Periapical Tissue Responses and Cementum Regeneration with Amalgam, SuperEBA, and MTA as Root-End Filling Materials

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Abstract

The purpose of this study was to compare the periapical tissue responses and cementum regeneration in response to three widely used root-end filling materials, amalgam, SuperEBA, and Mineral Trioxide Aggregate (MTA). These materials were placed using modern microsurgical techniques on endodontically treated dog premolars and molars. After 5 months, the cell and tissue reactions of surface-stained un-decalcified ground sections were evaluated by light microscopy and statistically analyzed. The major difference in the tissue responses to the three retrofilling materials were the degree of inflammation and types of inflammatory cells, number of fibrous capsule formations, cementum neoformation over these materials, osseous healing and resulting periodontal ligament thickness. MTA showed the most favorable periapical tissue response, with neoformation of cemental coverage over MTA. SuperEBA was superior to amalgam as a root-end filling material.

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Numerous materials have been used as retrofilling materials: amalgam, gutta percha, composite resins, glass ionomers, zinc oxide eugenol cements, such as IRM and SuperEBA, etc. Recently, MTA was introduced as the most tissue friendly retrofilling material (1–4). Amalgam has been used over 100 yr and is still most widely used material in restorative dentistry and apical retrofilling. However, in recent years many have questioned the safety and integrity of amalgam in general and as a retroflling material in particular, as it has many disadvantages: release of ions, mercury toxicity, corrosion and electrolysis, delayed expansion, and tissue tattoos (3–5).

In 1978, Oynick and Oynick (6) suggested SuperEBA cement as an ideal retrofilling material. SuperEBA is basically a reinforced zinc oxide cement and animal studies have shown it to be better than amalgam in terms of sealability, apical tissue reactions and regeneration of apical tissues (7, 8). In a retrospective study of human teeth the SuperEBA groups results were significantly better than the amalgam groups supporting the findings of the animal studies (6). Further, the apical healing of cases with only endodontic lesion in humans was 96.8% after 1 year when SuperEBA was used in conjunction with microsurgical techniques (9).

In the mid 1990s, MTA was introduced as a retrofilling material (1–4). The main constituents of MTA are very similar to Portland cement (10, 11). Results of MTA studies from dogs and monkeys demonstrated that MTA caused significantly less inflammation than amalgam. More importantly, cementum bridges formation directly over the MTA retrofillings was frequent observation (3, 4).

A survey of the literature indicates that there is a paucity of information on tissue reactions to widely used retrofilling materials in the same experimental model, so that the suitability of these materials for root end fillings can be critically compared.

Thus, this study was conducted to compare the periapical reactions as well as cementum regeneration in contact with amalgam, SuperEBA and MTA in dog teeth using the undecalcified ground section technique that is different from the usual histological technique using decalcification.

Materials and Methods

Five healthy female Beagle dogs weighing between 7 and 9 kg were used in this study, which was conducted in accordance with a University of Pennsylvania IRB-approved animal protocol.

Each animal was anesthetized with an intramuscular injection of 0.7 mg/kg of Ace Promazine (Aveco Co., Inc., Fort Dodge, IA) as a preanesthetic and an intravenous injection of 0.7 mg/kg of Propofol for short-term anesthesia. Subsequently inhalation general anesthetic of 2 to 3% Isoflourane was administered via an endotracheal tube throughout the surgical procedures. During the surgical phase, a dose of 2% Xylocaine with 1:50,000 epinephrine was injected into the surgical site to achieve maximum hemostasis. After recovery from the general anesthesia each dog was given 0.3 mg/kg Butorphenol Tartrate (Fort Dodge Laboratories Inc., Fort Dodge, IA) as an analgesic and was kept in a recovery area for observation.

Induction of periapical lesions: the pulp tissues of molars and premolars were removed from the canals and contaminated plaque paper points were placed into the canals and sealed with IRM for 2 wk. Periapical lesions formed between 4 to 6 wk and were verified radiographically. At this point the teeth were treated by conventional endodontics and the access cavities were restored with IRM.

As a surgical stage, full mucoperiosteal triangular flaps were made with vertical incisions at the mesial line angle of the cuspids and intrasulcular incisions extending to the mesial of the second molars. The cortical and cancellous bones at the apices were removed with a water cooled high-speed rotary instrument, creating osseous cavities of about 4×4 mm in diameter. After resection of each root end and removal of the radicular lesion, a 3-mm deep root-end cavity was prepared by ultrasonication (Obtura/Spartan, Fenton, MO). All surgical procedures and retropreparatons were done under an operating microscope.

The resected roots of 24 teeth were randomly assigned to three groups. In group A root-ends were filled with Amalgam (Tytin, Kerr Mfg., Co., Romulus, MI), in group B SuperEBA (Bosworth Co., Skokie, IL) was used and in group C the root end filling was MTA (Dentsply/Tulsa Dental, Tulsa, OK).

Four months after the last surgical procedure, the dogs were sacrificed with iv overdoses of sodium pentobarbital (Nembutal, Abbot Labs., N. Chicago, IL). The jaws were perfused with a mixture of 10% Buffered Formalin and 80% Ethanol via the carotid arteries. The jaws were then prepared for histological evaluation, which were done by a sawing and grinding technique, developed by Donath and Breuner (12) and Plenk (13) for the examination of undecalcified bone and teeth with attached soft tissue. After fixation for 1 month, the demineralized specimens were processed for embedding with methylmethacrylate monomer (No. 8060061 Merck, Darmstadt, FRG). After polymerization of the methacrylate, sections were cut from the block with an Isomet low speed saw (No. 11-1180, Beuhler Ltd., Lake Buff, IL). The final thickness of each specimen slide was approximately 80 microns in a buccolingual plane. The resulting ground sections were surface-stained with Giemsa solution (Giemsa's azur-eosin-methylene-blue, Merck AG, Darmstadt, FRG), and contact-microradiographs were prepared (13). The microscopic evaluation was made on a Nikon FXA microscope (Nikon, Japan) and black/white and color micrographs were taken.

The surface-stained ground sections were evaluated by two histologists for the degree of inflammatory cell response adjacent to the root-end filling materials. In addition, specimens were examined for periodontal ligament thickness and osseous healing and for fibrous capsule formation and cementum regeneration at the resected root ends. The specific parameters of evaluation and grading are presented in Table 1.

The distance from root-end fillings to regenerated bone was calculated with an image software at three points: the buccal margin, the center and the lingual margin of the root-end fillings.

A computer-assisted histometric analysis of the histology slides was done using Sigma Scan/Image software (Jandel Scientific Software, San Rafael, CA), while the statistical analysis was performed using Sigma Stat software (Jandel Scientific Software). Statistical values for each group of data were calculated with Chi-square.

TABLE 1. Scoring criteria for histologic evaluations

Acute inflammatory cells infiltrated	
1	<25%, PMN infiltrate adjacent to the resected root-end and root-end filling
2	>25% <50%, PMN infiltrate adjacent to the resected root-end and root-end filling
3	>50% <75%, PMNeinfiltrate adjacent to the resected root-end and root-end filling
4	>75%, PMN infiltrate adjacent to the resected root-end and root-end filling
Chronic inflammatory cells infiltrated	
1	<25%, plasma cells and lymphocytes infiltrate adjacent to the resected root-end and root-end filling
2	>25% <50%, plasma cells and lymphocytes infiltrate adjacent to the resected root-end and root-end filling
3	>50% <75%, plasma cells and lymphocytes infiltrate adjacent to the resect root-end and root-end filling
4	>75%, plasma cells and lymphocytes infiltrate adjacent to the resected root-end and root-end filling
Cementum regeneration on the resected root surface	
0	No presence of cementum regeneration
1	<25% cementum regeneration
2	<50% cementum regeneration
3	<75% cementum regeneration
4	75% < cementum regeneration
Cementum regeneration on the material	
0	No presence of cementum regeneration
1	<25% cementum regeneration
2	<50% cementum regeneration
3	<75% cementum regeneration
4	75% < cementum regeneration
Periodontal ligament (PDL) formation	
0	No PDL adjacent to the resected root end and root-end filling
1	PDL adjacent to <25% of the resected root end and root-end filling
2	PDL adjacent to <25%, <50% of the resected root end and root-end filling
3	PDL adjacent to >50%, <75% of the resected root end and root-end filling
4	PDL adjacent to >75% of the resected root end and root-end filling
Root end encapsulation	
1	No presence; no evidence of proliferation of tissue from severed PDL
2	Present; tissue proliferating from severed PDL encapsulates less than 50% of resected 2 root surface
3	Predominant; tissue proliferating from severed PDL encapsulates more than 50% of resected root surface
4	Event complete; tissue proliferating from severed PDL encapsulates entire resected root surface

Results

Statistical evaluations of the tissue response and osseous healing are shown Tables 1 and 2.

The tissue adjacent to MTA exhibited a minor degree of inflammatory cell infiltrates, which were primarily plasma cells, lymphocytes and some macrophages, but no PMN leukocytes (Fig. 1*C*). Adjacent to the SuperEBA fillings. Moderate numbers of inflammatory cell infiltrates were observed, that consisted of plasma cells, lymphocytes, macrophages and sometimes PMNs (Fig. 1*B*). In contrast, the tissue adjacent to amalgam showed a marked inflammatory cell infiltrate, which was composed primarily of PMNs, leukocytes, some macrophages and foreign body giant cell (Fig. 1*A*). There is a significant difference. The greater degree of PMN infiltrates at the amalgam site versus the MTA site is significant, as is the difference between SuperEBA and MTA ($p < 0.05$). When the root-end materials were evaluated for the degree of inflammation, MTA and SuperEBA showed less inflammatory reaction than amalgam as root-end materials. There is a significant difference between amalgam and SuperEBA, amalgam and MTA ($p < 0.05$).

A cementum-like material was observed growing over the MTA in seven of nine sections examined. (This difference too, between MTA and the other two materials was significant $p < 0.05$.) The two types of surface reactions over MTA were a crystalline-like structure (Fig. 2*A*) and newly deposited cementum (Fig. 2*B*, *C*) that started mostly from the adjacent dentin, but was sometimes also found in islands, and finally appeared as mineralized cellular cementum.

Cementum deposition on the resected root-ends occurred significantly more in the MTA group than the other groups ($p < 0.05$). There is no significant difference between the SuperEBA and Amalgam groups.

TABLE 2. Results of statistical analysis (Chi-sqare)

Dependent Variables	p-values	Comparisons
Degree of inflammation	0.0155	A vs. M, E vs. M
Abscess formation	0.0001	A vs. M, E vs. M, A vs. E
PMN infiltration	<0.0001	A vs. M, E vs. M, A vs. E
Plasma cell and lymphocytes infiltration	0.1281	
Macrophage infiltration	0.1327	
Cementum regeneration on material	0.0500	E vs. M
Cementum regeneration on resected root surface	0.0041	A vs. M, E vs. M
New bone formation on wound site	0.0037	A vs. M
New bone formation on material site	0.0191	A vs. M
PDL regeneration (score system)	0.0211	A vs. M
PDL regeneration (% system)	0.0411	A vs. M
Root-end encapsulation	0.0192	A vs. M
Woven bone formation	0.0003	A vs. M, E vs. M, A vs. E
Bone maturation	0.0006	A vs. M, E vs. M

A, Amalgam; M, MTA; E, Super EBA.

Thick fibrous tissue capsules were present around the inflammatory cell infiltrates over most amalgam and SuperEBA root-end fillings, but not over MTA root-end fillings. There is a significant difference ($p < 0.05$) between Amalgam and MTA root-end filling.

Discussion

The present study was performed using microsurgical techniques developed in the past decade. An operation microscope (Carl Zeiss, Oberkochen, Germany), ultrasonic tips (Obtura/Spartan, Fenton, MO) and other associated microsurgical instruments were used for the preparation and filling of the teeth. A comparison of the traditional apical surgery techniques and the microsurgical techniques has been well documented (14). These less invasive, more accurate microsurgical techniques cause minimum trauma to the surrounding tissues, eliminate lingual perforations, facilitate the complete preparation and filling of the canal complex, and result in faster healing of the surgical site. These were common problems associated with the traditional technique (14).

Many histological studies of dental tissues and materials were done on decalcified paraffin sections. The decalcification procedure frequently causes artificial separation of the resected dentin surface and root-end filling material and the newly formed cementum. In the present study these problems were eliminated by using a technique in which the specimens were not decalcified. Thus, the exact relationship between the resected dentin surface or root-end filling material and newly formed cementum could be examined closely. The technique used in this study was developed by Schenk (15), Donath and Breuner (12), and refined by Plenk (13).

This study was limited to the histological examination of the responses to the filling materials. The materials were chosen because they are used most frequently by clinicians. Amalgam has been and still is the most widely used material. SuperEBA was very popular in the 1990's and was slowly replacing amalgam as "the" material in endodontic practice. MTA is a relatively new material that became available in the late 1990's. This material appears to be the most promising to date, as it comes closest to being the ideal material for retrofilling and the results of reported studies are indeed impressive (1–4, 11).

Severe inflammatory responses to amalgam root-end fillings in dogs, that are characterized by the presence of many acute inflammatory cells, have been reported (3, 17), and the presence of a low-grade chronic inflammation following 10 to 15 wk after amalgam root-end fillings in ferrets has also been presented (18). Our histological findings agree closely with these findings, that amalgam causes an acute inflammatory reaction.

Similar to the results of the monkey studies by Torabinejad et al. (4), our study also showed that PMN leukocytes were frequently observed close to the amalgam together with macrophages. Foreign body giant cells and fibrous tissue capsules were found in close proximity to most amalgam root-end fillings in 5-months postoperative specimens. Results of the present study together with other studies clearly show that amalgam is not biologically suitable as a retrofilling material. The question is how amalgam retrofilling teeth are considered clinically successfully healed despite the significant inflammatory reaction of the periapical tissues. Possibly, clinical success of apical surgery is defined as being asymptomatic and showing reasonable radiographic healing, that can be achieved in the presence of an inflammatory reaction. As long as the body's forceful healing powers are stronger than the destructive processes, the inherent biological incompatibility of materials cannot be shown radiographically.

SuperEBA, when compared with glass ionomer cement, amalgam, IRM, and composite resin, is superior with the lowest number of inflammatory cells present (7). Further, the presence of giant cells on the surface of SuperEBA was reported (8).

In contrast, when comparing SuperEBA with IRM and amalgam it demonstrated a greater inflammatory response in the rat tibia preparation (19). In our present study, the SuperEBA group showed fewer inflammatory cell infiltrates than the amalgam group, but more than the MTA group. Plasma cells, lymphocytes, and Giant cells were found frequently with a small presence of PMNs.

Regeneration has been defined as the replacement of tissue components in their appropriate locations, in the correct amounts and the correct relationship to each other (20). This means the reformation of the bone in the surgical site, adjacent to a fully reconstituted periodontal ligament, attached to newly formed cementum over the resected root end and root-end filling material (11). Many root-end filling material didn't show newly formed cementum over the materials except for composite resin (21), MTA (3, 4), and Diaket (22, 11). Our study shows that there was no cementum growth over Amalgam and SuperEBA, but in most cases (7 of 9 sections) cementum grows over MTA. As shown in Fig. 2*B* newly formed cementum usually started from the margin of the resected dentin and gradually migrated over the MTA. We found two different calcified material deposits over MTA: the crystalline-like structure (Fig. 2*A*) and newly deposited cementum (Fig. 2*B*).

Trope et al. (7) showed the basophilic line adjacent to the SuperEBA as root-end filling material in only one case and suggests that this basophilic staining line could be considered as an early histologic sign of hard tissue formation that has been described by Schroder and Granath (23) in their pulp capping studies. In the present study close to

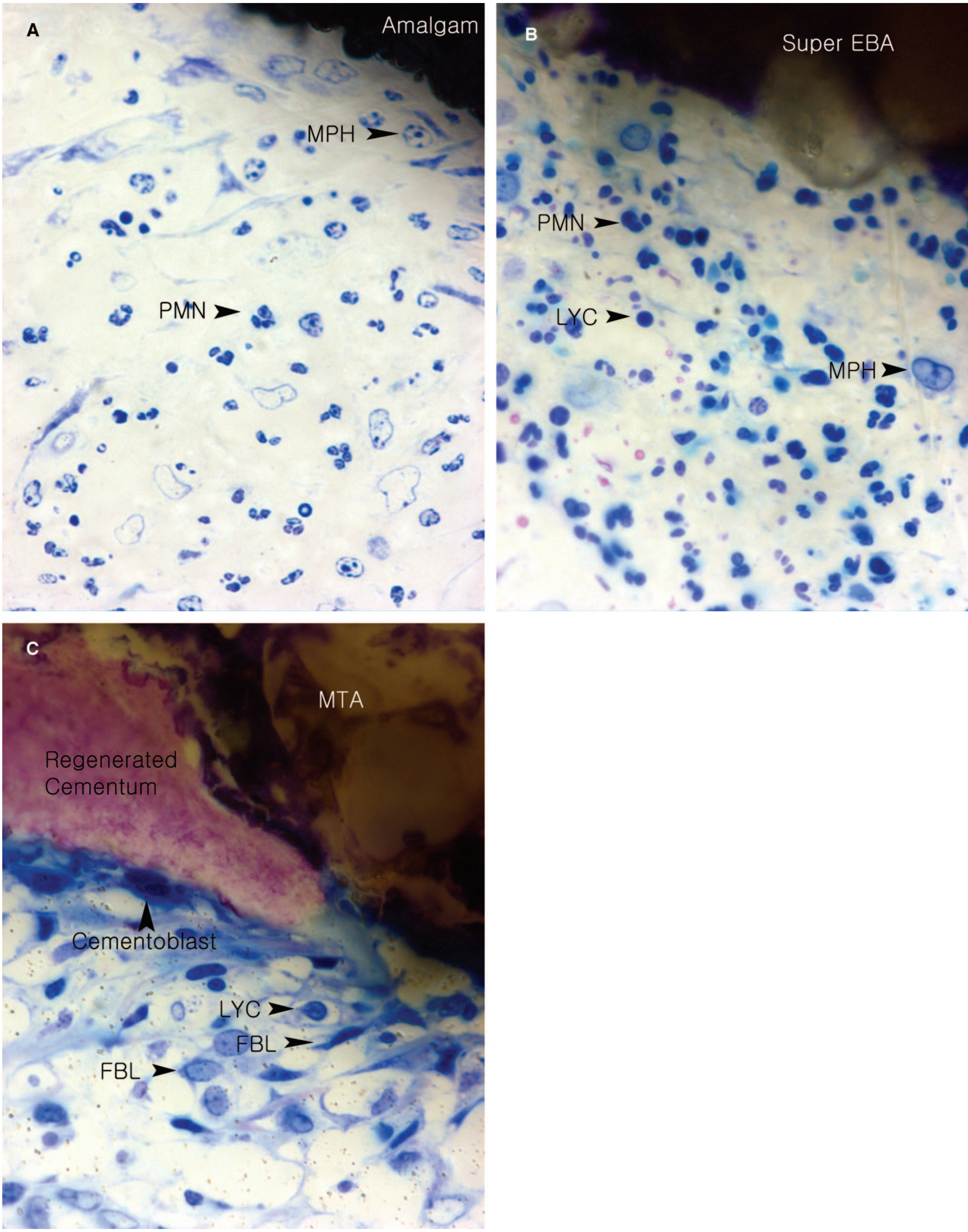

Figure 1. Inflammatory cell infiltrate on the root-end filling materials. Giemsa; ×800. (*A*) Amalgam specimen; marked acute inflammatory cell infiltrate mainly composed of polymorphonuclear leukocytes (PMN) and some macrophages (MPH). (*B*) SuperEBA specimen; moderate inflammatory cell infiltrate that consisted of PMNs, but also lymphocytes (LYC) and plasma cells and macrophages (MPH). (*C*) MTA specimen; granulation tissue with fibroblasts (FBL) with minor inflammatory cell infiltrate composed mainly of plasma cells and lymphocytes and some macrophages. Note the regenerated cementum on the MTA.

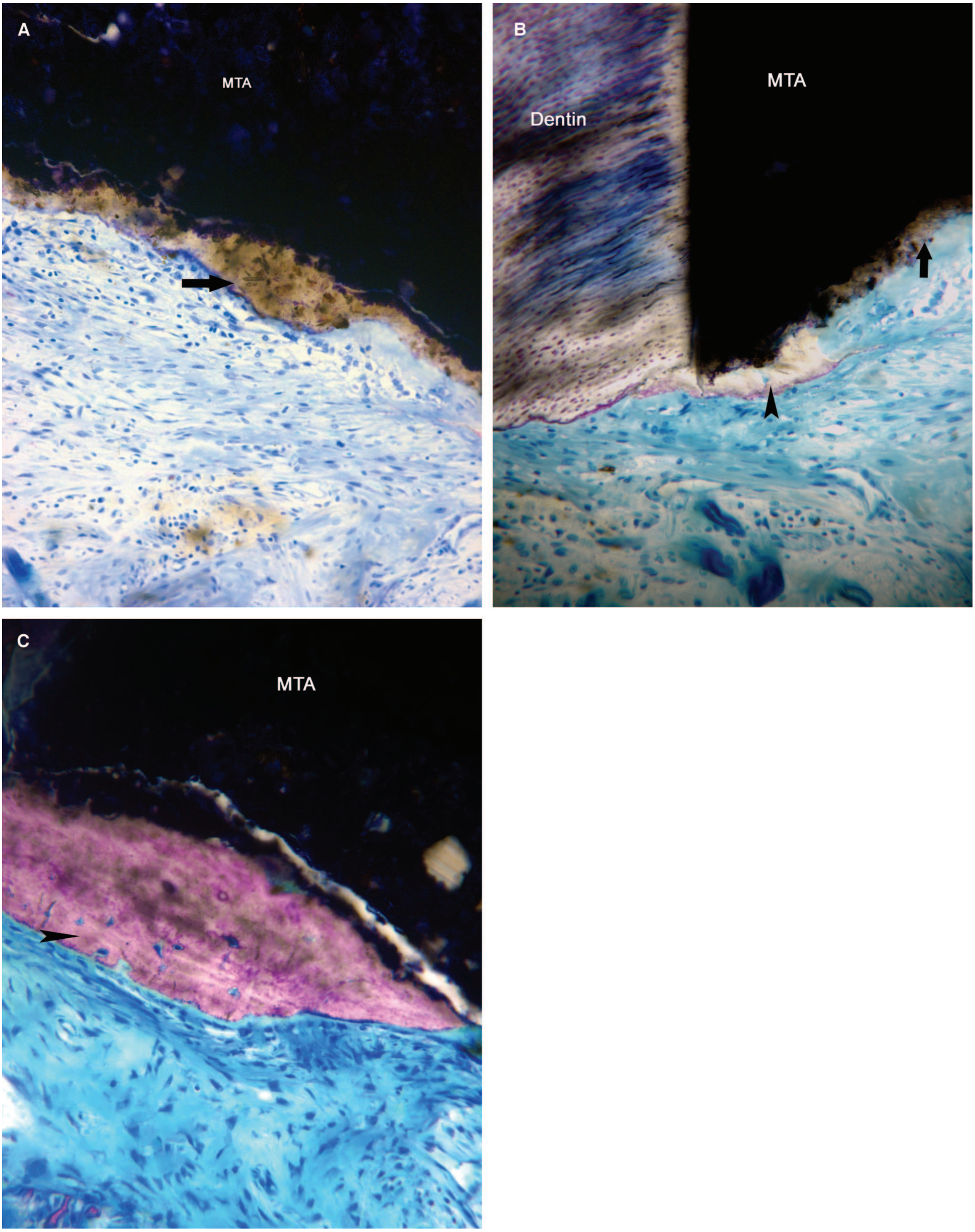

Figure 2. Cementum-like material deposition on the MTA root-end filling. (*A*) Crystalline-like structure growing over MTA (arrow) and beginning basophilic cememtum formation of cememtoblasts (CBL) in a fibrous tissue with some inflammatory infiltrates. Giemsa; ×200. (*B*) Newly formed cementum (arrow head) usually started from the margin of the resected dentin over crystalline-like structure (arrow) on MTA Giemsa; ×100. (*C*) Newly deposited, mineralized cellular cementum (arrow) growing over MTA Giemsa; ×200.

80% of sections showed these basophilic seams over the more crystalline-like structure on the surface of MTA (Fig. 2A).

From the SEM study of the dentinogenic effect of MTA in capping experiments, Tziafas et al. (24, 25) showed the formation of a superficial layer of crystalline structures onto the pulpal surface of MTA, and suggests a fibrodentinal nature of the newly synthesized matrix formed along the MTA-pulp interface. The source or origin of the new cementum is not clearly understood. Two possibilities exist; one derived from the remaining periodontal ligament (26, 29) or one from the growing connective tissue from bone (4). We observed that the pattern of newly formed cementum is not always from the margin of the resected dentin. In a couple of sections, a small island of cementum was found covering only MTA, totally isolated from the resected dentin. This finding suggests that cementum covering the resected root surface and MTA retrofilling may have originated from both periodontal ligament and alveolar bone. The layer of cementum over the MTA showed irregularities the same as the finding of Torabinejad et al. (4) and Regan et al. (11). However, in this study a complete cover of cementum over MTA could not be observed in any of the sections. Possibly, this is because of the short observation period of only 5 months.

Conclusion

The major differences among periapical tissue responses to amalgam, SuperEBA and MTA as root-end filling materials are the degree of inflammation and type of infiltrated inflammatory cells, frequency of fibrous capsules, the cementum formation over these materials and periodontal ligament thickness. MTA was the best material overall, although SuperEBA was better than amalgam as a root-end filling material. An important finding was that newly formed cementum coverage occurred only with the MTA group, suggesting that a biological barrier at the apex can be obtained only with MTA.

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