# The effect of orthodontic relapse on the proliferation of fibroblast and epithelial rests of Malassez in periodontal ligament of rat molars (A histopathological study)

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## ABSTRACT

Background: Relapse of previously moved teeth, is major clinical problem in orthodontics with respect to the goals of successful treatment. This study investigated the effect of orthodontic relapse on the proliferation of fibroblast and epithelial rests of Malassez cells in periodontal ligament of rat molars.

Materials and Methods: Sixteen ten-week- old male Wister rats were randomly divided into four groups composed of four animals each: Group I received no orthodontic force (control). In both Group II and Group III, uniform standardized expansive springs were used for moving the maxillary first molars buccally for periods of one and three weeks respectively. The spring initially generated an average expansive force of 20 g on each side. In Group IV the springs were left for three weeks, until the maxillary first molars moved buccally, after that the springs were removed and the animals were scarified after three weeks of relapse tooth movement. After the humanly scarification of animals, each maxilla in all groups was dissected into two halves each half including the three maxillary molars and processed for histological examination. The number of both fibroblast and ERM cells in each cluster was counted in the PDL of the pressure side of the mesio-buccal roots of the maxillary right and left first molars in all groups and the surface areas of the ERM clusters were also measured in all groups.

Results: The number of fibroblast was significantly increased at the end of active movement (Group III) and significantly very highly increased during the relapse period (Group IV). Regarding the ERM cells there were statistically significant increase in both the number of cells in each ERM cluster and the surface areas of the ERM clusters in Group III and highly significant increase in Group IV, while Group II showed no significant differences regarding all measurements.

Conclusions: It was concluded that fibroblast and ERM cells may play an important role during orthodontic relapse

Keywords: orthodontic relapse, fibroblast, epithelial cell rest of Malassez, rat. (J Bagh Coll Dentistry 2013; 25(4):114-119).

## INTRODUCTION

In orthodontics, relapse is a concept that is antagonistic to stability and can be defined as the tendency of treated teeth to return to their original position. Relapse in its various manifestations remains a phenomenon whose etiologies are still not well determined, unproved, and thus highly controversial. Its etiology is multifactorial. The major causative factor is the considerable residual forces remain in the tissues of the periodontium after teeth movements which tend to return the teeth into their original position [(1)]. Studies have shown that no means are yet available to help the relapse prediction following orthodontic treatment; therefore permanent retention could be the most reliable solution [(2)].

Tissue remodeling in orthodontics is mediated by a variety of cells. Teeth can be moved because cells around their roots are enticed by the mechanical force to remodel the tissues around them. Numerous studies have investigated the response of the dental supporting tissues to orthodontic force.

These studies attempted mainly to analyze the histological changes in paradental tissues during orthodontic tooth movement. Those studies showed extensive cellular activities in the mechanically stressed PDL involving fibroblasts, endothelial cells, osteoblasts, osteocytes, and endosteal cells [(3)]. To date, no attempt has been made to describe in detail histological changes during relapse and only a few studies have been published about the biological mechanisms behind the relapse process [(4)].

Fibroblasts are the principal cells of the periodontal ligament. They are aligned along the general direction of the fiber bundles and extend cytoplasmic processes that wrap around them. They are large cells responsible of protein synthesis and secretion. One of the main factors that avoid relapse is the rate of turnover or renewal of the periodontal collagen fiber turnover [(4)] which appears to be regulated by fibroblasts [(5)]. Therefore the periodontal fibroblasts may play a crucial role during orthodontic relapse.

Epithelial cell rests of Malassez (ERM) are development residues of Hertwig's root sheath, which is a double-layer structure made of epithelial cells that is present around the tooth root. In the periodontal ligament (PDL) of mature teeth, the epithelial cells form strands close to and

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along the cementum [6,7]. It was long believed that ERM comprised latent or quiescent cells devoid of structure and function, often associated with the genesis of cysts and tumors. However, these epithelial periodontal components are active, have a fundamental role in root development, root integrity even during orthodontic movement, protect against root resorption, and are involved in reparative and regenerative functions of the pulp and periodontal tissues including apexogenesis and periodontal healing. Compiling evidence suggests that ERM may be the source of cementoblasts [8-10]. Many investigations on the developmental and evolutionary biology of ERM with several clinical reports were supported a concept of ERM as a regulator of periodontal ligament biology as it relates to width, blood vessel homeostasis, and cementogenesis, as well as protection against resorption and ankylosis . There is evidence that ERM cells are stimulated during orthodontic tooth movement [9, 11].

No study examined the effect of orthodontic relapse on the fibroblast and ERM activities. Therefore the purpose of this study was to investigate the behavior and activity of these periodontal ligament cells against orthodontic relapse of rat molars. As a more knowledge about the effects of orthodontic relapse on discrete cell populations could strengthen the rationale of retention programs and thereby improve post orthodontic retention phase. Furthermore this study is an attempt to provide a base line for later histological, immunohistochemical and biochemical investigations on the fate of fibroblast and the ERM of the rat molars under different phases of orthodontic tooth movement and to serve as a part of a broader study of the biology of orthodontic relapse in accordance to the action of these cells.

# MATERIALS AND METHODS

## Sample

Sixteen ten-week- old male Wister rats weighing 250- 300 g were purchased from the animal house of the National Center of Drug Control and Research/ Baghdad- Iraq and used as experimental animals. The animals were treated ethically according to the guidelines of the animal care staff at the National Center for Drug Control and Research / Baghdad–Iraq.

The samples were randomly divided into four groups composed of four animals each:

» Group I (GI): no orthodontic force (control).

» Group II (GII): 7 days of orthodontic tooth movement.

» Group III (GIII): 21 days of orthodontic tooth movement.

» Group IV (GIV): 21 days after release of orthodontic force.

## Methods

Tooth movements were performed according to the methods used in a previous study by Al-Duliamy [12]. The rats in the control group receive no orthodontic force. In both (GII) and (GIII), uniform standardized expansive springs were used for moving the maxillary first molars buccally (Figure1).

The spring initially generated an average expansive force of 20 g on each side, and it exerted as continuous force of intermediate magnitude for molars expansion. In (GIV) the springs were left for three weeks, until the maxillary first molars moved buccally, after that the sprigs were removed and the animals were scarified after three weeks of relapse tooth movement.

## Histology

After the humanly scarification of animals under general anesthesia, each maxilla was dissected into two halves each half including the three maxillary molars. The specimens were then dehydrated and embedded in paraffin. Paraffin blocks were cut coronally at the area of maxillary first molar in 5 μ m thick serial sections with a microtome and mounted on microscope slides. The sections were stained with hematoxylin and eosin. The mesio-buccal roots of the maxillary right and left first molars of all groups were identified and examined. The root was divided into buccal and palatal sides, based on the mesio-distal axis of the root. Evaluation of the fibroblast and ERM cells numbers in clusters and the surface areas of these clusters was performed on the root surface of the pressure side of the mesio-buccal roots in all groups and in both halves of rat maxilla. The hematoxylin and eosin stained slides were examined by digital light microscopy to identify the fibroblast and ERM cells based on their morphology. Fibroblast was observed as elongated cell with little cytoplasm and dark staining flatted nucleus [5]. According to a method described previously [13], the ERM cells in their characteristic clusters were counted and the surface areas of the clusters were measured. All measurements have been done at 40X magnifications by using the inbuilt image processing software of digital microscope (Micros Crocus II MCX100LCD Produktions und HandelsgmbH) that was fed directly to a TV monitor with a real time live camera.

Measurements were blindly performed by two operators, after inter-operator calibration. The final count was designated to be the mean of these counts. Values for four sections, selected at five section intervals, were averaged for each animal.

### Statistical analyses

The mean numbers of fibroblast cells, ERM cells in clusters and the surface areas of these clusters on the pressure side of the mesio-buccal roots of the maxillary right and left first molars in all groups were statistically analyzed and compared with one-way analysis of variance (ANOVA) and least significant difference (LSD) test SPSS software.

In the statistical evaluation, the following levels of significance were used:

| | | |
|---|---|---|
| **P > 0.05** | **NS** | **Non-significant** |
| **0.05 ≥ P > 0.01** | ***** | **Significant** |
| **0.01 ≥ P > 0.001** | ****** | **Highly significant** |
| **P ≤ 0.001** | ******* | **Very highly significant** |

# RESULTS

### Fibroblast cells

From observations on Table 1, it is found that the highest mean value of the fibroblast cell number was found in Group IV while the lowest mean value of fibroblast cell number was found in Group I.

According to LSD (Table 2), there were no statistically significant difference between Group I and Group II in number of fibroblast cell, highly statistically significant difference between Group I and Group III and a very highly significant difference between Group I and Group IV.

### ERM cells

Typical appearance of the ERM was seen under digital light microscopy; they appeared in the form of clusters. The clusters consisted of numerous epithelial cells. These numerous epithelial cells showed oval or round cluster forms, and some cells showed a strand form. They were localized near the root surface rather than near the alveolar bone (Figure 2). From observations in Table 1, it was found that, the highest mean value of the ERM cell numbers were in Group IV. While the lowest mean value of the ERM cell numbers were in Group I.

On the other hand, the highest mean value of the surface areas of clusters were in Group IV. While the lowest mean value of the surface areas of clusters were in Group I. According to LSD (Table 2), there were no statistically significant difference between Group I and Group II in both the ERM cell numbers and the surface areas of clusters and statistically significant difference between Group I and Group III in both measurements and a very highly significant difference between Group I and Group IV. As there were a significant increased in number and cluster size of epithelial rests in areas of compression in Group III and Group IV (Figure 3).

# DISCUSSION

A rat model widely used in previous studies was also used in this experiment. According to Vignery and Baron [14], Tran Van et al. [15], Baron et al. [16] and Ren et al. [17], rat model has many similarities to the supporting structures in human teeth and it is generally good model to study orthodontic tooth movement because of its higher remodeling rate.

In the present study the pressure side in Group II and III represents the buccal side of the mesio-buccal roots of the maxillary right and left first molars while in Group IV after the removal of the expansive force it represents the palatal side of the mesio-buccal roots of the maxillary right and left first molars as the pressure here was as a result of rebound of the compressed periodontal ligament.

### Fibroblast cells

At the pressure side of the mesio-buccal roots of the maxillary right and left first molars of Group II, III and IV, the fibers of the PDL were compressed and between these fibers the fibroblast cell were arranged (Figure 2), this is in accordance with Krishnan and Davidovitch [4].

In comparison with Group I (control) there was no statistical differences in the number of fibroblast cell in Group II. One possible explanation for this is the fact that this time intervals are associated with inflammatory responses to forces. Furthermore according to Rygh [18] and Brudvik and Rygh [19], these early stages are associated local tissue necrosis of the compressed regions when no cells are present. It is to be expected that it takes time for these regions of the PDL to reorganize.

Regarding Group III**,** there was increase in the numbers of fibroblast cells. These finding confirmed by the result of Von B€ohl et al. [20] which reported that after the removal of the necrotic areas, the cells start the bone remodeling process.

Regarding the sample in Group IV, after release of the expansive force, the very highly significant differences in the number of fibroblast cell in this group may be explained by the remodeling of the extracellular matrix which play

an integral part in tooth movement in response to the orthodontic relapse force and this matrix mainly produced by fibroblast cells according to Krishnan and Davidovitch [4].

**ERM cells**

In the present study of rats, typical cluster of the ERM were seen in the compression area in all the experimental groups very near the root surface in round form and strands arrangement this is in accordance with Becktor et al. [21]. The reappearance of the ERM in the compressed areas is explained by the cells' ability to migrate from the surrounding undamaged areas of the PDL.

In comparison with Group I (control) the ERM cell numbers and surface areas of clusters showed no statistical differences in Group II ; this may be explained by the result of Rygh [18] and Brudvik and Rygh [19], regarding the initial phase after force application which accompany local tissue necrosis with cell free zone.

The increased numbers of ERM and surface areas of clusters in Group III where periodontal remodeling is accelerated suggest that these cells might be involved in remodeling activities during orthodontic tooth movement this is in consistent with the results of Talic et al. [13] and Consolaro and Consolaro [9]. Concerning the increased numbers of ERM and surface areas of clusters there was evidence that they were very highly significant in Group IV. This finding means that, relapse movement stimulates ERM cell proliferation.

The histological observations, along with previous findings [9] showed that orthodontic tooth movement stimulates ERM cell proliferation. On the other hand the present study observed for the first time that ERM could involve at least in part, in regulating tissue reorganization around the orthodontically moved teeth during retention phase after orthodontic tooth movement. These finding suggesting that the ERM may play a crucial role in enhancing post orthodontic stability which is most difficult elements of clinical orthodontic practice. Future studies are needed from a biomolecular basis and cell biological approach to clarify the specific role of the ERM in tissue reorganization during retention periods particularly collagen turnover and production of different mediators for tissue remodeling.

As a conclusion; it has been shown in this study that, there were highly significant increase in the numbers of fibroblast cells**,** numbers of ERM cells in clusters and the surface areas of these clusters during relapse tooth movement. This exaggerated response of these periodontal ligament cells indicate that the fibroblast and the ERM cells may play an important role during orthodontic relapse.

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**Table 1. Descriptive statistics and groups' comparisons regarding fibroblast and epithelial cells numbers and cluster surface area of the epithelial cells**

| Groups | Fibroblast cells number | | Epithelial rests cell number | | Clusters surface area (µm$^2$) | |
|---|---|---|---|---|---|---|
| | Mean | S.D. | Mean | S.D. | Mean | S.D. |
| Group I | 8.125 | 0.834 | 5.125 | 0.834 | 2.956 | 0.659 |
| Group II | 8.125 | 0.834 | 5.125 | 0.834 | 2.923 | 0.634 |
| Group III | 9.250 | 0.886 | 6.125 | 0.641 | 3.583 | 0.350 |
| Group IV | 11.625 | 0.744 | 6.875 | 0.641 | 5.681 | 0.866 |
| ANOVA | 31.889 | | 9.651 | | 33.656 | |
| p-value | 0.000 *** | | 0.000 *** | | 0.000 *** | |

**Table 2. LSD test after ANOVA test**

| Parameters | Groups | | Mean difference | p-value |
|---|---|---|---|---|
| Fibroblast cells number | I | II | 0 | 1 (NS) |
| | | III | -1.125 | 0.011 * |
| | | IV | -3.50 | 0.000 *** |
| | II | III | -1.125 | 0.011 * |
| | | IV | -3.50 | 0.000 *** |
| | III | IV | -2.375 | 0.000 *** |
| Epithelial rests cell number | I | II | 0 | 1 (NS) |
| | | III | -1 | 0.015 * |
| | | IV | -1.75 | 0.000 *** |
| | II | III | -0.921 | 0.023 * |
| | | IV | -1.75 | 0.000 *** |
| | III | IV | -0.75 | 0.073 (NS) |
| Clusters surface area (µm$^2$) | I | II | -0.033 | 0.902 (NS) |
| | | III | -0.627 | 0.036 * |
| | | IV | -2.725 | 0.000 *** |
| | II | III | -0.66 | 0.024 * |
| | | IV | -2.758 | 0.000 *** |
| | III | IV | -2.098 | 0.000 *** |

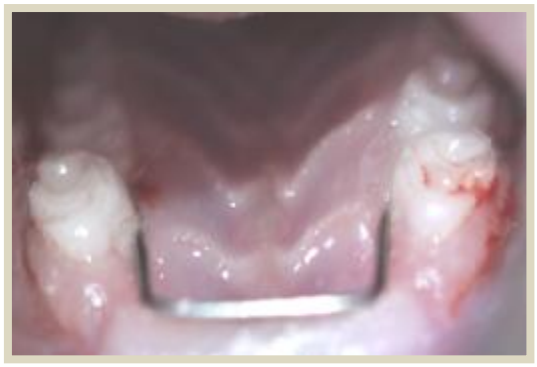

**Figure 1: The spring in its place in rat maxilla.**

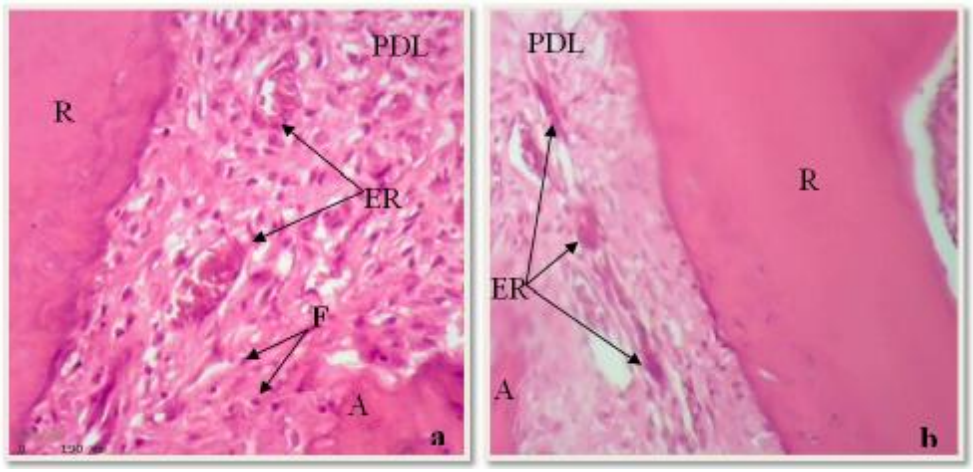


**Figure 2:Typical clusters of epithelial rests (ER) in compression side of PDL very near root (R) surface, alveolar bone(A), fibroblast cell (F). a: round cluster. b: strand form,(H & E Staining × 40).**

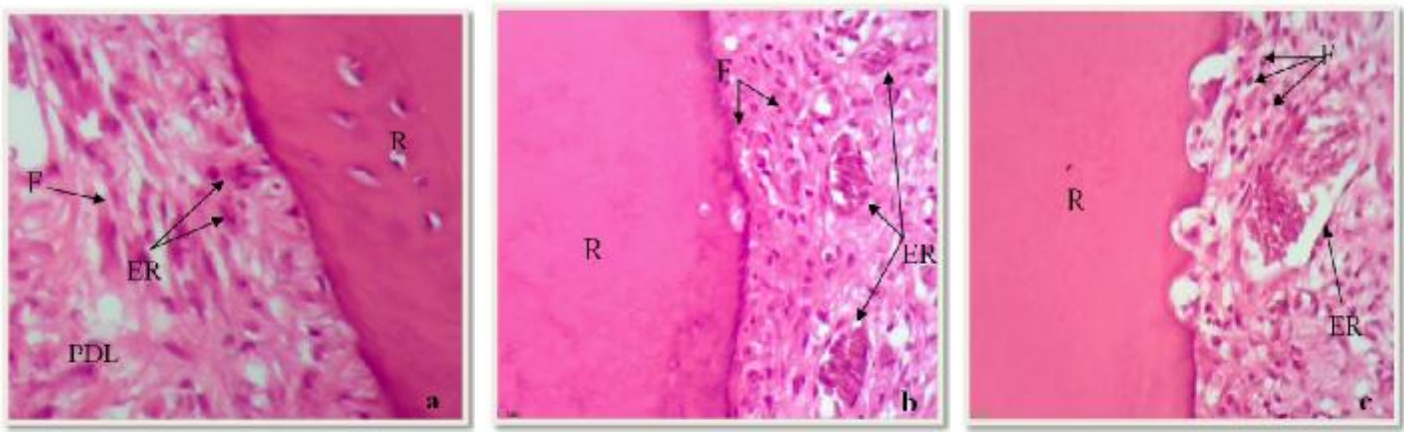


**Figure 3: Clusters of epithelial rests (ER) in PDL near root surface (R) and Fibroblast cell (F) in a: Group I (control) b: increased number of fibroblast and epithelial rests cell and cluster size in Group III and in c: Group IV (H & E Staining × 40)**