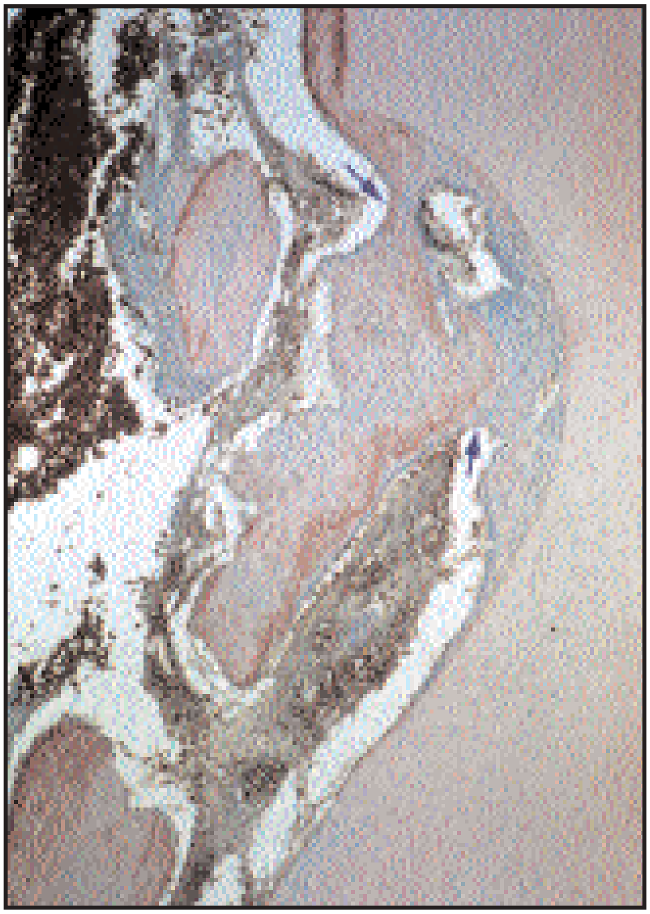

Two Patterns of Histologic Healing in an Intrabony Defect Following Treatment with Enamel Matrix Derivative: A Human Case Report

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Human histologic evidence of periodontal regeneration following treatment of intrabony defects with enamel matrix derivative (EMD) has yielded inconsistent results in recent case reports. A 46-year-old woman presenting one deep intrabony defect at the distal root of a mandibular first molar scheduled for extraction was selected for EMD therapy. During surgery, a notch was placed at the most apical level of calculus on the experimental root. Nine months postsurgery, a block section including the distal root and surrounding periodontal tissues was obtained and processed in a mesiodistal plane for histologic evaluation. Histologic analysis demonstrated two different patterns of healing along the proximal and furcal surfaces. Regeneration with new cellular cementum, bone, and periodontal ligament with functional fiber orientation was observed on the distal aspect of the root, whereas the furcal surface healed through ankylosis. This report underlines the biologic variability in wound healing following EMD therapy in periodontal intrabony defects and within the same defect. Host-specific intrinsic and/or extrinsic factors accounting for this variability remain to be investigated. (Int J Periodontics Restorative Dent 2005;25:xxx–xxx.)

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Human histologic evidence of regeneration of the periodontal supporting tissues—including cementum, connective tissue attachment, and alveolar bone—has been demonstrated with various treatment materials including autogenous bone[1–3]; demineralized freeze-dried bone allograft (DFDBA)[4,5]; Bio-Oss (Geistlich) alone,[6] in combination with Bio-Gide (Geistlich),[7] or in combination with autogenous bone and Bio-Gide[8]; and enamel matrix derivative (EMD; Emdogain, Biora).[9–14]

Several reports have demonstrated that EMD can yield periodontal regeneration on previously diseased root surfaces in humans,[10–14] but on an inconsistent basis.[12,15] In a multicenter 10-case series, histologic evaluation of the region coronal to the base of the calculus notch showed evidence of regeneration in 3 of 10 intrabony defects treated with citric acid conditioning and EMD.[12] In these 3 cases, connective fiber insertion into both new bone and new cemetum was demonstrated. A parallel arrangement of the connective tissue fibers of the periodontal ligament (PDL) was also observed. Both cellular and acellular new cementum were identified, deposited on both old cementum and dentin. Three other defects yielded connective tissue attachment/adhesion, whereas healing in the remaining 4 sites resulted in a long junctional epithelium.[12]

Sculean et al[11] report the formation of 1.88 mm and 4.82 mm of new cementum of a mixed cellular and acellular type with inserting collagen fibers covering the previously diseased root surface in two deep, combined one- and two-walled defects at two maxillary central incisors extracted 6 months following treatment with EMD. These two defects showed 1.77 mm of new bone and no bone regrowth, respectively.

In two human biopsies evaluated 6 and 9 months following treatment of one- and two-walled intrabony defects with EMD, only slight regeneration was evidenced at the most apical level of the notch prepared at the bottom of the intrabony defect.[15] A slight amount of new bone was observed, but there was no new cementum formation. No root resorption or ankylosis was observed in any of the above-mentioned human histologic reports.

The aim of the present case report is to present histologic wound healing observed 9 months following treatment of a human periodontal intrabony defect with EMD.

Method and materials

The patient selected for treatment with EMD was a 46-year-old female nonsmoker in good physical health and with no contraindication for surgical periodontal therapy. The patient was diagnosed with generalized moderate chronic periodontitis and presented one deep intrabony defect at the mandibular first molar that was considered to have a hopeless prognosis. The patient had received comprehensive periodontal therapy, including scaling and root planing in all quadrants and apically positioned flaps in the maxillary and mandibular left quadrants.

The initial therapy was performed on all teeth in the mandibular right quadrant, but not on the experimental tooth. No occlusal adjustment or odontoplasty was performed on the experimental tooth prior to therapy, as fremitus was not detected, nor were contacts observed in centric occlusion or excursive or lateral mandibular movements. The mandibular first molar was scheduled for extraction because of advanced bone loss, through-and-through furcation involvement, and a history of multiple periodontal abscesses. The patient was informed about the nature of the project, and she gave informed consent to have the distal root of the molar surgically removed, together with the surrounding periodontal tissues, following EMD therapy. This procedure was approved by the Ethics Committee of the University of Padova Institute of Clinical Dentistry.

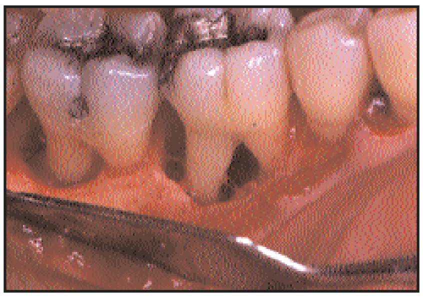
Pre- and postsurgical documentation consisted of clinical photographs, standardized periapical radiographs, and clinical measurements made just prior to surgery and 9 months postoperative. Clinical parameters included probing depth, recession, and clinical attachment level (CAL), with the cementoenamel junction (CEJ) as the reference point. All clinical measurements were carried out using a periodontal probe (XP23/UNC15, Hu-Friedy) and rounded to the nearest 0.5 mm (Table 1). Radiographic measurements to detect bone level gain between the two examinations were made by superimposing a transparent millimeter grid on the radiographs, using the amalgam apical margin and the roof of the furcation as reference points at the distal and furcal aspects, respectively. The radiographic distance between these landmarks and the bottom of the defect was measured and rounded to the nearest 0.5 mm (Table 1).

The surgical procedure and clinical measurements were performed by the same clinician. Reverse bevel sulcular incisions were made at the buccal and lingual surfaces of the first and second molars, with releasing incisions at the mesial line angles of the first premolar, and full-thickness flaps were reflected. Thorough removal of granulation tissue within the defect was performed, avoiding disturbance of the calculus present on the root surface. A notch serving as a landmark for the histologic measurements was made into the distal root through and along the apical extent of calculus with a No. $^1/_4$

Table 1 **Clinical and radiographic measurements (mm) at baseline and 9 months following EMD therapy**

Surface	Baseline clinical PPD	Baseline clinical REC	Baseline clinical CAL	Intraoperative osseous CEJ–crest	Intraoperative osseous CEJ–bottom of defect	Postoperative clinical PPD	Postoperative clinical REC	Postoperative clinical CAL	Radiographic Baseline	Radiographic 9 mo
Distal	10.0	1.0	11.0	3.5	12.0	3.0	3.0	6.0	13.0	8.5
Furcal	12.0	0.0	12.0	5.5	13.0	3.0	3.5	6.5	9.5	4.0

EMD = enamel matrix derivative; PPD = probing pocket depth; REC = recession; CAL = clinical attachment level; CEJ = cementoenamel junction.



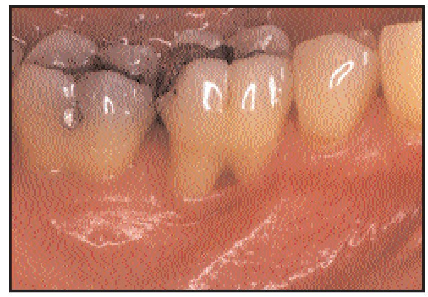


Fig 1 (left) *Intraoperative buccal view of circumferential intrabony defect at distal root of mandibular first molar associated with through-and-through furcation involvement.*

Fig 2 (right) *Buccal view of mandibular first molar 9 months postoperative, just prior to biopsy procedure. Note slight marginal gingival inflammation in interradicular area and soft tissues partially filling buccal entrance of furcation.*

round bur, using fiberoptic illumination and magnifying loops to enhance visibility. Following notch preparation, scaling and root planing were carried out using hand and ultrasonic instruments in an attempt to create a hard root surface coronal to the reference notch. Special care was taken to preserve the integrity of the notch during root surface instrumentation.

Defect morphology (Fig 1) and measurements from the CEJ to the alveolar crest and to the depth of the osseous defect were documented. The flaps were tested for closure, and the tissues were presutured using a horizontal internal mattress suture in the defect-associated distal space.[16] The root surface was treated for 15 seconds with topical application of 37% orthophosphoric acid, then thoroughly rinsed with physiologic solution according to the manufacturer's recommendations. Subsequently, the EMD was applied to coat the entire extent of the exposed root surface and slightly overfill the defect. The suture (No. 5-0 monofilament; Ethilon, Ethicon/Johnson & Johnson) was then tightened and supplemented by interrupted sutures[16] to achieve complete coverage over the EMD sites, flap positioning at the CEJ, and marginal tissue adaptation to the root surface.

The patient was given a nonsteroidal antiinflammatory drug for 3 days and antibiotics (doxycycline 100 mg two times daily) for 10 days postoperative and was instructed to rinse with chlorhexidine 0.2% three times daily for 6 weeks following surgery. The patient was seen 7 days posturgical for wound inspection and removal of nonstabilizing sutures. Remaining sutures were removed at 3 weeks. The patient was asked to avoid mechanical plaque control until healing had progressed sufficiently to allow normal oral hygiene measures. She was recalled for oral hygiene reinforcement and professional supragingival plaque control on a monthly basis for the first 6 months and every 3 months thereafter. Subgingival instrumentation was not performed at any of the recall appointments.

Nine months postoperative (Fig 2), a second surgical procedure was carried out to remove the distal root en bloc. Full-thickness flaps were reflected on the buccal and lingual aspects of the tooth, leaving undis-

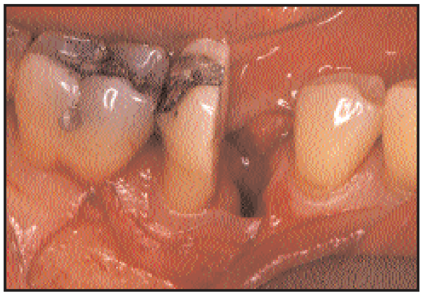


Fig 3a (left) *Buccal view of right mandibular area during biopsy procedure, following extraction of mesial root. Attempt was made to maintain the integrity of the marginal soft tissues at buccal, mesial, and distal aspects of distal root.*

Fig 3b (right) *Distal root immediately following its removal en bloc with surrounding periodontal hard and soft tissues.*

turbed the marginal buccal, distal, and furcal tissues. The two roots were then separated, and the mesial root was extracted (Fig 3a). Vertical incisions converging apically were made down to the bone surface through the marginal distal and furcal gingival tissues at approximately 1 mm and 2 mm, respectively, from the distal and mesial line angles of the root. The vertical incisions delimited a triangular area extending approximately 10 mm apical to the gingival margin. These incisions were subsequently extended through most of the buccolingual width of the alveolar bone up to the lingual surface of the distal root with a No. 700L cross-cut fissure bur mounted on a high-speed handpiece under sterile water irrigation. The root with the surrounding tissues, including the buccal alveolar plate along with wedges of proximal and furcal hard and soft tissues, were removed (Fig 3b). Bio-Oss was placed into the biopsy and extraction sites and covered with a Gore-Tex membrane (WL Gore) to augment the residual ridge and prepare the edentulous area for future implant placement. The buccal and lingual flaps were released through apical periosteal incisions and coronally respositioned to allow for complete coverage of the membrane.

The block specimen was immediately rinsed with sterile saline and fixed in 10% neutral buffered formalin solution for histologic processing. After fixation, the specimen was decalcified in 5% formic acid for about 4 weeks, embedded in paraffin, and sectioned step serially at levels 50 µm apart, along a plane parallel to the long axis of the root in a mesiodistal direction at a thickness of 5 to 6 µm. For the histologic analysis, four sections 50 µm apart, representing the most mid-distal area of the root, were stained with hematoxylin-eosin and trichrome stains and examined under a light microscope. Linear measurements were made to assess the newly formed cementum, connective tissue attachment or adhesion, and bone regrowth coronal to the reference notch. Qualitative histologic parameters, including root resorption, ankylosis, direction of fibers of newly formed connective tissue, type of cementum formation, and presence of inflammation, were also evaluated.

Results

Clinical and radiographic findings

The patient consistently demonstrated good compliance with periodontal maintenance throughout

Fig 4a (left) *Baseline periapical radiograph.*

Fig 4b (right) *Nine-month postoperative radiograph shows bone fill in both distal and furcal components of intrabony defect and well-defined crestal lamina dura in distal interproximal area.*

the 9-month postoperative healing phase. She maintained adequate levels of plaque control, except in the furcation area. This was partially filled with interradicular soft tissues, allowing only limited access for oral hygiene tools (Fig 2).

The intraoperative morphologic characterization of the defect at the distal root demonstrated a suprabony component associated with a deep intrabony circumferential defect involving all four surfaces of the root (Fig 1). Preoperative, CAL was 11.0 mm and 12.0 mm on the distal and furcal aspects, respectively. The corresponding gains in CAL at 9 months postoperative were 5.0 mm and 5.5 mm. Probing depth reduction of 7.0 mm and 9.0 mm was observed at the distal and furcal sites, respectively. Significant new hard tissue formation was observed radiographically, with a gain in bone height from the base of the defect of 4.5 mm and 5.5 mm at the distal and furcal surfaces, respectively (Fig 4). A well-defined crestal lamina dura was radiographically evident in the distal interproximal area. Tooth vitality was positive prior to surgery and 9 months postoperative. Increased mobility of degree 2 was observed at baseline and reduced to less than degree 1 prior to the biopsy procedure.[17]

Histologic and histomorphometric findings

Two distinct histologic healing patterns were evidenced along the proximal and furcal aspects of the distal root (Fig 5): regeneration at the distal surface and ankylosis at the furcal surface. **[AU: Edit OK?]**

Regeneration with new cementum, bone, and connective tissue attachment was observed coronal to the notch at the proximal aspect of the distal root (Figs 6 to 8). The newly formed cementum was uniformly thick, predominantly cellular in nature with embedded cementocyte-like cells, and deposited over the old cementum. In the coronal aspect of the root, an artifactual separation of the newly formed cementum from the root surface was present, most likely related to the histologic processing. No resorption lacunae were observed at the old-new cementum interface, indicating that the new cementum formation was not preceded by a superficial resorption of the old cementum surface. The intervening dense con-

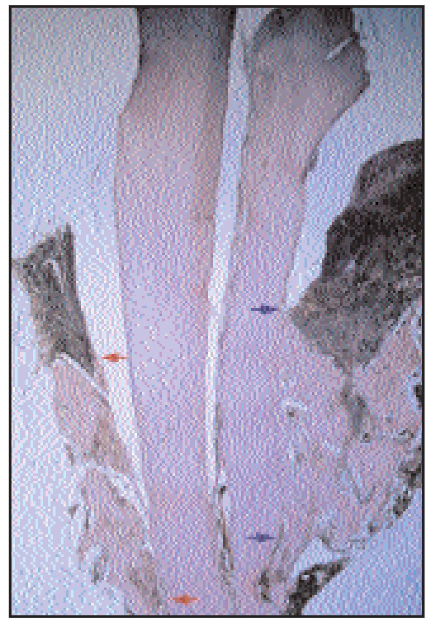


Fig 5 *Histologic overview of a central section shows both proximal (left) and furcal (right) aspects of distal root. Proximal surface demonstrates regeneration with new cementum, bone, and connective tissue attachment in area demarcated by* red arrows. *Healing through ankylosis can be seen at furcal aspect in area delineated by* blue arrows *(hematoxylin-eosin stain; original magnification × 3).*

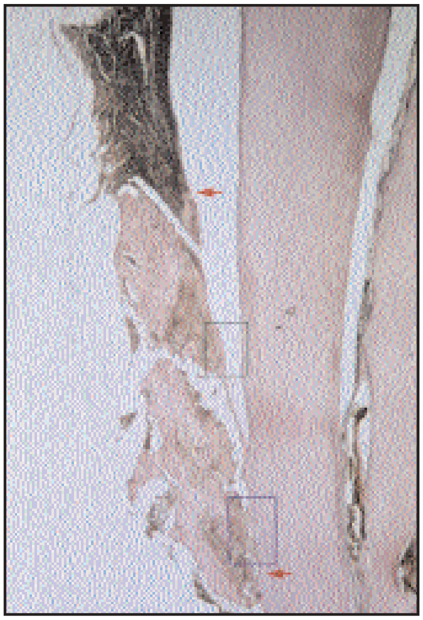


Fig 6 *Higher magnification of distal surface demonstrates new attachment apparatus coronal to base of calculus notch* (N). **[AU: Please indicate where "N" should be shown on the figure. (Also in Fig 9.)]** *Note artifactual separation between newly formed cementum and root surface in coronal portion of defect. Although the most coronal extension of new cementum cannot be clearly identified, bone regeneration appears to parallel new cementum deposition* (between arrows) *(hematoxylin-eosin stain; original magnification × 8).*

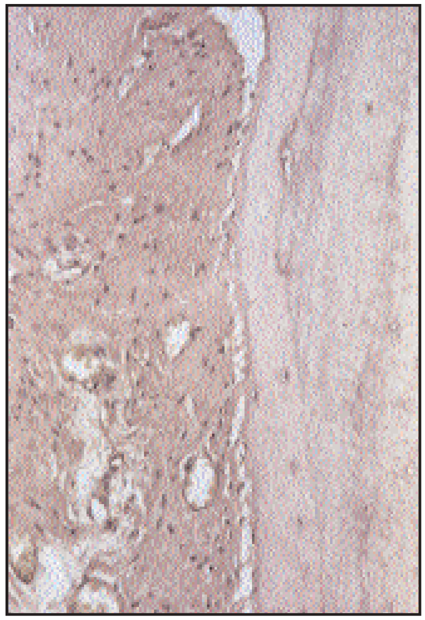


Fig 7 *Higher magnification of area within bottom frame in Fig 6 shows new cellular cementum, with inserting connective tissue fibers deposited over old cementum (hematoxylin-eosin stain; original magnification × 62).*

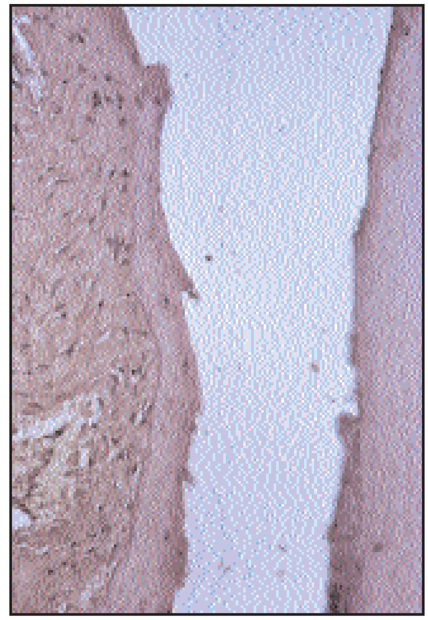


Fig 8 *Higher power of artifactual split area (top frame in Fig 6) demonstrates newly formed cellular cementum separated from root surface. Inserting connective tissue fibers can be observed at new cementum surface (hematoxylin-eosin stain; original magnification × 62).*

nective tissue layer between the new cementum and newly formed alveolar bone was inserted into both tissues and displayed functional fiber orientation. Although the most coronal extension of the newly formed cementum could not be clearly identified on any of the examined sections, new bone formation seemed to parallel cementum deposition throughout most of the defect. Histomorphometrically, new cementum covered nearly 3.22 mm, whereas bone extended 3.63 mm coronal to the base of the reference notch.

The marginal gingival connective tissue in correspondence with the junctional epithelium at the furcal root surface exhibited an inflammatory cellular infiltrate extending in isolated clusters within the supracrestal transseptal fibers (Figs 9 to 11). New dense cortical bone presenting a multilayered arrangement with numerous embedded osteocytes was observed in direct contact with the old cementum surface up to 4.17 mm coronal to the reference notch. In one specimen stained with hematoxylin-eosin, a small area of mineralized cellular tissue within the notch area was initially interpreted as cementum-like formation. The same root-associated cementum-like tissue appeared partially confluent with and indistinguishible from the alveolar bone in a section 50 μm away. Numerous small, shallow resorption lacunae on the old cementum surface and matching interdigitations at the bone–old cementum interface were observed, indicating that a resorptive activity preceded the subsequent mineralized tissue formation against the root surface.

Discussion

This human histologic case report demonstrated significant improvement of CAL and radiographic bone fill following treatment of a periodontal intrabony defect with EMD, but a dual histologic healing pattern with regeneration at the proximal aspect and ankylosis through most of the furcal surface of the same root.

The regeneration at the proximal aspect of the distal root can be compared to the histologic outcome described in other human case reports.[10–14] The functional fiber orientation of the PDL, predominantly cellular nature of the newly formed cementum, and its deposition on old cementum in the present case are in line with earlier findings.[11–13] The split between the newly formed and old cementum in the coronal aspect of the defect can be attributed to an artifactual separation. This is confirmed by the fact that the new cementum was firmly attached to the root surface in the apical portion of the defect. Nevertheless, it cannot be excluded that the artifactual microscopic split could have resulted from a weak attachment between the regenerative cementum and root hard tissues, similar to what has been observed with cementum formed under guided tissue regeneration conditions in human teeth.[18] In contrast with previous results that have demonstrated a discrepancy between bone regrowth and connective tissue attachment formation,[11–14] bone regeneration in the present report

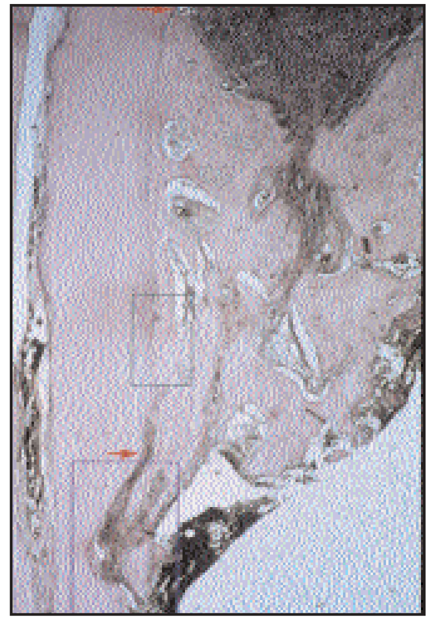


Fig 9 *Furcal surface of distal root shows regenerated bone ankylosed to root surface* (arrows) *coronal to calculus notch* (N) *(hematoxylin-eosin stain; original magnification × 6).*

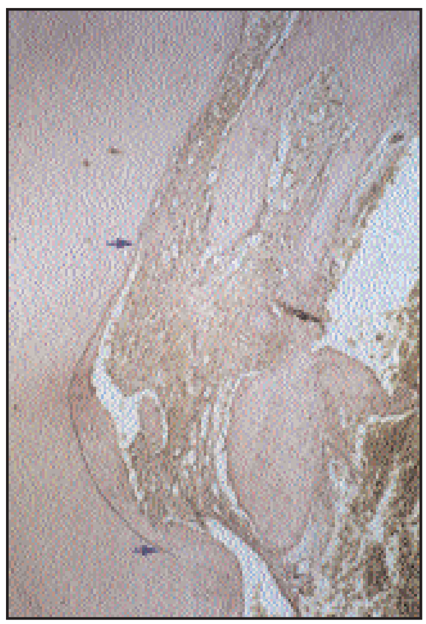


Fig 10a *Higher magnification of area within bottom frame in Fig 9 shows slender layer of what appears to be cellular cementum attached to root surface and thinning out coronally* (between arrows). *Connective tissue with parallel fiber orientation separates this tissue from newly formed bone (hematoxylin-eosin stain; original magnification × 25).*

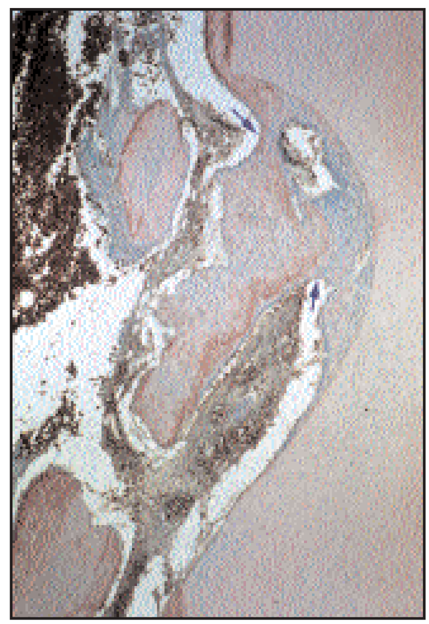


Fig 10b *Pinpoint fusion of bone and cementum-like tissue in isolated areas in notch* (arrows) *within section 50 μm away* **[AU: Away/apart from what point?]** *(trichrome stain; original magnification × 25).*

Fig 11 *Higher magnification (top frame in Fig 9) shows ankylotic union between newly formed bone arranged in multilayers and root surface. Old cementum shows numerous small, shallow resorption lacunae in perfect interdigitation with osseous profile (hematoxylin-eosin stain; original magnification × 50).*

appeared to parallel new cementum deposition.

The ankylotic pattern observed in the present report does not appear to be a transient phenomenon like the bony union demonstrated during early healing in rodents[19,20] following treatment of surgically induced fenestration defects and in monkeys[21] following replantation of extracted incisors. Unlike the early ankylosis that disappears at the later stages of wound healing with restoration of the PDL space, the ankylosis in this report was still detected 9 months postoperative along the entire root surface coronal to the reference notch, probably indicating its irreversible nature. The permanent character of this ankylosis and the unlikely possibility of developing reparative cementum and reestablishing a normal PDL space were further confirmed by the establishment of a regeneration healing pattern at the corresponding proximal surface of the same root at the same postoperative interval. Conversely, the absence of multinucleated cells at the root-bone interface in the present case suggests that the irregular root surface profile was established through a resorptive process at the earlier healing period and may corroborate the nonprogressive nature of the resorption-ankylosis phenomenon at the biopsy time point.

Bone tissue formation in direct contact with denuded root surfaces is not only limited to injury to the dentoalveolar structures,[22–25] but rather a common finding following certain periodontal procedures.[1,26–40] Various degrees of root resorption and ankylosis have been reported with a variety of therapeutic modalities and materials used in the treatment of periodontal defects, including fresh iliac bone marrow,[1,26–29] platelet-derived growth factor (PDGF) with citric acid application,[30] bone morphogenetic protein (BMP)-2,[31,32] BMP-3 (osteogenin) combined with DFDBA,[33] osteogenic protein (OP)-1,[34] and root surface demineralization.[35–40]

Beyond the well-known reversible or irreversible damage to the PDL cells implicated in ankylosis associated with dental trauma, a number of hypotheses were proposed to explain the ankylosis-inducing mechanisms in periodontal therapy-related studies. Replacement ankylosis observed in fresh iliac bone marrow–treated sites has been attributed to the presence of viable bone cells transferred with the graft into the periodontal defect and acting directly or indirectly on the regenerating periodontal tissues.[28] In biologically modulated periodontal therapy,[30–34] the occurrence of ankylosis is complicated to interpret because of the lack of cellular specificity, wide array of biologic activities of most molecular mediators, and complex interaction between the various molecular signals at a wound site. In citric acid therapy in animal models,[35–37,40] various factors have been advocated as potential ankylosis-inducing mechanisms. The incidence of ankylosis is greater at citric acid–treated than non–acid treated teeth,[40] at increased distance from the base of the treated lesion,[35,36] and in larger defects.[35] Furthermore, root resorption and ankylosis are significantly correlated with the magnitude of connective tissue attachment, suggesting that root resorption and ankylosis frequently occur following the accomplishment of connective tissue repair over extended portions of the treated root surfaces.[36,38]

In addition to the factors inherent to the surgical procedure and/or regenerative material, external stimuli such as occlusal forces and masticatory function have been implicated as potential regulators of cell turnover in the PDL and modifiers of the healing response.[19,41–43] Finally, research-related considerations such as the animal and defect models[37] and the submerged tooth model[31,33,44] have been reported to be more frequently associated with ankylosis.

The ankylosis at the furcal aspect is difficult to interpret in light of the existing in vitro, animal, and human histologic data related to EMD therapy. The in vitro findings relative to the mechanisms of action of EMD assessed in a variety of cells[45–58] have been, at least in part, contradictory. The discrepancies in these studies have been attributed to differences in EMD concentrations, cell lines and densities, growing conditions and experimental designs, and methods applied.[59] EMD has been shown to significantly stimulate proliferation of human PDL cells,[45,46,57] modulate matrix synthesis by human PDL cells,[46,47] enhance attachment and spreading of human PDL fibroblasts to culture plates coated with EMD,[48] and promote proliferation of transgenic mice cementoblasts.[49] Although caution should be applied when extrapolating from in vitro findings to actual wound healing processes, these effects of EMD on the PDL cellular lines, coupled with EMD's inhibitory effect on epithelial proliferation,[51,57] are in line with the requirements of periodontal regeneration and the results of clinical and animal studies showing that EMD may prevent ankylosis in transplanted and replanted teeth.[60–63]

Inconsistent results have also been reported relative to the specific effect of EMD on the differentiation, mitogenesis, and biosynthesis of bone cell lineages.[59] However, the majority of in vitro[49,50,52–56] and animal studies[64–68] suggest osteopromotive properties of EMD. Considering that the inherent multifunctional characteristics of EMD do not justify its selective behavior at a single wound site with a combined regeneration-ankylosis healing pattern, analysis of the extrinsic and/or intrinsic factors that could have generated different histologic outcomes at two adjacent aspects of the same root suggests that the ankylosis in the furcal site may be related to the following considerations.

First to be examined is the potential effect of acid remnants at the base of the defect at its furcation aspect to inflict severe chemical injury on the coronal portion of the PDL and interradicular crest, which provide the cellular elements for regeneration of cementum and PDL.[69,70] **[AU: Edit OK?]** This could have been caused by the difficult access to the bottom of the furcation-associated defect, resulting in partial washout of the orthophosphoric acid used to condition the root surface. The necrotizing effect of low-pH orthophosphoric acid may have impaired the vitality and subsequently the healing potential of precursor cells involved in cementum and connective tissue formation.[71–73] Root surface conditioning was used in the present study, as it has been advocated as a prerequisite of the EMD protocol to allow removal of the smear layer and precipitation of the EMD proteins onto a root surface free of organic remnants. Root surface preparation has been conventionally performed with orthophosphoric acid 37%,[15,74–79] citric acid[80,81] at pH 1, or ethylenediaminetetraacetic acid (EDTA) 24% at neutral pH.[15,79,82–92] The favorable therapeutic results obtained with all three agents and positive outcomes demonstrated without any chemical conditioning suggest that specific chemical root preparation may not be required to achieve significant adjunctive clinical improvements in EMD therapy. Further studies are needed to investigate the potential effects of root surface conditioning combined with EMD therapy.

Second, marginal inflammation in the furcation area could have altered the phenotypic expression of mesenchymal cells or altered the multifunctional effect of EMD on these cells. While the patient's overall interproximal plaque and bleeding scores were optimal throughout the follow-up period, the interradicular furcation site of the first molar showed persistent signs of inflammation because of access limitations.

Third, the root surface in the furcation area could have, as a result of different inherent characteristics related to the nature of cementum[93] and presence of concavities,[94] provided a different substratum for the repair tissues.

The ankylosis pattern at the reference notch in this report appears similar to the "pinpoint" fusion of bone and cementum observed in isolated areas following grafting of human intrabony defects with

DFDBA and osteogenin.[33] Similar to the present report, those authors indicate that bone and cementum remain fused for three to four serial sections and then become separated by a PDL space. They point out that this type of ankylosis is different from replacement-type ankylosis and speculate that it might occur when the PDL formation fails to keep pace with the rapidly forming cementum and bone.

With the limited information that can be drawn from this case report, it may be concluded that the use of EMD in intrabony defects yields satisfactory clinical and radiogaphic outcomes. However, under the existing clinical conditions and surgical modality applied in the present case, healing at 9 months postoperative was mediated through a dual histologic healing pattern, with regeneration at the proximal aspect and ankylosis through most of the furcal surface of the same root. Host-specific intrinsic and/or extrinsic factors accounting for this variability need to be further investigated.

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