

Ultrastructure of Human Junctional Epithelial Cells Subjected to Different Magnitudes of intrusive orthodontic Forces

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Abstract

Background: The present work aims to observe the structural characteristics and cellular features of Junctional Epithelium (JE) subjected to light and heavy intrusive orthodontic forces.

Materials And Methods: Twenty vital maxillary first premolars from orthodontic extraction cases were allocated in three groups. Control group: premolars were not subjected to any force.

Group I: light intrusive force was applied.

Group II: heavy intrusive force was applied. Extraction was done after 7 and 14 of force application. Transmission electron microscope (TEM) was used to examine JE cells.

Results: Control, TEM revealed common cellular features regarding JE cells. Evidence of cellular alterations of JE could be detected in both experimental groups after 7 days as they expressed various degrees of nuclear alterations, chromatin condensation, cytoplasmic organelles affection and loss of cellular junctions. Moreover, no serious deteriorations were reported in all groups such as, nuclear pleomorphism or apoptotic figures. Adaptive figures were recorded in all experimental groups with variable degrees. The basal junctional epithelial cells exhibited more enhancements in ultrastructural criteria than the superficial cells.

Conclusions: Human JE was a unique tissue. It is characterized by low cellular differentiation but high adaptability even after exposure to different magnitudes of Intrusive orthodontic forces.

Keywords: Junctional Epithelium, TEM, tooth intrusion, orthodontic force.

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I. Introduction

Orthodontic treatment seeks an acceptable functional and aesthetic occlusion with proper tooth movements.¹ Periodontal complications are reported to be one of the most common side-effects linked to orthodontics. Therefore, periodontal health is an important factor that may be used to evaluate the success of orthodontic therapy.² Biological changes in periodontium caused by orthodontic treatment should be considered by specialists in order to facilitate their collaboration and manipulation especially the increased number of adult patients seeking orthodontic treatment.³

Orthodontic intrusion is a common approach in treating different esthetic and functional problems, including gummy smile and deep bite.⁴

Intrusion was defined as "a translational form of the tooth movement directed apically and parallel to the long axis"⁵, or as "apical movement of the geometric center of the root in respect to the occlusal plane or a plane based on the long axis of the tooth."⁶

Intrusion commonly used in orthodontic treatment to improve sagittal and vertical incisor relationships, to correct interincisal angle and consequently, the gingival line and restore the esthetics of smiling.⁷

For many years, orthodontic intrusion was unfavorable and associated with serious side-effects from the periodontium and cementum, e.g. root resorption. However, lately, documented successful orthodontic intrusion is obtained and considered a safe procedure, provided that the magnitude and direction of forces are carefully managed.⁸

During orthodontic intrusion in patients with healthy periodontium, the gingival margin and the mucogingival junction moves apically 79% and 62% of total intrusion, respectively.⁹ While in periodontal affected teeth, clinical data suggest that orthodontic intrusion can improve the level of attachment provided absolute control of inflammation and bacterial biofilms.^{10&11} The use of light intrusive forces was also recommended to move teeth efficiently and may reduce the amount of root resorption.¹²

Gingival epithelium consists of three regions: oral gingival epithelium (OGE), sulcular epithelium (SE) and Junctional epithelium (JE). JE is a specialized type of gingival epithelium that is attached to the crown or root tightly like a collar. It is located at the junction of periodontal soft tissue and hard tissue. As, JE is a special structure at dento-gingival junction, it differs from other epitheliums (OGE, SE) in origin, cell morphology, proliferation and differentiation.^{13&14} JE cells have unique orientation which is parallel to the tooth surface. They have uniform shape either flat or spindle, with large intercellular spaces.¹⁵ Previous studies were conducted upon orthodontic extrusion movements and their effects on the periodontal tissues, and the width of the keratinized gingiva. No negative effects on the periodontal tissues were noted due to application of orthodontic tooth extrusion.¹⁶

However, it is unclear about JE cellular reaction, repair after different magnitudes of intrusive orthodontic tooth movement.

II. Aim Of The Work

The present work aims to investigate the cellular JE changes that might occur in response to light and heavy intrusive orthodontic forces. This could help orthodontists to evaluate periodontal integrity towards intrusive orthodontic forces of different magnitudes, and accordingly case prognosis.

III. Materials And Methods

Twenty vital and sound maxillary first premolars, from orthodontic male patients (15-22 years old) treated in the Department of Orthodontics, Faculty of Dental Medicine for Girls, Al-Azhar University, Cairo, Egypt, were included in the study. All of the included premolars were selected to be minimally malposed, out of any occlusal trauma and were planned for extraction during comprehensive orthodontic treatment. All cases were selected to be Angle's class I dento-alveolar bimaxillary protrusion with minimal crowding. All patients had good oral hygiene, no previous orthodontic treatment, no history of dental trauma, no missing permanent teeth (with exclusion of 3rd molars), non-smokers, with noninflammatory disease or compromised oral conditions. The patients were instructed to stop taking any anti-inflammatory drugs 1 month before bonding till extraction. The study proposal was approved by the Research Ethics Committee (REC), Faculty of Dental Medicine for Girls, Al-Azhar University, Cairo, Egypt. An informed consent was signed by the patient or his guardian after treatment and research procedures were explained to him/her.

Grouping

Only one premolar from each patient was included in the study and randomly allocated in one of three groups. First is the control group, in which the maxillary first premolars (4 teeth) were not bonded and extracted just prior to the leveling and alignment stage. Second, Group I, in which light intrusive force was applied to the test premolars (8 teeth). Third, Group II, in which heavy intrusive force was applied to the test premolars (8 teeth).

Appliances and Loading

All patients were treated with synergy low friction straight wire (LFSW) appliances with 0.022" slot (Rocky Mountain Orthodontics RMO Corp., Colorado, USA). In Group I, the test premolars were not bonded until the 0.012" NiTi initial archwire (Ortho Technology, Inc., Florida, USA) was fully engaged in all other bracket slots. Then, the bracket of the test premolar was bonded in a position which allows the bracket slot to be 1mm occlusal to the archwire level and parallel to it. At the same session, the archwire was forcefully engaged in the slot of the newly bonded bracket giving light intrusive force as measured clinically. Measuring the force was done before bonding of the test premolar by deflecting the archwire segment between canine and second premolar brackets 1 mm occlusally using a wire tucker. Then a force gauge dynamometer (White Oak Orthodontic Products, Pennsylvania, USA) was used to measure the force generated due to wire recoil on unloading.

In Group II, the test premolars were not bonded until a 0.16" x 0.22" NiTi archwire (Ortho Technology, Inc., Florida, USA) is passively engaged in all bracket slots. At this time, the bracket of the test premolar was bonded, the archwire was activated and the produced force was measured exactly as in Group I but the generated intrusive force was much higher in Group II.

Post-operative care

Patients were instructed to forego oral hygiene procedures gently using soft brush. Chemical plaque control is initiated after orthodontic wire fixation by 0.12 % Chlorhexidine Gluconate rinses, twice daily. Mechanical plaque control performed at weekly until the end of the study. In both Group I and Group II, gingival biopsy specimens less than 3 mm in depth detected by periodontal probing were isolated full length along the dento-gingival region and immediately above the alveolar crest after 7 and 14 days of intrusive force

application.^{14&17} Gingival tissues were carefully dissected during extraction of premolars, in buccolingual direction and cut into 1 x 1 x 2 mm where the longer side is the buccolingual, this will ensure proper orientation of the specimens during processing.

Preparation of the specimens for transmission electron microscopy (TEM)

Preparation and fixation

Gingival specimens were instantly immersed in a mixture of 2.5% Glutaraldehyde and 10% Formaldehyde (F/G solution) in a labeled jar for each group for 24 hours. After that, the specimens were prepared according to Bancroft and Stevens¹⁸ for electron microscopic examination. From each part, cross sections (1 mm thick) were cut and washed several times in phosphate buffer solution with pH 7.2-7.4. The specimens were post-fixed in 1% Osmium Tetroxide for one hour, and washed again in Phosphate Buffer. The specimens were loaded in ascending concentrations of Ethyl alcohol for complete dehydration. The specimens were embedded in (EPON 812) in flat rubber moulds to obtain the specimen blocks. Semi-thin sections were cut with a diamond knife, mounted on glass slides and stained with 1.0% Toluidine Blue for light microscopic examination (Semi-Thin sections). The area of interest, junctional epithelial cells (JE), was selected for Ultra-Thin sectioning. The cut sections were stained with Uranyl Acetate and Lead Citrate to be examined with transmission electron microscope (Joel Ltd., Tokyo, Japan). Then, the specimens were thoroughly examined and reported after masking the group number to make the evaluation unbiased.

IV. Results

The applied force

The applied force due to archwire unloading in the light intrusive force group (Group I) ranged from 18 to 35 with a mean of 26.25 ± 5.18 grams. In group II where heavier intrusive force was applied, the force was much higher and ranged from 86 to 122 grams with a mean of 104.5 ± 12.34 grams (Table 1).

Table 1. Descriptive statistics of the applied force (in grams) in Group I and Group II

	n	Mean	SD	SE	Median	Mode	Range	Min.	Max.
Group I	8	26.25	5.18	1.83	25.5	25	17	18	35
Group II	8	104.5	12.34	4.38	101.5	---	36	86	122

n: Number of cases *SD*: Standard deviation *SE*: Standard error

TEM evaluation

Control Group

Electron Micrographs of control group detected junctional epithelial cells (JE) very clearly. It appeared with neither keratinization nor epithelial ridges. JE had a clear boundary with SE and possessed its special morphology and orientation. JE cells were hyperchromatic flat or spindle and they were aligned parallel to the tooth surface. The basal layer and suprabasal layer were clearly identified. The intercellular spaces were obvious. JE cells were abundant in organelles with large nucleus Fig.1.

Group I

Electron micrographs of Junctional Epithelial cells after 7 days of light intrusive force application revealed vary structural alterations. These structural alterations could be presented by diffuse intracellular vacuulations, well-defined irregular cell and nuclear membranes and nuclear chromatin clumping especially peripheral. However, wide intercellular junctions (IJ) and normal nucleus cytoplasmic ratio were detected Fig.2. After two weeks of light force application ultrastructure of superficial junctional epithelial cells showed elongated superficial cells with well-defined nuclear membrane and vacuolated wide intercellular junctions. Well-defined nuclear membrane with open faced nuclei and regular nuclear chromatin condensation were detected. Evidence of attachment plaques appeared in 2 specimens of this group Figs.3 and 4. Basal Junctional Epithelial cells of Group I (14ds) expressed wide open faced nucleus with central constriction, regular nuclear membrane, swollen mitochondria, wide intercellular junctions Fig.5.

Group II

Electron micrographs of Junctional Epithelial cells after 7 days of heavy intrusive force application revealed moderate structural alterations. The intercellular junctions were totally lost. The cell membranes were hazy and ill-defined. Their cytoplasm was filled with numerous small vacuoles. The nuclear membranes were totally hyalinized and ill-defined. Diffused nuclear chromatin condensation was a common feature of this group samples Fig.6. After two weeks of heavy intrusive force application ultrastructure of junctional epithelial cells showed superficial cells wide intercellular junctions. The superficial cells attained fusiform morphology. The cells expressed an altered nuclear cytoplasmic ratio with few cytoplasm and cytoplasmic organelles. All

cells attained large nuclei with diffuse hyperchromatism, however, their nuclear membrane were regular and well-defined Fig.7.

Basal Junctional Epithelial cells of Group I I(14ds) showing open faced nucleus with slight chromatin condensation, irregular nuclear membrane, wide hyalinized intercellular junctions and cytoplasmic organelles degeneration Fig.8.

V. Discussion

Orthodontic intrusion has wide indications in dental practice, such as for an anterior open bite or molar elongation. Conventional orthodontic intrusive techniques are usually accompanied by undesirable reactions of the anchorage teeth, and their complicated mechanisms also make periodontist resists to do the procedure. Successful orthodontic treatment obtained by moving the teeth as efficiently as possible with no or least damage to teeth and their supporting tissues. In the present study, the effect of two magnitudes of intrusive orthodontic force on human junctional epithelial cells after an interval of one and two weeks was investigated. Independent variables of two magnitudes of intrusive force and their effect on selected area of junctional cells were studied with its relevance to clinical importance.

It was of prime importance to study the effects of the intrusive forces on junctional gingival tissue which is as normal and healthy as possible like in normal clinical conditions. Therefore, the inclusion criteria were posted very carefully to select healthy young gingival tissue which is not affected by any disease, trauma or medicine as possible. Ethical considerations aimed to minimize patients' discomfort during teeth intrusion. So pain killer with no anti-inflammatory effect (Paracetamol) were allowed for patients during the study. Regarding clinical application of intrusive force, it was set to resemble the force applied during leveling stage with 0.012" archwires and during finishing and detailing stage with the 0.016"x0.022" archwires. Hence, the brackets of the test premolars were bonded only 1mm occlusal to the archwire level which is a common malposition during both treatment stages. Although this mechanics may not apply pure intrusion and will be accompanied by little anticlockwise labiopalatal moment, this momental component seems to be much less than that expected during intrusion applied with cantilever arm from first molar to first premolar.¹⁹ The mean of applied light force (26.25 grams) was not far from the mean optimum intrusive force reported by Proffit et al⁸ for multirooted teeth (20 grams), while the mean of the applied heavy force was much higher (104.5 grams). The used bracket system was Synergy Low-Friction Straight Wire (LFSW) appliances with 0.022" slot (Rocky Mountain Orthodontics RMO Corp., Colorado, USA). Both the 0.022" slot and its design in Synergy brackets allow more archwire play especially at the mesial and distal ends of the slot. This makes the actually applied force very close to that measured by the dynamometer.

Extraction was done after intrusive force application by one and two weeks. This period was reported by other researchers¹⁴ to trace morphological changes of junctional cells. Direct excision minute sample of junctional gingiva apart of tooth surface was selected, in order to minimize the effect of any external factors, such as decalcification, except the applied intrusive force. Ultra-structural cellular details were optimally obtained by transmission electron microscope. Junctional epithelial cellular affection was evidenced in all experimental groups as they expressed various degrees of nuclear alterations, chromatin condensation, affection loss of cytoplasmic organelles and cellular junctions. Dental pulp cellular alterations were reported by other researchers due to intrusive force application for 21 days.^{20&21} Cells always try to adapt upon mechanical influences of their surroundings, so they perform a complex mechano-chemical response, which depends on mechano-transduction mechanisms. Adhesion molecules have been shown to play a main role in mechano-transduction, it now evidenced that the nucleus can act as a mechano-sensitive structure.²² In all experimental groups, superficial junctional epithelial cells expressed wide extracellular compartments or even loss of cellular junctions. This could be attributed to their sensitivity to any changes in their external environment such as intrusive force. Application of orthodontic force, induce mechanical strain which could stimulate the cells and their associated extracellular matrix. This stimulation has the ability to regulate integrin expression, focal adhesion proteins, cytoskeletal organization, cell morphology, cell adhesion to extracellular matrices, cell proliferation, and cell differentiation.²³

Generally, when the extracellular matrix is stressed, isometric tension develops in the cells within the matrix. This isometric tension is equal in magnitude to the mechanical tensional force exerted upon them by the extracellular matrix, leading to changes in their cellular cytoskeleton and architecture with activation of cellular transcription factors.^{23&24} This in turn influences the expression of genes involved in cell attachment, proliferation, differentiation, and apoptosis. However, No serious deteriorations were reported in JE cells of all groups such as, nuclear pleomorphism or apoptotic figures after application of different magnitudes of intrusion forces. This finding goes with previous study which denied any negative effect on gingival tissues due to extrusion force after 6 months.¹⁶ This may indicate their high ability of adaptation and regeneration. Moreover, neither inflammation nor swelling microscopically in the gingiva was reported and the epithelium remained attached to the cemento-enamel junction, even when the tooth was intruded.²⁵ Ultrastructurally, heavy intrusive

force had more impact upon junctional epithelial cells than light intrusive force in majority of the examined groups especially at 7 days. Moreover, slight difference was recorded between light and heavy forces after 14 days.

Instead, features of adaptation were recorded in all experimental groups with variable degrees. The basal junctional epithelial cells exhibited more enhancements in ultrastructural criteria than the superficial cells. Whereas, basal cells of group I subjected to light intrusive showed better structural morphology than those subjected to heavy force. This is evident by nuclear division seen in group I. Figs. 5 & 8 After 7 days of heavy and light force application, the cells were spherical and not fully stretched. At 14 days after force application, JE cells were fully stretched to be spindle-shaped. This feature was reported by other researchers at 7 days of junctional epithelial cell culturing.¹⁴ The delay may be attributed to the primary affection of the applied intrusive force. Electron-dense lamina like attachment plaque was evidenced in few samples of group I after 14 days of force application. While, in co-culture model, JE cells can form basement membrane-like and hemidesmosome-like structures in 2 weeks.¹⁴

VI. Conclusions

JE is a unique special stratified gingival epithelium of quit differentiation but high adaptation and regeneration ability even after experiencing different magnitudes of intrusive orthodontic forces after 14 days. Basal junctional epithelial cells subjected to light intrusive force expressed faster regeneration than those subjected to heavy intrusive force. The healing ability and adaptability of junctional epithelial cells make both light and heavy intrusive orthodontic force a viable procedure when indicated.

Recommendations

Further investigations are yet to be assessed regarding the long-term results and the level of attachment of the junctional epithelial cells upon tooth surface.

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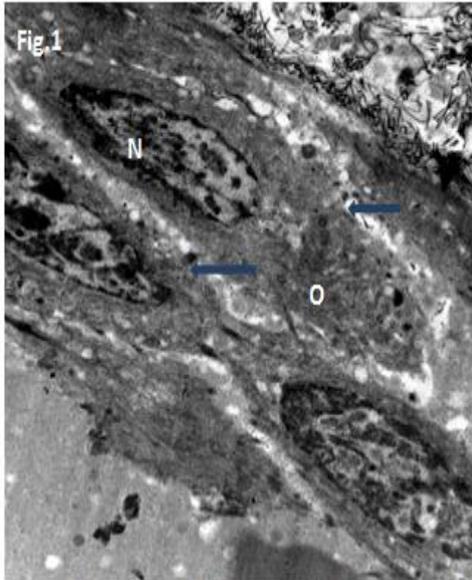


Fig.1 Electron micrograph showing Control Junctional Epithelial cells .They were hyperchromatic flat or spindle with large nucleus (N) and aligned parallel to the tooth surface. Notice:obvious intercellular spaces (arrows) and abundant organelles (O).Org.Mag.

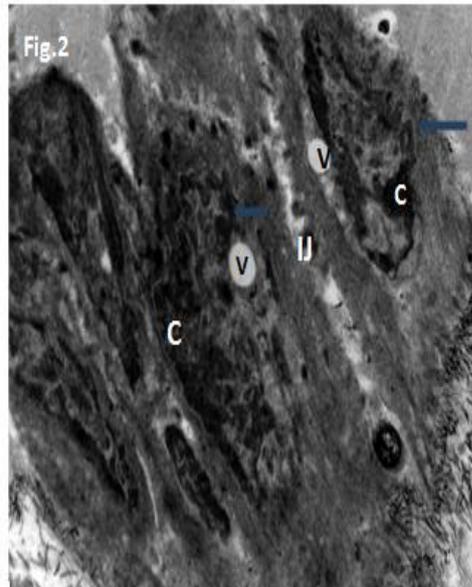


Fig.2 Electron micrograph showing Junctional Epithelial cells of Group I(7ds) showing wide intercellular junctions(IJ), normal nucleus cytoplasmic ratio, intracellular vacuolation (V), well defined irregular nuclear membrane (arrow) and peripheral chromatin clumping (C).

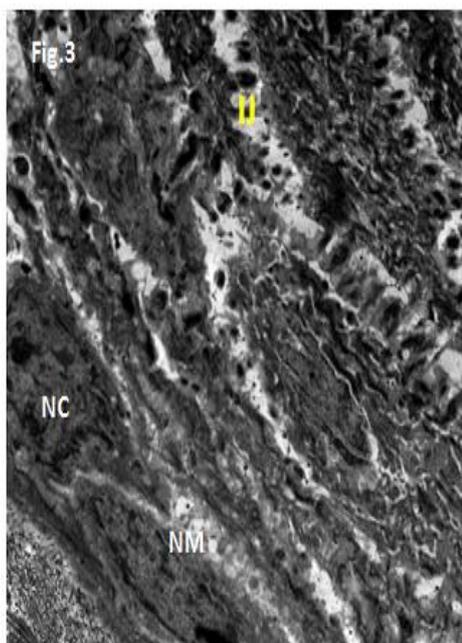


Fig.3 Electron micrograph showing Junctional Epithelial cells of Group I(14ds) showing vacuolated intercellular junctions(IJ),well defined nuclear membrane(NM), regular nuclear chromatin(NC). Org.Mag.

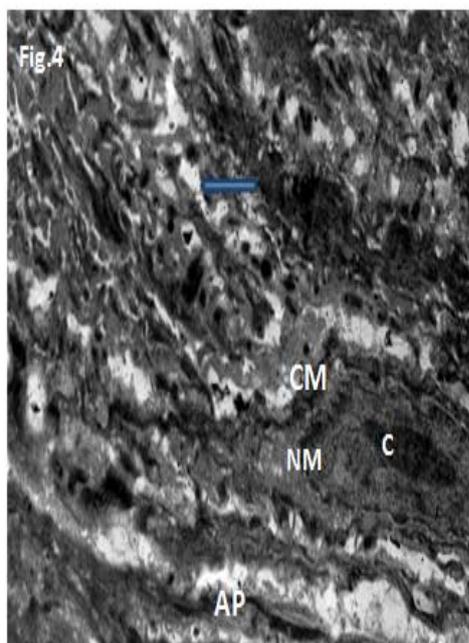


Fig.4 Electron micrograph showing Junctional Epithelial cells of Group I(14ds) showing intercellular vacuolations (arrow),irregular cell membrane (CM),irregular nuclear membrane(NM), central chromatin condensation(C),attachment plaque(AP).Org.Mag.

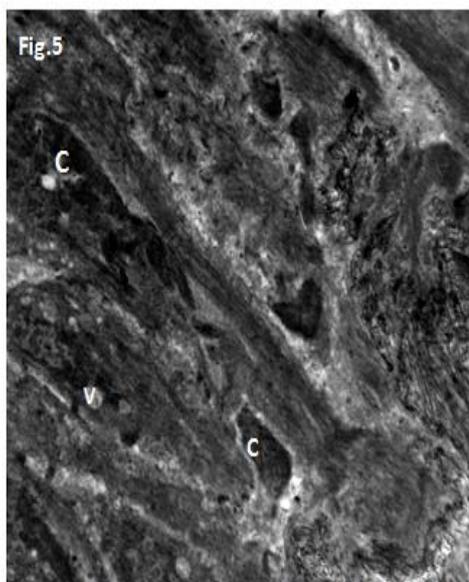


Fig.5 Electron micrograph showing Junctional Epithelial cells of Group II(7ds) showing lost intercellular junctions, illdefined cell membranes, intracytoplasmic vacuoles (V), illdefined nuclear membrane, diffused chromatin clumping(C) .Org. Mag.

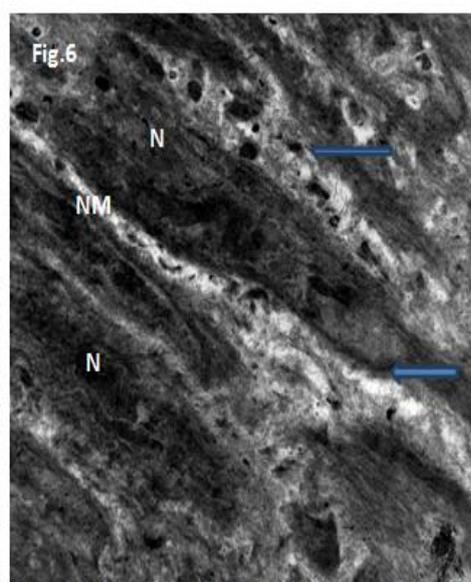


Fig.6 Electron micrograph showing fusiform Junctional Epithelial cells of Group II (14ds) showing wide intercellular junctions (arrows), fusiform cells, diffuse hyperchromatic nucleus (N), regular welldefined nuclear membrane (NM).Org. Mag.

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