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Guido Reni, *Ercole sulla Pira* (1617). Museo del Louvre, Parigi *(detail)*.

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Article

PHOTO-BIO-MODULATION IN ORTHODONTICS IN ORDER TO PROMOTE PROLIFERATION AND DIFFERENTIATION OF KERATINOCYTES: AN *IN VITRO* STUDY

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ABSTRACT

Gingival augmentation techniques proposed in the international literature do not exclude a surgical component, which determines consequent post-surgical discomfort and results are not always predictable. In recent years, the introduction of laser photobiomodulation has led to a less invasive approach, particularly in the treatment of periodontally compromised patients, limiting the surgical phase to seriously compromised cases, with regeneration techniques for the restoration of a correct periodontal tissue anatomy. The aim of this in vitro study is to establish the validity of laser photobiomodulation in order to develop the epithelial keratinized layer of the tissue by stimulating fibroblasts-keratinocytes organotypic cultures and fibroblasts and keratinocytes mono-cultures. We created two groups (test and control), each one composed of 3 fibroblast cultures, 3 keratinocyte cultures and 3 organotypic cultures. We performed laser irradiation of test group at 50 J/cm2 of fluency with one application every 48 h for a total of 5 applications. 48 hours after the last laser application, we investigated the presence and amount of keratins 5 and 8 with citofluorymetric and western blotting analyses. Analyses showed an increase in keratin synthesis in test group cultures, showing a remarkable increase in production of keratin 8 in co-cultures test. Laser photobiomodulation can considerably enhance keratin synthesis when applied with high energy doses and repeated applications to keratinocytes-fibroblasts co-cultures.

KEYWORDS: *photobiomodulation, low level laser irradiation, biostimulation, keratinization, keratinocytes, fibroblasts*

INTRODUCTION

Orthodontic treatment of ectopic dental elements is well documented in international literature (1). Often, when the tooth is repositioned at the end of orthodontic therapy, it is possible to observe a recession of vestibular gingival tissue that surrounds the dental element (2-5). In this case, gingival augmentation techniques, such as coronal advanced flaps

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(CAF) eventually combined with connective tissue grafts (CTG), are required. These techniques require a planning of one or more surgical phases with a resulting post-operative discomfort and results are not always predictable (6, 7).

In recent years, the introduction of laser therapy has led to a less invasive approach in the treatment of the compromised periodontal patient (8-11), confining the surgical component of the treatment only when the anatomy of the periodontal tissues is compromised and there is a need for guided tissue regeneration (12).

Similarly, the same advantages of using laser photobiomodulation may be useful to avoid mucogingival surgery in case of gingival recession of a repositioned ectopic tooth. In an essay we previously published (13), we examined the clinical effects of laser photobiomodulation on alveolar bone remodelling during orthodontic tooth movement and finally on formation of new keratinized gingiva. The results of this study concluded that laser photobiomodulation could promote the formation of attached gingiva around the crown of teeth erupted in oral vestibular mucosa.

Due to these clinical findings, we decided to investigate these results in vitro, applying the same laser photobiomodulation protocol we used in the clinical study to an in vitro reproduced epithelial tissue (14-17), in order to establish laser irradiation potential to increase keratin production and therefore the development of a keratinized layer.

MATERIALS AND METHODS

We used a kit composed of primary basal fibroblasts-keratinocytes cell lines (Matched Set-Cryopreserved Dermal Fibroblasts and Keratinocytes, Tebu-Bio™). These cells were isolated from human epidermis of a single donor and conserved in two separate vials.

Once in the lab, the cells were unfrozen and placed into their respective culture medium. The keratinocytes were placed in a growth medium (Human Adult Keratinocyte Growth Medium KM-2, Tebu-bio™) composed of MCDB153 and epidermal growth factor (rEGF), insuline, transferrin, bovine serum albumine, ethanolamine, phosphoethanolamine, hydrocortisone, calcium chloride, ephinefrine, bovine pituitary extract (BPE), penicillin, streptomycin and amphotericin B.

Fibroblast cells were placed in a growth medium (Euroclone™) composed of DMEM (4.5 g/L of D-glucose) and 10% fetal bovine serum (FBS), penicillin, streptomycin and amphotericin B. Both fibroblasts and keratinocytes media were changed 24 h after the culture set-up and again at a 48-h interval.

Before proceeding with the set-up of the experimental groups, cell cultures were cultivated until they achieved at least 70-80% of confluence. In order to obtain valid organotypic co-cultures that could reproduce the epithelial tissue, we tested various growth media, changing the concentration of the two types of media used for the fibroblast and the keratinocyte cells. Finally, we found that a 1:1 mixture of the two growth media (with a final 5% of FBS) permitted an ideal coexistence and survival of the two cell populations in the same cultures.

Fig. 1. *100x photography of organotypic fibroblast cultures.*

We were then able to create organotypic fibroblast-keratinocytes co-cultures by positioning keratinocytes cells on top of fibroblast cultures with the 1:1 mixture growth medium (Fig. 1). We prepared two identical experimental groups (test and control) dividing the cultures in 12-well transparent plastic multi-wells. Each well had a 1 cm diameter. At the time of the experimental group set-up, fibroblast cells were seeded at a density of 50000 cells per well, meanwhile keratinocyte cells were seeded at a density of 100000 cells per well, both for monolayer cultures and for organotypic co-cultures.

For each monolayer culture and for each co-culture we used the same culture medium used to set-up the organotypic co-cultures. Each experimental group was composed of 3 fibroblast cells cultures, 3 keratinocytes cells cultures and 3 fibroblast-keratinocyte cells co-cultures.

Fig. 2. *Laser biostimulation of test group cultures.*

Twenty-four h after the group set-up, we placed the cultures of the test group under sterile extractor fan and we irradiated each culture with a 980-nm continuous wave diode laser (Wiser Doctor Smile, Lambda™) every 48 h for 10 days. Laser energy was delivered through a particular hand-piece (Onda Piana, Lambda™) that keeps energy properties unaltered in a variable distance from 0 to 100 cm, and delivers the same amount of energy in every point in a 1 cm^2 area. The hand-piece was perpendicularly stabilized by means of a mechanical arm at a distance of 1 cm from the culture surface, and the laser device was set with the following parameters: output power 1 Watt, exposure time 50 s, energy density 50 J/cm^2 (Fig. 2). During the irradiation phase of this work, we changed the culture medium of both groups every 3 days.

Forty-eight h after the last irradiation, we collected data from the cultures of both groups by using flow cytofluorimetry (FACS) and western blotting. Using the analyses we estimated presence and quantity of keratin 5 and 8 using monoclonal antibodies reactive to cytokeratins 5 and 8 (KRT 5/8, Antibodies-online™). FACS analysis data was statistically processed with t-student analysis (95% confidence level).

RESULTS

Data obtained with FACS analysis show a low positivity to keratin 5-8 of fibroblast cultures of both test and control groups (1.5% and 0.5%, respectively), as expected. Both keratinocytes and organotypic cultures showed a statistically significantly difference of keratin 5-8 expression between test and control group (p=0.0007 and p<0.0001, respectively) in favor of the control group, while on the other hand, it showed an increase of positive cells in the irradiated group in both keratinocytes (84.2% in the test group and 82.9% in the control group) and organotypic cultures (6.1% in test group and 4.7% in control group) (Fig. 3).

Data obtained with Western blotting analysis showed a decrease in keratin 5 expression of irradiated keratinocytes cultures, but an increase in keratin 8 expression of the same cultures. This analysis also showed a small increase of keratin 5 expression of the organotypic cultures of the irradiated group, and a high increase of keratin 8 expression of the same cultures (Fig. 4, 5).

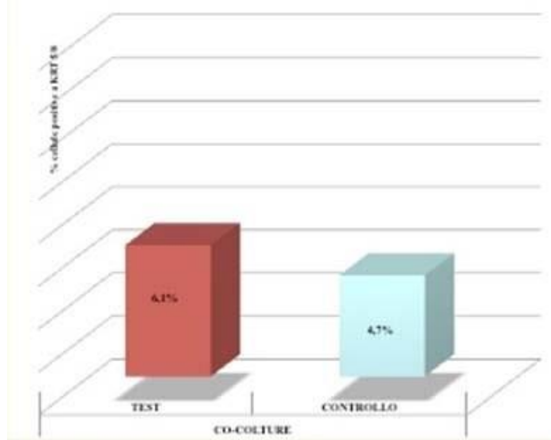

Fig. 3. *Example of FACS analysis in test group organotypic cultures.*

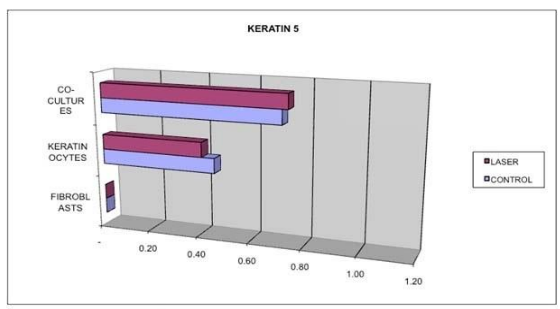

Fig. 4. *Western blotting analysis of keratin-5 expression.*

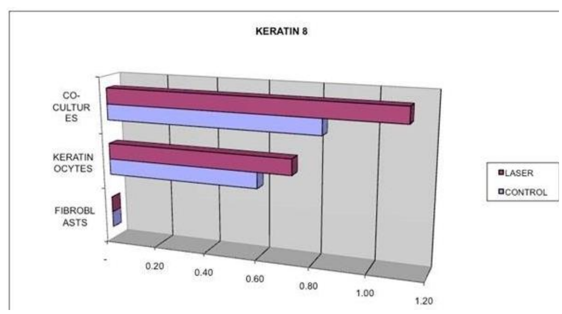

Fig. 5. *Western blotting analysis of keratin-8 expression.*

DISCUSSION

Studies that examine laser photobiomodulation applied to cell cultures are widely present in international literature (18-26). The energy density used in these studies is on the average 5 J/cm^2. It has been observed that a higher energy value density applied to cell cultures may lead to an inhibition in terms of cell vitality and proliferation, leading ultimately to cell death. However, we decided to use a relatively high energy density (50 J/cm^2) with repeated applications (every 48 h for 10 days).

The base hypothesis involves a sufficiently strong stimulus to push cells to increase the production of keratins, similar to the phenomenon of epidermal keratosis following repeated and constant traumatic events which lead to the formation of palmar or plantar callus. The possible periodontal clinical application is represented by the chance to increase the volume of the gingival keratinized layer around dental elements and/or implants.

The international literature is rich in studies that have evaluated laser photobiomodulation on cell cultures (27-31). These studies evaluate the effects of laser photobiomodulation in terms of vitality, proliferation, adhesion, migration, growth factors and protein synthesis with an application range of laser energy density of 0.12 to 20 J/cm^2. A literature review did not however enlighten to the effects of laser photobiomodulation on keratin production by either fibroblasts and/or keratinocytes. Despite using significantly higher energy density values than those suggested in international literature, we managed to maintain cell survival in all of our specimens. This was possible using a particular hand-piece assembled to the diode laser that lead to an optimal energy absorption of the cell cultures avoiding inhibitory or apoptotic effects that are often described when using an energy density higher than 20 J/cm^2.

We had an increase of the keratin-positive cell rate in test group culture compared to non-biostimulated cultures, both for keratinocyte and organotypic cultures while there was a decrease of the total positivity to keratin in the test group specimens. We could conclude that laser photobiomodulation caused a reduction of the number of cells expressing keratin, leading therefore to a minor total expression of keratin as observed through FACS analyses, but an increase of keratin-positive cells in relation with the total population of the test group specimens. Therefore, laser photobiomodulation was not able to obtain an increase of the total amount of keratin in the treated cultures, but stimulated a higher number of cells to synthesize keratin, compared to non-treated group.

The results of Western blotting analyses lead mainly to two conclusions: a decrease of keratin-5 expression was observed in the keratinocyte test group, a small increase of both keratin-8 expression of keratinocyte and keratin 5 in the organotypic cultures in the test group and a great increase of keratin-8 expression in the organotypic cultures in the test group. We can conclude that the difference in keratin expression of the two group specimens (i.e. a decrease of total expression in the test group) as shown by FACS analyses, may be explained by the fact that the keratinocytes used in this study are able to produce less keratin 5 than keratin 8 and that on the other hand, had a strong increase following laser photobiomodulation as shown by Western blotting analysis.

CONCLUSIONS

In conclusion, the increase of both keratin-5 and -8 in treated organotypic cultures suggest that a synergy between laser photobiomodulation and the mesenchymal compartment represented by fibroblasts is crucial to stimulate a higher synthesis of keratin in the test group organotypic cultures.

Author Contributions

G.C. designed the research study. G.C. performed the research. P.C. and G.C. wrote the manuscript. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

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Conflict of Interest

The authors declare no conflict of interest.

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BPI

Evaluation Study

TEMPOROMANDIBULAR JOINT DISCAL LIGAMENTS EVALUATION BY MRI

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ABSTRACT

To evaluate the discal ligaments of the temporomandibular joint (TMJ) by magnetic resonance imaging (MRI) and correlate them with disc displacement, twenty-six patients (21 females and 5 males; age range 14-54 years; mean age 34 years) had bilateral TMJ evaluated by means of MRI since they were affected by TMJ disorders. Every joint was studied at closed and opened mouth with TSE double echo T2/PD weighted parasagittal and paracoronal individualised sequences. The following parameters were evaluated: the presence of reducible disc displacement (RDD) or a not-reducible disc displacement (NRDD); signal intensity, morphology, thickness and length of the ligaments; imaging quality of lateral and medial discal ligaments in paracoronal scans, expressed in percentage and correlation between the kind of disc displacement (RDD and NRDD) and axis of best visualisation of the discal ligaments; in 69% of TMJs had RDD whereas 31% of cases had NRDD. Ligaments showed a comma-like shape, a low signal intensity on T2-weighted sequences and intermediate signal intensity on DP-weighted ones; their length ranged from 3 to 8 mm. On paracoronal scans, the lateral discal ligament was depicted in 56% of cases, while the medial ligament was clearly defined in 75%. The lateral ligament was clearly defined in 60% of TMJs with RDD and 47,1% of TMJs with NRDD, whereas the medial ligament was clearly defined in 77,1% of TMJs with RDD. and in 70,6% of TMJs with NRDD. MRI can identify soft tissues of TMJ. Coronal T2-weighted scans are useful for identifying the discal ligaments in healthy and RDD TMJs.

KEYWORDS: *temporomandibular, disk, joint, displacement, pain, imaging*

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INTRODUCTION

Temporomandibular joint (TMJ) movements are conditioned by biomechanical constraints such as dense connective tissue's discal ligaments and fibrous structures (1). In addition, collateral ligaments join the disk with the poles of the mandibular condyle guaranteeing optimal dynamic efficiency.

The most important anatomic structure of the TMJ is the articular disk interposed between the condyle and the temporal bone, preventing possible articular damage. The disk follows the movements of the condyle and guarantees optimal dynamic efficiency thanks to the presence of discal ligaments, too (2). Collateral ligaments originate from the articular disk's lateral and medial margins and insert, curving downwards, at the lateral and medial margins of the mandibular condyle.

Anatomical studies have revealed the presence of an insertion tubercle of the lateral ligament on the lateral face of the mandibular condyle; this is the only macroscopic structure of insertion of capsular or pericapsular ligaments on the condyle (3). During mouth-closing movements, the lateral ligament is the only anatomical structure that stops the disk from moving forwards and medially under traction exerted by the lateral pterygoid muscle (4). Parasagittal scans of the TMJ with T2-weighted sequences were the best to identify joint recesses, the retrodical tissue (5) and, indirectly, discal ligaments.

Few studies have examined the morphological and functional aspects of the discal ligaments, especially with MRI, although this technique is considered the gold standard in the static and dynamic (6) imaging of temporomandibular disorders (TMD) and in maxillofacial pathologies (7-8). The disc displays a linear arched shape on the coronal plane, with reduced thickness at its medial and lateral margins (9).

We aim to evaluate the discal ligaments by T2/PD-weighted sequences MRI with coronal and axial individualised planes.

MATERIALS AND METHODS

The study group consisted of 26 patients (21 females and 5 males; mean age: 34 years, range: 14-54 years) with TMD. Exclusion criteria were a history of previous trauma to the head or face and tumors or malformations of the maxillofacial region. Diagnostic, treatment, and consensus for the scientific use of data were obtained from all patients. The study was performed with respect to the Declaration of Helsinki of 2013.

1.5-T superconducting MR imaging unit (Avanto, Siemens, Erlangen, Germany) with TMJ surface coil was performed; every TMJ was studied at closed and open mouth. TSE double echo T2/PD-weighted parasagittal sequences (TR/TE 2000/105 and 2000/15 ms respectively, FoV 256x189 mm, thickness 3 mm, gap 0, 2 acquisitions) oriented perpendicular to the long axis of the condyle in the axial scout were performed. The opened-mouth sagittal images and the axial scout were used as localisers for coronal imaging with TSE double echo T2/PD weighted (TR/TE 2000/105 and 2000/15 ms respectively, FoV 256x154 mm, thickness 3.5 mm, 3 acquisitions). The section was centred on the condylar head parallel to the ideal line of collateral ligaments.

The following parameters were evaluated: number of TMJs showing a reducible disc displacement (RDD) or a not-reducible disc displacement (NRDD); signal intensity, morphology, thickness and length of the discal ligaments; imaging quality of lateral and medial discal ligaments in paracoronal scans, expressed in percentage and a correlation between disc displacement (RDD and NRDD) and the axis of best visualisation of discal ligaments.

In order to define the quality of images, these parameters were assessed by scoring images on a scale from 0 to 3, in which 0 corresponded to poor visualisation of the discal ligament, 1 to sufficient definition, and 2 and 3, respectively, to good and excellent definition.

RESULTS

As regards disc displacement, 35 (69%) TMJs have RDD, while 17 (31%) have NRDD (Table I). Both internal and external collateral ligaments showed a homogeneous low signal intensity on T2-weighted sequences and intermediate signal intensity on PD-weighted ones, similar to the disc. They presented a curve, comma-like shape with a thickness of 5 mm in the superior part and 1 mm at the insertion point; the length was from 3 to 8 mm (Fig. 1 and Fig. 2).

On paracoronal scans, the morphology of lateral discal ligament was depicted (score 2-3) in 56% of cases, sufficient in

36%, while in 8%, it was not visible (Fig. 3A). In regards to medial ligament, it was clearly defined (score 2-3) in 75% of cases, sufficient in 19%, and it was not well depicted in 6% of cases (Fig. 3B). In the case of RDD, the lateral ligament was clearly defined (score 2-3) in 21/35 (60%) cases while the medial ligament in 27/35 patients (77.1%). In TMJs with NRDD, the lateral ligament was clearly defined (score 2-3) in 47.1% (8/17) of cases and the medial ligament in 70.6% (12/17).

In the TMJs with RDD, the visualisation of the ligaments was better in the coronal plane (58% of cases) than in the axial one (15%) and similar in 27% of cases, while in the TMJs with NRDD, the visualisation of the ligaments was better in the axial plane (29% of cases) than in coronal plane (14%) and similar in 57% of cases.

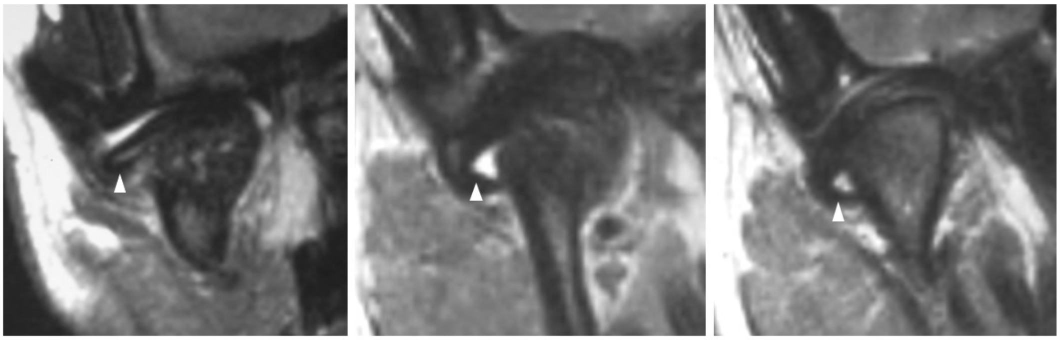

Fig. 1. *TSE T2 W MRI individualized coronal scans. The white arrowhead shows the lateral discal ligament.*

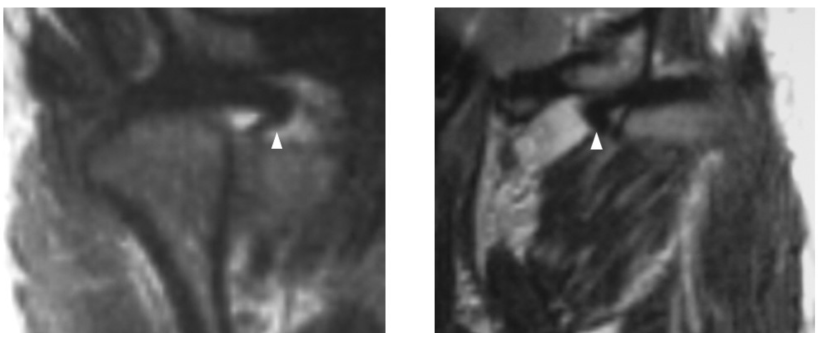

Fig. 2. *TSE T2 W MRI individualized coronal scans. The white arrow head show the medial discal ligament.*

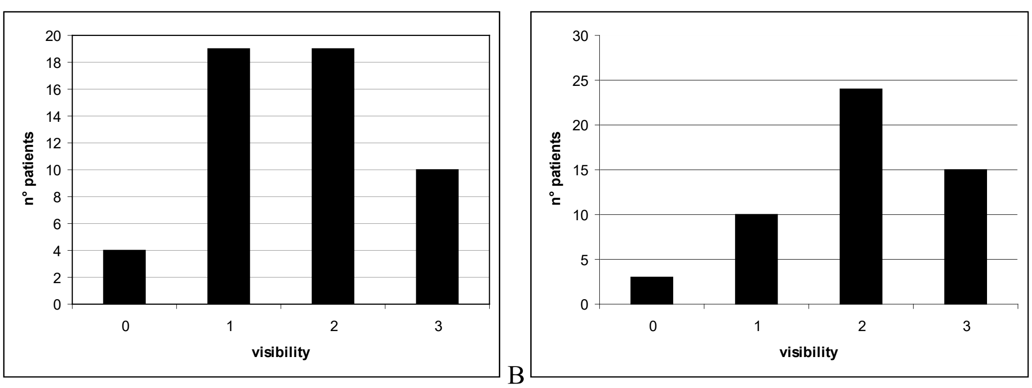

Fig. 3. ***A)*** *Lateral ligament visibility score distribution.* ***B)*** *Medial ligament visibility score distribution.*

Table I. *Visibility score (VS) versus reducible disc displacement (RDD) or a not-reducible disc displacement (NRDD) at open mouth.*

Patients 1-26	Lateral ligament VS	Medial ligament VS	RDD/NRDD at open mouth	Patients 27-52	Lateral ligament VS	Medial ligament VS	RDD/NRDD at open mouth
1	2	2	RD	*27*	2	1	RD
2	2	1	RD	*28*	2	2	NRD
3	3	3	RD	*29*	1	3	RD
4	3	3	RD	*30*	2	2	RD
5	3	3	RD	*31*	1	3	RD
6	1	2	NRD	*32*	0	3	RD
7	2	1	RD	*33*	2	3	NRD
8	3	2	NRD	*34*	2	3	RD
9	2	2	RD	*35*	2	2	RD
10	2	3	RD	*36*	2	2	RD
11	1	2	RD	*37*	3	3	RD
12	1	2	RD	*38*	3	3	RD
13	2	2	RD	*39*	0	0	NRD
14	1	2	RD	*40*	0	0	NRD
15	1	1	RD	*41*	2	3	RD
16	0	1	NRD	*42*	3	3	RD
17	1	2	NRD	*43*	1	1	NRD
18	2	2	RD	*44*	1	2	NRD
19	3	3	RD	*45*	1	0	RD
20	1	2	RD	*46*	1	1	RD
21	3	3	NRD	*47*	2	1	NRD
22	2	2	RD	*48*	2	2	NRD
23	1	1	RD	*49*	1	2	RD
24	1	2	NRD	*50*	1	2	NRD
25	1	2	RD	*51*	2	2	NRD
26	1	1	RD	*52*	3	2	NRD

DISCUSSION

Discal ligaments are well identified on paracoronal images. However, the medial ligament is more easily depicted than the lateral one: in fact, the disc undergoes antero-medial traction from the superior belly of the lateral pterygoid muscle (4), thus distending the medial part of the joint capsule, the inferior joint compartment and, therefore, the medial ligament.

With the antero-medial displacement of the disc, the lateral ligament is likewise subjected to medial traction. Thus it adheres to the cortical portion of the head of the condyle, which is isointense, making identification of lateral ligament more difficult.

Using a scan plane parallel to the ligament axis makes it possible to show the entire discal ligaments from their origin on the disc to their insertion on the condyle. In this way, it is easier to identify and distinguish the ligaments from the surrounding isointense structures (cortical bone of the condyle, disc and capsule wall) thanks to the synovial liquid, hyperintenses in the T2 weighted sequences while the ligaments structures are hypointenses.

Noteworthy, when the disc dislocation cannot be reduced, the disc is placed anteriorly instead of above the condyle when the mouth is open, so individualised "paracoronal" scans will be inclined anteriorly. In this way, scans will be parallel to ligaments. Our results agree with those of recent literature (10-13).

CONCLUSIONS

MRI can easily identify soft TMJ tissues. Disc and the retro-discal tissues are clearly visualised in parasagittal scans, while the condyle-discal ligaments are better visible in individualised scans parallel to the ligaments' axis.

Authors' contributions

DM acquisition of clinical and imaging data and interpretation of data; FC drafted the manuscript; MDG revised the manuscript; LB gave final approval of the version to be published.

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Case Report

SOCKET PRESERVATION AND DELAYED IMPLANT PLACEMENT IN THE POSTERIOR MAXILLA

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ABSTRACT

This report describes the rehabilitation of an upper molar with socket preservation and delayed implant placement. A 62-year-old man with a 2.6 cracked tooth was treated with alveolar ridge preservation (ARP), and after 4 months, a single implant was positioned to restore function and aesthetics. The ARP was performed with xenograft bone substitute material and a collagenic membrane. The implant was positioned with an insertion torque of 30 Ncm and restored with a metal-ceramic single crown after 3 months. At 1 year of follow-up, marginal bone levels were acceptable, and the patient had function restored. ARP is a valid treatment option when post-extractive implant placement could be difficult. Several factors may influence the socket anatomy, adversely affecting primary implant stability. ARP requires a two-stage surgery approach to rehabilitate missing teeth with dental implants.

KEYWORDS: *socket, bone, fixture, regeneration*

INTRODUCTION

Dental implants are widely used to rehabilitate missing teeth, from single-tooth replacement to complete arch rehabilitation (1, 2). Different causes can lead to teeth extraction, such as periodontal disease, trauma, periapical lesions, or other pathological scenarios (3). Once the tooth has been extracted, the alveolar socket undergoes a remodelling process that ends with bone resorption (4). A study by Van der Weijden reported that alveolar socket reduction is approximately 3.87 mm in width and 1.67 mm in height in the first 3 months of healing (5). Moreover, the primary roots are often covered by a thin layer of buccal bone that can be damaged during the extraction, resulting in a horizontal defect that must be corrected before or during the implant placement. In these terms, the post-extractive sockets' alveolar ridge preservation (ARP) during extraction plays a crucial role in achieving optimal implant placement and stability during years (6, 7). In recent years, bone regeneration has been widely investigated, and using bone substitute material (BSM) instead of bone block led to more predictable and valuable techniques that clinicians can use (8). BSM is a scaffold for new bone cells

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resulting from blood clot stabilization. BSM can be divided into 4 groups: autograft (from the same patient), allograft (from the same species), xenograft (from another species) and alloplastic (synthetic material). In addition, barrier membranes can be used to avoid epithelium cell repopulation and to give bone cells time and space to grow and form new bone (9). This report describes the rehabilitation of an upper molar with socket preservation and delayed implant placement.

CASE REPORT

A 62-year-old man complained about a failing metal-ceramic fixed prosthesis on tooth 2.6. At the clinical observation, the tooth showed a root canal treatment attempt without filling and a line crack between the roots (Fig. 1). The periapical X-ray showed signs of periapical lesions around the distobuccal and palatal roots (Fig. 2).

In accordance with the patient, the treatment plan was to extract the tooth and perform an ARP with BSM to prevent bone resorption. After antibiotic prophylaxis and anaesthesia, the tooth was gently removed, taking care not to damage the alveolar socket (Fig. 3). Mechanical debridement of granulation tissue was performed, and hydrogen peroxide was used to clean the socket. Xenograft BSM (BioOss, Geisthlic) was inserted into the post-extractive socket (Fig. 4). Care was taken not to pack the BSM at the bottom of the socket in order not to obstruct the blood clot. A dermal matrix collagen membrane (BioGide, Geistlich) was positioned onto the BSM graft and stabilized utilizing a single horizontal crossed mattress suture (Vycryl 4/0) (Fig. 5-6).

After 4 months, an open flap without vertical incision prosthetically driven surgery was performed, and a transmucosal implant (Standard Plus WideNeck 4,8 Ø x 10 mm, Straumann) was inserted following the manufacturer's indication (Fig. 7-8-9-10).

The insertion torque was 30 Ncm, and transgingival healing was obtained employing a 3 mm height healing cap. Single interrupted sutures were used to obtain wound closure around the implant. After the healing period of 3 months, osseointegration of the implant was checked by screwing the healing cap up to 35 Ncm. The healing cap was removed, and a closed tray impression was taken using polyvinylsiloxane material. A metal-ceramic restoration was realized and cemented onto a solid 5.5 mm height solid abutment. The patient was involved in a long-term maintenance protocol with professional hygiene and occlusion checks every 6 months. After 1 year of follow-up, the periapical x-ray showed good status of the peri-implant bone and no signs of loss of marginal bone (Fig. 11-12).

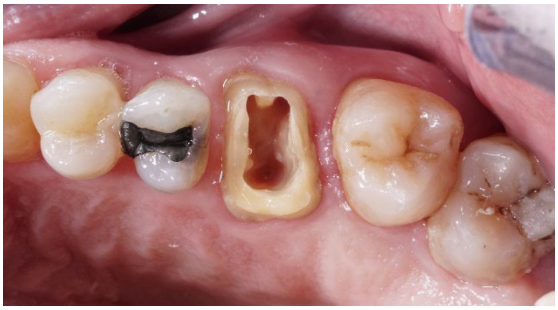

Fig. 1. *Clinical observation of tooth 2.6*

Fig. 2. *Periapical X-Ray of tooth 2.6.*

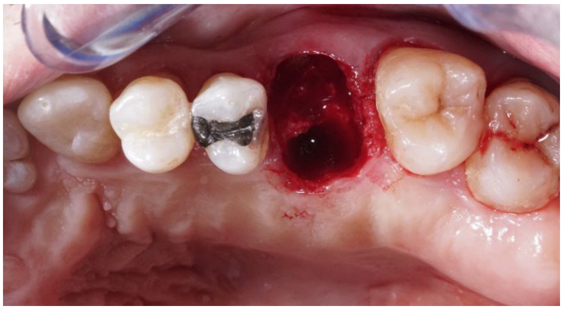

Fig. 3. *Extraction socket*

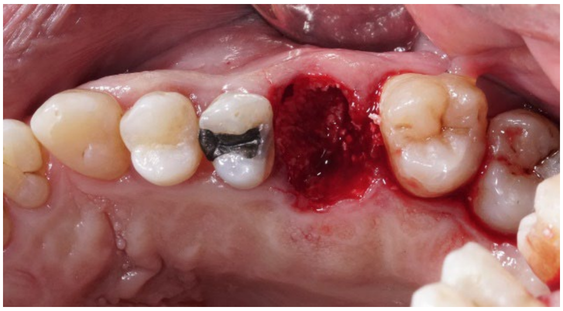

Fig. 4. *BSM graft inserted into the extraction socket.*

DISCUSSION

Tooth extraction always causes a cascade of histologic events that lead to the resorption of the alveolar process. The dynamics of resorption are the same for both jaws and are significantly greater on the buccal wall than on the palatal or lingual sides (10). As a result, the greater reduction is in terms of width and not in height. Together with xenograft BSM, ARPs were reported to regenerate approximately 12.5% to 24% of bone values after 4/6

Fig. 5. *Membrane stabilized by horizontal crossed mattress suture.*

Fig. 6. *Periapical X-Ray of socket filling with BSM graft.*

Fig. 7. *Periapical X-Ray after the healing period of ARP.*

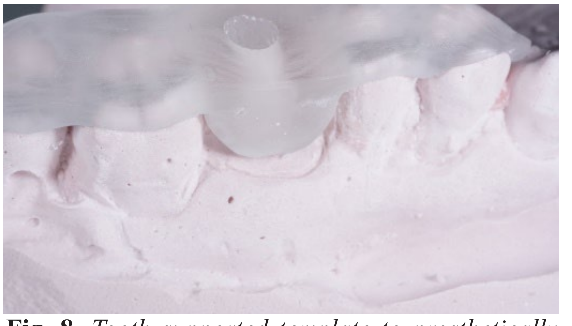

Fig. 8. *Tooth supported template to prosthetically driven placement of the implant.*

Fig. 9. *Implant placed*

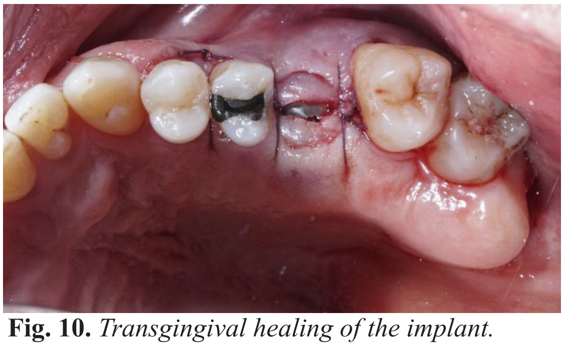

Fig. 10. *Transgingival healing of the implant.*

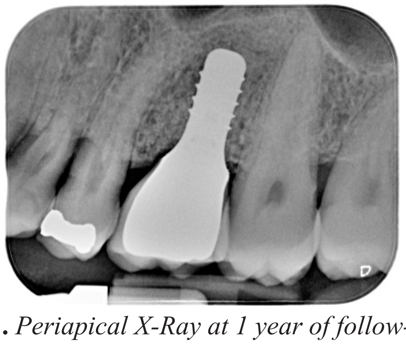

Fig. 11. *Periapical X-Ray at 1 year of follow-up.*

Fig. 12. *Metal-ceramic restoration at 1-year-follow-up.*

months (11). However, it must be pointed out that the final goal of ARP is not bone regeneration but bone preservation to place the implant. In these terms, an osteoconductive material positioned into a fresh extraction socket with a collagen membrane on top allows the blood cells to be stable and protected by epithelium cells (12). This concept is at the base of Guided Bone Regeneration (GBR), in which a barrier can stop the mucosa's epithelial cells from migrating into the socket, voiding interferences with the healing process (13). A systematic review by Willenbacher reported that autografts and xenografts showed promising results in preventing horizontal resorption (12). However, alloplastic material seems the best to prevent vertical shrinkage. Moreover, the horizontal keeping of bone significantly reduces the need for additional augmentation during implant positioning, as reported in the meta-analysis (14).

CONCLUSIONS

ARP is a non-invasive treatment that can be used by every clinician after tooth extraction. The possibility of using xenografts or alloplastic BSM facilitates the whole procedure without an autologous bone graft. The technique can be a valid clinical alternative to post-extractive implant placement, especially in the posterior sites when the drill pathway must be at the centre of the septum. The post-extraction implant placement can often be challenging because of septum fracture or periapical infection. Performing an ARP could prevent bone resorption and facilitate implant placement after healing.

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Case report

ROLE OF MAGNETIC MALLET AND PHOTO-BIO-MODULATION ON MESENCHYMAL STEM CELLS IN SINUS GRAFT SURGERY: A CASE REPORT

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ABSTRACT

Sinus membrane elevation is a common surgical technique to increase bone height in the posterior maxilla before dental implant placement. However, the biological mechanism of bone regeneration in the sinus membrane remains largely unknown. A case report of sinus lift elevation and implant insertion with a Magnetic Mallet is described, as well as pre and post-surgical use of laser for decontaminating the implant site. In addition, Schneider's membrane formation is reported, and the efficacy of laser-associated therapy in the sinus lift procedure is discussed.

KEYWORDS: *mesenchymal stem cells, maxillary sinus membrane, sinus lift, osteotomy, magnetic mallet, photobiomodulation*

INTRODUCTION

In many implant-prosthetic rehabilitations, bone augmentation in the posterior maxillary is very important. Since 1965 the elevation of the sinus floor through the lateral wall of the maxilla has been mostly successful, and for 30 years, this method has been recognized as a gold standard for internal ridge elevation. The starting point of this technique is a donor site: the autologous bone, mixed with biocompatible materials, is then used for floor elevation (1).

The invasiveness of the lateral approach is obvious: a donor site requires additional time and volume and determines the risk of morbidity in either the donor or recipient site.

A different approach was first investigated in 1986 by Tatum OH (2): he accessed the sinus floor through the ridge crest. The technique uses a series of osteotomes after reaching the sinus floor. The osteotome was used to crack the sinus floor bone and elevate the Schneiderian membrane, which was then pushed with antral curettes to create the space into which the graft material was packed.

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Another variation of this method was described by Summers RB (3), who created a more practical and efficient crestal approach. Two different ways were suggested:

A) osteotomy sinus floor elevation (OSFE) through the only use of the implant (tent effect);

B) bone-added osteotomy sinus floor elevation (BA-OSFE) through the positioning of an implant and the packing of graft bone particles.

Clinical observations of bone formation in sinus-lifting procedures without grafting bone substitutes were observed, but the biological nature of bone regeneration in sinus-lifting procedures is unclear. Therefore, comprehending the biological basis of the healing process is necessary to improve surgical techniques and reduce the risk of complications and failures.

MATERIALS, METHODS AND RESULTS

Case report

A 28-year-old male with no significant medical history presented for the replacement of missing second premolars extracted 3 years earlier. The results of blood tests, including a complete blood count, chemistry, and clotting profile, were all within normal limits.

The initial X-ray (Fig. 1) revealed a height of alveolar bone resorption edentulous area in the upper region of the left posterior maxilla (area of second premolar) with pneumatization of the maxillary sinus. The residual bone height was 3 mm, the alveolar ridge was about 7 mm wide, and there was the absence of significant vertical resorption of the alveolar ridge.

Fig. 1. *The initial X-ray. The residual bone height was 3 mm.*

The punch incision was preferred initially, but a full-thickness flap was raised because of the risk of miscalculating the exact alveolar bone height in the critical area under local anaesthesia. The bone was accessible and marked with an Er:YAG laser (Pluser-Lambda SPA, Vicenza, Italy, parameter 200 mj, 20 Hz, 600-micron tip). The implant site was prepared using the magnetic-dynamic mallet device (Meta Ergonomica, Turbigo, Milan, Italy). For the mallet technique, the following inserts were used for the required depth of 0.5 mm below the sinus roof: P.F. 10–160, F–200, F–230. Program power 2 to 4 was used. For the drilling technique were used lanceolate drill Ø –2.2 mm, Ø–3.0 mm, Ø–3.2 mm, Ø–3.4 mm, Ø–3.6 mm and a countersink Ø-4 mm.

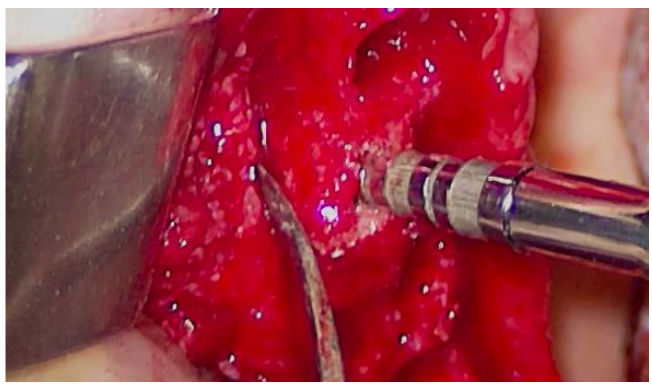

Fig. 2. *Magnetic Mallet: drills in action.*

Radiographs were taken with a depth gauge to determine the length of the preparation. To improve primary stability in cancellous bone, condensing the bone through radial reinforcement has been achieved by a series of bone condensing devices of magnetic mallets with a tapered tip. The magnetic mallet device fractured the sinus cavity floor with a 3.6 mm Ø drill and a countersink 4 mm Ø. A change in the resonance during malleting indicated a complete osteotomy. The Valsalva test to assess the patency of the membrane of Schneider was negative during all procedures. Before inserting the fixture, the surgical site was irrigated with SiOxyl+ solution, and a diode laser irradiated the cavity for 60 sec (2.5 W Peak Power, 0.5 W Average Power, T-on 20

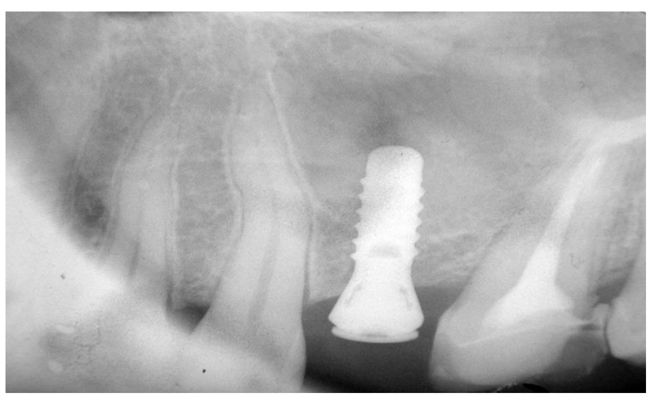

Fig. 3. *X-ray immediately after surgery.*

micron, T-off 80 micron, Frequency 10.000 Hz, tip 400 microns), in order to decontaminate the area and to improve the bone regeneration. A 10 mm long, 4.1 mm diameter and not submerged SLA implant screw ITI (Straumann, Basel, Switzerland) was used.

Manual screwing facilitated lifting the sinus membranes to the desired system height, and the initial stability was achieved. Finally, the surgical site was closed with a 4-0 resorbable suture. The implant position and the amount of sinus floor lifting were visible on radiographs (Fig. 2). Photobiomodulation (PBM) sessions were made for the first 4 months every 2 weeks, including day 0.

The patient received PBM every 14 days for 4 months using a multi-panel system consisting of eight cold lights, with a combination of penetrating wavelengths, from visible to infrared, simultaneously or singly activated on considering the effects needed, such as reduction of pain, inflammation, and oedema. PBM session releases 6 min of irradiation with 48 J/cm of fluency, calculated as the sum of the fluency produced by each cold light, 16 J/cm, multiplied for the three different light groups, 16 J/cm^2 x 3 = 48 J/cm^2. The fluency was calculated by considering 40mm as the distance from the three light groups to the patient's cheeks (delivered by lateral light groups) and lips (delivered by frontal light group) (Fig. 3).

We made two consecutive stages of irradiation in each PBM session, for a total duration of 12 min and 96 J/cm^2 of fluency delivered. A relaxation time of 1 min was made between each session. Each PBM stage was delivered every 14 days. Finally, the fluency delivered to the patients was 192 J/cm^2 per month. Amoxicillin, 500 mg for 8 hours, analgesics, and chlorhexidine mouthwash twice daily for 7 days have been prescribed to the patient. The sutures were removed after 7 days, and the patient was followed twice a month for 3 months (Fig. 4).

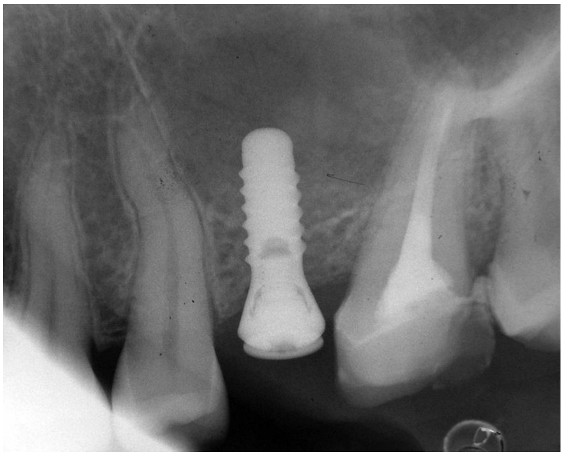

Fig. 4. *X-ray 3 months after surgery.*

Eight months after inserting the implant, X-rays showed the implant and the surrounding bone under the sinus membranes tented in the second premolar region in the upper left (Fig. 5). The system has been loaded and restored with a porcelain-metal crown. X-ray taken at 36 months (Fig. 6) showed a stable clinical situation around the plant's apex. In addition, a dome-shaped structure was observed at the site of the second premolar area.

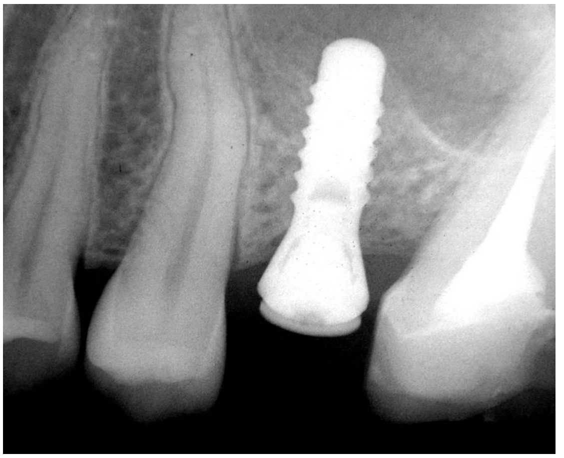

Fig. 5. *X-ray 8 months after surgery.*

DISCUSSION

The magnetic-dynamic technique has recently been introduced in oral bone surgery, such as dental extraction, split crest, sinus lift, and implant site preparation (4). The case reported shows the bone formation around the implant radiographically after the application of the OSFE technique realized by the magnetic mallet device.

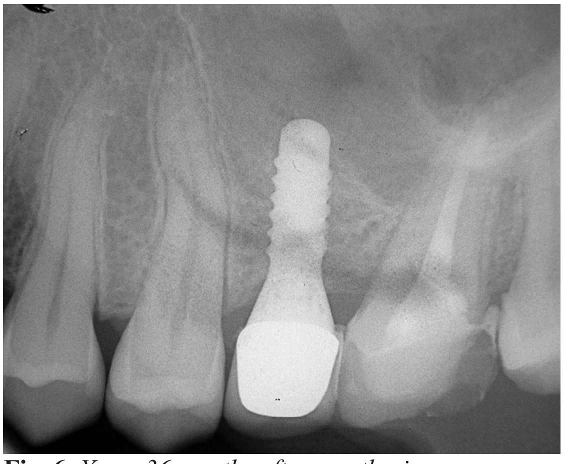

Fig. 6. *X-ray 36 months after prosthesis.*

Over time, the concept of bone formation associated with sinus lift has changed. At first, the hypothesis was that bone formation derived from the proliferation and differentiation of bone cells, induced by hormones (such as parathyroid hormone, sex steroids, calcitonin, D3 vitamin, glucocorticoids) growth factors (IGF-I and TGF-beta) and resorbed by the inflammatory process, with cytokines (IL-1, IL-4, IL-6, IL-11 and INF-gamma); in bone morphogenic units, the interaction of these factors on bone surfaces was considered one of the actors of bone remodelling process (i.e. bone formation and resorption) (1-3,5).

After the mesenchymal stem cells' discovery and the role that this cell's family play in the bone formation process were uncovered, there was also a change of perspective on the biological basis of bone regeneration during osteotome sinus lift elevation. In 2009, Ginnady P. and Gintaras J. (6) noted that in many studies, new bone formation in the maxillary sinus by mucosal membrane lifting had been obtained with and without graft material. Even if the mechanism of this specific bone gain is unknown, some studies are investigating the role of the Schneiderian membrane in this process. Using both in vitro and in vivo assays, Srouji et al. (7) explore the osteogenic potential of the human maxillary sinus Schneiderian membrane. Osteogenesis requires active osteoblasts (bone-forming cells) derived from mesenchymal progenitors (mesenchymal stem cells). This cell type can be found in bone marrow stroma, periosteum, and other sites such as adipose tissue and microvascular walls. However, their presence in the human maxillary sinus Schneiderian membrane was not proven. Therefore, flow cytometry analysis was conducted on cultures by looking for specific markers expressed by osteogenic mesenchymal progenitors (such as STRO-1, CD105, CD146, CD 166, CD 71, and CD 73).

Gruber already underlined the presence of stem cells in the sinus Schneiderian membrane (8) and their potential for osteogenic differentiation in vitro culture. A histologic examination of the explanted samples shows a pseudostratified columnar ciliated epithelium facing the sinus cavity with a richly vascularized lamina propria and a deeper layer of periosteum-like connective tissue lacking any evidence of the presence of osseous mineralization. The data from Srouji's et al. investigation (7) show evidence of osteoprogenitor cells within the Schneiderian membrane.

Histological study of the explants from which osteoprogenitor cells were isolated indicated the absence of associated bone fragments, dispelling the possibility that the osteoprogenitor cells may be carried over from the maxillary bone underlying the sinus membrane. The deep portion of the human maxillary sinus Schneiderian membrane represents an interface with the underlying bone and could be equated to a periosteum. The osteogenic progenitors revealed from the study could have originated from this profound portion of the explanted tissue. It is, therefore, reasonable to assume that a periosteum-like membrane also lines the maxillary bone, forming the sinus floor at the site where this interfaces with the maxillary sinus mucosa and that lifting of the sinus mucosa results in the lifting of this periosteum-like membrane as well; this could be considered a possible demonstration of the importance of the role of the sinus Schneiderian membrane in new bone gain in association with osteotome sinus lift elevation without bone graft.

In 2015 a research conducted by Guo et al. proved the role of stem cells in bone formation in the maxillary sinus Schneiderian membrane (9). This study indicates the presence of a similar pattern of protein expression in human mesenchymal stem cells isolated from the maxillary sinus Schneiderian membrane and bone marrow. Despite this evidence, we combined our protocol lasers' techniques to improve stem cells' proliferation and differentiation in osteoblasts (10,11) and prevent bacteria contamination (12) during surgical procedures. The laser technique used, without any thermal stress, is without risk. A high-power diode laser combined with H_2O_2, OHLLT/Oxygen High-Level Laser Therapy can reduce the number of bacteria inside the periodontal pockets, around the implants, and inside the alveolar bone after extractions. Finally, laser therapy after surgery improved the healing of hard and soft tissues, reducing the risk of complications during bone regeneration therapy.

CONCLUSIONS

A case of sinus lift elevation by using a Magnetic Mallet in addition to laser therapy is reported. The role of the human maxillary sinus Schneiderian membrane, and the cell population of the deepest part of the membrane, in determining bone gain where a blood clot is formed around the implant is described. Laser applications help the potential of new bone formation without bone graft in the maxillary sinus since laser reduces bacteria in the surgical field.

Author Contributions

G.C. designed the research study. G.C. and A.L. performed the research. A.L. and P.C. wrote the manuscript. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

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Conflict of Interest

The authors declare no conflict of interest.

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Case report

ANGULAR CHEILITIS DURING ORTHODONTIC TREATMENT

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ABSTRACT

Angular Cheilitis (AC) is a multi-factorial disorder clinically characterized as an eroded and erythematous non-vesicular lesion radiating from the angle of the mouth, which may be unilateral or bilateral in presentation. AC offers many etiological causes, from infectious to traumatic and iatrogenic forms. The recurrence of this manifestation represents an unpleasant and painful discomfort for the patients. Besides using antifungal drugs as the first choice option, alternative topical treatments, vitamin supplementation, and photodynamic therapy have experimented with actinic cheilitis. This report presents a case of AC in a 15-year-old girl undergoing orthodontic therapy for 18 months, treated with combined therapy of topical antifungals and low-level laser therapy.

KEYWORDS: *cheilitis, commissure, infection, pain, laser*

INTRODUCTION

Angular cheilitis (AC) is an inflammatory skin process of varied etiology occurring at the angle of the mouth, also known as "labial commissure" or "perlèche" (1). The etiology is highly varied, with many local and/or systemic causes responsible for first onset and recurrence (2) and several predisposing factors like microbiological changes, smoking, drug assumptions, mouth breathing, hematological deficiencies, and loss of vertical dimension in the elderly (3). Among the infective etiology, Candida albicans, Streptococcus aureus, and Streptococci are generally associated with AC; pseudo-hyphae and budding yeasts have been detected in 48% up to 93% of cases (4, 5). AC has also been correlated to immunocompromised individuals and atopic patients (6); the peak incidence is during the third, fifth, and sixth decades (7).

The labial commissures are interface points for the oral mucosa and the face's squamous epithelium. They are likewise a mechanically active hinge for the oral aperture that supports more action and forces than the rest of the lips. Therefore, the commissures are specifically susceptible to stress (8).

AC is clinically characterized by erythema, rhagades, ulcerations, and crusting of one or both lip commissures and

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peri-labial skin, responsible for an unpleasant and painful discomfort (9). Some authors suggest that when these clinical manifestations occur during orthodontic treatment, it could be due to a nickel allergy (10). Different therapy options are available, which should be chosen by the severity of the disease, the symptomatology, the frequency of recurrences, and the potential adverse effects of the medications. Low-level laser therapy (LLLT) has been widely employed for treating oral mucosa affections, such as aphthous stomatitis (11), because of its biomodulation and analgesic effect. Moreover, the healing process stimulates and promotes immediate pain relief without an overdose of medication or side effects are advantages to be considered (12). Hence, in the present work, we present a patient in orthodontic therapy diagnosed with AC treated with LLLT.

CASE REPORT

A 15-years-old girl presented bilateral erythematous lesions affecting mouth angles. She had been attending a specialist orthodontic practice for 18 months to treat her class II division 1 malocclusion using fixed appliances. A thorough medical history was taken to rule out other disorders, but no relevant information was provided. She had no known allergies and no courses of antibiotics recently; she was a non-smoker with competent lips. Extra-oral examination revealed two deep erythematous lesions affecting the mouth angles, which extended 5 mm from the vermillion border to the surrounding skin (Fig.1). These lesions were weeping slightly and were sore and tender on the mouth opening. These manifestations have been present for several weeks and were not preceded by prodromal symptoms associated with herpes labialis. Intra-oral examination showed no other disorders, and oral hygiene was found to be fair to good. Thus, a clinical diagnosis of AC grade 2 was performed, according to the scale proposed by Ohman et al. (13).

The patients gave a history of frequent recurrence of these extremely painful lesions since the beginning of the orthodontic treatment. She also reported having used various pharmacological aids, including topical anesthetics, topical antifungals, mouthwashes, and steroids, which took around one week to heal but still, the lesions recurred every 1-2 months. Therefore, we decided to treat the patient with combined therapy of topical antifungals and LLLT using a 940- nm diode laser (Biolase, USA). The laser was activated 1-2 mm away from the lesion and advanced slowly toward the area ending up 1-2 mm away from the lesion, moving continuously from the periphery of the lesion to the center, covering the whole area, and shifting away from the lesion if the patient sensed warmth. The setting was initially put at 0.6 W CW (1.2 W pulsed) for 30-45 seconds. The laser cycles were alternated to allow the tissue to cool down with free cycles of 15-20 seconds. The patient reported that lesions started healing earlier than in previous attacks; there was also an early reduction in pain. Healing was uneventful, and the patient experienced a spontaneous reduction in pain. The lesions healed within 7-8 days; a follow-up after one year showed no recurrence of the lesions. The patient reported no further signs of AC during the last 12 months. The patient's orthodontic treatment continued uneventfully.

DISCUSSION

AC can be a painful condition that affects the corners of the mouth (7). The case presented in this paper indicates that

Fig. 1. *Erythematous lesions revealed by extra-oral examination*

AC can also occur in healthy patients undergoing orthodontic treatment, even if these patients do not have a nickel allergy or are not immune compromised. Scientific evidence supports antifungals as the first-line treatment for AC. However, these trials clearly express pieces of evidence about the role of Candida albicans in the pathogenesis of AC, showing antifungal agents as a valid treatment choice option (14, 15). In 2016, Rocha and colleagues reported a case of AC at the right commissure treated by methylene blue – mediated photodynamic therapy, leading to immediate pain relief and re- epithelization of the site (16). Several studies reported the LLLT employment for treating and managing oral mucosa disorders, such as recurrent aphthous stomatitis and actinic cheilitis (11, 12, 17). Since no medications are required, no side effects and no risk of over-dosage could be encountered. Moreover, LLLT enables rapid wound healing without scarring providing high comfort for the patient and giving a very satisfactory cosmetic result (17). The topical treatment with antifungals seems to remain the most reliable therapeutic option against AC. Nevertheless, when LLLT was performed, there was a considerable healing improvement and a consequent analgesic effect. Therefore, although further studies are needed to assess whether new therapeutic approaches may replace conventional pharmacological treatments, the efficacy of the LLLT should be deepened not only for its efficacy against microorganisms but also for its biomodulation and analgesic effects, accelerating healing and improving the quality of life of the patients.

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