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CONTENTS

TREATMENT WITH NOVEL HYBENX® ROOT CANAL CLEANSER SUGGESTS BIOFILMS BLOCKED HEALING OF HUMAN WOUNDS: CASE SERIES

M. GITTERLE

Wound & Hyperbaric Medicine, Christus Santa Rosa, New Braunfels, TX, USA

Pathogenic biofilms are considered a potential major obstacle to healing chronic wounds. The six-month evaluation period in this study assessed the effect of a single treatment of the HYBENX® (HYB) Root Canal Cleanser on chronic wounds, especially its ease of use, safety, potential for shortening duration of Inflammatory Phase, and promotion of granulation. HYB gel was applied to the wound bed and periwound skin for 10 seconds. Gel was removed by cotton gauze pads and low pressure saline rinsing. Standard wound dressings based on wound etiology, location, and exudate characteristics were applied. Duration of these seven HYB-treated wound cases (two pressure ulcers, one surgical wound, four lower extremity ulcers—a venous reflux-associated calf ulcer, one diabetic foot ulcer, and two animal-induced wounds—brown recluse spider and bovine bite) ranged from 12 days to 10 years. Three cases had sinus tracts. After HYB application, sinus tract closure occurred at 7, 16, and 21 days. The 10-year recalcitrant wound from brown recluse spider bite healed in 97 days. Diabetic foot ulcer responded to HYB treatment plus additional strategies and healed in six months. These data support the hypotheses that pathogenic biofilm actively prevents the healing of chronic wounds, and biofilm can be disrupted with a single HYB treatment.

Chronic wounds present an increasing challenge to healthcare systems worldwide. Non-healing wounds have a disproportionately high treatment cost and are associated with high morbidity. In certain populations —notably the increasingly large diabetic population — such wounds can dramatically increase the amputation rate and mortality risk (1).

Key to meeting the challenge of chronic wounds is our emerging understanding of the complexity of the wound healing process (2). Necrotic tissue, slough, wound bioburden (3), and insufficient host molecular and cellular components (e.g. growth factors, protease inhibitors, extracellular matrix, angiogenic factors, antioxidants, oxygen saturation) can disrupt the normal healing process (2). Furthermore, the roles of the human microbiome— microorganisms which live on or within the human body— and of pathogenic bacteria and fungi in the non-healing of chronic wounds remain controversial (4-6). Bacteria in particular, but also a number of fungal species are now known to thrive on wounds in a polymicrobial community called "biofilm" (7) instead of a free-floating infection of a single species (5). Biofilms detected by scanning electron microscope were significantly more common in chronic wounds (60%) than acute wounds (6%) (3, 8).

Biofilm communities are highly resistant to host immune defenses and to conventional wound treatment modalities, including antiseptic cleansers and topical and systemic antibiotic therapies, for several key reasons (9, 10). Biofilm organisms exist in multiple "zones" within a complex extracellular

Key words: chronic wounds; diabetic foot ulcer; oral decontaminant; pressure ulcer; surgical ulcer; wound infection; adjunctive debridement agent

Corresponding author:
Prof. Furio Pezzetti,
Department of Experimental, Diagnostic and Specialty Medicine,
University of Bologna,
Via Belmeloro 8,
40126 Bologna, Italy
e-mail: furio.pezzetti@unibo.it

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polymeric substance (EPS) composed of proteins, polysaccharides and glycoproteins (5). EPS is strongly adherent to host tissue, contains channels for nutrients and waste removal, and can stimulate inflammation (5). Biofilm organisms comprise about 20% of the biofilm, and have various levels of metabolic activity and distinct roles, depending on their location (10, 11). For example, bacteria near the wound bed have very low metabolic activity and thus are very tolerant of many antimicrobials (11). Furthermore, biofilm bacteria are also capable of sharing resistance-conferring genes through direct, inter-species mRNA transfer inside the anatomic confines of the biofilm matrix. Due to the increasing prevalence of resistant organisms in the community, the risk of refractory, highly resistant biofilm populations has become a reality (12).

Such biofilm communities are known to alter wound conditions to favor the proliferation and success of the biofilm organisms, which subverts several wound conditions compatible with healing (5). An 11-clinician panel from the wound therapeutics research community strongly agree that biofilms play a major role in an increasing number of complex non-healing, chronic wound cases (13). Furthermore, they suggest that clinicians should consider different treatment paradigms that can lead to finding cost-effective, safe methods for removing biofilm in non-healing wounds (4). Disruption and removal of dead tissue, biomass, and biofilm from the wound bed is currently undertaken by various types of debridement.

Debridement methods include autolytic, biosurgery (e.g. larval therapy), enzymatic, hydrosurgical, mechanical, surgical, and ultrasonic (3). Each debridement method has specific advantages and disadvantages. The choice of debridement technique depends on the patient's wound type, most effective debridement method, anatomical site of wound, amount of necrotic tissue, patient's comorbidities, and preference of the patient (adherence, accessibility) (3). A recent position document suggests that management of biofilms may need to include debridement, cleansing, and antimicrobial treatment at regular intervals to prevent reformation (14).

Despite use of debridement methods and standard wound management, unsuccessful healing of many chronic wounds, especially diabetic foot ulcers, are associated with amputation rates as high as 21% (15). Since the standard of care did not promote healing in many chronic wounds, we hypothesized that the current modalities for removing biofilm were not sufficient in all chronic wound cases and that biofilms still hindered the progression of wound healing. In our search for efficacious agents against biofilms, we noted a substantial similarity of biofilm-related complications in non-healing wounds with biofilm-associated oral pathologies, including dental abscesses, plaque, and periodontitis (15).

HYBENX® (HYB) Root Canal Cleanser is an FDA-cleared dental device, and is used to cleanse infections from the root canal. The same product, marketed as HYBENX® Oral Tissue Decontaminant in Europe and Canada as a class I medical device, is used as an oral adjunctive debridement agent to treat biofilm-associated oral pathologies (16-19). HYB is a hygroscopic and denaturing sulphur-containing solution that denatures, desiccates, and coagulates the biofilm matrix and microbes (20). It can induce a protective layer of denatured, coagulated tissue debris over the ulcer wound bed which subsequently dissolves (19, 20). Furthermore, in a prospective, randomized trial, patients with recurrent aphthous stomatitis who received a single HYB application on the oral ulcer showed a significantly greater decrease in pain score on days 1 and 2 than those treated with multiple SaliCept applications (20). The single HYB application healed the oral ulcer in 50% of the patients with recurrent aphthous stomatitis by day 8, similar to the 44% healing rate of patients treated with multiple SaliCept applications (20). Both treatments were deemed safe as the adverse events appeared unrelated to treatment (20) .

Thus, we hypothesized that HYB used in dental offices to cleanse root canals in the US and to remove biofilms from teeth, tooth pulp, and periodontal tissues in Europe and Canada provides a distinct mode of action and may improve efficacy in at least some chronic wounds in our patients. Safety data were considered carefully, and no evidence was identified that might raise concern

over patient or clinician safety in the use of HYB as a topical treatment modality for human wounds (20, 21). Use in the oral cavity on oral lesions (20) would be expected to have at least as high uptake as an application to wounds located on extremities. Clearly, a series of investigations in patients with chronic wounds represents an opportunity to compliantly utilize an approved modality in an off-label application that could possibly improve the rate of healing chronic wounds.

In June of 2014, my team began a series of investigations with HYB as a treatment modality for use in chronic wound therapy in a hospital-based outpatient clinic population. These investigations were motivated by a need for improved strategies for addressing biofilm-related challenges in wound healing, promising results of HYB in treating biofilm-associated oral diseases, and the poor outcomes associated with standard modalities in chronic wounds.

MATERIALS AND METHODS

Patients

Patients with chronic wounds of varying etiologies were offered the opportunity to receive an application of the FDA-approved oral decontaminant, HYB. Over a six-month period, HYB was applied to wounds of varying etiologies in more than 30 patients, and observations were made in respect to changes in wound characteristics, including granulation tissue quality, velocity, and coverage, bioburden characteristics, epithelialization, wound drainage, periwound skin, and patient experience.

HYBENX® application

HYB gel was obtained from Epien Medical, Inc. (St. Paul, Minnesota). Our application strategy was simple. Loose debris was removed manually, and the application area was dried as much as possible. A mild topical anesthetic (EMLA cream, LET (lidocaine, epinephrine and tetracaine gel), or viscous lidocaine) was applied to the wound bed and surrounding edge. HYB gel was applied to the center of the wound bed from a 3cc syringe, and quickly spread with a gloved finger over the full wound bed and a variable width of periwound skin (range: 0.5 to 5 cm from wound bed). After 10 seconds, the HYB gel was wiped off with cotton gauze pads and was followed by low pressure saline rinsing of residual product. Total application and removal time was less than one minute. Following HYB application, standard wound dressings were applied as appropriate, with dressing selection based on wound etiology, location, and exudate characteristics. Follow-up of the patients' wounds were carried out at standard intervals.

RESULTS

We chose to present seven HYB-treated wound cases (two pressure ulcers, one surgical wound, four lower extremity ulcers—a venous reflux-associated calf ulcer, one diabetic foot ulcer, and two animal-induced wounds—brown recluse spider and bovine bite) for the following reasons. The cases represented the broad range of applications for which HYB was found to be useful. Secondly, the photographic documentation in these cases was effective in visually demonstrating the typical changes in wound bed characteristics seen following HYB application.

Patient comfort was not a significant issue as long as common methods for mild topical anesthesia were used prior to HYB treatment. Application of mild topical anesthesia successfully prevented the brief discomfort that occurred during application, and the following 2 minutes. Generally, HYB application was very well tolerated, and any discomfort was minor and brief (< 5 min).

Pressure ulcers

Case 1. A 40-year-old morbidly obese, non-diabetic male presented with a pressure ulcer on his left buttock (Fig. 1A). Prior care before referral to our clinic (neosporin, duoderm) had failed to maintain or stimulate the healing process of the pressure ulcer over a 60-day period. The wound was initially characterized by essentially 100% fibrin and slough coverage. After a single HYB application, which was associated with brief, minor pain, topical therapy was continued (duoderm), along with a continuation of offloading measures that had already been instituted. At the one-week follow up visit, substantial improvement in granulation coverage was noted (Fig. 1B). On the first and second weekly

post-application visit, a clear improvement in proliferative activity was noted (Fig. 1B, C). The wound went on to complete closure within 49 days of HYB application in spite of sub-optimal offloading due to the patient's occupational circumstances (Fig. 1D). The patient also reported that he had noticed significant improvement in wound pain following HYB application. No excisional debridement was required following HYB application.

Case 2. A 33-year-old female with spina-bifida, severe scoliosis and paraplegia presented with a stage IV pressure ulcer at the thoracolumbar spine area. It measured 7.6cm x 7.5cm x 5cm (depth) in size and had a 4 cm deep sinus tract which extended to lumbar spine bone, with evident osteomyelitis (Fig. 2A). The healing process had not been adequate over the prior 10 months despite the following treatments: supine and seated offloading, intensive nutritional support, serial debridement, and application of various dressings, which included Hydrofera Blue, Calcium Alginate with Silver, Silvasorb, Medihoney Alginate, Santyl, and negative pressure wound therapy (NPWT) with granufoam sponge, depending on the concurrent wound characteristics. Treatment involved a single HYB application. The patient was also treated with culture-directed antibiotic therapy for osteomyelitis, offloading measures, aggressive nutritional support, Hydrofera Blue, NPWT, Medihoney Gel, Santyl, Fibracol, Prisma, Endoform, 10 applications of Grafix Core, Iodosorb,

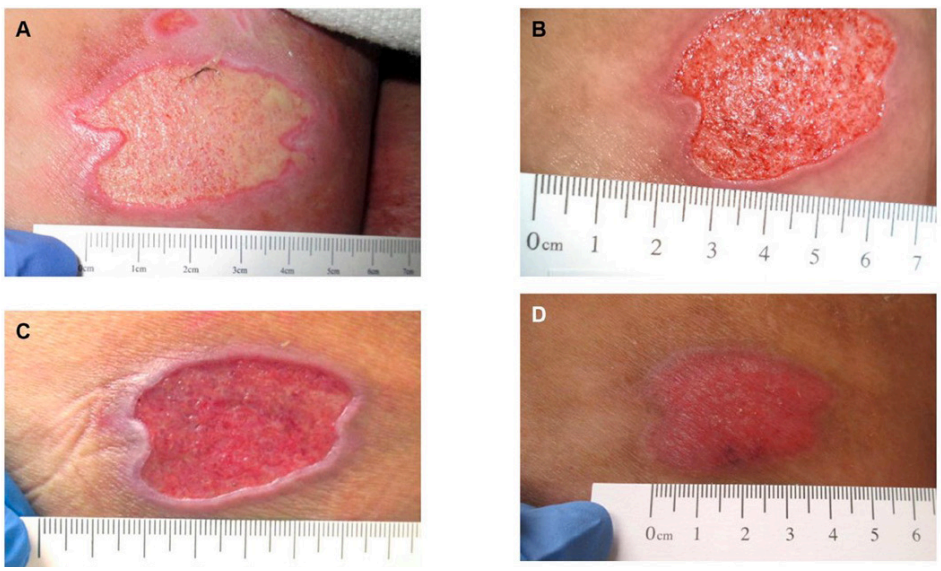

Fig. 1. *Pressure ulcer on left buttock of case 1, a 40-year-old morbidly obese, non-diabetic male.* ***A****) Wound prior to HYB application.* ***B****) One week post-HYB, note granulation coverage.* ***C****) Two-week visit.* ***D****) 49 days post HYB.*

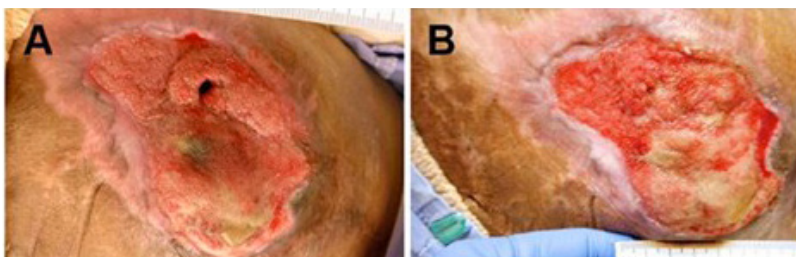

Fig. 2. *Stage IV pressure ulcer in case 2, a 33-year-old female with spina-bifida, severe scoliosis and paraplegia.* ***A****) Wound prior to HYB application.* ***B****) One month, note closure of sinus tract.*

and Medihoney Colloid. Of note, resolution of a previously worsening, 4 cm deep sinus tract to bone was noted within 7 days of HYB application. Over the subsequent three weeks, granulation coverage improved, and edge characteristics became more favorable to closure (Fig. 2B). The sinus tract remained closed. After 16 months, the wound depth has decreased to 0.2cm and closure was at 78%. The patient continues to await arrangements for plastics closure. Clinically, rapid resolution of the deep sinus tract was felt to be largely due to effects of HYB application; i.e. eradication of sinus tract biofilm, and stimulation of granulation tissue.

Surgical wound

Case 3. A 72-year-old female presented with a non-healing surgical T-spine wound at four days after diagnosis of wound dehiscence (surgical closure 21 days prior). The wound bed initially showed a paucity of granulation, heavy slough, and very poor edge characteristics (Fig. 3A). The patient was also diagnosed with severe protein calorie malnutrition, chronic kidney disease, and hyperlipidemia. Treatment involved nutritional repletion and a single HYB application followed by packing with Aquacel Ag ribbon every other day for 6 weeks, which was followed by five sequential Grafix Core Amniotic

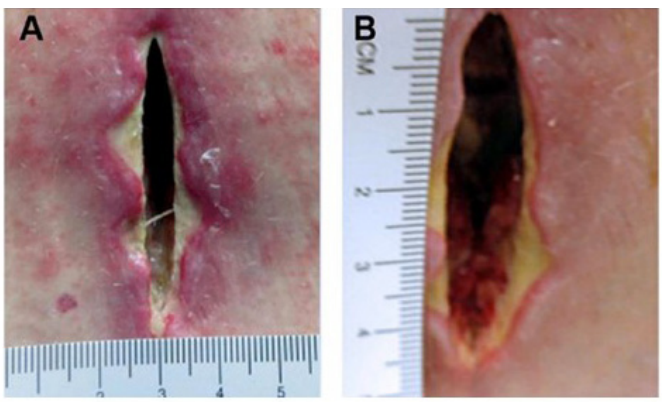

Fig. 3. *Non-healing surgical T-spine wound in case 3, a 73-year-old female.* ***A****) Wound prior to HYB application.* ***B****) Two weeks post-HYB treatment.*

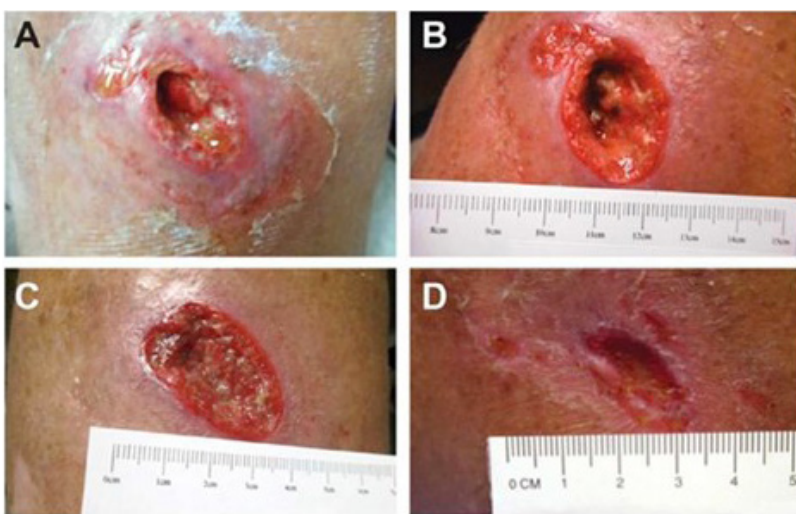

Fig. 4. *Fracture-induced lower extremity ulcer in case 4, a 62-year-old female with venous insufficiency.* ***A****) Wound prior to HYB application.* ***B****) One week post-HYB treatment, note granulation coverage.* ***C****) Three week visit, note closure of sinus tract.* ***D****) 90 days.*

Allograft applications. The wound bed showed a very rapid transition to proliferative status to nearly 100% granulation within two weeks (Fig. 3B). The wound had healed (complete closure) by 77 days.

Lower extremity ulcers

Case 4. A 62-year-old female presented with a calf ulcer and 2.5 cm sinus tract post tibial plateau fracture. The ulcer was at the site of a hematoma associated with the injury, and had been present for 45 days. The patient had a history of venous reflux and prior treatments (Hydrofera Blue) (Fig. 4A). The patient received the described HYB treatment, and Hydrofera Blue alternating with Iodosorb wound dressings were applied. After one week, the wound showed significant improvement in granulation (Fig. 4B). At three weeks post-HYB, sinus tract closure was achieved and granulation coverage was nearly 100% (Fig. 4C). Full healing was apparent at 90 days post-HYB (Fig. 4D). The extended course was chiefly due to the patient's incipient venous insufficiency.

Case 5. A 72-year-old male presented a diabetic foot ulcer, overlying a 1st metatarsophalangeal (MTP) gouty tophus, of three months duration (Fig. 5A). The patient has Type II Diabetes, gout, and secondary lymphedema. Prior treatments had included two Hydrofera Blue applications for one week, then Iodosorb three times per week (TIW) until HYB application. After a single HYB

Fig. 5. *Diabetic foot ulcer in case 5, a 72-year-old male with type II diabetes, gout, and secondary lymphedema.* ***A)*** *Wound prior to HYB application.* ***B)*** *14 days, note granulation coverage.* ***C)*** *22 days.* ***D)*** *Six months.*

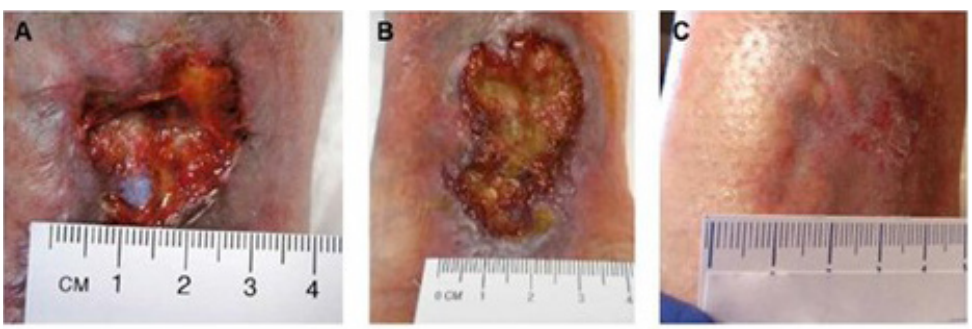

Fig. 6. *Brown recluse spider induced ulcer of 10-year duration in case 6, a 59-year-old female.* ***A)*** *Wound prior to HYB application.* ***B)*** *Three weeks post-HYB.* ***C)*** *97 days post-HYB.*

application, the patient received the following treatments listed in sequential order: Iodosorb (TIW for three weeks), Endoform (TIW for three weeks), Hydrofera Blue (TIW for four weeks), Prisma (TIW for one week), Calcium Alginate with Ag for two weeks, and Fibricol for two weeks. The patient was also treated with NPWT, hyperbaric oxygen therapy (2.0 ATA for 90 minutes, 54 treatments), live-cell placental allograft (Grafix Core), aggressive diabetes management, and compression dressings. Improved granulation coverage and quality, and improved edge characteristics were observed at 14 days (Fig. 5B). Epithelialization was very active at the wound edge at 22 days post-application (Fig. 5C). Final closure was noted after six months (Fig. 5D). Note the diabetic foot ulcer quickly progressed to 100% granulation and showed strong improvement in edge characteristics following HYB application, which has been a typical finding.

Animal-induced wounds in lower extremities

Case 6. A 59-year-old female with chronic recurrent right medial calf ulceration at the site of self-reported "Brown Recluse Spider Envenomation" (Fig. 6A). The ulcer had been present for 10 years at the time of initial evaluation, and prior treatments in our facility had included Medihoney Alginate weekly for 4 weeks, followed by Hydrofera Blue for one week. Patient had evident venous reflux, which was at least partially responsible for non-healing. After a single HYB application, the wound was treated with Hydrofera Blue alternating with Iodosorb, followed by every-other-weekly application of EpiFix cryopreserved amniotic allograft. At three weeks post-HYB application, drainage had significantly diminished, wound depth was reduced, and better edge integration was noted (Fig. 6B). Complete healing occurred on day 97 (Fig. 6C), despite the more than 10-year duration of the ulcer prior to initiating HYB treatment.

Case 7. A 62-year-old female presented with a non-healing bovine bite wound on her right thigh of two-month duration (Fig. 7A). Comorbidities included hypertension, hyperlipidemia, chronic obstructive pulmonary disease, and obesity. Prior treatments included 23 days of topical therapy with mupirocin ointment prescribed by the patient's primary physician, with no improvement. A debridement was performed on the initial visit, one week prior to HYB application. Treatment involved a single HYB application along with NPWT and ciprofloxacin. In this case, rapid improvement was observed in granulation coverage, and more notably, rapid closure of a 4 cm sinus tract in 16 days (Fig. 7B). NPWT was clearly helpful, but closure rate was much greater than would be expected with NPWT alone, as the wound showed 99% closure in 14 days after HYB application (Fig. 7B). The patient moved and was lost to follow-up after the 14-day visit.

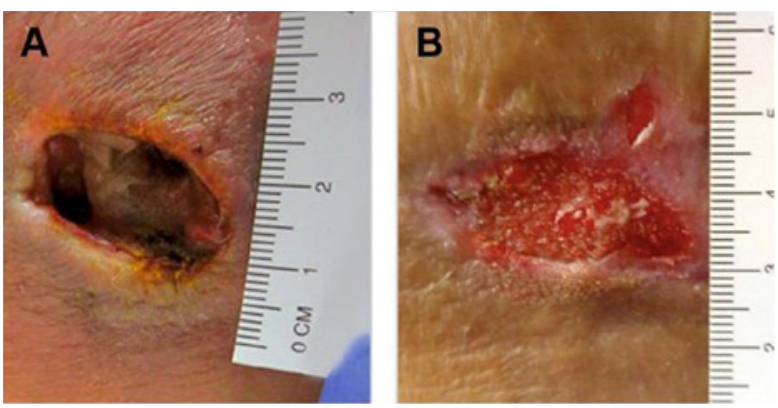

Fig. 7. *Bovine bite induced ulcer of two-month duration on case 7, a 62-year-old female.* ***A****) Wound prior to HYB application.* ***B****) Two weeks post-HYB treatment.*

DISCUSSION

The three principal challenges in wound care are to shift a wound as rapidly as possible from the Inflammatory Phase of wound healing to the Proliferation Phase, to maintain appropriate proliferative characteristics through obliteration of dead space, and to finally reach 100% epithelialization. In our hospital-based wound clinic population, we frequently manage chronic wounds, such as lower extremity venous ulcers and diabetic foot ulcers of more than one to two years duration. These patients have generally undergone extensive prior treatment with various wound care modalities, including debridement, topical therapies, specialty dressings, multiple courses of antibiotic therapy, prior attempts at revascularization, and compression modalities. One possible explanation for recalcitrant chronic wounds is the presence of a pathogenic biofilm that is not sufficiently controlled by the provided debridement and antimicrobial therapies (4, 8, 22). Biofilms on decubitus ulcers, diabetic foot ulcers, non-healing surgical wounds, and venous leg ulcers contained multiple bacterial and fungal species that differed only slightly among wound types and patient demographics (23).

Recently, an 11-clinician panel from the wound therapeutics research community provided clinical recommendations for detection and treatment of biofilm in various types of non-healing chronic wounds, including dehisced surgical wounds, diabetic foot ulcers, pressure ulcers, and venous leg ulcers (4). Mechanical debridement was strongly recommended for non-healing burns, diabetic foot ulcers, pressure ulcers, and venous leg ulcers and weakly recommended for dehisced surgical wounds (4). They strongly recommended the use of antimicrobial dressings for non-healing burns, diabetic foot ulcers, pressure ulcers, and dehisced surgical wounds and weakly recommended them for venous leg ulcers (4). The panel did not indicate specific products, which may vary in efficacy for different types of non-healing wounds, because the shared evidence and experience was not sufficient to support specific recommendations (4). The panel suggests that clinicians should consider different treatment paradigms that include finding cost-effective, safe methods for removing biofilm in other disease states and assessing the methods in non-healing wounds (4), which encourages case studies of adjunctive debridement agents.

The purpose of this six-month evaluation period of the HYB Root Canal Cleanser was to assess its effect on chronic wounds, especially its ease of use and safety, and the evident duration of the Inflammatory Phase and shift to Proliferation Phase. The seven presented cases indicated that a single HYB application reduced the need for subsequent extensive debridement and facilitated a more rapid transition to proliferative characteristics and closure than any other single modality. It also improved overall comfort of the patients, similar to HYB-treated patients with aphthous stomatitis (20, 24) or patients with abscesses (19).

We have found HYB treatment helpful in wounds of various etiologies, including abscess wounds, arterial ulcers, complicated traumatic wounds, diabetic foot ulcers, pressure ulcers, surgical dehiscence and non-healing surgical wounds, venous ulcers, vasculitic ulcers, and wounds related to osteomyelitis with exposed bone.

By utilizing HYB after the initial debridement and diagnostic procedures, we often noted a rapid transition to 100% granulation coverage. These data are consistent with the complete resolution of inflammation in the HYB-treated periodontal abscesses in all five cases within 16 to 30 days (19). Inflammatory characteristics, including periwound inflammation, excessive wound drainage, and inflammatory debris accumulation resolved relatively quickly in these seven cases after HYB application. As in HYB-treated oral biofilm-related pathologies (19), anti-inflammatory mechanisms of HYB probably included the denaturation, desiccation, and removal of the biofilm bacterial populations and the polysaccharide matrix, which harbors pro-inflammatory cytokines and matrix metalloproteases (MMPs). MMPs are proteases known to be deleterious to stable fibroblast proliferation and collagen deposition, and overproduction of these enzymes is typical of wounds in the inflammatory state.

Edge characteristics have also consistently improved. Edge transition is an important determinant of wound bed proliferation, and ultimately epithelialization. We found that HYB

application is associated with more favorable transition characteristics from periwound to wound bed, favoring a saucer-like characteristic most compatible with rapid wound closure.

No deleterious effects were observed on the wound bed or periwound skin characteristics, such as hypersensitivity, necrosis, diminished granulation velocity, or reduced keratinocyte migration, in agreement with patients treated with HYB for aphthous stomatitis (20). These findings agree with abundant dental experience with the product (25), where HYB has been well tolerated, and induced less side effects in oral applications in humans than in controls (21), and veterinary medicine. In contrast to the pain induced by surgical debridement, patient comfort has not been a significant issue, as long as common methods for mild topical anesthesia are used prior to HYB application. Generally, HYB application is very well tolerated, and any discomfort has typically been quite brief. In a prospective study of patients with oral lesions, HYB treatment significantly reduced pain from aphthous stomatitis on days 3 to 6 but not the full study (days 1-6) (24). Mild discomfort had resolved by day 3 in HYB-treated patients with abscesses (19).

Our observations in this patient population has established HYB as a clear breakthrough in clinical wound treatment: its use in our experience has been associated with relatively rapid progression to 100% granulation tissue and subsequent stable closure in patients who had previously failed to heal in spite of standard-of-care treatment.

It is the opinion of our team that our successes with HYB are most likely due to its unique efficacy in addressing a fundamental impediment to wound healing common to all problem wounds; namely, the presence of complex biofilms. Conventional approaches to eradication of biofilm have often failed *in vitro* and *in vivo*, for the following reasons:

- Resistance of biofilm organisms to standard culture techniques utilized to identify free-living (planktonic state) organisms;
- Sharing of antibiotic resistance genes between biofilm species;
- Self-shielding of biofilm organisms through the production of extracellular polymeric substance (EPS), a matrix of high-molecular-weight saccharide polymers that shields biofilm organisms from conventional attack by other cleansing agents, antiseptics, and antibiotics;
- Physical attachment of the biofilm matrix to host tissue by the EPS matrix, rendering biofilm *physically difficult to remove.*

In contrast, HYB represents a true innovation, as it consistently destroys both the biofilm matrix and biofilm organisms in a definitive biophysical manner: denaturation and desiccation (20). Development of bacterial resistance is considered impossible. Denaturation and desiccation destroys biofilm matrix components structurally and causes the bacteria and fungi to osmotically implode, while the application of HYB appeared to leave the host tissue, including fibroblasts and the developing collagen matrix, unharmed. We feel that this is a fundamentally sound approach to biofilm management that is universally applicable to the biofilm problem in wound healing.

The role of pathogenic biofilm in chronic wound healing has been controversial. The HYB-induced denaturation and removal of biofilm from non-healing wounds stimulated steady progression to granulation and epithelialization in the wound. Based on currently available evidence, we feel it is likely that the HYBENX® Root Canal Cleanser will become a standard treatment modality in wound care. These data strongly support the hypothesis that pathogenic biofilms play an active role in preventing the healing of chronic wounds.

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FRENULA IN THE ORAL CAVITY: AN OVERVIEW OF DIAGNOSIS, PROGNOSIS AND CLINICAL MANAGEMENT IN GROWING PATIENTS

M. PASINI[1], S. CARUSO[2], L. LARDANI[2], R. GATTO[2], M.R. GIUCA[1] and M. SEVERINO[2]

[1]Unit of Dentistry and Oral Surgery, University of Pisa, Italy; [2]Department of Life, Health and Environmental Sciences, University of L'Aquila, Italy

This study described the clinical complications and management of frenula. The frenula of the oral cavity are natural anatomical elements consisting of a triangular fold of mucous membrane, which extends in the sagittal direction from the gingiva in the submucosa of the lip, cheeks and tongue. Histologically, the oral frenula consist of a fibrous tract formed by collagen fibers placed in bands associated with thin perivascular nerve structures. These structures are coated by a stratified squamous epithelium. There are syndromes that cause repercussions in the oral frenula: Ehlerdanlos syndrome, Pallister-Hall syndrome, Opitz syndrome, Oral-facial-digital syndrome, Pediatric hypertrophic pyloric stenosis, Ellis-van Creveld syndrome, Holoprosencephaly; there are also non-syndromic conditions. Clinical examination must include the inspection and palpation integrated by the pull of the lips and the verification of the tongue protrusion capacity. Consequences of the upper and lower lip frenula are described as well as the relative surgical procedures (frenotomy and frenectomy).

The frenula of the oral cavity are natural anatomical elements consisting of a triangular fold of mucous membrane, which extends in the sagittal direction from the gingiva in the submucosa of the lip, cheeks and tongue. The frenula are divided into congenital, present at birth, and acquired, consisting of bridles or cicatricial retractions due to iatrogenic interventions.

Depending on their position, the frenula may be: median (upper and lower central incisors level); lateral (in the vestibular region at the level of the upper and lower premolars) or lingual (linking the ventral surface of the tongue to the floor of the mouth). Histologically, the oral frenula consist of a fibrous tract formed by collagen fibers placed in bands associated with thin perivascular nerve structures. These structures are coated by a stratified squamous epithelium. It was believed that the frenula essentially consist of muscle fibers with contractile capacity arising under the depressor muscle and the orbicularis muscle of the lips. However, the studies conducted by Henry et al. (1976), who examined, through biopsy, eleven labial frenula classified as abnormal and three normal frenula of autopsy reports, demonstrated the absence of muscle tissue and that there were no histological differences between normal and abnormal labial frenula.

The upper labial frenum is developed in the third month of fetal life. It consists of a band of fibrous tissue that initiates in the palatine papilla and, through the alveolar process of the maxilla, goes into the inner part of the upper lip, taking the

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Corresponding Author:
Dr. Silvia Caruso, D.D.S., Ph.D.
Department of Life, Health and Environmental Sciences
University of L'Aquila
Square Salvatore Tommasi, 1
67100 Coppito (Aq), Italy

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name of the maxillo-labial frenum. This type of insertion and the size of the frenum are such that the maxillary appears divided into two symmetrical parts. At birth, after the development in height of the alveolar processes, the recession of the frenum from its papillary attack begins, resulting in the welding of the maxillary processes. When deciduous incisors appear, the detachment of the papilla is completed and the frenum tends to retract in the apical direction and to atrophy. Finally, after the birth of the permanent central incisors, the final retraction and atrophy of the frenum take place. In 98% of cases, central incisors erupt separated from the median intermaxillary suture, presenting a diastema that will decrease later only with the development of the lateral incisors and canines. In case of retained canine there can be problems for this diastema. The presence of an inter-incisive diastema is therefore physiological in primary school children up to 11-12 years of age (1). Failure of apical migration of the frenula insertions involves the permanence of a band of fibrous tissue, which is interposed between the central incisors. The fibrous tissue can create problems in flap surgery such as maxillary sinus lift (2). In the fibrous connective tissue of the periodontal ligament, there are some bone thalamic fibers, having a similar structure to that of the elastic fibers (3). Their position follows the directions of trans-septal fibers, perpendicular to the oblique fibers and outside the ligament, the same direction as those fibers associated with the occurrence of peri-implantitis (4). After an ultrastructural examination, these fibers appear to be constituted by fibrils, surrounded by amorphous material, placed parallel to the longitudinal axis of the fiber, and they become thicker by increasing in volume as a result of abnormal mechanical stimuli. The fibers of this type attach to the alveolar bone and intersect between the fibers of the bone, forming a border that is often recognizable with accurate radiographic investigations used for the positioning of the fixtures (5-7). The epithelial tissue that makes up the frenum is orthokeratinized, stratified and squamous and without any expansion to the underlying connective tissue. Connective tissue is mainly composed of collagen fibers with a meandering course that starts from the attached gingiva and goes inside the body of the frenum. In addition, there are some areas of connective tissue woven with a similar tissue from the labial mucosa. Some fat cells arranged in clusters are mainly in the areas of strewn connective tissue. The connective tissue mainly consists of elastic fibers arranged in dense parallel bands, which run through the frenum for its entire length, originating from the periosteum and passing above the alveolar processes. The elastic fibers also constitute the wall of the blood vessels of greater caliber. In the body of the frenum, in some cases, the salivary glands may be present.

Recent epidemiologic studies (8, 9) reported that the anatomical variations of the lingual frenum might be justified by the persistence of sublingual tissue in the midline that did not undergo apoptosis during embryonic development. The lingual frenum shows the same constitution as the oral mucosa; however, the presence is also reported of bundles of skeletal muscle fibers and high density of type 1 collagen fibers in the short frenum and in the frenum with ankyloglossia.

Syndromes associated with alterations of oral frenula

Some syndromes which are cited below can cause repercussions in the oral frenula.

Ehlerdanlos syndrome is a hereditary disease of the connective tissue characterized by hyperlaxity, mild skin hyperextensibility, tissue fragility and extra-muscular-skeletal symptoms. It can determine the absence of the lower labial and lingual frenum (10).

Pallister-Hall syndrome is a dominant autosomal pleiotropic malformation disease with hypothalamic hamartoma, pituitary dysfunction, bifid epiglottis, polydactyly, and, rarely, kidney abnormalities and urogenital malformations. The oral manifestations include micrognathia, microglossia and an abnormal frenum extending from the buccal mucosa to the alveolar ridge (11).

Opitz syndrome is a congenital condition characterized by the association of various anomalies: craniofacial (including: ocular hypertelorism, broad nasal bridge, cracking of the palate), genitourinary with hypospadias, malformations of the larynx,

pharynx, and/or trachea that can cause difficulty in swallowing and breathing. They can also be present in the heart malformations and mental retardation. Regarding the frenum, this syndrome shows the same anomalies similar to those of Pallister-Hall syndrome (12).

Oral-facial-digital syndrome is a rare neurodevelopmental disorder of the ciliopathies group. It is lethal in males and it is characterized by several anomalies: external defects (craniofacial and fingers) and possible involvement of the central nervous system and internal organs (kidney, pancreas and ovaries) in females. It determines the presence of a hypertrophic lingual frenum that is not fully differentiated from the floor of the mouth (13).

Pediatric hypertrophic pyloric stenosis is characterized by the obstruction of unknown mechanical nature of the pylorus, i.e. the valve located between the stomach and the duodenum. Also determines the absence or hypoplasia of the mandibular frenum that is an important diagnostic tool in the detection of this disease (14).

Holoprosencephaly is an autosomal dominant condition characterized by brain malformation that causes cyclopia, single nostril, single central incisor and premaxillary agenesis, absence of maxillary labial frenum (13).

Ellis-van Creveld syndrome is an associated achondroplasia with alterations of the development of the skin, hair and teeth, polydactyly and cardiac septal defects. The fusion of the medial portion of the upper jaw to the gingival margin lip with the elimination of the maxillary labial vestibule, or the presence of numerous frenula that bind the upper lip to the gum represent significant findings of the syndrome. In addition, an abnormal attachment of the frenum in the mandibular labial vestibule has been noticed (6)

In addition, there are other conditions that may cause alterations in oral frenula, although not always, like drug hypertrophy (15, 16). For example, the ankylosis of the upper labial frenum may show a characteristic of familiarity, such as other diseases like central giant cell granuloma. Moreover, there may be acquired frenula consisting of bridles or cicatricial retractions due to secondary abnormal healing after surgical and/or periodontal techniques particularly difficult, or wrong thermal, chemical, electrical burns, used for example for the treatment of periodontitis.

Clinical examination

Clinical examination should be run through the inspection and palpation, and integrated by the pull of the lips and the verification of the tongue protrusion capacity. In general, these analyses are a part of the clinical screening of patients before the beginning of a functional interceptive orthodontic treatment, or temporo-mandibular disorders prevention. The aspects to analyze are: the insertion level, the length, the consistency (soft or fibrous), the type of insertion (single or unraveled), the degree of mobility of the tongue and lips, the amount of attached gingiva, and the ischemia of the papilla at the lip stretching. In addition, a radiographic examination allows to investigate the presence of any disorder underlying the frenum that may justify any diastema (tooth, both the normal range or supernumerary, odontogenic tumors). In these cases orthopantomographs, or three-dimensional radiographic reconstructions as those used, for example, for the temporary anchorage devices in orthodontics are indicated. In addition, UV ray images at the interincisal septal bone could reveal the presence of the trans-septal fibers that run parallel within the median palatine suture (in cases in which there is a permanent diastema from tectum-labial frenum, the deepest fibers of which cross the intermaxillary suture, fitting on the side of the interincisal palatal papilla. Many classifications have been proposed over the last few years which provide four basic types of attachment: mucosal frenum (when frenulum inserts the top of the mucous membrane and not on the gums); gingival frenum (when frenulum fits in the attached gingiva a bit lower); papillary frenum (when frenulum fits in the highest part of the gingiva which is called “gingival papilla”, then even lower); piercing frenum or tectum-labial (when frenulum fits tenaciously to the gingival papilla and also in the gingiva of the palate; it is usually very fibrous with a broad base). The frequency of the various types of frenula is shown in Table II.

From an anatomical point of view, pathological frenula are distinguished in four forms: fan-shaped plica insertion on the lip; fan-shaped plica in interincisal space; fan-shaped plica shaped both on the lip and in the interincisal space; and extended frenum with fat tissue. Among all these types, the most common pathological situation is an interincisal frenum with papillary or trans-papillary insertion, in correspondence of a median diastema and recessions treatable with conservative therapy (17, 18). However, it is now certain that no frenum may be called into account for the presence of a diastema before having considered all other possible causes.

Consequences of the upper and lower lip frenula

In the early years of the 1900's, it was thought that the presence of the frenum might determine the presence of a diastema. Angle in 1907 (19) claimed the correlation between the upper labial frenum and interincisal diastema. For these reasons, the orthodontic treatment was preceded by a frenectomy intervention. Later, there were some different opinions regarding this point. Meenakshi (20) pointed out that at the age of 6, the frenum is normal in 98% of children and at the age of 12 it is normal in only 7% of cases. In 1937, Broadbent described the various phases of normal eruption, resulting, in most cases, in a spontaneous correction of interincisal diastema (21).

In 1953, Ceremello highlighted that the presence of the frenum is not connected with either the presence or with the width of the diastema (22). Therefore, it is fundamental to assess other potential causes of the diastema such as bad habits, dysgnathia, agenesis, supernumerary tooth, usually adopted to establish the orthodontic treatment needed. After this, the frenum is a possible etiological factor of the diastema. There are many controversies about the role of the morphology of the trans-septal fibers in orthodontic treatment, mostly regarding the results and the incidence of orthodontic relapses. These fibers, for their contracting ability, contribute to the closure of a diastema; however, the trans-septal fiber chain can be interrupted: if inter-radicular space is large and the suture is not yet closed; if the fibers can penetrate squarely within the bony depression; and if the interruption of the fibers can be associated to a wide frenum.

Table I. *Frequency of types of attachment of the upper and lower frenula.*

Attachment	Upper Frenum	Lower Frenum
Mucosal	46.%	92.1%
Gingival	34.3%	6.5%
Papillary	3.1%	0.2%
Piercing Papillary	16.1%	1.2%

According to the literature (23, 24), there is a distinction between "simple" frenum, in which the fibers have a continuous course, and "permanent" frenum, in which trans-septal fibers bend squarely in order to gather along the palatal suture. In the first case, orthodontic treatment is ideal, while in the second case orthodontic treatment must be preceded by surgical resection of the fibers. A strong correlation between pathological frenum and diastema relapse after orthodontic treatment, so it is possible to detect a recurrence of 63.4% of patients that were not treated with surgery and recurrence of 6.4% of patients that were treated with frenectomy. Ceremello argues that the removal of the frenum should always be postponed to orthodontic treatment and eruption of the canines, as there are no forces that tend to reduce the interincisal diastema, and therefore the possibility of its spontaneous healing are drastically reduced.

Periodontal consequences

Periodontal and perimplantitis implications of frenula involve the lower median frenum and frenula in general as causes of inflammation, following a tension and retraction mechanism at the gingival margin known as pull syndrome (i.e. an ischemia of the area corresponding to the papillary attachment or a traction of the papilla) (25). Conducted studies demonstrated that the presence of the frenum is not sufficient to determine the accumulation of plaque and therefore the inflammatory phenomenon (26). In these cases, other factors should be investigated, such as smoking habit, as being responsible for periodontal disease.

In fact, an accurate hygiene and the removal of

local irritants is sufficient to reduce inflammatory signs and to arrest or even lead to a reduction of recessions, as proved in periodontal patients (27). Despite recent studies attributing to the frenum only a contributing factor role in the genesis of recessions, it must be considered that the marginal insertion of the frenum might cause tissue stability problems.

Prosthetic consequences

The presence of labial frenula might interfere with the marginal seal mechanisms (28) or create abnormal muscular forces to during clenching, that could have a role in the maintaining of a prosthesis during chewing and phonation. Frequently, in a partial or total edentulous patient, it may occur that, due to the resorption of the alveolar process, the frenum is placed in the ridge, interfering with impression materials and the prosthetic stability. In these situations optical impressions are very useful (29-32); the dentist will decide whether to change the prosthesis or to surgically remove the frenum.

Breastfeeding and tooth decay consequences

In nursing infants, possible interference during breastfeeding has been documented when there is a very large frenum (33). Moreover, if there are frenula with insertions in the free or marginal gingiva, it was hypothesized that the presence of frenula might be an obstacle to oral hygiene procedures and therefore can predispose to a higher risk of developing vestibular cervical caries (34). In fact, it has been observed that movements of the lips cause the frenum to pull on the

Table II. *Description of criteria for defining tongue ankylosis.*

Year	Author	Description of criteria
1982	Jorgenson et al.	Lack of tongue protrusion due to the frenum, frenum extends to papillated surface of tongue, tongue frenum fissures tip during movements.
1990	Fleiss et al.	Tongue tip cannot reach top of gums; tongue tip cannot swing from one corner of mouth to the other; when notching displays tongue protruded; tongue cannot be protruded beyond lower gum.
1990	Notestine	Frenum <1 cm in length; heart-shaped tongue when protruded; when tight feeling finger placed under tongue along the midline; tongue cannot reach gum line when protruded. Marmet et al. (1990): Inability to bring tongue over lower gum ridge; normal breastfeeding sucking motion inhibited; heart-shaped tongue when protruded.
1992	Harris	Short, thick and fibrous frenum.
1996	Masaitis and Kaempf	Maternal nipple trauma; heart-shaped tongue when protruded; inability to bring tongue over lower gum ridge.
2000	Messner and Lalakea	Frenum abnormally short; decreased mobility of tongue tip. Messner et al. (2000): short frenum
2002	Ballard et al.	appearance: 1. raised tongue, 2. frenum elasticity, 3. length of frenum with raised tongue, 4. Upper insertion point, 5. lower insertion point. Feature: 1. lateralization, 2. elevation, 3. protrusion, 4. expansion, 5. cupping, 6. wave motion, 7. flick.
2004	Griffiths	Frenum thick; heart-shaped tongue when protruded
2005	Hogan et al.	Frenum extending along 25-100% of tongue's total length.

fibers inserting into the free marginal tissue, which in turn, can lead to food and plaque accumulation (35).

Ankyloglossia

Ankyloglossia is a congenital oral anomaly that may decrease mobility of the tongue tip and is caused by an unusually short, thick lingual frenum, a membrane connecting the underside of the tongue to the floor of the mouth (36). There is no known etiology for this structural alteration of the tongue, however, there is a greater frequency in men and it is estimated that the percentage of prevalence is between 4-10.7% of the world population. The evaluation of the lingual frenum plays a fundamental role in the field of pedodontics with regard to the impact on oral motor function, the correct articulation of words and on the development of maxillary bone structures, with consequences on teeth occlusion, breathing and, as previously observed in literature, on body posture. In particular, the alterations of the frenum of the tongue may have consequences in the field of dentistry, speech therapy, pediatrics. There are no clear indications for the exact timing to perform a curative surgery because of lack of anatomical and diagnostic globally-accepted criteria regarding the restriction of the frenum and consequences of breastfeeding, growth and motor functions. The ankyloglossia is present at birth and it can be intercepted through a clinical evaluation and intraoral physical examination (37, 38). There are two different types: partial, when the short frenum causes a partial limitation of tongue movements, and total when there is a fusion between the floor of the mouth and tongue with almost complete limitation of movement of the tongue. The criteria for the diagnosis of ankyloglossia have been proposed by several authors. In general, the diagnostic criteria include the presence of a short frenum and decreased lingual mobility (some of these are described in Table II). The shortness of the frenum causes a reduction of the lingual movement or ankyloglossia and it is classified by distinguishing four grades based on Kotlow's classification: class I: mild ankyloglossia (12-16 mm); class II: moderate ankyloglossia (8-12 mm), class III: severe ankyloglossia (4-8 mm), class IV: complete ankyloglossia (<4 mm) (39). The consequences of ankyloglossia include difficulty in nursing infants to assume milk correctly (40). Nipple pain is reported between 60-80% in nursing mothers in the postpartum period. Usually this pain passes in 14 days, but persistent pain is reported in a percentage ranging from 36-80%. This pain is reported in a higher percentage in mothers with children affected of ankyloglossia than children with normal tongue. It was demonstrated that the presence of a short lingual frenum may cause difficulties in swallowing. Moreover, the literature data indicate that the frenotomy procedures are able to determine an improvement in breastfeeding and a resolution of nipple pain. The impact at logopedic level is illustrated by Messner and Lalakea who showed correlations between ankyloglossia and difficulty in pronunciation of the following phonemes: / S /, / z /, / t /, / d /, / l /, / j /, / zh /, / ch /, / th /, / dg /, besides difficulty in pronouncing "r". The frenum surgical therapy showed an improvement of language, particularly in combination with speech therapy lessons. However, literature shows that not all children affected of ankyloglossia suffer from language disorders. The repercussions on the malocclusion are represented by the possible formation of an anterior open bite, formation of spaces in the mandibular incisors and a decrease in the diameter of the upper jaw with the possible occurrence of cross-bite and mandibular asymmetry in growing patients. In addition, it was observed that as the grade of tongue-tie increased, its association with Classes I and II malocclusion decreased and that there is an association with skeletal class III. The mechanism is explained by the abnormal thrust of the tongue on the mandible and not on the lingual spot at the level of the lingual retro-incisor papillae, which influence the development and growing process of the mandible and the Temporo-mandibular joint (41, 42). Periodontal effects include a possible gingival recession localized on the lingual aspect of the lower incisors, that results as not being caused by other factors, such as a dental cross-bite (43). The therapeutic strategy is to control bacterial biofilm and, only in the event of persistence of the problem, in the surgical procedure of elimination of the frenum.

Surgical procedures: frenotomy and frenectomy

The literature shows that the appropriate timing for the execution of the surgical procedure is variable, depending on individual factors, considering the severity and extent of the frenum and in particular on the consequences that the frenum has determined and may determine. For the upper labial frenum, the patient should wait for the eruption of the upper canines. For other frenula, a specialist evaluation to assess surgery is indicated after six years of age, because before this age, spontaneous regressive phenomena leading to its atrophy are usually present. An earlier assessment may be required if there are severe alterations in the language due to the lingual frenum and if there is a fusion between lingual raphe and mouth floor. Surgery can be carried out at 4-5 years of age, in collaborating patients without dental fear, or by the adoption of conscious sedation techniques, or particular types of anesthesia, in particular when there are obvious difficulties in the language. Otherwise, it is better to wait for the age between 8 and 10 years to achieve greater compliance and in order to better assess any complications to be treated in collaboration with orthodontics. Pathological frenula cannot run into changes, so a timely interception and treatment is important. The frenotomy consists in cutting the frenum, with disconnection of the frenum on one of its sides to eliminate the tension forces exerted by the frenum itself. This is a partial removal of the frenum and its apical repositioning. Recurrence leading to revision occurs with rates of 3% to 13%. Frenectomy consists of complete excision of the frenum from the apex of its attachment to its base, including the deep periosteal insertions (44). These two techniques can be performed with the traditional surgical technique with a scalpel or other instruments such as the electrosurgical unit and laser. Complications of surgery are tongue bites, hemorrhage and infections. The surgical technique requires anesthetic infiltration although it is reported that laser techniques require a lower dose of anesthetic (45). The infiltration with a local anesthetic must be limited to avoid a reduction of the display of the anatomy of the frenum (46). Regarding the labial frenum, the lip should be turned outside in order to clearly display insertions. Furthermore, it is important to maintain a hemostatic control: aspiration is to be limited in favor of compression with saline wet gauze in order to reduce bleeding and obtain a good operative vision. The surgical technique must also consider insertion of the frenum (47). Upper labial frenum with vestibular insertion: the frenum is picked off at the insertion on the alveolar process and lip-insertion. It is removed with a scalpel, surgical scissors or laser, and at this point it forms a diamond-shaped wound. Muscle insertions at the bottom of the wound are removed; preserving the periosteum and the wound margins can be undermined with scissor opening movements to facilitate wound closure. Upper labial frenum with palatal insertion: two insertions are performed. At the palatal insertion, a horizontal incision is performed and the frenum is removed, separating it from the periosteum in the direction of the vestibule. Suture is then performed. Frenectomy of the upper lip with a z plastic consists of the elimination of the frenum and the execution of two parallel oblique incisions between them, with the angle at 60 degrees from the center line, drawing a Z. The flaps from the lower floors are separated and transposed by closing the wound by primary intention and deepening the fornix. Frenectomy with vestibuloplasty: two oblique V-shaped incisions are performed upwards, around the frenum, partial thickness. Supraperiosteal dissection is performed, the frenum is eliminated and the mucosa of the lip at the level of the deepest point of the vestibule is sutured to the periosteum, just below the nasal spine. This technique is used for hypertrophic frenula and to deepen the vestibule; healing will take place by secondary intention. Removal of the lower lip frenum: the operational techniques of frenotomy and frenectomy are the same of those for the upper lip. Usually it is better to match the vestibuloplasty in order to increase the keratinized gingival band. If the keratinized gingiva is absent a gingival graft after surgery to remove the frenum is recommended.

Surgery of the lingual frenum: surgery must consider the proximity of the anatomical structures that are not to be injured, in particular, sublingual caruncle, nerve branches of the lingual nerve, Wharton's duct opening. Surgery starts with

anesthesia infiltration and exposure of the lingual frenum, holding the tip of the tongue with gauze. Then, in the case of frenotomy there is the cut of the frenum, or in the case of frenectomy, there is the complete removal of the frenum, by pinching the ends of the frenum with hemostats. It is recommended to control bleeding with gauze and saline rather than with a surgical aspirator. At the end of the surgery, a rhomboid wound remains. In the frenotomy intervention, suture is generally not performed to avoid stretching forces. In the frenectomy with traditional techniques (scissors and scalpels), the sutures must be absorbable, undermining the lateral margins of the wound and separating the muscle layers from the mucous ones. After surgery of the lingual frenum, in order to reduce the chances of recurrence and the development of stretching forces, myo-functional exercises can be done (48-50).

The prescription of lingual myotherapy exercises, which consist of repeated movements of the tongue, prescribed before and immediately after surgery, seem to improve the lingual mobility, while reducing the stretching forces and reducing the recurrence; patient compliance has to be high to achieve the expected results. In addition, exercises to improve the language, when needed, are recommended after surgery.

Advantages of laser techniques

The laser allows to make precise cuts in conjunction with a more or less marked action of hemostasis by ensuring better visibility, depending on its optical affinity with the target tissue (hemoglobin and water). The laser technique also involves the use of fewer instruments, a reduced amount of anesthetic, and possibly the absence of post-operative suture. Healing occurs in the first 7-10 days after surgery, and after 15-20 days the laser surgical wound appears quite comparable to that obtainable by primary intention, with the added benefit that it does not show any signs of scar retraction, often seen in areas healed after being sutured. All these requirements are necessary to obtain a post-operative course free from infectious and inflammatory complications, and fast healing tissue, with minimal discomfort for the patient and resulting in lower use of analgesic medicines. In oral surgery several type of lasers have been used: Argon (514 nm), diodo laser (810-930 nm), Nd:Yag (1064 nm), Erbium (2780-2940 nm), CO2 (10600 nm).

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FACIAL SKELETAL ALTERATIONS IN MOUTH BREATHING PAEDIATRIC PATIENTS: CEPHALOMETRIC EVALUATIONS

G. CECCANTI[1], S. CARUSO[2], M. PASINI[1], M. R. GIUCA[1], L. LARDANI[2] and M. SEVERINO[2]

[1]Unit of Dentistry and Oral Surgery, University of Pisa, Italy; [2]Department of Life, Health and Environmental Sciences, University of L'Aquila, Italy

The morphogenesis of the dento-maxillofacial district is related to epigenetic factors and intrinsic genetic factors, and to local and general environmental factors, which interfere with the neuromuscular balance. Among the several local environmental factors that can interfere with a harmonious and balanced craniofacial growth, there are spoiled habits, such as mouth breathing. Mouth breathing represents the most serious condition, since not only plays a fundamental role in the development of the entire dento-maxillofacial apparatus, but also lead to negative consequences at the systemic level as far as pulmonary, circulatory, nervous, endocrine and digestive functions. The orthodontist plays an important role in the diagnosis, since the most significant symptoms are of orthodontic nature such as, for example, the alteration of the shape of the dental arches, of occlusion, and growth vectors of the maxillary. The aim of this study is to determine whether the tendency to develop a condition of skeletal hyper-divergence with high vertical anterior dimensions of the middle and lower facial third, are related to the degree of patency in the nasopharyngeal canal. Cephalometric data from 50 mouth-breathing subjects and 30 prevalent nasal-breathing subjects were analyzed, looking for a correlation between the degree of skeletal divergence of the jaw (the FMA angle in particular) and the degree of patency of the nasopharyngeal canal (AD-PTV). Data confirmed the correlation between facial morphology and the reduction of the nasopharyngeal space in mouth breathing subjects. The correlation between skeletal class II malocclusion and the reduction of the sagittal diameters of the rhino-pharyngeal canal wasn't relevant. Finally, although the table of percentages shows an increase in the diameters of the upper airways in older subjects, demonstrating the physiological atrophy during the growth, this correlation is not statistically significant. This study demonstrated a significant correlation between small nasopharyngeal canal size and facial skeletal hyper-divergence was also demonstrated.

Literature clearly shows how nasal or mouth breathing plays a fundamental role in craniofacial development (1-3) and oral health status, with caries (4), white spots with hypo-mineralization (5) and periodontal diseases (6) similar to those observed in other systemic diseases as, for example, in adolescents with diabetes (7) or in smoking patients (8). This influence is evident in the case of mouth breathing patients with the characteristic facial morphology, the so-called "adenoid facies" (9).

In literature, it was also associated also to snoring and atopic state, dysfunctional characteristics of

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Corresponding author:
Dr. Silvia Caruso, D.D.S., Ph.D.
Department of Life, Health and Environmental Sciences,
University of L'Aquila,
Piazza Salvatore Tommasi 1,
67100 Coppito (Aq), Italy

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visual defects, and with skeletal maturation (10-13). A particular "adenoid facie" is also observed in some syndromic cranio-facial anomalies (14).

Dental treatments in children with mouth breathing are also more difficult, and should be performed by expert clinicians, and with appropriate technique to control pain because of children's difficulty to breath with nose, as some cases of dental fair and anxiety could be associated to their mouth breath condition (15-19). In addition, some temporo-mandibular diseases in young adults could be related to a particular facial morphology characterized by palatal diameter reduction and mouth breathing (1, 20, 21). For all these reasons the presence of mouth breathing is often associated to a high grade of orthodontic treatment need and is considered a major problem in orthodontics (1, 22). Thus, interceptive orthodontic treatments should be directed to correct this aspect. Treatments for palatal expansion can influence the facial morphology and the oropharyngeal space, as well as other functional appliances, and modified monobloc functional appliance are proposed to manage obstructive sleep apnea in children (23-27). In clinical practice, however, some subjects show present an "adenoid facies", but not an evident mouth breathing. We then asked ourselves whether the tendency to develop a condition of skeletal hyper-divergence and a high vertical dimension of the middle and lower facial third could be related to the greater or lesser degree of patency of the nasopharyngeal canal, and, ultimately to problems of posture (28-30).

The skeletal hyper-divergence and a high vertical dimension of the middle and lower facial third are associated to hypotonic orbicular muscles in children, and to abnormal activations of neck muscles during clenching (31-33).

In order to verify this hypothesis, we analyzed the data of our study sample (group 1=50 mouth breathing subjects), looking for a correlation between the degree of skeletal divergence of the jaw (in particular, the FMA angle) (30), adopted to evaluate the results of functional treatments, and the degree of patency of the nasopharyngeal canal (AD-PTV). We also studied the relationship of the latter with the skeletal class (ANB Angle), always related to the group of mouth breathing patients.

The possible association between a determined facial skeletal anomaly (divergence and skeletal class) and a reduction of the nasopharyngeal space (especially in growing patients) is certainly interesting from the diagnostic and prognostic point of view. In order to collect more data to demonstrate it, we compared the results of our study group related to skeletal divergence (FMA and AR-GO-ME), skeletal class (ANB angle and Wits value) and patency of the nasopharyngeal canal (Ptv-AD, SNP-AD1, SNP-AD2) with those of the control group (group0 = 30 nasal breathing subjects). Many authors in literature demonstrated the correlation between nasopharyngeal space reduction and mouth breathing (3, 34).

Since we believe that the cephalometric evaluations can be helpful for the diagnosis and possible therapy of adeno-tonsillar hypertrophy and knowing that these lymphatic structures can undergo changes also in a volumetric sense during the growth period (for example, the physiological atrophy of the adenoid tonsils), we used the data to verify the variations of the nasopharyngeal size in two different groups of patients (one group from 7 to 10 years old and another group from 10 to 13 years old), comparatively to the average reference values, which are indicated by the authors of the cephalometric techniques for measuring the degree of nasopharyngeal patency used by us. Therefore, in order to evaluate the nasopharyngeal space in the two groups, we used two cephalometric techniques (Linder-Aronson-Henrikson and Ricketts-Schulhof) in order to compare and to verify the reliability of both these methods.

MATERIALS AND METHODS

We examined patients who autonomously came for a dental visit at the pedodontics clinic of the University Dental Clinic of Santa Chiara Hospital in Pisa (June 2012/ December 2013). All the parents of the young patients signed an informed consent and a medical record with questions focused on detecting spoiled oral habits and nasal obstruction problems.

We selected a sample of patients who, after an

intra- and extra-oral physical examination, presented characteristic symptoms of a mouth breathing condition. Among these patients, we excluded those with serious systemic pathologies and syndromes, those who had already performed an orthodontic therapy, those who underwent tonsillectomy and adenectomy. Furthermore, the patients selected were aged between 7 and 13 years old.

Our sample consisted of 50 subjects, 23 males and 27 females. For the aim of the study, we divided the sample into two age groups, the first consisted of subjects aged between 7 and 10 (average age 8.9) and the second one consisted of subjects aged between 10 and 13 (average age 11.5).

Subsequently, we selected, by a randomized method, among all the patients, 30 subjects who had no evident problems of nasal obstruction and who represented the control group. These patients (14 males and 16 females) did not have either serious systemic diseases or syndromes and should have not undergone adenectomy and/or tonsillectomy. The control group was also composed of subjects aged between 7 and 13, subsequently divided into two age groups (average ages of 8.5 and 11.7, respectively), equally to the control group.

For the purpose of our research, all the selected subjects (both from the study group and the control group) were asked to perform skull teleradiograph in latero-lateral projection, on which the cephalometric study was performed. We measured the nasopharyngeal dimension according to two cephalometric techniques, Linder-Aronson and Henrikson technique 9, modified by Schulhof 10 and Ricketts, and Schulhof technique 10. We also measured the skeletal divergence of the mandible compared to the middle third facial and the relationship between the two maxillaries on the sagittal plane (skeletal class).

In order to evaluate these two parameters, we used the cephalometric analysis according to Tweed 11 and through teleradiographies in particular, we detected the amplitude of the FMA angle in order to evaluate the mandibular divergence, the angle of the ANB angle and the Wits index for the skeletal class.

Since we obtained two values for the skeletal class, we decided to evaluate two values for the skeletal divergence so we also observed, from the analysis of Biork and Jarabak, the goniac angle (Ar-Go-Me) that is normally useful to determine the characteristics of mandibular growth in qualitative and quantitative aspects, i.e. the direction and growth potential, but it also contributes to a better definition of facial morphology.

In conditions of skeletal normodivergency of the jaw, the average value of the angle is 25° ± 3°; we consider it hyper-divergence with values higher than 28° and hypo-divergence with values lower than 22°.

We also considered the ANB angle, i.e. the resultant of the difference between the SNA angle and SNB angle. The mean ANB value, indicating skeletal I class ratios between the jaws, is 2° ± 2°; values higher than 4° indicate a skeletal class II, while values below 0° indicate a skeletal class III.

Since an anomalous position of the anterior skull base can invalidate the validity of the ANB value, we took into consideration another cephalometric parameter, highlighted by the Tweed technique: the Wits index expressed by the linear distance (in mm) between points AO and BO, identified by the orthogonal projection respectively of the points A and B on the occlusal plane, defined by the straight line that connects the midpoint of the molar interscupation with the midpoint of the overjet and incisive overbite. The Wits index also indicates the antero-posterior ratios between the two jaws: it has positive values if BO is distal to AO and negative if AO is distal to BO. The mean value is 2 ± 2 mm, which indicates a class I skeletal relationship between the two jaws; values higher than 4 mm indicate a skeletal class II, while values below 0 mm indicate a skeletal class III.

The subjects of the control group and study groups were divided into three subgroups according to the two values related to the skeletal divergence: normodivergent, hypodivergent and hypodivergent. The same thing was done for the two values expressing skeletal class: class I, class II and class III. Subjects were once again subdivided into three more subgroups, according to the dimensions of the nasopharyngeal space: reduced space, normal space and increased space according to the reference values of the cephalometric techniques of the authors we have chosen (Linder- Aronson-Henrikson-Schulhof and Ricketts-Schulhof).

We then compared the data of the values described above of the subjects of the study group with the subjects of the control group. We compared these values within the study group: in particular, we wanted to verify whether

there was any correspondence between the dimensions of the nasopharyngeal space (Ptv-AD) and the skeletal divergence (FMA), and again between the dimensions of the nasopharyngeal space (Ptv -AD) and the skeletal class (ANB).

The last comparison was made among the three measurements of the nasopharyngeal space size values (for Ptv-AD and for SNP-AD1 and SNP-AD2) and between the two subgroups, in order to verify whether there was any correlation between the age increase and a possible increase in upper airway size, justified by the physiological hypertrophy of the adenotonsillar lymphatic complex during growth.The statistical tests used are CHI2 and Fisher's test with p value £0.001.

RESULTS

The study group consisted of 50 mouth breathing subjects (23 males and 27 females) divided into two age groups: the first included patients aged between 7 and 10 years old and the second between 10 and 13 years old. The control group consisted of 30 nasal breathing subjects (20 males and 10 females), also divided into two age groups.

All the subjects (both study and control group) were divided into three subgroups based on the cephalometric values related to the FMA angle: normodivergent (25° ± 3°), hypodivergent (<22°) and hyperdivergent (> 28°).

The same was performed for the goniac angle (ArGoMe): normodivergent (130° ± 5°), hypodivergent (<125°) and hyperdivergent (> 135°). Although the hyperdivergents resulted in higher percentages (FMA 44%; ArGoMe 36%) in the group of mouth breathing subjects compared to nasal breathing subjects (FMA 23%; ArGoMe 20%), the correlation is not statistically significant (both for FMA and for ArGoMe both with 0.25 <P> 0.10) (Table I, II).

All subjects (both those of the study group and those of the control group) were divided into 3 subgroups based on the cephalometric values related to the ANB angle: class I (2° ± 2°), class II (> 4°), class III (<0°) (Table III, IV). The class II mouth breathing subjects were 38%, unlike those of the control group which were 23%.

Table I. *Frequencies of distribution of normal, low and high angle of mandibular divergence in the whole sample.*

	FMA (normal angle)	**FMA (low angle)**	**FMA (high angle)**	**TOT**
Nasal breathing subjects (control group) (n = 30)	18 (60%)	5 (17%)	7 (23%)	30
Mouth breathing subjects (study group) (n = 50)	22 (44%)	6 (12%)	22 (44%)	50
TOT	40	11	29	80

Table II. *Frequencies of distribution of normal, low and high Ar-GoMe angle in the whole sample.*

	Ar-GoMe (normal)	**Ar-GoMe (low)**	**Ar-GoMe (high)**	**TOT**
Nasal breathing subjects (control group) (n = 30)	20 (67%)	4 (13%)	6 (20%)	30
Mouth breathing subjects (study group) (n = 50)	23 (46%)	9 (18%)	18 (36%)	50
TOT	43	13	24	80

We used the same method for Wits measurement: class I (2 ± 2 mm), class II (> 4mm), class III (<0mm). Based on these Wits values, the percentages of classes II in the two groups is the same (30% each). Following these comparisons, the correlation between mouth breathing subjects and a specific skeletal class (both of ANB and Wits) is not significant (Table III, IV).

We compared the linear measurements of the nasopharyngeal space (using the Ptv-AD, SNP-AD1 and SNP-AD2 values) of mouth breathing subjects with the ones of nasal respiratory subjects. The substantial difference was found in patients with nasopharyngeal space reduction (in all three measurements): in fact, in mouth breathing subjects, the percentages of subjects with values lower than the norm were respectively 52%, 48% and 52% compared to those of the control group which were 10% for the three measurements.

Therefore, the statistic correlation is significant with P <0.001, calculated both with Chi2 and with the exact Fischer test (Tables V-VII), demonstrating the close correlation between reduction of the upper airways and FM in mouth breathing.

In order to verify, from a cephalometric point of view, whether the tendency to develop a condition of facial skeletal hyper-divergence is related to the higher or lower degree of patency of the nasopharyngeal canal, we compared the values of mouth breathing subjects related to hyper-divergence (FMA divided into normo, hypo and hyper-divergent) with the values of the same group related to the nasopharyngeal space (Ptv-AD divided into subjects with normal, reduced and increased space).

The frequency table shows that out of a total of 50 patients, 26 subjects had a reduced nasopharyngeal space and 20 of them were hyperdivergent; while, among 30 nasal breathing subjects, 3 of them showed nasopharyngeal space reduction, of which 2 were hyperdivergent. Therefore, the statistic correlation between reduced nasopharyngeal canal patency and facial skeletal hyperdivegence is significant for the study group with P <0.001, calculated both with Chi2 and with the exact Fischer test (Table VIII).

We accepted the research hypothesis, while for

Table III. *Frequencies of distribution of skeletal classes in the whole sample (ANB angle).*

	ANB (class I)	**ANB (class II)**	**ANB (class III)**	**TOT**
Nasal breathing subjects (control group) (n = 30)	21 (70%)	7 (23%)	2 (7%)	30
Mouth breathing subjects (study group) (n = 50)	26 (52%)	19 (38%)	5 (10%)	50
TOT	47	26	7	80

Table IV. *Frequencies of distribution of skeletal classes in the whole sample (Wits index).*

	Wits (class I)	**WITS (class II)**	**WITS (class III)**	**TOT**
Nasal breathing subjects (control group) (n = 30)	19 (63%)	9 (30%)	2 (7%)	30
Mouth breathing subjects (study group) (n = 50)	28 (56%)	15 (30%)	7 (14%)	50
TOT	47	24	9	80

the control group we had to accept the null hypothesis with 0.25 <P> 0.10 (calculated with Chi2 and with the exact Fischer test) (Table IX).

In order to verify, from a cephalometric point of view, the possible existence of a correlation between the skeletal class and the degree of patency of the nasopharyngeal canal, we compared the values of mouth breathing subjects related to the facial skeletal class (ANB divided into 1st class, 2nd class and 3rd class) with values of the same group related to the nasopharyngeal space (Ptv-AD divided into subjects with normal, reduced and increased space).

The frequency table shows that, out of a total of 50 patients, 26 subjects had a reduced nasopharyngeal space and, among them, 42% (11 patients) were hyperdivergent; by operating the same comparison in the group of nasal breathing subjects (30 people), we found out that the subjects with nasopharyngeal space reduction were 3, among which none of them was hyper-divergent. The statistic correlation between the reduced patency of the nasopharyngeal canal and the sagittal skeletal relationship of the two jaws is not very significant for the study group with 0.05 <P> 0.025, calculated both with Chi2 and with the exact Fischer test; with the Yates correction, the P-value improves but it does not go below 0.01. The

Table V. *Frequencies of distribution of data concerning nasal-pharyngeal space in whole sample.*

	Ptv-AD (lower)	**Ptv-AD (normal)**	**Ptv-AD (higher)**	**TOT**
Nasal breathing subjects (control group) (n = 30)	3 (10%)	26 (87%)	1 (3%)	30
Mouth breathing subjects (study group) (n = 50)	26 (52%)	19 (38%)	5 (10%)	50
TOT	29	45	6	80

Table VI. *Frequencies of distribution of data concerning SNP-AD1 in the whole sample.*

	SNP-AD1 (lower)	**SNP-AD1 (normal)**	**SNP-AD1 (higher)**	**TOT**
Nasal breathing subjects (control group) (n = 30)	3 (10%)	26 (87%)	1 (3%)	30
Mouth breathing subjects (study group) (n = 50)	24 (48%)	22 (44%)	4 (8%)	50
TOT	27	48	5	80

Table VII. *Frequencies of distribution of data concerning SNP-AD2 in the whole sample.*

	SNP-AD2 (lower)	**SNP-AD2 (normal)**	**SNP-AD2 (higher)**	**TOT**
Nasal breathing subjects (control group) (n = 30)	3 (10%)	26 (87%)	1 (3%)	30
Mouth breathing subjects (study group) (n = 50)	26 (52%)	22 (44%)	2 (4%)	50
TOT	29	48	3	80

statistic correlation is worse in the control group (0.90 <P> 0.25) (Tab X, XI).

DISCUSSION

This study carried out on 50 subjects, aged between 7 and 13 years old, who were clinically diagnosed with mouth breathing, found a significant reduction in the size of the nasopharyngeal space, evaluated both with the cephalometric technique of Richets and Schulhof (Ptv-AD) and Linder-Aronson and Henrikson (SNP-AD1 and SNP-AD2), compared to 30 subjects, aged between 7-13 years (control group).

Furthermore, there was a significantly positive correlation between the reduction of the nasopharyngeal space and mandibular divergence in mouth breathing subjects; this correlation was wasn't observed in the control group.

Regarding the relationship between the size of the upper airways and the sagittal skeletal position of the jaws, the presente study showed a slightly non-significant correlation in mouth breathing subjects between skeletal class II (ANB) and a small nasopharyngeal space (Ptv-AD).

No significant difference between the study and

Table VIII. *Cross-tabulation between FMA values and Ptv-AD in mouth breathing subjects (n=50).*

	Ptv-AD (lower)	**Ptv-AD (normal)**	**Ptv-AD (higher)**
High FMA	20	2	0
Normal FMA	4	13	5
Lower FMA	2	4	0
TOT	26	19	5

Table IX. *Cross-tabulation between FMA values and Ptv-AD in nasal breathing subjects (n=50).*

	Ptv-AD (lower)	**Ptv-AD (normal)**	**Ptv-AD (higher)**	**TOT**
High FMA	2	5	0	7
Normal FMA	0	17	1	18
Lower FMA	1	4	0	5
TOT	3	26	1	30

Table X. *Cross-tabulation between ANB values and Ptv-AD in mouth breathing subjects (n=50).*

	Ptv-AD (lower)	**Ptv-AD (normal)**	**Ptv-AD (higher)**	**TOT**
class I	10	11	5	29
class II	11	9	0	19
class III	5	0	0	5
TOT	26	19	5	50

the control groups was observed for the skeletal divergence, assessed by measuring the value of FMA and the Gonial angle, differently from what several authors reported in the literature (3, 10, 29, 30).

Our study doesn't confirm a significant increase in the nasopharyngeal space in older subjects (10-13 years) respect to younger patients (7-10 years), justified by the physiological adeno-tonsillar atrophy during growth, although these data are near the level of significance in the group of mouth breathing subjects. It should be noted that in literature it was showed that the orthodontic treatment is not able to change airway space in adolescent (35). So, it could be argued that this influence can be possible only with orthopedic-functional treatment in children.

In addition, an increase in the size of the upper airways in mouth breathing subjects is evident from the frequencies relative to Ptv-AD and SNP-AD1 and SNP-AD2 in subjects with different age.

The fact that many of our experimented correlations were not statistically significant (unlike what was widely reported in the literature) can be attributed to the selection of the subjects of the study group. In fact, regarding the diameters of the nasopharyngeal space measured with Ptv-AD, SNP-AD1 and SNP-AD2, it is necessary to underline that the authors Linder-Aronson and Henrikson, Ricketts and Schulhof of cephalometric techniques found the mean values referred to subjects aged from 6 to 16 years old.

Diversely, in our study, patients are aged from 7 to 10 years old in one group (average age 8.9) and from 10 to 13 years old (average age 11.5) in the other group. In addition, this study was carried out on bi-dimensional radiographic information, while a three-dimensional visualization of the upper dental arch and palatine could improve the morphological analyses of this area, as previously observed.

A deeper analysis in this field should consider also some cephalometric variables describing cervical posture, as palatal expansion was proved to influence breathing and cervical lordosis, and, from a clinical point of view, a check of occlusal functions and Temporomandibular joints. Furthermore, the authors considered observed some differences between males and females, although not statistically significant, differently from other studies in literature, which evidence that some orthodontic problems are differently diffused in males and females and the resolution of oral breathing could reduce future dental problems (1, 24-35). Finally, in the present study, the diagnosis of mouth breathing was carried out only by a clinical evaluation, while specific instrumental examinations could have resulted in an overestimation of the pathognomonic signs.

Mouth breathing is a spoiled habit that interferes with the neuromuscular balance of the young patients and could play a preponderant role as an etiopathogenetic factor of dysgnathia. It is often associated with a more complex clinical picture, called upper airway resistance syndrome. The most severe signs regard the dento-maxillo-facial apparatus, are: the adenoid facies, the reduction of the transverse diameters of dental arches, the retrognathia or mandible, and the vertical growth of the middle and lower third of the face (36-38).

Table XI. *Cross-tabulation between ANB values and Ptv-AD in nasal breathing subjects (n=30).*

	Ptv-AD (lower)	**Ptv-AD (normal)**	**Ptv-AD (higher)**	**TOT**
class I	0	20	1	21
class II	2	5	0	7
class III	1	1	0	2
TOT	3	16	1	30

The orthodontist plays a fundamental role during the diagnosis. It is important to make early diagnosis in order to intercept and prevent malocclusions by trying to identify and eliminate (where possible) all those environmental factors (including chronic mouth breathing) that can alter the balance of craniofacial growth. This study showed the correlation between the reduction of the nasopharyngeal space and the cranio-facial morphology in mouth breathing subjects.

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REGENERATIVE ENDODONTICS PROCEDURE OF AN IMMATURE PERMANENT MANDIBULAR MOLAR WITH A NECROTIC PULP USING BIODENTINE: A 16 MONTHS RADIOGRAPHIC FOLLOW-UP

E. AMBU[1], S. CARUSO[2], R. GATTO[2], S. TECCO[3] and M. SEVERINO[2]

[1]Private practice, Bologna, Italy; [2]Department of Life, Health and Environmental Science, University of L'Aquila, L'Aquila, Italy; [3]Dental School, Vita-Salute San Raffaele University, Milan, Italy

An ideal scenario for a tooth in necrosis with immature root would be to continue root development after the regeneration of pulp tissue. We report a case, where the regenerative endodontic procedure (REP) was done using biodentine as a scaffold in a immature mandibular molar tooth.

Necrosis of an immature first molar is a very bad occurrence since even if treated successfully the affected tooth will have an uncertain long-term survival, eventually calling for further future restorative or prosthetic procedures (1-11). The most common localization of carious lesions is the occlusal portion of the first permanent molars. This occurrence is very well prevented by the correct placing of pits and fissure sealants aside all necessary hygienic protocols and procedures (12-14). Another important role in caries incidence can be played by a reduced salivary flow due to pathologies or drug induced (15, 16).

An ideal scenario for a tooth in necrosis with immature root would be the regenerative endodontic procedure (REP) to continue root development after the regeneration of pulp tissue. Teeth with a necrotic pulp and immature apices are capable of regenerating tissues of pulp-dentin complex and can regain tooth vitality with a proper procedure, made by a proper disinfection and a suitable scaffold. In these cases, the traditional apexification procedure can be a very long procedure. In addition, the use of calcium hydroxide has been shown, both in clinical studies and in experimental studies, to produce weakening and fracture of the coronal part of the roots (17). The benefits of REP may be the root elongation, the apex closure, and the thickening of the tooth walls, to achieve greater resistance to fracture (18). The survival outcomes of immature teeth treated with either REP or apexification methods show a more successful by the REP with a retrospective view, with clinical outcome comparable to the traditional apexification (19, 20). Thus, the process of mineralization of the tooth can resume physiologically (21, 22).

MATERIAL AND METHODS

Radiographic examination of the present case revealed incomplete endodontic treatment with immature root and wide-open apex in the lower right first molar. A periapical radiolucency was also noted around the apices. Based on clinical and radiographic findings, a diagnosis of necrotic pulp and periapical symptomatic periodontitis was confirmed. So, a regenerative endodontic procedure was planned.

Procedure

At the first appointment, the canal was not

Key words: regenerative endodontic procedure, biodentine, immature root

Corresponding author:
Prof. Simona Tecco, D.D.S., Ph.D.,
Dental School Vita-Salute San Raffaele,
Via Olgettina 58,
20132 Milan, Italy
Tel.: +39-329-7838023
e-mail: tecco.simona@unisr.it

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instrumented, but only disinfected with sodium hypochlorite at 5.25%. Then, a sterilization of infected root-canal dentine by topical application of a mixture of ciprofloxacin, metronidazole and minocicline (tri-antibiotic paste: minocycline 100 mg, metronidazole 200 mg, cicloproxacina 500mg) was performed (23, 24).

At the second appointment, after 2-3 weeks, a new conditioning of the dentin was performed, because the smear layer can create unfavourable conditions for the survival, migration, adhesion and proliferation of stem cells from apical papilla (SCAPs) (25, 26). In addition, the exposure of collagen fibres and a demineralization can produce an environment favourable to the cell's proliferation (25). Furthermore, the dentin conditioning causes the liberation of growth factors present in the dentin, as the transforming growth factor-β (TGF-β), the bone morphogenetic protein 2 (BMP2) and angiogenic factors such as platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF) and fibroblast growth factor 2 (FGF2) (25). A bleeding with an endodontic probe was provoked a clot formation (blood clot) was expected.

The blood clot is a physical component which is able to support cell growth and differentiation, and supply signalling molecules and growth factors for the maturation and differentiation of stem cells (27). The clot or the regenerate tissues should not be placed in contact with endodontic cements or reconstruction materials such as amalgam, composite resins (28) or glass-ionomer cements (29). The success of every endodontic therapy is always highly influenced by the biocompatibility and the sealing ability of the used material (30).

An intermediate layer of biocompatible material, such as MTA or calcium hydroxide should be placed coronally for tissue protection (31). The hermetic seal of the environment can be also achieved with Biodentine. Biodentine is generally indicated in root and furcation perforations, internal and/or external resorptions, apexification, and retrograde surgical filling. In the present case, a layer of Biodentine was placed coronally, on the clot, to perform an airtight seal of the environment. The tooth was then monitored by X-ray for 16 months.

RESULTS

The X-ray was taken after 1, 4, 8, 12 and 16 months. Clinically, the tooth was asymptomatic and not sensitive to percussion. After 8 months, a pulp sensibility tests elicited a positive response, which was comparable to the contralateral molar. At this

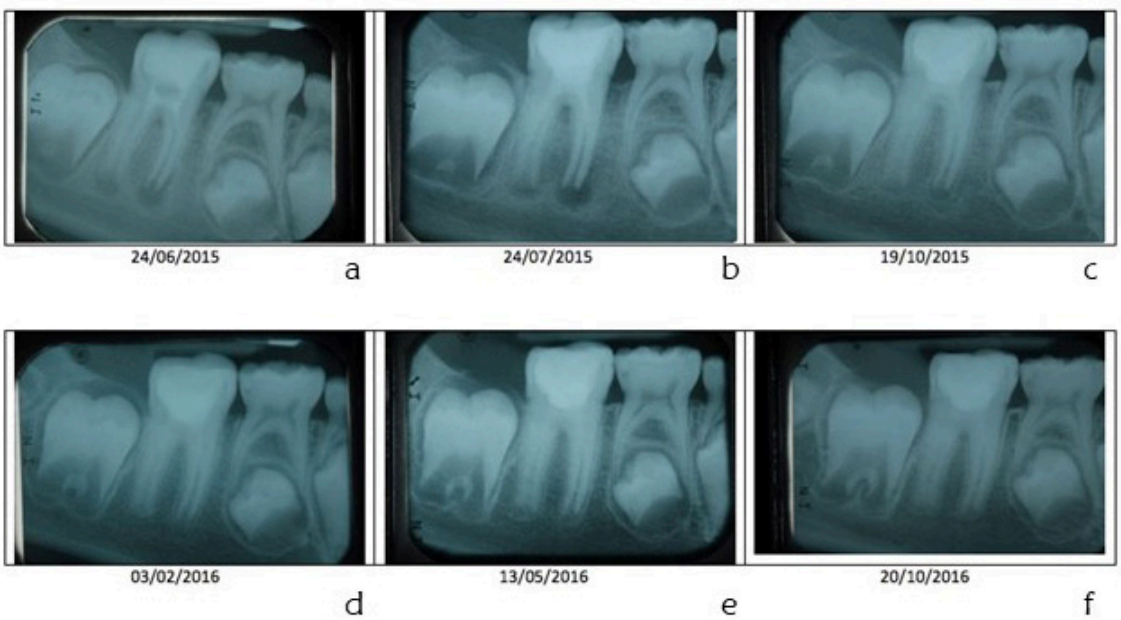

Fig. 1. *Radiographic examination at T0 (**a**) and reviewed at months 1 (**b**); 4 (**c**); 8 (**d**); 12 (**e**); 16 (**f**).*

time, the X-ray examination revealed the complete resolution of the periapical lesion, with thickening of the dentinal walls, root lengthening and apical closure, also showing a continuous lamina dura (Fig. 1).

DISCUSSION

Regenerative endodontics involves biological procedures directed towards replacement of damaged structures of the pulp-dentin complex, in young permanent tooth. As all regenerative procedures, a correct diagnosis and the evaluation of other pathological conditions are fundamental (32-34).

Case reports, including the present study, demonstrate that tooth in necrosis with immature root are capable of regenerating tissues of pulp-dentin complex and can regain tooth vitality with a proper procedure, made by a proper disinfection and a suitable scaffold. The concept of scaffold is very well known in bone regenerative procedures (35). The hidden treasure for regenerative endodontics is in apical papilla, giving the regenerative thrust and the cellular landscape of SCAPs. The limitations of a regenerative endodontics may be a possible discoloration of the crown, the creation of antibiotic-resistant bacterial strains, and allergic reactions (18). To reduce discoloration, it can be eliminated the minocycline. But in cases of mandibular molars this problem is less important, respect to incisors.

For molar and incisor staining some treatments can also be proposed. Some clinical complications can occur during the procedure, such as the absence of bleeding during the second phase. This can be due to the experience of the operator. To avoid this, it is mandatory to use only anaesthetic without vasoconstrictor. (36) In addition, there may be the collapse of the seal given by the MTA layer. In these cases, it may be helpful to use a collagen matrix (31). The present case was followed up clinically and radiographically for 16 months. Although no computed tomography images were taken, the differences in bone density, root length, and lesion size are well detectable through traditional dental x-ray.

The dentin hypersensitivity observed during the follow up was managed with laser desensitizing. Laser devices are of great help in reducing hypersensitivity even if they are mostly indicated for soft tissues surgery (36).

A low number of case-reports are published about REP applied to permanent mandibular molars, as the great part of cases describe data about incisors, and there aren't follow-up x-rays of 16 months from REP.

Future studies should investigate the suitability of these teeth to withstand orthodontic forces, as this treatment is highly indicated for young patients. The data in the literature, in fact, show how orthodontic forces and orthodontic devices can create high loads to teeth and affect even the TMJ functionality (37-44).

The present case confirms that REP is a viable treatment modality for immature mandibular molars with open apices and necrotic pulps, an effective treatment option to save teeth with compromised structural integrity.

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EFFECT OF PRESSURE AND LIGHT CURING OF COMPOSITE MICRO HARDNESS

V. CAMPANELLA[1], G. GALLUSI[1], V. DI TARANTO[1], C. CUGINI[1], R. NARDI[1], A. MEA[1], G. MARZO[2] and A. LIBONATI[3]

[1]Department of Clinical Sciences and Translational Medicine, Dental School, University of Rome "Tor Vergata", Rome, Italy; [2]Department of Life, Health and Environmental Sciences, Dental School, University of L'Aquila, L'Aquila, Italy; [3]Department of Surgical Sciences, Dental School, Catholic University of Our Lady of Good Counsel of Tirane, Tirane, Albania

The aim of this study is to evaluate the micro hardness Vickers of a composite micro hybrid polymerized under constant pressure. Twelve experimental samples were made equally divided into two groups: an experimental group and a control. Enamel plus HRi (Micerium) microbiotic composite resin, UD3 colour, was inserted into a syringe heater (ENA HEAT Composite Heating Conditioner) so that the material could be brought to a temperature of 39°C. A defined amount of composite resin is taken from the syringe with a Heidemann spatula and placed between two slides, previously cleaned with 90 ° alcohol. The samples are then inserted one at a time into a special device for constant pressure application. Vickers hardness measurements were made on the top of surfaces. The mean value of the samples belonging to the experimental group is 56.81 ± 0.71. The mean value of the control samples is 52.02 ± 2. The results obtained allow us to state that applying a constant pressure during the cementation phase of indirect adhesive restorations allows to obtain better mechanical characteristics of the composite used as a cementing agent.

The cementation phase of indirect restorations in composite resin or in ceramic material involves the use of a thin layer of composite material, as stable highly loaded and sliding material, as a cementing agent. The use of resinous cements is crucial for the longevity of the indirect aesthetic restoration. Compared with conventional cements, they increase retention, determining a lower incidence of micro-fractures, minimal solubility, biocompatibility, temporomandibular (1-3) and oral diseases (4-9), and are useful to control dentinal sensibility. On the other hand, however, these require pre-treatment of the dentinal surface to modify or remove the smear layer, and numerous steps to create a uniform hybrid layer. The validity of the adhesive cementation is strictly correlated to the characteristics of the substrate, as different substrates can be observed in the presence of caries (10, 11), or a previous metal restoration, or in cases of tooth with enamel hypo mineralization, or in cases of periodontal diseases (12-16), or in case of orthodontic treatments (17, 18) . It also depends on the physical-mechanical properties of the cement, to the effectiveness of the adhesive techniques and to the position of the margin. Contamination by oral fluids significantly compromises the adhesion of the composite on the dental substrate: it is therefore essential that the cementation step is carried out by performing the isolation of the dental elements with the rubber dam. The cementation of indirect restorations can be performed either with exclusively light-cured materials or with dual-cured materials, which are therefore self-curing and light-curing (19). An analysis of the literature highlights the advantages and disadvantages of each class of materials.

Key words: micro hybrid composite, pressure, polymerization, hardness, Vickers hardness test

Corresponding author:
Prof. Vincenzo Campanella,
Department of Clinical Sciences and Translational Medicine,
Dental School, University of Rome "Tor Vergata",
Via Montpellier 1, 00133 Rome, Italy
e-mail: vincenzo.campanella@uniroma2.it

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The good chemical and micromechanical adhesion of the material to the mineralized tissues of the tooth allows to have more conservative dental preparations and generally little retentive in mechanical terms and less periodontal and oral disease (20-23). Numerous clinical protocols suggest to proceeding with the cementation of indirect restorations (24, 25) by exerting a constant pressure during the polymerization phase of the cementing agent, with the finger or with manual instruments.

The behaviour of cements and composite resins is reported in the literature in terms of marginal adaptation and adhesion values to the enamel-dentinal tissue, when subjected to this pressure (26). In fact, it has been demonstrated that subjecting the inlays to constant pressure improves adhesion (25). The pressure allows excess cement to flow along the prepared walls, avoiding the formation of additional material thicknesses, which would prevent the correct restoration of the restoration inside the cavity; the pressure maintained in a constant way leads to counteract the "plunger effect", which follows the compression of the composite and would determine an upward push of the restoration (27). Furthermore, the applied pressure can reduce the formation frequency and the porosity extension in the cementing material layer, resulting in a better micro-adaptation of the cement to the cavity walls (28).

Moreover, recent studies have shown a greater formation of weak intermolecular interactions (hydrogen bonds, Van der Waals forces, ionic exchanges) between the cement and the adhesive surface, which can therefore increase the retention of the restoration (29). The pressed material, despite the c-factor remains adhered to the cavity walls and the contraction can also be exploited to the advantage of the clinician promoting a better marginal adaptation of the restoration. Recent studies have observed that hardness, as well as other mechanical properties, is a measure of the extent of polymerization of a composite (30).

The behaviour, both physical and mechanical, and the stability over time of the resin materials are closely related to the degree of conversion of the monomer into polymer, due to the extension of polymerization and therefore to the amount of double bonds, in methacrylate groups, engaged in the polymerization process (31, 32). As the degree of polymerization increases, the matrix hardness, flexural strength, fracture, compression, tensile, wear and creep resistance are defined as deformation following a constant load (26, 33). The technological advancement, within the mechanical characteristics of composite resins, has therefore made direct and indirect restorations increasingly resistant to masticatory and long-lasting loads, with a clinical behaviour compatible also with parafunctional masticatory loads as in bruxes patients (3), or in cases of great restoration as for example in children after pulpotomies or pulp regeneration. In 2003, and then in 2018, Campanella (25) demonstrated how the "PUP" (Polymerization Under Pressure) permit to overcome the limits shown by the technique of realization of indirect adhesive restorations: the application of constant pressures during the polymerization phase of the composite, used as a cement, allowed, in fact, a complete fitting of the inlay within the prepared cavity, succeeding in obtaining a marginal precision never reached before. This pressure also allowed greater control of polymerization shrinkage observed an increase in the adhesion values and the marginal adaptation of dual-cured composite cements (34). In addition to the validity of the cementation procedures under constant pressure, it has been shown that the increase in application time is able to improve the adhesive properties and the marginal adaptation of the composite cement to the dental tissues. An advantage that can be clinically useful also for controlling dentinal hypersensitivity (26, 29). Although they are parameters in the cementation of indirect restorations, there is no standardization of the intensity and time-application variables of pressure, so much so that in many protocols it is defined as "light" or "practicable with a finger", without its value in Pascal is scientifically defined.

In this regard, whose objective was to evaluate the effects of the application on the experimental samples of two different pressures, one double the other.

The results of the study confirmed a positive effect due to the increase in strength intensity applied during cementation of the restoration, showing an

increase in the adhesion and marginal adaptation values of cement.

The aim of the study is to evaluate and validate the possible correlation between the application of a constant pressure and the increase in the conversion degree of a micro hybrid composite. The degree of conversion, achieved by the material at the end of the light curing, is strictly related to the mechanical properties and, in particular, to the hardness of the resin.

The experimental samples were then subjected to the Vickers hardness test at low load, so that a possible increase in hardness values could be observed, following the application of a pressure during the photopolymerization of the composite material.

MATERIALS AND METHODS

Sample preparation

Twelve experimental samples were made, equally divided into two groups: an experimental group and a control (Fig. 1). Enamel plus HRi (Micerium) microbiotic composite resin, UD3 color, was inserted into a syringe heater (ENA HEAT Composite Heating Conditioner) so that the material could be brought to a temperature of 39 °C; this expedient makes the resin more fluid and reduces the stress in the extrusion phases from the syringe. In addition, the biomechanical characteristics are enhanced by increasing the mobility of resinous monomers and free radicals and reducing the risk of separation between matrix and filler, and the risk of bubble formation is eliminated.

A defined amount of composite resin is taken from the syringe with a Heidemann spatula and placed between two slides, previously cleaned with 90° alcohol. In an in vivo condition, an inlay should be cemented after local anesthesia (35) and isolation of the surgical field with rubber dam. The samples are then inserted one at a time into a special device for constant pressure application.

This device consists of a frame and a metal piston, above which there is a support for positioning the weight (2 kg). The 6 samples belonging to the experimental group are subjected to pressure during the whole polymerization phase (30 sec): through a modified optical fibre tip, in fact, a LED lamp (≈1100mW) is connected to the piston. In this way the pressure will be kept constant by the tip itself and the lamp will be in direct contact with the sample, thus limiting the influence on the degree of conversion of the composite to other parameters, such as the distance

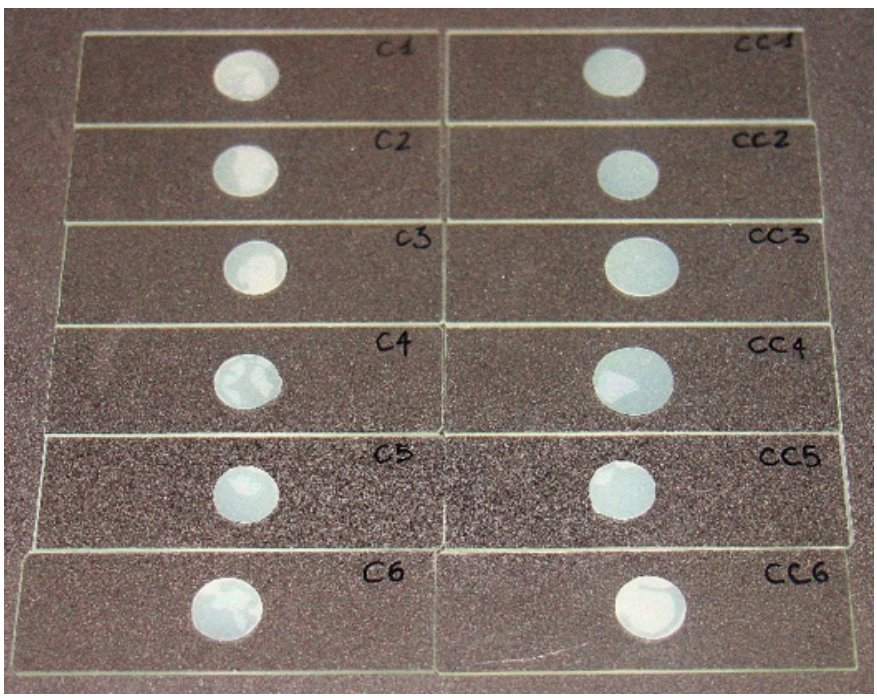

Fig. 1. *Composite resin samples between two slides.*

between composite and light source. The samples of the control group, on the other hand, after being subjected to pressure for 10 seconds, are removed from the device and light-cured without any pressure being applied. From the above procedure, composite discs with a thickness of about 200-250μm were obtained, which were then stored for 24 h. The conservation took place inside containers that, once closed, were able to prevent the penetration of light to limit the influence of a subsequent irradiation of the material on the degree of polymerization.

Vickers test

The tests are carried out using durometers: they are pressed on the sample and, by measuring the size of the impression by the microscope, produced by the penetrator on the material, a value is obtained that expresses the resistance offered to penetration. For the Vickers test a square pyramid-tipped indenter is used; the angle at the vertex has amplitude equal to 136°, so the durometer, meeting the surface of the sample, draws an angle of 22°. The impression obtained is therefore square in shape, symmetrical and easy to read. Any asymmetries, calculable as the difference between the diagonals of the impression, must be less than 5%. Vickers and Knoop tests can be performed using reduced loads: for micro-hardness tests the force used is between 0.0098-9.81 N.

On each sample different indentations are made, and it is necessary that these are positioned correctly, in order to obtain clearly defined impressions without appreciable distortions. Therefore, the distance between the edge of the sample and the centre of the indentation must be at least 2.5 times the half of the two diagonals, while the distance between the centres of different impressions will be at least 2.5 times the diagonal of the impression. The penetration depth is generally equal to 1/7 of the length of the diagonal impressed. The FM-700, FUTURE-TECH micro durometer was used. The machine is equipped with a double microscope optics, with 10x and 50x magnification, and a micro Vickers test indentation. A setting was selected for the application on the samples of a load of 300g for 15 sec. Focused on the sample, a surface portion was selected to be submitted to the indentation,

Fig. 1. *Composite resin samples between two slides.*

Experimental Group		*D1 (μM)*	*D2 (μM)*	*HV*
	1	98,17	98,16	57,2
SAMPLE 1	*2*	98,73	99,10	56,9
	3	100,61	101,59	54,4
	1	99,57	99,57	56,1
SAMPLE 2	*2*	99,28	96,35	58,1
	3	98,05	100,25	56,6
	1	99,52	99,54	56,2
SAMPLE 3	*2*	98,06	96,76	58,6
	3	97,35	97,36	57,6
	1	99,20	99,96	56,1
SAMPLE 4	*2*	98,94	99,09	56,7
	3	99,45	98,70	56,7
	1	98,79	97,88	57,5
SAMPLE 5	*2*	97,48	98,36	58
	3	98,01	98,14	57,8
	1	100,22	100,66	55,1
SAMPLE 6	*2*	100,18	99,09	56
	3	98,86	98,91	56,9

which was, therefore, as free as possible without defects. Under the microscope, with a magnifying glass at 50x, the impression left by the penetrator on the material was read. The alignment of parallel bars, observable inside the lens, at the vertices of the obtained square allows the measurement of the distance between the two points, therefore of one of the two diagonals of the square. The procedure was repeated by measuring the distance between the other two vertices, thus obtaining the length of the second diagonal.

Once the measurement of the two diagonals (d1 and d2) has been determined, the machine has automatically

Table I. *Results of micro-hardness tests on experimental group.*

Control Group		*D1 (µM)*	*D2(µM)*	*HV*
	1	103,70	105,45	50,9
SAMPLE 1	*2*	103,95	103,62	51,6
	3	106,18	106,62	49,1
	1	103,68	105,73	51,5
SAMPLE 2	*2*	102,61	102,53	52,9
	3	102,37	102,98	52,8
	1	103,02	103,70	52,1
SAMPLE 3	*2*	103,46	103,08	52,2
	3	102,52	102,44	53
	1	100,10	99,84	54,3
SAMPLE 4	*2*	99,68	100,44	55,6
	3	101,26	101,82	54
	1	109,18	102,65	49,8
SAMPLE 5	*2*	100,89	103,40	53,3
	3	101,58	104,66	51,3
	1	106,71	110,70	47,1
SAMPLE 6	*2*	101,23	101,44	54,2
	3	104,87	104,38	50,8

Table II. *Results of micro-hardness tests on control group.*

Experimental group	HV Average	Standard Deviation (σ)
Sample 1	56,17	± 1,54
Sample 2	56,93	± 1,04
Sample 3	57,47	± 1,20
Sample 4	56,5	± 0,34
Sample 5	57,77	± 0,25
Sample 6	56	± 0,9

Table III. *Media and standard deviation of the experimental group*.

Experimental group	HV Average	Standard Deviation (σ)
Sample 1	56,17	± 1,54
Sample 2	56,93	± 1,04
Sample 3	57,47	± 1,20
Sample 4	56,5	± 0,34
Sample 5	57,77	± 0,25

Table IV. *Average and standard deviation of the control group*.

Control group	HV Average	Standard deviation (σ)
Sample 1	50,53	± 1,29
Sample 2	52,4	± 0,78
Sample 3	52,43	± 0,49
Sample 4	54,63	± 0,85
Sample 5	51,4	± 1,85
Sample 6	50,7	± 3,55

supplied, through the mathematical calculation, the value of the Vickers hardness. On each sample 3 indentations were made for a total of 36 indents. The measurements were all performed by the same operator.

RESULTS

The micro-hardness values obtained from the samples belonging to the experimental group and those of the control group have been reported below (Table I, II). In addition, the mean and standard deviations within the same sample and the same sample group were calculated. (Table III, IV). The expected value for the control samples is 52.02 ± 2. The Student's t statistical test allowed to state that the difference between the means is significant for $p < 0.05$. The null hypothesis is therefore to be rejected.

DISCUSSION

The hardness of a composite is directly proportional to the amount of double bonds formed during polymerization and its estimate is an indirect method for assessing the quality of the polymerization (30).

The hardness tests, in fact, provide information about the degree of conversion of the resin comparable to those obtained with the direct method of Fourier infrared spectroscopy (31). The significance of the results obtained confirms the existence of a correlation between the application of a constant pressure on a composite material, during the polymerization phase, and the consequent increase of its mechanical characteristics, in particular of the hardness Photopolymerization is a process of forming long

polymer chains starting from monomers contained within the paste, consequent to the activation, by a light source, of a catalyst. Frequently, as a catalyst, Canforochinone is used: activated by irradiation with visible light with a wavelength between 400 and 470 nm, it reacts with tertiary amines, also contained in the organic phase of the resin, freeing free radical species. Free radicals are able to activate other catalysing molecules or resinous monomers; each photo initiator then gives rise to an activation nucleus from which the polymerization reaction spreads in a multicentric manner, which rapidly extends to the whole material ("initial stage" and "propagation" of the polymerization). The application of the pressure and its maintenance during the polymerization phase of the resin could hypothetically result in a greater proximity between monomers and activators, with a consequent more effective polymerization. This greater degree of conversion would be able to justify the significant difference between the hardness values shown by the experimental and control samples.

The inorganic part of the composite resin is constituted by the filler, very fine mineral particles incorporated in the matrix. The function of the filler is to limit the contraction caused by the approach of the monomers during the polymerization phase and to increase the physical-mechanical properties of the material. The composite resin used for the experimentation is classified according to the filler content as micro hybrid, since there are particles of different sizes within the matrix. The smallest particles are able to fill the voids that are formed with the approach of the larger particles and this causes an increase in mechanical properties. The pressure and the thinning of the resulting material can cause a strong decrease of the space in which the particles are freely distributed, thus causing an increase in the surface hardness. Furthermore, by subjecting the composite resin to the pressure, greater surface homogeneity is obtained, and the presence of defects is limited, which could affect the hardness values obtainable from the Vickers test.

Previously it has been shown that the application of a pressure on composite materials causes an increase in the mechanical properties of the material: Brosh (28) has observed that the pressure exerted causes an increase in the stiffness and resistance to the diametrical traction of the resin. The increase in these properties has been correlated precisely to the lower presence of defects within the composite material, which are normally responsible for fractures and deformations. From a clinical point of view, this characteristic can be useful in clinical situations as for example restorations in patients with an excessive masticatory loading (subjects with malocclusion (17, 18) or muscular excessive loading (1-3) or oral diseases (23, 35-39) or in children who need great restorations after pulpotomy or pulp regeneration.

The results obtained allow us to state that applying a constant pressure during the cementation phase of indirect adhesive restorations allows to obtain better mechanical characteristics of the composite used as a cementing agent. Moreover, the measurement of the thicknesses of the samples, obtained by exerting pressure and keeping it constant during the polymerization of the material, demonstrates how this expedient can hesitate, even in clinical practice, in achieving greater restoration precision by limiting the thickness occupied by the cementing agent. The observation of the surface of the experimental samples under an optical microscope makes it possible to affirm that the application of the pressure allows to obtain more homogeneous and more defect-free surfaces. However, it is necessary to solve critical aspects of the experimentation in order to obtain the most accurate hardness values possible:

- The use of a dedicated software could standardize the reading of the indentations, thus limiting the influence of the operator and above all of his experience of using the measuring microscope on the values obtained;
- Alternatively, observation at SEM rather than at an optical microscope could at least improve estimation of the size of the impression;
- The improvement of the polymerization conditions of the material would allow to increase the degree of polymerization and the hardness values;
- The number of samples to be tested should be increased.

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DENTINAL SUBSTRATE VARIABILITY AND BONDING EFFECTIVENESS: SEM INVESTIGATION

V. CAMPANELLA[1], G. GALLUSI[1], R. NARDI[1], A. MEA[1], V. DI TARANTO[1], E. MONTEMURRO[1], G. MARZO[2] and A. LIBONATI[3]

[1]Department of Clinical Sciences and Translational Medicine, Dental School, University of Rome "Tor Vergata", Rome, Italy; [2]Department of Life, Health and Environmental Sciences, Dental School, University of L'Aquila, L'Aquila, Italy; [3]Department of Surgical Sciences, Dental School, Catholic University of Our Lady of Good Counsel of Tirane, Tirane, Albania

Though the literature reports a well-documented scientific evidence of the performance of modern adhesive systems on normal dentin, few researches are available on the interaction between adhesives and compromised dentin. In this study, the authors analyzed the microscopic structural aspects of the adhesive interface on two common altered dentin substrates: dentin contaminated by metallic oxides and caries affected dentin. Thirty teeth were selected and divided in three groups of ten teeth according to the different dentin substrate they had: (A) normal dentin, (B) caries-affected dentin and (C) dentin contaminated by metallic oxides. The experimental cavity preparation was realised exposing in each sample, portion of pathologic dentin and portion of sound dentin as control. Each cavity was restored with micro-hybrid composite, with total etching and a two-step adhesive system. Each sample was sectioned in two halves analysed with two SEM micro-morphological methods: direct observation and decalcification. SEM investigations were able to show the difference in thickness and morphology of the adhesive interface with the two different dentin variables. Caries affected dentin determined the formation of less distinctive resin tags with few micro-tags. Discoloured dentin showed a poor infiltration with few resin tags probably due to alterations in the tubular structures. The micro-morphological variability of dentin substrate influences the clinical efficacy of bonding restorations determining significant differences in the qualitative and quantitative aspects of resin tags.

The distinction and identification of the dentin substrate the adhesive procedures by which represent the starting point for an optimal adhesion (1-3) both in restorative and in prosthetic dentistry (4, 5). Though the literature reports are well documented scientific evidence of the performance of the modern adhesive systems on normal dentin (6, 7) only few researches are available on the interaction between adhesives and compromised dentin. In this study the authors analysed the microscopic structural aspects of the adhesive interface on some substrates frequently observed in the clinical practice: dentin contaminated by metallic oxides and caries affected dentine under carious processes.

The substrate on which most frequently the clinician attempts to gain adhesion is represented by pathologically or physiologically altered dentin (8-13). Two common altered dentin substrates are:

Key words: Dentinal Substrates, Resin-tags, Micro-morphology, Discoloured Dentin, Caries Affected Dentin, Adhesion to dentin

Corresponding author:
Prof. Vincenzo Campanella,
Department of Clinical Sciences and Translational Medicine,
Dental School, University of Rome "Tor Vergata",
Via Montpellier 1, 00133 Rome, Italy
e-mail: vincenzo.campanella@uniroma2.it

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caries-affected dentin and discoloured dentin. A particular clinical situation is also represented in case of hypo mineralization in children (14, 15), with hypersensitivity (16) after orthodontic treatments (17, 18). The caries-affected dentin is characterised from the obliteration of the dentinal tubules and from acid-resistant mineral deposits (19). An altered dentin with obliteration of tubes is also reported in subjects with teeth wear, due, for example, to a non-physiological muscular loading (20-22) and temporomandibular diseases (23, 24). Recent micro structural observations of the adhesive interface in caries-affected dentin showed the presence of a "acid-base resistant zone" underlying the hybrid layer; this zone is wider in the dentin that reacted to carious processes than in the normal dentin and it's hypothesized, though it's not been cleared yet, if it can influence the width of adhesive penetration (25) capable of causing pulp suffering due to greater ease in detaching the restoration, causing pulp necrosis (26) and oral diseases (13, 20, 27, 28).

Recent studies on adhesion to caries affected dentin demonstrated the alteration of a normal adhesive interface, which determine an Investigations of microtensile strength and SEM micromorphology showed that caries affected dentin produces thicker adhesive interfaces, possibly due to a deeper infiltration of resin into partially demineralised intertubular dentin. These studies reported also a better behaviour of etch and rinse adhesives can be compared to one step self-etching bonding systems (6-8).

Other authors investigated the micromorphology of the adhesive interface in caries-affected dentin using Goldner's trichrome stain, a classic bone stain, and light microscopy. The results of their research suggested that, in caries affected dentin there is an increase in the exposure of collagen zone and a decrease in the quality of the adhesive infiltration when compared with noncarious dentin (8).

Another altered dentin substrate, frequent in the clinical practice, is dentin contaminated by oxidative processes under old amalgam restorations. The frequency of this kind of substrate is directly correlated to the modern trend to replace old metal restorations with aesthetic materials (10). Researches on the causes of the removal of old metallic restorations and placing new adhesive ones, showed that the main reasons for undergoing this process were, secondary caries under the old restoration, fracture of residual tooth structures and aesthetic needs of the patient (6, 7). The increased aesthetic requirements of the actual dentistry is causing the disappearance of amalgam even though several scientific papers continue to assess its long life and efficacy (12, 14); for these reasons it is of crucial interest to investigate the characteristics of the dentin under amalgam restorations which will be our adhesive substrate for the new restoration after amalgam removal.

The dentin contaminated by metallic oxides is defined in literature as "discoloured dentin", since the products of the oxidation of the amalgam penetrate into the dentinal tubules and determine the formation of dark pigmentations. Although it is contaminated by the metal oxides, the structure of the dentin substrate remains intact with unaltered tubular structure and hardness (19). However, from a clinical point of view, it is not clear the role of this areas for the dentinal sensitivity (14-16). Products of the amalgam oxidation are stored in the gap between restorative material and dentinal walls creating a site with local high metallic ions concentration (14). Some authors suggested that dentin contamination by metal oxides is associated with a partial demineralization (19) and the most frequently chemical species found in the stained areas are Sn and Zn in the form of complexes as Ca-P-Sn or Ca-P-Zn (29-34). Metal oxides have been also hypothized to affect periodontal health status (35-38).

Sn and Zn deposits in partially demineralised dentin are the reason for the eventual higher radiopacity of dentine under amalgam restoration observed sometimes in x-rays (14-16, 19). Often, these areas of high radiopacity, are interpreted as sclerotic dentin or presence of liners under the restoration. Scientific researches demonstrated that the cause is the deposits of tin, coming from corrosion of conventional amalgams, and zinc, as Zn is the most soluble component of amalgams (19). Recent amalgam formulations contain high

percentage of copper, Cu, and it was found that the metallic deposits in the dentin are mainly Cu and Zn instead of Sn (15).

Amalgam oxides penetrate into dentins, creating variable dimensions dark areas on the floor of the preparation (probably even depending from wetness coming from vital dentin tissue); for this reason many authors talk about "discoloured dentin" as a partially demineralised and non-carious tissue which is not mandatory to be completely eliminated during cavity preparation.

Some authors hypothesised that the presence of metallic ions in the discoloured dentin prevents carious recidivisms (39, 40) and it was demonstrated that Sn, Zn and Cu ions could reduce acid formation by plaque (41). A possible effect of minerals on oral microflora has also been hypothised (40). From the analysis of the literature is evident the presence of few sporadic studies conducted in the last years on oxidised dentin.

In a recent study was investigated that the microtensile bond strength of the adhesion of discoloured dentin in comparison to normal dentin (42). In this paper the authors found minor values of adhesion in discoloured dentin even if the SEM appearance of the substrate showed a normal shape and number of dentinal tubules. The authors suggested that the lower values of adhesion (5, 42) are due not to morphological changes of the substrate but to three possible mechanisms: precipitation of plasmatic proteins as a consequence of amalgam corrosion with reduction of the infiltration by adhesive resin, large deposits of metallic ions over collagen fibres that interfere with polymerization of the adhesive resin, and the hypothesis of metallic ions can behave as heavy metals and therefore reduce the solubility of smear layer by acid conditioners.

Aim of our work is to investigate the morphological characteristics of the adhesive interface on altered dentinal substrates with the null hypothesis, that there is no difference in the adhesive interface to normal dentin, discolored dentin and caries affected dentin.

MATERIALS AND METHODS

Thirty teeth were selected and divided in three groups of ten. According to the different dentin substrate they had sound dentin, caries-affected dentin and dentin contaminated by metallic oxides. The sample selection has been conducted through preliminary radiographs in order to visualize the presence and the extension of the substrates. To simplify the section cutting procedures the radicular portion has been removed and the sample has been fixed on Plexiglas stubs. The cavity preparation was realised exposing, in each sample, the pathological dentin and a portion of normal dentin as control.

Each cavity was restored with micro-hybrid composite (Point 4 Kerr) after adhesive procedures with total etching (37% ortophosphoric acid) and application of a two steps adhesive system (Optibond Solo plus Kerr). The curing unit used was a halogen lamp Coltolux 75 (Colthene)**.** Each sample was sectioned in two halves analysed with two SEM micromorphological methods: direct observation and decalcification.

For the direct SEM observation, the samples were lapped, cleaned, dehydrated and gold sputtered. For the other SEM analysis, the samples were immersed in a 33% HCl solution for 48 hours as suggested in literature, dehydrated and gold sputtered.

In *in vivo* studies the procedure to be performed is the same but the use of local anesthesia is necessary (46).

RESULTS

The images of the samples of normal dentin show an adhesive interface with copious resin tags, several microtags originating from resin infiltration through lateral ramifications of dentinal tubules, indicating a good performance of the adhesive system.

The microphotographs of the samples of caries-affected dentin highlined the partial obliteration of dentin tubules due to a hypermineralisation of the peritubular dentin with the formation of long and thin resin tags. Therefore, when this is compared, the control area on normal dentin, the resin tags appear irregular, thin, with a small number of ramifications and with a hardly conic base. These evidences suggest a lower efficiency of acid etching technique on a more mineralised dentin.

The morphological aspect of the adhesive interface in dentin contaminated by metallic oxides presents small and few resin tags with a conic

base and a small number of microtags. Another characteristic of the adhesive interface of this group is the irregular aspect of the resin tags probably due to the difficulty of the resin impregnation through the tubules containing metallic oxides.

DISCUSSION

The microscopic structural analysis verified the efficacy of the etch&rinse technique through microscopic proof of a hybrid layer of a constant width in all the samples. The dentin substrate variability conditions in a qualitative and quantitative way, the morphology and the distribution of resin tags, as highlighted by the zone of transition between normal and altered dentin. The samples of oxidised dentin showed few and short resin tags; these ultra-structural aspects suggest an intrinsic fragility of resin tags generated in a substrate contaminated by metallic oxides.

The samples of caries-affected dentin present resin tags, long and thin and with an irregular distribution along the adhesive interface. According to the literature that describes caries-affected dentin as a less receptive substrate to acid demineralisation, the samples of this group showed dentinal tubules partially or totally obliterated (19). The decalcified samples of caries-affected showed an incomplete decalcification that suggest the presence of acid-resistant components (22).

A common micro-morphological aspect in altered substrates is the rare presence of micro-anastomosis between resin tags. This finding reveals the difficulty of the adhesive monomers to diffuse through the dentinal tubules for their previous obliteration due to caries or metallic oxides (14, 15, 19).

From the research conducted the adhesive system tested has shown variable performances on different dentin substrates. This variability suggests the impossibility to foresee the real adhesion obtained in the clinical practice. The adhesion that can be obtained is probably dependent on the dentin substrate more than on the characteristics of the adhesive system used. Furthermore, clinical variables are represented by a different substrate found in the same tooth where the clinician may has found normal, caries affected, sclerotic and oxidised dentin at the same time. In conclusion, given the local variability of the dentinal substrates it is impossible to foresee the general behaviour of an adhesive system that depends on the possibility to infiltrate the tubular system and the collagen fibers. The further analysis of these themes requires microscopical researches with the aim to evaluate not only the formation of resin tags but also the real efficiency to impregnate the collagen fibers.

At the present time, the clinical practice might be oriented in extending horizontally the cavity preparation on sound dentin in order to obtain a predicable adhesion.

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MANAGEMENT OF PEDODONTIC PATIENTS IN MODERATE SEDATION IN CLINICAL DENTISTRY: EVALUATION OF BEHAVIOUR BEFORE AND AFTER TREATMENT

S. MUMMOLO[1], S. SAPIO[2], A. FALCO[2], O. L. VITTORINI[3] and V. QUINZI[1]

[1]Department of Life, Health and Environmental Sciences, University of L'Aquila, L'Aquila, Italy; [2]Private Practice, Italy; [3]Department of Medical, Oral and Biotechnological Sciences, G. d'Annunzio University of Chieti-Pescara, Italy

The aim of this study is to evaluate the success of functional rehabilitation of one dental session in patients with severe disabilities such as severe autism, baby bottle syndrome and severe odontophobia, and to assess how patient compliance pre- and post-intervention changes in moderate sedation. Between the beginning of 2016 and the end of 2018, 20 pedodontic patients with severe odontophobia, severe autism and baby bottle syndrome who needed dental treatment came to our attention. Patients were aged between 3 and 12 years, including 9 males and 11 females, including 10 odontophobics, 6 cwith severe autism and 4 with baby bottle syndrome. During the first visit, these patients were evaluated with a rating scale to evaluate the need to carry out dental intervention in a moderate sedation regime. We were able to treat 18 of the 20 patients, and carried out a total of 45 extractions, 4 oral hygiene sessions, 60 dental caries, 29 root canal treatments and 13 sealing pits and fissures. Before and after treatment, each patient's mood was assessed by using a test. Control visits were performed after 30 days, 3 months and the last one after 6 months. After the last follow-up visit, the patient was asked to complete the assessment test of his mood so that it could be compared with the one completed before the intervention. Complete functional and aesthetic rehabilitation of the patient's oral cavity was carried out in a single session. Emotional state and patient compliance was improved one year after surgery in about 67% of patients, especially in children with odontophobia. Children with severe autism showed less collaboration in post-intervention assessment. The approach with moderate sedation was effective for the elimination of pain and for the treatment of preventive care, also improving the compliance and the mood of young patients.

Often in clinical practice, the dentist has to deal with non-collaborating patients. This situation can occur with adult patients, but above all it is easily found in the management of younger patients such as adolescents and children. Very often the anxiety of dental care arises in childhood (1) and is associated with poor oral health, such as tooth decay and periodontal and oral pathology problems (2-4), with consequences also on dental occlusion (5, 6), and cranio-facial development (7-9). Dental fear and anxiety (DFA), as reported by several authors, is a very common condition among adolescents and children, with a prevalence of about 9-10% and with a tendency to decrease with age (10-14). There are multiple causes, some of which include traumatic dental experience experienced in the past or experiences associated with pain during a previous visit (14-16). This situation can lead the

Key words: tooth, sedation, dentistry, rehabilitation, odontophobia

Corresponding Author:
Dr Stefano Mummolo,
Department of Life, Health and Environmental Sciences,
University of L'Aquila,
Piazza Salvatore Tommasi, 11, L'Aquila, Italy

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young patient, in the most serious cases, to refuse dental visits, and to poor oral hygiene (17, 18) with, consequently, a disastrous oral situation (19, 20), with problems of caries (21, 22), and an high index of orthodontic treatment need and teeth for prosthetic treatment (23, 24). Therefore regardless of the etiological cause that led to this condition, the important thing is to know the best way to treat and to manage these patients, considering that one of the most important factors for the success of a treatment is a correct approach. The digital impression to minimize the sense of suffocation is useful in these cases (25-28). This condition should not be underestimated; WHO recognizes odontophobia as a real disease which affects about 20% of the population. Successful treatment can have benefits on the oral health of young patients, also regarding dental occlusion and development (7), and from a psychological point of view can lead to greater compliance with the figure of the dentist. Another category of patients with whom the dentist has to interact are patients with severe disorders such as autism or other syndromes. These conditions, when they are present in a mild form, allow, even if in a difficult way, the dental treatment. Contrarily, when such pathologies present themselves in a very severe form, it may become impossible to treat the patient who refuses all contact with the figure of the dentist. But how to intervene in these cases? How to take a non-collaborating or severely disabled patient to trust the dentist and undergo dental care? The proposed approaches are varied: there are non-pharmacological techniques for the simplest cases of patients with low levels of anxiety and dental fear. Some authors also propose the use of music therapy with the aid of a music therapist before starting the dental intervention (29), especially listening to audio stories seem to distract the child more by reducing his anxiety (30).

An approach of this type may be less effective in the most severe cases where it will be necessary to take a pharmacological approach (31) and is more effective to apply to pharmacological sedation which is very useful in adults in surgical procedures (32-39). According to the American Dental Association (ADA) there are different levels of sedation: minimal sedation; moderate sedation; deep sedation; general anaesthesia. As reported by the American Academy of Pediatric Dentistry and the American Academy of Pediatrics, in a recent report released in 2016 (8, 40), the goals of sedation are: to protect the patient's safety and well-being; to minimize physical discomfort and pain; to control anxiety, minimize psychological trauma and maximize the potential for amnesia; change the behaviour and/or movement in order to allow the safe completion of the procedure; returning the patient to a state where discharge from the medical/dental check is safe, as determined by recognized criteria. Careful assessment of the patient is absolutely necessary before resorting to sedation to avoid complications during the procedure. Therefore, the general and family medical history is of great importance in order to frame the patient in a risk category (41) and to evaluate whether or not sedation is possible. It refers to the classification of the American Society of Anaesthesiologists

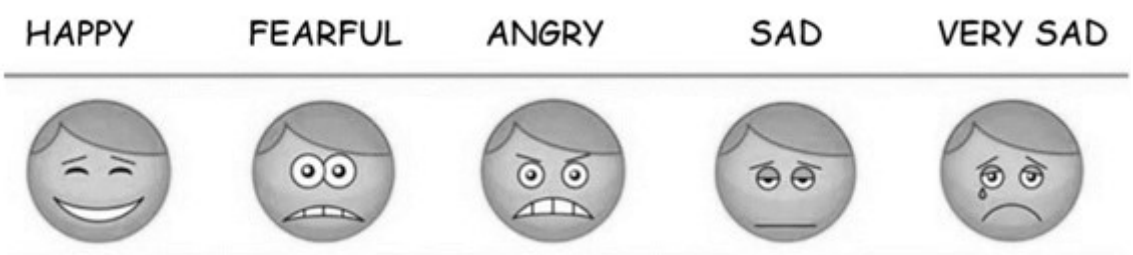

Fig. 1. *Scale used to assess the patient's mood.*

(ASA) which classifies 6 categories of patients. Only patients of the ASA 1 and ASA 2 classes are eligible for sedation. In the case of ASA 3 and ASA 4 patients, however, their health conditions do not permit sedation in the outpatient setting but require special structures and hospitalization. Routes of administration for a pharmacological sedation are different: oral, nasal, intravenous. Some authors recommend the combined use of drugs in order to obtain a safer and more predictable level of sedation by exploiting the advantages of two drugs (e.g. Protoxide and Midazolam). More important is, that through sedation not only can the dental problems be resolved, but it is also possible to increase the compliance of the child in the appointments after the intervention, reducing the anxiety of the young patient. The use of sedatives can decrease the negative impact of invasive dental treatments compared to a sedative-free approach, therefore in our work we analyse and report our experiences in the management of complex cases of children with serious dental problems and a low collaboration rate. The aim of the present study is therefore to evaluate the success of functional rehabilitation in one session in patients with severe disabilities such as severe autism, baby bottle syndrome and severe odontophobia, also assessing how patient compliance pre- and post-intervention changes in moderate sedation.

MATERIALS AND METHODS

This study followed the ethical principles of the Declaration of Helsinki. Between the beginning of 2016 and the end of 2018, 20 pedodontic patients with severe odontophobia, severe autism and baby bottle syndrome who needed dental treatment came to our attention. Patients were aged between 3 and 12 years, including 9 males and 11 females, including 10 odontophobics, 6 with severe autism and 4 with baby bottle syndrome. During the first visit, these patients were evaluated with a rating scale, compiled by us, to evaluate the need to perform dental performances in a moderate sedation regime. This treatment is preferably performed in the operating room due to the advantage of having an environment ready to better manage any emergencies. The operating room where the dental services were performed is designed exclusively for outpatient surgery, but not for surgery

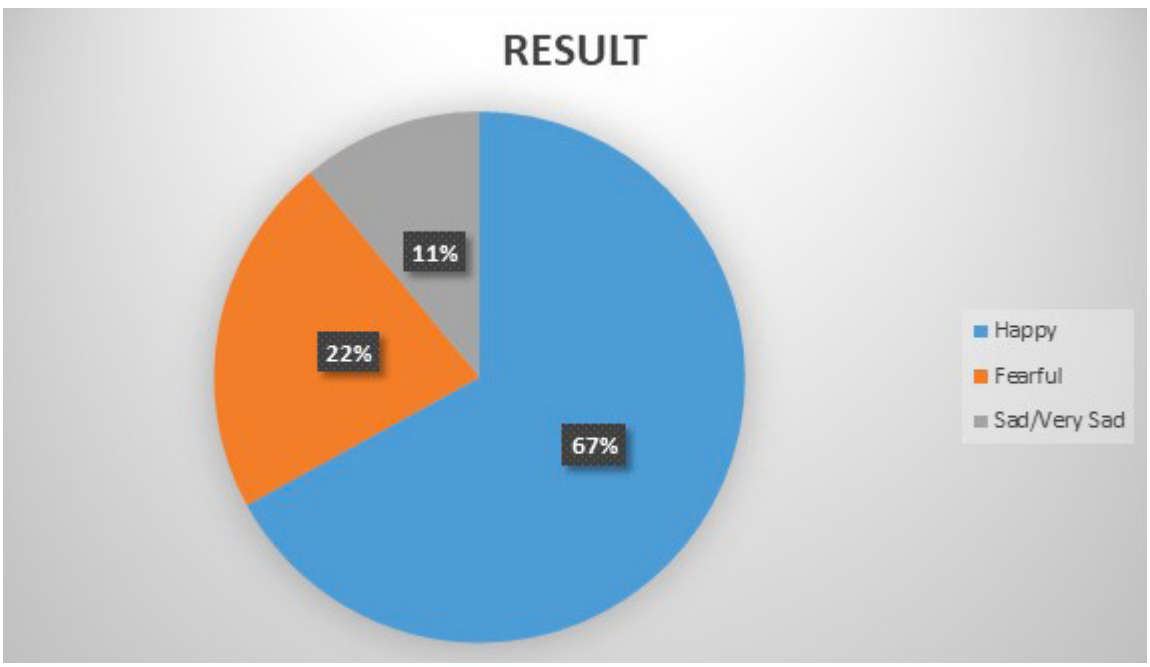

Fig. 2. *Results about the patient's mood during the treatment.*

under general anaesthesia. The parameters that we considered important to consider for the realization of the scale are the following: (i) possibility of dialogue with the patient; (ii) possibility to perform a visual inspection and evaluation of the dental arches; (iii) possibility to perform an X-ray or an orthopantomograph; (iv) evaluation of pain through visual analogic scale, and the patient's inability to chew and wash; and (v) patient who has never undergone dental treatment. These parameters give a score that, added to the other items, defines the degree of necessity for this intervention (Table I). Patients who score a value of 3 to 5 are strongly indicated for sedation treatment and are therefore referred for a visit with the anaesthesiologist. Patients who score 1 to 2 are considered borderline patients and therefore require careful behavioural re-evaluation on subsequent visits. The conditions of the young patients who came to our observation did not allow the children to undergo previous treatment and dental visits, so the young patients presented a disastrous oral situation that needed urgent treatment (26). Among these we also wanted to include patients with Baby bottle syndrome because this syndrome often hides important psycho-social conditions and bad eating habits that affect the oral health of the child. These 20 patients were evaluated through our rating scale and all presented scores between 3 and 5, therefore it was considered appropriate in these cases to use sedation for greater compliance, avoiding creation of a situation of severe stress for the child and restoring the dental clinical picture in a single operative session. Each patient's parents/guardians were informed of their child's clinical situation and once they accepted the treatment plan, were referred by the anaesthesiologist for a visit. A card was also filled out by the child where he had to indicate, where possible, his mood, thus marking the corresponding "face" (Fig. 1). Table II shows the data related to the treated patients. The values of our rating scale, the age of the patient, the sex, the clinical picture and the treatments performed are indicated. The anaesthesiologist performed a mild sedation through the oral administration of benzodiazepines before accompanying the patient to the surgical area; subsequently, a moderate sedation was performed through the administration of intravenous benzodiazepines. After that the patient was constantly monitored by the anaesthesiologist through the assessment of blood saturation and heart rate. Passing through a mild state of sedation allows more collaboration to enter the surgical room. Furthermore, the patient with mild sedation will be more likely to take the vein for the second stage of anaesthesia, which would be impossible to obtain otherwise in these patients. At the end of the intervention the parent of the child was dressed in sterile shoes, gown and sterile cap and accompanied to the surgical area so that the child sees a familiar figure immediately after the operation. The young patients together with their guardians/parents were accompanied to the post-surgical observation room where a suitable and relaxing environment was created to allow the complete recovery of the child who was monitored and any pharmacological instructions to be followed in the post-operative were given. Follow-up visits were scheduled after 30 and 90 days and the last after 6 months. During the last follow-up visit, the patient's mood evaluation test was completed again to make a comparison with the pre-operative one.

Table I. *Parameters included in the scale to evaluate the need of sedation.*

Possibility of dialogue with the patient	Affermative: 0 Negative: 1
Possibility to perform a visual inspection and evaluation of the dental arches	Affermative: 0 Negative: 1
Possibility to perform an Rx or an OPT	Affermative: 0 Negative: 1
Evaluation of pain, the patient's inability to chew and wash	Affermative: 1 Negative: 0
Patient who has never undergone dental treatment	Affermative: 1 Negative: 0
Total	

RESULTS

Despite sedation, it was not possible to treat all patients, however 18 out of 20 patients were treated correctly and successfully. The two cases of patients who could not be treated refer to a 13-year-old boy with a severe form of odontophobia and a particular psychological-familial situation and a 4-year-old autistic child who was unable to be administered with the dose. of benzodiazepine necessary for anxiolysis.

Table II. *Treatments performed.*

Rating scale	Age	Gender	Clinical presentation	Treatments
5	10	F	Autism	Oral Hygiene, 5 EX , 2TC, 3 DC
4	3	M	Baby bottle	4TC, 2 EX, 4 DC
5	13	M	Odontophobia	No Treatments
4	8	M	Odontophobia	2 TC, 2 EX
4	4	M	Autism	No Treatment
4	6	F	Odontophobia	1 TC, 2 EX, 3 DC, 4 SEA
5	4	F	Baby bottle	8 DC, 2 EX, 2 TC
3	11	F	Odontophobia	Oral hygiene , 7 EX, 3 TC, 4 DC
4	7	F	Autism	2 SEA, 2 DC, 3 EX
5	9	M	Odontophobia	Oral hygiene , 4 EX, 1 TC, 3 DC
3	8	M	Odontophobia	1 TC, 2 EX, 2 DC
4	10	F	Autism	4 DC
3	5	M	Baby bottle	6 DC, 3 TC
4	10	F	Odontophobia	5 EX, 2 TC, 3 DC
5	7	M	Odontophobia	1 TC, 3 SEA, 2 DC
3	4	F	Baby bottle	8 DC, 1 TC
5	12	F	Autism	Oral hygiene , 7 EX, 2 DC
4	6	M	Odontophobia	4 SEA, 3 TC, 2 DC
3	10	F	Autism	1 TC, 2 DC
5	9	M	Odontophobia	4 EX, 2 TC, 2 DC
DC= Dental caries; TC= Root canal treatments; EX= Extractions; SEA= Sealing pits and fissures				

In total, 45 extractions were performed, 4 oral hygiene sessions, 60 dental caries, 29 root canal treatments and 13 sealing pits and fissures. In the treated patients (18 out of 20), it was possible to carry out all the necessary interventions successfully, the pain symptomatology was completely removed and the masticatory function was restored. Already in the post-operative period there was a greater serenity in many children in whom dental pain was eliminated thanks to the intervention. The young patients were reviewed for a follow-up visit 30 days, 90 days, and 6 months post-surgery. During the check-ups, the treatments performed, the state of health and the general oral hygiene of the patient were re-evaluated (27). We found a progressive improvement of the collaboration during the control visits, and on two of the 18 patients treated, it was possible to carry out further treatment with the help of nitrous oxide without resorting to intravenous sedation, in the operating room with the anaesthetist. The positive data is that most of the patients treated in the surgical room, during the first visit did not consent to an inspection of the dental arches, while during the check-ups, after the operation in the operating room, it was possible to check with a good collaboration of the patients who granted the oral inspection. At 6 months from the intervention, the patient was asked to fill in the assessment test of his mood so as to evaluate the change of the patient's approach to the visit and the figure of the dentist. When the child does not show an angry, or sad/very sad and not even scared mood, he goes to mark the "happy" face that indicates a positive mood on the part of the young patient.

We found that after 6 months, about 67% of children (12) indicated a "happy" mood on the evaluation table. About 22% (4) indicated a "frightened" mood because of the fear of having to undergo dental treatment again, the remaining 11% of young patients (2) indicated a "sad" or "very sad" mood " (Fig. 2).

DISCUSSION

Our results are in line with those obtained by other authors who evaluated the change in behaviour of the young patient on subsequent visits to treatment in moderate sedation. Therefore, according to our experience, we believe that in these categories of pedodontic patients a therapeutic approach in moderate sedation and the aid of the anaesthesiologist is of considerable help and in some cases the only solution. This category of patients often comes to our attention with the refusal to collaborate in treatment but with extremely compromised clinical situations that require immediate and decisive intervention. Thanks to the sedation with the aid of the anaesthetist, it is therefore possible to perform long and complex treatments for a situation that would otherwise require a series of stressful sessions for the child, for example surgical interventions, or the bonding of a palatal expander, or for TMJ disorders. In this way, it is possible to limit everything to a single session and there is also a change in the attitude in many of the patients treated with regard to the dental figure and the visit. Already immediately after the intervention, the young patients, if interviewed, report that they do not remember any treatment performed and this amnesia, due to benzodiazepines, can encourage greater collaboration even during subsequent follow-up visits, as they have no memory of the traumatic event. From our clinical practice it emerges that after 6 months of treatment many of the children, if evaluated with the scale of their mood, no longer indicate a "fearful" or "sad" mood, but tend to have a more serene and positive approach to the visit and the figure of the dentist. In our opinion, these data are also influenced by the child who knows that no further treatment should be performed on subsequent follow-up visits, so he is more serene and inclined to be in a positive mood. We found greater difficulty in evaluating and less improvement in collaboration with patients with severe autism, as opposed to dental phobia patients.

Moderate sedation approach with the assistance of the anaesthesiologist is of considerable help to the clinician and it allows to perform oral treatments in one session in those patients with severe disabilities such as severe autism, baby bottle syndrome and with severe odontophobia. In addition, this approach helps to increase the compliance of the young patient.

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CONVERGENT IMPLANT TRANSMUCOSAL COLLAR AND HEALING ABUTMENT: AESTHETICS INFLUENCE ON SOFT TISSUES. A CLINICAL STUDY

A. BARLATTANI Jr[1], M. MARTELLI[2], F.M. CERUSO[2], M. GARGARI[1,2] and L. OTTRIA[1]

[1]Department of Clinical Science and Translational Medicine, University of Rome "Tor Vergata", Rome, Italy; [2]Department of Dentistry "Fra G.B Orsenigo – Ospedale San Pietro F.B.F.", Rome, Italy

Two conditions are necessary for an correct and functional prosthetic implant rehabilitation: maintaining pre-implant soft tissue health and stability of bone tissue, in terms of implant osseous-integration and maintenance of optimal crestal attachment levels. In addition to these parameters - necessary for the longevity of the restoration - one of the main aspects of therapy is the achievement of a final aesthetic that reproduces as faithfully as possible the natural anatomy of the lost tooth and the associated soft tissues. To achieve this last objective, an implant system was designed and used by our group. This implant is characterized by a convergent trans-mucosal emergence fixture associated with a progressive closing system of trans-mucosa healing pillars (healing abutment). These guarantee, together with the micro and macrostructure of the implant, an immediate and highly aesthetic condition of the peri-implant soft tissues, and in the same time an optimal seal on the convergent neck of the implant itself.

Periodontium integrity guarantees health and longevity of the dental elements (1). The periodontium consists of the superficial covering tissues, such as the gingiva, and the deep supporting tissues such as the periodontal ligament, the alveolar process and the radicular cementum (2). The tooth is anatomically placed half in the oral cavity - a septic environment in continuity with the outside, rich in commensal and opportunistic bacteria (3) - and half in the alveolar bone - an aseptic environment. Between these two compartments it is necessary that there is a hermetic seal, which avoids a transmission of bacteria at the level of the underlying bone tissue, therefore at a systemic level. This seal is called biological width (4). When the seal fails, bacterial infiltration occurs at the level of the gingival sulcus, with consequent destruction of the dental support apparatus. If this acute inflammatory process is not controlled and contrasted, the tooth element will be removed (1). This leads to an aesthetic and functional deficit, especially in the posterior sectors, with gnathological alterations (5), and above all repercussions or damage at the level of the temporomandibular joint (6, 7). The evolution of implantology, and the simplification and standardization of surgical protocols meant that these sites could be rehabilitated (8-11). From the product point of view, there are many implant solutions available on the market. Research has always been based on the design of implant systems that could guarantee a muco-connective seal similar to that present on the natural tooth. Using a macro classification, there are two types of implants:

- *Bone Level implants*, with implant-abutment connection at bone level
- *Tissue Level Implants*, with transmucosal implant-abutment connection

To date, in the literature, the presence of an implant system able to guarantee a stable and

Key words: trans-mucosal implant, convergent collar, healing-abutment, peri-implant soft tissue, aesthetic implant-prosthesis

Corresponding Author:
Liliana Ottria, *MD, DDS, MSc*
Department of Clinical Science and Translational Medicine,
University of Rome "Tor Vergata", Rome, Italy
e-mail: lilianaottria@gmail.com

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hermetic marginal seal against bacterial penetration has not been demonstrated (12). A fundamental role is determined by the implant-abutment connection. This infiltration can result in peri-implant bone disease and cause a restoration failure (13).

Implant connections

The precision of the connection is necessary to create a seal between the abutment and the implant. If this seal is not created, in the connection gap a reservoir of accumulating bacteria is formed which is able to move toward the nearby peri-implant tissues (14). As a consequence, the formation of ICT (inflammatory connective tissue) can occur, because the inflammatory cells are continuously activated by the resident bacterial cells in the peri-implant tissue (15). However, the accuracy of the connection cannot be absolute. It must always be considered that in an industrial production of two mechanical components that must be coupled, a margin of dimensional error (± 10 μm) must be tolerated. Moreover, it should be taken into account that two mechanical components during mastication undergo elastic deformations, therefore it is easy to understand why the system cannot be perfectly sealed (16). In their study, Canullo *et al.* (17) studied four different types of implant-abutment connections: external hexagon, double internal hexagon, internal hexagon with external collar and conical connection. A microbiological analysis on these connections showed that all were contaminated after 5 years of functional loading (17).

To date, the literature unanimously states that this infiltration can be controlled, but not excluded completely, using a platform switching type implant-abutment connection. This consists of an internal conical connection with abutment at 15° coronally divergent with a diameter at the point of connection, reduced compared to the plant base of 0.3 for each side (17, 18). An *in vivo* histological study on animals conducted by Berglundh *et al.* focused on hard and soft tissues in relation to a platform switching connection, demonstrating how soft tissue followed the transmucosal secondary component recreating a peri-implant seal. In detail, the study showed that at 2 weeks many fibroblasts were present in the tissue-implant interface, and at 4-6 weeks a mature epithelial barrier was found, whereas the underlying connective tissue matrix was mostly composed of fibroblasts and collagen fibers, both parallel and perpendicular to the long axis of the implant. After an 8-week healing period, the histometric analysis showed that the epithelium barrier was about 2 mm high, while the underlying connective tissue measured about 1.5 mm (19).

Prama® transmucosal implant

A valid alternative to that described above seems to be provided by the PRAMA® (20) converging neck transmucosal implants. These implants guarantee a valid connective tissue (21) and epithelial seal, thanks to the convergent transmucosal neck unitarily to an internal hexagon connection located at the transmucosal level, which eliminates the problem of bacterial accumulation at its level. This placement of the connection ensures that there is no trauma at the level of the soft tissues at every stage of therapeutic programming, and there is no consequent bacterial infiltration as the connection overhangs the peri-implant connective seal. By doing so, the risk of bacterial infiltration can occur only during the surgical implant insertion phase (22). Albeit minimal, with traumatism the simple disassembly is also considered of a traditional abutment with

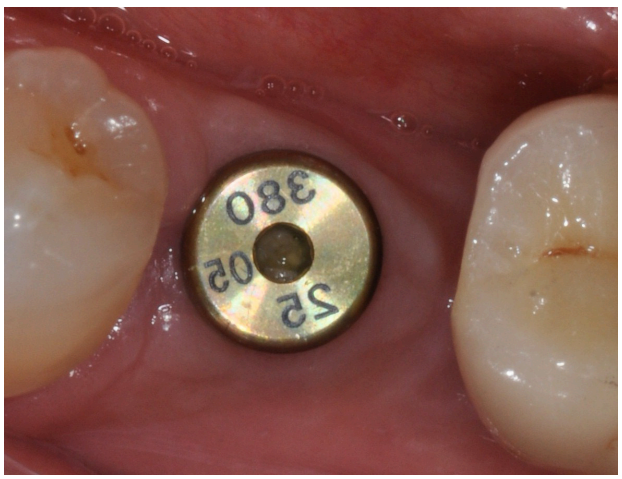

Fig. 1. *Healing abutment 3.80-25-05 mm (CH 05) at 15 days from surgery. Mandible single edentulous clinical case. Implant site 1.6.*

bone level connection, and subsequent assembly of imprint transfer (23). An *in vivo* animal study conducted by *Borges at al.* 2018 demonstrated how the disconnection and subsequent reconnection of abutments resulted in a loss of peri-implant soft and bone tissue height, compared to controls with abutments connected at the time of implant insertion and never disconnected thereafter (24).

PRAMA® implant is a system consisting of an implant fixture and a transmucosal single-piece component. The endosseous implant is very similar to all the implant systems on the market (25) for materials (ZirTi), and micro- and macro-structure. The peculiarity resides in the trans-mucosal neck, synthesis of a horizontal and vertical platform switching model characterized by a short cylindrical route of 0.8 mm, and a subsequent hyperbolic convergent portion 2 mm high. The surface of the neck is not smooth, but rather grooved in order to create a functional surface, defined UTM (Ultrathin Threaded Microsurface). This grooved surface guides the fibroblasts, allowing them to create circular collagen fibers that migrate towards the narrower diameter of the neck, creating a stable biological seal (25). A short epithelial groove is found in the periodontal survey. The convergent morphology of the PRAMA® neck therefore allows to recover the portion of soft tissue, that otherwise – in a traditional implant – would be lost because occupied by the titanium abutment. This convergent geometry enables to fill the volumes around the crown portion of the neck with a clot and with important growth factors that can be transformed into thick and stable soft tissues. This thickening is achieved immediately and successfully with the use of healing abutments.

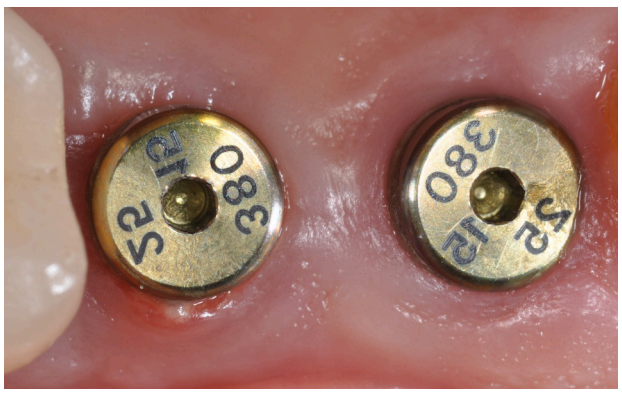

Fig. 2. *Healing abutment 3.80-25-15 mm (CH 15) at 30 days from surgery. Upper maxillary edentulous saddle clinical case. Implant site 1.4 and 1.5.*

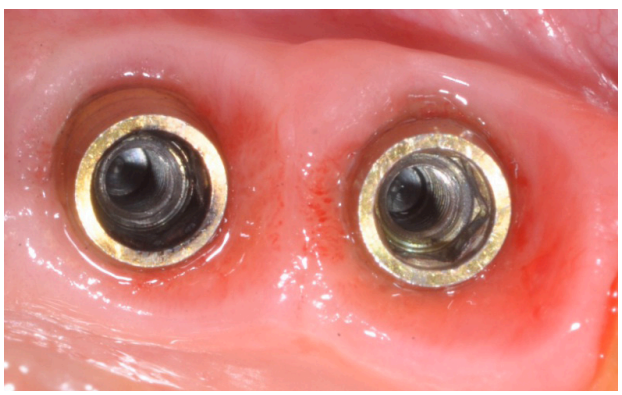

Fig. 3. *Soft tissue conditioned with Prama® convergent collar implant system and healing abutment. Implant site 4.4 and 4.5.*

Healing abutment

Taking advantage of the B.O.P.T. prosthetic concepts (26), titanium Healing Abutments have been designed. These Healing Abutments (like a prosthetic restoration on a vertical preparation to finish (27)), clench to the trans-mucosal neck of the implant at 0.50 mm (CH 05) (Fig. 1) or 1.5 mm (CH 15) (Fig. 2). The prosthetic protocol suggests the use of CH 05 healing abutment at the moment of the surgical implant insertion, and then to replace it with the CH 15 healing abutment, approximately after 25 days after the first placement. This procedure does not involve any risk of bacterial infiltration, since the implant connection is supragingival. The anatomical configuration of the healing abutment, when closing on the implant neck, resembles the physiological dental enamel overcontour (24), allowing the deviation of soft tissues from the neck. The space between the implant neck and the healing abutment is filled by the clot and growth factors. The result is a conditioning of the soft tissue with thickening of the peri-implant mucosa, which will optimally house the subsequent prosthetic restoration (28-32), and guarantee an excellent aesthetic and biological result in terms of peri-implant seal (33-37) (Figs. 3, 4). This procedure, associated to an accurate protocol of domiciliary hygiene, does not involve any risk of bacterial infiltration and contributes to prevent

tissue peri-implant disease (36-41); furthermore this method is suitable for treating patients with systemic and oral cavity diseases (42-51).

MATERIALS AND METHODS

The study was conducted at the Odontostomathology Operative Unit "Fra G.B.Orsenigo" Ospedale Villa San Pietro F.B.F. Rome, Italy. Six patients were selected (3 male and 3 female) and three single edentulous cases and three edentulous saddle cases were treated. From a total of nine implants, five were implanted in the upper maxillary and four in the mandible; of which two post-extraction. All other sites had an edentulous condition for three to six months. Exclusion criteria were: patient under the age of 18, denied consent to treatment, smoker, poor oral hygiene, systemic diseases. All interventions were performed by the same surgeon.

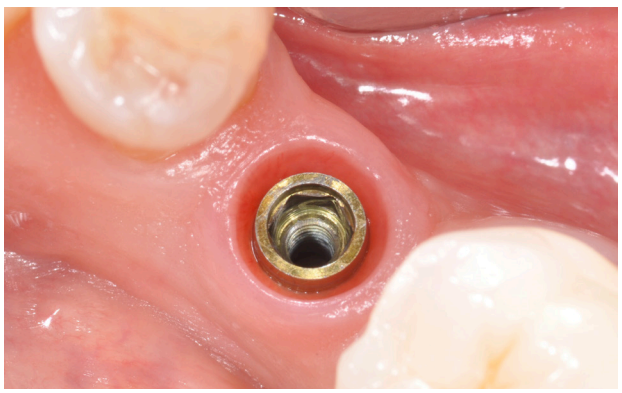

Fig. 4. *Soft tissue conditioned with Prama® convergent collar implant system and healing abutment. Implant site 3.5.*

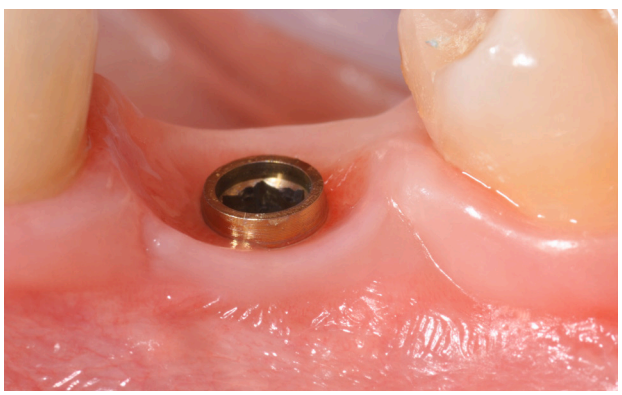

Fig. 5. *Vestibular view. Soft tissue conditioned with Prama® convergent collar implant system and healing abutment. Implant site 4.6.*

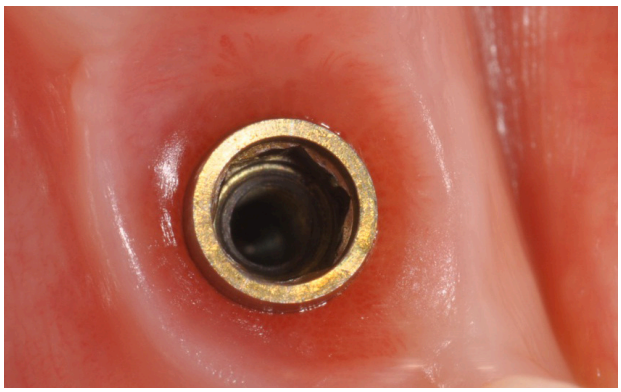

Fig. 6. *Occlusal view. Soft tissue conditioned with Prama® convergent collar implant system and healing abutment. Implant site 4.6.*

Fig. 7. *Vestibular view. Aesthetic definitive restoration, screw in ceramic metal crown. Implant site 4.6.*

After local infiltration with Mepivacaine Hydrochloride + Adrenaline 1:10000, the patient was asked to rinse, for about a minute, with a 0.12% chlorhexidine solution. An incision was made on the ridge without cuts of releases with subsequent dissection of the muco-periosteum flap. The preparation of the implant site was carried out following the surgical protocol of the manufacturer (Sweden & Martina®): preparation drills in sequence (for specific diameter and length) with surgical micromotor setting at 900-1100 rpm, followed by insertion of the first mechanical implant at 25 rpm 35Ncm, then manual. All the mechanical phases occurred under abundant irrigation of 0.9% P/V physiological NaCl pre-cooled solution. All the implants used were Sweden & Martina® PRAMA, of variable length (10 mm, 11.5 mm, 13 mm) and diameters (3.8 mm and 4.25 mm). Once the implants were inserted,

all of them had a final tightening torque greater than 45 Ncm, thus guaranteeing excellent primary stability. The convergent implant neck was fully positioned in the extra-osseous seat. The surgical phase iwas completed with the insertion of the specific Healing Abutment for specific CH diameter and height 05 (0.5 mm). The surgical site with suture 4-0 Vicryl® was sutured, re-approaching the flap and evaluating the congruity and integrity of the free margins. Twenty-five days after the initial operation, the Healing Abutment was replaced with a new one of the same diameter and height as the previous but progressive CH 15 (1.5 mm). All the cases studied were completed with screwed prosthesis in ceramic metal.

RESULTS

To date, the analysed system has proven valid, both aesthetically and functionally (Figs. 5, 6, 7). Stable conditionings of the soft tissues were obtained. All cases achieved good aesthetics, with a physiological gum reformation, and sufficient thickening to reconstitute the classic 'orange peel' appearance typical of healthy gums. There was no bleeding during the survey. The radiography observed a limited crestal bone resorption, confirming an absence of pathological bone remodeling, attributable to bacterial over-infections. Since the study was carried out on a limited number of samples and based on a purely macroscopic analysis of the clinical results, further confirmation is postponed to future histological analysis.

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TRANSGINGIVAL IMPLANTS WITH A CONVERGENT COLLAR (PRAMA). SURGICAL AND SCREWED PROSTHETIC APPROACH. A CASE REPORT

F.M. CERUSO[1*], L. OTTRIA[2*], M. MARTELLI[1], M. GARGARI[1,2] and A. BARLATTANI JR[2]

[1]Department of Dentistry "Fra G.B. Orsenigo - Ospedale San Pietro F.B.F.", Rome, Italy;
[2]Department of Odontostomatological Sciences - University of Rome "Tor Vergata", Rome, Italy

*These authors contributed equally to this work

Objective. This article presents a case report of transmucosal implant with a convergent collar (PRAMA) inserted in the anterior maxillary esthetic area. The purpose of this study is to evaluate soft and hard tissue after 12 months. Methods. One implant was placed in aesthetic area. The implant was immediately loaded with a provisional screw. After 3 months the definitive screwed prosthesis was placed. The patient was reassessed at 12 months post implant placement. During the examination, the soft-tissue texture, color and amount of keratinized tissue were checked. Results. No statistically significant horizontal dimensional changes of the alveolar ridge were observed between each timepoint. Mean soft tissue levels significantly improved between baseline and 12 months. Conclusions. The reduced buccal width of the transmucosal component gives more space to the gingival thickness, promotes stability and gives a better seal. The use of transmucosal implant create a shift of the inflammatory cell infiltrate away from the crestal bone level.

The maintenance of soft and hard tissue stability over time has become part of the long-term outcome assessment for implant-supported restoration in the esthetic area (1). To maintain soft and hard tissue stability (2) it is essential to avoid microbiological contamination around the vital bone and the treated part of the implants (3-5) even in regenerated bone, maintaining adequate mechanical property of the implant (6, 7).

Different strategies have been applied in order to move the microbiological contamination away from the vital bone, mostly based on chemical and mechanical removing of plaque, through mouthwashes and pro-biotics or contrasting risk factors such as smoking, or controlling the systemic health status correlated to periodontal diseases (8-16).

Some techniques used for chronic periodontitis could be useful, as photodynamic treatment or laser. Recently developed strategies are platform switching and soft tissue-level implant.

In platform switching, the mismatch between the abutment with the implant neck, may reduce the vertical component of the biologic width by introducing a horizontal distance to harbor the inflammatory cell infiltrate (17).

A completely different approach was suggested by the soft tissue level implant concept, intended to relocate the implant-abutment interface vertically and its associated bacterial infiltration above the crestal bone (3).

A new implant system (Prama, Sweden and Martina, Due Carrare Italy) with an innovative

Key words: transmucosal implants, Prama, immediate loading, biologic width.

Corresponding Author:
Francesco Mattia Ceruso,
Department of Dentistry,
"Fra G.B. Orsenigo - Ospedale San Pietro F.B.F.",
Rome, Italy
e-mail: hello_982@yahoo.it

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trans-mucosal surface morphology (BOPT) was recently developed (18). The Prama transgingival neck is characterized by a cylindrical part 0.80 mm high and by a hyperbolic portion 2 mm high, especially designed to guarantee a real continuity with the post.

When the corresponding tapered abutment is connected to the implant, the result resembles a tooth prepared with a feather-edge design with no finish line. This is in accordance with the principles of the biologically oriented preparation technique (BOPT).

In prosthodontic there are several prosthetic approaches (19); the Biologically Oriented Preparation Technique (18) (BOPT) is an interesting prosthetic approach designed originally for periodontal treated teeth, its concepts were transferred to Prama Implants who offer all the characteristics needed for an implant-prostheses rehabilitation with benefits of both hard and soft tissues.

The aim of this study is to evaluate the clinical use of biologically oriented preparation technique (BOPT) with transmucosal implants (PRAMA, Sweden and Martina, Due Carrare, Italy)

Case report

A 68-year-old woman came to our attention with a missing tooth. The tooth 5.3 had fallen spontaneously (Fig. 1). Tooth loss was due in part to root resorption and partly to bone resorption.

Preoperative analyses of anatomical features were performed using a panoramic radiograph and a CT scan. Before the surgery Alginate impressions and laboratory casts for both maxillary and mandibular arches were taken. We had the provisional prosthesis, with large holes at the implant site, before starting the surgery. We had used the provisional as a surgical guide.

Antimicrobial prophylaxis was administered with the oral use of 2 g Augmentin 1 h before surgery and twice daily for 5 days post-operatively. After administration of local anesthesia (2% articaine/adrenaline 1:100.000) one implant (PRAMA 4.25mm x 11.50 mm, Sweden and Martina, Due Carrare, Italy) was placed in a sub-crestal position leaving the gold portion of the implants above the bone (Fig. 2). In this way, periodontal soft tissues heals along the collar of Prama implants.

Prama implant was instantly loaded with a provisional fixed in mouth with composite resin, to screw-retained temporary abutments (Sweden and Martina, Due Carrare, Italy) (Fig. 3, 4).

Proper occlusal contacts were checked with an 8 microns aluminum foil (Hanel Shimstock, Coltene/Whaledent). Nylon 4-0 sutures (Atramat, Int Farmaceutica, Mexico) were used to enhance periodontal soft tissue healing.

Appropriate analgesics were prescribed, and

Fig. 1. *The tooth 5.3 fell spontaneously, its loss was caused partly by root resorption and partly by bone resorption.*

Fig. 2. *The PRAMA® implant (4,25 mm x 11,50 mm) in sub-crestal position, with its gold portion above the bone.*

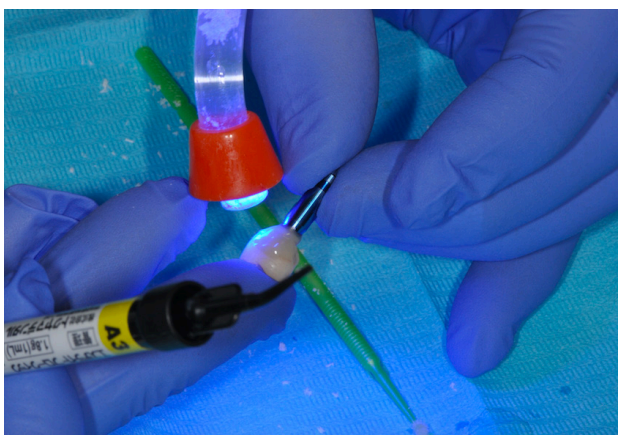

Fig. 3. *The PRAMA® implant immediately loaded with a temporary, fixed with composite resin on a provisional screwed abutment.*

Fig. 4. *The PRAMA® implant immediately loaded with a temporary, fixed with composite resin on a provisional screwed abutment.*

postoperative oral hygiene instructions were given to the patient. The sutures were removed two weeks after dental implant placement. After radiographic control (Fig. 5), at 12 weeks the operative sequence was carried out to achieve the definitive prosthesis:

1) preliminary appointment:
   - a conventional alginate impression is made, and study models are cast;
   - a rigid custom tray is manufactured with a window cut through over the implant.

2) next appointment:
   - the screwed provisional was removed;
   - appropriate impression copings were selected and fitted;
   - the open tray was tried in, the impression coping should emerge level with the window. This permits easy removal of the impression copings, while ensuring that the copings are

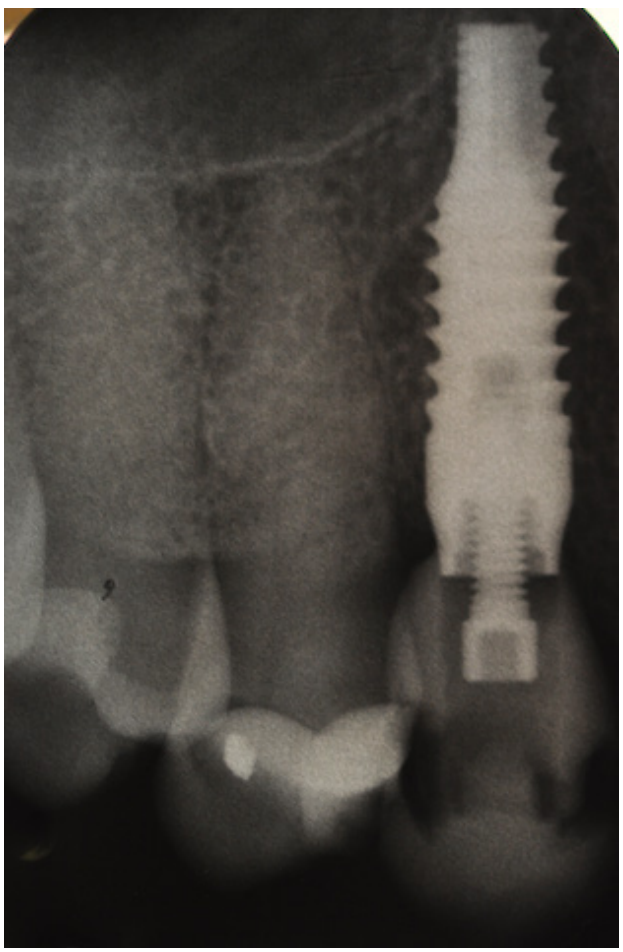

Fig. 5. *Radiographic control after 12 weeks, before placing the screwed final crown in metal-ceramic.*

supported by enough impression material;
- the window was sealed with wax;
- the open tray impression was created with additional silicones (VPS) of different viscosities and an IOS digital impression (20-23). The tips of the impression coping should be felt through the wax covering the window. Once the impression set, the impression copings were unscrewed through the window and impression is removed from the mouth.
- the screwed provisional was replaced.

The final metal-ceramic screwed restoration was carried out considering the most important aesthetic and functional canons:

1) centric contacts - even distribution occlusal contacts with small and centered over the implants;

Fig. 6. *After 12 months, the control of the final restoration in metal-ceramic. The texture of the soft tissue, its color and the amount of keratinized tissue were checked.*

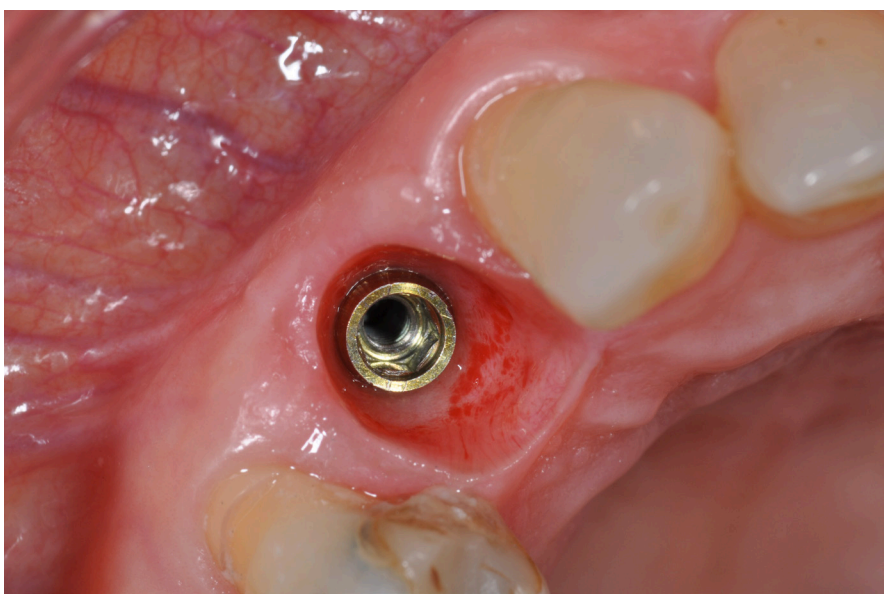

Fig. 7. *After 12 months, the control of the final restoration in metal-ceramic. The texture of the soft tissue, its color and the amount of keratinized tissue were checked.*

2) eccentric contacts - anterior guidance only, distributed over multiple teeth (24, 25);

3) angle of tooth contact - shallow as possible to minimize shear forces but still disclude posterior teeth;

4) jaw-to-jaw position - centric relation as defined by Dawson to control tooth contacts;

5) VDO - alter if necessary to create proper tooth form and guidance (26, 27).

The patient was reassessed 12 months post implant placement. During the examination, the soft-tissue texture, color, and amount of keratinized tissue were checked (28, 29) (fig.6 e 7).

RESULTS

At the 12-month follow-up, the implant was clinically osseointegrated and at baseline, interproximal radiographs revealed no bone defect around the implant. No statistically significant horizontal dimensional changes of the alveolar ridge were observed between each timepoint.

Mean soft tissue levels significantly improved between baseline and 12 months. The use of trans-mucosal implant creates a thick gingival tissue around implant, and a shift of the inflammatory cell infiltrate to the central axis implant and away from the crestal bone level. The creation of this mechanical barrier serving as a defense mechanism prevents bacteria penetration from the oral environment and is a viable technique to reduce bone resorption also in case of patients with oral pathology (30-45).

DISCUSSION

Within the limits of this clinical case report, the clinical use of biologically oriented preparation technique (BOPT) using Prama implants guaranteed an excellent gingival seal and an optimal gingival esthetic already after six months. It has been improving after 12 months.

With the BOPT technique, the clinician and the laboratory technician can interact with the surrounding tissues modifying their shape and scalloped architecture regardless of any preexisting dental or gingival limitation.

The reduced buccal width of the transmucosal component gives more space to the gingival thickness and promotes stability and give a better seal.

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FULL-DIGITAL WORKFLOW TO REHABILITATE A HIGH VALUE AESTHETIC ZONE: A CASE REPORT

P. CAROSI[1], A. PINTO[1], V. QUINZI[2], V. MAZZETTI[1], P. CARDELLI[1] and M. LAURETI[1]

[1]Department of Chemical Science and Technologies, University of Rome "Tor Vergata", Rome, Italy;
[2]Department of Life, Health and Environmental Sciences, University of L'Aquila, L'Aquila, Italy

Replacing missing teeth in the aesthetic area is a clinical challenge which must be solved by a multidisciplinary approach in order to obtain an esthetic and functional implant-prosthetic rehabilitation. First therapeutic choice should be the less invasive option, in accordance with patients' expectations. The present clinical case presents the rehabilitation of the upper incisor group using a full-digital workflow to reduce operative time, costs and patient discomfort. The use of computer-guided-surgery and 3D technologies, as intraoral scanners or cone-beam-computed-tomography, allows the clinician to visualize all patient's information when planning the rehabilitation in order to obtain a more predictable and a less invasive surgery.

The current standard of care for oral rehabilitation by means of implants, restorative (1), endodontic (2) and prosthetic indirect restorations (3) is no longer only the replacement of missing teeth in terms of function, but also the achievement of satisfactory aesthetic (4, 5). The positioning of the implants nowadays is optimal due to a prosthetically guided project which is mandatory to choose the type of implant (6), and to achieve these objectives (7-9), after considering the periodontal status of the patient, and eventually after periodontal and oral treatments (10, 11).

When inflammatory processes end with tissue deficiency it is not possible to perform implant rehabilitation. To overcome this problem, new procedures have been tested. In fact, platelets concentrates have been considered as a valid alternative to synthetic or animal membrane during the Guided Bone surgery (12).

The implant rehabilitation has to be carefully planned in advance (13), because having dental implants placed flapless and immediately loaded leads to the advantages of reduced treatment times and costs (14, 15).

On the other hand, there were also procedures that, differently from flapless surgery, required the use of diode laser that was less invasive and reduced surgery-related pain experienced by patients (16). Furthermore, laser can also be used in soft tissue remodeling, for example in case of gingival overgrowth drugs-induced (17).

The innovation of technologies such as cone beam computed tomography (CBCT), allows volumetric bone imaging with lower radiation doses (5, 18-21), facilitating the preoperative acquisition (21, 22) such as the available bone volume and quality, the presence and location of relevant anatomical structures and pathologies, and their relationship with future rehabilitation (21, 23-31).

Secondly, Computer-aided design/Computer-assisted manufacturing (CAD/CAM) technology (32,

Key words: dental implant, implant guided surgery, intraoral scanner, digital workflow, immediate loading

Corresponding author:
Dr. Alessandro Pinto,
Department of Chemical Science and Technologies,
University of Rome "Tor Vergata",
Via Montpellier 1,
00133 Rome, Italy
e-mail: pintosmile@virgilio.it

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33) gives the possibility to utilize implant planning software. There are several software programs which help in the planning of implant-supported rehabilitations (6, 8). With coDiagnostix (Dental Wings, Montréal, Canada), a 3D digital planning file is created for the ideal implant positioning. This planning is transferred to surgery, and operators will use a custom-made surgical template with metallic sleeves to guide the surgeon to precisely position each dental implant in the position previously virtually planned, together with specific surgical instruments in combination with a guided drill stop kit. In addition, a prefabricated prosthesis made by the digital implant and prosthetic planning can be immediately delivered to the patient. Often mucoperiosteal flaps are adopted by surgeons when operating freehand to visualize the recipient site more clearly. This is not always necessary if computer-guided implant placement is carried out. Blood circulation is preserved due to flapless or mini-flap surgical procedures in the soft tissue improving and accelerating the regeneration process (5, 34, 35).

Fig. 1. *Pre-operative intraoral view.*

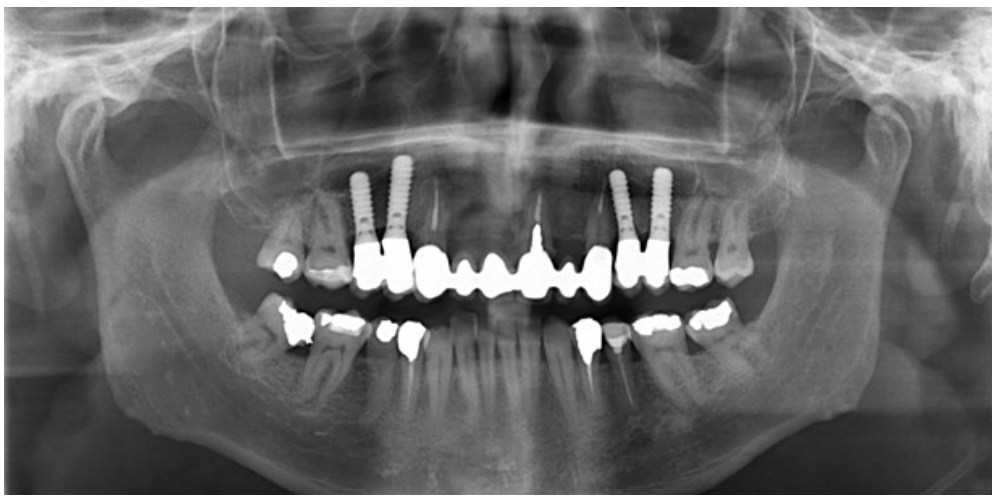

Fig. 2. *Pre-operative panoramic.*

Inconveniences such as dimensional instability of materials and storage of dental stones can be eliminated using the digital impression technique which provides a better accuracy of definitive restoration (9, 36-39). Three-dimensional (3D) data taken from intraoral scanners can be matched to the file digital imaging and communication in medicine (DICOM) of the CBCT to plan ahead both the surgical and the prosthetic procedures. The same analyses can be also used for temporary implants, or for the temporomandibular joint investigation, or to analyze the cranio-facial structure for orthodontic purposes, as for example the palatine area and the airway space or for surgical interventions. Generally, removable prostheses are used during the healing period. These prostheses are considered awkward by patients.

The above techniques could reduce the healing period without risking an unsuccessful implant and a longer period for complete rehabilitation. As suggested by surveys of the scientific literature, immediate loading procedures for edentulous arches have gained wide popularity among clinicians as well as great acceptance by patients (10). High primary implant stability (high value of insertion torque) and lack of micromovements are considered two of the main factors necessary for achieving a predictable high success rate (40). The presence of micromovements could lead to encapsulate the implant by the soft tissue, causing its failure (41). To avoid the risk of encapsulation, it is recommended to keep the implants load-free during the healing period: 3 to 4 months in mandibles and 6 to 8 months in maxillae (42). The purpose of this article was to report the rehabilitation of a high aesthetic area using a full-digital workflow to plan implants and immediate prosthetic rehabilitation.

Clinical report

A 54-year-old man came to the dental clinic reporting functional and aesthetic complications in relation to an old fixed dental prosthesis going from tooth 1.3 to tooth 2.3 (Fig.1). Clinical and radiographic examinations revealed a good status of the two canines (Fig. 2). The tooth 2.1 showed a good status, but the patient referred pain in the fornix zone, and it had grade 1 mobility. The patient asked for a fixed rehabilitation that included also the two canines. The best treatment plan was to extract tooth 2.1 and insert two dental implants in 1.2 and 2.2 positions and two new crowns on teeth 1.3 and 2.3.

The study of the case started making an intraoral scan of both arches with an intraoral scanner (IOS) (Trios, 3Shape, Copenhagen, Denmark) (Fig. 3). The result is a standard tessellation language (STL) file that was used to print a 3D diagnostic cast in printable resin.

Fig. 3. *Intraoral scan.*

Through CBCT quality and quantity of maxillae bone can be visualized in a 3D rendering and studied to plan implants positions. The digital planning of dental implants' position was made by the matching of several files: two STL files, one from the intraoral scan, one from digital wax-up made by the dental technician, one DICOM file from CBCT.

The digital matching of all these data on the coDiagnostix software (Fig. 4) led to a more predictable and aesthetic implant-supported rehabilitation. Once the implant planning was completed, the software allowed to print directly the surgical template that will guide the implant insertion.

After local anesthesia, a flapless surgical technique was used to insert the two dental implants, avoiding the elevation of a muco-periosteal flap. Two tapered implants (Straumann Bone-Level-

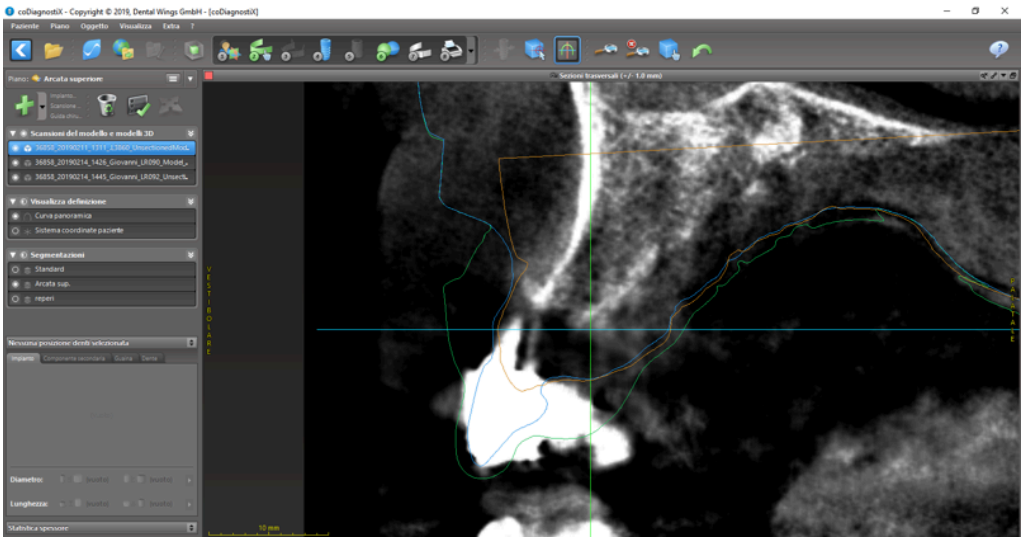

Fig. 4. *Digital matching.*

Fig .5. *Scan bodies placed after surgery planning.*

Fig. 6. *Temporary restoration.*

Fig. 7. *Temporarily screwed 2 hours after surgery.*

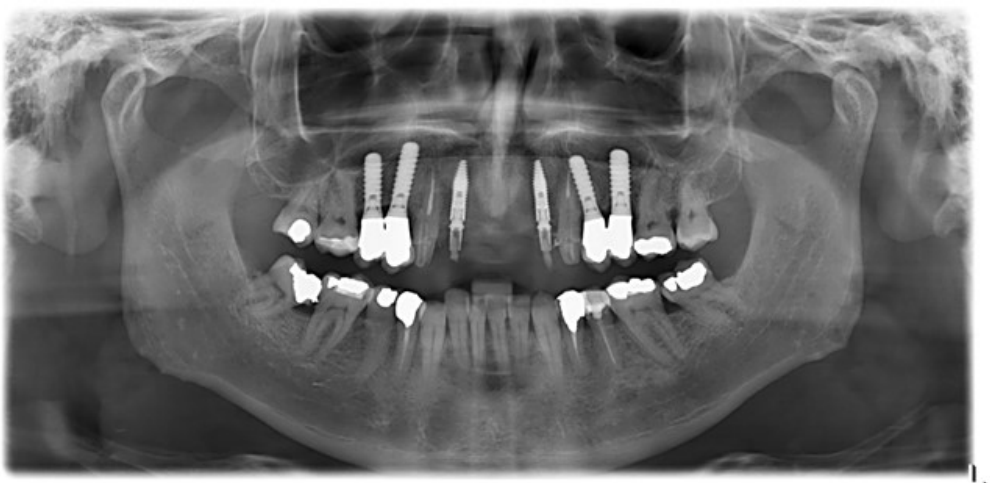

Fig. 9. *Final OPT.*

Tapered 3,3 ø, Straumann, Basel, Switzerland) were positioned (Fig. 5). The insertion torque was 35N for both implants.

The coDiagnostix software offers the possibility to export a STL file of the pre-operative intraoral scan with the scan bodies positioned where the planned dental implants should be after the surgery (Fig. 6). This file can be processed by the dental laboratory to obtain the temporary restoration without impression.

The temporary restoration carried out by the digital planning is directly relined in the patient mouth. The provisional included also the two canines to give the right position to the restoration (Fig. 7).

Once the temporary restoration was finished and polished, it was screwed with a 15N torque on the two dental implants (Fig. 8). The occlusal scheme was controlled to obtain a "nonoccluding" provisional in occlusion and in excursions to avoid trauma during the healing period. In addition, the patient was instructed to follow a soft diet and to wear a nightguard (Fig. 9).

DISCUSSION

This article describes a treatment protocol to rehabilitate partially edentulous maxillae with a partial implant-supported fixed prosthesis. The treated site was completely healed as dental extractions were made several years ago.

In this clinical case a full-digital workflow was used to plan the surgery and the immediate provisional. The digital workflow has been a hot topic for the last 10 years in dentistry and many authors investigated about that and reported that there is more efficiency than the conventional workflow in terms of cost and time (32). However, it requires an adequate and multi – disciplinary learning curve to minimize the errors involved. The digital work-flow includes also a complete analysis of face (43), occlusion (44) and temporo-mandibular status of the patient (9).

As a result of the digital workflow, computer-guided surgery was performed in this clinical case. The 5th International Team for Implantology Consensus Conference analyzed data about computer-guided surgery accuracy from several studies. The result is an inaccuracy at the implant entry point (between the planned implant position and the position at which the implant was inserted) of, on average, 1.12 mm and an inaccuracy of, on average, 1.39 mm at the apex of the implants. However, these results are in the acceptable range (45).

The dental laboratory printed the surgical template from the digital planning. This template was tooth-supported as it rests on occlusal surfaces of the adjacent teeth in order to provide support during the implant placement. This type of surgical template is reported to be more accurate than bone- and mucosal-supported guides. D'haese et al. showed an inaccuracy at the entry point of 0.87 mm and an inaccuracy at the apex of the implants of 0.95 mm (46-48).

The computer-guided surgery allows the clinicians to choose between conventional mucoperiosteal flap surgery or flapless surgical procedures. We decided to do the flapless surgery and to avoid elevating flaps mainly to reduce postoperative complications such as pain, swelling, infections or dehiscence (49). The control of the bacterial load after the surgical procedure can be obtained easily through a regular home oral hygiene procedure and mouth rinse, to prevent periimplantitis (50-53). The procedure appears indicated for special patients with systemic or local diseases (54, 55), or conditions which influence the periodontal status, or smoking patients that show high risk of periodontal diseases (33). There are, for sure, disadvantages such as inability to visualize the true topography of the underlying bone increasing the risk of complications such as unwanted bone perforations. However, there are also other advantages, as the preservation of hard tissues, the maintenance of the vascular supply and the reduced clinical work load (49).

This clinical case reports a functional rehabilitation, but also an aesthetic rehabilitation. Facing aesthetic is always a great challenge for all clinicians. Immediate loading of dental implants gives aesthetic and psychological benefits to patients and can be considered a successful procedure (31). However, Albrektsson (42) reminds us that there are two main factors necessary to achieve a good result: high primary stability and lack of micromovements.

Rehabilitate the anterior maxillae is always a hard test for every clinician. Digital planning seems to improve predictably and accuracy in immediate loading restoration maintaining a high aesthetic value.

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COMPARISON OF THE ACCURACY OF INTRAORAL SCANNING AND CONVENTIONAL IMPRESSION TECHNIQUES ON IMPLANTS: A REVIEW

L. ARCURI[1], C. LORENZI[1], A. VANNI[2], N. BIANCHI[1], A. DOLCI[1] and C. ARCURI[1]

[1]Department of Chemical Science and Technologies, University of Rome "Tor Vergata", Rome, Italy; [2]Unit of Dentistry and Oral Surgery, University of Pisa, Italy

The aim of this review was to collect available evidence and evaluate accuracy outcomes of dental implant impression techniques, and to compare the accuracy of conventional implant impression *versus* digital implant impression. Inclusion and exclusion criteria were defined by the authors before the start of the study. The inclusion criteria were: all studies published in English language; studies no older than five years; analyzing the accuracy of digital *vs* conventional technique impression on implants. The exclusion criteria were: publications that reported the same data as later publications by the same authors and systematic reviews; commentaries and letters to the editor; case report and case series. The search resulted in 106 titles. Following the first stage of screening, after the records identification through database manual searching, 112 potentially relevant studies were identified. After the second stage screening, 33 full text publications were obtained and analyzed and 17 were excluded. Afterwards, 18 articles resulted eligible after full text reading and a cross search of the articles' references was accomplished; 3 articles were consequently added. At the end only 7 articles were included in the quantitative analysis. Within the limitations of this systematic review, digital impression on dental implants offers a comparable accuracy compared with conventional impressions technique. More clinical trials are recommended to investigate the accuracy of these scanners and their validity in clinical use.

Dental implants have improved the care of partially and completely edentulous patients for several decades (1). Dental implants are an alternative treatment to conventional dentures for patients who have lost their teeth, for example for restorative and endodontic failure (2, 3), and they can provide long-term stability and comfort also in patients with oral lesions (4-9). They require an initial operation to facilitate the fixation of the dental implant into the bone (10-13) then in a second step a prosthesis is connected to the screw. From a biomechanical perspective, a poor-fitting prosthesis connected to the dental implant can increase stresses and tensions in the surrounding bone, leading to marginal bone loss, which may in turn lead to implant loosening and subsequent implant failure (14). The passive fit of prosthodontic frameworks is critical to prevent mechanical and biological complications in multiple implant rehabilitation. Misfitting prostheses may negatively affect the long-term success rate of full-arch fixed implant rehabilitations, particularly in immediate loading procedures (15-19).

Impression deformations and errors during dental laboratory procedures greatly contribute to generate misfits in implant prosthodontics (19-21) and in indirect complex rehabilitation (22). The position of dental implants is recorded and transferred

Key words: dental implant; digital impression; implant impression; digital dentistry; intraoral scanning; intraoral scanner

Corresponding Author:
Dr. Lorenzo Arcuri,
Department of Chemical Science and Technologies,
University of Rome "Tor Vergata",
Rome, Italy
e-mail: lorenzo.arcuri.1990@gmail.com

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to a working stone cast for the manufacturing of implant-supported prosthesis. The correct transfer of each implant position in relation to neighboring implants or teeth is paramount for the design and fit of implant-supported prosthesis and therefore for long-term success of implant therapy avoiding mechanical and biological complications (23).

An accurate implant impression is an integral prerequisite for obtaining an accurate master cast which is the key for the fabrication of an accurately fitting prosthesis (24, 25). The transfer of implant positions with conventional, intraoral optical or extraoral optical methods is the starting point for the production process of implant-supported prosthesis. Multiple studies examined and compared the accuracy of different implant impression techniques. However, intraoral implant positions must be transferred to an extraoral reference model for the assessment of the accuracy of intraoral impressions. The technique with the least assumed error is used to create a reference model and novel methods are compared with the previously created reference model (23, 26, 27). Dental stone intraoral impressions are still the preferred method in implant dentistry prostheses and have long been successful in clinical practice, despite the fact that dental stone is likely to undergo size variations resulting from ongoing chemical reactions (26), and because of secondary reactions while setting (27). Because this impression procedure is the foundation of prosthetics, errors at this stage will affect the rest of the process (28, 29).

With current computer-aided design – computer aided manufacturing (CAD/CAM) techniques, digital technologies once limited to the scanning and milling of tooth-supported fixed prostheses can now support the implant impression process (30-32). Direct intraoral scanning of an implant cannot only create a three-dimensional (3D) virtual model to design and mill the restoration but can also be used to fabricate a milled model with a removable implant analogue (33). Digital impression procedures have been recently introduced in fixed and implant prosthodontics as, by their nature, these procedures may eliminate the errors with conventional impressions and stone casts (34-38).

Digital impressions using an intra-oral optical scanner (IOS) eliminate tray selection, dispensing and polymerization of impression materials, and disinfection and shipping to the laboratory, while patient comfort is an additional advantage (21, 23). A digital impression can be created in one of two ways: via direct intra-oral scanning or indirectly via scanning casts made from conventional impressions. If scanned directly from the mouth, digital data can be sent electronically to a milling unit that uses this data to fabricate a digital model, thus, intermediate steps involved in conventional impression-taking are bypassed. Accuracy is described by precision and trueness (ISO 5725-1) (39), and is a key aspect in function and aesthetics of indirect restorations (1, 22).

Achieving passive fit of implant prosthesis is identified as an important factor for the long term success of implant supported fixed dental prostheses (IFDP) (40, 41). Although passive fit is yet to be clearly defined by clinical criteria, clinical studies have reported that the acceptable misfit between implant platform and prosthesis varies from 59 to 150 μm (42, 43). Various factors can contribute to misfit of the IFDP, such as the accuracy of implant impression, the master cast fabrication, and the prosthesis fabrication procedures. Understanding the impact of these factors allows the fabrication of an accurate master cast, which reduces the possibility of framework misfit (44-51).

The introduction of digital impressions using IOS into the fields of fixed and implant prosthodontics aims to aid in achieving this goal while it has the advantages of the elimination of tray selection, reduced risks of distortion during impression-making, pouring, disinfecting, and shipping to the laboratory, potentially increased patient comfort and acceptance and, finally, electronic storage as digital information, leading to better efficiency and reduced costs (24). In a digital workflow for the fabrication of implant prostheses, deviations in accuracy (trueness and precision) have been reported when making a digital impression in similar fashion to the conventional approach (40, 52-54). The aim of this review was to collect available evidence and evaluate accuracy outcomes of dental

implant impression techniques, and to compare the accuracy with the conventional implant impression *versus* digital implant impression.

MATERIALS AND METHODS

Inclusion and exclusion criteria were defined by the authors before the start of the study. The inclusion criteria were: all studies published in English language; studies analyzed not older than five years; studies analyzing the accuracy of digital *vs* conventional technique impression on implants. The exclusion criteria were: publications that reported the same data as later publications by the same authors; systematic reviews; commentaries and letters to the editor; case reports; and case series.

Types of interventions

All the works examined compared a reference model with digital and conventional impression on implants. Accuracy was evaluated by superimposing the digital files of each test group onto the digital file of the controls with inspection software.

Outcome measures

Each study evaluated the distortion of conventional or digital impression in angular or linear deviation or in mean of volumetric deviation from the master model. Even though not all the studies adopted the same criteria, they were always well specified in the publications included.

Research Question

Pico question. We formulated a specific question according to the Participants, Interventions, Control, and Outcomes (PICO) format. The question was: "Are (O) digital impressions as accurate (I) as conventional method (C) for dental implant restoration (P)?".

An electronic search of publications was established from three electronic databases: Cochrane, PubMed, Cochrane and Web of Science. The search strategy used a combination of controlled vocabulary and free-text words. The detailed search design and strategies, including keywords, developed for each database are presented below.

Search strategy

The search strategy involved searching electronic databases (MEDLINE, EMBASE, and COCHRANE LIBRARY) and was supplemented by cross-checking the bibliographies of relevant review articles. We used two filters to follow data for the research: publication dates about 5 years, and publications in English. The following combination of words was used: (dental prosthesis retention) AND dental impression technique[MeSH Terms]) OR dental impression materials[MeSH Terms]) OR dental impression) OR (dental and impression)) OR digital vs conventional impression) OR (digital and conventional impression)) AND digital dentistry) OR digital) AND dentistry) NOT natural tooth)) AND (digital vs conventional impression) AND (digital and conventional impression)) AND dentistry[MeSH Terms]) AND dental prosthesis) OR dental prosthesis) AND implants) OR Dental implants single tooth[MeSH Terms]) AND (dental implants) AND (implants and prostheses[MeSH Terms])) OR dental prostheses, implant supported[MeSH Terms]) AND implant impression) AND accuracy) OR implant) AND impression) AND "last 5 years"[PDat] AND English[lang]).

Selection criteria and data extraction

The search resulted in a great number of published studies on the topic, so a three-stage screening process was performed by two independent reviewers (C.L. and N.B.) and disagreement between the two reviewers was resolved after additional discussion. All the titles were screened to eliminate irrelevant publications, review articles and animal studies. All abstracts of publications selected during the first screening were analyzed, excluding studies comprised in the exclusion criteria. Through analysis of the whole selected full texts, the study eligibility was based on the predetermined inclusion and exclusion criteria. A table with data from all the included studies was created and the results were discussed.

RESULTS

Identified articles

The search resulted in 106 titles. Following the first stage of screening, after the records identification through database manual searching, 112 potentially relevant studies were identified. After the second stage screening, 33 full text publications were obtained and analyzed and 17 were excluded.

Afterwards, 18 articles resulted eligible after full text reading and a cross search of the articles references was accomplished; 3 articles were consequently added. In the end only 7 articles were included in the quantitative analysis (Table I).

DISCUSSION

Accuracy of digital impressions has not been widely studied to date. Verification of accuracy of a digital impression in the implant field should be a prerequisite for the clinical application of any new technology. The primary purpose of this systematic review was to evaluate the accuracy of digital implant impression techniques compared with conventional techniques. In general, from 7 included studies, 2 (52, 53) reported digital impressions that were less accurate than the conventional ones, 3 reported digital impressions that were more accurate (14, 53) than the conventional ones, and another 2 found almost similar accuracy between digital and conventional impressions (26, 42). All included studies made comparisons between digital and conventional methods, however, almost each study had its unique methodology and study design, which can be stratified into 3 main approaches (i.e., measuring distance and angulation errors, evaluating 3D or 2D discrepancies). The scanners used were different and various brands were applied for conducting the experiments (e.g., iTero, TRIOS, True Definition, CEREC OMNICAM). On the other hand, various conventional impression techniques were also used (e.g., splinted, non-splinted, open-tray, closed-tray, direct impression). The included studies used direct digitization for scanning the master models, so the indirect digitization was not considered as representative of the digital impression in this study. The digitalization of the master model was carried out using different scanners in each study as shown in Table II. However, conventional impressions were digitized in all the studies in order to make comparisons.

Papaspyridakos et al. (24) compared a digital impression system (Trios) with several conventional

Table I. *Study selection process.*

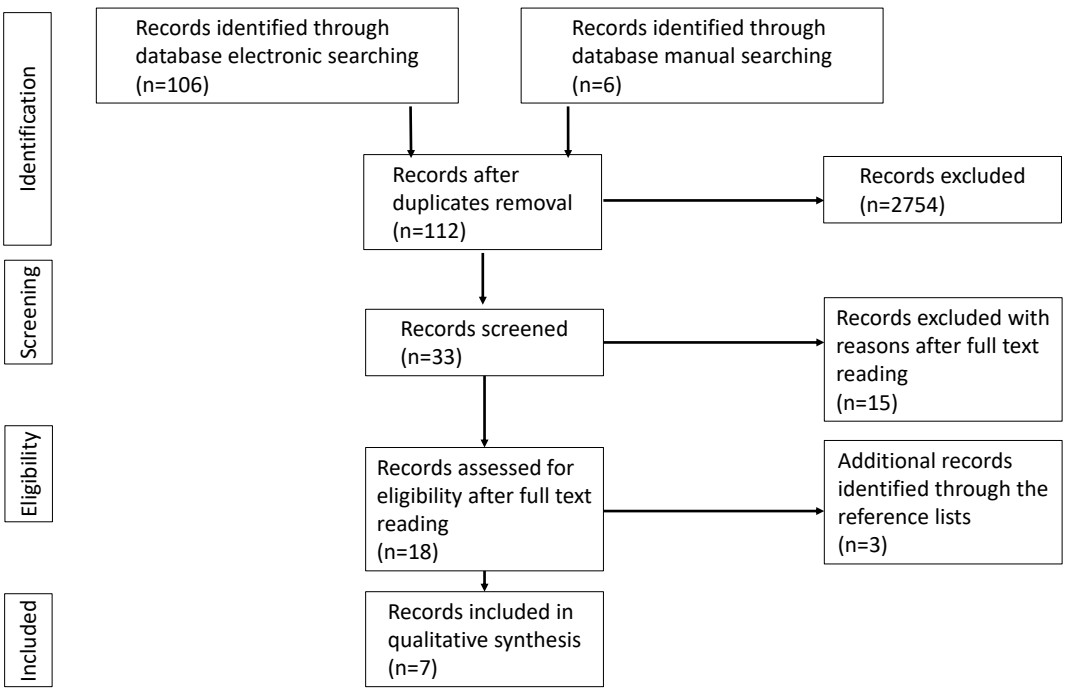

Table II. *Characteristics and main findings of included studies.*

	N of implants	N of impressions	Type of study	Master cast	Conventional impression method	Digital impression scanner	Master cast and conventional impression digitalization		measured values
Sarah Amin et al	5	30 (n= 10 for each of the three group)	In vitro	A stone master cast representing an edentulous mandible using five internal connection implant analogs	Splinted open-try technique with Polyether	-CEREC Omnicam. -3M True Definition	High-resolution reference scanner (Activity 880 scanner; Smart Optics, Bochum, Germany)	Geomagic	Differences in accuracy between the three groups
Amelia A. Chew et al	2 for each of the two model	40 (n= 5 for each of the eight group)	In vitro	Two-implant master models were used to simulate a three-unit implant-supported fixed dental prosthesis. The models have different implant levels—bone level (BL) and tissue level (TL).	Closed-try conventional impression with Polyether	-TRIOS Color (3Shape) -iTero -True Definition Scanner (3M ESPE)	Coordinate measuring machine (CMM)	-	linear distortions (dx, dy, dz), global linear distortion (dR), angular distortions (dΘy, dΘx), and absolute angular distortions (AbsdΘy, AbsdΘx) between the master models, test models, and .stl files of the digital scans.
Sang J. Lee et al	1	60 (n=30 for each of the two group)	In vitro	A customized maxillary model (Models Plus, Kingsford Heights, Ind) con- taining a single implant (Bone Level, Regular Crossfit; Straumann, Basel, Switzerland) located in the maxillary left second premolar position was prepared and used as a reference model.	Vinyl polysiloxane	iTero	Lava Scan ST; 3M ESPE, Seefeld, Germany	Geomagic	The divergences in the x- , y -, and z-axes between each reference and test data- set at 10 specified contact locations were measured.
Wei-Shao Lin et al	2 for each of the four model	80 (n=10 for each of the eight group)	In vitro	Four customized epoxy resin master casts simulating a Kennedy Class II mandible were fabricated, and two implant analogs were placed in the posterior edentulous mandible with different amounts of me- siodistal divergence (0, 15, 30, and 45 degrees) in each master cast. The distance between the implant restor- ative platforms was 10 mm, and the platforms of the implant analogs were placed 1 mm coronal to the cast surface	Polyvinyl siloxane	iTero	Cagenix, Cagenix Inc	-	Distance and angle measurements were taken
Amin Marghalani et al	2 for each of the two model	60 (n=10 for each of the six group)	In vitro	Two partially edentulous mandibular casts with 2 implant analogs with a 30-degree angulation from 2 different implant systems (Replace Select RP; Nobel Biocare and Tissue level RN; Straumann)	A splinted open-tray implant-level conventional impression technique was used for the Nobel Biocare implant models (NC group) and the Straumann implant models (SC group).	-CEREC Omnicam. -True Definition Scanner (3M ESPE)	Activity 880 scanner; Smart Optics	Geomagic	Accuracy was evaluated by superimposing the digital files of each test group onto the digital file of the controls with inspection software.
Panos Papaspyridakos et al	5	50 (n=10 for each of the five group)	In vitro	A stone cast of an edentulous mandible with five implants was fabricated to serve as master cast (control) for both implant- and abutment-level impressions	For the conventional polyether impressions of the master cast, a splinted and a non-splinted technique were used for implant-level and abutment-level impressions	TRIOS	IScan D103i	Mimics	measured deviations between the control dataset and the test data-set
Paulo Ribeiro et al	4 for each of the two model	70=40 (n= 10 for each of the four groups) for the first group and 30 (n=10 for each of the three group)	In vitro	Two different master models with 4 implants equidistant in the intermentoneal region were created, one with parallel implants (model 1) and the other with non-parallel implants (model 2).	Polyether -Closed tray impressions with replacement abutments -Open tray impression groups for dragging copings, without splinting -Open tray impressions for ferrules	True Definition Scanner (3M ESPE)	Imetric IScan D104i, Porretruy, Switzerland	Geomagic	deviations in three dimensions

	The mean of the RMS values for the overall 3-D deviations		Linear distortion dx		Linear distortion dy		Linear distortion dz		Angular Distortion on Y-axis		Angular Distortion on X-axis		Volumetric deviations in distance measures		Median value
	Digital	Conventional	Digital	Conventional	Digital	Conventional	Digital	Conventional	Digital	Conventional	Digital	Conventional	Digital	Conventional	Digital
Panos Papaspyridakos et al	-Omnicam 46,41 -True definition 19,32	167,93													
Panos Papaspyridakos et al			BONE LEVEL: TRIOS -0,032 iTero -0,028 True Definition -0,029 TISSUE LEVEL TRIOS -0,040 iTero -0,008 True Definition 0,018	Bone level conventional: -0,016 Tissue level conventional: -0,026	BONE LEVEL: TRIOS 0,034 iTero 0,009 True Definition 0,023 TISSUE LEVEL TRIOS 0,000 iTero 0,030 True Definition 0,020	Bone level conventional: 0,012 Tissue level conventional: 0,002	BONE LEVEL: TRIOS -0,037 iTero -0,030 True Definition 0,013 TISSUE LEVEL TRIOS 0,007 iTero -0,029 True Definition 0,024	Bone level conventional: -0,016 Tissue level conventional: -0,011	BONE LEVEL: TRIOS 0,105 iTero 0,191 True Definition 0,315 TISSUE LEVEL TRIOS 0,089 iTero 0,230 True Definition 0,206	Bone level conventional: 0,058 Tissue level conventional: 0,186	BONE LEVEL: TRIOS 0,206 iTero 0,154 True Definition 0,226 TISSUE LEVEL TRIOS 0,066 iTero 0,160 True Definition 0,195	Bone level conventional: 0,090 Tissue level conventional: 0,196			
Panos Papaspyridakos et al									0,088	0,093	0,034	0,011	Buccal cusps -0,012 Lingual cusps -0,014 Fossae -0,017 Contact areas 0,005	Buccal cusps -0,018 Lingual cusps -0,019 Fossae 0,016 Contact areas 0,001	
Panos Papaspyridakos et al									0 deg 1,598 15 deg 2,261 30 deg 0,722 45 deg 1,248	0 deg 0,612 15 deg 0,710 30 deg 0,718 45 deg 0,810			-0,530	0,006	
Panos Papaspyridakos et al															Omnicam for Nobel Biocare implant system 20 True Definition for Nobel Biocare implant system 15 Omnicam for Straumann implant system 26 True Definition for Straumann implant system 17
Panos Papaspyridakos et al															Group1 7,42 Group 2 17,65 Group 4 13,05 Group 5 8,23
Paulo Ribeiro et al			**Model 1 (without angulation)**: Group D (using the 3MTM True Definition Scanner) = 15 **Model 2 (with angulation):** Group G (using the 3MTM True Definition Scanner) = 32	**Model 1 (without angulation):** -Group A (closed tray impressions with replacement abutments) = 20 -Group B (open tray impressions for dragging copings, without splitting) = 36 -Group C (open tray impressions for ferrules) = 33 . **Model 2 (with angulation):** -Group E (closed tray impressions with replacement abutments) = 26 -Group F (open tray impressions for dragging copings, without splitting) = 17	**Model 1 (without angulation)**: Group D (using the 3MTM True Definition Scanner) = 17 **Model 2 (with angulation):** Group G (using the 3MTM True Definition Scanner) = 22	**Model 1 (without angulation)**: -Group A (closed tray impressions with replacement abutments) = 22 -Group B (open tray impressions for dragging copings, without splitting) = 36 -Group C (open tray impressions for ferrules) = 26. **Model 2 (with angulation):** -Group E (closed tray impressions with replacement abutments) = 21 -Group F (open tray impressions for dragging copings, without splitting) = 15	**Model 1 (without angulation)**: Group D (using the 3MTM True Definition Scanner) = 15 **Model 2 (with angulation):** Group G (using the 3MTM True Definition Scanner) = 32	**Model 1 (without angulation)** -Group A (closed tray impressions with replacement abutments) = 26 -Group B (open tray impressions for dragging copings, without splitting) = 21 -Group C (open tray impressions for ferrules) = 11. **Model 2 (with angulation):** -Group E (closed tray impressions with replacement abutments) = 24 -Group F (open tray impressions for dragging copings, without splitting) = 15							

		Valori misurati
	Conventional	
Panos Papaspyridakos et al		Differences in accuracy between the three groups
Panos Papaspyridakos et al		linear distortions (dx, dy, dz), global linear distortion (dR), angular distortions (dΘy, dΘx), and absolute angular distortions (AbsdΘy, AbsdΘx) between the master models, test models, and .stl files of the digital scans.
Panos Papaspyridakos et al		The divergences in the x- , y -, and z-axes between each reference and test data- set at 10 specified contact locations were measured.
Panos Papaspyridakos et al		Distance and angle measurements were taken
Panos Papaspyridakos et al	Conventional for Nobel Biocare implant system 39 Conventional for Straumann implant system 22	Accuracy was evaluated by superimposing the digital files of each test group onto the digital file of the controls with inspection software.
Panos Papaspyridakos et al	19,38	measured deviations between the control dataset and the test data-set
Paulo Ribeiro et al		deviations in three dimensions

(polyether splinted and non-splinted) impression techniques for the registration of five implants in edentulous mandibles. They reported that there were no significant differences between the digital and conventional impression methods and concluded that digital impressions could be used for implant impressions in edentulous jaws. Ribeiro et al. (14) demonstrated that for a model with four parallel implants, the deviations of the digital impressions were smaller than those associated with the conventional techniques. This improvement was not observed when using a model with four angled implants, however, where the conventional techniques yielded similar results. Therefore, digital impressions of full-arch models were able to achieve the accuracy of conventional impressions in an *in vitro* model. Another study (43) superimposed digital and conventional impressions of 5 implanted full-arch master models and found almost similar 3D deviations between the 2 groups. Only the digitally fabricated single unit framework showed bigger marginal discrepancies and inferior passivity-of-fit than the conventional ones. This is in contrast to an *in vitro* study by Lee et al. (33), which showed that milled models from a digital impression were comparable to gypsum models from conventional impression in a single implant impression. The in *vitro study* by Marghalani et al. (43), comparing Nobel Biocare and Straumann by using the conventional impression technique, the Straumann system had significantly better accuracy than the Nobel Biocare system. This may be attributed to the larger impression copings providing more stability to the splint. Using the Omnicam technique, the Straumann system was significantly less accurate than the Nobel system, but both systems had accuracy within clinically acceptable levels. However, using the True Definition technique, no statistically significant differences were found between the 2 implant systems. Chew et al. and Lin et al. (41, 52) also found that 3D discrepancies of conventional impressions are lower than a digital one for bone-level test groups. Lin (41) demonstrate that at interimplant angulation 0 to 15°, digital impressions were less accurate than conventional ones but the accuracy would be improved with increasing interimplant angulation up to 30 and 45°.

Within the limitations of this systematic review, digital impression in implants offers a comparable accuracy compared with conventional impression techniques. Two of the analyzed studies found that accuracy of digital impressions is lower than a conventional technique. The others articles encourage the use of intraoral scanners in the implant field. More clinical trials are recommended to investigate the accuracy of these scanners and their validity in clinical use.

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HELICOBACTER PYLORY AND ORAL DISEASES

F. GABRIONE[1], A. BARLATTANI Jr[2], P. AVANTAGGIATO[3], M. BALDONI[4] and M. GARGARI[2]

[1]Private practice, Milan, Italy; [2]Department of Clinical Science and Translational Medicine, University of Rome "Tor Vergata", Rome, Italy; [3]Universidad Europea Madrid, Madrid, Spain; [4]Department of Medicine and Surgery University Milano Bicocca, Italy

***Helicobacter pylori* (*H. pylori*) gastric infection is considered one of the most common human infections. It occurs in half of the world population and is the most common cause of adenocarcinoma of the distal stomach. The risk of developing gastric cancer is believed to be related to differences among *H. pylori* strains and the inflammatory responses mediated by host genetic factors. The accepted evidence is that the *H. pylori* strains reach the stomach by ingestion through the mouth and, because of its non-invasive nature, the stomach is the definitive site for colonization. One of the key issues related to the eradication of gastric *H. pylori* has been the importance of oral hygiene and periodontal procedures. Dental plaque control and periodontal therapy can prevent gastric *H. pylori* infection recurrence for patients with gastric diseases associated with *H. pylori*.**

Helicobacter pylori (*H. pylori*) gastric infection is considered one of the most common human infections (1). It occurs in half of the world population (2, 3) and is the most common cause of adenocarcinoma of the distal stomach (4). The risk of developing gastric cancer is believed to be related to differences among *H. pylori* strains and the inflammatory responses mediated by host genetic factors (3-6). *H. pylori* is known to be acquired early in life (7, 8), and is likely to be transmitted from person to person. The transmission is supported by crowded living conditions, accompanied by poor hygiene and intra-familial clustering. Nevertheless, the exact manner of transmission is not completely understood (4-10). The accepted evidence is that the *H. pylori* strains reach the stomach by ingestion through the mouth, and because of its non-invasive nature, the stomach is the definitive site for colonization (10). Evidence of transmission via the oral route includes the high prevalence of *H. pylori* among African children whose mothers premasticate their food before offering it to them (8), and to the higher prevalence of the infection in people who share chopsticks (11). The presence of *H. pylori* in the oral cavity was first reported in 1989 when the bacterium was cultured from dental plaque of a patient with gastric disease associated with *H. pylori* infection (12).

Recently, questions have been raised concerning the possibility that the oral cavity is an *H. pylori* reservoir participating in infection transmission or a nidus for reinfection after eradication therapy or that it plays a role in oral disease. However, the results of the studies on this subject are controversial. Different groups have found discrepant results, ranging from 0-100% positivity for *H. pylori* in the oral cavity by polymerase chain reaction (PCR) methods (13, 14). In addition, inconsistent correlation between *H. pylori* positivity in the oral mucosa and in the stomach has been found. Many factors likely account for this variability, including the genetic background of the study population, cultural habits, socioeconomic level and differences in the accuracy of the methods used as well as the strain of bacteria.

H. pylori was detected in different niches of the

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Corresponding Author:
Dr. Gabrione Federica,
Via Carlo Marx 111/A,
20099 Sesto san Giovanni (MI), Italy
e-mail: federicagabrione20@gmail.com

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oral cavity: periodontal areas, mucosa and saliva. Periodontal pockets (15-17), implant sites (18-28) and prosthetic restoration (29-32) are matter for investigation. High prevalence of *H. pylori* was detected in the oral cavities of periodontitis patients (33). *H. pylori* was identified in subgingival plaque of chronic periodontitis, in dental plaque of periodontitis and gastric disease patients (34, 35), in dental plaque of healthy patients (36) and, with a higher significant prevalence, in saliva and subgingival biofilm of patients with chronic periodontitis *versus* periodontally healthy non-dyspeptic patients (37). Another study related an association between poor oral health and the high prevalence of peptic ulcers caused by *H. pylori* (38). There was also an association between the presence of *H. pylori* in the dental plaque and gastritis (36). Oral lesions (17, 28, 39-43) and imperfect dental restoration (25, 26, 44) could be additional reservoirs. A positive link was observed between *H. pylori* infection and periodontal disease (45), and an association was established between the supragingival colonization of *H. pylori* and oral hygiene parameters such as the presence of plaque and gingival bleeding (46).

Gastric infections caused by *H. pylori* are treated with systemic therapy (antibiotics and proton pump inhibitors). Elimination of the infection is known to reduce the risk of ulcer relapse. However, the recurrence of the infection can occur. In view of the difficulties in achieving complete eradication of *H. pylori* from the gastric mucosa, it is essential to identify potential reservoirs of this microorganism that could be responsible for refractoriness to eradication therapy (gastric therapy or gastric polytherapy).

It has been demonstrated that *H. pylori* in the oral cavity affected the outcome of eradication therapy (47) and was associated with a recurrence of gastric infection (47). Oral colonization of *H. pylori* in dental plaque can persist after eradication therapy. Therefore, even if eradication therapy is successful, dental plaque might be a sanctuary for *H. pylori*, leading to gastric recurrence (48). *H. pylori* in dental plaque may represent a risk factor for gastrointestinal reinfection and ulcer relapse after antibiotic therapy. The periodontal pocket may be important as a natural reservoir for *H. pylori*, because it can provide microaerobic conditions (49). Oral cavity of gingivitis or chronic periodontitis patients who are positive for *H. pylori* in their stomachs can harbour *H. pylori* after systemic eradication (33). Recently, a meta-analysis confirmed the close relationship between the infection of *H. pylori* in the oral cavity and the stomach. *H. pylori* in the oral cavity is more difficult to be eradicated than in the stomach and may be a source of reinfection (50). Oral health and a pleasant smile are a goal of every person and a civil society. Therefore all dental disciplines must contribute to this goal and all dentists must guarantee their patients the best possible care.

CONCLUSION

One of the key issues related to the eradication of gastric *H. pylori* is the importance of oral hygiene and periodontal procedures. Specifically, the question of the effect of periodontal treatment on gastric *H. pylori* elimination is relevant clinically and not very well documented. Dental plaque control and periodontal therapy can prevent gastric *H. pylori* infection recurrence for patients with gastric diseases associated with *H. pylori*.

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WHY PATIENTS WITH CARDIOVASCULAR RISK SHOULD GO TO DENTIST: IS THERE SUFFICIENT EVIDENCE OF INFLUENCE OF PERIODONTAL THERAPY ON CARDIOVASCULAR DISEASE?

L. OBERTI[1], P. AVANTAGGIATO[2], A. PELLATI[3] and L. ARCURI[4]

[1]Private practice, Milan, Italy; [2]Universidad Europea Madrid, Madrid, Spain; [3]Department of Morphology, Surgery and Experimental Medicine, University of Ferrara, Ferrara, Italy; [4]Department of Clinical Science and Translational Medicine, University of Rome "Tor Vergata", Rome, Italy

Cardiovascular disease (CVD) is a common cause of death, representing 29% of the mortality all over the word. More than 70 million Americans have been diagnosed with various forms of CVD, including high blood pressure, coronary artery disease (acute myocardial infarction and angina pectoris), disorders of peripheral arteries. There is strong evidence that periodontal disease (PD) is associated with an increased risk of CVD. In addition, many patients with CVD are also affected by PD, which can be mild or severe. The aim of this manuscript is to investigate the effects of periodontal therapy on the management of CVD. Thirty-four randomised controlled trials and reviews were included in this manuscript to test the effects of different periodontal therapies for patients with CVD. In conclusion, we may affirm that there is some lack of knowledge on the relationship between PD and CVD, however there is sufficient evidence to justify a periodontal treatment to prevent CVD; in fact, PD is very prevalent in the middle-aged population and can have a significant impact on the cardiovascular function.

For decades doctors and dentists have turned their attention to their respective fields of action. However, recent studies have strongly suggested that oral health may be indicative of systemic health. Currently, this gap between medicine and dentistry is rapidly shrinking, because there are substantial data supporting the idea of a close association between periodontal disease (PD) and systemic conditions, such as cardiovascular disease, diabetes, adverse reactions in pregnancy, osteoporosis, oral lesions (1-10). Therefore, there is reason to hope that the strong evidence identified to date by these studies could lead to significant improvements in treatment of periodontal infections (6, 11-20) and may bring about an improvement of systemic conditions also. For this reason, researchers must continue not only to discover more information on the correlation between PD and systemic diseases, but also focus their attention on the positive associations that may arise from the treatment of the oral cavity as a way to increase the healing of systemic diseases.

The term periodontal disease (PD) is generally used to describe diseases affecting the gums and tooth support tissues, causing damage to the connective tissue and alveolar bone (21). PD is caused by specific bacteria of the biofilm formed by plaque. The bacteria leak into the periodontal ligament space, causing anaerobic infection and creating a cascade of events which end with the production of inflammatory mediators and bacterial metabolites (22). At least two potential metabolic pathways could develop from the periodontal pocket and as a result

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Corresponding Author:
Dr. Oberti Luca,
Via IV Novembre 20,
24048 Treviolo (BG) Italy
e-mail: luca.oberti@outlook.it

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of this condition could lead to a localized infectious-inflammatory disease involving other organs. The first step is the passage of periodontal pathogens and their products through the ulcerated epithelium into the bloodstream, leading to bacteremia and/or causing a systemic immune response. The second step is the passage of inflammatory mediators from the periodontal pocket into the bloodstream (22).

The pathological mechanisms by which PD may contribute to the pathogenesis of systemic inflammatory diseases are currently the topic of intensive research. Thousands of different kinds of bacteria and various viruses are associated to the composition of the plaque and are involved in the etiopathogenesis of PD. The most frequently identified include three species called "red complex": *Porphyromonas gingivalis, Tannerella forsythia, and Treponema denticola* (23).

Periodontal disease and cardiovascular disease

Cardiovascular disease (CVD) is a common cause of death, representing 29% of the mortality all over the word. More than 70 million Americans have been diagnosed with various forms of CVD, including high blood pressure, coronary artery disease (acute myocardial infarction and angina pectoris), disorders of peripheral arteries etc. The main cause of these CVDs is atherosclerosis, which is responsible for 50% of deaths in the United States, Europe and Japan (24).

After eliminating other risk factors, some studies indicate that severe PD is associated with an increased risk of CVD ranging from 25% to 90%; 8.91% of patients with CVD are also affected by PD, which can be mild or severe. While 66% of subjects with CVD also suffer from PD (25). These data must be explained also by the fact that CVD and PD share similar risk factors such as smoking, diabetes mellitus, obesity and hypertension (26). In literature some studies demonstrated a mild-moderate association between CVD and PD (27-29).

In one study, Ziegler et al. (30) demonstrated that the depth of pathological periodontal pockets is related to diastolic blood pressure in obese adolescents. This association was unaffected by other risk markers for cardiovascular events or periodontal disease. In addition, Kumar reported that a persistent infection, such as chronic periodontitis, may influence changes in the systemic levels of high-sensitivity C-reactive protein (HsCRP), LDL and HDL, which potentially have an impact on inflammation-associated atherosclerotic processes, such as CVD (31). Another study affirmed that PD was a significant risk factor in people aged over 40 years, who had coronary artery disease proved by coronary angiography (32). Another clinical trial provided new evidence regarding the positive effect of periodontal treatment on risk markers for recurrence of cardiovascular events in stable coronary artery disease patients (33). A recent randomized controlled trial concluded that the use of Triclosan-toothpaste had influence on biomarkers of cardiovascular risk. However, it is unclear whether this influence is clinically significant. The reason why PD therapy could modify CVD management is still unknown (34).

On the contrary, a recent study found insufficient evidence to support or refute whether periodontal therapy can prevent the recurrence of CVD in the long term in patients with chronic periodontitis. No evidence on primary prevention was found (35). Likewise, in the study previously cited, Thomopoulos stated that from the clinical point of view, advising periodontal prevention or treatment targeting on the prevention of CVD is unjustified. By contrast, oral hygiene including periodontal health might contribute to an overall well-being and healthy lifestyle and therefore may, at least partially, contribute to cardiovascular prevention (36).

Hypothesis on mechanism of action

The presence of pathogenic bacteria correlated with periodontal disease may be localized in plasma or ateromasic plaques (37, 38). Investigating these plaques, Cairo et al. identified various bacteria: *T. forsynthensis* (79%), *F. nucleatum* (63%), *P. intermedia* (53%), *P. gingivalis* (37%) and *A. actinomycetemcomitans* (5%) (39). These bacteria can also be found in the carotid, coronary and occluded arteries of patients who have Buerger disease (40). The pathogens induce the release of inflammatory cytokines; even normal chewing

contributes to the spread of pro-inflammatory cytokines from the mouth into the bloodstream (TNF, IL-1 and PGE2) (41). Animal studies have been able to demonstrate atherosclerosis induced by pathogenic bacteria of PD (42).

The presence of acute or chronic infections may play a significant role on CVD onset, causing increased vascular inflammation and thrombosis promotion (43, 44). These infections may be considered a secondary pathogenic pathway (45, 46), among which PD might be the most common.

There are two main hypothesised ways for pathogenesis: i) direct invasion by the bacteria into the endothelial vascular wall; and ii) release, in response to infection, of inflammatory mediators with atherogenic systemic effects. These micro-organisms, in particular the species *P. gingivalis*, have demonstrated the ability to interact with the surface of endothelial cells and induce smooth cell proliferation (47, 48). In addition, platelets could be activated and aggregated by periodontal pathogens present in the atheromas through collagen-like platelet aggregation-associated protein expression. The activated and aggregated platelets could induce atheroma formation and thrombosis, finally leading to CVD (49).

In patients with CVD, the serological inflammatory markers are significantly higher, suggesting that CVD may contribute to inflammation of the soft tissues, causing gingivitis, periodontitis or pericoronitis. A high proportion of patients with hypertension develop PD, and many antihypertensive medications may cause xerostomia that increase oral mucosa inflammation (50). As previously cited, the reason why PD therapy could modify CVD management is still unknown, but a recent study reports that microparticles could be the missing link between PD therapy and improvement in PD management. In fact, the rational of considering periodontitis as risk factor for systemic disease is the passage of inflammatory cytokines and/or bacteria into the bloodstream, thus causing systemic disease in distant organs. Membrane microparticles are released by multiple cells in inflammatory environments. Recent data suggested the role of these microparticles in the pathogenic process of many systemic diseases that can be also associated to periodontitis. Periodontitis could therefore be a chronic reservoir of microparticles, hence partially elucidating the interaction with systemic disease initiation or progression (51).

Other studies confirm that periodontal therapy improves serological markers of CVD. In fact, when PD is active, the levels of systemic inflammation markers are increased and, when PD is treated, the level of systemic inflammatory mediators significantly decrease (52, 53). Oral health and a pleasant smile are a goal of every person and a civil society. Therefore all dental disciplines must contribute to this goal and all dentists must guarantee their patients the best possible care (54-61).

CONCLUSIONS

There is some lack of knowledge on the relationship between PD and CVD. There is sufficient evidence to justify periodontal treatment to prevent CVD. There are factors that can affect both diseases as genetic factors (IL-6, IL-10, Vitamin D-receptor), environmental factors (smoking, diabetes, stress etc.), and impaired immune response. However, PD is highly prevalent in the middle-aged population and can have a significant impact on cardiovascular function.

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CALCIUM SULPHATE FOR CONTROL OF BLEEDING AFTER ORAL SURGERY IN ANTICOAGULANT THERAPY PATIENTS

L. OBERTI[1], A. PIVA[2], G. BELTRAMINI[3,4] and V. CANDOTTO[4]

[1]Private practice, Milan, Italy; [2]Universidad Europea Madrid, Spain; [3]Dental and Maxillo-Facial Surgery Unit, IRCCS Ca Granda Ospedale Maggiore Policlinico di Milano, Milan, Italy; [4]Department of Biomedical, Surgical and Dental Sciences, University of Milan, Milan, Italy

Control of bleeding after oral surgery is mandatory in patients taking anticoagulants. There are different haemostatic measures to prevent post-surgical bleeding. The aim of the present paper is to study the use of a haemostatic agent, calcium sulphate (CaS) (P30, Ghimas, Bologna, Italy) for controlling post-surgical bleeding in a group of patients treated with warfarin therapy for thromboembolic states. Twenty teeth (12 mandibular molars, 8 maxillary molars) in 20 patients (14 male and 6 females) with a mean age of 54.3 years (±10.3 years) were included in the study. The patients were divided into 2 groups; in the study group of 10 patients calcium sulphate was used in layers to fill the socket after extraction, while for the 10 patients in the control group put a gauze with tranexamic acid was put in the extraction site immediately after extraction, and half an hour after extraction. The outcome was bleeding in subsequent days. Bleeding at post-operative day 1 was significant in 5 patients of the control group, however, in the study group treated with calcium sulfate there was no bleeding in any patient (p value 0.0055). CaS demonstrated to be a good haemostatic agent for controlling bleeding after oral surgery in patients taking anticoagulants.

Hemostasis is a defense mechanism composed of different systems in order to preserve the integrity of blood vessels, prevent blood loss and ensure a good flow of the circulatory bloodstream (1, 2). Alterations of hemostasis include a wide range of potential causes such as conditions of deficiency, hereditary and metabolic disorders, and cancer. Hemostatic control is of paramount importance in oral surgery (3-11) and in perio-surgery (12-15). Nowadays, the most common cause of bleeding disorders is the use of drugs (3). Patients with oral pathology diseases (16-23) can chronically use drugs. There are many drugs administrated for the prevention of thromboembolic events, and it is very important for dentists to learn about these drugs, their mechanism of action, complications and methods to prevent such complications. Tissue damage is usually associated with vascular insult resulting in a more or less profuse bleeding (2). The rupture of the vascular endothelium activates hemostasis, exposing different proteins to the blood stream (2, 24-26).

The adoption of appropriate hemostatic measures allows to maintain antiplatelet or anticoagulant therapy, avoiding thromboembolic risk. The incidence of adverse events with uncontrolled bleeding is 0 to 3.5 (27). As a hemostatic measure some authors recommend tranexamic acid, used for post-surgical rinsing to stabilize the blood clot, since it inhibits the stabilization of plasminogen and fibrinolysis (28-31) and appears to have few side effects (nausea,

Key words: calcium sulphate; bleeding; haemostasis; warfarin; anticoagulants; thromboembolic states

Corresponding Author:
Dr. Oberti Luca,
Via IV Novembre 20,
24048 Treviolo (BG),
Italy
e-mail: luca.oberti@outlook.it

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diarrhea, orthostatic hypotension) (29). The rinses are recommended 2 times a day for 2 days. Other authors (30, 31) recommend to use sterile gauze impregnated with tranexamic acid. In this case, the risk of clot dissolution is less than rinsing, due to mechanical compression of the wound.

Other widely used hemostatic agents are: intralveolar oxidized cellulose (Surgicel®), reabsorbable collagen sponges (Octocolagen®, Gelatamp® from Roeko), fibrin adhesive (Tissucol®) or tissue adhesives (Tisuacryl® from Dentsplay) and, of course, sutures. The aim of our study is to use a hemostatic agent, Calcium sulphate (CaS) (P30, Ghimas, Bologna, Italy) for controlling post-surgical bleeding in a group of patients treated with warfarin therapy for thromboembolic states. CaS is used for controlling bleeding during endodontic surgery and surgical-orthodontic treatment of impacted teeth.

MATERIALS AND METHODS

Twenty teeth (12 mandibular molars, 8 maxillary molars) in 20 patients (14 men and 6 woman) with a mean age of 54.3 years (±10.3 years) were included in the study. The patients were selected from patients of 3 dentists working in private practice during 2014. All patients showed an (International Normalized Ratio) (INR) > 3.0 at the moment of surgery due to therapy with warfarin. All patient signed an informed consent form. All patients showed good periodontal health at the moment of extraction. No patient stopped warfarin therapy prior to surgery. The patients were divided into 2 groups: for 10 patients of the study group CaS was used to fill the socket after extraction, while in the 10 subjects of the control group a gauze with tranexamic acid was put in the extraction site immediately after extraction and half an hour after extraction. All dental surgeries were performed in the morning, in order to be able to resolve any bleeding complications in the course of the day. Dental extractions were performed under local anesthesia using articaine with adrenaline 1.100.000 infiltrate in the subperiostium. Patients were asked to wait one hour in waiting room and bleeding was monitored. According to Scully and Wolff protocol (32), the following instructions were given to patients (32):

- Apply pressure with a piece of gauze for 30-40 minutes (only for control group);
- Avoid oral rinses during the first 24 hours;
- Follow a soft and cold diet during the first 24 hours;
- Avoid suctioning movements;
- Avoid touching the socket region with the tongue or manipulation of the operated zone.

All patients returned for post-operative follow-up at days 1, 3, 5, 7. The outcome was bleeding in subsequent days.

Chi-squared test according to Statistical Package for Social Science (SPSS 8.0) was employed for statistical analysis. The value of $p<0.005$ was considered as significant.

RESULTS

Bleeding on post-operative day 1 was significant in 5 patients of the control group, however, in the study group treated with CaS, there was no bleeding in any patient. No fresh clot or blood oozing was present on day 3 in the control group. At 7 day there were no differences in the two groups (Table I).

Statistical analysis

A statistically significant difference in the adequate hemostasis was present in the study group in respect to the control group (*p* value 0.0055).

DISCUSSION

Some authors have compared different methods of hemostasis in patients taking anticoagulants without observing significant differences in the various methods (33, 34). Even the use of sutures is a very effective method. Some authors prefer resorbable sutures as they do not require removal (33-35), on the contrary, other authors prefers non-resorbable; in fact, resorbable sutures remain longer in the oral cavity causing bacteria retention and greater likelihood of post-surgical infections (36-38). Oral health and a pleasant smile are a goal of every oral surgery, therefore all dental disciplines

Table I. *Number of patients showing bleeding in post-operative follow-up.*

	day 1	day 3	day 5	day 7
CaS	0	0	0	0
Tranex acid	5	0	0	0

must contribute to this goal (39-44) and all dentists must guarantee their patients the best possible care.

A significant percentage of the population receives anticoagulant therapy for the prevention and treatment of thromboembolic states such as deep vein thrombosis, pulmonary embolism, cerebrovascular disorders, cardiac disorders and many other prothrombotic states. Failure of hemostasis is one of the main problems of dentists after teeth extraction. In case of excessive bleeding, healing can be delayed as well as increase the risk of infections. The methods most commonly used are local hemostatic measures: sutures, antifibrinolytic agents, intralveolar oxidized cellulose, reabsorbable collagen sponges, fibrin adhesive or tissue adhesives.

In the present study, we describe good hemostasis in patients taking warfarin, using CaS. CaS is highly absorbent and is used for controlling bleeding after oral surgery in patients under therapy with anticoagulants. After application in post-extraction, wound bleeding ceases in 1- 3 minutes. CaS is a resorbable material and is completely reabsorbed in 1-4 months, maybe because CaS reabsorb blood proteins during the intrinsic coagulation pathway. The resorption of Factor XII may be important in clot creation. Our results are similar to the study of Scarano et al. with a statistically significant difference in the adequate hemostasis between the group using CaS and the group managed with sutures ($p = 0.0056$) (38).

There are no standard protocols for the treatment of post-operative bleeding in these patients. In any case, it is important to assess the thromboembolic risk related to the discontinuation of anticoagulation or antiplatelet therapy compared with minor complications of post-surgical bleeding. We must therefore formulate a customized protocol for each patient according to local and systemic conditions.

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ORAL REHABILITATION OF MAXILLA: TECHNICAL CONSIDERATION

D. LENTO[1], A. PIVA[2], G. BELTRAMINI[3,4] and S. MUMMOLO[5]

[1]Private practice, Milan, Italy; [2]Universidad Europea de Madrid, Madrid, Spain; [3]Dental and Maxillo-Facial Surgery Unit, IRCCS Ca Granda Ospedale Maggiore Policlinico di Milano, Milan, Italy; [4]Department of Biomedical, Surgical and Dental Sciences, University of Milan, Milan, Italy; [5] Department of Life, Health and Environmental Sciences, University of L'Aquila, Italy

Oral rehabilitation of edentulous maxilla is particularly difficult because of the lack of bone in correspondence of maxillary sinuses, therefore, the surgeon is forced to place implants in sites where bone is more prevalent. In addition, patients require more frequent oral immediate rehabilitation in order to reduce the discomfort related to wearing a total denture. A viable solution to provide stability and retention of the prosthesis in a short time is represented by the technique of intraoral welding supporting total denture or fixed prosthesis. This goal may be achieved thanks to the technique of welding titanium bars onto implant abutments. In fact, the procedure can be performed directly in the mouth, eliminating the possibility of errors or distortions due to prosthetic procedures. This paper describes a case report and the most recent data regarding long-term success and high predictability of intraorally-welded titanium bar in immediate loading implants.

Immediate loading of implants is a proven technology in oral surgery. It has been demonstrated that it is possible to reduce healing time and immediately load implants with prosthesis on the same day of surgery, exposing implants to occlusal and muscular forces, without failures (1-4). In cases of immediate loading, adequate fixation and immobility of the implants are of utmost importance to prevent the risk of micromovements with regard to surrounding bone (3). In fact, perimplant bone reshapes itself according to the force exerted, and an optimum distribution of forces, both at the level of implants and of prosthesis, is an essential requirement for a successful oral rehabilitation. Thyerefore, in cases of immediate loading, adequate fixation and stability of implants are very important conditions to prevent the risk of micro-movements and loss of implants. In this context, a rigid splinting seems to have an important role in the response of periimplant tissues, since it is able to reduce stress on the implant.

The immediate fixation of implants can be achieved by intraoral welding of abutments. Intraoral welding allows to obtain immediate retention of osseointegrated implants. This method consists of welding a titanium bar directly to the abutments in the oral cavity before immediate loading. With this method, many steps related to prosthetic procedures are eliminated, reducing patient discomfort and achieving functional and aesthetic results (5, 6).

Degidi and coll. (1-7) introduced this method and have published numerous studies on immediate loading of multiple implants by welding a titanium bar directly onto abutments in order to create a metal-reinforced temporary or definitive restoration. In 2006 Degidi and coll. (1) published a new

Key words: welded titanium bars; immediate loading implants; oral rehabilitation

Corresponding Author:
Dr Stefano Mummolo,
Department of Life, Health and Environmental Sciences,
University of L'Aquila,
Piazza Salvatore Tommasi, 11, L'Aquila, Italy
e-mail: stefano.mummolo@univaq.it

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method defined syncrystallization. This technique consists of splinting multiple implants with a rigid titanium bar welded onto abutments. It presents the advantage of immediate restoration on the same day of surgery, stability and retention of implants in the early stages of bone healing, and less implant fractures due to reducing time of restoration. The authors finally concluded that the most reliable devices for immediate oral rehabilitation are one-piece implants and intraoral welding procedure of abutments. In fact, there is no reason to use the two-piece fixtures if they are immediately restored. The abutment-implant junction may represent a critical point in surgery, being a via for bacteria penetration and proliferation, thereby causing perimplant bone resorption (7). Then implants could be inserted not parallel. In a one-piece implant the neck can be curved, otherwise in the two-piece implant this is not possible. In today's dental literature, most frequently, aesthetics are addressed with fixed restorations. This technique gives the opportunity to provide the patients with very good aesthetic outcomes of a hopeless dentition utilizing dental implants, intraoral welded titanium components, and acrylic resin dentures. These prostheses provide excellent facial support, phonetics, aesthetics, smile line, and function. Intraoral welded titanium frameworks offer many advantages for the patient, clinician, and dental technician. This technique was then proven in atrophic mandibula and maxilla, even if the implants are placed also in zygomatic bone (5, 6). In fact, it has been demonstrated to successfully rehabilitate the edentulous atrophic maxilla with a permanently fixed prosthesis supported by an intraoral welded titanium framework attached to standard and zygomatic implants on the day of surgery (5, 6). Furthermore, the intraoral welding technique seems to have no adverse effect on marginal bone loss and implant survival (7-10).

In the traditional protocols, the osteointegration of implants was obtained before the exposition to occlusal forces. More recent protocols reduce the healing period and, in the case of immediate loading for a full-arch replacement, expose immediately the implants to occlusal and muscular forces. The exposition to these mechanical stresses, before biological integration, may lead to the implant micromovements and finally to an early implant failure. The intraoral welding technique proved to be very effective even in patients who had lost their teeth due to periodontal diseases, related or not to systemic diseases (11-16), and in subjects with previous precancerous lesions and oral carcinoma (17-23).

In conclusion, we may affirm that an optimal biomechanical stress distribution, both at the level of the provisional superstructure and at the level of the implant infrastructure, should be the primary goal of the rigid temporization of multiple immediately loaded implants. In casea of immediate loading, adequate fixation and immobility of the implants are of utmost importance to prevent the risk of micromovements in regard to the surrounding bone. In this context, rigid splinting seems to have a significant impact on the peri-implant tissue response since it is able to reduce the mechanical stress exerted on each implant. If rigidity is lost, implant failure is likely to occur due to uncontrolled masticatory forces. Consequently, rigid splinting considerably increases the stability of the prosthetic restoration and the ability to keep the micromovements below a critical threshold.

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SILICA DIOXIDE COLLOIDAL SOLUTIONS IS EFFICIENT IN THE TREATMENT OF CHRONIC PERIODONTITIS: A CASE CONTROL STUDY

E. MARCHETTI[1], A. PIVA[2], A. PELLATI[3] and V. QUINZI[1]

[1]Department of Life, Health and Environmental Sciences, University of L'Aquila, Italy; [2]Universidad Europea de Madrid, Madrid, Spain; [3]Department of Morphology, Surgery and Experimental Medicine, University of Ferrara, Ferrara, Italy

The objective of this study was to compare the efficacy of supportive periodontal therapy (i.e. scaling and rooth planning, SRP) alone versus a chemical device silica dioxide (SiO_2) colloidal solutions (SDCS) used in association with SRP in the treatment of chronic periodontitis in adult patients. A total of 20 patients with a diagnosis of chronic periodontitis (40 localized chronic periodontitis sites) in the age group of 35 to 55 were selected. None of these patients have previously received any surgical or non-surgical periodontal therapy and demonstrated radiographic evidence of moderate bone loss. Two non-adjacent sites in separate quadrants were selected in each patient to monitorize treatment efficacy (split mouth design). Clinical pocket depth (PD) and microbial analysis (MA) were analyzed at baseline and 15th day. SPSS program and paired simple statistic T-test were used to detect significant differences. Total bacteria loading, *Tannerella Forsitia* and *Treponema Denticola* loading were statistically reduced when SiO2 is locally delivered. SDCS gel is an adjuvant therapy which should be added to SRP in the management of moderate to severe chronic periodontitis.

Periodontal disease is one of the prevalent hillness in adult population (1-4). It is characterized by a symptom triad: tooth mobility, foetor ex ore, gingival bleeding. If left untreated the disease can lead to tooth loss. Pathogenesis of periodontal disease is multifactorial and bacterial have a preminent role (5). The main pathogens implicated in periodontal disease are anaerobic gram-negative bacteria of which the most aggressive were identified in the "red complex" group: *Porphyromonas gingivalis, Tannerella forsythia*, and *Treponema denticola* (6).

The aim of periodontal treatment is to eliminate oral infection, and prevent the progression of the disease (7). Many studies have widely demonstrated that the non-surgical therapy including scaling and root planing (SRP) associated with a good level of oral hygiene can prevent the onset of periodontal disease and allow for proper maintenance of oral health (8, 9).

The aim of the present study is to evaluate the effectiveness of a new chemical device (i.e. silica dioxide colloidal solutions, SDCS) used in association with SRP in the treatment of chronic periodontitis in adult patients.

MATERIALS AND METHODS

A total of 20 patients with a diagnosis of chronic periodontitis were randomly selected. Patients were qualified for the study if they have two non-adjacent sites located in separate quadrants which required periodontal treatment, in the age group of 35-55. Subjects have

Key words: Periodontitis; scaling; root planing; silicium; drug; delivery

Corresponding Author:
Dr. Marchetti Enrico,
Department of Life, Health and Environmental Sciences,
University of L'Aquila,
L'Aquila
Italy

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not received previously any surgical or non-surgical periodontal therapy. The patients were excluded from the study if they meet any of the following criteria: (1) pregnancy; (2) an history of taking antibiotics or using antibacterial mouth rinses for past 6 months; (3) teeth with furcation involvement; (4) smoking, and drug or alcohol abuse. This study has been approved by the ethics Committee of the hospital. Subjects participating in the study volunteered will follow a detailed verbal description of the procedure and by signing consent forms.

Clinical methods

A total of 20 patients (i.e. 40 sites) were selected and grouped into two categories: control and test (split mouth design). The control group (20 sites) was treated with SRP without using SDCS (control site). The test group (20 sites) was treated by SRP plus SDCS (test site). All patients underwnt to SRP at the baseline measurement. Prior to SRP clinical pocket depth and microbial analysis was performed in each selected site. Then SRP was done at both sites using ultra sonic scaler. After SRP, SDCS was adjunct in the test site of each enrolled patient. After 2 weeks, microbiological samples were collected again from from both sites in each patient. For bacteria analysis, sites were isolated using cotton rolls. Sterile absorbable paper points (size 60) were used for the collection of subgengival samples and were immediately transferred to microbiological lab for processing. *Porphyromonas gingivalis, Tannerella forsythia*, and *Treponema denticola* and total bacterial loading were evaluated.

Real-Time Polymerase Chain Reaction

Probes oligonucleotides were designed basing on 16S rRNA gene sequences of the Human Oral Microbiome Database (HOMD 16S rRNA RefSeq Version 10.1) counting 845 entries. All the sequences were aligned in order to find either consensus sequence or less conservate spots. Two real-time polymerase chain reaction (PCR) runs were performed for each sample. The first reaction quantified the total amount of bacteria using two degenerate primers and a single probe matching an highly conservated sequence of the 16S ribosomal RNA gene. The second reaction detected and quantified the three red complex bacteria, i.e. *P. gingivalis, T. forsythia and T. denticola*, in a multiplex PCR. This reaction included a total of six primers and three probes that were highly specific for each specie. Oligonucleotide concentrations and PCR conditions were optimized to ensure sensitivity, specificity and no inhibitions in case of unbalanced target amounts. Absolute quantification assays were performed using the Applied Biosystems 7500 Sequence Detection System. The amplification profile were initiated by a 10 min incubation period at 95°C to activate polymerase, followed by a two- step amplification of 15 s at 95°C and 60 s at 57°C for 40 cycles. All these experiments were performed including nontemplate controls to exclude reagents contamination.

Plasmids containing synthetic DNA target sequences (Eurofin MWG Operon, Ebersberg Germany) were used as standard for the quantitative analysis. Standard curves for each target were constructed in a triplex reaction, by using a mix of the same amount of plasmids, in serial dilutions ranging from 101 to 107 copies. There was a linear relationship between the threshold cycle values plotted against the log of the copy number over the entire range of dilutions (data not shown). The copy numbers for individual plasmid preparations was estimated using the Thermo NanoDrop spectrophotometer.

The absolute quantification of total bacterial genome copies in samples allowed for the calculation of relative amount of red complex species. To prevent samples and polymerase chain reaction contamination, plasmid purification and handling was performed in a separate laboratory with dedicated pipettes.

Statistical analysis

SPSS program and paired simple statistic T-test were used to detect significant differences.

RESULTS

A statistical significant differences was detected between (A) Total Bacterial Loading pre and post SDCS treatment, (B) *T. forsythia* pre and post SDCS treatment and (C) *T. Denticola* and post SDCS treatment. Notheworthy, no statistical significant difference was detected between control and test site as regard pre-treatment bacteria concentration as well as pre vs. post-treatment bacteria concentration in control site (i.e. only SPR). When p-value is less than 0.05 then the difference between the twocompared bacterial loadingsis statistically significant.

DISCUSSION

It is well understood that most destructive types of periodontal diseases occur due to the presence of pathogenic micro-organisms colonizing the subgingival area and the suppression or eradication of these microbes result in improvement in periodontal health. Mechanical debridement is effective in both disturbing the biofilm and reducing the bacterial load. However, sometimes mechanical instrumentation may not be sufficient to control the disease due to tissue invasive pathogens, or other tooth related anatomic factors (10-13). In such conditions, adjunctive use of a chemical device provides an additional benefit in controlling the disease. This material can be potentially used in protecting the healing process in oral surgery (14-24). Support periodontal therapy is widely used, but a greater effectiveness is demonstrated by the administration of topical antimocrobicals in association. The advantages of topical therapy involve the use of antimicrobical agents directly into the periodontal pocket, minimizing the adverse effects related to systemic therapy (9). Some patients with oral pathologies (4, 21, 25-33) can use additional drugs so that there is no risk of drug interaction. The potential benefits of local drug delivery include improved patient compliance, an easier access to periodontal pocket and a lower dosage of antimicrobial agent. Periodontal cleaning is of paramount importance in prosthetic patients (34-37). The most commonly used methods for local drug delivery are local gengival irrigations (5). The antimicrobial agents used as local drug delivery agents include tetracycline, ofloxacin, clindamycin, chlorhexidine, etc. (9). These local drug delivery devices have been used either alone or as adjunct with SRP. These local antimicrobials are administered directly into the periodontal pocket and the effectiveness of these chemical device is related to their bactericidal activity and the subsequent reduction of gingival inflammation (38).

The topical use of chemical device along with mechanotherapy dramatically improves clinical results, and at the same time is free from its inherent adverse effects and disadvantages. Local delivery of chemical devices into the pocket achieves a greater concentration of the drug locally, proving bactericidal for most periopathogens, and at the same time, exhibits negligible impact on the microflora residing in other parts of the body.

Our study evaluated the efficacy of SDCS gel in the management of moderate to severe chronic periodontitis. The results of this investigation demonstrated an overall improvement in most of bacterial parameters. Microbiological testing was thought appropriate to evaluate the effect of SDCS gel on subgingival microbial population, the primary etiological factor for periodontitis. Several methods have been used for microbiological testing in periodontitis (39). However, many techniques have not been fully accepted due to low sensitivity or specificity. Moreover sometimes they are slow, expensive and laborious. Recently, a rapid and sensitive test (LAB SRL, Ferrara, Italy) was developed to detect and quantify the three bacterial species more involved in periodontitis: *Porphyromonas gingivalis, Tannerella forsythia*, and *Treponema denticola.*

It is well known that both *P. gingivalis* and *T. denticola* occur concomitantly with the clinical signs of periodontal destruction (40). They appear closely 'linked' topologically in the developing biofilm, shown an in vitro ability to produce a number of outer membrane- associated proteinases. They are considered the first pathogens involved in the clinical destruction of periodontal tissues. Moreover both them and *T. forsythia,* show an higher prevalence in disease than in health suggesting that these bacterial are associated with the local development of periodontitis. Based on this reported data, red complex triad was investigated and SDCS was able to significantly reduce their concentration. Oral health and a pleasant smile are a goal of every person and a civil society.

In conclusion, SDCS gel is an adiuvant therapy which should be added to SRP in the management of moderate to severe chronic periodontitis.

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ORAL REHABILITATION OF EDENTULOUS JAWS WITH ONE-PIECE IMPLANTS: A CASE SERIES

D. LENTO[1], P. AVANTAGGIATO[2], M. BALDONI[4] and M. DI GIROLAMO[4]

[1]Private practice, Milan, Italy; [2]Universidad Europea de Madrid, Madrid, Spain; [3]Department of Medicine and Surgery University Milano Bicocca, Italy; [4]Department of Clinical Science and Translational Medicine, University of Rome "Tor Vergata", Rome, Italy

The popularity of one piece implants has increased considerably between patients and dentists. The advantages of one-piece immediate loading for rehabilitation of edentulous mandibles is to reduce the number of interventions and timing of prosthetic. These parameters can be better controlled with a one-piece implant. Twenty-one patients with one-piece implants inserted in totally edentulous mandibles were considered for this retrospective study. Inclusion criteria were: Good oral hygiene, absence of lesions of the oral mucosa, no smoking or smoking less than 20 cigarettes a day, drinking less than 2 glasses of wine a day, good general health no pregnancy. Twenty-one (12 females 9 males) patients were enrolled in this retrospective study. The mean follow-up was 1 years. A total 84 one-piece implants (Biohorizon, Italy) were inserted in edentulous mandible. Implants diameter was 3.0 mm in all fixtures. Implants length was equal and longer than 12 mm in 44 and 40 fixtures respectively. 48 were inserted in females 36 in males (range 33-67; mean age 58.3). One-piece immediate loading implants has no difference in survival rate respect to two-piece implant and delayed loading for rehabilitation of totally edentulous mandibles. In conclusion one-piece immediate loading implant is a reliable device for mandible rehabilitation.

Dental implants have been used clinically in a routine manner to restore jaws (1-13). Over the past decade changes in dental implant design and surface configuration combined with an improved understanding of the biological and biomechanical aspects have improved the clinical outcome of implant treatments. These advancements have led to the one- stage surgical procedures in conjunction with earlier loading, especially in the completely edentulous mandible. Today there is evidence, although based on a small number of studies and relatively low patient numbers, that immediate loading can lead to survival rates comparable to conventionally loaded implants (14).

The ultimate goal of an immediate loading protocol is to reduce the number of surgical interventions and to decrease the timeframe between surgery and prosthetic delivery without sacrificing implant success rates. From this point of view prosthetic rehabilitation is paramount importance (15-18). These new protocols will ultimately diminish patients' reservations and result in increased acceptance of implant therapy (19).

Before embracing the procedure as a routine treatment, the immediate loading technique needs to be validated with a significant number of clinical cases, extended follow-up, and a clear definition of limitations. Because implant macrogeometry/microgeometry and the loading mode play a crucial role during the healing phase, it is important when documenting immediate loading cases to identify clearly the type of implant and rehabilitation used.

Key words: one-piece implants, edentulous mandibles, prosthetic rehabilitation

Corresponding Author:
Dr. Di Girolamo Michele,
Department of Clinical Science and Translational Medicine,
University of Rome "Tor Vergata",
Rome, Italy

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The advantages of immediate loading is to reduce the number of interventions and timing of prosthetic (20). Furthermore, the success of immediate loading is related to the primary implant stability and loading control (21). In fact primary stability was always considered fundamental to acquire osseointegration. To facilitate the immediate loading protocol, the implant stability at the time of placement is essential and implant surface modifications have significant role in measuring the success of osseointegration. The primary implant stability at placement is the mechanical phenomenon related to the quality and quantity of the bone at the recipient site, the type and design of the implant and the surgical technique used. These parameters can be better controlled with a one-piece implant (21). In fact, although the two piece implants have shown great success for a long time, the two stages surgical procedures, the infiltration of bacteria in the micro-gap between abutment and implant, and the screw fracture after loading, are considered complications, that could be overcame by the use of one-piece implants (22). In addition, the one piece implant allows a minimally invasive flapless surgery very well accepted by patients (23). The immediate prosthetic of a one-piece system allows for better tissue healing , better adhesion of the gingival mucosa to form a collar healthy and adherent to the implant, avoiding a second surgical procedure (24). The prosthetic procedure of one-piece implant enables the physiology of the natural tooth. The one-piece implant enables a borderline preparation following the contour of the gingival margin leading to a better preservation of mucous seal (25). One piece immediate loading implants have a survival rate similar to delayed loading implants (26). Since immediate loading of one piece implants has become a procedure for rehabilitation of edentulous patients, we decided to perform a retrospective study on one piece implants inserted in edentulous mandibles.

MATERIALS AND METHODS

Sample

Twenty-one patients with one-piece implants inserted in mandible were considered for this retrospective study. The study population were selected from patients of 3 dentists working in private practice and undermitted to implant therapy from 2012 to 2014. The main criterion in subject selection was the presence of complete edentulous condition, untreated or previously treated, even due to periodontal teeth requiring extractions, but with an unsatisfactory perception concerning of denture. Inclusion criteria were: good oral hygiene, absence of lesions of the oral mucosa, no smoking or smoking less than 20 cigarettes a day, drinking less than 2 glasses of wine a day, good general health (no prior chemotherapy or radiotherapy, immunosuppression or corticosteroid therapy, no liver, blood or kidney disease), no pregnancy. The exclusion criteria were as follows: bruxism, smoking more than 20 cigarettes/ day, consumption of alcohol higher than 2 glasses of wine per day, localized radiation therapy of the oral cavity, antitumor chemotherapy, liver, blood and kidney diseases, immunosuppressed patients, patients taking corticosteroids, pregnant women, inflammatory or autoimmune diseases of the oral cavity. All patients signed an informed consent for participation in the study.

Outcomes

The first outcome was implants survival rate (ISR) 1 years after insertion and prosthetic. The second outcome was the peri-implant bone resorption (PBR). It was evalueted in relation to resorption of bone more than 1,5 mm in the first year after implant insertion.

Data collection

All patients underwent a radiographic X-ray (Sirona Orthophos X5, Siemens, Italy) with a software for image processing (Sidexis XG, Siemems, Italy) (Fig. 1). A CT scan (Gendex, Kavo Italia) before implants insertion was performed when necessary. Peri-implant crestal bone levels were evaluated by the calibrated examination of radiographic x-ray after surgery and at the end of follow-up period (1 year). The measurements were carried out medially and distally to each implant calculating the distance between the implant neck and the most coronal point of contact between implant and bone. The measure has been put in relation with the level of bone measured immediately after the insertion of the implant, which was the point of reference for subsequent measurements. Peri-implant crestal bone levels were evaluated by the calibrated examination of radiographic x-ray after surgery and at the

end of follow-up period (1 year). The radiographs with measures were save in a compressed in a TIFF format. Each file was processed with a windows XP professional operating system using Photoshop 8.0 (Adobe, United States) and shown on 17'' Asus computer display. The Sidexis software allows radiographic measurements corresponding to the real ones. The difference between the implant abutment junction and the bone crestal level was defined peri-implant bone resorption (PBR).

Surgical protocol

All patients followed the same surgical protocol. The anesthesia of the jaw was obtained by the injection of articaine and post-surgical analgesic treatment was performed with 100 mg of ketoprophene 3 times a day if necessary. An antimicrobical prophylaxis was administred with 1 gr. Amoxycillin twice daily for 5 days starting one hour before surgery. One-piece implants (Biohorizon, Italy) were inserted in mandibles (fig.2). All implant necks were positioned at crestal level. A provisional prosthesis was provided within 48 hours and a definitive within 4 weeks. All patients agree to follow a strict oral hygiene protocol and recall.

T-test analysis was used to calculate implant survival rate (ISR) and peri-implant bone resorption (PBR).

RESULTS

Twenty-one (12 females 9 males) patients were enrolled in this retrospective study. The mean follow-up was 1 years. A total 84 onepiece implants (Biohorizon, Italy) were inserted in edentulous mandibles. Implants diameter was 3,0 mm in all fixtures. Implants length was equal and longer than 12 mm in 44 and 40 fixture respectively. 48 were inserted in females 36 in males (range 33-67; mean age 58,3).

Ten implants were lost after 1 year follow-up. Implant failures have been carefully examined. We did not observe particular characteristics of the implant sites that could affect implant success. The age of patients and the position of the implant did not influenced implant failures. Primary stability and placement of the implant neck to the bone crest level have been respected in each lost implant. The bone quality was adequate in every patient in whom the

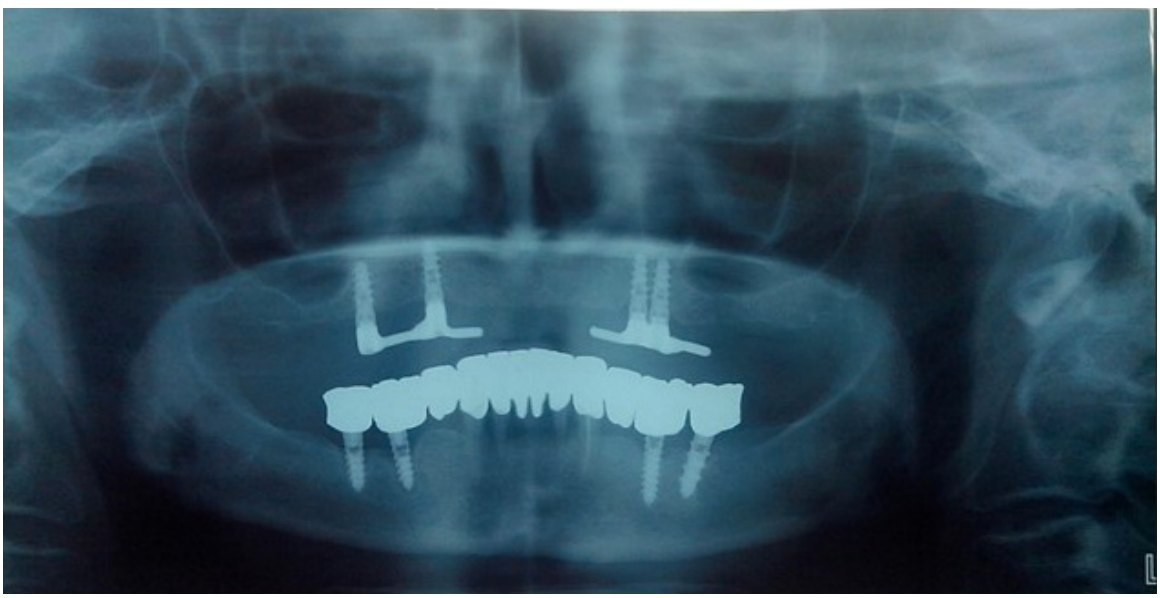

Fig. 1. *Panoramic X-ray showing inferior rehabiltation on implants and natutal teeth.*

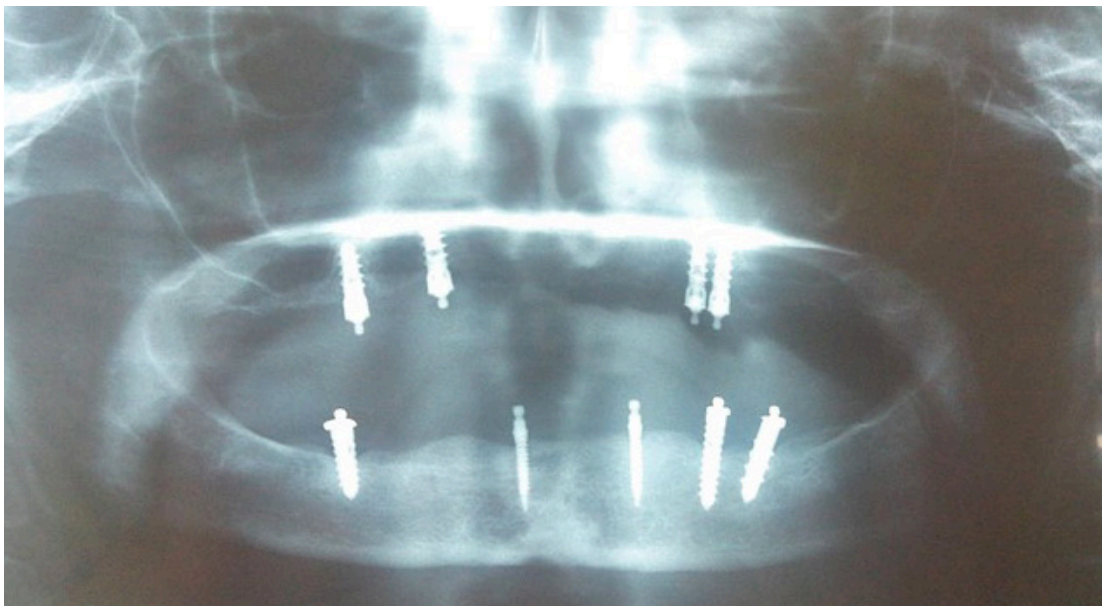

Fig. 2. *Panoramic X-ray after inserting new implants: in the mandible were used 2 one-piece implants.*

implant was lost. In any case, the loss of the implants did not affect the prosthetic rehabilitation. Implant survival rate (ISR) was 80,5%.

Peri-implant bone resorption was investigated in the remaining 74 implants. Only 2 implants have more than 1,5 mm of crestal bone resorption after 1 years.

DISCUSSION

One-piece immediate loading implants have becoming a widespread procedure for oral rehabilitation of edentulous jaws. Many factors can influence the implants survival: surgical technique, bone quality, the type of implants, system factors related to the occlusion. Related conditions such as residual teeth (27-29), periodontal status (30-32) and mucosal health (33-41) should be considered prior to place fixtures. The surgical technique should provide for the primary stability, bone quality and quantity are key factors for implant success also. Mandible has a good bone quality and good bone quality is a predictor of implants survival rate. One- piece implant design avoids manipulating soft gingival peri-implant tissues, allowing adhesion of connective tissue to bone and implant-abutment neck. The advantages of one piece implants are manifold: functional and esthetic rehabilitation, reduced operating time, less armamentarium, less damage to surrounding tissue and a better use of the space where the bone thickness is reduced. The management of partial edentulous space requires a multidisciplinary approach. Implant success depends on the amount and quality of bone tissue and the primary stability that allows a correct osseo-integration. The type of implant includes design, surface characteristic, diameter and length of implant. Occlusion is related to prostetic design. Patient's compliance is better in one-piece implant respect to two stage procedure. Infact patients suffer from pain and inflammation more frequently with traditional delayed prosthetic. Osso-integration of two-piece implant needs 3-6 months before loading, so the prosthetic phase can begin. Besides patients need minimal post-surgery medications thank to flapless procedure, and flapless surgery allows blood supply to new bone reducing resorption. A single stage with one-piece implants provide a better osseo-integration avoiding micromovements of fixture,

a good soft tissue healing and less discomfort for patient. The limitation of the study is that there is no a comparison in the survival rate of one-piece respect to two piece. However one piece immediate loading implants has no difference in survival rate respect to two piece implant and delayed loading (26). In literature there is an early previous study describing one piece immediate loading implants to restore a totally edentulous jaws with a median follow-up of 5 year (14). The authors confirm that the immediate loading technique may be most advantageous strategy for edentulous patients. Our study adds further data to the immediate loading successful rehabilitation of edentulous mandibles. Oral health and a pleasant smile are a goal of every person and a civil society. Therefore, all dental disciplines must contribute to this goal and all dentists must guarantee their patients the best possible care.

In conclusion one-piece immediate loading implant is a reliable device for mandible rehabilitation. Patient compliance to oral rehabilitation is definitely promoted by one-piece implant with careful planning and a minimally invasive technique.

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WHY DENTISTS AND DENTAL HYGIENISTS SHOULD IMPROVE PROGRAMS FOR INDUCING SMOKING CESSATION? THE EFFECT OF TOBACCO SMOKING ON PERIODONTAL HEALTH

G. GALLUSI[1], P. AVANTAGGIATO[2], L. BAGGI[1] and E. MARCHETTI[3]

[1]Department of Clinical Sciences and Translational Medicine, Dental School, University of Rome "Tor Vergata", Rome, Italy; [2]Universidad Europea de Madrid, Madrid, Spain; [3]Department of Life, Health and Environmental Sciences, University of L'Aquila, Italy

Periodontal diseases (PD) affect about half of the adult population all over the world. PD is caused by bacterial infection inducing an inflammatory response with progressive destruction of the periodontal tissues and finally the lost of teeth. Tobacco smoking (TS), alcohol consumption, and systemic conditions such as diabetes, osteoporosis, malnutrition and stress are considered additional risk factors. This short review examines the potential causal association between TS and PD. There are many studies for a higher level of PD among smokers. Greater levels of clinical alveolar bone loss, tooth mobility, probing pocket depth and tooth loss are more frequent in smokers than in non-smokers. The modification of the periodontitis micro-flora in smokers influences the development of PD. Also there are data suggesting smoking effects on both host responses in humans. Response to periodontal treatment is different in smokers and non-smokers. Various clinical studies have demonstrated that TS is a major risk factor for poor response to periodontal therapy. TS is a factor that has the potential to negatively affect healing and the outcome of implant treatment. It is mandatory for dentists and dental hygienist to promote smoking-cessation programs as well as educate our community on the benefits of not smoking.

Periodontal diseases (PD) affect about half of the adult population all over the world (1-5). PD is caused by bacterial infection inducing an inflammatory response with progressive destruction of the periodontal tissues and finally the lost of teeth. Tobacco smoking (TS), alcohol consumption, and systemic conditions such as diabetes, osteoporosis, malnutrition and stress are considered additional risk factors. PD therapy is important to reduce local inflammation and bacteraemia. Recently has been stated that PD appears to increase the risk of cardiovascular disease, pulmonary disease, preterm and low birth weight (6).

TS is not only a risk factor of PD, but it is now recognized as the most important cause of different types of cancer and related pathologies (7-14). The number of cigarette smokers is slowly declining, but TS is rising in developing countries, and it will probably continue to rise in future. The World Health Organization estimates that the number of tobacco smokers worldwide is more than 1 billion and is expected to increase to 1.7 billion by 2025 (15). Based on these trends, it is predicted that, by 2030, approximately 10 million people will die annually as a result of tobacco use, with 70% of such deaths occurring in low- and middle-income countries (16).

There are many studies stating that PD is more severe among smokers. Clinical signs of PD such as alveolar bone loss, tooth mobility, clinical attachment level and tooth loss are more frequent in smokers than in non-smokers. Bone loss may affect pillars of bridge restoration (17-22).

Key words: tobacco smoking; periodontal disease; tooth loss; alveolar bone loss; oral microflora

Corresponding Author:
Dr. Gallusi,
Department of Clinical Sciences and Translational Medicine
Dental School, University of Rome "Tor Vergata"
Rome, Italy

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Smoking and periodontal disease

There are many studies evidencing that TS has a negative effect on the progression of PD and slows healing after periodontal therapy. These studies are unanimous in demonstrating a positive relationship between TS and periodontitis severity. These findings were confirmed by a recent systematic review, which showed a causal association between TS and tooth loss and an evident decrease in the risk of tooth loss after smoking cessation[5]. The relationship between TS and PD is evident even if smokers, paradoxically, show less clinical sings of gingivitis such as bleeding on probing and oedema. It is presumed that TS may decrease gingival bleeding and crevicular fluid volume as a result of changes in the proportion of blood vessels and vascular alterations in periodontal tissues (23).

TS may influences the microbiological findings of periodontal pockets of smokers, in fact in smoker's periodontal pockets, were found more anaerobic bacteria respect to non-smokers (24). An anaerobic environment could conceivably promote the growth of Gram-negative periodontal pathogens in the sub gingival plaque. However, there is no significant difference in oral bacteria between smokers and non-smokers. May be that TS modifies the immune response to the aggression of oral bacteria. Though studies stated that TS increases the number of neutrophils, the first line of defence against bacterial infection, smokers have decreased activity of neutrophils including chemotaxis, phagocytosis, adherence and its capacity to produce cytokines. In addiction TS influences lymphocyte count and production of antibody.

TS effects on periodontal tissues

Nicotine is the most studied molecule of tobacco and the most pharmacologically active metabolite in TS. TS has been shown to have various psychological effects on smokers. TS can penetrate the body by absorption through the oral mucosa and skin or inhalation via the lungs. TS is also highly addictive. Only 2.5 per cent of the 34 per cent of smokers who quit TS are successful (25). TS has influence on PD. TS acts blocking fibroblast growth and production of fibronectin and collagen (26). Periodontal cells exposed to TS have also been shown to have decreased growth and protein content, damaged cell membranes, and atypical shapes (27). TS has been implicated as a risk factor for alveolar bone loss (28). One hypothesis has been the possible stimulant effect of TS on osteoclastic activity, the cells most responsible for bone resorption (29). TS may stimulate osteoclast differentiation and the resorption of calcium phosphate, the major inorganic component of bone. It is thought that TS osteoclast stimulation may partially explain the increased rapidity of alveolar bone loss and refractory disease incidence in smokers. Influence of TS on progression bone loss may be exacerbated by other factors such as specific sub gingival bacteria loading (30). TS has also been thought to delay apoptosis (31). A delay in cell death has been thought to contribute to tumour production. Also, this delay would allow for osteoclasts to continue the resorptive process longer than their normal life cycle. These factors may also contribute to the accelerated alveolar bone loss of smokers.

Smoking and host response

Since TS does not appear to significantly change the periodontal microflora, some researchers have suggested that TS may have an effect on the host response. It has been hypothesized that TS may alter the host response in two ways:

- TS could impair the normal functions of the host response in neutralizing infection, resulting in destruction of the surrounding healthy periodontal tissues.
- TS may cause tissue destruction by a direct toxic effect.

Several studies have shown that the effect of TS on periodontal tissues may involve both of these processes. For example, smokers tend to have depressed numbers of T-helper lymphocytes, which are important cells of the immune system to regulate cell-mediated immunity and the activity of B lymphocytes (32). In addition, the host requires functional neutrophils to deal effectively with bacterial infections. TS has been shown, by several studies, to have a deleterious effect on various neutrophil functions. For example, TS seems to

decrease the chemotaxis and phagocytosis of both oral and peripheral neutrophils (33). Neutrophils are cells characteristic of acute inflammation, and are attracted to the site of the insult with a process called chemotaxis. Once at the site of injury, neutrophils engulf microorganisms destroying them and their noxious effects. It has been shown that ineffective neutrophil action can favour to more severe PD. Finally the actual immunoinflammatory mechanisms that could precisely explain the intensified severity and progression of PD in tobacco users have not been completely explained.

Disease masking

Clinical signs of PD, in smokers, are masked. Gingival tissues in smokers are less oedematous and bleeding respect to non-smokers. The term disease masking is used because the vasoconstrictive properties of TS hide the inflammatory and destructive changes of PD. The periodontal tissues are suffering from vasoconstriction with consequence of decreased blood flow to the gingiva. This hides the clinical signs of PD by reducing gingival inflammation, erythema, and bleeding (24).

Nicotinic stomatitis, or smoker's palate, is another mucosa alteration that is characteristic of smokers. It is characterized by a whitish and hyperkeratosis mucosa of the palate with erythematous salivary glands with inflammation of the orifices due to chronic irritation. Patients with nicotinic stomatitis have no symptoms, so this alteration is diagnosed when patient goes to dentist for toothache or other problems. Nicotinic stomatitis tends to regress after smoking cessation.

Smoking cessation and periodontitis

It is evident that TS increases tooth loss, but we don't know if smoking cessation improves periodontal health. A recent study about TS and tooth loss among veterans showed that the rate of tooth loss among men who quit smoking was about 50% lower than the rate among current smokers but still significantly higher than the rate among non-smokers (34). In a 12-year follow-up study of 1031 Swedish women, prospective rates of tooth loss were similar in never smokers and former smokers who had abstained from smoking an average of 10 years before entering the study (35). These findings support the idea that smoking cessation may stop progression of periodontal bone loss and attachment loss (36). The results of few studies suggest that PD and tooth loss risk is reduced after smoking cessation, but however in a more severe form respect to non-smokers. May be that once the alveolar bone is lost, it is no possible to regenerate it spontaneously. In addition smokers who quit smoking appear to be more health conscious than those who continue to smoke, and follow PD therapy to maintain oral health (37)

Smoking and periodontal therapy

Response to periodontal treatment is different in smokers and non-smokers. Various clinical studies have demonstrated that TS is a major risk factor for poor response to periodontal therapy, and favours delay in healing to nonsurgical and surgical periodontal therapies, as well as to regenerative and plastic periodontal procedures. PD refractory to therapy is more common in smokers and TS is a risk factor for disease progression after active periodontal therapy (38).

Studies have shown that smokers present a worse clinical response to scaling and root planning than non-smokers (39-41). As the TS reduces the effect of scaling and root planning, the use of adjunctive antimicrobial therapy in smokers with chronic periodontitis has not been demonstrated to improve clinical response (42).

Smoking and implant dentistry

The insertion of implants in smokers significantly affected the failure rates, the risk of postoperative infections as well as the marginal bone loss (43-52). TS is a factor that has the potential to negatively affect healing and the outcome of implant treatment (53).

Smoking and periodontitis: current perspective

In conclusion, even if it is stated that TS worsens the progression of PD, due to the direct heat from the cigarette, the vasoactive response from nicotine, or a change in the host response, the mechanism by which smoking induces periodontal attachment loss is currently unknown. TS has not only been shown to increase the severity of PD, but also to decrease the response of the gingival tissues to periodontal

therapy, resulting in a greater incidence of refractory disease. In addiction TS leads to a number of systemic manifestation including increased risk of coronary artery disease; diabetes; osteopenia; and premature, low-birth-weight babies. On the other side smoking cessation can lead to a regression of clinical signs of PD, with improved oral and systemic health. Smoking is the major preventable risk factor for PD. So, it is mandatory for dentists and dental hygienists to promote smoking-cessation programs as well as educate our community on the benefits of not starting smoking.

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COMPLICATIONS IN SINUS LIFT

V. CANDOTTO[1], G. GALLUSI[2], A. PIVA[3], M. BALDONI[4] and M. DI GIROLAMO[2]

[1]Department of Biomedical, Surgical and Dental Sciences, University of Milan, Milan, Italy; [2]Department of Clinical Science and Translational Medicine, University of Rome "Tor Vergata", Rome, Italy; [3]Universidad Europea Madrid, Spain; [4]Department of Medicine and Surgery University Milano Bicocca, Italy

Prosthetic rehabilitation of atrophic maxillary implants often requires grafting owing to vertical and transversal bone deficiency. The use of this procedure in order to insert implants was introduced by Tatum and published as a clinical study by Boyne and James. It can be performed via a lateral or crestal approach. This surgery is now widespread, with good results in terms of bone augmentation and implant osseointegration. Nevertheless, there is a small proportion of patients who have infectious complications with an incidence ranging from 3.5% to 10.5%. We observe that maxillary sinus augmentation is a successful preprosthetic technique for augmentation of the edentulous posterior maxilla. Preoperative assessment of the anatomy of the sinus reduces the rate of complications considerably

Prosthetic rehabilitation (1-4) of the atrophic maxillary implants often requires grafting owing to vertical and transversal bone deficiency. The use of this procedure in order to insert implants was introduced by Tatum (5) and published as a clinical study by Boyne and James (6). This procedure is suitable for the rehabilitation of both a posterior tooth and a completely edentulous maxilla in regions with loss of alveolar bone and sinus pneumatisation (7).

As stated in the Babson College Consensus Conference, in 1996, this procedure is considered a safe and predictable method, provided that the maxillary sinus is healthy. In particular, it is considered mandatory to respect the natural homeostasis of the maxillary sinus and to perform surgery in the presence of an efficient ciliary movement, a normal sinusal mucosa and a pervious sinus ostium (8).

Several surgical techniques have been used to improve bone volume, with various graft materials available (9-16). The location and amount of bone atrophy determine which surgical option is to be selected. The sinus lift procedure is a technique which allows graft placement between the Shneiderian membrane and the sinus floor. Two approaches are commonly used: the lateral window technique and the osteotome intrusion technique (17, 18). The latter is indicated when at least 5–6 mm of alveolar bone is present, showing a gain of 4–8 mm in bone height, and there is sufficient bone to stabilize the implant (19). It can be performed via a lateral or crestal approach. This surgery is now widespread, with good results in term of bone augmentation and implant osseointegration. Nevertheless, there is a small proportion of patients with infectious complications, with an incidence ranging from 3.5% to 10.5% (20).

Intraoperative complications

The most common intraoperative complication was damage of varying degrees to the Schneiderian membrane during the opening of the bone window or its dissection. The reported incidence in the literature ranges from 7% to 56% of cases (21, 22).

Key words: sinus lift; bone augmentation; complications; maxillofacial surgery

Corresponding Author:
Dr. Valentina Candotto
Department of Biomedical, Surgical and Dental Sciences,
University of Milan,
Via della Commenda 10,
20122 Milan, Italy
e-mail: candottina@libero.it

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Various methods have been proposed to deal with these complications, from leaving them untreated to suturing the Schneiderian membrane, sealing with reabsorbable membranes, and using glues obtained from autologous fibrin gel (8, 23, 24). In general, small perforations may regenerate spontaneously. Perforation of the sinus membrane may represent an avenue for the entry of bacteria, with the resulting contamination and infection of the sinus graft (24). Injury to the arteries during preparation of the lateral window will result in bleeding. To prevent this, the position of the artery should be evaluated preoperatively with cone-beam computed tomography (25).

Postoperative complications

The most frequent complication is wound infection, followed by postoperative sinusitis, followed by, in rate of occurrence, the exposure of the bone graft onlay and exposure and ultimate loss of the graft. It is often resolved with systemic antibiotics, local measures, vasoconstrictors, and aerosol sprays. Some cases may call for nasoendoscopic exploration. In addition to exploration of the nostrils and sinus cavities, this allows one to observe the status of the sinus membrane and whether the graft is exposed. As a therapeutic method, it also allows washouts, drainage, and local application of antibiotics or antiseptics (17).

DISCUSSION

Sinus lift surgery is a proven and reliable technique because of the low rate of postoperative complications observed and the success rate of implants placed into the grafted area. Nonetheless, as with any surgical procedure, it is not exempt from risk (19). To perform this surgical technique it is necessary to evaluate the oral cavity status of the patients, such as periodontium (26-29), remaining teeth (30-33) and mucosal health (34-41).

Proussaefs et al. (22) demonstrated that sinus membrane perforations repaired with a resorbable collagen membrane resulted in less bone formation, less bone surface contact with residual graft particles, more soft tissue formation, and a reduced implant survival rate compared to non-perforated sites (25). On the other hand, Froum et al. (8) reported that sinuses with membrane perforation repaired by a collagen membrane showed significantly greater vital bone percentages compared to the non-perforated sinus group (42).

Perforations could be repaired with a collagen membrane; the loss of graft material, resorption, or failure of graft maturation due to the deterioration of the sinus membrane might lead to graft failure.

We observe that maxillary sinus augmentation is a successful pre-prosthetic technique for augmentation of the edentulous posterior maxilla. Preoperative assessment of the anatomy of the sinus reduces the rate of complications considerably.

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TONGUE REHABILITATION THROUGH THE FROGGY MOUTH DEVICE: CASE SERIES

P. MANZINI[1,2,3], M. ROSMARINI[3], E. FRATI[3], M. SEVERINO[4] and S. CARUSO[4]

[1]Department of Neuroscience-Head of Orthodontics Service, Maxillo-Facial Surgery ASST Mantua, Italy;[2]UNIMORE, Mantua, Italy; [3]Orthodontist and Experts at Zero-20-32.com; [4]Department of Life, Health and Environmental Science (MeSVA), University of L'Aquila, L'Aquila, Italy

Based on the knowledge supporting the position of the tongue in relation to the palate to be the guiding factor of oro-dental growth and the key factor in long-term orthodontic stability after treatment, 6 different case reports show how the Froggy Mouth device changes treatment strategy in rehabilitation of dysfunctional swallowing by relying on the subcortical process, following the principles established by pioneering studies on memorization mechanisms conducted by Eric Kandel (year 2000 Nobel Prize winner in medical field for his research on memorization process).

Over the past 30 years scientific literature regarding young age interceptive orthodontic therapy (1-8) has shown that the tongue's position in relation to the palate guides oro-dental growth (8) and allows for long-term stability after a treatment (8-10), influencing also the temporo-mandibular joint (2, 7, 8, 11-13). Specifically, when physiological position and function of the tongue (5, 14) (the correct pressure on the palate during deglutition) send a correct message to the brain, a correct response (efferent message) is generated i.e. the harmonious antero-posterior/transversal growth of the jaws (15), and reliable long-term stability in case of corrective orthodontic treatment (8, 16-20). On the other hand, if the tongue applies asymmetric and/or no pressure on the palate (21) during its function, the message sent to the brain will be incomplete, if not outright destabilizing for the occlusal balance, as for example in case of reduced palatal dimension (10, 19, 22).

Swallowing dysfunction is related to oral breathing and can influence the bacterial loading of the saliva (23, 24), influencing the periodontal status (25) and the caries and decalcification appearances during early childhood, mostly for incisive and molar teeth (26-28). Functional alteration in the maxillofacial area will manifest during child development age (6, 29, 30) as asymmetric development of the palate or as lack of three-dimensional growth of the upper and lower jaw, which also affects the masticatory muscle function (31), as well as the facial muscles (32), while it will determine a relapse of orthodontic treatment in adults.

Physiological swallowing during early years of life

In what can be identified as a suction-deglutition physiological process in the infant's mouth, up until teeth start erupting the tongue completely fills the oral cavity (16), making contact with the buccal mucosa on the sides and with the labial mucosa anteriorly. Saliva and food passage are determined by the negative pressure (difference in pressure between anterior and posterior regions of the mouth) present in the oral cavity, and movement is performed by the contact

Key words: dysfunctional swallowing; interceptive orthodontics; orthodontic relapse; long-term memory

Corresponding Author:
Dr. Pietro Manzini,
Department of Neuroscience-Head of Orthodontics Service,
Maxillo-Facial Surgery ASST Mantua.
Mantua, Italy
e-mail: pietro-manzini@libero.it

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and contraction of the lips under the direct control of the facial nerve.

As within 3 years of age primary dentition erupts, adult type deglutition changes together with the introduction of alternate unilateral mastication. A physiological deglutition at this stage takes place when dental arches bite and the tongue elevates against the palate thanks to the action of the styloglossus muscle and the upper jaw (16, 30).

Involuntarily repeated every minute with its 17 muscles, the tongue will exercise a very important role by applying pressure to the inner surface of the teeth stimulating antero-posterior/transversal maxillary growth, while dental occlusion will allow the mandible to follow an adequate and compatible growth pattern (6, 8, 30). When the suction-deglutition mechanism continues beyond age 9 months, labial-mental muscle contraction will undermine development, resulting in skeletal disorders varying in the three dimensions of space such as incisive gap, narrow and forward extended upper jaw, mandibular retrusion or deviation (14, 16, 30).

Epidemiology of swallowing praxia at age 3-4 years

Statistics show these medical conditions involve 40-46% of children aged 3 to 4 years (30). A French national study conducted in the late 80s on health conditions of children aged 4 years found only 60% met criteria of correct deglutition (absence of suction-deglutition mechanism) (16). The data clearly suggest that it is necessary to re-establish proper health conditions in the orofacial district starting as early as age 3-4 years (6, 16, 30).

However, the 'quality' of treatment is crucial for a correct outcome in harmonious growth of dentoskeletal structures in the three dimensions of space. Nevertheless, the involvement of the trigeminal nerve, which participates in the control of breathing, centers through its sensory nucleus, favoring recovery of nasal rebreathing (6, 18) while contraction of the tensor tympani muscle, leading to dilation of the Eustachian tube, allows for an improved aeration of the middle ear, resulting in a reduction of serous otitis cases.

Quality of deglutition rehabilitation

Fellus - following studies performed by Eric Kandel (year 2000 Nobel prize winner in the medical field) on the passage from short- to long-term memory - concluded that atypical deglutition rehabilitation requires stimulation of neural network and sub-cortical nervous areas (3, 4, 8, 16, 21, 33).

When lips are stretched and posterior teeth are in occlusion, the trigeminal nerve controls the action of the upper posterior portion of the tongue that moves the initial motor dynamics posteriorly: styloglossus muscle tension reduces the tongue's width inducing its super posterior elevation causing its tip to 'automatically' (without any specific sensory references) relocate in a physiological area.

On the other hand, the path to healing will be harder and unpredictable if rehabilitation requires precise control of voluntary movements searching for proprioceptive sensation of the tongue in contact with the retro-incisal papilla (3, 4, 29), or on the palatine spot (16). Recent study (1, 8) found that the rehabilitation therapies relying on cortical control and short-term memory fail in 80% of cases: just think about the automatic risk of the lowering of the posterior portion of the tongue due to the elevation of its tip preventing the styloglossus muscle to perform its adequate function in the adult type deglutition dynamics.

The Froggy Mouth device

The "Froggy Mouth" device, to be worn in a sagittal position between lips and teeth to keep distance between upper and lower lip, prevents bilabial contact and the chance to have a negative pressure in the oral cavity and therefore the occurrence of suction-deglutition. Froggy Mouth is a new appliance which helps young children from 3-4 years old to use the best swallowing method for a toothed patient through the subcortical way and no longer the suction deglutition method. Froggy Mouth is not placed inside the mouth but between the lips. It prevents the upper lip from touching the lower one, thus, avoiding the negative pressure applied inside the mouth thanks to a water-tight joint around the lips. Indeed, it forces the child to find a new way for the deglutition in the lower part of his brain by raising the upper back of the tongue to the palatal bone. This appliance must be worn 15

minutes per day and should not be worn during the night like «trainers». Indeed, the quality of the sleep is critical for young children. It must be worn in front of a screen (TV or movie/series on a computer screen) in order to catch the patient's full attention and stimulate his neuron circuits which are related to automatic functions. When lips are relaxed and teeth bite together, the styloglossus muscle's action reduces the tongue's width and induces its super-posterior region's elevation. Wearing this device, every time a child swallows saliva or food, he will spontaneously find the need to posteriorly elevate the tongue: having the styloglossus muscle reduce the tongue's width, its tip will naturally position in a physiological area without the need of a specific sensory reference.

The device must be worn 15 minutes/day (to activate trigeminal nerve control of neural networks generating automated movements) while performing recreational activity, such as watching tv, playing videogames, or using pc, to benefit from a positive prevalence recognized by the limbic system, which will facilitate its acquisition and the automatic engrams of the new type of swallowing. The device can be worn at any age as three sizes are available: for 4-7-year-old children (size S); for 8-11-year-old children (size M) and for 12-year-old children (size L).

Case reports

Fig. 1 shows the appliance. Fig. 2 reports the children. The clinical photo of six children are reported in Figs. 2-18. Case 1 is a child with anterior cross-bite (Fig. 3), who wore the appliance for 5 months before correcting the crossbite (Fig. 4).

Fig. 5 reports the second case, a female child, with end-to-end occlusion, who wore the appliance for three months before correcting the occlusion (Fig. 6-7).

Fig. 8 reports the clinical case 3, a child with an anterior open bite, who wore the appliance for 1 year (Fig. 9). Fig. 10 reports the fourth case, a disabled child with bilateral crossbite, and openbite, and with dysfunctions. The patient wore the appliance for five months, obtaining a considerable clinical result (Fig. 11).

The fifth clinical case is described in Fig. 12, a child with agenesis of the two upper second incisive, and with an excessive overjet. After one month of treatment, the occlusion appears clinically improved (Fig. 13).

Fig. 14 shows the sixth clinical case with

Fig. 1. *Froggy appliance*

Fig. 2. *Partial correction of anterior cross bite two, three and five months after beginning of Froggy treatment*

Fig. 3. *Class III anterior cross-bite treated with Froggy appliance*

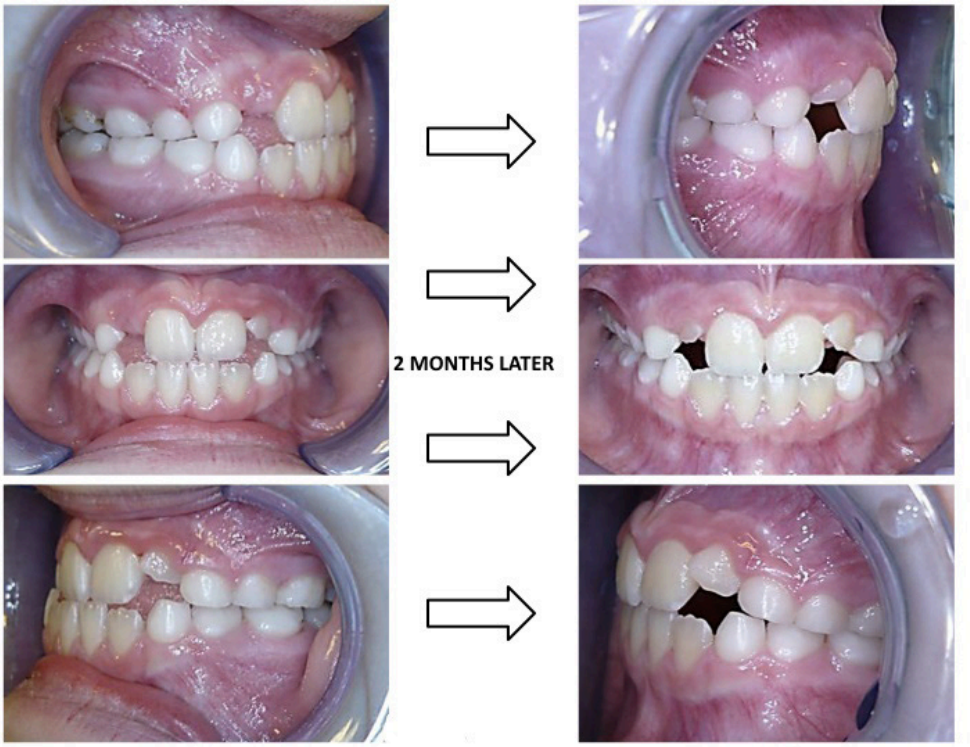

Fig. 4. *Two months later*

Fig. 5. *Seven months later*

excessive overjet, labial frenula asymmetry, and limited amount of mouth opening. Fig. 15 shows the direction of teeth movements. In Fig. 16 the improvement of the occlusion is clear.

CONCLUSIONS

The 6 case reports, classified according to the influence of dysfunctional swallowing, outline the most effective orthodontic prevention policy: restoring normal oral functions will determine harmonious and eumorphic growth during child development stages, while preventing relapse in adult corrective treatments and could reduce future dental problems (8, 34-42). Froggy Mouth is perfectly adapted to young children and it can even be used alone if the deformity is slight: the simple fact to rebalance the functions will allow a spontaneous correction of slight deformity when the child still has deciduous teeth (43-45).

The clinical evidence indicates that the Froggy Mouth is effective in the myofunctional correction of the atypical swallowing mechanism, providing the clinician with a new therapeutic approach for neuromuscular re-training of atypical deglutition and dysfunctional deglutition in patients during their growth phase. However, further scientific evidence is needed to support the results of this investigation.

The automatic movements created by long-term memory using a Froggy Mouth device restored functional physiological balance in a short period of time, preventing appearance/aggravation/consolidation of dysmorphia in patients aged 3 years. Moreover, and above all, it made this balance permanently stable, preventing the most frequent negative outcome in orthodontics i.e., the relapse, and probably other postural problems that are hypothetically associated to malocclusion, or temporomandibular joint morphological alterations (3, 4, 9, 12).

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TEMPOROMANDIBULAR DISC DISPLACEMENT WITH REDUCTION TREATED WITH ANTERIOR REPOSITIONING SPLINT: A 2-YEAR CLINICAL AND MAGNETIC RESONANCE IMAGING (MRI) FOLLOW-UP

G. MINERVINI, L. NUCCI, A. LANZA, F. FEMIANO, M. CONTALDO and V. GRASSIA

[1]Multidisciplinary Department of Medical-Surgical and Dental Specialties, University of Campania Luigi Vanvitelli, Naples, Italy

Clicking may appear in the initial, middle, or final phase of mandibular opening. Magnetic Resonance Imaging (MRI) is the most appropriate diagnostic imaging for diagnosing disc position. With anterior repositioning splint (ARS), disc recapture is achieved through a change in the position of the condyle to encourage adaptation of the retrodiscal tissues. Three patients reported pain and sounds during movement and clicking in the final phases; also, the MRI confirmed anteriorized disc position and the treatments consisted of an ARS. The post-treatment examination confirmed a normal opening without deviations and deflections. After 2 years, the conditions were stable, and the MRI showed thickening of the retrodiscal tissues, including extra fibrous tissue, resulting in a pseudodisc. Treatment using ARS can stimulate tissue fibrosis and the formation of a pseudodisc. MRI is the gold standard for diagnosis and treatment planning of disc displacement cases.

Temporomandibular disorders (TMD) are defined as a musculoskeletal disorder affecting joint masticatory muscles and their associated structures, often in conjunction with dental occlusion (1). It has been estimated that 8-15% of women and 3-10% of men currently suffer from TMD (2), with an increase in frequency related to age (3-5). The etiology has been associated with several factors including trauma, emotional stress (6-8), parafunctional habits (6), muscle dysfunction, discal or bone morphology alteration, and malocclusion (deep bite with insufficient horizontal overlap and occlusal contacts in the anterior teeth) (5, 7, 9, 10). Several stages of clinical dysfunction arising from the relationship between the articular disc and condyle have been described (2, 11). These include; 1): disc displacement with reduction; 2): disc displacement with reduction and with intermittent locking; 3): disc displacement without reduction and with limited opening; 4): disc displacement without reduction and without limited opening; 5): posterior disc displacement (2, 12-14).

Most often, the disc displaces in the anterior direction, as reported in the literature (11, 15-17). Diagnosing TMD is complex and elaborate, and clinical examination alone is sometimes not sufficient (7). In fact, clinical examination alone may accurately diagnose anterior disc displacement with reduction only 43–75% of the time (18). Combining clinical examination with other imaging methods could improve the evaluation of the disc and condyle joint before and after treatment (18). Current literature reviews indicate that magnetic resonance imaging (MRI) is one of the most appropriate diagnostic imaging tools for diagnosing disc position and morphology (7, 19, 20) due to its lack of ionizing radiation, non-invasive nature, and excellent soft

Key words: temporomandibular joint, magnetic resonance imaging, pseudodisc, anterior repositioning splint, disc displacement with reduction, TMD

Corresponding Author:
Prof. Felice Femiano,
Multidisciplinary Department of Medical-Surgical and Dental Specialties,
University of Campania Luigi Vanvitelli,
Via de Crecchio 6, 80138 Naples, Italy
e-mail address: felice.femiano@unicampania.it

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tissue visualization (7, 18, 19, 21-23).

In disc displacement with reduction, the articular disc is often displaced anteromedially or laterally (7, 18, 21, 24) to the condylar head (9, 12, 18). This retrusion position of the condyle can produce stretching and slow elongation of the intracapsular ligaments (9, 19, 25, 26), which contributes to the anterior displacement of the disc (9). However, in this case, ROM is not limited due to the disc's reducing during condylar translation (2), which may result in a clicking sound (2). Clicking may appear in the initial, middle, or final phase of mandibular opening (12), with the latter indicating that the bilaminar zone is affected (5, 9).

Management of disc displacement with reduction becomes necessary when pain or dysfunction is present (2) and conservative therapeutic approaches are not effective (6). Conservative treatment such as cognitive behavioral therapy (15), physical therapy, pharmacological therapy, and occlusal splints should be considered for first-line management (6, 27, 28). Different types of occlusal splints have been recommended for the treatment of disc displacement with reduction (29-31), most of which induce a slight vertical condylar distraction that eliminates the influence of the occlusion (19). An occlusal splint that is specifically used to correct a partial or complete anterior disc displacement is called an anterior repositioning splint (ARS) (19). With this device, disc recapture is usually achieved through a temporary change in the position of the condyle (usually forward and downward) in the articular fossa to encourage adaptation of the retrodiscal tissues (6, 11, 18, 25). The blood vessels and nerves of this tissue eventually become replaced with more fibrous tissue, resulting in a pseudodisc (2, 4, 11, 15, 19, 22). The stability of the disc reduction depends on the convexity of its posterior band and the functional integrity of its inferior stratum (19). Orthodontic or prosthetic rehabilitation can improve the stability of the reduction (9, 19). Although many TMD reports involve pseudodiscs, few cases have included pre- and post-treatment MRI evaluation. The objective of this study was to describe three cases treated with an ARS and report how the retrodiscal tissue became a functional pseudodisc (2, 4, 11, 15, 16, 19) over a 2-year MRI follow-up.

Case reports:

The functional examination was carried out as follows. The patient was asked to open their mouth slowly until pain was first felt; if the patient's range of motion (ROM) was restricted, an attempt was made to increase the ROM passively by gently placing a downward force on the lower incisors. In this, instance it is helpful to test the "end-feel" of the ROM. A soft end-feel suggests a muscle-induced restriction, whereas a hard end-feel is more likely to be associated with intracapsular sources. Next, the severity of the patient's occlusion was examined, and the location of the mandibular incisor directly below the midline between the maxillary central incisors was noted. Then, the patient performed maximum left and right laterotrusive movement with the mandible to reveal ROM in each direction. During opening and closing, a finger was placed behind the condyle to palpate the posterior and lateral aspects of the joint. Bilateral sagittal temporomandibular joint (TMJ) MRI scans of the mouth opening and maximum intercuspation were obtained by means of a 1.5 Tesla (T) super-conductive magnetic resonance system (GE Medical, Milwaukee, WI, USA) equipped with TMJ coils. The MRI protocol included a proton density–weighted spin echo sequences (repetition time (RT) 500, echo time 21, matrix: 256 × 128, FOV: 10 cm, NEX: 4). A separate specialist who was not informed about the functional examination results evaluated the MRI scans. When a diagnosis was achieved, written informed consent was signet and a treatment plan with an ARS was developed.

To design the ARS, the clinician first guided the patient's mandible forward until the disc was reduced and the click disappeared (Fig. 4A, B); this closure position was then transferred to a semi-adjustable articulator with a wax bite. An appliance was made for the upper dental arch such that it covered the whole lingual surface and capped the labial surface of the mandibular teeth to keep the mandible forward. The patient was instructed to wear the appliance full time (24 hr per day except for meals) for 5-7 days a week. After an average of 9 weeks, the clinical signs and symptoms of TMD disappeared, and the appliance was worn only at

night. Subsequently, the appliance was gradually converted to a nonrepositioning design (2). Clinical records and MRI scans were obtained at an average follow-up of 2 years to compare the condyle/disc position and morphology.

Case 1

The first patient, a 62-year-old female, presented with an Angle Class II, division 1 malocclusion, an increased overjet (5 mm), and overbite (7 mm) (Fig. 1A). The patient reported pain and sounds during mandible movement and a condylar palpation revealed clicking in the final phase (22 mm) of opening, which was more prominent on the left side. The mandibular opening was slightly restricted (26 mm) with a soft end-feel, probably due to muscle restriction caused by the intracapsular displacement (Fig 1C). The maximum laterotrusive movement on the left was 10 mm, and that on the right was 4 mm, suggesting left condyle impairment. The MRI images confirmed the presence of a left TMJ anteriorized disc position in maximum intercuspidation (Fig 1D). A combination treatment of splint therapy and transcutaneous electrical nerve stimulation (TENS) for the management of myofacial pain and to resolve the muscular contraction was prescribed.

Case 2

The second patient was a 57-year-old female with an Angle Class II, division 1 malocclusion, an increased overjet (5 mm), an overbite (8 mm), and a relative transverse maxillary contraction (Fig. 2A). The upper midline was deviated to the right with respect to the facial midline. In the upper arch was a fixed prosthesis from the upper left canine to the upper right canine (Fig. 2A), and the lower incisors presented with an abraded incisal edge. The patient reported a history of pain and sounds during jaw movements, especially on the left side. The functional examination showed limited mouth opening (21 mm) with a hard end-feel sign indicative of intracapsular changes (Fig. 2B). No TMJ clicking was observed during left condyle movement, and on the other side, normal condilar translation was reported. The patient was diagnosed with articular locking and disc displacement with reduction and intermittent locking. The TMJ closed lock was physically reduced via jaw manipulation so that TMJ MRI scans of both the mouth opening and maximum intercuspation could be obtained. After manipulation treatment, the functional examination revealed clicking in the final phase of opening (28 mm) in the left TMJ, whereas in the right TMJ, an almost normal condilar translation was observed. The MRI images confirmed a left TMJ anteriorized disc position during maximum intercuspidation (Fig. 2D), whereas a less anteriorized disc position was present on the right (Fig. 2F). The treatment plan after jaw manipulation consisted of an ARS, which the patient wore full time for 5–7 days, followed by part-time use. It was suggested that the upper permanent prosthetic device be removed and replaced with a newer device to increase mandibular movement and avoid relapse, but the patient refused this treatment option, so the fixed prosthesis was filed as a temporary solution.

Case 3

The last patient was a 63-year-old female with Class II malocclusion, an increased overjet (9 mm) and overbite (8 mm) (Fig. 3A), disc displacement with reduction and intermittent locking, and an upper midline deviation to the left with respect to the facial midline. The patient reported pain and sounds during mouth movements. The mandibular opening was limited (22 mm), with a hard end-feel and mandibular deflection (Fig. 3B). Functional examination found no clicking in the right TMJ, and the left TMJ showed normal condilar translation; however, laterotrusive movement on the right side was very compromised (3 mm). TMJ MRI scans of both the mouth opening and maximum intercuspation were obtained after disc reduction via jaw manipulation. Images confirmed the presence of a right TMJ anteriorized disc during maximum intercuspidation (Fig. 3F), whereas less severe disc displacement was present in the left TMJ (Fig. 3D). When the TMJ closed lock was physically reduced via jaw manipulation the ARS could be worn. Due to the disc dislocation, the patient was required to wear the ARS full time; this induced an anterior mandible position (Fig. 4A, B) but produced muscle fatigue,

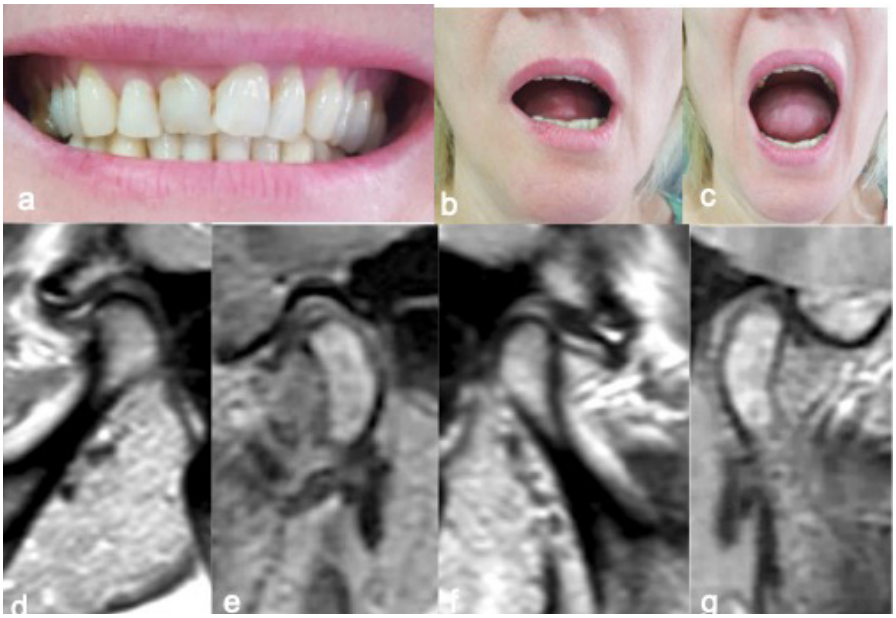

Fig. 1. *Case one. (**a**): The patient presented an increased overbite; (**b**): Mandibular pre-treatment opening was slightly restricted probably due the muscle restriction caused by the intracapsular derangement; (**c**): The functional post-treatment examinations confirmed that the objectives of the treatments were to eliminate the pain and sound, the mandibular opening is normal and without deviations; (**d, f**): Pre-treatment MRI image showed both disc anteriorized, with a worst position on the left TMJ; (**e, g**): Post-treatment imaging showed a recover of good condyle-disc position on the right side (**g**), while the left TMJ the disc results yet anteriorized, but very thickened in the retrodiscal zone (**e**).*

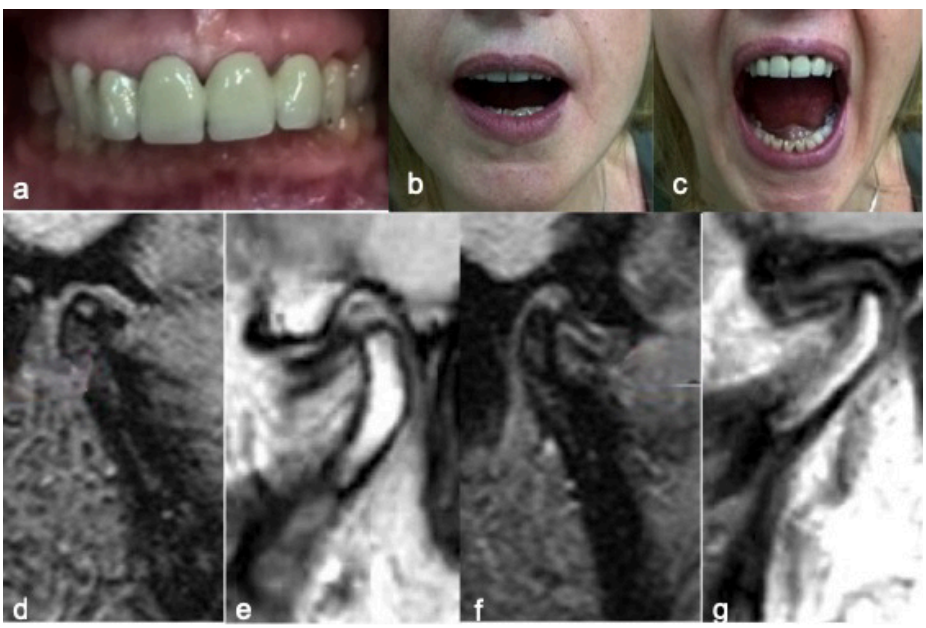

Fig. 2. *Case two. (**a**): The patient presented an increased overbite and there was an incongruous fixed prosthesis from upper left canine to upper right canine; (**b**): Functional pre-treatment examination showed limited opening with mandible deflection, in fact the opening pathway is shifted to one side and becomes greater with opening; (**c**): At the functional post-treatment examinations mandibulars opening is normal and without deviations; (**d**): Pre-treatment MRI images confirmed at left TMJ presence of anteriorized disc position in maximum intercuspidation; (**f**): less anteriorized disc position was present at right TMJ; (**e, g**): In the follow up images discs were still anteriors to the condyles, but were visible a thickening in the retrodiscal zone probably due to the retrodiscal functionalization.*

Fig. 3. *Case three. (**a**): The patient presented an increaed overjet and overbite; (**b**): The mandibular pre-treatment opening is limited, there was a mandible deflection, the opening pathway is shifted to one side and becomes greater with opening; (**c**): The objectives of the treatments were to eliminate the pain and sound, the mandibulars opening is normal and without deviations; (**d**): Pre-treatment, in the left one the disc appared less dislocated; (**f**): In the third that was the more complex case for the management, the MRI were obtained also after the jaw manipulation and the images confirmed at right TMJ presence of anteriorized disc position in maximum intercuspidation; (**e, g**): After 2 years MRI image shows a retrodiscal tissue modification, we hypothesized due the fibrosis caused by retrodiscal tissue functionalization.*

Fig. 4. *Anterior Repositioning Splint (ARS). With ARS, disc recapture is usually achieved through a temporarily change in the position of the condyle (usually forward and downward); in fact the appliance was made on the upper dental arch, where it covered the whole lingual surface and capped the labial surface of the mandibular teeth to keep the mandible forward.*

so an auxiliary treatment with TENS was started. The results suggested that partial disc capture was achieved in the affected TMJ based on bilateral MRI scans (Fig. 1E, G, 2E, G, 3E, G). The functional post-treatment examination confirmed pain and sound reduction and normal mandibular opening without deviations and deflections (Fig. 1C, 2C, 3C). After 2 years, the patient's condition was stable, and the MRI scans showed thickening of the retrodiscal tissue and posterior attachments, including extra fibrous tissue, resulting in a pseudodisc (Fig. 1E, G, 2E, G, 3E, G). In the first case, pre-treatment MRI images showed that both discs were anteriorized (Fig. 1D, 1F), with greater severity on the left (Fig. 1D). The post-treatment scans showed a corrected condyle disc position on the right side (Fig. 1G), whereas on the left, the TMJ disc remained anteriorized and very thickened in the retrodiscal zone (Fig. 1E). For the second patient, the MRI images initially showed that both discs were anteriorized (Fig. 2D, F), with greater severity on the left; in the follow-up images, the discs were still anterior to the condyles, but there was visible thickening of the retrodiscal zone, probably due to retrodiscal functionalization (Fig. 2E, G). The third case was more complex, but after jaw manipulation, the MRI images revealed that both discs were seriously dislocated (Fig. 3D, F), with the right one more severely compromised; after 2 years, the MRI images showed retrodiscal fibrous tissue modification due to retrodiscal tissue functionalization (Fig. 3E, G).

DISCUSSION

The exact mechanism of occlusal splint treatment is still controversial (25, 27, 32-35) and not well understood (7).

ARS treatment for patients with TMJ disc displacements aims to restore the normal physiologic relationship between the condylar head of the mandible and the articular disc (22, 25, 27, 32-34). When designing an ARS, the clinician guides the patient's mandible forward until the disc is reduced (2). Full-time use of the ARS with mandible projections of 4–5 mm tend to produce discomfort, auditory symptoms, posterior open bite, and pain in the posterior area of the jaw from stretching the temporal muscle. To avoid some of these side effects, the literature suggests that an anterior displacement of 3 mm produces the best functional result for disc recapture while minimizing patient discomfort (9). Another important treatment approach is the part-time use of the devices to avoid significantly changing the number of occlusal contacts or causing any skeletal problems (15). In fact, some clinicians instruct the patient to wear the appliance full time for 5-7 days, then gradually convert to night-time wear only (2), whereas others suggest gradual conversion of the appliance to a nonrepositioning design (2).

Katzberg and Westesson suggested that disc recapture is best achieved when mandibular repositioning is guided by disc visualization via MRI (33). Conventional MRI is accompanied by practical drawbacks, nevertheless is still regarded as the standard of reference for TMJ imaging (36). During the last decades, the real-time imaging in clinical medicine promises to shorten the diagnostic procedures (37-46). The recent development of a real-time MRI method has reduced image acquisition times. The technique allows a noninvasive visualization of dynamic processes at high spatial resolution. Krohn et al described as a real-time MRI promises a more precise and reliable diagnosis of DD and may further prevent misinterpretation of static images (28) unfortunately it is a technique still not widespread.

Future objective will be to visualize the functionalization of the retrodiscal tissue and the formation of the pseudodisc with the use of the real-time MRI. The mandible advancement obtained through the ARS produces a partial disc reduction or recapture on areas of the condyle without structural arrangements produced by the morphological alterations caused by disc displacement, as verified in anatomic studies and MRI (9, 34, 47). Hosoki et al. presented a case report in 1995 in which MRI images revealed new TMJ functional disc remodeling within 18 months of ARS treatment. Kurita et al. (32) also evaluated disc repositioning after ARS using MRI and concluded that about 70% of the anteriorly displaced discs that showed both middle to late opening movement click and closing movement

click near maximum intercuspation were recaptured with ARS (18, 32, 34). In addition, Simmons and Gibbs used MRI to study ARS recapture of the TMJ disc and reported the recapture of discs in 25 of 26 reducing displacements, but no recapture was achieved in partially reducing or non-reducing joints (32).

The success of repair depends on the amount of disc displacement, the extent of damage to the tissue (18, 19), the amount of load on the joint, and the tissue repair capabilities (18) (35). Based on the MRI images, the discs that failed to be recaptured were displaced more severely than those that were successfully recaptured (32), and medially and anteriorly displaced discs seem to be more difficult to recapture (25).

However, in acute disc displacement without reduction but with limited opening, the TMJ closed lock must be physically reduced via jaw manipulation before an ARS is used (11). In fact, it is difficult to recapture a displaced disc in patients with long-term dysfunction and late-opening clicks of the joints (38). ARS removes the influence of the teeth on the joint position by slight distraction of the joint which, in turn, enables damaged connective tissue to heal (7). Even partial maintenance of a normal disc/condyle relationship (only at night) decreases pressure on the retrodiscal tissues, allowing for healing and adaptive changes to occur.

Part-time wear allows contact between the condyle and retrodiscal structures during normal function, which can help to stimulate fibrosis and the formation of a pseudodisc, providing protection to previously damaged structures (15). Downward and forward mandibular positioning with an ARS prevents the condyle from articulating with the vascularized and well innervated retrodiscal tissues in the posterior attachment (2, 6, 11), allowing adaptive changes to occur (6). Yano et al. found that ARS therapy not only repositioned the displaced articular discs but also led to condylar bone remodeling manifested as a “double contour” on MRI images of the condylar heads. The “double contours” appear more often on the posterior bevel (sagittal plane) as well as the medial and middle area (coronal planes) of the condyles (11). The therapeutic lower jaw position determined by the clinician is always anterior to maximal intercuspidation by the patient (19). Therefore, for the stability of disc recapture and the prevention of clicking recurrence after removal of the appliance, a stable occlusion must be provided after splint therapy by orthodontic, prosthetic, or surgical means, however this permanent stabilization procedure is potentially irreversible, complex, and expensive (9, 15, 18, 32, 33). To determine whether the corrected disc-condyle relationship is stable, treated patients must be followed up longitudinally (18, 35).

Management of disc displacement with reduction becomes necessary when pain or dysfunction are present (2).

The success of treatment depends on the amount of disc displacement, the extent of damage to the tissue (18, 19), the amount of load on the joint, and the tissue repair capabilities (16, 18). Treatment of patients with disc displacements of the TMJ using a disc repositioning device can stimulate tissue fibrosis and the formation of a pseudodisc, which provides protection to the previously damaged structures (15). MRI is the gold standard for diagnosis, treatment planning, and follow-up of disc displacement cases due to its excellent soft tissue visualization. However, to determine whether the corrected disc-condyle relationship is stable, treated patients must be followed up longitudinally (18, 22).

In conclusion, ARS is a valid appliance to resolve cases of different complexity in a reasonable time, with long term results.

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TECHNICAL NOTE: SURGICAL GUIDE FOR COMPUTER-AIDED ENDODONTIC SURGERY

P. AVANTAGGIATO[1], A. PIVA[1], A. SALAMINI[2], A. AVANTAGGIATO[1], R. TERRONI[3] AND F. CARINCI[4]

[1]DMD, Private practice, Ferrara, Italy; [2]ENG, Sintesi Sud s.r.l., Ariano Irpino (AV), Italy; [3]Dental Technician, Private practice, Ferrara, Italy; [4]Department of Morphology, Surgery and Experimental Medicine, University of Ferrara, Ferrara, Italy

Introduction: CBCT combined to intra-oral scansion are the means to build an endodontic surgical guide to perform an easier and safer access to the apex in endodontic micro-surgery. Method: A 38-year-old woman presented chronic apical periodontitis, which involved the three roots of 16 that was treated by endodontic therapy several years ago elsewhere. The palatine channel was retreated, the mesio- and disto- buccal roots were instead treated with apicoectomy. A surgical template was printed by a 3D printer to obtain greater precision in the surgical access. Discussion: Endodontic microsurgery has evolved over the years. New tools have been introduced to improve therapy, even if the basic principles have not changed. In fact, according to the literature, it is necessary to cut at least 3 mm of root to be sure of eliminating the anatomical variations and the accessory channels. Several Authors have devoted themselves to creating an endodontic surgical template, some in vitro and others in vivo. The present paper introduces a new method that allows a more conservative osteotomy and greater precision the surgical access. Conclusions: Further investigation are needed to test and improve the effectiveness of the treatment but this technique seems very promising because it is less invasive for the patient and simplifies the work for the dentist who can perform micro-surgery in an easier and faster way.

Computer-guided surgery is already present in dentistry from several years, since nowadays it is becoming a common treatment in implantology as it allows a faster and less invasive surgery (1, 2).

The same it can be obtained in endodontics. For example, it can be hypothesised that a template can help to find the root channels (3), but the most important potential of this technique is in endodontic microsurgery.

Surgical endodontics produces successful outcome in 89% of cases when magnification devices, appropriate cavity and correct retrofilling materials are used (4). However, tooth position can affect the success of this therapy, especially for the access. Sometimes one of the challenges during endodontic surgery is to distinguish the root tip from the others and from the surrounding bone, particularly if the lesion extends lingually and the bone is not fenestrated (5). Moreover, mental foramen and maxillary sinus are the anatomical structures that have to be managed to avoid complications.

The use of CBCT is mandatory to perform an exact diagnosis of type, and extension of the peri-apical lesion, shape and anatomy of the roots and of the channels system. Its use permits to orient the efforts of the surgeon to obtain a precise shape

Key word: apicoectomy, endodontic surgery, endodontic guide, root-end surgery, computer-aided surgery

Corresponding author:
Francesco Carinci
Department of Morphology, Surgery and Experimental Medicine,
University of Ferrara,
Via Luigi Borsari 46, Ferrara, Italy
Tel.: +39.0532.455874- Fax: +39.0532.455874
e-mail: crc@unife.it

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of the cavity once the root end is resected. In fact, the presence of an isthmus or an accessory channel can be evidenced before the direct inspection of the resected root during apicoectomy. Furthermore, CBCT combined to intraoral scansion are the means to build an endodontic surgical guide to perform a facilitate access to the apex. The aim of the present paper is to demonstrate the use of a new customized device as surgical guide in apicoectomy.

MATERIALS AND METHODS

A 38-year-old female patient reported a feeling of pressure in the region of a previous endodontic treated first molar. The clinical examination evidenced presence of painful sensation at the vertical percussion. Initially a 2D X-ray was taken, which showed no anomalous signs. For this reason, a CBCT x-ray was performed. From the 3D radiograph it was possible to establish the diagnosis of chronic apical periodontitis, which involved the three roots of the tooth. The orthograde re-treatment was possible only for he palatine channel, while it was not safe to retreat the mesio- and disto-buccal roots because the tooth was already retreated. With the consent of the patient it was decided to attempt the surgical approach and due to the anatomical complexity of the element an endodontic surgical template was projected.

To this aim, a digital impression of the patient's mouth has been taken using an intra-oral scanner (Trios 3 Color Pod. 3Shape Copenhagen, DK). Then a CBCT of the patient using NewTom Giano HR Professional (software NNT NewTom Verona Italy).

Subsequently the file Dcm obtained from the intra-oral scanner was imported on 3SHAPE Implant Studio, a software used for implant planning and implant guided surgery, together with the Dicom file of the CBCT of the patient. Thanks to this software these two images can be matched in a single tridimensional image, choosing 3 points in common. Once localised on CBCT the mesiobuccal and distobuccal roots of the first right upper molar, it was measured. their diameter.

From implant library of the 3Shape software it was chosen an implant type with the same dimension of the root or little more. At this point it was simulated to place these two implants exactly in correspondence of mesio-buccal and disto-buccal root apexes, "tricking" the software (Fig. 1).

The software Implant Studio always loads a preview of the project, so it was adjusted it using a sleeve that has 3.6 mm of inner diameter (we measure 3.28 mm for each root). The case was refined, and a print of the surgical guide

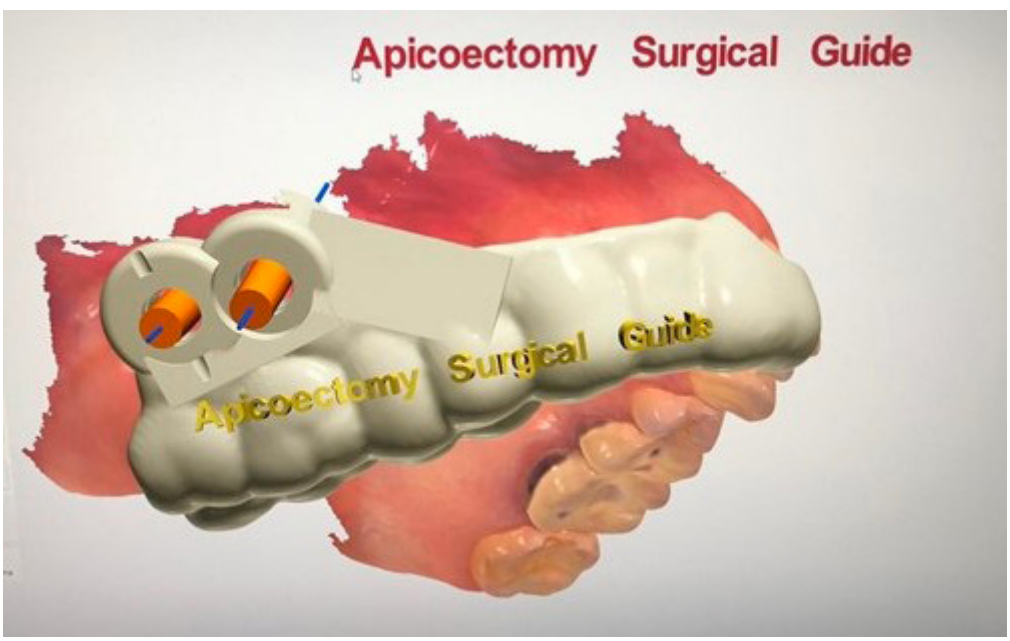

Fig. 1. *3Shape software output.*

was obtained with Photocentric HR2 printer (Fig. 2). The surgical guide was tested and adapted on the 3D model of the patient, previously printed with Photocentric HR2. The procedure was explained to the patient, the informed consent was signed.

Local anesthesia was performed (Mepivacaine 2% and 1: 100,000 adrenaline) followed by a half-moon shaped flap. Once the flap was raised and the bone was exposed, the surgical guide was positioned

Osteotomy was performed through the surgical guide until the appreciation of the two apexes of the tooth. The apex of the mesio-buccal root was found easily, whereas the apex of the disto-buccal root was very close to the mesio-buccal root of the second upper right molar. Once the access to the roots was obtained, the guide was removed and the last 3 mm of roots were resected. The apicoectomy was performed perpendicular to the long axis of the tooth with minimal bevel. Ultrasonic tips were used to obtain a cavity that was filled with a bioceramic cement. Finally, the flap was sutured. Antibiotics were prescribed in the following 5 days. Bruising and edema were very moderate.

DISCUSSION

Some Authors tried to develop a surgical guide to solve cases of Endodontic surgery. Peng et al. (6) created a template for apicoectomy for an in vitro study, using an extracted anterior tooth. As in this case, both the CBCT and the scan, using an optical scanner were used. These two technologies were combined to obtain a very precise template based on the patient's bone, his teeth and his gum. The software used was Geomagic Studio 2012 and the template was printed with a 3D printer in polylactic acid (PLA).

Strbac et al. (7) used the patient's CBCT and his intraoral scan obtained by a optical scanner (iTero; Align Technology Inc, San Jose, CA) and processed the two files with a software used in guided implanvology (coDiagnostiX Version 9.6; Dental Wings Inc, Montreal, Canada) (5). In the present work dental and mucous support were used, like in the previous study, but compared to the other cases of the literature, the entry hole was reduced to promote healing. In fact according to Von Arx et al. (8) a good presence of bone tissue permits a better repositioning of the flap and improves tissue healing.

The difference with the present case-report lies in the entry hole, which is smaller. Moreover the shape is different, as Strbac et al. created a loop,

Fig. 2. *surgical guide.*

while in the present study a little round hole for each root was produced, to minimize the osteotomy. Furthermore, the software and the optical scanner were different. Strbac et al. used an implant software too but anchor pins were positioned to signal the root apex (7). According to the principles of endodontic microsurgery, the Authors considered appropriate to design the surgical template in order to resect the last 3 mm of root because it was shown that accessory channels and anatomical variability are recomposed in this value (9).

The apicoectomy surgical guide is a tool that allows a more conservative surgical access. It is also quite simple to create.

Further investigation is needed to test and improve the effectiveness of the treatment but this technique seems very promising because is less invasive for the patient and simplifies the work for the dentist who can perform micro-surgery in a easier and faster way.

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SILICA SOLUTIONS (SL) IS EFFICACY IN THE TREATMENT OF CHRONIC PERIODONTITIS: A CASE CONTROL STUDY

D. MUCCHI[1] and M. BALDONI[2]

[1]Private Practice, Ferrara; [2]Department of Medicine and Surgery University Milano Bicocca, Italy

Aim: The objective of this study was to compare the efficacy of supportive periodontal therapy (i.e. scaling and rooth planning, SRP) alone versus a chemical device silica dioxide (SiO2) colloidal solutions (SL) used in association with SRP in the treatment of chronic periodontitis in adult patients. Materials and Methods: A total of 20 patients with a diagnosis of chronic periodontitis (40 localized chronic periodontitis sites) in the age group of 35 to 55 were selected. None of these patients have previously received any surgical or non-surgical periodontal therapy and demonstrated radiographic evidence of moderate bone loss. Two non-adjacent sites in separate quadrants were selected in each patient to monitorize treatment efficacy (split mouth design). Clinical pocket depth (PD) and microbial analysis (MA) were analyzed at baseline and 15th day. SPSS program and paired simple statistic T-test were used to detect significant differences. Results: Total bacteria loading, Tannerella Forsitia and Treponema Denticola loading were statistically reduced when SiO2 is locally delivered. Conclusion: SL gel is an adiuvant therapy which should be added to SRP in the management of moderate to severe chronic periodontitis.

Periodontal disease is one of the prevalent hillness in adult population. It is characterized by a symptom triad: tooth mobility, foetor ex ore, gingival bleeding. If left untreated the disease can lead to tooth loss. Pathogenesis of periodontal disease is multifactorial and bacterial have a preminent role (1-22). The main pathogens implicated in periodontal disease are anaerobic gram-negative bacteria of which the most aggressive were identified in the "red complex" group: Porphyromonas gingivalis, Tannerella forsythia, and Treponema denticola (23-35).

The aim of periodontal treatment is to eliminate oral infection, and prevent the progression of the disease (36-47). Many studies have widely demonstrated that the non-surgical therapy including scaling and root planing (SRP) associated with a good level of oral hygiene can prevent the onset of periodontal disease and allow for proper maintenance of oral health (48-53).

Aim to the present study is to evaluate the effectiveness of a new chemical device (i.e. silica dioxide colloidal solutions) used in association with SRP in the treatment of chronic periodontitis in adult patients.

MATERIALS AND METHODS

A total of 20 patients with a diagnosis of chronic periodontitis were randomly selected. Patients were qualified for the study if they have two non-adjacent sites located in separate quadrants which required periodontal treatment, in the age group of 35-55. Subjects have not received previously any surgical or non-surgical periodontal therapy. The patients

Key words: periodontitis, scaling, root planing, silicium, drug, delivery

Corresponding author:
Dr D. Mucchi,
Private Practice,
Via Cavallotti 65,
44021 Codigoro, FE, ITALY

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were excluded from the study if they meet any of the following criteria: (1) pregnancy; (2) an history of taking antibiotics or using antibacterial mouth rinses for past 6 months; (3) teeth with furcation involvement; (4) smoking, and drug or alcohol abuse.

This study has been approved by the ethics Committee of the hospital. Subjects participating in the study volunteered will follow a detailed verbal description of the procedure and by signing consent forms.

Clinical methods

A total of 20 patients (i.e. 40 sites) were selected and grouped into two categories: control and test (split mouth design). The control group (20 sites) was treated with SRP without using SL (control site). The test group (20 sites) was treated by SRP plus SL (test site). All patients underwent to SRP at the baseline measurement. Prior to SRP clinical pocket depth and microbial analysis was performed in each selected site. Then SRP was done at both sites using ultra sonic scaler. After SRP, SL was adjunct in the test site of each enrolled patient. After 2 weeks, microbiological samples were collected again from both sites in each patient.

For bacteria analysis, sites were isolated using cotton rolls. Sterile absorbable paper points (size 60) were used for the collection of subgingival samples and were immediately transferred to microbiological lab for processing. *Porphyromonas gingivalis*, *Tannerella forsythia*, and *Treponema denticola* and total bacterial loading were evaluated.

Real-Time Polymerase Chain Reaction

Probes oligonucleotides were designed basing on 16S rRNA gene sequences of the Human Oral Microbiome Database (HOMD 16S rRNA RefSeq Version 10.1) counting 845 entries. All the sequences were aligned in order to find either consensus sequence or less conservative spots. Two real-time polymerase chain reaction (PCR) runs were performed for each sample. The first reaction quantified the total amount of bacteria using two degenerate primers and a single probe matching a highly conservated sequence of the 16S ribosomal RNA gene. The second reaction detected and quantified the three red complex bacteria, i.e. *P. gingivalis, T. forsythia and T. denticola*, in a multiplex PCR. This reaction included a total of six primers and three probes that were highly specific for each specie. Oligonucleotide concentrations and PCR conditions were optimized to ensure sensitivity, specificity and no inhibitions in case of unbalanced target amounts. Absolute quantification assays were performed using the Applied Biosystems 7500 Sequence Detection System. The amplification profile was initiated by a 10 min incubation period at 95°C to activate polymerase, followed by a two-step amplification of 15 s at 95°C and 60 s at 57°C for 40 cycles. All these experiments were performed including non-template controls to exclude reagents contamination.

Plasmids containing synthetic DNA target sequences (Eurofin MWG Operon, Ebersberg Germany) were used as standard for the quantitative analysis. Standard curves for each target were constructed in a triplex reaction, by using a mix of the same amount of plasmids, in serial dilutions ranging from 101 to 107 copies. There was a linear relationship between the threshold cycle values plotted against the log of the copy number over the entire range of dilutions (data not shown). The copy numbers for individual plasmid preparations was estimated using the Thermo NanoDrop spectrophotometer.

The absolute quantification of total bacterial genome copies in samples allowed for the calculation of relative amount of red complex species. To prevent samples and polymerase chain reaction contamination, plasmid purification and handling was performed in a separate laboratory with dedicated pipettes.

Statistical analysis

SPSS program and paired simple statistic T-test were used to detect significant differences.

RESULTS

A statistically significant differences was detected between (A) Total Bacterial Loading pre and post SL

treatment, (B) *T. forsythia* pre and post SL treatment and (C) *T. Denticola* and post SL treatment (see Table I). Noteworthy, no statistically significant difference was detected between control and test site as regard pre-treatment bacteria concentration as well as pre vs. post-treatment bacteria concentration in control site (i.e. only SPR). When p-value is less than 0.05 then the difference between the two compared bacterial loadings is statistically significant (see last column in Table I).

DISCUSSION

It is well understood that most destructive types of periodontal diseases occur due to the presence of pathogenic micro-organisms colonizing the subgingival area and the suppression or eradication of these microbes result in improvement in periodontal health. Mechanical debridement is effective in both disturbing the biofilm and reducing the bacterial load. However, sometimes mechanical instrumentation may not be sufficient to control the disease due to tissue invasive pathogens, or other tooth related anatomic factors. In such conditions, adjunctive use of a chemical device provides an additional benefit in controlling the disease.

Support periodontal therapy is widely used, but a greater effectiveness is demonstrated by the administration of topical antimocrobicals in association. The advantages of topical therapy involve the use of antimicrobical agents directly into the periodontal pocket, minimizing the adverse effects related to systemic therapy (54-65). The potential benefits of local drug delivery include improved patient compliance, an easier access to periodontal pocket and a lower dosage of antimicrobial agent. The most used methods for local drug delivery are local gingival irrigations (66-74). The antimicrobial

Table I. *Total bacterial loading pre and post SL treatment.*

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
Pair 1	CBT1 - CBT2	-1325978	1,3E+07	3071472	-7778900	5126944	-,432	18	,671
Pair 2	CBTS1 - CBTS2	1974667	2661685	610632,4	691775,9	3257558	3,234	18	,005
Pair 3	PG1 - PG2	67807,53	188312,2	43201,77	-22956,0	158571,1	1,570	18	,134
Pair 4	PGS1 - PGS2	146377,9	443168,4	101669,8	-67222,5	359978,2	1,440	18	,167
Pair 5	TD1 - TD2	-62,6842	61310,98	14065,70	-29613,6	29488,26	-,004	18	,996
Pair 6	TDS1 - TDS2	71305,05	134958,8	30961,68	6256,9798	136353,1	2,303	18	,033
Pair 7	TF1 - TF2	32294,74	83725,10	19207,86	-8059,47	72648,94	1,681	18	,110
Pair 8	TFS1 - TFS2	70521,11	144430,5	33134,62	907,8438	140134,4	2,128	18	,047
Pair 9	CBT1 - CBTS1	-159089	5454840	1251426	-2788238	2470059	-,127	18	,900
Pair 10	CBT2 - CBTS2	3141556	1,3E+07	2961350	-3080009	9363120	1,061	18	,303
Pair 11	PG1 - PGS1	-124696	368553,3	84551,92	-302333	52941,09	-1,475	18	,158
Pair 12	PG2 - PGS2	-46125,5	256030,5	58737,43	-169528	77277,23	-,785	18	,443
Pair 13	TD1 - TDS1	-77013,0	177212,4	40655,32	-162427	8400,6546	-1,894	18	,074
Pair 14	TD2 - TDS2	-5645,26	31338,33	7189,5070	-20749,9	9459,3306	-,785	18	,443
Pair 15	TF1 - TFS1	-33451,8	128754,0	29538,19	-95509,3	28605,60	-1,132	18	,272
Pair 16	TF2 - TFS2	4774,5263	78364,59	17978,07	-32996,0	42545,05	,266	18	,794

agents used as local drug delivery agents include tetracycline, ofloxacin, clindamycin, chlorhexidine, etc. (75-86). These local drug delivery devices have been used either alone or as adjunct with SRP. These local antimicrobials are administered directly into the periodontal pocket and the effectiveness of these chemical device is related to their bactericidal activity and the subsequent reduction of gingival inflammation (87-98).

The topical use of chemical device along with mechanotherapy dramatically improves clinical results, and at the same time is free from its inherent adverse effects and disadvantages. Local delivery of chemical devices into the pocket achieves a greater concentration of the drug locally, proving bactericidal for most periopathogens, and at the same time, exhibits negligible impact on the microflora residing in other parts of the body.

Our study evaluated the efficacy of SL gel in the management of moderate to severe chronic periodontitis. The results of this investigation demonstrated an overall improvement in most of bacterial parameters.

Microbiological testing was thought appropriate to evaluate the effect of SL gel on subgingival microbial population, the primary etiological factor for periodontitis. Several methods have been used for microbiological testing in periodontitis (99-105). However, many techniques have not been fully accepted due to low sensitivity or specificity. Moreover, sometimes they are slow, expensive and laborious. Recently, a rapid and sensitive test (LAB SRL, Ferrara, Italy) was developed to detect and quantify the three bacterial species more involved in periodontitis: *Porphyromonas gingivalis, Tannerella forsythia*, and *Treponema denticola*.

It is well known that both *P. gingivalis* and *T. denticola* occur concomitantly with the clinical signs of periodontal destruction (106-111). They appear closely 'linked' topologically in the developing biofilm, shown an *in vitro* ability to produce a number of outer membrane-associated proteinases. They are considered the first pathogens involved in the clinical destruction of periodontal tissues. Moreover, both them and *T. forsythia*, show a higher prevalence in disease than in health suggesting that these bacterial are associated with the local development of periodontitis. Based on this reported data, red complex triad was investigated, and SL was able to significantly reduce their concentration.

SL gel is an adjuvant therapy which should be added to SRP in the management of moderate to severe chronic periodontitis.

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