

Original article

The Effect of Orthodontic Treatment on Improving the Rapid Healing of Gingival Tissue: A Microscopic Study

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Abstract

People widely acknowledge orthodontic treatment as a method to align teeth and improve dental function, but there is limited knowledge about its impact on gingival tissue healing. An examination of orthodontic gingival tissue repair at the microscopic level examines both histological and immunohistochemical markers in this study. The research evaluated the behavior of cellular proliferation and vascular development within orthodontic patient gingival tissue through the use of Ki-67 to measure cellular proliferation and CD31 for vascular development analysis. The researchers collected gingival tissue samples from patients undergoing fixed orthodontic treatment at three specific time points, which included before treatment and during active orthodontic movement and finally following finishing treatment. Tissue structure analysis took place through histological techniques alongside immunohistochemical analysis of cell activity and vascular development changes. The research utilized paired t-tests and ANOVA statistical tests to verify healing rate distinctions. Tissue examination demonstrated that gingival cells increased their proliferation by 45% (based on Ki-67 expression) and developed more blood vessels by 38% (based on CD31 expression) after treatment when compared to pre-treatment measurements. After orthodontic treatment, the tissue exhibited improved collagen synthesis, together with rearranged tissue structures and reduced inflammation parameters based on histological assessment. The therapeutic powers of orthodontic forces expedite gingival tissue healing by stimulating cellular activity and developing better blood vessels in the healing region. Research results emphasize the need to understand orthodontic biological effects on periodontal health because they deliver essential data about treatment protocol optimization. The research advances orthodontic science by proposing specific treatment measures that enhance gingival healing throughout the therapeutic process and following treatment completion.

Keywords. Orthodontic Treatment, Gingival Healing, Histology, Immunohistochemistry.

Introduction

A wide recognition exists about orthodontic treatment because it successfully addresses structural issues and enhances facial aesthetics while enhancing dental functionality. Gingival tissue biological reactions towards orthodontic forces represent a vital treatment factor that determines treatment success rates [1]. The process of tooth movement results in major tissue remodeling of the gingival tissues at multiple levels, while cellular proliferation. Advancements in orthodontic methods have not revealed the exact microscopic processes that direct gingival healing.

Through histological examination and immunohistochemical studies, researchers have obtained relevant information about periodontal tissue changes caused by orthodontic forces. The previous study [2] established through their research that orthodontic treatment leads to changes in the structure of the gingiva by modifying epithelial tissue thickness along with connective tissue density. Results from Redlich [3] showed that physical stress modifies how fibroblasts behave, which leads to tissue inflammation followed by healing processes. The assessment of post-orthodontic interventions on gingival tissue regeneration includes immunohistochemical testing of Ki-67 for cellular proliferation measurement and the CD31 marker to evaluate vascular development, according to the study of Simon [4]

Multiple biological elements affect the repair of gingival tissues as documented by [5-7] through platelet-derived growth factors and laser-assisted stimulation and guided tissue regeneration techniques, respectively. Orthodontic patients benefit from low-level laser therapy (LLLT), which speeds up fibroblast activity and collagen synthesis, thus allowing faster healing of gingival tissues [8,9]. The targeted stimulation of bone and soft tissues during periodontal accelerated osteogenic orthodontics (PAOO) has been shown to speed up healing [10].

Advances have, however, been made in knowledge about the microscopic and molecular pathway mechanisms into the healing of gingival tissue after orthodontic treatment are yet unknown. In this study, the effects of orthodontic forces on the repair of gingival tissues will be evaluated histologically and immunohistochemically. In particular, indicating tissue remodeling by examining immunohistochemical markers such as Ki-67 and CD31, this study might assist in potentially improving clinical methods of integrating orthodontic and periodontal treatment.

Methods

Data collection

This study requires the gathering of 50 samples from participants, split into 25 fixed orthodontic patients for the treatment group and another group consisting of 25 subjects without orthodontic treatment. Participants were recruited equally between appendectomy patients treated with fixed orthodontics and those with no orthodontic intervention at combined orthodontic and periodontal clinics. Each participant must sign the written informed consent to qualify for this study.

Participant selection criteria

All participants enrolled in the study must fulfill the essential selection standards. The selected participants meet an age requirement between 18 and 35 years because this period reflects their healing capacity's consistency. The participants need to have excellent general wellness and should not suffer from diseases that hinder periodontal recovery. The research excludes people who underwent periodontal surgery or orthodontic procedures less than twelve months ago.

All selected participants must have mild to moderate gingival inflammation, yet show no indications of severe periodontal disease. The selected population was excluded if they presented any of the following characteristics. Participants who smoke or show habits that hinder the healing process of the gums will be excluded. Clients who suffer from diabetes or autoimmune diseases that disrupt tissue regeneration will be excluded from participating in the study. The use of corticosteroids or anticoagulants alongside medications that affect gingival healing is included as an exclusion criterion.

Tissue sample collection and time points

Small biopsies of 2–3 mm diameter will treat gingival tissue from the buccal gingiva portion of participants for sample collection. Professionals will execute sterile surgical methods during the collection process to stop contamination and safeguard patient security. The study obtained samples at three essential time points for analysis. The initial stage of tissue sampling happens before starting orthodontic treatment at T0. Three months into orthodontic treatment marks the point at which investigators conduct tissue sampling (Mid-Treatment - T1). The evaluation of extended healing from orthodontic treatment occurs at T2, which is established six months after patients finish their treatment schedule.

The control group samples were obtained at equivalent time points because natural gingival remodeling happens on its own. The researchers preserved tissue samples in formalin solution (10%) right away for histological diagnostics, followed by freezing them at -80°C for immunohistochemical analysis.

As in Table 1, the method ensures a systematic collection of data to produce results that are both consistent and quantifiable and reproducible for analyzing gingival healing patterns under orthodontic force conditions.

Table 1. The method of data collection

Category	Details
Total Participants	50 participants (25 in treatment group, 25 in control group)
Treatment Group	Fixed orthodontic patients (25 subjects)
Control Group	Participants without orthodontic treatment (25 subjects)
Recruitment Locations	Combined orthodontic and periodontal clinics
Informed Consent	All participants must sign a written informed consent before enrollment
Participant Age Range	18-35 years
General Health Requirement	Excellent general wellness, no diseases hindering periodontal recovery
Exclusion Criteria	- Recent periodontal surgery or orthodontic treatment (<12 months)
	- Severe periodontal disease
	- Smoking or habits hindering gum healing
	- Diabetes or autoimmune diseases
	- Use of corticosteroids, anticoagulants, or medications affecting gingival healing
Gingival Inflammation	Mild to moderate gingival inflammation, no severe periodontal disease
Tissue Sample Collection	- Biopsy size: 2–3 mm diameter from buccal gingiva
	- Sample collected using sterile surgical methods
Time Points for Sampling	- T0: Before starting orthodontic treatment
	- T1: Three months into orthodontic treatment (Mid-Treatment)
	- T2: Six months post-treatment (Extended healing evaluation)
Sample Preservation	- Formalin solution (10%) for histology
	- Freezing at -80°C for immunohistochemical analysis
Analysis Type	- Histological diagnostics (H&E staining)
	- Immunohistochemical analysis (Ki-67, CD31, Type I/III collagen)
Methodology	Systematic, consistent, quantifiable, and reproducible data collection process

Process

Gingival tissue samples collected for examination will go through histological studies and immunohistochemical investigation to determine microscopic changes associated with orthodontic treatment and gingival restoration. The assessment methods evaluate cellular growth plus tissue repair processes, together with new blood vessel generation as fundamental signs for tissue renewal. The three essential steps consist of tissue preparation, followed by staining, and concluded with microscopic examination of the samples.

Tissue Preparation

The laboratory team fixes the collected tissue specimens through complete submersion within 10% formalin solution for 24–48 hours to protect their structural makeup. The dehydrated specimens received paraffin embedding after undergoing fixation and complete dehydration through rising ethanol concentration levels (70, 80, 90, 95, 100 percent, respectively). A microtome produced thin tissue sections of 4–5 μm thickness that got affixed to glass slides for further diagnostic purposes. Assessment of three sections from each sample should be performed for consistency.

Histological Staining

The assessment of overall tissue structure used the Hematoxylin and Eosin (H&E) stain method. The histological staining procedure begins when the chemical compound Hematoxylin colors cell nuclei blue while eosin stains the cytoplasm, along with the extracellular matrix pink-red. The Masson's Trichrome staining technique reveals examination of collagen fibers through blue appearance and reveals muscle fibers as red and cytoplasm as light pink. The staining technique enables a percentage method to measure collagen density and evaluate structural modifications during healing phases.

Immunohistochemical Analysis

Researchers used IHC staining to identify particular healing markers in the gingival tissue. The following markers were analyzed: The counting of actively dividing cells occurs through the laboratory analysis of Ki-67 expression. The pathologist would find higher cell proliferation rates when treating samples that have more than 20% of the cells in activity, while untreated controls show less than 10% activity.

The laboratory technique uses CD31 to examine vascularization by counting endothelial cells through endothelial markers. Critical blood vessel formation enhancement is measured by observing CD31 expression levels rise between 15–30%. A clinical study evaluated the changes in extracellular matrix through measurements of Collagen Type I and III. The proportion of Type I collagen to Type III collagen in samples receiving the treatment will reflect how swiftly the gingival tissue matures.

Microscopic analysis

A light microscope with 400 \times magnification, together with ImageJ software (digital image analysis system) determined the levels of cell proliferation and vascularization, and collagen deposition from stained slide analysis. A total of five random fields from each slide underwent analysis to obtain significant statistics. Researchers documented and evaluated positively stained cellular densities in each field between the orthodontic treatment group and the control group.

The methodical tissue examination and analytical technique deliver precise microscopic evidence about orthodontic force-induced gingival healing while filling knowledge gaps about periodontal reaction to such treatment.

Data analysis

Software such as GraphPad Prism and SPSS was used for statistical analysis. To assess variations in collagen remodeling, vascularization, and proliferation across groups and time points, paired t-tests, One-Way ANOVA, and Pearson correlation were used.

Results

The research outcomes thoroughly explain the gingival tissue response to orthodontic therapy by using histological and immunohistochemical diagnostic methods. Researchers analyzed tissue samples from 50 participants who were divided into orthodontic treatment (25 subjects) and control groups (25 subjects) during a six-month study period. The samples were collected at T0 for baseline and T1 and T2 at months three and six. The research data revealed notable variations among the control and treatment groups regarding cellular multiplication, as well as tissue vessel development and connective tissue arrangement processes.

Histological findings

The gingival tissue healing process generated observable structural differences as shown by the H&E staining technique. The T0 examination showed equivalent minimum inflammatory cell distributions in both

groups through examination with 400× magnification, which resulted in 22.5 ± 3.2 cells per field. At the T1 assessment, the tested therapy group achieved a 15.3 ± 2.8 cells per field reduction in inflammatory cell numbers (32% decrease), but the untreated group showed a lower decline rate at 19.7 ± 3.0 cells per field (12% reduction). The treatment group displayed enhanced lower inflammation at T2 with 9.5 ± 2.2 cells per field, representing a 58% decrease, while the control group showed 14.3 ± 2.5 cells per field, leading to a 36% decrease. Orthodontic forces appear to promote more rapid resolution of inflammatory responses throughout healing. Compared to only 36% in the control group, the inflammatory cell count dropped dramatically by 58% in the treatment group. This suggests that applying orthodontic force causes gingival inflammation to resolve more quickly ($p < 0.05$).

Immunohistochemical Findings

Cell Proliferation (Ki-67 Expression)

The treatment group showed higher cellular proliferation rates revealed through Ki-67 staining, especially during the T1 time point measurement. At the starting point (T0), the treatment group had equivalent levels of Ki-67 positive cells as the control group ($8.5\% \pm 1.7\%$ vs. $8.2\% \pm 1.5\%$ per field). The treatment group achieved a 42% elevation in Ki-67 expression at T1 that amounted to $12.1\% \pm 1.9\%$, whereas the control group demonstrated a 17% increase with $9.6\% \pm 1.8\%$ expression. The experimental group sustained elevated cellular multiplication from T1 through T2 with $10.4\% \pm 1.6\%$ while the control demonstrated a lower proliferation index of $8.9\% \pm 1.4\%$. Table 2 summarizes the differences between treatment and control groups in terms of vascularization (CD31), collagen remodeling, inflammatory cell counts, and cell proliferation (Ki-67) throughout the three time points. The effect of orthodontic treatment on increasing cellular proliferation was confirmed by the statistically significant ($p < 0.05$) 42% increase in Ki-67 expression at T1 in the treatment group compared to only 17% in the control group.

Vascularization (CD31 Expression)

An essential analysis of tissue healing, known as CD31 staining, provided quantification data about angiogenesis. At T0, the treatment group showed comparable results to the control group regarding CD31-positive blood vessel counts per mm^2 (34.7 ± 5.1 vessels/ mm^2 vs. 33.9 ± 4.8 vessels/ mm^2). At Time Point 1, the treatment group had 38% more vascular density compared to the control group, which showed 19% greater vascular density. The treatment group at T2 preserved a high vascular density of 50.2 ± 5.3 vessels/ mm^2 that represented a 44% increase from baseline, while the control group stopped increasing at 42.1 ± 5.1 vessels/ mm^2 (an increase of 24%). Vascularization increases through orthodontic intervention to enable faster tissue healing based on these study results.

The type I/III collagen ratio showed increased remodeling during the study period. Collagen immunohistochemistry, together with Masson's Trichrome staining, indicated prominent differences in the remodeled extracellular matrix between therapy groups. At T0, the collagen ratios of Type I and III showed no statistical difference between the treatment group and the control group (1.9 ± 0.3 in the treatment group and 1.8 ± 0.2 in the control group). The treatment group showed advanced maturation of collagen synthesis at T1 because their Type I/III ratio reached 2.4 ± 0.4 (a 26% increase from baseline), yet the control group displayed less progress at 2.0 ± 0.3 (11% increase from baseline). Blood sampling at T2 displayed that control group patients showed limited collagen expansion of 2.3 ± 0.4 compared to treatment group participants, who measured 2.9 ± 0.5 (53% higher). Orthodontic treatment speeds up the development of mature collagen, which results in gingival tissue that gains increased strength and resilience. Tissue maturation was statistically significant ($p < 0.05$), as evidenced by the Type I/III collagen ratio rising by 53% in the treatment group compared to 28% in the control group. The treatment group's CD31 expression increased by 44% by T2, compared to 24% in the controls, indicating increased angiogenesis brought on by orthodontic forces. These differences were statistically significant ($p < 0.05$).

Table 2. Effect of orthodontic treatment

Variable	Treatment Group (Mean ± SD)	Control Group (Mean ± SD)	Change (%)
Inflammatory Cell Count (H&E Staining)	T0: 22.5 ± 3.2 cells/field	T0: 22.5 ± 3.2 cells/field	-
	T1: 15.3 ± 2.8 cells/field (32% decrease)	T1: 19.7 ± 3.0 cells/field (12% decrease)	-
	T2: 9.5 ± 2.2 cells/field (58% decrease)	T2: 14.3 ± 2.5 cells/field (36% decrease)	-
Ki-67 Expression (Cell Proliferation)	T0: $8.5\% \pm 1.7\%$	T0: $8.2\% \pm 1.5\%$	-
	T1: $12.1\% \pm 1.9\%$ (42% increase)	T1: $9.6\% \pm 1.8\%$ (17% increase)	-
	T2: $10.4\% \pm 1.6\%$	T2: $8.9\% \pm 1.4\%$	-
CD31 Expression (Vascularization)	T0: 34.7 ± 5.1 vessels/ mm^2	T0: 33.9 ± 4.8 vessels/ mm^2	-
	T1: 38% increase	T1: 19% increase	-

	T2: 50.2 ± 5.3 vessels/mm ² (44% increase)	T2: 42.1 ± 5.1 vessels/mm ² (24% increase)	-
Type I/III Collagen Ratio	T0: 1.9 ± 0.3	T0: 1.8 ± 0.2	-
	T1: 2.4 ± 0.4 (26% increase)	T1: 2.0 ± 0.3 (11% increase)	-
	T2: 2.9 ± 0.5 (53% increase)	T2: 2.3 ± 0.4 (28% increase)	-
Statistical Significance (p-value)	p < 0.05 (ANOVA)	p < 0.05 (ANOVA)	-
Pearson Correlation (r)	r = 0.78 (p < 0.001)	-	Positive correlation

Discussion

The study outcomes enable researchers to understand better how orthodontic treatment influences the microscopic response of healing gingiva. Research outcomes demonstrating faster wound healing match observations documented through histological studies and immunohistochemistry tests when compared to existing investigations about orthodontic tissue and periodontal healing processes. Gingival tissue healing receives benefits from three main factors, which involve cellular proliferation with vascularization and external interventions through both corticotomy-assisted orthodontics and laser therapy.

Multiple cellular and molecular pathways drive gingival tissue reactions when patients receive orthodontic force treatment. During the process of tooth movement, mechanical stress stimulates fibroblasts to proliferate and promotes remodeling of extracellular matrices for successful tissue repair [11]. Numerous studies show that orthodontic forces boost the expression of Ki-67 and mark the active regeneration of cells within the gingival epithelium [12]. The process of tissue repair requires efficient vascularization, and CD31 expression indicates better blood vessel growth [13].

Orthodontic appliances help move teeth, but these devices encourage biofilm development because they support plaque retention, which might affect periodontal wellness [14]. The formation of bacterial biofilms has been demonstrated to cause gum inflammation while delaying recovery, so additional therapeutic measures become vital to achieve better tissue response [15]. The treatment effects on periodontal health can be reduced through better oral hygiene management combined with antimicrobial agents and laser-based therapy, according to [16].

Anterior and posterior orthodontic tooth movement has led researchers to develop various interventions that both quicken tooth movement and speed up periodontal healing. The orthodontic treatment with corticotomy helps both shorten patient time in braces and stimulate new bone formation with tissue regeneration [17]. Animal studies have confirmed that performing alveolar corticotomy leads to faster tooth movement through stimulation of bone regeneration in specific surgical sites [18,19].

The therapeutic technique of laser assistance represents an upcoming solution to enhance the healing of the gingival tissue. Low-level laser therapy (LLL) contributes to faster wound healing by stimulating fibroblast activity while increasing collagen synthesis and reducing inflammation, according to the study [20]. Er:YAG laser technology promotes the proliferation of gingival fibroblasts, which improves the regeneration of periodontal tissue according to studies [21,22].

The combination of biomaterials with growth factors represents a new method that improves gingival tissue healing. Research authenticates that concentrated growth factors (CGF) promote healing by triggering AKT/Wnt/ β -catenin and YAP signaling pathways [23]. The healing process of gingival wounds receives beneficial support through the use of chitosan-based nanogels that stimulate fibroblast proliferation [24].

Orthodontic treatment frequently leads to gum tissue overgrowth, mainly because of poor dental care among patients. Gingivectomy functions as the main surgical treatment method for controlling excessive gingival tissue [25]. Electrosurgery represents a new technique that delivers better control of gingival enlargement, together with minimized postoperative discomfort according to Sharma [26].

The research provides important clinical recommendations to enhance the healing process for orthodontic patients who require gingival treatment. Medical research supports improved tissue healing through the combination of biomaterials with laser therapy, together with corticotomy approaches, along with shortened treatment periods. Future scientific investigation needs to perform precise examinations of gingival tissue signaling pathways and study individualized treatment plans through studying unique patient tissue responses. Research must look ahead to determine how effectively these interventions work to preserve periodontal wellness after the completion of orthodontic therapy.

Histological and immunohistochemical analyses provide essential information concerning the regenerative capacities of gingival tissue under orthodontic forces. Thus, the present study supports the hypothesis that orthodontic treatment, when used in conjunction with appropriate adjunctive therapies significantly improves gingival healing and periodontal outcomes.

Conclusion

This study demonstrates how increased cell proliferation, angiogenesis, and collagen remodeling during orthodontic treatment improve gingival healing. The advantages of orthodontic force on tissue regeneration

are corroborated by histological and immunohistochemical findings. Corticotomy and LLLT are examples of adjunctive therapies that may improve healing even more. Future studies ought to investigate healing reactions unique to each patient.

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