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Original Article

Histomorphometric Analysis of Periodontal Tissue Regeneration by the Use of High Density Polytetrafluoroethylen Membrane in Grade II Furcation Defects of Dogs

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Abstract

Statement of Problem: There are limited histomorphometric studies on biologic efficacy of high density tetrafluoroethylen (d-PTFE) membrane.

Objectives: To investigate the healing of surgically induced grade II furcation defects in dogs following the use of dense polytetrafluoroethylene as the barrier membrane and to compare the results with the contra lateral control teeth without the application of any membrane.

Materials and Methods: Mandibular and maxillary 3rd premolar teeth of 18 young adult male mongrel dogs were used for the experiment. The furcation defects were created during the surgery. 5 weeks later, regenerative surgery was performed. The third premolar teeth were assigned randomly to control and test groups. In the test group, after a full thickness flap reflection, the d-PTFE membrane was placed over furcation defects. In the control group, no membrane was placed over the defect. 37 tissue blocks containing the teeth and surrounding hard and soft tissues were obtained three months post-regenerative surgery. The specimens were demineralized, serially sectioned, mounted and stained with Hematoxylin and Eosin staining technique. From each tissue block, 35-45 sections of 10 µm thickness within 60µm interval captured the entire surgically created defect. The histological images were transferred to computer and then the linear measurement ranges of the defects area, interadicular alveolar bone, epithelial attachment and coronal extension of the new cementum were done. Then, the volume and area of aforementioned parameters were calculated considering the thickness and interval of the sections. To compare the parameters between the control and test teeth, we calculated the amount of each one proportionally to the original amount of defects.

Results: The mean interradicular root surface areas of original defects covered with new cementum was 74.46% and 29.59% for the membrane and control defects, respectively ($p < 0.0001$). Corresponding values for epithelial attachment were 16.03% and 48.93% for the membrane and control defects, respectively ($p < 0.005$). The mean volume of the new bone formed in the inter-radicular defects was 61.80% and 35.93% for the membrane and control defects, respectively ($p < 0.02$).

Conclusions: The present study provided a biological rationale for high density polytetrafluoroethylen (d-PTFE, n-PTFE) as a barrier membrane for enhancement of the bone and cementum regeneration in grade II furcation defects subjected to regeneration therapy.

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Introduction

One of the inflammatory diseases is periodontitis that leads to alveolar bone and periodontal ligament destruction. There are different approaches to treatment of this kind of disease. In recent years, however, the use of regenerative procedures has become more common, aiming at restoring the lost periodontal support. It has been documented that for the formation of a new connective tissue attachment, the progenitor cells reside in the periodontal ligament [1].

Consequently, it should be expected that a new connective tissue attachment would be predictably achieved if such cells aggregate in the root surface during healing. During GTR, a membrane is placed over the defect to prevent epithelial cells, connective tissue cells and bone cells from contacting the root surface and also to provide a space for in-growth of periodontal ligament tissue [2,3]. Different types of membranes have been introduced for the regenerative therapy. The first non-resorbable membrane ever used was made of expanded polytetrafluoroethylene(e-PTFE). It is a biocompatible polymer that resists against enzymatic and microbiological attack [4,5]. Ingrowth of bacteria occurs due to the highly porous structure of ePTFE when the membrane is exposed in the mouth [6]. Exposure results in high rates of infection; this frequently requires an early removal of the device. The highly porous structure allows the soft tissue ingrowth, which complicates the removal. So the removal of the membrane needs sharp dissection and extensive surgery. Expanded PTFE must be completely buried and the primary closure must be maintained to ensure the predictability of regenerative surgery [7]. Other non-resorbable membrane which is made of 100% non-porous, dense, non-expanded and non-permeable PTFE is high-density polytetrafluoroethylene membrane (n-PTFE, d- PTFE). The primary advantage of the dense PTFE is the ability to remain exposed in the mouth while protecting the underlying defect and the bone graft. Since the n-PTFE membrane is soft and flexible, it is easy to handle. Primary closure is not required, and if the membrane is exposed, it may be removed without the need to second surgery. If the primary closure technique is used, the membrane may be easily removed through a small incision in a flapless technique [8,9]. Dense PTFE is also available with titanium reinforcement. It can increase the stiffness of the material for use in defects where space-making is required. The embedded titanium framework allows the membrane to be shaped to fit with a variety of defects without rebounding. It also provides extra stability in large, non-space-making osseous defects [10,11].

Bioabsorbable membranes have been introduced to avoid a second surgery for the membrane removal. The main disadvantage of bioabsorbable membranes is the unpredictable resorption period. The primary closure is also needed when using bioabsobable membranes in regenerative surgery [12-15].

There are different ways to assess the GTR therapy outcome. The most valid one is histologic method. One of the disadvantages of histologic studies is the use of linear measurement. By the use of histomorphometric method, the area of attachment and the volume of regenerated tissue can be measured. There are few studies in the literature using d-PTFE membrane in which histomorphometric method has been used [16].

In 2003 Stephan *et al*. compared porous and non-porous teflon membrane in treatment of vertical osseous defect. They used clinical reentry method to assess the amount of regenerated tissues and found no statistically significant difference between the two groups [17].

In 2008, Hoffmann *et al*. used d-PTFE membrane for socket preservation. 12 month after the extraction, evaluation showed significantly higher amounts of bone regeneration following the use of d-PTFE membrane [18].

The only study in which the d-PTFE membrane was used for class II furcation treatment was the one conducted by James *et al*. in 2001. They compared porous (e-PTFE) and non- porous (d-PTFE) membrane and used clinical reentry method for assessing the tissue regeneration. They concluded that there was no statistically significant difference between the two groups [7].

The aim of this study was to evaluate the periodontal regeneration histomorphometrically after using the high-density polytetrafluoroethylene membrane in grade II furcation involvement in Mongrel dogs.

Materials and Methods

In the current study, 18 one year old Mongrel dogs, each weighing approximately 22 kg were included. The third premolars in each jaw of the dogs were selected for study and the following indices were assessed:

plaque index, gingival index and probing depth, amount of gingival recession and width of attached gingival on the buccal aspect of the third premolars measured by Williams' periodontal probe.

All surgical procedures were carried out under general anesthesia. 0.5 mg/kg acepromazin 2% was injected intra-muscularly half an hour before the surgery. Induction of the general anesthesia was done by 20 mg/kg sodium pentobarbital. After the intubation, general anesthesia was maintained by 2% halotan and 50% oxygen during the procedure.

Creation of furcation involvement

After the induction of general anesthesia, a sulcular incision was done on the buccal aspect of the third premolars and a full thickness flap was elevated. After removing the supracrestal periodontal tissue, the distance between CEJ and alveolar bone was measured. This distance on different teeth was 1-1.5 mm. A grade II furcation involvement was created by the high speed rotary instrument and bur in width and height of 4mm. For preventing the reattachment of periodontal ligament to the root surface denuded from the alveolar bone, impression paste was placed in the space created in advance. After one week, the stitches were removed. Impression paste was maintained in the area for 5 weeks.

After 5 weeks since the creation of furcation defect, the plaque and gingival indices were measured again. Afterwards, the impression paste was removed. Supra-gingival scaling was performed by ultrasonic instrument. Brushing was done every other day during the following week after removal of the impression paste.

GTR surgical procedure

One week after the plaque control program, GTR surgery was performed. A full thickness flap was reflected after induction of general anesthesia. Then, debridment and root planning were carried out. The depth and width of the furcation defects were assessed and 0-0.5 mm increase in defects was found. For having a reference as the primary level of the bone to future microscopic assessment, a groove was created next to the most coronal part of the alveolar bone.

Up to this stage, the procedure was the same between the test and control teeth. After creation of the groove, one of the third premolars of the jaws was selected as the control and the other one in the same jaw was chosen as the test randomly. In the control teeth, the flap was returned to its place and sutured. In the test teeth, the membrane (industrial d-PTFE sheets were used after sterilization) was placed over the defect so that it covered the defect completely and also 4-5 mm of the alveolar bone. Then the flap was returned to its place and sutured.

After the surgery, oral amoxicilline was prescribed 1gr per day for 7 days.10 days after the surgery, the stitches were removed. In the test teeth, the membranes were maintained for 5 weeks. During this period, the plaque control was done by 0.2% chlorhexidine mouthwash. Chlorhexidine was applied on the teeth and gingiva by the cotton pellet. For removing the membranes, no incision was needed and they were removed easily by a forceps.

Three months after the regenerative surgery, the dogs were sacrificed by extra dose of anesthetic drug. The third premolar teeth with the surrounding tissue were removed with two vertical dissections at the mesial and distal parts of the teeth and one horizontal dissection. The samples were kept in formalin for 72 hours and then in formic acid for 21 days to be decalcified. After that, the blocks were formed with paraffin. Mesial-distal serial sections were provided from each block in 10 micron width and 60 micron interval. 35-45 sections from each block were prepared for the microscopic assessment. Finally, histomorphometrican analysis was carried out. The histologic images of histological sections were transferred with the computerized image analysis Axio-Vision (Carl Zeiss, Germany) to the computer. Then, linear measurements of the defects area range, interadicular alveolar bone range, epithelial attachment range and coronal extension of the new cementum were done. Then, volumetric measurements of the aforementioned parameters

Table 1: The mean, standard deviation and P-value of BN ,NC,E,V,CT in the test and control teeth in the lower jaw using Paired T-test

P-value	Standard deviation	Mean	Parameters
0.114	0.1551	0.6352	BN_T
	0.2399	0.4167	BN_C
0.002	0.0938	0.7642	NC_T
	0.2451	0.3304	NC_C
0.003	0.0559	0.1072	E_T
	0.2916	0.5537	E_C
0.633	0.0809	0.0698	V_T
	0.0711	0.0497	V_C
0.811	0.044	0.059	CT_T
	0.0634	0.0662	CT_C

were calculated by considering the thickness of histologic sections and interval of these sections. To compare the afore-mentioned parameters between the test and control teeth, the amount of each one was calculated proportional to the volume and area of original defects. The following parameters were assessed in the defect zone:

- Ratio of the volume of the new bone formation (BN)
- Ratio of the area of the root covered with the new cementum (NC)
- Ratio of the area of the root with epithelial attachment (E)
- Ratio of the area of the root with the connective tissue attachment without new cementum formation (CT)
- Ratio of the area of the root without any attachment(V)

Results

The test and control teeth in each jaw were compared according to the new bone formation (BN), new cementum (NC), the length of the root with the connective tissue attachment without cementum formation (CT), epitheial attachment (E), and the length of the root in the defect area without any attachment (V). The test and the control teeth were compared by the afore-mentioned factors in both jaws. For this analysis, paired T-test and Wilcoxon signed rank test were used.

In the lower jaw, the volume of the new bone formation was greater in the test teeth in comparison with the control ones but the difference was not statistically significant (p = 0.114). No significant difference was

Table 2: The mean, standard deviation and P-value of BN ,NC,E,V,CT in the test and control teeth in the upper jaw using Paired T-test

P-value	Standard deviation	Mean	Parameters
0.103	0.2247	0.5983	BN_T
	0.2342	0.2938	BN_C
0.010	0.2614	0.7222	NC_T
	0.2713	0.2564	NC_C
0.299	0.2570	0.2211	E_T
	0.3211	0.4156	E_C
0.057	0.0132	0.0101	V_T
	0.2752	0.2598	V_C
0.523	0.0678	0.0466	CT_T
	0.077	0.0682	CT_C

Table 3: The mean, standard deviation and P-value of BN ,NC,E,V,CT in the test and control teeth in both jaws using Paired T-test

P-value	Standard deviation	mean	Parameters
0.017	0.1845	0.6180	BN_T
	0.2373	0.3593	BN_C
0.000	0.1848	0.7446	NC_T
	0.2511	0.2959	NC_C
0.005	0.1825	0.1603	E_T
	0.3030	0.4893	E_C
0.115	0.0656	0.0419	V_T
	0.2162	0.1477	V_C
0.513	0.0546	0.0532	CT_T
	0.0675	0.0671	CT_C

also found in V (*p* = 0.63) and CT (*p* = 0.811). The new cementum formation was significantly higher in the test teeth (*p* = 0.002). On the contrary, the epithelial attachment was more in the control teeth (*p* = 0.003). In both paired T-test and Wilcoxon signed ranks test, the results for these factors were the same (Tables 1 and 4).

In the upper jaw, the new cementum formation in the test teeth was considerably more compared to the control teeth (*p* = 0.010). On the other hand, the amount of the root surface in the defect area had no attachment and the root surface with epithelial attachment was more in the control teeth. There was no significant difference in the other factors. (*p*-value for V was 0.057 and for E was 0.299) (Table 2).

As shown in Table 3, the comparison of the control and test teeth in both jaws revealed that the volume of the new bone and cementum formation in the test teeth was greater compared to the control teeth (*p*-value for NC was 0.0001 and for BN it was 0.017). There was no significant difference in the V and CT. (*p*-value for V was 0.115 and for CT it was 0.513). The amount of epithelial attachment was greater in the control teeth (*p* = 0.005).

Moreover, the control and test teeth in the upper and lower jaws were compared. The results obtained from all factors were the same in the control and test teeth in the lower and upper jaws, except for the amount of the root's lack of attachment which was greater considerably in the control teeth in the upper jaw compared to the control teeth in the lower jaw (*p* = 0.028).

Table 4: Data obtained from comparing the control and test teeth in each jaw using Wilcoxon signed ranks test (T and C represent test and control teeth and L and U represent lower and upper jaws)

P-value	Parameters
0.093	BN_{TL}-BN_{CL}
0.012	NC_{TL}-NC_{CL}
0.012	E_{TL}-E_{CL}
0.463	V_{TL}-V_{CL}
1.00	CT_{TL}-CT_{CL}
0.176	BN_{TU}-BN_{CU}
0.018	NC_{TU}-NC_{CU}
0.310	E_{TU}-E_{CU}
0.046	V_{TU}-V_{CU}
0.398	CT_{TU}-CT_{CU}

Discussion

The ultimate goal of periodontal therapy is regeneration of perioduntium. One of the methods believed to have predictable results in treatment of periodontal defects including grade II furcation involvement is GTR. In several studies, it has been shown that the teeth undergoing GTR treatments have gained more clinical attachment compared to the controlled teeth. There are several factors that affect GTR treatment. They include meticulous plaque control, prevention of barrier membrane infection and bacterial accumulation and provision of an adequate and stable space under the barrier membrane [16].

Several materials have been introduced to be used as barrier membranes. The gold standard for GTR materials is e-PTFE. But as mentioned earlier, there are several problems with e-PTFE including bacterial contamination due to its high porosity, need for the primary closure and the complicated removal [6,7,19]. Another non-resorbable membrane is n-PTFE which is less porous. This membrane is believed to have better handling properties and does not need any primary closure. This membrane can also be easily removed through a flapless surgery [18].

Also, different types of bioabsorbable membranes have been introduced. The main advantage of these membranes is that they permit a single- step procedure, thus alleviating patient discomfort and expenses for a second procedure, and avoiding the risk of additional morbidity and tissue damage. The main disadvantage of resorbable membranes is unpredictable resorption time. These membranes also need primary closure [14]. n-PTFE membranes are less costly compared to e-PTFE and resobable membranes.

According to the results of this study, n-PTFE led to an increased regeneration of the bone and cementum in grade II furcation defects in the maxillary and mandibular third premolars in dogs ($p < 0.05$).

The means of bone regeneration in the test and control teeth were 61.8% and 35.9% of the primary defects, respectively. The mean of cementum regeneration in the test and control teeth were 74.5% and 29.6% of the root surfaces' lack of tissue attachment in primary defects. The non-significant results of bone regeneration in each jaw can be explained by inadequate number of the teeth in each jaw, so when the teeth of the maxilla and mandible were assessed, this difference was significant.

In many histologic sections of the test teeth, bone formation was still in an active phase. So it can be assumed if more time had been given for the bone to be generated, more amounts of bone would have been regenerated and more differences between the test and control teeth would have been observed.

It was not possible to perform optimum oral hygiene during the healing phase after regenerative surgery. This could lead to gingival inflammation and release of the inflammatory mediators which can decrease the rate of new bone formation. This can have more effects on the test teeth as a result of barrier membrane presence. On the other hand, the dog teeth have very short root trunk and there is not an adequate space between CEJ and furcation. This can lead to lack of optimum adaptation of the membrane that influences the result of GTR treatment [16]. So we can suggest that the GTR surgery with d-PTFE membrane might yield better results in human.

There are several means for assessment of the periodontal regeneration. In most studies [5,7,9,17,18,19, 20], the outcome of regenerative periodontal surgery was evaluated by measuring the attachment level using periodontal probe, radiographic analysis or reentry operations. However, such methods did not provide any reliable proof for the attachment (i.e. formation of the cementum with inserting collagen fibers coronal to the attachment level before treatment). The most valid means of assessment of the periodontal regeneration is histological method. As this method is invasive and hard to be done, the usage of this method is limited. One of the drawbacks of the histologic measurement is the use of linear measurement instead of measuring the area of attachment and volume of the regenerated tissue [16]. With the use of histomorphometric method, the attachment area and volume of the regenerated tissue can be measured [16,21]. But as histomorphometric studies are time-consuming and difficult to perform, there are few previous studies using this method especially in grade II furcation defects. Treatment of furcation defects is very challenging because it is usually hard to obtain thorough membrane adaptation and optimal oral hygiene [16].

The review of the literature revealed that most of the studies found no difference or no statically significant difference between e-PTFE and d-PTFE membranes [4,5,7,17,18,19,20]. In 1995, Bartee *et al*. evaluated n-PTFE (d-PTFE) membrane as a barrier material to facilitate guided tissue regeneration in the rat mandibles. They created bilateral through-and-through defects in the mandibular angle of 12 rats. The experimental sites were covered by n-PTFE and the opposite defects served as the control sites. After 10 weeks, complete ossification was observed on the d-PTFE treated sites by histological evaluation while little osseous regeneration was observed on the control defects [9]. In 2001, Lamb *et al*. compared porous and non-porous teflon membranes in class II furcation defects using clinical reentry evaluation method. 24 patients with periodontitis and one class II furcation defect were included in this study and randomly divided into two groups. In one of the groups, porous Teflon membrane was used during GTR surgery and in the other one non-porous Teflon membrane was used. 9 months after the initial surgery, the second surgery for evaluation of healing outcome was performed. No statistically significant difference was found between the two groups [7]. In 2003, Walters *et al*. conducted a study relatively similar to Lamb *et al*.'s research. They included 24 patients with periodontitis and divided them into two groups. Also, they used reentry method for evaluation of regenerative surgery outcome to compare porous and non-porous teflon membrane with this difference that they used the membranes to treat vertical osseous defects. They found no statistically significant difference between the two groups [17]. In 2007, Macedo *et al*. evaluated the possibility to obtain guided bone regeneration with two types of physical barriers (calcium sulfate and PTFE nonporous barrier) in surgical defects created in the rat parietal bones. After 7,14, 30 and 45 days, four animals were sacrificed in each period and microscopic analysis was done. They found that PTFE barrier was more effective when compared to calcium sulfate during bone regeneration, involving transcortical shallow defects [22]. In 2008, Hoffmann *et al*. used d-PTFE membrane to preserve the alveolar bone after tooth extraction. 276 extraction sites were evaluated. Measurements were taken post-extraction and 12 months after the surgery. The distance from the cemento-enamel junction of the adjacent teeth to the bone level was measured. Hard tissue biopsies were taken from 10 representative cases during implant placement 12 months after the socket preservation. The results of this study showed that the use of a d-PTFE membrane allowed for a significant regeneration of the volume of the sockets following tooth extraction. Histologic evaluation indicated that the newly formed tissue in the extraction sites was mainly regular trabecular bone with areas of bone marrow and typical cells. The authors mentioned absence of a control group as a limitation of this study [18].

In 2000, Marouf compared e-PTFE and d-PTFE effectiveness in guided bone regeneration surgery. They used elderly rabbits as the experimental animals. Two non-self-healing defects were created in each rabbit calvarium. One of the 2 defects was fully covered with e-PTFE membrane and the other one was covered with d-PTFE. The specimens were obtained at 4,8 and 16 weeks and examined with light microscopy. They observed greater speed and quantity of bone regeneration in the defects covered with e-PTFE. However, in the defects covered with d-PTFE, marginal bone regeneration was observed, so it can be postulated that if more time had been given, more bone regeneration would have been observed and the difference would have been less significant between the two groups. [6]

Conclusions

As this study showed, with the use of d-PTFE membrane in guided tissue regeneration surgery, an increase of periodontal tissue regeneration was gained in comparison to the control teeth. This can provide a biologic reason for using this type of membrane in GTR surgery.

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