Dentin and osteodentin matrix formation in apicoectomized replanted incisors in cats

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The reestablishment and rate of osteodentin and dentin matrix formation in 27 apicoectomized replanted and 20 control incisors in cats were studied after Procion H8-BS vital staining. In control teeth the pattern of matrix formation differed in the various pulpal zones, with a higher rate of matrix formed toward apical areas, most dominantly in maxillary incisors. Osteodentin formation could be traced after a lag period of more than 10 days after replantation. Thirty and 60 days postoperatively osteodentin matrix was found in the total pulpal length in 83% and 73% of the teeth, respectively. A common finding was a tubular osteodentin matrix in the pulpal apical third in the replanted teeth. Tubular osteodentin matrix was, however, present most incisally in some teeth 60 days postoperatively. Internal resorption corresponding to outer cervical lesions dominated the pulpal reactions in the maxillary replanted teeth after 60 days. It is concluded that under the present experimental conditions the pulp tissue possesses a high healing potential and that the osteodentin formation reflects the pulpal healing pattern after replantation traumas. The results also indicate that successful pulpal healing depends on unexposed dentinal tubules. □ *Dental pulp; Procion H8-BS; pulp reactions; reparative dentin*

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A common reaction pattern after traumatic injuries to the dental pulp is the formation of irregular dentin or so-called osteodentin. Several histologic (1–3) and roentgenologic (4, 5) studies have thus shown excessive hard tissue formation in the pulp of replanted teeth. Anderson et al. (2), however, have described relatively great morphologic variations in the pulpodentinal tissue responses.

Maintained pulp vitality, especially in young teeth, after replantation is important because further root development may be facilitated. The deposition of calcified tissue on the dentinal walls may strengthen the existing root structure, and inflammatory resorptions on the root surface may be avoided.

There is some histologic evidence from both human and animal experiments that the pulpal tissue and the odontoblasts may survive the replantation trauma (1, 6, 7). The survival or healing capacity of the pulp has partly been related to the extent of apical contact between pulp and periodontal tissues (5, 8).

An adequate indication of the vitality of pulp cells has been their ability to continue hard-tissue formation, a criterion widely used for functioning pulp tissue in post-traumatic roentgenologic evaluation (5, 9). However, relatively little experimental evidence is available concerning the incidence and reestablishment of the hard-tissue matrix elaboration in the pulp after a general trauma or a replantation trauma (2, 7).

The aim of the present investigation was therefore to study the matrix-producing capacity of pulpal cells, or their successors, and their potential for reestablishment of matrix production after a replantation trauma. The physiologic dentin formation in control cat teeth was also studied. In addition, the apical pulpal width was observed and the influence of the extraction procedures on the pulpal healing was examined.

Visualization of hard-tissue formation in control and replanted apicoectomized cat teeth was performed by vital staining with Procion brilliant red H8-BS, to semiquantify the hard tissue formation at 10, 30, and 60 days. This vital staining technique has previously been used to investigate hard tissues in growing bone and teeth (10–12), with reliable results.

Materials and methods

Mature third incisor teeth from 12 cats, 7–9 months of age, were used in the experiment. Twenty-seven of the incisors were replanted, and 20 contralateral teeth served as controls. One tooth fractured during the extraction procedure. Preoperative radiographs were taken to verify that root development was completed.

Experimental procedure

The animals were anesthetized by intraperitoneal injections of mebumal, 50 mg/kg body weight. Additional intravenous doses of 2–4 mg mebumal were given if necessary. An in vivo label, Procion Brilliant Red H8-BS (ICI, Manchester, U.K.) at a concentration of 100 mg/ml sterile distilled water, was administered intravenously in a femoral vein at a dose of 100 mg/kg body weight. One hour after dye injection the teeth were extracted with elevator and forceps. On removal, the teeth were wrapped in a sterile saline-saturated surgical sponge. The apicoectomy was performed by preparing a circumferential groove in the dentin with a round bur in a slow-speed hand piece, cooled with sterile saline. The procedure was then completed with a scalpel. The roots were flushed with saline and replanted within 10 min after extraction. No fixation of the teeth was used. An antibiotic, Ditardopen Leo Vet® (Løwens Kjemiske Fabrik, Norway), 1 ml/kg body weight, was administered intramuscularly after tooth replantation and on the 2nd day postoperatively. The animals were kept on a soft diet for 1 week.

After 10, 30, and 60 days the animals were given an overdose of mebumal, followed by perfusion with 1% glutaraldehyde and 4% formaldehyde after flushing with saline containing 0.003% heparin. The teeth with surrounding tissues were removed and postfixed for 24 h in the same fixative. The specimens were demineralized in 0.5 M ethylenediamintetraacetic acid containing 0.25 M sucrose, pH 7.2. The end point of the decalcification was determined radiographically. The demineralized teeth were embedded in paraffin and sectioned serially in a longitudinal direction at 5 μm. The sections were alternatively prepared for routine histology, stained with hematoxylin and eosin, or left unstained for examination in ultraviolet light.

Registration procedure

Procion-labeled material. The sections were examined in a Leitz fluorescent microscope, using a BG 12 5-mm excitation filter and a 490 barrier filter. For the registration procedure a micrometer eyepiece with a magnification of ×125 was used.

For measurement of the postoperative dentin and osteodentin formation at different tissue levels, the pulp in the apicoectomized replanted teeth were subdivided transversally in equal zone lengths in coronal, cervical, and intermediate zones. In the control teeth an additional apical zone was present (Fig. 1). The total pulpal length was measured. Sections from the most central part showing the entire pulpal length were used for registration. The sections were considered acceptable for measurements when the bulk of the preexperimental dentinal tubuli could be observed without interruption in each of the zones described above.

The greatest amount of postexperimental dentin and osteodentin formed in control and replanted teeth corresponding to levels A, B, C, D, and E (Fig. 1) were measured in micrometers in the directions of preexperimental dentinal tubules, for calculation of mean values. Recordings of less than 4 μm were not registered as matrix formation. Teeth with pulpal necrosis were excluded when the means for osteodentin formation were calculated.

All evaluations for one specimen were

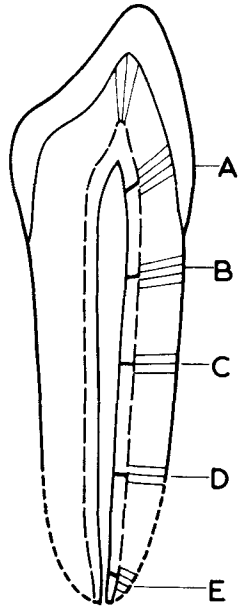

Fig. 1. Schematic drawing of a cat third incisor with the different levels for dentin/osteodentin matrix registration. A = incisal; B = cervical; C = midroot; D = apical third; and for the control teeth, E = apical (levels indicated with heavy line). Corresponding pulpal zones: AB = coronal zone; BC = cervical zone; CD = intermediate zone; and DE = apical zone of the control teeth.

made independently on two adjacent sections in accordance with the described criteria. In replanted teeth with internal resorptions the formed matrix could not be registered at all pulpal levels owing to resorbed tissue. Differences were tested with Wilcoxon's rank-sum test, when appropriate. Statistical significance is defined as $p < 0.025$. The registrations were made by the same investigator.

To evaluate the measuring error, duplicate registrations with a minimum time interval of 7 days were carried out at 21 pulpal levels of 5 randomly selected control teeth. The reproducibility was expressed as the percentage of the two readings giving the same result in the two evaluations. The readings were identical in 88% of the registrations. In 8% the difference between the first and second registration was 1–8 μm and in 4% more than 8 μm.

The apical diameter of the pulp corresponding to the start of the experimental period was estimated from the sections. In the replanted teeth the apical pulpal diameter was measured at the level of apicoectomy (D) and for the control teeth at level E. Postexperimental formation of cementum at the root surface was also registered, as was the presence of internal and external resorptions.

Hematoxylin- and eosin-stained material. The tissue reactions in the odontoblast layer and the main pulp tissue and the morphology of the mineralized repair tissue were studied within the different pulpal zones (Fig. 1) at the various observation periods. For the histologic examination the following criteria were used: a) odontoblast layer: normal structure, reduced, or missing; b) the main pulp tissue: normal tissue structure, altered tissue structure with presence of connective tissue cells without inflammation or scattered inflammatory cells, connective tissue stroma missing with presence of leukocytes, tissue necrosis; c) mineralized tissue: normal dentin, osteodentin, cell inclusions, dentinal tubules; presence of hard tissue formation within the main pulp; and d) internal resorptions, ankylosis, and external root resorptions as well were registered.

Results

Injection of Procion dye did not cause observable toxic manifestations in the cats or visible alterations in the microscopic structure of the regular dentin formed in the control teeth after dye injection. The Procion dye administration produced a narrow well-defined fluorescent band in the organic matrix of the dentin in the entire length of the pulp (Fig. 2). This was a consistent finding in all teeth except for five experimental teeth with internal resorptions. The mean apical diameter of control and replanted teeth at the beginning of the experimental period is given in Tables 1 and 2. The pulpal length in the control teeth varied between 3.7 and 5.5 mm, whereas the corresponding values for the experimental teeth after apicoectomy were 3.5 to 5.5 mm.

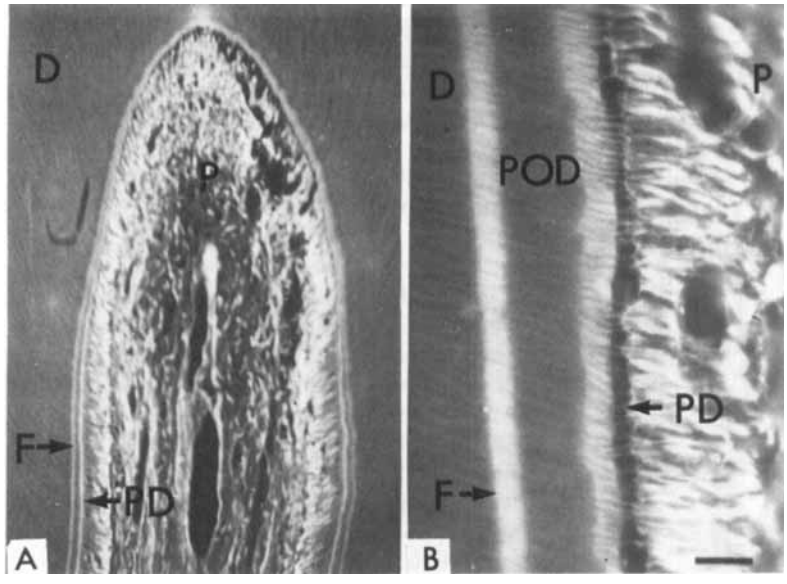

Fig. 2. Procion H8-BS-labeled control maxillary third incisor. 2A. Coronal zone. 2B. Intermediate zone. Fluorescent band (F) corresponding to the dye injection of 60 days. Most coronally a scanty amount of dentin matrix is formed. D = preexperimental dentin; POD = postexperimental dentin; PD = predentin; and P = pulp tissue (ultraviolet light). Bar = 20 μm.

The calculated mean values of post-experimental dentin and osteodentin formation in control and replanted teeth are given in Tables 1 and 2. Seven measuring sites in six maxillary and one mandibular tooth were not accessible for registration owing to curved roots in the apical region. In these teeth the pulpal diameter at the apical level could not be recorded as well. The measurements of postoperative dentin and osteodentin formation from the same pulpal level on two adjacent sections in each tooth were identical in 80% of the recordings. In 9% the difference between the two recordings from the same level was less than 8 μm but more than zero, and in 11% the difference was more than 8 μm.

Dentin and osteodentin matrix formation

Control teeth. Ten, 30, and 60 days after Procion dye injection dentin formation had occurred along the entire length of the pulp in all specimens. After 10 days the matrix elaboration was rather scanty in all pulpal zones of the two lower incisors. The mean values of dentin formation at levels A, B, C, D, and E after 10, 30, and 60 days are shown in Table 1. A considerably greater amount of dentin matrix had been produced in the maxillary than in the mandibular incisors 60 days after Procion administration, except for the most incisal level (A), where no significant difference was found.

In the mandibular incisors there was no difference in matrix production at the different pulpal levels after 10 and 30 days. After 60 days, however, there was a tendency toward increased matrix production in the apical areas (Table 1). The apical pulpal diameter in the maxillary teeth in general exceeded the corresponding recordings for the mandibular teeth (Table 1).

Replanted teeth. Out of a total of 27 replanted teeth, 7 teeth lacked formation of postoperative osteodentin matrix. In four of the teeth, this was due to pulpal necrosis. Four maxillary and one mandibular tooth showed internal resorption to various extents 60 days postoperatively. In these teeth osteodentin matrix had been elaborated before, and in adjacent areas simultaneously with, the ongoing resorption (Fig. 3).

The mean values of osteodentin matrix formation during the different experimental periods are given in Table 2. Ten days postoperatively no formation of osteodentin

Table 1. Mean dentin matrix formation (μm) at various pulpal levels (A, B, C, D, and E) at different observation periods (10, 30, and 60 days) in mandibular and maxillary control teeth. Range in parentheses

Jaw	Observation period, days	Teeth, *n*	Registrations, *n*	Apical pulpal diameter, μm	Pulpal levels				
					A	B	C	D	E
Mandibular	10	2	4	164 (104–223)	<4 (<4–<4)	<4 (<4–<4)	<4 (<4–<4)	<4 (<4–<4)	<4 (<4–<4)
	30	2	4	262 (169–354)	30 (25–35)	36 (19–54)	25 (18–31)	27 (13–40)	35 (30–39)
	60	6	12	280 (98–420)	16 (11–32)	27 (16–42)	36 (19–56)	37 (23–51)	45 (27–56)
Maxillary	10	2	4	179 (104–254)	14 (13–14)	13 (10–15)	15 (14–16)	14 (12–15)	20 (13–27)
	30	2	4	343 (339–347)	32 (32–32)	52 (39–64)	88 (63–112)	78 (74–81)	70 (69–70)
	60	6	12	679 (402–1016)	14 (11–27)	50 (38–72)	102 (57–141)	146 (99–199)	193 (140–226)

Table 2. Mean osteodentin matrix formation (μm) at various pulpal levels (A, B, C, and D) in apicoectomized replanted teeth. The apical pulpal diameter is represented by level D. Range in parentheses

Jaw	Observation period, days	Teeth, *n*	Registrations, *n*	Apical pulpal diameter, μm	Pulpal levels			
					A	B	C	D
Mandibular	10	2	4	210 (200–220)	<4 (<4–<4)	<4 (<4–<4)	<4 (<4–<4)	<4 (<4–<4)
	30	6	12	210 (112–305)	16 (9–22)	21 (16–33)	20 (<4–35)	58 (17–114)
	60	6*†	10	269 (196–404)	89 (48–139)	65 (32–93)	57 (18–97)	123 (81–219)
Maxillary	10	2†	2	262 (262–262)	<4 (<4–<4)	<4 (<4–<4)	<4 (<4–<4)	<4 (<4–<4)
	30	6††	8	279 (133–530)	2 (<4–8)	13 (8–15)	18 (15–20)	39 (19–80)
	60	5*	8‡	542 (501–582)	18 (<4–34)	14 (<4–28)	92 (92–92)	96 (83–109)

* Internal resorption (four maxillary and one mandibular tooth).
† Each sign represents one tooth with pulp necrosis.
‡ Number of registrations varies between two and eight at different pulpal levels.

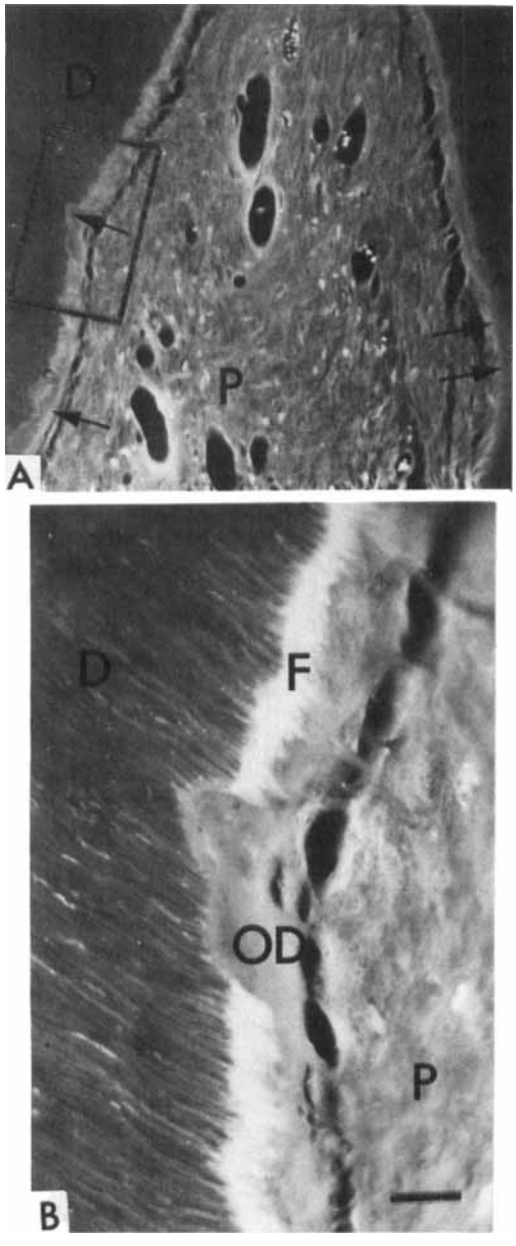

Fig. 3. A. Areas of internal resorption (arrows) in coronal zone 60 days after replantation of maxillary incisor. 3B. Higher magnification of framed area in A. Fluorescent band (F) in preexperimental dentin is resorbed. Tissue loss is repaired with osteodentin matrix (OD) in localized areas. D = dentin; P = pulpal tissue (Procion label, ultraviolet light). Bar = 20 μm.

matrix could be registered in the replanted teeth. Thirty days after Procion labeling 10 out of 12 teeth showed matrix formation in all pulpal zones. In two upper incisors, however, the matrix elaboration was scanty and inconsistent at level A and could not be measured with the present recording method (Table 2). Owing to pulpal necrosis, two maxillary teeth showed no matrix formation. Sixty days after replantation all teeth (n = 11) except 1 lower incisor showed formation of osteodentin postoperatively. Eight teeth showed matrix elaboration in the total pulp length. In four maxillary and one mandibular incisor internal resorption was present to various extents (Fig. 3). For this reason, the registration of osteodentin at levels A, B, C, and D in these teeth was in some instances impossible. The mean values of osteodentin formation in maxillary teeth in the 60-day group therefore represents relatively few observations and varies between two and eight registrations (Table 2). A striking finding in all maxillary and one mandibular tooth was a deep external tissue loss on the root surface in the gingival areas probably initiated by the extraction trauma. These external defects were partly filled in with repair tissue (Fig. 4). A consistent finding was that matrix formation subjacent to the affected dentinal tubules of the external lesion was reduced or absent (Fig. 5). Reduced matrix

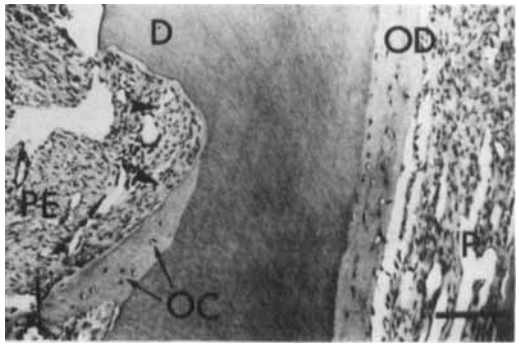

Fig. 4. External lesion in cementum and dentin loss in cervical area (arrowheads) after extraction procedure of replanted mandibular incisor. Dentin cavity partly covered with osteocementum (OC) 60 days after replantation. P = pulp tissue; OD = osteodentin matrix; D = dentin; PE = gingival connective tissue (hematoxylin and eosin stain). Bar = 100 μm.

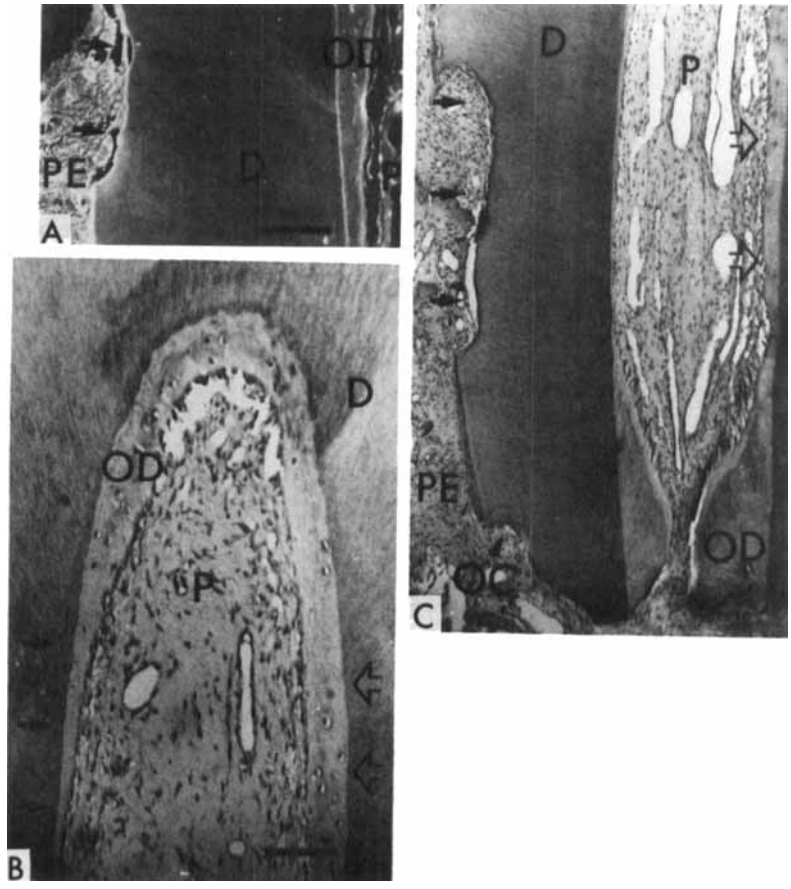

Fig. 5A and C. Reduced osteodentin formation in areas subjacent to external dentinal cavities (arrows) 60 days after replantation. 5B and C. Osteodentin formation coronally to the external cavities and in adjacent pulpal areas (open arrows) is not reduced. P = pulp tissue; OD = osteodentin; D = dentin; PE = periodontal tissue; OC = osteocementum. (A, Procion label, ultraviolet light; B and C, hematoxylin and eosin; A and B, bar = 100 μm.)

formation was also occasionally observed in relation to external root resorptions (Fig. 5).

Fig. 6 illustrates the amount of dentin and osteodentin matrix formation in control and apicoectomized replanted mandibular teeth at different pulpal levels 10, 30, and 60 days postoperatively. The most extensive matrix production was found in the experimental teeth after a 60-day period, but already at 30 days postoperatively a considerable amount of osteodentin matrix had been produced in the entire pulpal length. The amount of matrix formed at levels A, B, and C 30 days after replantation was similar to that seen in the control teeth.

In all experimental teeth the formation of osteodentin matrix started postoperatively after a delay of at least 10 days (Table 2). After this lag period, a continuous matrix elaboration was registered throughout the entire pulp (Table 2). An osteodentin matrix production averaging more than 2 μm daily occurred at the apical level (D) in the mandibular teeth. Level D showed a significantly higher matrix production both 30 and 60 days after replantation compared with the matrix formation at the other levels (A, B, and C) except for level A after 60 days (Fig. 6).

Histologic observations

Pulp tissue. In control teeth the histologic structure was in accordance with a normal cellular arrangement—that is, a regular odontoblastic layer without a cell-free zone in the subodontoblastic region (Fig. 7).

In experimental teeth a consistent histologic reaction pattern could be observed in the pulp at the different observation periods. Ten days after replantation the odontoblastic

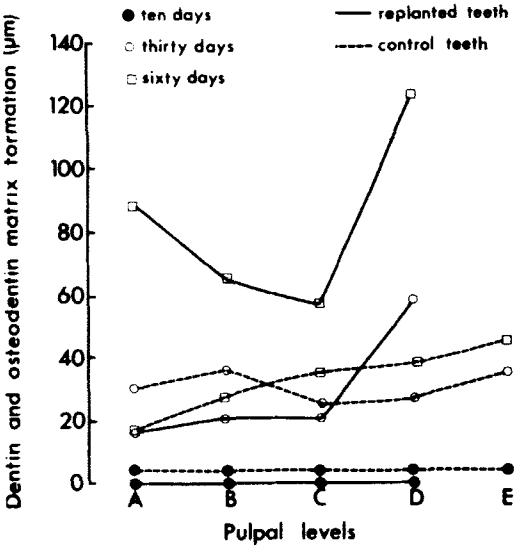

Fig. 6. Dentin and osteodentin matrix formation in mandibular control and apicoectomized replanted incisors at different pulpal levels (A, B, C, D, and E) 10, 30, and 60 days after replantation.

layer was missing in all but one tooth, in which a reduced odontoblastic layer was present in the intermediate zone. One pulp was necrotic. In three teeth the main pulp showed connective tissue cells in the intermediate and partly in the cervical zone, with large blood vessels running longitudinally. In the coronal zone the tissue structure was lost, and only scattered inflammatory cells were present. Thirty days after replantation, the odontoblastic layer was present but reduced in the total length of the pulp except for in two teeth, which showed pulpal necrosis. In the intermediate zone of two mandibular teeth a relatively normal odontoblastic layer was found. Large blood vessels were present in the entire length of the pulp. A cell-rich connective tissue without inflammatory cells was observed. Ingrowth of bony tissue had occurred in two pulps from one animal. Sixty days postoperatively a normal odontoblastic layer was a common finding in the intermediate zone of the mandibular incisors, and even most incisally in two teeth (Fig. 8). Otherwise, a reduced odontoblastic layer was present. Compared with the control teeth, the connective tissue

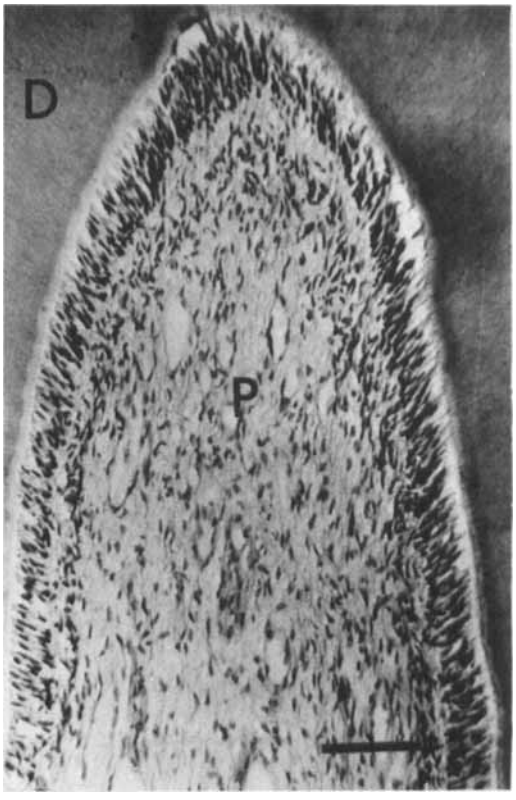

Fig. 7. Coronal zone in a mandibular control pulp. No cell-free zone is present in the pulp tissue. D = dentin; P = pulpal tissue. (Hematoxylin and eosin; bar = 100 µm.)

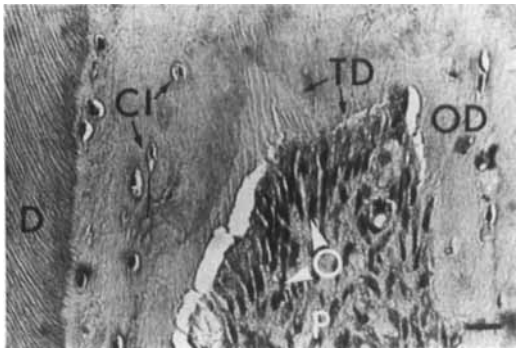

Fig. 8. Coronal pulpal area of mandibular incisor 60 days after replantation. Odontoblast-like cells (O) are present. A tubular dentin matrix (TD) is formed related to the odontoblast-like cells. D = dentin; OD = osteodentin; CI = cell inclusions; P = pulpal tissue. (Hematoxylin and eosin; bar = 20 µm.)

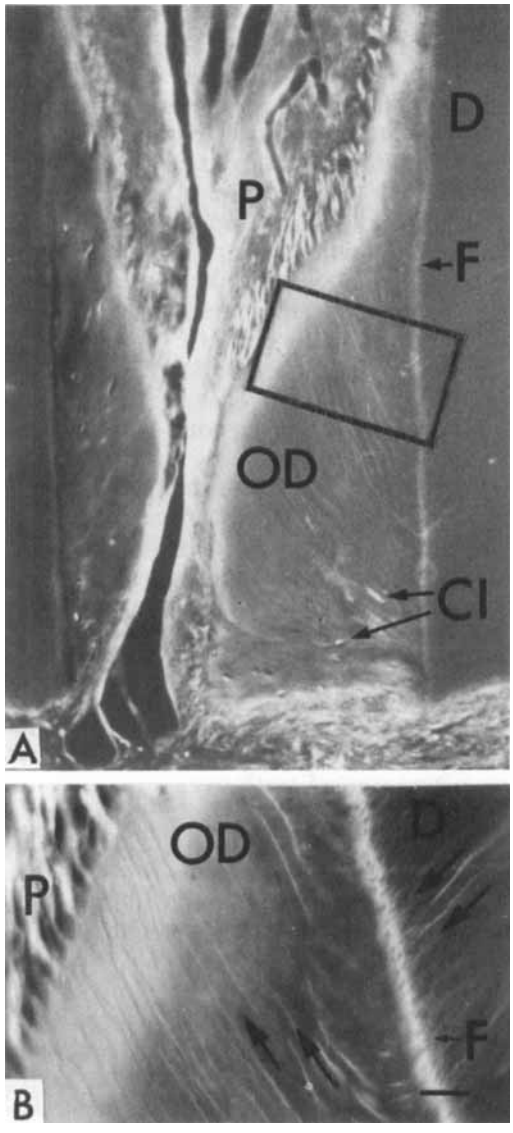

Fig. 9A. Large amount of osteodentin matrix (OD) with few cell inclusions (CI) formed in intermediate zone 60 days after replantation. 9B. Higher magnification of framed area in A. Note change in tubule direction (arrows) in osteodentin matrix formed after replantation compared with preexperimental tubules. D = dentin; P = pulpal tissue; F = fluorescent band. (Procion H8-BS label, ultraviolet light; bar = 20 µm.)

in the pulp showed a lower cell density. Large blood vessels were present in the entire pulp.

In the upper incisors the reaction pattern was dominated by internal resorption with areas of intact pulpal connective tissue combined with areas of inflammation and loss of tissue structure (Fig. 3). One lower incisor showed a necrotic pulp.

Mineralized tissue. In control teeth dentin matrix morphology was not altered by the Procion dye administration in any of the teeth (Fig. 2). In experimental teeth cell inclusions were a frequent finding in the osteodentin matrix formed postoperatively (Fig. 8). Thirty days after replantation dentinal tubules could be demonstrated in the intermediate zone in two teeth, whereas after 60 days dentinal tubules were present in the intermediate zone in four lower incisors and also most incisally in two of these teeth. The direction of the postoperatively formed tubules was different from that of the dentinal tubules formed preoperatively (Figs. 8 and 9).

Root cementum and root surface. In control teeth, 10 days after replantation, a small amount of new cementum had formed at the apical zone of the root surface. Thirty and 60 days after Procion dye injection, postoperatively formed cementum was present in the entire root length.

In replanted teeth no cementum formation could be observed 10 days after replantation.

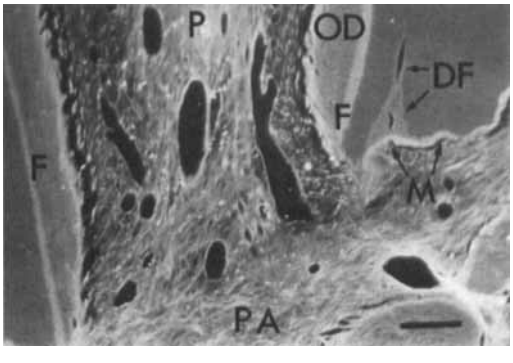

Fig. 10. The apicoectomized dentinal root areas covered with hard-tissue matrix (M) 30 days after tooth replantation. Dentinal fracture (DF) partly filled in with hard-tissue matrix. P = pulpal tissue; F = fluorescent band; OD = osteodentin; PA = periapical tissue. (Procion H8-BS label, ultraviolet light; bar = 100 µm.)

Thirty days postoperatively new cementum was formed in the entire root length in 75% of the teeth, and all replanted teeth showed cementum formation in the apical two thirds of the root. Two teeth showed ankylosis, and external resorption was present in 25% of the replanted teeth. Sixty days after replantation new cementum was present in the entire root length in all teeth. External resorption was registered in five teeth (four maxillary and one mandibular incisor).

Remodelling of the cut apical dentinal root areas with new cementum was a consistent finding 30 and 60 days after replantation (Fig. 10). External root lesions were present cervically in all teeth with pulpal necrosis. Such lesions were occasionally found in teeth with a vital pulp 30 days after replantation and in all maxillary and one mandibular tooth in the 60-day group. Apposition of new cementum was registered in some of those lesions (Fig. 4).

Discussion

The results clearly demonstrate a high healing potential of the dental pulp after tooth replantation under the experimental conditions used, as 20 of 23 teeth showed osteodentin formation during 30-day and 60-day postoperative periods.

The findings confirm previous observations (2, 13, 17) that osteodentin or dentin matrix formation is a reliable determinant of pulpal cell function after a general trauma. A positive relationship was found between the histologic evaluation of cellular morphology and osteodentin matrix formation.

Pulpal repair in replanted teeth is dependent on early and adequate reestablishment of the vascular system (14, 15). The size of the area of pulpal tissue in contact with the vascularized tooth socket has been shown to be important for pulpal repair (5). In the present study the apicoectomy procedure seemed not to increase the dimension of the apical contact area. The intention of improving postoperative healing by means of the apicoectomy can therefore be debated. A similar but somewhat delayed healing pattern after apicoectomy compared with the healing in replanted immature teeth has been reported (3, 16). In spite of this proposed negative effect the pulpal cells in the present experiments have regained their ability to form matrix in the total pulpal length in 83% of the teeth during a 30-day period.

Procion H8-BS proved to be a suitable tool as a dentinal marker for comparison of pre- and post-operative dentin matrix.

Relatively severe pulpal tissue changes compared with the controls were observed 10 and 30 days after replantation. A differentiated evaluation of the histologic reaction pattern is therefore considered to be of limited value (7).

The mean values for the amount of dentin and osteodentin formed after replantation were based on measurements on two representative adjacent sections from each tooth. The recordings can thus not be interpreted as strictly correct values but as a semiquantitative measure of dentin and osteodentin formed postoperatively.

The significantly different pattern of the rate of matrix formation in mandibular and maxillary control teeth over a period of 60 days demonstrates that teeth from different jaws should not be used uncritically in comparative studies of dentin or osteodentin formation. The difference in pulpal apical diameter in maxillary and mandibular incisors (60 days (Table 1)) supports the view that the developmental stage of the teeth may be different in the two jaws.

In the most apically situated pulpal tissue of the replanted teeth the rate of matrix formation showed a nearly linear increase postoperatively after a few days' lag period. This may indicate that after a short period of adjustment the pulpal cells have regained their ability to produce matrix. The observation is in accordance with findings in transplanted mouse teeth (18).

The tubular nature of the postoperative osteodentin matrix in the intermediate zone most likely implies a minor depressing effect of the replantation trauma on the pulpal cells in the area with survival, or rapid replacement of the odontoblasts. The frequent cell inclusions in the osteodentin matrix in cervical and coronal zones, however, may be

related to a more severe cell disturbance, as suggested in previous studies (2, 16).

The observation that the inclination of the dentinal tubules changes postoperatively agrees well with previous findings (3).

In accordance with observations in replanted human teeth (1, 7), odontoblast-like cells could be identified most incisally in the pulp, lining the osteodentin matrix. Surprisingly, a similar degree of cell differentiation was absent in the other parts of the coronal and the cervical zones, which presumably had been revascularized earlier. In particular, a considerably higher rate of matrix formation was found at the incisal level than in the cervical level in the mandibular teeth 60 days postoperatively. The biologic events behind these observations remains unknown. However, both revascularization and reinnervation are supposed to influence posttraumatic hard tissue formation (9). It has also been suggested (19) that the lack of a functional nerve supply may cause increased matrix formation in teeth. Nerve reactions and their influence on pulpal healing after traumatic injuries need further investigations.

The mild initial inflammatory response observed in coronal and cervical zones 10 days after replantation in this study could be due to tissue degeneration without significant bacterial contamination. The absence of inflammatory cells 30 days postoperatively, however, suggests that no irritants causing cellular inflammatory reaction were present at this stage of pulpal healing.

Pulpal inflammation has probably been depressed owing to antibiotic administration and controlled experimental conditions. Systemic antibiotic treatment has been shown to prevent bacterial invasion of the pulp in replanted teeth (20).

As shown in numerous studies, a replantation trauma may imply combined injury to pulp and periodontium. Progressive internal resorption associated with dentinal tubules that communicated with external cervical lesions was a consisting finding in the maxillary incisors 60 days postoperatively. In the corresponding pulpal tissue severely inflamed areas could be observed. Internal resorption related to external cervical lesions has also previously been reported after replantation (21, 22). Wedenberg (23) concluded that development of progressive internal resorption requires an initiating traumatic factor and thereafter a continuous bacterial stimulation. Our findings support these conclusions. Both internal resorption and reduced or lacking osteodentin matrix formation related to exposed dentinal tubules could be caused by long-lasting external irritating agents. Plaque bacteria in exposed dentin cavities have been shown to be able to induce injury without invasion of the pulpal tissue (24). Bacterial extracts as well may cause a reduced capacity of the fibroblasts to form collagen in vitro (25). In replanted human premolars with pulp obliteration, few complications have been reported (4) when coronal restorations were avoided. These findings underline that an intact tooth surface is important for successful pulpal healing in replanted teeth.

The repairing cells after replantation have often been assumed to proliferate from the peridontal connective tissue (16). Our findings, that the cells within the pulpal cavity were able to produce a tubular matrix, whereas hard-tissue repair on exposed dentin on the root surface never was tubular in nature, do not support this assumption but lead to the conclusion that the repairing cells within the pulp are of pulpal origin, derived from surviving pulpal cells. For the most apically situated pulpal cells, nutrition by diffusion is sufficient to meet the metabolic requirements until revascularization is established.

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