G.A. Scardina, A. Cacioppo, F. Seidita, G. Garofalo*, M. Lotti, P. Messina

Department of Orthodontics, University of Palermo, Palermo, Italy
**Private Practice, Palermo, Italy*

e-mail: scardina@odonto.unipa.it

Evaluation of gingival microcirculation in patients undergoing fixed orthodontic treatment: a pilot study

ABSTRACT

Aim *Among the many biological effects which occur during orthodontic movement, we decided to investigate gingival microcirculation. The aim of the study was to evaluate the biological microvascular response to the application of orthodontic force in vivo.*

Materials and methods *Forty patients (case group) between 9-22 years of age (average± DS 12±3.01) were selected for the study (M/F ratio: 20/20). They needed fixed orthodontic treatment due to several types of malocclusion. Forty healthy subjects (control group) were also recruited (M/F ratio 20/20; average age 12 years ± 4.01; Mean±SD =10.04±1.7). A videocapillaroscopic examination was performed on each patient on the buccal alveolar mucosa at the pre-treatment time (t0), 1 month after the beginning of the treatment (t1), after 2 months (t2), after 6 months (t3), and after 12 months (t4).*

Results *Capillary density increases significantly from t0 to t1. Between t1 to t2, t2 to t3 the density underwent further increase. Between t3 and t4 (69.22 ± 3.63) the density showed no increase. In the control group no statistical differences were observed.*

Conclusion *Videocapillaroscopy allows the in vivo evaluation and quantification of the microcirculatory changes consequent to the application of orthodontic force, managing to detect subclinical changes in angiogenesis. In fact, the study revealed an increase in the density of the capillaries which is directly proportionate to the application time of the orthodontic device, i.e. the exogenous mechanical force. This research offers new perspectives for the future of monitoring fixed orthodontic therapy.*

Keywords Orthodontic treatment; Periodontal microcirculation.

Introduction

Following the application of a force on a tooth, a series of changes are observed in the periodontal tissue and at a metabolic-molecular and microvascular level.

During dental movement two areas of the tooth and periodontal complex are considered: a compressed one, usually known as "pressure area" or "resorption area", and a relatively extended one, known as "tension area" or "area of apposition" [Blondini et al., 2008; Lanteri, 2002]. In the pressure area an initial collapse of the vessels is produced, which determines an important reduction in the capillary blood flow and periodontal space. During this phase of dental movement we can consider four main effects: deformation of the extracellular matrix (ECM) and hydraulic variations; cellular deformation; cellular activation and differentiation; remodeling of the tissue [Henneman et al., 2008]. The remodeling of the tissue occurs in both soft and hard tissues (mainly bone) [Proffit, 2007; Melsen et al., 2007].

During orthodontic movement, the vascular component has an essential role, supplying nutrients to the cells for their activity, as well as allowing the various metabolic factors to reach the cells. In addition, it allows migration of cells with an amplificatory and regulatory role in the process of tissue remodeling from the vascular system.

The aim of this study was to evaluate the gingival microcirculatory modifications in vivo in patients undergoing fixed orthodontic treatment.

Materials and methods

Forty patients (case group) were recruited for the study (M/F ratio 20/20; average age 12 years ± 3.01; Mean±SD: 11.03±2.8). They needed fixed orthodontic treatment due to different types of malocclusions. Forty healthy subjects (control group) were also recruited (M/F ratio 20/20; average age 12 years ± 4.01; Mean±SD: 10.04±1.7).

The criteria for inclusion in the study were as follows:

- Good general health.
- Good periodontal health (Silness and Löe Plaque Index ≤1; Gingival Index≤1).

- Optimal oral hygiene.
- Presence of teeth 1.3, 1.2, 1.1, 2.1, 2.2, 2.3.
- No previous orthodontic treatment

All recruited subjects did not have any systemic pathologies. They took no drugs that could influence oral microcirculation and they were non-smokers.

The research was carried out after the subjects, or their parents or guardians in the case of minors, had given their written informed consent. The case group underwent routine clinical and instrumental examinations that precede orthodontic treatment:

- General examination.
- Dental examination (extra- and intraoral).
- Functional examinations (with special focus on occlusal and muscular balance)
- Alginate impression for the preparation of plaster study models for analysis of space and Bolton index.
- Extra- and intra-oral photographic analysis
- X-ray examination (ortopantomography, lateral teleradiography of the skull)
- Cephalometric analysis

All the patients were treated with McLaughlin, Bennet and Trevisi technique.

The selected patients were submitted to oral videocapillaroscopic examination [Scardina et al., 2005; Scardina and Messina, 2006; Scardina and Messina, 2007; Scardina et al. 2011]:

- before orthodontic treatment (t0);
- 1 month after the beginning of treatment (t1);
- after 2 months (t2);
- after 6 months (t3);
- after 12 months (t4).

The control group underwent oral videocapillaroscopic examination with the same timing:

- t0
- after 2 months (t1);
- after 6 months (t2);
- after 12 months (t3).

The oral videocapillaroscopic examination was performed using the videocapillaroscope Videocap 200, produced by DS Medical S.r.l. (Milan, Italy), and related software (Videocap software 8.0). It was performed under standardised environmental conditions: constant temperature (24±1 °C); illumination using neon light for medical use (white point 6500 °K) and test always performed by the same operator (GAS).

The examined area was the masticatory mucosa of the front teeth (from tooth 1.3 to 2.3), taking a minimum of five images at the five times established in the research protocol and evaluating the capillary density parameter per mm^2.

Videocapillaroscopy

Oral videocapillaroscopy is a simple, repeatable, non-invasive analysis that is performed *in vivo* and is well-tolerated by the patient. This computerised technique is used with specific software (DS Medigroup, Milan Italy) that allows the acquisition and the elaboration of the data. The videocapillaroscope is made up of a central unit, a fibre optic probe with video-optic terminal and a high resolution colour monitor. The lens has a focal spot of 1.811 mms and magnifications which vary from 10 to 1,000X. For this study, a 200X magnification was used. The effects of the orthodontic force on the periodontal microcirculation were thus monitored over time.

The capillaroscopic images were then stored and used for the measurement of the capillary density by means of the Videocap software. The data obtained, related to the surface area, analysed with the Mann-Whitney test for comparison of non-parametric ordinal data, with the p level set at 0.05.

For the statistical analysis the software P.A.S.T was used [Scardina and Messina, 2006]. This is a freeware developed by Øyvind Hammer, D.A.T. Harper and P.D. Ryan in 1995 and updated to the latest version, release 1.97, in January 2010.

Results

The times t0-t1, t1-t2, t2-t3, t3-t4, t0-t2, t0-t3, t0-t4 were compared in the case group. The times t0-t1, t1-t2, t2-t3, t3-t4, t0-t2, t0-t3 were compared in the control group. For every correlation, the differences relative to the vascular density parameter were evaluated.

The results showed statistical differences in the case group and no statistical differences in the control group.

Capillary density increased from t0 to t1; t1-t2, t2-t3, t0-t2, t0-t3, t0-t4 in the case group.

No statistical differences were observed from t3 to t4 (Tables 1, 2, 3).

Discussion

This *in vivo* study analysed the changes in gingival microcirculation during application of an orthodontic force, in comparison to an initial time in which there was no application of an external mechanical force.

Investigations about variations of the microcirculation subsequent to orthodontic force have been made by other authors, but using animal models and, often,

Timing	t0	t1	t2	t3	t4
Mean ± SD	43.08 ±3.81	56.34 ±2.75	69.68 ± 3.63	78.42 ± 4.01	76.22 ± 2.8

TAB. 1 Capillary loop density means and standard deviations, related to timings (case group).

in vitro [Verna et al., 2000]. Thanks to the use of the videocapillaroscope, in this article it was possible to obtain data from patients *in vivo*.

It is important to notice that in an investigation on humans he results obtained are particularly reliable and more applicable compared with those obtained from studies on animal models. In addition, *in vivo* results allow to capture the dynamism and the intrinsic variability typical of the living organism, which is hardly obtainable in the case of histological sections and in vitro models.

The obtained results reveal, at times following the application of an orthodontic force, a relatively linear increase in capillary density in the site examined (except t_3 to t_4), and therefore a numerical increase in the capillaries per site (Fig. 1, 3). This behaviour clearly indicates a process of angiogenesis, i.e. the formation, growth and proliferation of blood vessels, whose aim is to respond to the new functional demands of the tissue [Ren et al., 2008; Noda et al., 2009].

During orthodontic movement, the vascular component has an essential role, supplying nutrients to the cells for their activities. In addition, it allows the migration of immune system cells that have a regulatory role in the process of tissue remodelling [Ku et al., 2009; Ren and Vissink, 2008; Kaku et al., 2008; Thi et al., 2007; Kohno et al., 2003; Krishnan and Davidovitch, 2009; Anastasi et al., 2008]. The formation of new vessels thus responds to a functional demand of the tissue, which, at the same time, even though in different sites, activates processes of bone apposition and resorption.

The instrumental data showing the increase in capillary density per mm^2 underscores, in our opinion, the metabolic needs of the tissue: the request for nutrients, the elimination of waste products, gas exchange, the migration of cells such as endotheliocytes, osteoblasts, osteoclasts, but also immune system cells, fundamental for the regulation of the processes underway through their numerous mediators.

It should be noted that in this study no reduction of the vessels was detected during the first examination (t0-t1). This was different from the observations of other authors [Von Böhl and Kuijpers-Jagtman, 2009] who correlated it to that particular phenomenon of tissue necrosis called "hyalinization". The entity of this modification is dependent on various factors, such as intensity [Maltha et al., 2004] and duration of application of the orthodontic force. In addition, the increase in capillary density enabled us to understand how the application of a mechanical force on the tooth does not damage microcirculation, but causes an alteration in a proliferative process.

The results of our research permits to correlate the actual presence of orthodontic movement with microvascular modifications, which can be detected through variations in an angiogenetic sense. This correlation can be useful to the clinician who requires an evaluation on the actual success of a movement. In fact, the application of an orthodontic force can often be ineffective to the aims of the dental movement. The videocapillaroscopic evaluation permits to obtain

FIG. 1 Capillaroscopic pattern of gingival mucosa at t0 in the case group.

FIG. 2 Capillaroscopic pattern of gingival mucosa at t1 in the case group.

FIG. 3 Capillaroscopic pattern of gingival mucosa at t2 in the case group.

Timing Comparison	t0 - t1	t1 - t2	t2 – t3	t3 – t4	t0 – t2	t0 – t3	t0 – t4
Significance	S	S	S	NS	S	S	S

TAB. 2 Results of Mann Whitney test applied to timing comparison: t0-t1; t1-t2; t2-t3; t3-t4; t0-t2; t0-t3; t0-t4 (case group).

Timing Comparison	t0 - t1	t1 - t2	t2 – t3
Significance	NS	NS	NS

TAB. 3 Results of Mann Whitney test applied to timing comparison: t0-t1; t1-t2; t2-t3 (control group).

instrumental data, otherwise not obtainable, on the effectiveness of the mechanical force. Visualisation of microvascular variations in a proliferative sense will in fact be a favourable sign of the success of a dental movement, even if it is not visible and not clinically objectivable. *Vice versa*, if some time after the application of the force no increase in capillary density is noticed, we can conclude that the orthodontic force applied is not effective.

These considerations also suggest possible future research, aimed at studying the correlation between intensity of the applied orthodontic force and capillaroscopic patterns. The possibility of standardising the videocapillaroscopic examination in the field of orthodontics would in fact support conventional examinations [Krishnan, 2007]. The use of videocapillaroscope for the diagnosis and follow-up of the orthodontic treatment, thus allowing a reduction in the quantity of Xrays for the patient, with a considerable benefit from a biological point of view. In addition, the radiological examination does not permit visualization of early modifications at the periodontal level, while, with a suitable standardisation of the methodology, the videocapillaroscope could detect microvascular modifications that are correlatable, for example, to gingival recessions or radicular resorption.

This perspective still needs further research, applied in the field and on a wider scale, in order to legitimately introduce videocapillaroscopy among the routine examinations. In our opinion, this research opens a new perspective on understanding the mechanisms that lead to tooth movement, starting from the application of mechanical energy; for the clinician, the use of the videocapillaroscope can be a support for the diagnosis and follow-up in orthodontics.

References

- Anastasi G, Cordasco G, Matarese G, Rizzo G, Nucera R, Mazza M, Militi A, Portelli M, Cutroneo G, Favaloro A. An immunoistochemical, histological, and electron-microscopic study of the human periodontal ligament during orthodontic treatment. Int J Mol Med 2008;21:545-554.
- Henneman S, Von den Hoff JW, Maltha JC. Mechanobiology of tooth movement. Eur J Orthod 2008;30:299-306
- Kaku M, Motokawa M, Tohma Y, Tsuka N, Koseki H, Sunagawa H, Arturo Marquez Hernandes R, Ohtani J, Fujita T, Kawata T, Tanne K. VEGF and M-CSF levels in periodontal tissue during tooth movement. Biomed Res 2008;29:181-7.
- Kohno S, Kaku M, Tsutsui K, Motokawa M, Ohtani J, Tenjo K, Tohma Y, Tokimasa C, Fujita T, Kawata T, Tanne K. Expression of vascular endothelial growth factor and the effects on bone remodeling during experimental tooth movement. J Dent Res 2003;82:177-82.
- Krishnan V, Davidovitch Z. On a path to unfolding the biological mechanism of orthodontic tooth movement. J Dent Res 2009;88:597-608.
- Krishnan V. Orthodontic pain: from causes to management – a review. Eur J Orthod 2007;29:170-179.
- Ku SJ, Chang YI, Chae CH, Kim SG, Park YW, Jung YK, Choi JY. Static tensional forces increase osteogenic gene expression in three-dimensional periodontal ligament cell culture. BMB Rep 2009; 42:427-32.
- Lanteri C. Ortognatodonzia. Ed. Masson; 2002.
- Maltha JC, van Leeuwen EJ, Dijkman GE, Kuijpers-Jagtman AM. Incidence and severity of root resorption in orthodontically moved premolars in dogs. Orthod Craniofac Res 2004;7:115–121.
- Melsen B, Cattaneo PM, Dalstra M, Kraft DC. The Importance of Force Levels in Relation to Tooth Movement. Semin Orthod. 2007;13:220-233.
- Noda K, Nakamura Y, Kogure K, Nomura Y. Morphological changes in the rat periodontal ligament and its vascularity after experimental tooth movement using superelastic forces. Eur J Orthod 2009;31:37–45.
- Proffit WR. Contemporary Orthodontics. Mosby Elsevier; 2007.
- Ren Y, Maltha JC, Stokroos I, Liem RSB, Kuijpers-Jagtmane AM. Effect of duration of force application on blood vessels in young and adult rats. Am J Orthod Dentofacial Orthop 2008;133:752-757.
- Ren Y, Vissink A. Cytokines in crevicular fluid and orthodontic tooth movement. Eur J Oral Sci 2008;116:89-97.
- Scardina GA, Pizzigatti ME, Messina P. Periodontal microcirculatory abnormalities in patientes with sistemi sclerosis. J Periodontol 2005;76:1991-1995.
- Scardina GA, Messina P. Microvascular characteristics of the human filiform papillae: a videocapillaroscopic study. Ann Anat 2006;188:183-186.
- Scardina GA, Messina P. Microvascular periodontal alterations: a possible relationship between periodontitis and rheumatoid arthritis. Clin Hemoreol Microcirc 2007;37:229-235.
- Scardina GA, Cacioppo A, Pisano T, Messina P. In vivo evaluation of labial microcirculation in diabetics: a comparison with healthy subjects. Panminerva Med 2011;53:81-5.
- Sfondrini G, Gandini P, Sfondrini MF, Cacciafesta V, Fraticelli D. Ortognatodonzia Terapia, Ed.Martina; 2008.
- Thi MM, Iacobas DA, Iacobas S, Spray DC. Fluid shear stress up-regulates vascular endothelial growth factor gene expression in osteoblasts. Ann N Y Acad Sci 2007;1117:73-81.
- Verna C, Dalstra M, Melsen B. The rate and the type of orth tooth movement is influenced by bone turnover in a rat model. Eur J Orthod 2000;22:343-352.
- Von Böhl M, Kuijpers-Jagtman AM. Hyalinization during orthodontic tooth movement: a systematic review on tissue reactions. Eur J Orthod 2009;31:30-6.