days

The histological evaluation for CPS-1 revealed the inflammatory reaction was more severe than in 30 days. A moderate inflammatory reaction consisting of an infiltrate of polymorphonuclear leukocytes, lymphocytes and macrophages was observed at the open ends of the polyethylene tube. Many mast cells were also observed surrounding areas of blood vessels (Fig. 4a). The median inflammatory reaction score of the CPS-1 group was 2 ($P > 0.05$). Tissue reactions against CPS-2 and Acroseal decreased after the 60-day period. The test materials were surrounded by capsule-

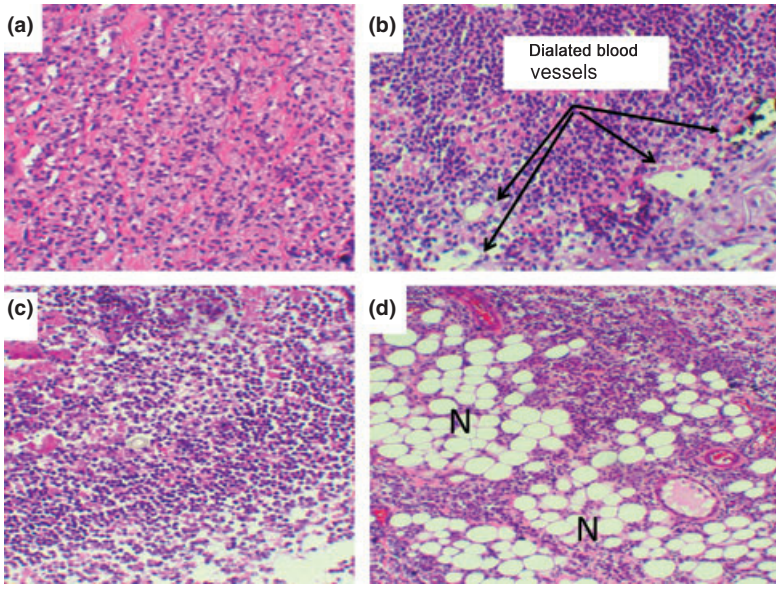

Figure 1 (a) Control (empty tube) at 7 days. Note the heavy inflammatory infiltrate with polymorphonuclear leukocytes (×100 H/E). (b) CPS-1 at 7 days intense mononuclear infiltrate of macrophages, dilated blood vessels (arrows) (H/E × 100). (c) CPS-2 at 7 days severe inflammation dominated by mononuclear cells (H/E × 100). (d) Acroseal at 7 days showed liquefactive necrosis (N) (H/E × 100).

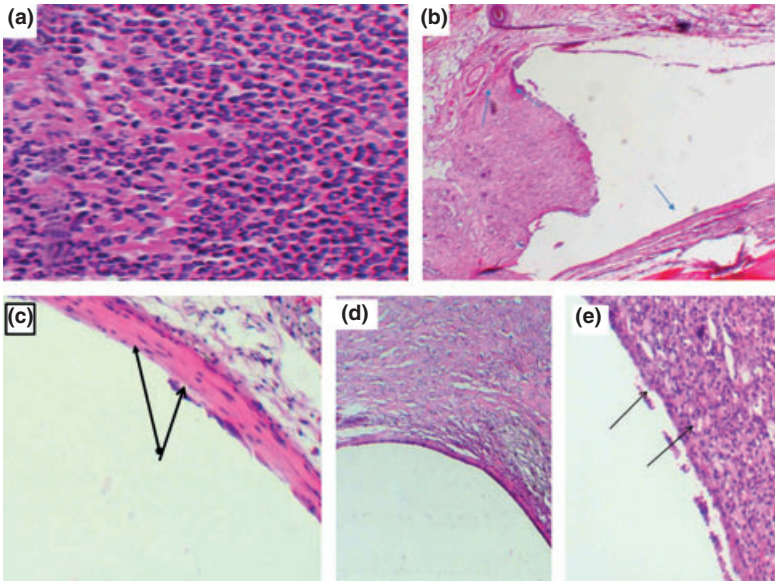

Figure 2 (a) Inflammatory infiltrate of plasma cells, lymphocytes and neutrophils in control (empty tube), CPS-1, CPS2 and Acroseal at 15 days (H/E × 120). (b) Control (empty tube) at 15 days thick fibrous capsule at the open end (H/E × 100). (c) CPS-1 at 15 days moderate inflammation, thick capsule (arrows) (H/E × 120). (d). CPS-2 at 15 days. Note thick fibrous capsule (H/E × 100). (e) Acroseal at 15 days few inflammatory cells, thick fibrous capsule formation (arrows) (H/E × 100).

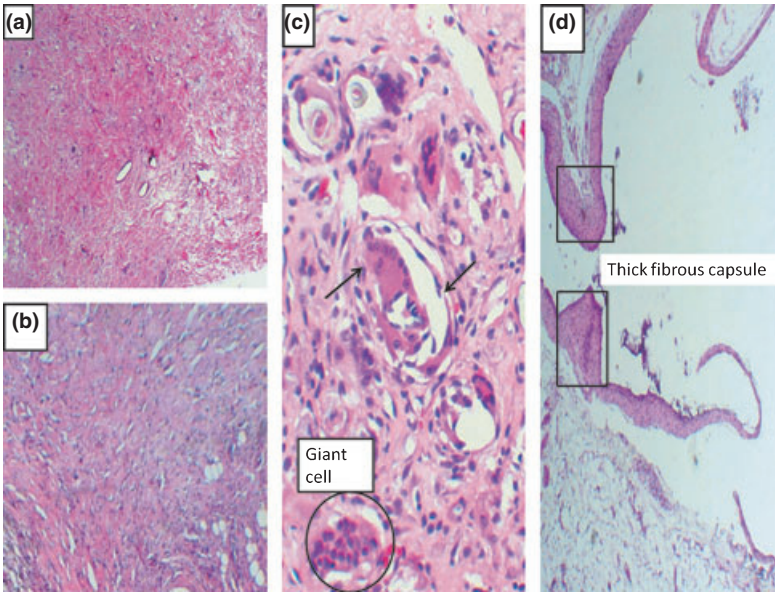

Figure 3 (a) Control (empty tube) at 30 days. Decreased number of mononuclear cells (H/E × 100) (b) CPS-2 at 30 days a moderate inflammatory infiltrate of lymphocytes (H/E × 100). (c) CPS-1 at 30 days. Note multinucleated giant cells and hyperaemic blood vessels (arrows) (H/E × 200). (d) Thick fibrous capsule formation of all implanted materials and control (empty tube) at 30 days (H/E × 100).

like fibrous tissue (Fig. 4b,c). The median inflammatory cell score of the control group, CPS-2 and Acroseal was 1 ($P > 0.05$). The mean thicknesses of fibrous tissue were 3.34, 4.32, 2.53 and 2.63 μm in the control group, CPS-1, CPS-2 and Acroseal, respectively ($P < 0.05$).

Ninety days

CPS-1 was associated with a moderate chronic inflammatory infiltrate consisting of macrophages, lymphocytes and polymorph nuclear leukocytes. At the open end of the tubes, the granulomatous tissue was walled off by thick fibrous encapsulation, red cells and vacuolated dead cells (Fig. 5a). The median inflammatory score of CPS-1 was 2 ($P > 0.05$). For CPS-2 and Acroseal, there was no significant difference between the degrees of inflammatory infiltrate for the various materials. The median inflammatory reaction of both groups was 1 ($P > 0.05$). The median inflammatory score of the control group was 0 ($P > 0.05$). Mean values of fibrous tissue thickness were 2.03, 4.50, 2.43 and 2.58 μm in the control group, CPS-1, CPS-2 and Acroseal, respectively (Fig. 5b–d).

There was no difference in tissue reaction between CPS-1, CPS-2 and Acroseal groups, and the control groups for the first two observation periods ($P > 0.05$). However, there was a highly significant difference between the CPS-1 group and the CPS-2, Acroseal and control groups at the last two observation periods (60 and 90 days) ($P < 0.01$). There were highly significant differences between the observation periods within the four groups at 7, 15, 30, 60 and 90 days.

The mean and standard errors of thickness of fibrous capsule are shown in Table 2. There was no significant difference between the control group, CPS-1, CPS-2 and Acroseal groups for the first four observation periods ($P > 0.05$). However, there were highly significant differences between the CPS-1 group and the control group, CPS-2 and Acroseal at the last observation period (90 days) ($P < 0.01$). The control group (empty tube) was associated with the thinnest ($P < 0.01$) fibrous capsule.

Discussion

The biocompatibility of dental materials is an important requirement because the toxic components in these materials would produce irritation or even degradation of the surrounding tissue, especially when accidentally extruded into the periradicular tissues (Huang *et al.* 2005). Recommended tests for biocompatibility of endodontic materials include *in vitro* cytotoxicity assays (Schwarze *et al.* 2002a,b) and local toxicity reactions by subcutaneous (Kaplan *et al.* 2003) or intraosseous implantation (Bhambhani & Bolanos 1993) of materials in laboratory animals and human subjects (Bernath & Szabo 2003). Although cell culturing methods give valuable information about the response of specific cells to a test material, they do not provide the full picture of how a tissue reacts to the material under *in vivo* conditions (Sundqvist & Figdor 2003). *In vivo* implantation experiments provide more complete and

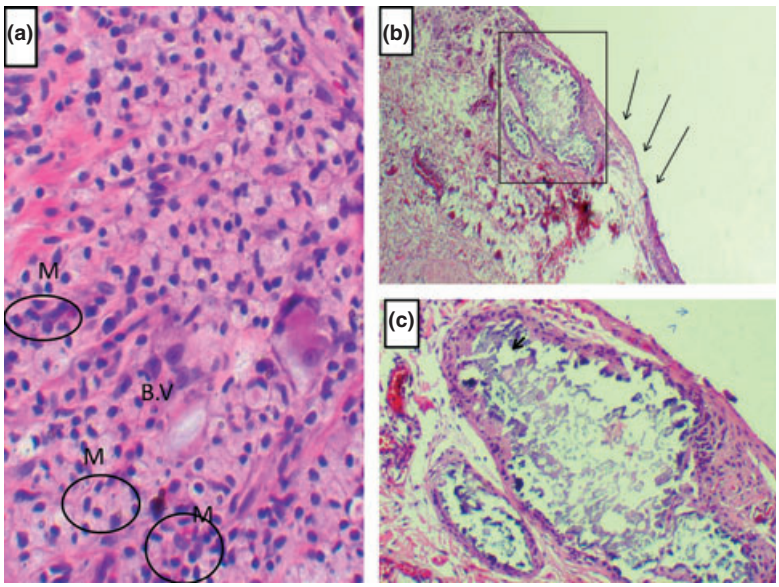

Figure 4 (a) CPS-1 at 60 days moderate inflammatory reaction of lymphocytes and plasma cells, perivascular mast cells (M) in close proximity to blood vessel (B.V) (H/E × 200). (b) CPS-2, Acroseal and control (empty tube) at 60 days. Note the formation of a capsule-like fibrous tissue (arrows) (H/E × 40). (c) A reduced number of inflammatory cells (H/E × 120).

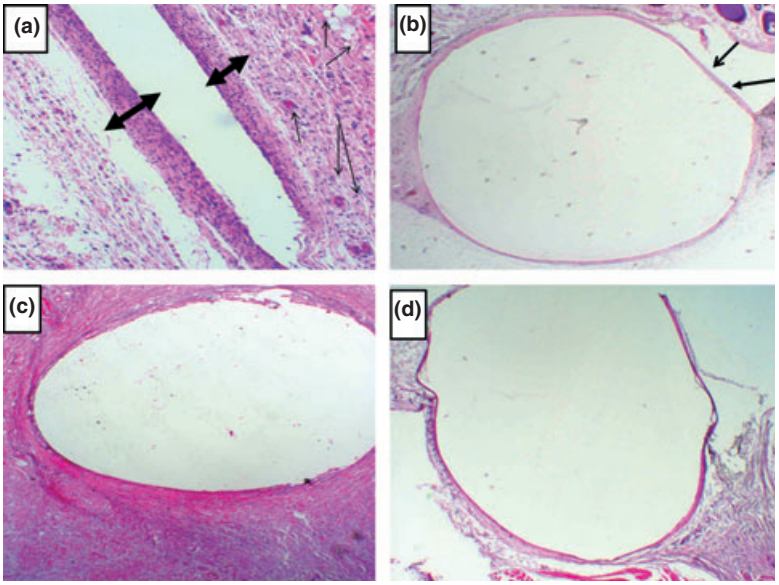

Figure 5 (a) CPS-1 at 90 days thick fibrous encapsulation (arrows) at the open end of the tube, red cells and vacuolated dead cells (arrows) (H/E × 100). (b) CPS-2 at 90 days mild inflammatory response, thin fibrous capsule (arrows) (H/E × 100). (c) Acroseal at 90 days. Note the thin fibrous capsule (H/E × 100). (d) A salient fibrous capsule at the open end of control group (empty tube) at 90 days (H/E × 100).

clinically relevant information on the long-term tissue response (Langeland 1978).

Several reports (Quinalan *et al.* 2002, Sanders & Rochefort 2003) have stated that inflammation is an important criterion when evaluating biocompatibility. It is known that the measurement of fibrous capsule thickness is a good marker of inflammation (Quinalan *et al.* 2002, Sanders & Rochefort 2003). The current study was therefore based on the assumption that there was a meaningful relationship between inflammation and fibrous capsule thickness. The observations of decreased inflammatory cell numbers and increased thickness of the fibrous capsules provide evidence of a direct relationship between these two parametric values for CPS-2 sealer and Acroseal at 7, 15 and 30 days.

In the present study, the inflammatory reactions of the control group and all tested materials (CPS-1, CPS-2 and Acroseal) at 7 days were more severe than at 15 and 30 days. This condition is expected to be caused partly by the surgical trauma produced during the placement of the tubes in addition to the toxic effects of the implanted material and the tube material itself (Zmener *et al.* 1988). At this time, the tissue was disorganized and infiltrated with neutrophils, which is consistent with the findings of other studies (Spang berg & Langeland 1973).

The inflammatory reaction for CPS-2 sealer and Acroseal become milder by the 30th day. A stronger action of the sealers in the beginning and attenuation of the inflammatory response over time has been reported elsewhere (Kolokuris *et al.* 1996, Gomes *et al.* 2004), and the results of this study concur with these studies after a 30-day period.

Subcutaneous implantation of CPS-2 and Acroseal elicited a similar pattern of irritation and connective tissue response, as measured by inflammatory reaction scores and fibrous capsule thickness. The response of tissues to the effects of CPS-2 and Acroseal was examined at 7, 15, 30, 60 and 90 days. Seven-day results of the tested materials revealed severe inflammatory responses, but these subsided by the 60th day. Additionally, the condition of the fibrous connective tissue around the materials indicated that they were well tolerated and induced the formation of a collagenous capsule with negligible or no inflammation.

There were no significant differences for fibrous tissue thickness between CPS-2 and Acroseal at 7th, 15th, 30th, 60th and 90th days ($P > 0.05$). This situation could be explained by the presence of inflammatory cells (macrophages, giant cells and neutrophil leukocytes) owing to the implantation process. Both sealers (CPS-2 and Acroseal) resulted in a reduction in

the inflammatory reaction leading to a less thick fibrous capsule at the 60th and 90th day. Based on the results of the present investigation, it appears that both CPS-2 and Acroseal can be accepted as biocompatible root canal filling materials in this experimental model.

On the other hand, CPS-1 elicited a severe inflammatory reaction at 7 days, which decreased by the 15th and 30th day; however, at 60 and 90 days, a persistent inflammatory response was observed. CPS-1 caused a greater inflammatory response compared to CPS-2 and Acroseal groups with the appearance of a few giant cells at (15 and 30 days of implantation). Giant cells are known to participate in the reaction to foreign bodies and are associated with the presence of material that the body finds difficult to break down. In the present study, CPS-1 sealer broke down slowly, illustrated by the dispersed material and subsequent endocytosis by the foreign body giant cells, which might have been the cause of the persistent chronic inflammation. Additionally, the CPS-1 sealer was associated with the presence of mast cells at 60 days, mainly in the surrounding area of blood vessels; this suggests a late hypersensitivity reaction to the composition of the implanted material. Although the late hypersensitivity reaction to endodontic materials is not frequently reported (El Sayed *et al.* 1995), these adverse systemic reactions that occur more frequently than reported in the literature should not be dismissed (Ebner & Kraft 1991). According to Bratel *et al.* (1998), endodontic materials can induce a late hypersensitivity reaction even in subtoxic concentrations. Additionally, the observed increasingly thickened fibrous connective tissue capsule forming around the tested samples of CPS-1 sealer and the comparisons between revealed groups that there were significant differences for fibrous tissue thickness between 7, 30 and 90 days, respectively. At the 90-day period, there was a marked increase in the thickness of fibrous connective tissue around the material which suggests that CPS-1 was not well tolerated by the tissue.

The difference in inflammatory response for CPS-1 sealer is related to a number of factors including the variation in chemical composition and the quantity of chemotoxic leachables migrating from the unreacted polyacrylic acid, which has a low pH and may leak gradually to the surrounding humid connective tissue during the setting process (Miyazaki *et al.* 1993, Safavi *et al.* 2002). This is especially true at the initial assessment period with the effects becoming milder as the toxic material is neutralized by local lymphatic drainage. Later, harmful effects of a material are considered to be more important than its initial effects (Yaltirik *et al.* 2004). The results of the histometric evaluation indicate that the least favourable tissue reaction was observed with CPS-1 sealer. The inflammatory responses caused by CPS-2 and Acroseal sealer, however, were acceptable at the 90th day demonstrating that they were well tolerated by the tissues. The inflammatory reaction was severe during the first experimental period, and finally after 90 days, a picture of complete healing was observed. On the contrary, CPS-1 initially caused severe inflammation of the adjacent tissues, and a moderate chronic inflammation remained after 90 days. Biocompatibility is as important as the physical and chemical features when selecting a material for root canal filling because of its potential contact with vital tissues.

Conclusion

Within the limitations of this *in vivo* study, CPS-2 sealer and Acroseal had a favourable biocompatibility level base.

References

Abd-el Fattah WI, Selim MM (1991) Thermal behavior and structural variations of both chemically precipitated and biological hydroxyapatite. *Journal of Ceramica Acta* **3**, 613–8.

Bermudez O, Boltong MG, Driessens FCM, Planella JA (1994) Optimization of a calcium phosphate cement formulation occurring in the combination of monocalcium phosphate monohydrate with calcium oxide. *Journal of Materials Science: Materials in Medicine* **5**, 67–71.

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

Bhambhani SM, Bolanos OR (1993) Tissue reactions to endodontic materials implanted in the mandibles of guinea pigs. *Oral Surgery, Oral Medicine, Oral Patholgy, Oral Radiology and Endodontics* **76**, 493–501.

Bratel J, Jontell M, Dahlgren U, Bergenholtz G (1998) Effects of root canal sealers on immunocompetent cells *in vitro* and *in vivo*. *International Endodontic Journal* **31**, 178–88.

Costa CA, Teixeira HM, do Nascimento AB, Hebling J (2000) Biocompatibility of two current adhesive resins. *Journal of Endodontics* **26**, 512–6.

Ebner H, Kraft D (1991) Formadehde-induced anaphylaxis after dental treatment? *Journal of Contact Dermatitis* **24**, 307–9.

El Sayed F, Seite-Bellezza D, Sans B, Bayle-Lebey P, Marguery MC, Bazex J (1995) Contact urticaria from formaldehyde in

a root-canal dental paste. *Journal of Contact Dermatitis* **33**, 353.

Fukase Y, Eanes ED, Tagaki S, Chow LC, Brown WE (1990) Setting reaction and compressive strength of calcium phosphate cements. *Journal of Dental Resarch* **69**, 1852–6.

Geurtsen W (2001) Biocompatibility of root canal filling materials. *Australian Endodontic Journal* **2**, 12–21.

Gomes BP, Pedroso JA, Jacinto RC *et al.* (2004) *In vitro* evaluation of the antimicrobial activity of five root canal sealers. *Brazalian Dental Journal* **15**, 30–5.

Gutmann JL, Witherspoon DE (2002) Obturation of the cleared and shaped root canal system. In: Cohan S, Burns RC, eds. *Pathways of the Pulp*, 8th edn. St. Louis: CV Mosby, 298–301.

Hauman CHJ, Love RM (2003) Biocompatibility of dental materials used in contemporary endodontic therapy: a review, part 2: root-canal-filling-materials. *International Endodontic Journal* **36**, 147–60.

Ho YC, Huang FM, Chang YC (2006) Mechanisms of cytotoxicity of eugenol in human osteoblast cells *in vitro*. *International Endodontic Journal* **39**, 389–93.

Hong CY, Lin SK, Kok SH, Wong MY, Hong YC (1990) Histological reactions to a newly developed calcium phosphate cement implanted in the periapical and periodontal tissues. *Journal of the Formosan Medical Association* **89**, 297–304.

Huang FM, Tsai CH, Yang SF, Chang YC (2005) Induction of interleukin-6 and interleukin-8 gene expression by root canal sealers in human osteoblastic cells. *Journal of Endodontics* **31**, 679–83.

Ioannis K, Nikolaos E (1998) *In vivo* comparison of the biocompatibility of two root canal sealers implanted into the subcutaneous connective tissue of rats. *Journal of Endodontics* **24**, 82–5.

Jarcho M (1986) Biomaterial aspects of calcium phosphates: properties and applications. *Dental Clinics of North America* **30**, 25–47.

Kaplan AE, Ormaechea MF, Picca M, Canzobre MC, Ubios AM (2003) Rheological properties and biocompatibility of endodontic sealers. *International Endodontic Journal* **36**, 527–32.

Khashaba RM, EL-Din Sanad M, EL-Khodary A (2006) Effect of certain additives on some properties of three formulated calcium phosphate cements. PhD Thesis. Cairo University, Cairo, Egypt.

Khashaba RM, Chutkan NB, Borke JL (2009) Comparative study of biocompatibility of newly developed root canal sealers on fibroblasts derived from primary human gingival and a mouse l929 cell line. *International Endodontic Journal* **42**, 711–8.

Khashaba RM, Lockwood PE, Lewis J, Messer RLW, Borke JL (2010) Cytotoxicity, calcium release, and pH changes generated by novel calcium phosphate cement formulations. *Biomedial Materials Research* **93**, 297–303.

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

Langeland K (1978) Correlation of screening tests to usage tests. *Journal of Endodontics* **4**, 300–3.

Lee J, Kim Y, Bae K (2002) Biocompatibility of two newly-developed resin-based root canal sealers. *Journal of Endodontics* **28**, 263.

Miyazaki K, Horibe T, Antonucci JM, Takagi S, Chow LC (1993) Polymeric calcium phosphate cements: analysis of reaction products and properties. *Dental Materials* **9**, 41–5.

Modaresi J, Yavari SA, Dianat SO, Shahrabi S (2005) A comparison of tissue reaction to MTA and an experimental root-end filling material in rats. *Australian Endodontic Journal* **31**, 69–72.

National Institutes of Health (2006) Public Health Service policy on human care and use of laboratory animals. Available at htpp://grants.nih.gov/grants/olaw/references/phspol.htm.

Nguyen TN (1991) Obturation of the root canal system. In: Cohen S, Burns RC, eds. *Pathways of the Pulp*, 5th edn. St. Louis: CV Mosby, 193–281.

Quinalan CA, Zisterer DM, Tipton KF, O'Sullivan MJ (2002) *In vitro* cytotoxicity of a composite resin and compomer. *International Endodontic Journal* **35**, 47–55.

Safavi KE, Pascon EA, Langland K (2002) Evaluation of tissue reaction to endodontic materials. *Journal of Endodontics* **28**, 749–53.

Saidon J, He J, Zhu Q, Safavi K, Spangberg LS (2003) Cell and tissue reactions to mineral trioxide aggregate and Portland cement. *Oral Surgery, Oral Medicine, Oral Patholgy, Oral Radiology and Endodontics* **95**, 483–9.

Sanders JE, Rochefort JR (2003) Fibrous encapsulation of single polymer depends on their vertical dimension in subcutaneous tissue. *Biomedial Materials Research* **67**, 1181–7.

Schwarze T, Fiedler I, Leyhausen G, Geurtsen W (2002a) The cellular compatibility of five endodontic sealers during the setting period. *Journal of Endodontics* **28**, 784–6.

Schwarze T, Leyhausen G, Geurtsen W (2002b) Long-term cytocompatibility of various endodontic sealers using a new root canal model. *Journal of Endodontics* **28**, 749–53.

Spang berg L, Langeland K (1973) Biological effects of dental materials. *Oral Surgery, Oral Medicine, Oral Pathology* **35**, 402–14.

Sugawara A, Nishiyama M, Kusamo K *et al.* (1992) Histopathological reaction of calcium phosphate cement. *Dental Materials* **11**, 11–6.

Sundqvist G, Figdor D (2003) Endodontic treatment of apical periodontitis. In: Ørstavik D, Pittford TR, eds. *Essential Endodontology*, 5th edn. Oxford: Blackwell Scientific Publications, 242–77.

Yaltirik M, Ozabos H, Bilgic B, Issever H (2004) Reactions of connective tissue to mineral trioxide aggregate and amalgam. *Journal of Endodontics* **30**, 95–9.

Yoshikawa M, Hatami S, Tsuji I, Toda T (1997) Histopathological study of a newly developed root canal sealer

containing tetracalcium-dicalcium phosphates and 1.0% chondroitin sulfate. *Journal of Endodontics* **23**, 162–6.

Yuan H, Li Y, de Bruijn JD, de Groot K, Zhang X (2000) Tissue responses of calcium phosphate cement: a study in dogs. *Biomaterials* **21**, 1283–90.

Zmener O, Guglielmotti MB, Cabrini RL (1988) Biocompatibility of two calcium hydroxide- based endodontic sealers: a quantitative study in the subcutaneous connective tissue of the rat. *Journal of Endodontics* **14**, 229–35.

Zmener O, Guglielmotti MB, Cabrini RL (1990) Tissue response to an experimental calcium hydroxide-based endodontic sealer: a quantitative study in subcutaneous connective tissue of the rat. *Endodontics and Dental Traumatology* **6**, 66–72.

Zmener O, Banegas G, Pameijer CH (2005) Bone tissue response to a methacrylate-based endodontic sealer. A histological and histometric study. *Journal of Endodontics* **31**, 457–9.

