

Regeneration of bifurcation in beagle dogs: Histologic evaluation of two regeneration materials

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هدفت الدراسة إلى معرفة تأثير مادة إيمدوغاين وغشاء الغايدور القابل للامتصاص على شفاء وإعادة البناء في إصابات مفترق الجذور من الصنف الثاني. أُحدثت عيوب تجريبية في مفترق الجذور من الصنف الثاني (بارتفاع 3-5 ملم وعمق 2-5 ملم) في الضواحك عند أربعة من كلاب البيجول. خضعت الكلاب (1) و (2) لجراحة أحادية المرحلة تضمنت أحداث العيوب وتطبيق إيمدوغاين أو غايدور أو لا شيء (مجموعة مقارنة). أما الكلاب (3) و (4) فحُضعت لجراحة ثنائية المرحلة تضمنت أحداث عيوب وبعد حوالي شهرين جرى وضع الغشاء أو لا شيء (مجموعة مقارنة). دُبحت الكلاب بعد 6 أشهر وجرى تحضير مقاطع للأسنان المتروعة الكلس تحضيراً للفحص النسيجي. لم يلاحظ تشكل الملائم اللاحلوي بشكل ثابت عند استعمال إيمدوغاين في حال الجراحة الأحادية، أما في الجراحة ثنائية المرحلة فكان تشكله أعلى وذلك في نموذج حيوانات التجربة المستعملة.

The aim of this study was to compare Emdogain and Guidor resorbable materials in the regeneration and/or healing of experimental Class II furcation lesions. Class II premolar furcation defects 3-5 mm high and 2.5-5 mm deep were experimentally induced in four beagle dogs. Dogs 1 and 2 were assigned to one-stage surgery where the defect created was immediately followed by the placement of either Guidor or Emdogain or nothing (control). Dogs 2 and 4 were assigned to a two-stage approach where the creation of the defect was followed one month later with the membrane placement or nothing (control). After six months of healing animals were sacrificed. Subsequently, decalcified sections were prepared from all animals and used for histological evaluation. Accelerated cementum was not a constant finding with the use of Emdogain. It was concluded that the two-stage surgery approach is a more predictable experimental model for the healing of Class II furcation.

Introduction

The ultimate goal of the application of reconstructive surgical techniques in the treatment of periodontal bone defects is the regeneration of periodontal ligament, root cementum and alveolar bone. However, numerous studies have found that irrespective of the chosen technique, complete restoration of lost periodontal tissues is difficult and often impossible to achieve.¹⁻³ Histological studies of specimens subjected to guided tissue regeneration procedures often reveal an artificial defect between regenerated cementum and root dentin.⁴ Guided tissue regeneration (GTR), using non-resorbable or resorbable membranes that enable periodontal regeneration by blocking epithelial cell proliferation into the pocket, is a commonly accepted method used to treat Class II furcation and intrabony defects.^{4,5,6}

Wikesjö *et al.*⁷ developed an animal model where supra-alveolar periodontal defects were surgically created by the removal of 5 to 6 mm of alveolar bone from the cemento-enamel junction. The resulting circumferential supra-alveolar periodontal defect was used for immediate

reconstruction. This procedure resulted in a critical size defect where spontaneous bone and cementum regeneration did not exceed 25% of the defect over an eight week healing period.

The influence of space provision by guided tissue regeneration has been evaluated in this model. Alveolar bone and cementum regeneration approximates 75% and 40% of the defect height in 5 mm supra-alveolar periodontal defects, respectively, following an eight week healing interval.⁸ Therefore, the critical size, supra-alveolar, periodontal defect model represents a test candidate for reconstructive therapies in periodontal evaluation. Enamel matrix derivative (EMD) is available as a commercial formulation, and has recently been introduced as a new method for regenerative periodontal treatment.⁹ EMD is composed principally of amelogenin and related proteins that are derived from porcine tooth bud.¹⁰ These proteins are important for the development of acellular cementum, periodontal ligament, and alveolar bone.¹¹ The clinical efficacy and safety of EMD in animal and human periodontal osseous defects has been demonstrated.¹² Histological evidence has shown that treatment with EMD resulted in true periodontal regeneration.¹³ However, to date, studies evaluating EMD for use in the treatment of

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furcation defects or comparing it to other regenerative materials are lacking.

This present study aimed first to test the surgically induced Class II furcation treated immediately or one month following its creation and secondly to histologically evaluate the effectiveness of resorbable membranes (Guidor) and EMD (Emdogain) in the management of the surgically induced Class II furcation in beagle dogs.

Material and Methods

Four male beagle dogs, 24 to 48 months old were used in this study. Prior to the creation of the defects, a preparatory period of 5 to 8 weeks permitted the establishment of a clinically healthy gingiva by means of scaling and an oral hygiene regimen.

In each dog, three experimental quadrants were used. In each of these quadrants, the experimental teeth were the first and third premolars. A total of six experimental teeth were therefore used in each dog. At each experimental site (upper right, upper left and lower right), a defect imitating a Class II furcation was created.

Rompone and Kataral intramuscular injection in a dose of 1mg/kg sedated each dog. The gingiva and the mucosa of each surgical site were infiltrated with local anesthesia (Xylocaine, 1.8ml). Following sulcular incision and elevation of buccal flap, alveolar bone around the furcation of each experimental tooth was removed with chisels and water-cooled rotating burs. The created defects were 3 to 5 mm high and 2.5 to 5 mm deep. Immediately following bone reduction around the furcation, the root surfaces were planed with curettes. The dogs were then randomly assigned to one of the two groups. The grouping was based on the type of surgery, namely the one-stage and the two-stage surgery procedures.

Dogs 1 and 2 were assigned to the one-stage surgery procedure approach where the defect created was immediately followed by the placement of either Guidor or Emdogain or no membrane (control). Dogs 3 and 4 were assigned to the two-stage surgery procedure. The two-stage surgery procedure consisted of the creation of the defect followed one month later with the membrane placement. The control group received no membrane.

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*** Emdogain, Biora AB, Medeon Science Park, SE-205, Malmo, Sweden

Guidor Group

This group was treated according to the principles of guided tissue regeneration by the use of Guidor. A mucoperiosteal flap was elevated in order to gain access to the defect. Following careful debridement and root planing, measurements of the defects were made. A bioabsorbable Guidor* matrix barrier made of amorphous polylactic acid consisting of two layers of material separated by an interspace of suitable configuration was chosen, trimmed and positioned to cover the defect at least 2 to 3 mm of the surrounding bone (Fig. 1). The membrane was firmly secured to the defect tooth using the attached ligature tied in a figure eight. Periodontal releasing incisions were necessary in order to achieve complete coverage of the Guidor membrane. The flap was closed with individual sutures passed through notches made at the coronal part of the tooth and adapted with light cure composite to ensure the coronally repositioned flap.

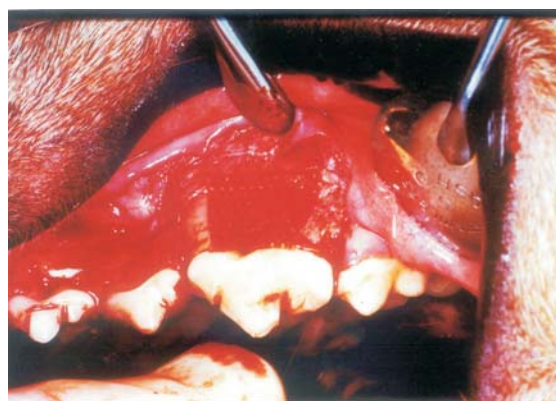


Fig. 1. Placement of the guidor membrane covering the entire Class II furcation defect.

Emdogain Group

Following surgical exposure, the root defects were conditioned for 2 minutes with a 24% EDTA** gel according to the instructions given by the manufacturer. The EDTA residues were removed by copious rinsing with sterile saline. The defects were filled with the EMD*** gel consisting of components (a vehicle solution and Emdogain in a spongy form) which were stored in the refrigerator and mixed freshly before each surgical procedure according to the instructions given by the manufacturer. Emdogain® Gel is composed of a

number of proteins that self-assemble to create a matrix. The dominant protein in this matrix is amelogenin. Emdogain® Gel used in this study was available in 0.3 ml to treat a single defect. The EMD gel was then applied into the root surface and into the defect with a sterile syringe. The flap was sutured and repositioned coronally as in the precedent group (Fig. 2).

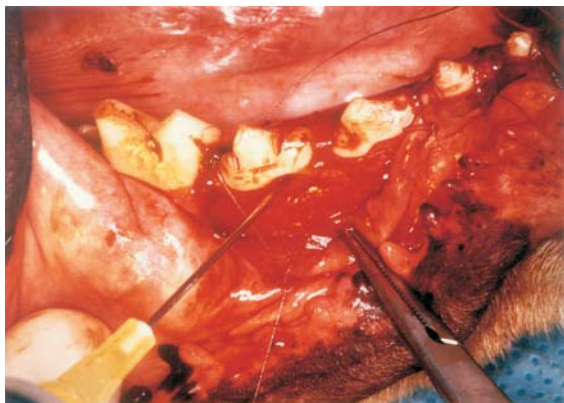


Fig. 2. Application of the Emdogain solution to the furcation area.

Control Groups

Following debridement of the furcation defect, the flap was repositioned coronally to obtain primary closure for dogs 1 and 2. For dogs 3 and 4, the flap was coronally repositioned one month following the creation of the defect to obtain primary closure.

Animal Sacrifice

All dogs were sacrificed six months following experimental surgery by an intravenous injection of concentrated sodium pentobarbital.[!] During sacrifice, mechanical perfusion of 10% neutral buffered formalin was carried out through the carotid arteries to ensure immediate and adequate fixation of the tissue.

Histological Procedures

Following sacrifice, tissue blocks including teeth and surrounding tissue were removed with an electric saw.^{!!}

Twelve teeth with their surrounding periodontal tissues were dissected and further

[!] Sleepaway, Fort Dodge Lab., Fort Dodge, IA, USA

^{!!} Dewalt, DW3401, Italy

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fixed by immersion in 10% neutral buffered formalin for 48 hours. After 48 hours of fixation, the specimens were washed and then decalcified in 5% neutral buffered EDTA for 3 to 4 months. The endpoint of decalcification was radiographically determined. Decalcified specimens were sectioned 7µm thick and stained with hematoxylin solution and eosin.

Statistical Analysis

A non-parametric statistical analysis method was used because the results of the experiment were recorded in an ordinal subjective manner. The degree of response was recorded as: 0 = absent, 1 = mildly present, 2 = moderately present and 3 = severely present.

Non-parametric tests included Chi-square test for ordinal histologic findings in all specimens.

Results

Examiner's Calibration

Analysis of data for intra examiner reliability and the examiner consistency of the readings of histological structures read in the slides showed that the percent agreement was 86.5%.

In the analysis of data for inter examiner reliability (two examiners) the results showed that the percent agreement was 83.4%.



Fig. 3. Emdogain specimen (one-stage surgery). This photomicrograph illustrates complete regeneration of connective tissue attachment and new bone formation with absence of epithelial invasion. Magnification 4X.

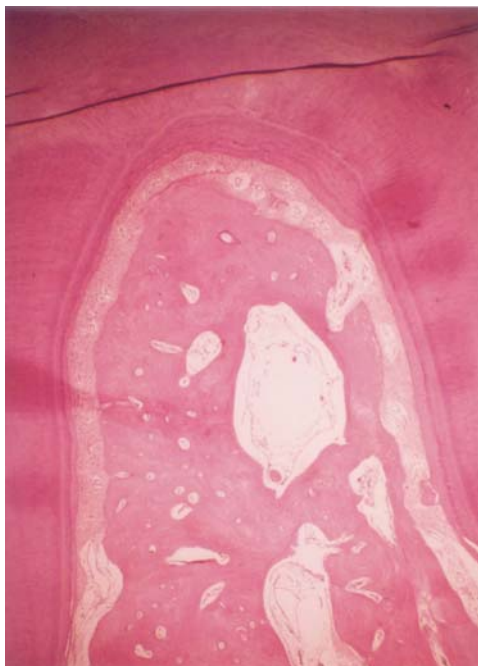


Fig. 4. Emdogain specimen (one-stage surgery). This photomicrograph illustrates the deposition of a thin layer of acellular cementum in the apical area. Magnification 10X.

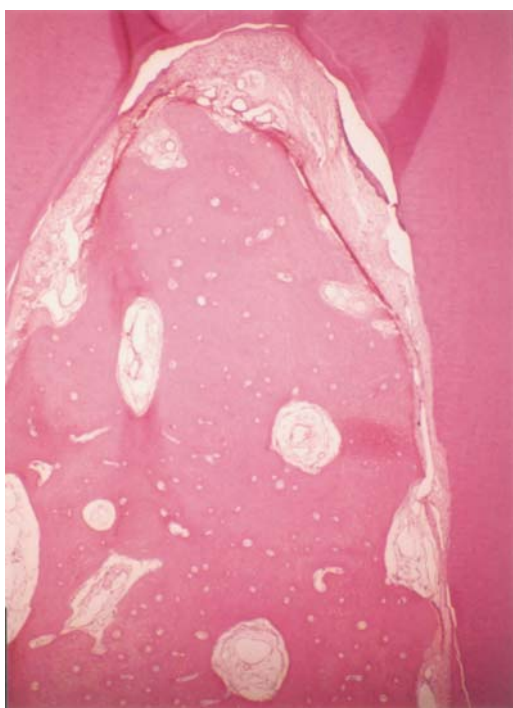


Fig. 5. Guidor specimen (one-stage surgery). This photomicrograph shows an almost complete regeneration of connective tissue and bone in the furcation area. Note the presence of a small epithelium. Magnification 4X.

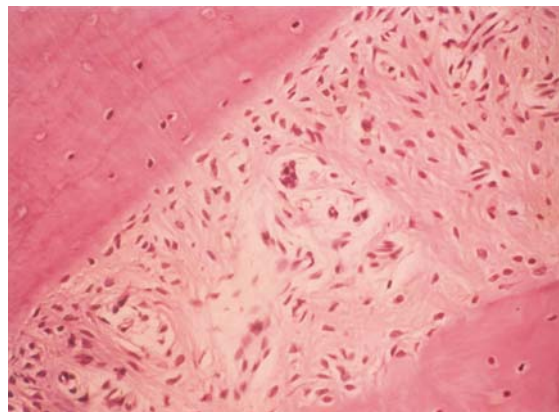


Fig. 6. Control specimen (one-stage surgery). This photomicrograph shows cementogenesis and the presence of cementoblast lining the root surface. Magnification 10X.

One Stage Surgery

In the Emdogain group, the light microscopic observations revealed that most of the surgically treated defects healed with connective tissue interfacing the cementum (Fig. 3). New cementum of the cellular nature was found on the entire root surface while in one specimen a layer of acellular cementum was visible in the apical area (Fig. 4). Regeneration of bone into the furcation showed a complete fill.

In the Guidor group, the healed furcation defect was occupied by various amounts of mineralized bone, bone marrow, periodontal ligament and connective tissue. In one specimen, epithelial ingrowth at the most cervical area was observed (Fig. 5)

The control group was marked by connective attachment to the root as well as new bone in the furcation area. New cementum of a cellular nature was seen as a homogenous layer deposited on top of the old cementum (Fig. 6)

Two Stage Surgery

In the Emdogain group, the light microscopic observations were marked by an incomplete regeneration (Fig. 7). The presence of epithelium, which interfaced the root of furcation was seen. A thin layer of cementum was observed at the apical extent of the furcation.

The previously exposed roots in the Guidor group were covered with new cementum, which was in direct contact with the instrumented surface. The new cementum was of cellular nature. The most coronal portion of the furcation was devoid of mineralized bone (Fig. 8).

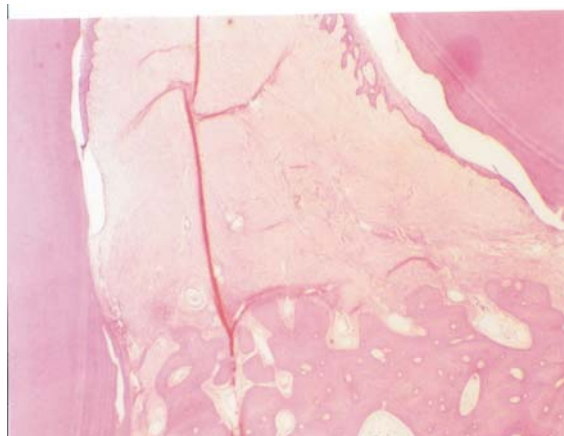


Fig. 7. Emdogain specimen (two-stage surgery). Note that the regeneration of connective tissue attachment is confined to the apical area. Magnification 4X.

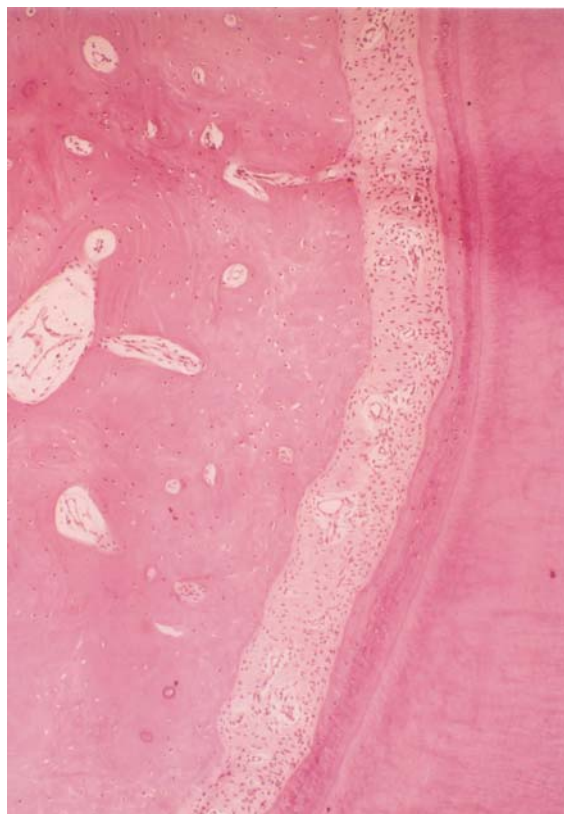


Fig. 8. Guidor specimen (two-stage surgery). This photomicrograph shows a highly cellular periodontal ligament with numerous cementoblasts. Magnification 4X.

The control group was marked in the coronal part of the furcation by the presence of chronic inflammatory cells invading the connective tissue area. The epithelium invaded the furcation area

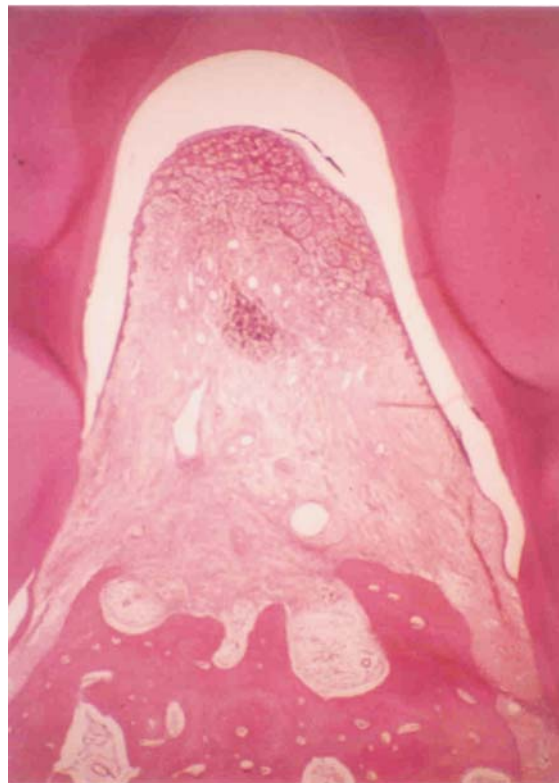


Fig. 9. Control specimen (two-stage surgery). This photomicrograph shows an incomplete repair and epithelial invasion in the furcation area. Magnification 4X.

preventing any connective tissue attachment. Newly formed cellular cementum was only seen in the apical area (Fig. 9).

Bone Regeneration

In the Emdogain and control group, there was no significant difference in bone regeneration between type one and type two surgery. In the Guidor group, animals treated by one-stage surgery yielded more bone regeneration ($X^2=35.9$, $P<0.0001$) when compared to those treated by two-stage surgery (Table 1).

Periodontal Ligament

The Emdogain ($X^2=31.9$, $P<0.0001$) and control ($X^2=21.0$, $P<0.0001$) groups showed more significant presence of periodontal ligament in one-stage surgery than two-stage surgery. No significant difference in presence of periodontal ligament between the two types of surgeries in the Guidor group was observed (Table 2).

Table 1. Descriptive statistics of the bone regeneration in one- and two-stage surgical procedures in all three groups

Degree of Response	Emdogain®		Guidor®		Control	
	sxl n	sxll n	sxl n	sxll n	sxl n	sxll n
0	2	0	0	6	0	0
1	8	11	0	15	0	3
2	11	17	6	14	19	26
3	9	12	24	5	11	11
Total	30	40	30	40	30	40
Chi-square Test	2.817		35.953		2.716	
P Value	.421		<0.0001		.257	

Table 2. Descriptive statistics of the periodontal ligament regeneration in one- and two-stage surgical procedures in all three groups

Degree of Response	Emdogain®		Guidor®		Control	
	sxl n	sxll n	sxl n	sxll n	sxl n	sxll n
0	2	5	1	6	0	5
1	0	15	4	5	6	20
2	0	9	6	6	12	14
3	28	11	19	23	12	1
Total	30	40	30	40	30	40
Chi-square Test	31.919		2.690		21.000	
P Value	<0.0001		0.442		<0.0001	

Table 3. Descriptive statistics of cementum regeneration in one- and two-stage surgical procedures in all three groups

Degree of Response	Emdogain®		Guidor®		Control	
	sxl n	sxll n	sxl n	sxll n	sxl n	sxll n
0	2	2	1	0	0	5
1	0	8	4	18	6	8
2	2	21	17	5	12	3
3	26	9	8	17	12	24
Total	30	40	38	40	30	40
Chi-square Test	31.160		18.647		13.533	
P Value	<0.0001		<0.0001		<0.005	

Cementum

Guidor ($X^2=18.6$, $P<0.0001$), Emdogain ($X^2=31.6$, $P<0.0001$) and control ($X^2=13.5$, $P<0.0001$) specimens yielded more cementum in two-stage surgery (Table 3).

Table 4. Descriptive statistics of connective tissue presence in one- and two-stage surgical procedures in all three groups

Degree of Response	Emdogain®		Guidor®		Control	
	sxl n	sxll n	sxl n	sxll n	sxl n	sxll n
0	0	8	14	2	12	2
1	4	3	8	8	13	6
2	2	5	4	8	5	18
3	15	24	4	22	0	14
Total	30	40	30	40	30	40
Chi-square Test	2.180		21.811		30.259	
P Value	.536		<0.0001		<0.0001	

Table 5. Descriptive statistics of epithelium tissue presence in one- and two-stage surgical procedures in all three groups

Degree of Response	Emdogain®		Guidor®		Control	
	sxl n	sxll n	sxl n	sxll n	sxl n	sxll n
0	24	12	9	5	12	0
1	4	3	12	16	12	6
2	0	5	3	8	2	11
3	2	20	6	11	4	23
Total	30	40	30	40	30	40
Chi-square Test	22.909		4.113		32.843	
P Value	<0.0001		.250		<0.0001	

Connective Tissue

The results were identical to the previous group except for the Emdogain group (Table 4).

Epithelial Invasion

Emdogain treated ($X^2=22.9$, $P<0.0001$) and control specimens ($X^2=32.8$, $P<0.0001$) showed more significant epithelial invasion in two-stage

surgery than one-stage surgery. No statistical significance difference in epithelial invasion between the two types of surgeries was observed in the Guidor group (Table 5).

Discussion

The results of this study demonstrated that the surgical treatment of Class II furcation in dogs with the one-stage surgery experimental approach healed with complete connective tissue closure. On the other hand, Class II furcation treatment with the two-stage surgery approach varied from epithelialization to complete repair. These findings corroborate results from a previous study in animals.¹⁴

Emdogain and Guidor in the animals treated with the two-stage surgery approach yielded some positive results when compared to the controls. Bone formation and periodontal ligament regeneration were significant in both experimental groups. Other authors have reported similar results.^{12,15} Guidor showed a superior regeneration in agreement with studies where it was used for regeneration purposes.⁵

Quantification of the cementum in this study showed no significant difference among the three groups. Little or no acellular cementum could be detected with Emdogain treatment. This is in contrast to Hammarström study where acellular was a constant finding when Emdogain was used.¹² As indicated by Araujo *et al.*,¹⁶ Emdogain may have no effect on cementum formation especially in the coronal part of furcation. In this study when acellular cementum was found, it was always in the apical portion and related only to the one-stage surgery procedure. It has also been shown that the enamel protein on the root surface at the time interval when connective tissue cells appear could not be detected thus leading to the assumption that Emdogain has no effect on cementum formation.¹⁷ In the furcations examined in the control group following type I surgery, the newly formed cementum was of a cellular nature. In the test groups, the cementum that had formed in the coronal and apical area of the furcation was similar to the cementum observed in the control group. Only in one specimen treated with Emdogain, and in the apical portion, could a thin acellular cementum be detected.

These observations are in agreement with findings made from other studies using identical models,¹⁸⁻²⁰ and confirmed that following guided tissue regeneration, the instrumented root surface

will host a reparative type, cellular cementum with comparatively few inserting fibers. This view was confirmed by a recent study where enamel matrix derivative has been shown to be a significant stimulator of human periodontal ligament cells.²¹

The morphological characteristic of the newly formed cellular cementum observed in Guidor and Emdogain groups were obviously different to the features of newly formed cementum following Emdogain application described by Hammarstrom⁹ from experiment in the monkey and, Heijl²² and Mellonig²³ in humans.

The observation made in the present study does not seem to confirm that enamel-like proteins when applied into an instrumented root surface create an environment conducive for the formation of acellular cementum. On the other hand, the observation that in most of the specimens a new cellular cementum with inserting collagen fibers was consistently formed on the previously exposed root surface points to the possible potential of Guidor and Emdogain for enhancing cementogenesis. The cellular, extrinsic/extrinsic fiber cementum observed in the entire root surface is in agreement with findings previously reported from an experiment evaluating the dynamics of periodontal wound healing.²⁴

A possible explanation for the better results obtained with Guidor in the present study could be the mechanical disturbance of the blood clot during the early phase with the use of Emdogain. Histological and clinical studies have demonstrated that early healing events such as wound stabilization and plaque control removal are crucial for the outcome of regenerative periodontal treatment.²⁵ The importance of blood clot protection and stabilization, especially during early wound healing, may explain the finding that in the furcations treated with Guidor new attachment occurred more consistently than in the furcations treated with Emdogain. Another factor for the better healing in Guidor specimens could be due the space created under the flap. It has been shown that the amount of bone regeneration is dependent of the available space and, thus, a collapse of the periodontal flap may in turn result in a limited amount of bone formation.²⁶ It is well established that GTR results in neosteogenesis when the healing is undisturbed by external factors.²⁷ In the present study, no membrane (Guidor) exposure was observed during healing. This observation could also explain the significant amount of connective

tissues observed following Guidor placement.

Such a diversity of data indicates that complete understanding of the mechanisms guiding periodontal tissue regeneration is still a problem to be solved by periodontologists. On the other hand, therapeutic effects and better availability of new more effective techniques promise greater possibilities in the treatment of periodontitis.

Conclusions

Within the limitations of this study, the following can be concluded:

1. The findings of the present study are in accordance with the current concepts of guided tissue regeneration using Guidor and Emdogain in the treatment of Class II furcation in beagle dogs.
2. The two-stage surgery experimental procedure was a more predictable experimental model than the one-stage surgery approach for the healing of Class II furcation defects in beagle dogs.
3. Acellular cementum was not a constant finding with the use of Emdogain in the treatment of Class II furcation defects.

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