The Effect of Splinting upon Healing after Tooth Replantation

—A Histological Study in Rat Molars—

by

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Abstract

The purpose of this study was to evaluate, in replanted rat upper first molars, through histological analysis, the effect of splinting upon postoperative events related to the tooth and its supporting structures.

Introduction

The replantation of avulsed teeth represents a possible form of therapy for 16% of the traumae on permanent dentitions and 7 to 13% of those related to deciduous teeth (1).

Recent publications deal with the biological responses which occur after dental replantations which seem to behave differently from replants or autografts of other organs [2].

Amongst the postoperative events observed after dental replantation, the literature agrees that external root resorption surely is the most significant, not only due to its frequency, but also because it represents, per se, one of the causes of failure [3,4,5,6].

As it seems, those resorptions are associated with the inflammatory process of the periodontal ligament due to surgical trauma [5,6,7,8].

The extra-alveolar time also seems highly significant, as it concerns the survival time of the replant [9,10], increasing the degree of resorption of cement and dentin [3,11] and reducing the possibilities of revascularization of the pulp [12].

Although the exact mechanism, through which external root resorption occurs, has not yet been well established, it is possible that means of reducing or preventing it be determined before its causes are entirely known [13].

In that sense, an important feature related to the operative procedures is doubtlessly the immobilization of the replanted tooth [7,9,14,15].

The evidence relative to the effect of splinting is more subjective than objective. Experience has demonstrated that a non-rigid splint probably leads to a functional arrangement of periodontal fibers, while a rigid splint, maintained for too long,

results in a non-functional periodontal arrangement where the fibers run parallel to the root surface, producing areas of ankylosis [14].

In the same manner, short periods of splinting, around one week, seem to promote superior results in terms of resorption and ankylosis, in comparison with longer periods of immobilization [9,15,16].

It is the aim of this study to define, on objective bases, the effect of splinting of the replanted tooth on the postoperative events related to the tooth and its supporting structures.

Materials and Methods

Seventy-two male albino rats weighing 100 to 110 grams were used in this study. They were maintained, before and during the whole experimental period, on a solid diet,* with the exception of the first 24 hours postoperatively and were given water "ad libitum" [17].

The animals were divided into two groups of 36, manipulated under general anesthesia induced with sodium pentobarbital,** in an approximate dosage of 40 mg/kg of body weight.

Group I—Under general anesthesia and using a 1/2 spherical bur rotating at low speed on the occlusal surface of the upper left first, second and third molars, a sulcus was made in the proximal/distal direction with a width similar to the diameter of the bur and an approximate depth of 0.5 mm, corresponding to the width of the enamel at that location [18].

When the sulcus was ready, after cleansing with physiologic solution and application of merthiolate solution*** as an antiseptic, the upper left first molar of all the animals was extracted using instruments specially adapted to this task.

After immediate replantation of that tooth, the occlusal surface of the three molars was degreased using an anionic detergent and, after acid etch of the enamel surfaces, fluid resin was applied to the three molar teeth as the splint base and was immediately built up with composite [11,19].

The manipulation and insertion of those materials were those conventionally described by the manufacturer of the composite system used,**** plus the information from KOMATSU et al. [20] and GAYNOR [19].

The 18 animals corresponding to the three longer periods were again anesthetised on the 10th postoperative day and the splint was cut up in the portion between the distal surface of the first molar and the proximal surface of the 2nd molar using a 1/2 spherical low speed bur, liberating the upper first molar.

Group II—The procedures were similar to those used for the previous group in relation to anesthesia and dental extraction. The molars were immediately replanted and maintained in their sockets without splinting.

The animals from the two groups, after manipulation, received an application

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of 16,000 I.U. of penicillin G. Benzatine.*

Six rats, in each group, were sacrificed at 2, 5, 10, 20, 30 and 60 postoperative days, and the left maxilla was removed, including the molar teeth and the adjacent hard and soft tissues.

The obtained specimens followed routine laboratory procedures; four of them were embedded so that they could be cut in a longitudinal direction and two were embedded to be cut transversely. The blocks were cut semiserially, 6 μm thick, and stained with hematoxylin and eosin for histological study.

Results

The results for the two groups will be described in terms of the different structures which were studied: mucous membrane, periodontal ligament, cement and dentin, pulp and alveolar bone.

a) *Mucous Membrane:*

For the two initial periods, the gingival epithelium presents, for both groups, similar features.

For the 2 day period, degenerate epithelial cells can be seen and the underlying connective tissue presents great numbers of polymorphonuclear neutrophils.

After 5 days, the gingival epithelium displays discrete proliferation in the apical direction and, in the underlying connective tissue, an intense neutrophilic infiltrate can be seen. Epithelial attachment is not observed in either group when those two periods are analyzed.

After 10 days, the epithelium continues its apical proliferation and attachment is not observed in either group. The adjacent connective tissue still presents an intense inflammatory infiltrate composed of great numbers of lymphocytes and some neutrophils. For the splinted teeth a moderate infiltrate of lymphocytes is observed.

At 20, 30 and 60 days, the gingival epithelium can be seen close to the cement (Fig. 1) and attachment is observed at the level of the cement/enamel junction or slightly below that limit for the splinted teeth. The underlying connective tissue presents, for those three periods, a small number of lymphocytes.

For the teeth which were not splinted, the gingival epithelium, after 20 and 30 days, continues its apical growth. After 60 days, reattachment occurred in only two cases, far below the cement/enamel junction and, in the remaining specimens, the epithelium remains far below that limit and reattachment cannot be seen. For these teeth, an intense neutrophilic infiltrate is observed as late as the 20 day period. After 30 days, the mucous membrane connective tissue displays an intense infiltrate of lymphocytes and histiocytes, which turns out to be moderate in the final period.

b) *Periodontal Ligament:*

For the two initial periods, both groups present a sound and well vascularized periodontal membrane close to the root apex and alveolar bone (Fig. 2). Closer to the medial and cervical thirds less vascular and degenerate areas can be seen, especially adjacent to the cement. The inflammatory infiltrate, present in the periodontal

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Fig. 1 Group I. 20 days. Gingival epithelium attached at the level of the cement/enamel junction. H.E. 250 X.

Fig. 2 Group I. 2 days. Transversal section of the tooth, at the cervical third, showing some fibroblasts with reduced nuclei in the periodontal membrane close to the cement. H.E. 250 X.

Fig. 3 Group I. 30 days. Transversal section of the tooth, at the middle third, showing periodontal fiber reattachment. H.E. 160 X.

Fig. 4 Group I. 60 days. Middle third of the root, showing periodontal fiber reattachment. H.E. 160 X.

Fig. 5 Group II. 5 days. Transversal section, at the middle third, showing small resorption areas of the cement and dentin. H.E. 160 X.

Fig. 6 Group I. Resorption areas partially repaired by newly formed cement. H.E. 160 X.

Fig. 7 Group II. 30 days. Transversal section of the tooth, at the cervical third, showing areas of degenerate periodontal membrane close to the cement. Pronounced root resorption can be noted. H.E. 160 X.

Fig. 8 Group I. 10 days. Alveolar bone wall, at the cervical third, with severe resorption and great numbers of osteoclasts. H.E. 160 X.

ligament, is mainly composed of neutrophils for the non-splinted group and lymphocytes in the splinted group.

After 10 days, the splinted teeth display a sound periodontal ligament containing newly formed fibroblasts and capillaries with the exception of small areas close to the gingival margin. The connective tissue is mildly infiltrated by lymphocytes and histiocytes.

The teeth from Group II, at that time, present several degenerate areas, especially close to the cement. The remaining areas are well vascularized and newly formed fibroblasts are observed. A moderate infiltrate of lymphocytes and histiocytes was also found.

For the splinted teeth, a sound periodontal ligament is observed after 20 days. The two final periods, 30 and 60 days, display reattachment areas related to newly formed cement (Figs. 3, 4).

The non-splinted teeth still present, at the 20 day period, a mild infiltrate of lymphocytes, histiocytes and, at some points, neutrophils, as well as degenerate areas at the cervical third or even closer to the medial and apical thirds. After 30 and 60 days, those areas can be found close to the cement and no reattachment is observed. An intense infiltrate of lymphocytes and histiocytes still exists.

c) *Cement and Dentin:*

Two days after replantation both groups present sound cement and dentin. After five days small areas of resorption can be seen (Fig. 5).

After 10 days external root resorption is more severe in both cases in relation to the latter period. The sites of resorption occur especially at the cervical half of the root in relation to the buccal and proximal surfaces. Comparing the two groups, the alterations of cement and dentin are more pronounced, at this moment, for the non-splinted teeth.

After 20 days, the teeth from Group I present larger areas of resorption, if compared to the latter period, but points of cementogenesis can be seen. After 30 and 60 days only areas of inactive root resorption can be seen, partially repaired by newly formed cement (Fig. 6).

The non-splinted teeth present, after 20 days, areas of active resorption, more pronounced than those observed for the latter period. Some of these areas are partially repaired with newly formed cement. Although some of those repaired sites persist after 30 and 60 days, in other locations the resorption is still active, with the presence of multinucleated cells (Fig. 7). These areas are especially large after 60 days.

d) *Pulp:*

The dental pulp, in most instances, shows signs of integrity at the apical portion for the splinted teeth. This can be seen from the initial period until 60 days.

The non-splinted teeth, meanwhile, present a vital pulp at the apical portion in 50% of the specimens after 2, 5 and 10 days. After 20, 30 and 60 days postoperatively the pulp is, in general, necrotic.

e) *Alveolar bone wall:*

Both groups display, after 2 and 5 days, resorption of the alveolar crest, with the presence of osteoclasts, especially at the proximal surface. After 10 days the

resorption is intense at that location (Fig. 8).

After 20 days, for both groups, the alveolar bone wall presents, through its whole length, areas of active or inactive resorption, repaired, in the latter case, by newly formed bone.

After 30 and 60 days the resorption is not active, although some areas of activity could be seen for the non-splinted teeth. As a whole, bone resorption was more extensive and deeper for these teeth.

Discussion

The extra-alveolar time has been pointed out as significant on postoperative alterations after dental replantation, resulting in a greater index of external root resorption [10,21,22] and reducing the chances of revascularization of the pulp [12, 14]. For this reason, we preferred the immediate replantation of the extracted teeth.

In relation to splinting time, the literature suggests that longer periods of immobilization may be harmful to periodontal healing, especially in the case of teeth with short extra-alveolar periods [9,15,16]. So, we thought 10 days would be an advisable period for splinting in this study.

The kind of splint we used seems to be relatively free of trauma and well tolerated by the experimental animal, due to its small size. The composite, though, must be confined as much as possible to the sulcus made on the occlusal surface, reducing possible occlusal interference.

The healing process, in terms of epithelial attachment, seemed faster and better for the splinted teeth in this study. For these teeth, that epithelium was positioned closer to the cement surface after 10 and 20 days than it was observed for the teeth from Group II.

In fact, the splinted teeth presented, after 30 and 60 days, epithelial reattachment at the level of the cement/enamel junction or a little below that limit.

On the other hand, the migration of the mucous membrane epithelium, in the apical direction, already passed the cement/enamel limit after 20 days in the case of the non-splinted teeth. After 60 days, epithelial reattachment occurred in only two cases and yet far below that limit. In the remaining specimens, from Group II, reattachment was not observed.

The migration of the epithelial attachment in the apical direction, in a greater or lesser degree, has been frequently described as a postoperative alteration after dental replantation [7,23].

That migration seems to be related to the periodontal inflammatory reaction or to a phylogenetic tendency of the epithelial tissue to invade connective tissue [7,23,24,25].

The time needed for epithelial neoformation of the mucous membrane is directly proportional to the operative trauma [26], but one cannot state that epithelial proliferation and its apical migration would be responsible for connective fiber destruction or that it would depend on the degeneration of those fibers, since the two phenomena occur at the same time [27].

GOLDMAN [28], however, suggests that the prevention of epithelial migration depends on a healthy periodontal ligament,

We observed, in this study, that epithelial reattachment occurred for the splinted teeth, which indeed presented a healthier periodontal ligament than the non-splinted teeth.

The differences in the final position of the epithelial attachment between the two groups, may be due to the mobility of the non-splinted teeth, which would prevent an approximation between epithelium and cement and posterior reattachment.

While without revascularization, the ligament, close to the cement, may maintain its integrity in areas where nutrition by diffusion is efficient [29], or it may undergo degeneration at some points as we observed in this study.

The lesser vascularization of the medial portion of the periodontal ligament, which we observed in the initial periods, was also found by CASTELLI et al. [30]. According to those authors, that happens because apical vascularization is much more extensive, while revascularization at the level of the middle third has to be done primarily on account of labial and palatine sources which are poorer.

Conversely to those writers we were able to observe, in the initial periods, a lesser vascularization and greater degeneration of the periodontal ligament at the cervical level. Those differences may be due to a probable different pattern of cervical vascularization of the teeth used in this study, or else to a more intense surgical trauma.

The persistence of the inflammatory process, observed for the non-splinted teeth, may be due, as it seems, to mobility and the consequent more intense trauma to the adjacent structures during mastication.

Cementogenesis was a significant feature in this study at places which presented connective fiber reattachment of the periodontal ligament. In fact, the relationship between cementogenesis and fiber reattachment has been frequently described [31, 32, 33].

Those areas were not observed for the non-splinted teeth which, besides that, also presented degenerate areas of the ligament, still after 60 days. It is very probable that this occurrence may be one of the consequences of their movements during the early phases of the healing process.

On the other hand, splinting for a short time seems to create better conditions for periodontal healing and even conduct for the functional arrangement of the periodontal fibers after dental replantation [9, 14, 15, 16].

We observed, in this study, that the regions more directly related to surgical trauma, which are the cervical, labial and proximal areas, presented resorption points already in the initial periods. The more severe trauma on those regions can be easily explained if we consider that the extractions were done with an extractor under lever type movements with greater support on those areas. It is probable that the intensity of the trauma determines the number of surviving cementoblasts per unit area which, associated with factors as the distance between a potential resorption site and an area containing great numbers of surviving cementoblasts, as well as the pulpal "status," would determine the kind of postoperative changes that would occur and the degree of its progress or repair [5].

The presence of inflammatory infiltrates related to areas of external root re-

sorption was observed, primarily, for the non-splinted teeth which presented more severe pulpal alterations. These observations seem to agree with the results from ANDREASEN [6].

In this study, resorption lacunae, partially repaired by newly formed cement, were observed for both groups. This agrees with the description from ANDREASEN [11]. The resorption was more extensive for the non-splinted teeth, which still presented evidence of active resorption for the two final periods, conversely from the splinted group. These differences seem to be due to the absence of splinting.

Those results differ from the observations of ANDREASEN [9], who did not observe any beneficial effect from splinting on replanted teeth in monkeys after short extra-oral periods. It is possible that those differences are due to the fact that the author used longer periods of splinting (2 and 8 weeks), besides other treatments, as extra-alveolar root filling.

On the other hand, the same author [15] describes beneficial effects from splinting, for one week, upon the periodontal healing process of human teeth. NASJLETTI et al. [16] also describe, after replantation in monkeys, a lesser degree of resorption and ankylosis due to a shorter period of immobilization.

The pulpal revascularization we observed, at the level of the apical third, has been described elsewhere [34,35].

So, for the longer periods, the splinted teeth presented, as a rule, sound pulpal tissue at least at the apical level, while the non-splinted teeth presented, for the same periods, pulpal degeneration, probably due to the movements consequent to the absence of immobilization, which would prevent the attempts of early revascularization during the initial periods. According to ANDREASEN [11] and RAND [36], the immobilization of the replanted tooth would be necessary to reduce damage to the pulp and supporting structures, besides protecting the blood clot, increasing the chances of revascularization of the tooth.

The resorption of the alveolar crest, lately repaired at least partially through newly formed bone tissue, was more extensive for the teeth from Group II, maybe due to the absence of splinting.

For both groups, those resorptions occurred, primarily, at the level of the vestibular and proximal surfaces, probably because those regions suffered greater surgical trauma and, maybe, as it is the case of the proximal surface, due to the technique used to extract the teeth.

Anyway, the process of resorption of the alveolar crest may have a close relationship with operative and masticatory trauma with the consequent presence of an inflammatory reaction within the periodontal ligament [7].

Some writers suggest that functional stimuli might inhibit ankylosis, depressing osteogenesis and stimulating fibrous healing. An early process of ankylosis, if present, would be removed by a later process of resorption, as it may happen in teeth splinted for a short time [5,8,9,15,16] or at the cervical region of non-splinted teeth [37].

Maybe those facts can explain the absence of ankylosis in this study since we were not able to relate it to the presence of epithelial rests of MALASSEZ, which, according to LÖE and WAERHAUG [38] and NASJLETTI et al. [32] would also be restrict-

ing factors of dento-alveolar ankylosis.

Although biologically ideal healing of all the structures of the replanted tooth cannot be obtained at the present level of knowledge, it is very important that procedures, well based on biological studies, be offered to the clinician as a means of treatment of avulsed teeth.

Summary and Conclusions

Seventy-two male albino rats were used in order to analyze the effect of splinting upon periodontal healing after molar replantation. The animals were sacrificed after 2, 5, 10, 20, 30 and 60 days. The authors concluded that splinting of the replanted teeth:

- a) Contributes to limiting the apical migration of the mucous membrane epithelium and favors epithelial reattachment.
- b) Contributes to reducing the degree of external root resorption and the degree of resorption of the alveolar bone wall.
- c) Creates better conditions for healing of the periodontal ligament and favors the revascularization of the pulp.

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