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CONTENTS

THE EFFECTS OF A NEW DECONTAMINANT SOLUTION ON ROOT CANAL BLEEDING DURING ENDODONTIC TREATMENT: A RANDOMIZED CONTROLLED STUDY

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Blood contamination of the canal during preparation and obturation can be a problem in Endodontics; this may result in apical microleakage. The purpose of this investigation was to observe and evaluate the hemostatic properties of biofilm decontaminant material (sulfonic/sulphuric acid solution, HybenX, EPIEN Medical) used in teeth with necrotic pulp and unstoppable bleeding after root canal shaping. A prospective study was designed with 2 randomized parallel groups: decontaminant material (experimental group) and sodium hypochlorite 5% (control group). The analysis of the root canal bleeding was evaluated by the clinician before and after the application of the sulfonic/sulphuric solution or sodium hypochlorite 5%, by measuring the millimeters of blood on a sterile paper point introduced in the root canal. Sixty patients with necrotic pulp and unstoppable bleeding were enrolled in this study and randomly divided into 2 groups: decontaminant material in 30 patients (experimental group) or sodium hypochlorite 5% in 30 patients (control group). *T*-test showed that the percentage change in millimeters of blood detected in the root canal was statistically greater for experimental group [mean difference: 0.74 (IC: 0.66-0.82); p<0.0001]. The hemostatic properties were better in the experimental group than in the sodium hypochlorite 5% group (control). Further research may be needed to confirm the results of this study.

The necessity to dry the root canal before proceeding to its filling, in order to increase adherence of the filling material to the root canal's walls, is agreed by everyone as a matter of fact (1-3). Indeed, humidity can alter the cement hardening properties, increasing or decreasing the working time (4-6) and inhibit its penetration into the dentinal tubules (7). Blood and/or other exudates represent a state of humidity in the canal: during the performance of a root treatment, pathologic situations can induce a bleeding in the canal that can sometimes be difficult to control, prevent an adequate drying of the canal and lead to the necessity to finish the treatment in more than one session.

Blood presence can inhibit effectiveness of irrigant solutions (sodium hypochlorite), due to a high concentration of albumin (8). The most frequent cause of bleeding is irreversible pulpitis; in this case, the blood comes from the canal system and often needs a prolonged rinsing and waiting time to stop. Other causes of bleeding can be due to iatrogenic factors (over instrumentation, dentinal chips extrusion, irrigation beyond the apex, perforations, stripping) or anatomical factors, such as minor or ignored canals still containing bleeding pulp. Sometimes, bleeding can be connected to the presence of exudate from an acute or chronic apical lesion.

Key words: calcium hydroxide, endodontic treatment, root canal bleeding, root canal exudation

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In most cases, the bleeding stops after canal cleaning and shaping; in all other situations (apical periodontitis, long and over instrumentation, dentinal chips extrusion irrigation past the apex, perforations and stripping) drying the canal may be difficult. Besides, a higher risk of bleeding can be found in patients affected by congenital coagulopathies (coagulation factors deficit) acquired coagulopathies or drug induced (antiplatelet, warfarin or new generation anticoagulants).

To prevent bleeding in patients with coagulation problems, it is necessary to comply with protocols coded for each disease, while no coded procedures are reported in literature for local causes of bleeding. From a clinical point of view, the problem is not worthy of interest and only practical, not experimental, suggestions can be found, mainly about hemostatic drugs generally used in dentistry (pulpotomy, surgical endodontics): lidocaine 1:50.000, ferric sulfate, calcium hydroxide, sodium hypochlorite. There are no coded protocols yet to control root canal bleeding with sure and predictable results.

A new device (HYBENX®, EPIEN Medical, Saint Paul, MN, USA) has been developed with the purpose of destroying dental biofilm. The material is a mixture of hydroxybenzenesulfonic acid (37%) and hydroxymethoxybenzene acids (23%), sulphuric acid (28%), and water (12%). The product is currently marketed by the producer both for use in Periodontics (HybenX Oral Tissue Decontaminant) and for Endodontics (HybenX Root Canal Cleanser). The two forms of the product, which have the same chemical composition but differ in consistency, viscous gel for periodontal use and liquid gel for endodontic use.

This device has been successfully used in periodontology. Recent studies (9, 10) have demonstrated the effectiveness of the oral tissue decontaminant material in the treatment of clinical cases showing acute periodontal abscess without the use of systemic or local antibiotics. Similar favorable effects were obtained in the treatment of peri-implant mucositis and peri-implantitis (11, 12). In addition, a randomized controlled trial (RCT) also demonstrated the beneficial effects of the material in the treatment of oral aphthae (13).

Based on this scientific information, a clinical and microbiological study was planned, using the decontaminant device in cases of teeth with necrotic pulp with the aim to destroy the dental biofilm of root canals. The ability of the decontaminant device to stop bleeding immediately after canal instrumentation was discovered accidentally during the procedures of this still unpublished trial.

Due to this surprising evidence, the authors were interested in evaluating the coagulation property of the decontaminant material. The purpose of this study was to test the reduction of root canal bleeding in terms of significant percentage change for millimeters of blood in the canal at 2 different time points (before and after treatment).

MATERIAL AND METHODS

A single center, participants and biostatistician blind, two-arm, randomized, placebo controlled clinical trial study was performed following the CONSORT statement (14) in the Endodontics Department, University Hospital Florence Careggi, Florence, Italy.

Study population

The study population consisted of patients that were treated between April 2017 and December 2017 at the Endodontics Department of the University Hospital of Florence Careggi, Italy. Subject inclusion criteria were: patients aged between 20 and 60 years, able and willing to sign a consent form, single-rooted teeth with necrotic pulp confirmed by electric vitality test, associated with healthy periodontium, physiologic sulcus depth (<3 mm), and absence of bleeding on the probing of the involved teeth.

Exclusion criteria were: patients with systemic diseases, using anticoagulants, antibiotics, or anti-inflammatory therapies in the last 30 days, patients with an allergy to sulphur in any form and pregnancy. All subjects were informed of the nature and potential risks and benefits of their participation in the study. They also received information on the duration of the procedure and the possible intraoperative and postoperative complications.

The study protocol was submitted and approved by the Ethics Committee of the University Hospital

of Florence (SPE16.302 University Hospital of Florence). Patients provided written and signed informed consent in accordance with the Helsinki Declaration of 1975, as revised in 2000, 2008 and 2013. Each recruited patient was examined, and routine preoperative information with respect to the number of appointments, duration of the procedure, and possible intraoperative and postoperative complications was given.

Treatment

The root canal therapy procedure was standardized and limited to one experienced endodontist (R.P.). Treatments were performed at the Endodontics Departments of the University Hospital of Florence Careggi, Italy, under local anesthesia using 1.8 mL mepivacaine hydrochloride with 1:200000 epinephrine (Optocaine; Molteni Dental SRL, Scandicci, Italy), and the teeth were isolated with rubber dams. After preparing the access cavity, the working length was determined with an electronic apex locator (Propex II Dentsply Maillefer Instruments, Ballaigues, Switzerland) with a size 10 k-file and confirmed taking a periapical radiograph with the 10 k-file inserted in the root canal. Root canals were shaped using ProTaper Universal NiTi files (Dentsply Maillefer Instruments, Ballaigues, Switzerland) in the following sequence: S1, S2, F1, F2, and F3 until each instrument reached the working length.

After each instrument, the root canals were rinsed with NaClO (Niclor 5, Ogna, Italy) using a syringe with a side-vented 30 G needle. ProTaper NiTi instruments were driven with an endodontic motor (XSmart Endo Motor, Dentsply Maillefer Instruments, Ballaigues, Switzerland) with a 16:1 contrangle set up as suggested by the manufacturer.

After the root canal shaping was performed, the root canal was dried with 4 sterile paper points. Quantifying root canal bleeding with paper points can be challenging, since the size of the paper point (if smaller than the apical constriction), will pass the foramen and draw blood or fluid from the periapical area. To avoid this inconvenience, a fifth 30 size-sterile paper point was introduced in the root canal, up to the working length, for 10 seconds to detect blood presence. With a caliber on the sterile paper point, the millimeters of blood present within the root canal were measured.

Only the teeth that at this point showed 1 or more millimeters of blood within the root canal were included in the present study and randomized to the 2 experimental groups to reach the estimated *a priori* sample size. In the patients excluded from the study, the ordinary endodontic treatment was carried out.

Before further treatment, the teeth were allocated to an experimental (HybenX) or a control group (sodium hypochlorite 5%) according to an uneven block randomization designed by the statistician (A.N.). For each tooth, closed envelopes were opened in a consecutive order, assigning the tooth to either the experimental or the control group.

HybenX was approved as a Class I CE medical device by the Italian Ministry of Health (no. 483768) on February 7, 2012.

Experimental Group

The decontaminant material was introduced inside the root canal using the pre-dosed syringe, and activated for 20 sec, with a sterile paper point, with an up and down movement up to the working length. Finally, the canal was rinsed with sterile water using a syringe with a side-vented 30 G needle 1 mm shorter than the working length.

The root canal was then dried with 4 sterile paper points. Finally, a fifth 30 size-sterile paper point was introduced in the root canal, up to the working length, for 10 seconds to detect the presence of blood. The millimeters of blood inside the root canal were measured again according the previous criteria.

Control Group

The root canal was irrigated with 5% sodium hypochlorite with a syringe and a side-vented 30G needle, activated for 20 sec with a sterile paper point with an up and down movement up to the working length to ensure a flow of irrigant solution throughout the canal.

Finally, the canal was rinsed with sterile water using a syringe with a side-vented 30 G needle 1 mm shorter than the working length. The root canal was then dried with four sterile paper points. Finally, a fifth sterile paper point was introduced in the root

canal, up to the working length, for 10 seconds to detect the presence of blood; the millimeters of blood inside the root canal were measured again according the previous criteria.

Sample Size Estimation

A total number of 60 patients (30 patients per group) were evaluated to reject the null hypothesis of equality between the two experimental groups in terms of root canal bleeding based on the following assumptions:

- Power of approximately 90% in rejecting the null hypothesis of equality
- Expected means at the baseline of 4 mm for both of the two groups
- Expected means gain after using the decontaminant material 2.5 mm (reduction of 40%) and 4 mm after using 5% sodium hypochlorite (no reduction)
- Standard deviation of 1.7 mm in both the experimental group
- Overall significance level = 5% two-sided.

Statistical Analysis

Data were analyzed using SAS Version 9.3 software (SAS Institute, Inc., Cary, NC). A preliminary Levene's test was performed to verify the homogeneity of variance for the percentage change in the two groups. A t-test was performed to verify the null hypothesis by using a type I error equal to 0.05. In order to confirm the firmness of the results a Wilcoxon non-parametric test was also carried out.

RESULTS

The initial study sample consisted in 72 consecutive patients with necrotic pulp. After the root canal instrumentation, 12 patients were excluded because they did not present root canal bleeding. Sixty patients (26 women and 34 men) with a mean age of 37.6 years (standard deviation, 10.1 years) (Table I) with a tooth with necrotic pulp and unstoppable root canal bleeding after instrumentation were enrolled in this study, and randomly divided into two groups according to the intervention used in order to control the bleeding:

1. Experimental Group: 30 teeth (14 mandibular teeth, 16 maxillary teeth)

2. Control Group: 30 teeth (11 mandibular teeth, 19 maxillary teeth)

All the efficacy analyses have been performed on the intention to treat (ITT) population. Any deviation from the predetermined randomization list occurred.

Descriptive statistical analysis of millimeters of blood detected in the root canal at baseline and after the treatment in both groups is shown in Table II. No relevant differences in terms of root canal bleeding at the baseline were detected between experimental and control group. Levene's test assessed the equality of variances for the measured variable calculated for the two groups. T-test showed that the percentage change in millimeters of blood detected in the root canal was statistically significant greater for experimental group (Table III). Wilcoxon test, performed to support the

Table I. *Demographic and baseline characteristics.*

	Total	HybenX	Control
Gender			
Men	34	18	16
Women	26	12	14
Age	37.6±10.1	40.1±9.9	35.2±10.2
Smoking habit			
Smokers	41	22	19
Non-smokers	19	8	11
Arch			
Maxillary	35	16	19
Mandible	25	14	11
Position			
Incisive	21	12	9
Canine	18	8	10
Premolar	21	10	11

result of the t-test, confirmed that experimental group was statistically significantly better than control group in percentage change of millimeters of blood in the root canal ($p<0.001$).

DISCUSSION

The results of the present study show the effect of a decontaminat device on the ability to dry the root canal in the presence of serum-hematic blood or exudates.

In case of root canal treatment, more frequently in case of over-instrumentation, a serosanguineous exudate is seen when a sterile paper point is placed into the apical extent of the canal. The persistence of this exudate may significantly affect final sealing with the persistence of microleakage.

Optimum sealing conditions were obtained when totally dry canals were filled with a single gutta-percha cone and a ZOE- based sealer. When Methacrylate-based Endodontic Sealers are used, the root canal walls must have a slight degree of humidity to promote adhesion to the root canal walls, but all materials exhibited some evidence of dye penetration. Root canals that remained totally wet (flooded), showed a higher degree of leakage. In these cases, liquids (irrigant solution, blood, and exudate) cannot be displaced completely in spite of the hydrophilic properties of the sealers. Liquids permeation during the polymerization process might result in the entrapment of water droplets within the sealer-dentin interface. This might result in bond disruption and further increased leakage (15). Furthermore, inflammatory exudate and blood itself are rich in proteins such as albumin, which can partially inactivate the antibacterial activity of commonly used disinfectant in root canal treatment (16).

There are no clinical studies that analyze how the problematic exudate can be controlled during ordinary endodontic treatment; in Literature, most of the studies concern the bleeding in case of pulpotomy. In cases of bleeding from the pulp calcium hydroxide, anesthetic solution with 1:50.000 epinephrine or ferric sulphate placed on a sterile paper point, are recognized as effective hemostatic agents (17-21).

It is generally believed that calcium ions play a key role in the regulation of platelet function (22). Furthermore, the use of calcium hydroxide as a root canal dressing can help to promote platelet aggregation probably because calcium binds to the phospholipids that appear secondary to the platelet activation and provides a surface for assembly of various coagulation factors (23).

According to Mohammadi (24), in a "weeping canal" condition, the most successful way of stopping the exudate is by drying the canal with sterile paper points and placing calcium hydroxide paste in the canal. It is the basic pH of calcium hydroxide that modifies the acidic pH of periapical tissues and changes it into a more basic condition that is responsible for this process. Other theories have been proposed:

a) Ca(OH)2 has a caustic action which can cauterize chronically inflamed tissue (25);

Table II. *Descriptive statistics.*

Group	Variable	N	Mean
1 Experimental	Baseline (mm)	30	3.40
	After treatment (mm)	30	0.26
	% Change	30	0.95
2 Control	Baseline (mm)	30	3.73
	After treatment (mm)	30	3.03
	% Change	30	0.21

b) Ca(OH)2 has an antibacterial effect may have an ability to inactivate toxins (26).

None of the methods with files and conventional irrigant solution (NaOCl 3%-EDTA 17%-Saline solution) used were efficient in removing the entire dressing from the canal walls, leaving 25 to 45% of the surface of the walls covered with the calcium hydroxide dressing, although the instrumentation of root canals allowed irrigation needles to reach most areas (27).

The study revealed that considerable amounts of calcium hydroxide remain at canal walls, and apical regions may interfere with the sealing efficiency from a mechanical point of view; it has also been shown to interact with zinc oxide-eugenol based sealers, influencing the setting reaction of the sealer and blocking the gutta-percha entrance, along with placement up to the working length and in to dentinal tubules. ZnOE cements, when in contact with calcium hydroxide, are completely disorganized as the zinc oxide-eugenol reaction is compensated for the preferential interaction of calcium hydroxide with eugenol. The presence of such remnants at critical areas, like the apical region, may adversely affect the clinical performance of the sealer and possibly the long-term prognosis of root canal therapy (28).

During a still unpublished clinical and microbiological study, using a decontaminant device (sulfonic/sulphuric acid solution) in order to destroy the dental biofilm of root canals, the authors were surprised to verify the immediate cessation of bleeding after using the material. Therefore, due to this outcome, the authors were interested in evaluating the coagulation properties of the decontaminant material during endodontic treatment.

Based on clinical observations, the sulfonic/sulphuric acid solution is able to stop bleeding in the root canal completely and permanently after rinsing it for 20 sec. The sulfonic/sulfuric acid solution is an aqueous solution, and thus it can be removed easily from the root canal with sterile water irrigation. In addition to this, since the device is purple in color, it allows the operator to verify its complete removal from the canal lumen in the drying phase with absorbent paper points. This enables the practitioner to proceed to root canal filling without the use of calcium hydroxide dressings.

Thus, the procedure can be concluded in a single visit. The importance of proceeding with treatment in a single appointment has always been a controversial aspect in Literature. In a review by Morerira et al. all the factors interacting in the endodontic success are evaluated between therapies in a single visit *vs* multiple visits. This review article stated that although there is no statistically significant difference between the two methods, a trend of superiority of the single visit therapy is observed in patients with periapical periodontitis in terms of incidence of postoperative complications and treatment efficacy (29). For this reason, having a device capable of stopping the bleeding that may be present inside the root canal at the end of the shaping and cleaning phase could represent an important tool in everyday endodontic practice.

This solution generates a final effect due both to

Table III. *T-Test on percentage change as (baseline - after treatment) x 100/baseline.*

Group	%Change estimates [95%IC]	H0:LSMean1=LSMean2 Pr > |t|
1 Experimental	0.95 [0.90 – 1,00]	<.0001
2 Sterile Saline Water	0.21 [0.15 – 0.27]	

		Difference Between Means	95% Confidence Limits for LSMean(i)-LSMean(j)	
		0.74	0.66	0.82

the keratolytic effect of hydroxybenzenes and the hygroscopic and denaturing ability of the sulfonate group and sulfuric acid (30). The final effect is associated with the interaction between the sulphate group and water molecules, since the sulphate group shows internal polar structure, while oxygen atoms display a strong charge on negative surface on the outside surface. The combination between large negative surfaces of the sulphate group with the positive surfaces of water molecules creates an electrostatic interaction, known as a hydrogen bond, where the positive charge of hydrogen atoms on the surface of the water molecule is attracted to the negative charge of the oxygen atoms surface (30-36). A possible explanation for the reduction of intracanal bleeding is that it is based upon the rapid desiccation of debris at the damaged vascular bed interface within the root canal. The product's sulphate groups molecularly remove bound water from the detritus at this interface causing instant denaturation of the tissue debris. This could lead to coagulation in the vascular bed and subsequent hemostasis. The effect is probably not tied to conventional blood factor hemostasis chemistry.

This chemical property also produces the denaturation of biofilm, with rapid subtraction of water from the matrix by sulfonic and sulfuric acids, leading to the detachment of biofilm materials from the root surface and facilitating root instrumentation and disinfection (37-51).

One possible limitation of this study was that there was no clinician blinding. Another limitation could be represented by the fact that, even though the measurement of the millimeters of blood in this study has been made with paper point with the same size of the latest instrument used to shape the canal (size 30), the bleeding registered with the sterile paper point could come totally or partly from the periapical inflamed tissues. On the other hand, the authors cannot completely ruled out that the haemostatic action of the material is a consequence of the leakage of minimal quantities of product into the periapical tissues. Further studies with larger patient samples are needed to confirm the findings of the present study.

Within the limits of the present study, the results show that the decontaminant material administered in teeth with necrotic pulp, dry the root canal significantly in the presence of serum-hematic blood or exudates**.**

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This study is registered on clinicaltrials.gov with number NCT03336853.

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ANALYSIS OF ORAL MUCOSA EROSIVE-ULCERATIVE LESIONS BY REFLECTANCE CONFOCAL MICROSCOPY

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***In vivo* Reflectance Confocal Microscopy (RCM) allows to optically biopsy vital tissues, non-invasively and in real time. It results in horizontal virtual slices at a microscopic resolution and correlating with conventional histopathology. The aim of the present work is to describe RCM cellular and architectural findings in oral mucosae affected by erosive-ulcerative diseases, thus highlighting in vivo the well-known histological peculiarities. A series of conventionally diagnosed Recurrent *Aphthous stomatitis* (RAS) and *Pemphigus Vulgaris* (PV) erosive and/or ulcerative oral lesions underwent RCM imaging to establish the application of RCM imaging to this kind of inflammatory non-tumoral lesions. A total of 12 RAS-related lesions and 8 PV-related lesions were considered. RCM imaging was capable to visualize their microscopic peculiarities, mainly inflammatory infiltrate, vessel dilation (RAS) and acantholytic cells, intraepithelial clefts and inflammatory cell carpets (PV). Despite RCM may result unnecessary to diagnose oral lesions referred to RAS and PV, its capability to highlight their main microscopic features could be advantageously used to monitor the healing or worsening of the clinical situation as well as the responsiveness/refractoriness to therapy.**

In vivo confocal microscopy (CM) is a real time imaging technique able to image, layer by layer, horizontal virtual slices (on confocal planes) of the vital tissues from the surface to the submucosa/superficial dermis and *vv*; it works at microscopic resolution (magnification up to 350x) and well correlates with standard histology (1).

Recently, CM has been largely applied in stomatology and its main principles are based either on the detection of the fluorescent light emitted by an endogenous marker or an exogenous substance applied to the living tissue, illuminated by a specific wavelength (Fluorescence CM, FCM) (2) or on the mechanism of backscattering of light emitted by vital structures with different refractive indexes (3-4). Born in dermatology (5-6), RCM has recently started to be applied in stomatology (3, 4, 7-12).

Recurrent *Aphthous stomatitis* (RAS) is a disease characterized by small round or ovoid ulcers usually extremely painful for the first 4-5 days, estimated to affect up to 25% of the worldwide population (13). The third and fourth life decades show a peak of RAS expression, the first episode usually occurs during the childhood or in later life stages (14). RAS is three times more frequent in white people than in Afro-American people, with females more affected

Key words: confocal microscopy, imaging, in vivo, recurrent Aphthous stomatitis, Pemphigus Vulgaris, optical biopsy

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than males (13). A series of trigger factors have been considered involved in RAS etiopathogenesis: genetic predispositions, food allergies and deficiencies, hormonal disorders, oxidative stress, mechanical trauma, viral and bacterial infections, systemic diseases (15).

Pemphigus Vulgaris (PV) is an autoimmune disease, potentially life threatening, which leads to manifestation of blisters and erosions (16). It affects skin and mucous membranes in which pathology reveals intraepithelial acantholysis due to the presence of circulating antibodies (IgG) against desmoglein 3 composing desmosomes (17). Normally PV involves people during their fifth or sixth decades of life, with Japanese, Ashkenazi Jews and populations with Mediterranean ancestors being more affected than other groups (17). Etiopathogenesis seems to involve both genetic and environmental causes (15, 16).

The aim of the present work is to describe RCM cellular and architectural findings in oral mucosae affected by these kind of erosive-ulcerative diseases, thus highlighting *in vivo* the well-known histological peculiarities, as well as other non-invasive real time imaging techniques diagnostic procedure (19-21).

MATERIALS AND METHODS

A series of patients affected by RAS or PV were considered, after conventionally diagnosis (clinical suggestions and lab tests) (15). The related vesicular, erosive and/or ulcerative oral lesions underwent RCM imaging with a handheld reflectance confocal microscope (VivaScope 3000®; Lucid, Rochester, NY, USA), commercially available for real-time *in vivo* imaging, to scan them from the surface to the sub-mucosa. The confocal findings were collected, described and compared with the literature, when present (19). This study has been approved by the ethical committee of the University of Campania "Luigi Vanvitelli" (#689/2014), where patients were recruited from the Oral Pathology Unit, after written informed consent was obtained, in accordance with the Declaration of Helsinki and its later amendments.

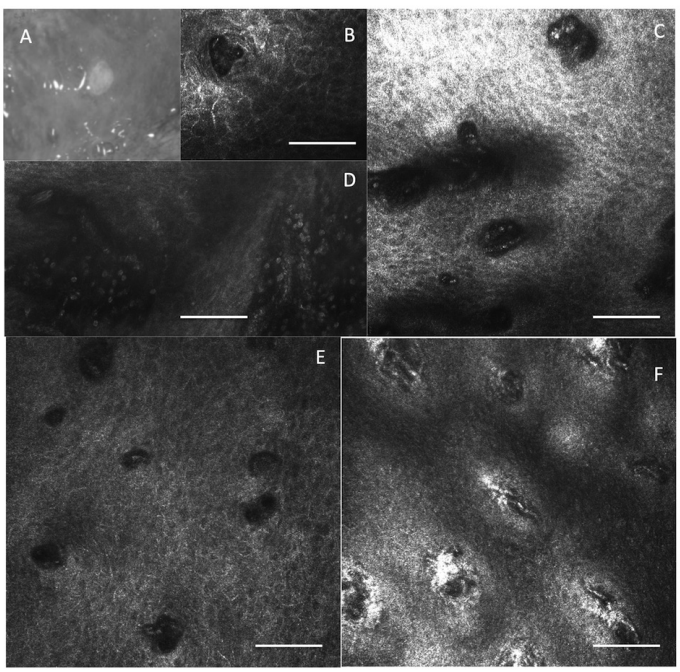

Fig. 1. *Twenty-two-year old male, non-smoker, affected by multiple small RAS-related ulcers. (**A, B**): Clinical and corresponding RCM images of one of the ulcers, located at the ventral tongue; (**C-F**): RCM images of multiple RAS ulcers of the lip mucosa/fornix in the same patient at different depths; (**C, D**): multiple ulcerative dark areas filled with inflammatory infiltrate; (**E, F**): small convoluted horizontal blood vessels within the papillae below the lesions. Scale bars: 100 μm.*

RESULTS

A total of 12 erosive-ulcerative lesions in 4 patients affected by RAS (2 females, 2 males, 28-41-years-of-age) and 8 erosive lesions in 3 patients suffering PV (3 females, 62-68-yearsof-age) were considered for RCM imaging. No intact vesicles were found. Four RAS lesions were located on lingual margins/ventral tongue and eight on cheek or lip mucosae and fornix, while the PV sites considered for RCM imaging were checks (4 lesions), lip mucosae (2 lesions) and gingiva (2 lesions).

Recurrent Aphthous stomatitis (RAS)

RCM imaging of ulcers related to RAS showed dark areas due to lack of substance fulfilled by a sparse-to-dense inflammatory infiltrate. At the epithelial- connective tissue junction (CEJ) grey-to black areas corresponded to inflammatory infiltrate that obscured the junction, while, at the sub-mucosal layers, small horizontal blood vessels, highly bright, were seen (Fig. 1).

Pemphigus Vulgaris

On RCM imaging, PV-related oral erosions were basically characterized by the identification of acantholytic cells as roundish bright keratinocytes, separated from each other and dispersed in strongly dark areas obscuring the images (intraepithelial clefts); in addition, diffused carpets or speckled areas of inflammatory cells at the lower layers, often disable the visualization of the sub-mucosal structures; in some cases, horizontally oriented enlarged blood vessels and inflammatory cells were visible below the sub-epithelial layers (Fig. 2).

DISCUSSION

Confocal microscopy is useful in stomatology to gain microscopic details both from the living tissues (3, 4, 7-11) and on adequately processed specimens (22, 23). *In vivo* RCM is not only useful in oral oncology as diagnostic aid for discovering both early and clear signs of oral cancer and its precursors (24-29), but also in monitoring the inflammation degree

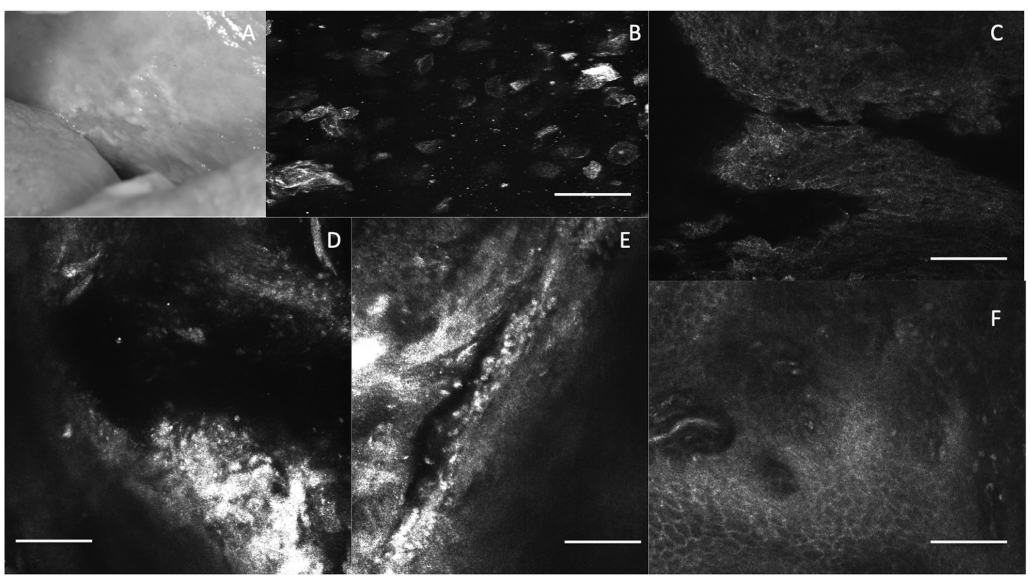

Fig. 2. *Sixty-five-year-old female, non-smoker, affected by oral PV. (**A**): Clinical details of the erosive areas at the cheek mucosa RCM imaged; (**B**): Acantholytic cells dispersed within the intraepithelial clefts; (**C, D**): At progressive depths, the cleft boundaries are strongly identifiable by the contrast in the different refractivity, and appeared progressively more fulfilled by inflammatory cells; (**E**): In some points, the clefts are quite linear; (**F**): At the CEJ, the boundaries of the papillae are obscured by the inflammatory infiltrate and enlarged horizontal blood vessels and diapedesis of inflammatory cells within the tissue. Scale bars: 100 μm.*

and epithelial and sub-epithelial changes occurring in infective, inflammatory and/or dysimmune diseases affecting oral mucosae (30-53). Previous literature reported few cases of PV imaged by RCM, limiting to 12 cases of desquamative gingivitis PV-related (7) and 1 case of multiple buccal mucosa erosions with a histological diagnosis of PV (19). Our results on PV imaging agreed the findings reported by Alessi et al. (7) and Ardigò et al. (19) both on gingival and buccal lesions (acantholysis, inflammatory infiltrate, dark cleft, enlarged horizontal blood vessels). Concerning RAS, literature did not report previous experiences, presumably because RCM imaging is considered unnecessary to diagnose oral lesions referred to RAS. Despite this fact, the present work has applied RCM imaging to RAS ulcerations in order to depict them in a unconventional way. Since the results confirmed RCM capability to highlight RAS (and PV) main microscopic features, it is reasonable to suggest RCM use to monitor the healing or worsening of the clinical situations as well as the responsiveness/refractoriness to therapy.

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ANALYSIS OF LIP PIGMENTATIONS BY REFLECTANCE CONFOCAL MICROSCOPY: REPORT OF TWO CASES

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Oral mucosa pigmentations belong to a heterogeneous variety of lesions, which are usually divided into two groups: exogenous or endogenous pigmentations. The pigmented lesions most frequently found in the oral mucosa are the amalgam tattoo, the melanotic macula and the nevus. All these lesions may affect every part of the oral mucosa, and they may represent a hard diagnostic challenge for the clinician; the clinical objective examination is not sufficient to make a correct diagnosis. Reflectance Confocal Microscopy provides a real-time microscopic evaluation of tissue layers, and is widely considered a useful auxiliary tool in monitoring skin and mucosa lesions. In this context, Reflectance Confocal Microscopy imaging is a valid aid in the management of oral mucosa pigmented lesions, to corroborate and support the diagnostic process.

Oral pigmented lesions consist in macular or tumefactive accumulations of melanin, hemosiderin, or exogenous metals that can be detected on oral mucosa in focal, multifocal or diffuse patterns (1). These lesions clinically manifest as blue, purple, brown, grey or black pigmentations. Some of them are localized benign lesions but others may be a sign of systemic diseases and even genetic conditions; furthermore, some require immediate intervention since they represent serious medical conditions, such as oral mucosal melanoma (2).

Among benign pigmentations, melanotic macules are the most common mucosal lesions of melanocytic origin, usually unifocal, affecting any mucosal area and in particular gingivae, palate and the lower lip (3). In particular, it seems that adult females are more likely to develop melanotic macules compared to males. These pigmentations, typically appearing as small and well-circumscribed lesions, do not result from increase in melanocytes number. Instead, the underlying mechanism consists in a functional melanocytic hyperactivity so that there is an increased melanin production (4); no treatment is required except for aesthetic concerns (5).

Another kind of reactive melanocytic pigmentation is smoker's melanosis, in which melanin production may represent a protective mucosal reaction to the irritant action of smoke. This condition has been reported in 21.5-30% of smokers and it usually presents as an irregular pigmentation in a diffuse pattern affecting the anterior labial lower gingiva, though tongue, lip, buccal mucosa and hard palate (6). As regards melanocytic nevi, these pigmentations represent a different kind of

Key words: confocal microscopy, imaging, in vivo, oral pigmentations, lentigo simplex, optical biopsy

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lesion as they are characterised as benign neoplastic lesions that develop as a consequence of melanocytic proliferation. Oral mucosa may be affected by this condition, although much less commonly than in the skin (7).

Differently from cutaneous nevi, malignant degeneration of the oral ones has not been reliably verified. Oral nevi are typically brown or blue asymptomatic macules, which are often unifocal and well circumscribed (8). The hard palate, buccal mucosae and vermilion of the lip are more likely to be affected (9). As regards mucosal melanoma, this is a rare malignant neoplasm, with a poor 5-year survival rate of approximately 15-40% (10). Primary oral melanomas represent less than 1% of total melanoma cases. These malignancies typically occur in the age range of 7-95 years and, in some studies, males tend to be more frequently affected than females. Oral melanoma is usually asymptomatic and may present as an irregular-shaped and dark brown to black-coloured macule. These lesions may exhibit a focal or diffuse pattern of pigmentation, whereas others may lack pigment and are defined as "amelanotic melanomas" (11).

In regards to melanotic macules, lesions affecting labial mucosae, known as labial melanotic macules (LMM) may be difficult to differentiate from melanoma located on the oral mucosa and the muco-cutaneous junction as well (12). Benign melanotic macules represent the most frequent cause of lip pigmentation, however their diagnosis can be troublesome, hence a close follow-up or a lip biopsy may be needed to establish a diagnosis with confidence (12). Even those macules are characterised by an increase in the number of melanophages leading to a hyperpigmentation of the basal keratinocytes.

Reflectance confocal microscopy (RCM) is a novel non-invasive imaging technique that represents a diagnostic aid in many skin pathologies (12-14). It provides a virtual biopsy of vital tissues in real time, resulting in microscopic horizontal virtual slices that well correlate with histopathological examination (15). RCM, in fact, may represent a valid support for the clinician in the perspective of a non-invasive diagnostic strategy (16-19). The most common RCM machines can capture images with a field of view of at least 400 x 400 µm, optical sections of 1-5 µm and lateral resolutions of 0.1-1 µm and can reach a depth of 200-300 µm (10). Despite the widespread use of this imaging technique in skin pathology, it is still rarely applied to the study of oral mucosal lesions (20-27).

The purpose of the present work is to describe 2 cases in which RCM was used in the study of labial pigmented lesions in order to investigate the potential utility of this technique as a clinical adjunct. The patients were recruited from the Oral Pathology Unit of the University of Campania "Luigi Vanvitelli" and the study has been approved by the ethical committee of the University of Campania "Luigi Vanvitelli" (#689/2014).

Case reports

Case 1

A 65-year-old female patient previously treated for oral candidiasis (28, 29), presented with melanotic areas on both the upper and lower lip, and with no symptoms reported. The patient's past medical history revealed diet-controlled hypertension, previous colon cancer and polyps, and even the presence of untreated thyroid nodules, hence medical history suggested the clinical suspicion of Peutz-Jeghers syndrome. Clinical examination showed several irregular shaped melanotic lesions that appeared to be brown uniformly pigmented and located on the muco-cutaneous junction. At RCM examination, no signs of malignancies (10, 12, 13) were found (Fig. 1). Supra-basal layers preserved their honeycombed architectural pattern, at the connective-epithelial junction (CEJ), the tissue appeared diffusely grainy and papillae clearly rimmed, regular in size and homogeneously arranged; sparse fusiform or stellate dendritic cells (in each observation they were less than 2 per papilla) surrounding papillae were identified.

Case 2

A 71-year-old male patient with an over 40-year smoking history, periodically followed up for a past history of oral cancer (30-36) and under treatment for periodontal disease (37-58) presented with irregular

shaped pigmentations on the lower lip. Patient's medical history also revealed hypertension. Clinical examination showed a brownish pigmentation of the vermillion, referred as asymptomatic but subjected to repeated traumatisms (Fig. 2). RCM examination did not show signs of malignancies (13): supra-basal

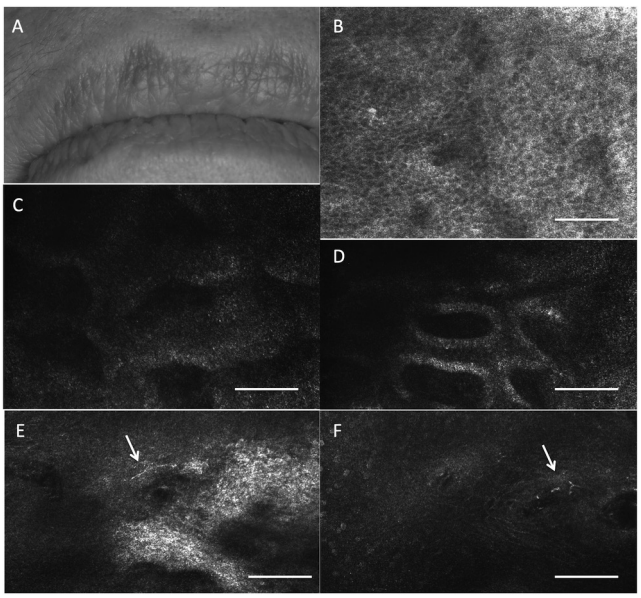

Fig. 1. *Benign melanotic macule of the upper lip. (**A**): Clinical appearance; (**B**): Within the supra-basal layers, the lesion preserved its honeycombed pattern; (**C, D**): At the epithelial connective tissue junction, the tissue is grainy and papillae appeared rimmed, regular in size and homogeneously arranged; (**E, F**): Few fusiform and stellate dendritic cells (less than 2 per papilla) surrounding papillae were identified (white arrows). Scale bars: 100 micron.*

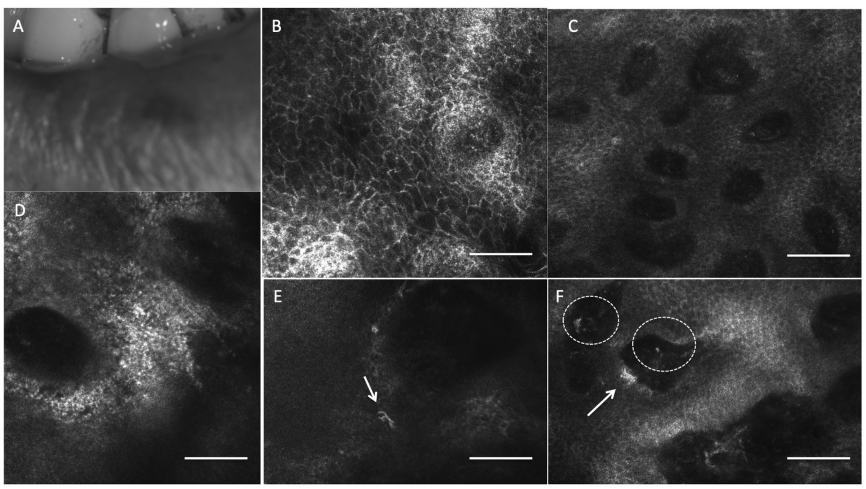

Fig. 2. *Benign melanotic macule of the vermillion. (**A**): Clinical appearance; (**B**): Within the supra-basal layers, the lesion preserved its frosted glass pattern; (**C**): tall connective tissue papillae, typical in vermillion, are identifiable as regularly preserved; (**C, D**): At the epithelial connective tissue junction, papillae appeared rimmed, regular in size and homogeneously arranged; (**E, F**): Stellate dendritic cells surrounding papillae were identified (white arrows) and small bright particle were found inside the papillae (dotted circle). Scale bars: 100 micron.*

layers preserved their frosted glass architectural pattern, and tall-connective tissue papillae were already present due to their higher interdigitation physiologically present at this site (18, 24). At the CEJ, the tissue appeared diffusely grainy grey and papillae were clearly rimmed, regular in size and homogeneously arranged; sparse stellate dendritic cells (in each observation they were less than 2 per papilla) surrounding papillae were also identified as well as small bright particle inside the papillae.

DISCUSSION

Based on the previous reports that identify and distinguish pigmented lesions of the lips (9, 12, 13), the present work aimed to adopt their identification criteria to define the nature of three pigmented lesions of the lip/vermillion, observed in two patients. The RCM findings agreed with the definition of benignant pigmented lesions, also called *lentigo simplex*. In the smoker patient, the lesion observed may be better ascribed to a smoker's melanosis, due to his history of smoking and the traumatisms. When the "RCM Lip Score for Diagnosis" was calculated according to Uribe et al. (13), the three lesions considered gave a result of zero. Despite these cases confirmed what literature have previously reported, we suggest the need for more extended studies and meanwhile, to follow up the pigmented lesions and/or to perform biopsy with conventional histopathological analysis for the certain diagnosis.

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TOPICAL TOLUIDINE BLUE-MEDIATED PHOTODYNAMIC THERAPY FOR THE TREATMENT OF ORAL LICHEN PLANUS

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Photodynamic Therapy (PDT) is a minimally invasive approach that has shown promising results in management of oral, head and neck lesions. PDT can be used alone or in combination with other conventional treatments (surgery, chemotherapy, radiotherapy). Oral Lichen Planus (OLP) is a mucosal and cutaneous chronic disease characterized by an autoimmune insult of basal keratinocytes. We aim to evaluate the feasibility of topical toluidine blue-mediated PDT for the treatment of oral cavity multifocal homogeneous white lesions by oral lichen planus without dysplastic features.

Photodynamic therapy (PDT) is currently inserted among low laser therapy techniques as a minimally invasive treatment for malignant and premalignant lesions (1). Resolution of the lesion tissue results both from direct cytotoxic damage and microcirculation damage; the part of the treated lesion is detached from the surrounding healthy tissue impeding normal healing and restoration of the site (2). The therapeutic effects of PDT are due to the production of free radicals of oxygen. In clinical practice, a photosensitizing molecule (also called "fluorophore"), which tends to accumulate in mitochondria, lysosomes and cytoplasmic membranes, is administered and subsequently activated by a light source directed on the area to be treated (3). The action of the light beam damages the subcellular structures in which the fluorophores have accumulated through mechanisms of apoptosis, necrosis and microcirculation-induced collapse, triggered by the production of radical oxygen (4, 5).

Oral Lichen Planus (OLP) is a mucosal and cutaneous chronic disease (6-8) characterized by abnormalities in the growth and differentiation of basal keratinocytes whose surface antigens are modified because of primary autoimmune damage (9). The autoimmune damage leads to a delay in the growth of mucosal epithelium that is responsible for hyperkeratosis and acanthosis (10). The clinician has to approach this kind of lesion by making a correct differential diagnosis with other white lesions of the oral mucosa (11-15), such as oral candidiasis (16-18) and white sponge naevus (19). Two different approaches can be used to manage patients with white oral lichen lesions: wait and see approach and medical therapy (20). Del Corso et al., in their study, conclude that excision is recommended to treat dysplastic lesions and vaporization for non-dysplastic lesions (21, 22). A further strategy is provided by the

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use of drugs, both topically and systemically. In 2002, a randomized clinical trial evaluated the efficacy of tazarotene, a topical retinoid, compared to a control group receiving a placebo; a significant reduction in hyperperatoric lesions was observed from OLP (23).

The wait and see approach is usually based on a "conservation" or on a process of "active surveillance"; avoiding risk factors, performing clinical visits for short follow-ups and practicing serial biopsies, are three of the cornerstones of this strategy (24). Aim of this preliminary work was to verify the validity of topical toluidine blue-mediated PDT in the resolution of oral cavity multifocal homogeneous white lesions by oral lichen planus and without dysplastic features.

Toluidine blue (TB) is a chromophore of the family of phenothiazines, with a strong absorption band in the spectrum region between 620nm and 660nm, and then finds its way in a "phototherapeutic window" (600 - 750nm) in which light penetration in the tissues is maximized. Specifically, the maximum absorption peak is 630 nm. TB binds to the nucleic acids of DNA and RNA, acting as a nuclear marker and highlighting areas corresponding to more marked mitotic activity of the cell, typically attributable to dysplastic and potentially malignant lesions (25).

MATHERIALS AND METHODS

For this study, 5 patients were enrolled from the Oral Mucosal Pathology Division located at the Multidisciplinary Department of Surgical and Dental Specialties at the University of Campania "Luigi Vanvitelli". The study was approved by the ethical committee of the University of Campania "Luigi Vanvitelli" (#0005947/2016).

Inclusion criteria

Patients selected for this study were previously subjected to incision biopsy of the contralateral lesion, and had received a histologic diagnosis of OLP without dysplasia.

Exclusion criteria

Subjects who did not agree to undergo treatment were excluded. Patients with systemic diseases, drug consumption, pregnancy, photosensitivity, aged 21 years or less, who had lesion/lesions with dysplasia and who received treatment for OLP at least 1 month previous to beginning the study, were excluded. Lesions adjacent to amalgam filling site were not eligible to the study.

Five patients, 3 women and 2 men (Table I), with multifocal OLP lesions were involved, and after written consent statement, underwent a photodynamic therapy cycle associated with blue toluidine. Each cycle consisted of a variable number of therapeutic sessions based on the persistence of the lesion. Between one session and the other, there was an interval of at least 14 days. Our study evaluated the potential of topical application of TB as photosensitizing agent on white multifocal lesions.

The sites to be stained by TB application were treated according to the following operating steps:

- preparation of target site with topical application of 2% acetic acid on cotton pellets for one minute;
- drying of the affected area with gauze;
- performing small touches with a TB soaked pellet on the lesion;
- performing a second dab with acetic acid at 1% for one minute.

PDT was then performed with FotoSan® 630 device (CMS Dental, Elmevej, Denmark), consisting of a handpiece, a charger and an external power supply. Due to its small size and ergonomic and lightweight design, the device has proven to be particularly useful for oral cavity application, including areas that are normally not easy to handle, due to their difficult accessibility. During this time period (2 min and 30 sec for each session), a suction hopper was placed in the mouth of the patient so that it did not wash away the dye from the stained areas. The patient was also asked to keep his eyes closed during all the phases in which the lamp was activated.

All the stages of our study, starting from the moment the patient came to our attention for the first time, were provided with photographic documentation collection. This allowed, between each session, and at the end of the therapeutic cycle, to monitor the response of the lesions to the PDT over time and to evaluate the clinically observable effects for each patient. Before starting the therapeutic sessions (T0), and at the end of each session, lesions were measured with a millimetre scale. At the end of the PDT cycle, patients returned to control after 7 days and if still present, the remaining lesion was again measured (T1). Furthermore, after 2 weeks, each patient

Table I. *Demographic data from included patients and location of the treated lesion; lesions size at T0, during follow-up at subsequent sessions (T1-T5) and final response ("RECIST").*

	Gender	Age	Location
AG	F	63	Upper left adherent gingiva
VG	M	71	Upper right adherent gingiva
CG	F	63	Upper right adherent gingiva
GN	M	68	Upper left adherent gingiva
MTB	F	62	Upper right adherent gingiva

T0	T1	T2	T3	T4	T5	Tf
10 mm	7,5 mm	7 mm	2mm	0	---------	*RC*
22 mm	20 mm	18 mm	16 mm	14 mm	12 mm	*PR*
10 mm	6 mm	3 mm	0	---------	--------	*RC*
13 mm	6 mm	3 mm	0	---------	--------	*RC*
12 mm	9 mm	6 mm	2 mm	0	--------	*RC*

was stained again on the treated site with TB, to allow us to appreciate the correct healing of the tissue; if still necessary, the remaining part of the lesion was then submitted to a further PDT session and one week later, at subsequent follow-up (T2), lesion was measured again.

At the end of the full cycle of treatment (Tf), we evaluated tissue healing (Complete response - CR), remaining entity of the lesion (Partial response - PR) or persistence of entire lesion (Stable disease - SD) in accordance with the Tumor Responsiveness Assessment Criteria (known as the "RECIST" acronym).

RESULTS

We selected all patients whose lesions were not strongly stained by TB, considering that the lesion cells were not dysplastic. In 1 subject, a partial resolution (PR) of the lesion was obtained. The rate of reduction of the lesion was about 54% (T0=22mm; Tf=12 mm). TB-related PDT produced total disappearance of clinically visible lesions (RC) in 4 cases out of the 5 treated (Table I).

DISCUSSION

The focus of the scientific community has deepened on research in identifying a minimally invasive and repeatable therapeutic strategy, in the perspective of a non-invasive diagnostic and interventional iter (26-36), capable of determining the complete healing of premalignant oral lesions, such as OLP (37, 38). PDT has been successfully used in the management of a variety of pathologies from different anatomical sites. These include the head and neck, brain, lungs, hepatobiliary tree and other gastrointestinal (39) and urological pathologies, skin, gynaecological conditions and in vascular anomalies (40-63). The limit of topical therapy is related to transcutaneous drug absorption, which limits the possibility of application to more superficial lesions. In fact, with the topical PDT it is possible to reach a maximum treatment depth of 1-2 mm so that this technique can only successfully intervene on very superficial lesions of less than 1 mm (44-48). Topical PDT can be considered a valuable auxiliary mean for OLP lesions treatment due to its highlighted advantages such as high selectivity of the dye in identifying cells with proliferative capacity and easy and quick execution, well tolerated by the patient. On the other hand, some disadvantages were noticed, such as the small size of light spot, which can adversely affect the result of extensive lesions' treatment. Preliminary clinical results obtained in patients with small to medium size lesions definitely encourage the future use of this technique for the treatment of more extensive lesions, when a few technical issue are resolved, such as the size of the light spot. The facility and

speed of execution, low cost and high comfort for the patient, which characterize this technique, support the value of PDT with TB in topical treatment of oral lichen planus white lesion.

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ASSOCIATION BETWEEN DENTURE STOMATITIS, *CANDIDA SPECIES* AND DIABETIC STATUS

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Candida species **are commensal yeasts of the oral cavity, which, under predisposing systemic and/or local circumstances, are responsible for a wide variety of clinical manifestations, globally known as oral candidiasis. Candida-associated denture stomatitis is an oral candidiasis particularly affecting the oral mucosa covered by a dental prosthesis, with several degree of severity. Diabetics suffer oral candidiasis more frequently than healthy individuals do and if they are denture wearers, the risk increases. Since various controversies still remain regarding the interrelationship among diabetes, oral *Candida spp.* strains involved in denture stomatitis and the presence of dentures, the present review aims to investigate the differences in *Candida* species frequencies and degree of denture stomatitis severity existing among diabetic and non- diabetic individuals, with and without dentures.**

Candida is a fungus usually existing as commensal species in the oral cavity. Occasionally, under systemic and/or local circumstances it becomes an opportunistic pathogen, thus leading to a variety of oral infections defined as "oral candidiasis".

Among the local factors mainly predisposing the occurrence of this kind of infection, the presence of a denture not adequately sanitized, along with poor oral hygiene are responsible for the so-called Candida-associated "denture stomatitis" (DS) (1), whose severity has been clinically classified by Newton et al. into tree types (2).

- Type I: localized erythematous;
- Type II; diffuse erythematous;
- Type III: hyperplastic granular.

DS prevalence among denture wearers ranges from 15% to over 70%; it is higher among elderly denture users, women, and those subjects reporting poor-fitting dentures, poor denture hygiene, continual night-time wearing of removable dentures, accumulation of denture plaque and bacterial and yeast contamination of denture surface (3). The adherence of *Candida* via a biofilm formation to the porous and rough surface of denture materials seems to facilitate colonization (4).

The hyphal-forming *C. albicans* types have shown to be the most common *Candida* strain responsible for oral candidiasis, due to its ability at epithelial binding, disruption and invasion (5). Further studies have focused on the increased detection of other *Candida species (Candida spp.)*, correlating them with the misuse of antifungals that has induced drug resistances (6) and several local factors and systemic

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conditions (7, 8). In addition, further subgroups of denture wearers are affected by diabetes. This systemic disorder contributes to diversify the fungal flora of the oral mucosae covered by the dentures and this must be investigated to establish the most effective therapy/prophylactic procedures to cure and prevent DS recurrence in these subjects.

Diabetes mellitus (DM) is a chronic disease of abnormal carbohydrate, fat and protein metabolism due to the decreased insulin secretion and/or insulin-resistance (9) associated with a significantly higher frequency and recurrence of oral *Candida* infections, mainly due to the hyperglycaemic status stimulating *Candida* growth and to the neutrophil dysfunction occurring in diabetics (10). However, various controversies remain regarding the interrelationship among diabetes, oral *Candida spp*. strains involved in DS and the presence of dentures.

Hence, the present review aims to investigate the scientific literature to establish if substantial differences in the Candida species frequencies and degree of DS exist among diabetics and non-diabetics individuals, with and without dentures.

Diabetes, oral candidiasis and Candida spp.

A wide number of studies investigated the relationship among salivary and/or hematic glucose and oral *candida* carriage, reporting the following:

- (*i*) salivary glucose levels (SGL) significantly higher in both uncontrolled and controlled diabetics compared with non-diabetics (11);

- (*ii*) SGL significantly correlated with haematic glucose levels (HGL) in the entire study population (11);

- (*iii*) median levels of *Candida spp.* colony forming units (CFUs) significantly higher in diabetics (the highest levels found in the uncontrolled diabetes individuals) (11, 12) and significantly associated with haematic glucose levels for the entire study population. In addition, the individuals that still have or have reported a previous history of prediabetes seems to be highly susceptible to oral *Candida* infections than that of healthy controls (13).

Candida spp. overall prevalence is higher in diabetics (average 67%, range from 41 to 100%) than in non-diabetics (average 44% ranges from 35.5% and 50.4%) (10, 14-19) and, although *C. albicans* is usually the most frequent species both in diabetics and non-diabetics, followed by other *non-albicans Candida* (NAC) (the most reported are *C. krusei*, *C. glabrata*, and *C. tropicalis,* and *C. kefir*), diabetics seem to have NAC levels higher than non-diabetics (13-15). Conversely, Sharma et al reported different frequencies in the *Candida spp.* by isolating *C. parapsilosis* as the highest occurring yeast in 30 Indian diabetics (20).

Candida species in otherwise healthy denture wearers with and without denture stomatitis

When considering otherwise healthy denture wearers (DW+), i.e. without diabetes and any other systemic condition, *Candida spp.* prevalence was reported as widely heterogeneous. Gauch et al. reported 89% of *Candida spp.* prevalence (78% *C. albicans* alone or associated with NAC, and 11% only NAC) (21), Loster et al. reported a prevalence of 58% (predominantly *C. albicans* accounting for 79% of total) (22, 23), while Bianchi et al. found *Candida spp.* in 83.3% of DW+ and in 53.5% of the DW- (24). Further authors, such as Altarawneh et al, explored the differences among *Candida spp. (albicans/NAC),* mucosal wetness/salivary flow and sub-denture mucosal inflammation in otherwise healthy DW+ with and without denture stomatitis (DS+ and DS-, respectively) (5). They excluded dry mouth as primary factor promoting *Candida* infection and reported that *C. albicans* yeasts were significantly 4-fold more present in saliva and 21-fold more cultured from the denture surfaces of the DS+.

Candida species in diabetic denture wearers

Several studies aimed to find differences in *Candida spp.* frequencies and overall prevalence between diabetics DW+ and DW-, or, conversely, between DW+ with and without *diabetes mellitus* (DM+ and DM-, respectively).

In works reporting groups of DM+/DW+ and DM+/DW-, (both without clinical signs and symptoms of DS), authors such as Gonçalves et al., isolated yeast strains from 53% of the whole diabetics subjects enrolled (10). DM+/DW+ group reported higher *Candida spp*. prevalence (74.7%) than the

control group DM+/DW- (16.3%); interestingly, the most frequent *Candida spp*. in the study group DM+/DW+ were NAC (52%), while *C. albicans* was isolated in 45% of the total. Similar results have been reported by Manfredi et al. (25).

Conversely, Dorocka-Bobkowska B et al. (16) and Motta-Silva et al. (17) focused on DW+ affected by DS, with and without *diabetes mellitus*. In both works, *Candida spp*. were isolated more in the subjects DW+/DM+ (100% and 85%) than in the individuals DW+/DM- (50% and 40%). Despite the different prevalence, *Candida spp*. frequencies were similar in both groups (*C. albicans*, followed by *C. glabrata*, *C. tropicalis* and *C. parapsilosis*). Furthermore, DW+/DM+ statistically reported:

- (*i*) higher incidence of extensive inflammation and oral complaints (burning sensation and dry mouth);

- (*ii*) the founding of *C. dubliniensis*, isolated only in the DW+/DM+ group (4.2%) (16); - (*iii*) a greater production of exoenzimes such as the proteinases by *C. albicans* (17).

The frequencies of the three *Candida spp.* more often isolated (*C. albicans, C. tropicalis and C. glabrata*) have been further investigated by Sanitá et al. that published a study on *Candida spp.* identification from the dentures of the individuals in the three groups: healthy subjects (DW+/DS-/DM-), non-diabetics with DS (DW+/DS+/DM-) and diabetics with DS (DW+/DS+/DM+) (18).

These three strains were found alone in some cases or mixed in other cases. *C. albicans* frequency was higher in the DS+ groups than in healthy group (in 100% of DW+/DS+/DM+, in 93% of DW+/DS+/DM- and in 64% of DW+/DS-/DM-); *C. tropicalis* were found in 20% of DW+/DS+/DM-, 28% of DW+/DS+/DM- and only in 2% of DW+/DS-/DM-, similar to *C. glabrata,* isolated in 10%, 20% and 14%, respectively.

Considering the overall DS patients (with and without diabetes) according to the degree of mucosal inflammation, the frequency distribution of *C. tropicalis* significantly increased relative to the highest degree of inflammation, while the prevalence of *C. albicans* and *C. glabrata* were not influenced by the degree of inflammation. The frequency of mixed species populations in the overall DM+ individuals (32.5%) was significantly higher than that in the DM- (14.4%). Similarly, Perić et al. showed in a cross-sectional study on DW+ subjects with *Candida*-positive DS that 57% of them were exclusively positive to *C. albicans*, while, the remaining 43% were affected by *C. albicans*+NAC or mixed species (predominantly found in younger individuals), such as *C. krusei* (48.6%), *C. tropicalis* (37.1%), and *C. glabrata* (34.3%). They also reported the higher frequency of NAC species in those patients suffering DS type III (by Newton's classification) (26).

DISCUSSION

Various studies have reported that individuals affected by *diabetes mellitus* (both type I and 2, both controlled and uncontrolled, both pre-diabetes status and past history of hyperglycaemia) seem to be higher susceptible to oral *Candida* infections than healthy individuals (11-13). *Candida spp.* have also been found more prevalent in denture wearers, both as carriers (in absence of DS) and CFUs (in presence of DS) (21-24), particularly those DW diabetics (10). In details, the evidence highlighted by Altarawneh et al. (5), reporting *C. albicans* 21-fold more cultured from the denture surfaces of the DS+, suggests the importance of prophylactic measures to reduce DS occurrence in denture wearers.

When the denture wearer is also affected by DM, *Candida spp.* isolation is often highly characterized by NAC species than *C. albicans* (10, 25). Usually, the most frequent species isolates in this cluster of subjects are: *C. albicans*, *C. glabrata*, *C. tropicalis*, *C. parapsilosis*. (16, 18). *C. tropicalis* and in general NAC species, significantly increased relative to the highest degree of inflammation (18, 26), thus suggesting that severe DS may be sustained by NAC infections. Since it has been reported that *C. tropicalis* and *C. glabrata* have the ability to cause fungemia in humans and that they are associated with a higher mortality risk than *C. albicans* (18), the presence of these species must be accurately identified in order to approach an adequate eradication of the oral infection. Sharma et al found *C. parapsilosis* as the highest yeast occurring in 30 Indian diabetics. (20).

However, this result must be contextualized and re-considered in further studies on more numerous subjects from other countries.

The pathogenic mechanisms of virulence of *Candida spp.* in denture wearers are various: while *C. albicans* in denture wearers has been proven to be more proteolytic than those from controls (17), NAC species such as *C. tropicalis, C. glabrata,* and *C. krusei* tenaciously adhere to acrylic prosthetic surfaces (26). *C. dubliniensis* was found in DW+/DM+ subjects by Dorocka-Bobkowska et al. (16) and, ubiquitously both in DM+ and DM- individuals by Zomorodian (15) and Sharma (20); other authors probably failed to isolate and report *C. dubliniensis* in the diabetics due to the use of phenotypic methods, making difficult the distinction between them and, therefore, underestimating its presence.

However, the confirmation of *Candida spp.* positivity in diabetic denture wearers reporting clinical signs and symptoms of DS, occurred in less than a half of them (19), thus suggesting that *Candida spp.* are not responsible alone for these clinical conditions, but perhaps the drugs used to control diabetes too. For these reasons, it is recommended to confirm the presence of *Candida* with a proper procedure to avoid any unjustified antifungal treatments and to differentiate them from other oral lesions and pathologies of different aetiology (27-34).

Lastly, scientific literature reports the associations between chronic and/or recurrent *Candida* infections and oral cancer arising, thus leading clinicians and patients to not-underestimate the gravity of these kind of infections and their effects on the oral mucosae (35-67).

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CLINICAL FITTING OF A CAST METAL POST AND CORE OBTAINED BY MEANS OF AN INTRAORAL OPTICAL SCANNING (IOS) AND DIGITAL WORKFLOW

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Customization of post-and-cores using computer-aided-design and computer-aided-manufacturing (CAD-CAM) requires the scanning of a pattern and the subsequent digital design. This case report describes the production of a CAD-CAM customized post-and-core designed from an intraoral scan and milled from a metal block. The use of an intraoral scanner (IOS) for post-endodontic rehabilitation could lead to a faster and more efficient CAD-CAM customized post-and-core realization. The use of a high resistance material such as metal is paramount in cases with high loss of coronal structure. The patient has been treated with bisphosphonate (BP) for years. The risk of osteonecrosis of the jaw after extraction was high.

A metal cast post and core is traditionally used to provide the necessary retention when restoring severely damaged teeth (1). Cast post and core has been considered the gold standard for years, especially in the situation of an insufficient ferrule effect and irregular shape of the root canal (1-8). Sometimes the dental coronal walls are completely missing and the restoration retention is assigned only to the radicular portion (9). In these cases, ferrule effect is not present and core retention by build-up is not sufficient, consequently it could be necessary to plan a metal cast post and core to restore the missing dental structure (10).

With the introduction and continuous development of Computer Aided Design - Computer Aided Manufacturing (CAD-CAM) technologies in the field of dentistry, different solutions to make individual post and core have been proposed. Most of them are based on the digitalization of a resin pattern made through a traditional technique (11-17).

The purpose of this case report is to emphasize the possibility of using an intraoral digital impression and digital workflow even in cases where traditional techniques and materials, such as metal, are required.

Clinical report

A 64-year-old Italian woman presented to dental office for a rehabilitation of the lower jaw. She reported being in good health and wanted to come back to a good masticatory function (Fig. 1). The patient had been treated for osteoporosis with Bisphosphonate (BP) (Prolia, Amgen, Thousand Oaks, CA, USA) for 10 years and for heart stroke with anticoagulant (Coumadin, Bristol-Myers Squibb, New York, NY, USA) for 5 years. Osteonecrosis of the jaw is an important complication in patients treated with BP after dento-alveolar surgical interventions as teeth extractions (18). At examination, posterior teeth were missing on both sides of lower jaw. All residual teeth had no problems, except tooth 4.3 that presented an

Key words: intraoral scanner, digital impression, digital workflow, Cad-Cam, post and core

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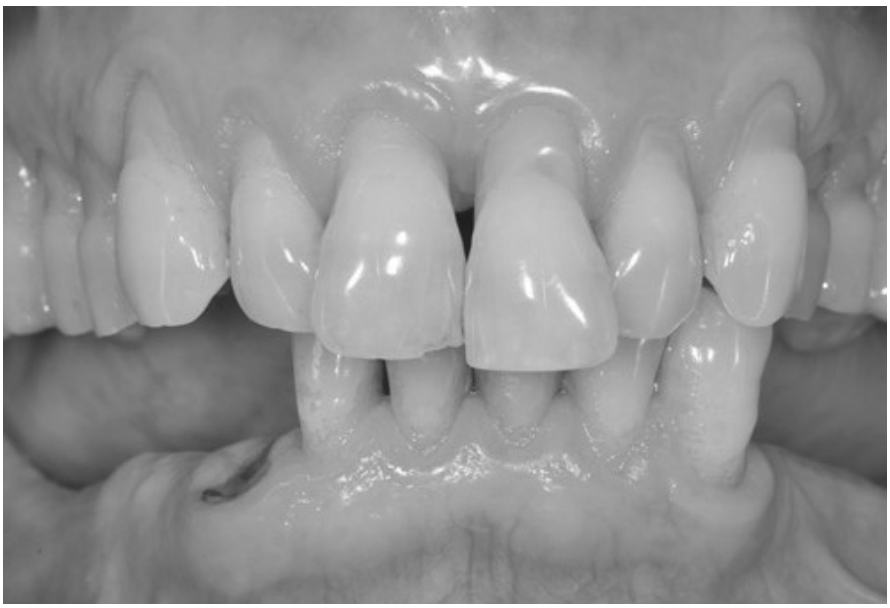

Fig. 1. *Pre-operative intraoral view.*

old metal-ceramic crown. X-rays showed good apical sealing, no infection, a fiber post and a residual part of core. The fiber post was not placed in the correct angulation (Fig. 2).

Although there are techniques to regenerate bone in the post-extractive alveolus, with the intent of preserving the volume of the ridge, it was not possible to proceed with extraction (19-23).

Due to a pharmacological situation, implants were excluded as treatment options. The patient decided to treat her situation with a mobile partial denture.

In addition to the mobile partial denture, the treatment included a metal post with ball-attachment as a support (24). To prepare the post space, diamond burs and drills (LARGO, Dentsply Sirona, York, Pennsylvania, USA) were used.

Two impressions were made to create two metal posts: the first one using a traditional impression and the second one using an intraoral scanner (IOS). Milled metal posts were made from both impressions. The post with the best fit was chosen for final cementation. The first impression was taken

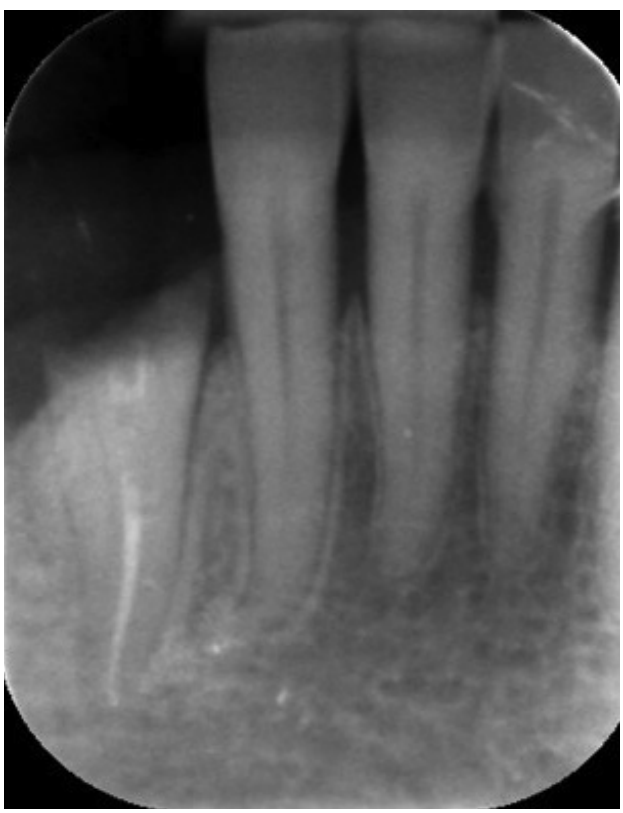

Fig. 2. *Pre-operative intraoral X-ray.*

with Polyvinylsiloxane material (Flexitime, Kulzer, Hanau, Germany) in the conventional manner (Fig. 3), while the second impression was taken with an IOS (Trios, 3Shape, Copenhagen, Denmark) without using Scan Post (Fig. 4).

A Lab-scan (D2000, 3Shape, Copenhagen, Denmark) was used to scan the traditional impression. Both posts were designed using CAD module in a CAD software (Dental System, 3Shape, Copenhagen, Denmark). The information was transmitted to a 5-axis milling machine (Coritec 650i, Imes-Icore, Eiterfeld, Germany) to be milled from a metal alloy disc. Ball attachments were sintered on top of the posts in a furnace after milling.

Three different clinicians evaluated fitting the two posts intraorally using Light Silicone (Xantropren VL, Kulzer) and decided to cement the post obtained by means of the iOS (Fig. 5, 6).

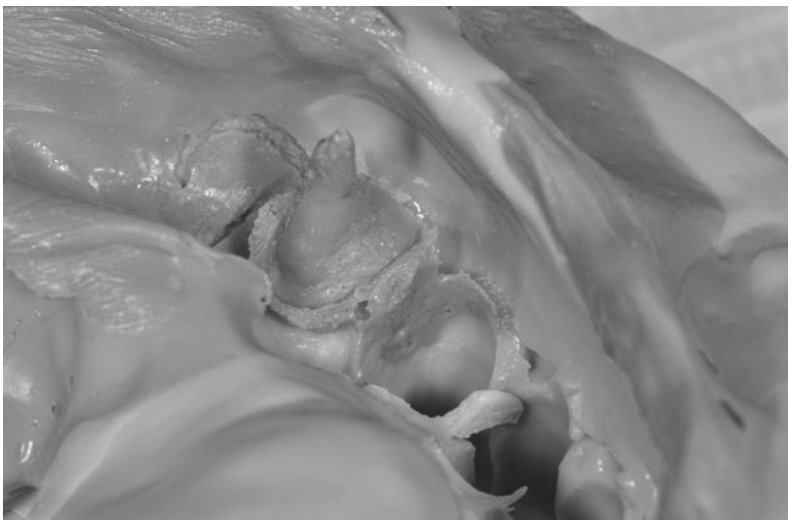

Fig. 3. *Polyvinylsiloxane impression.*

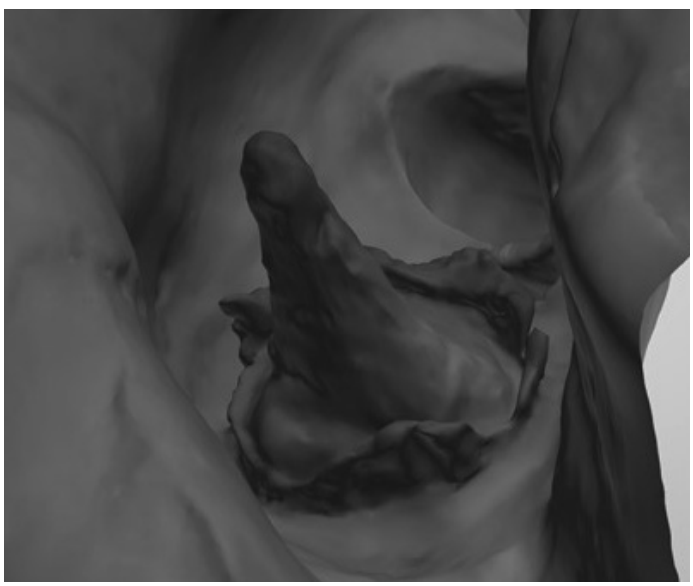

Fig. 4. *Intraoral scanner impression.*

The post was cemented (Fig. 7) using a glass ionomer cement (Ketac Cem, 3M, Maplewood, Minnesota, USA). An x-ray was performed to check the fit of the post in the root canal (Fig. 8).

DISCUSSION

Nowadays, the use of a metal cast post is being examined because of the internal strength that it can develop into the post space. The strength comes from a high elastic module that is much higher than the one of the radicular dentin. This high discrepancy could lead to root fracture. In the last years, the companies are developing materials with elastic module close to the one of the dentin. CAD-CAM technology has improved the use of these materials as they can be easily milled or printed to create prostheses.

The Rehabilitation of endodontically treated teeth through a complete digital workflow is still challenging. It is possible to make a direct scan of the post space only within specific conditions (24-46) otherwise there is the necessity of using a scan support as the scan post. The other techniques involving digital technologies are still hybrids.

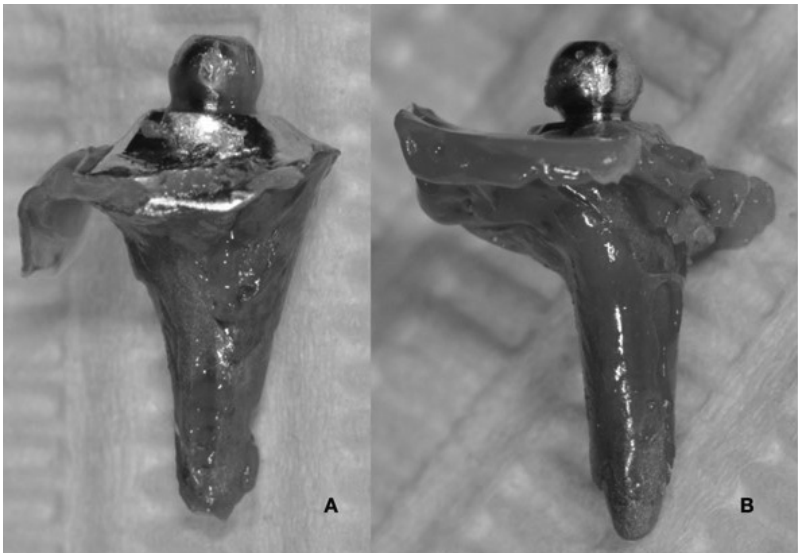

Fig. 5. *Fitting test of the two posts. (**A**): Intraoral scanner-based Post; (**B**): Traditional impression-based post.*

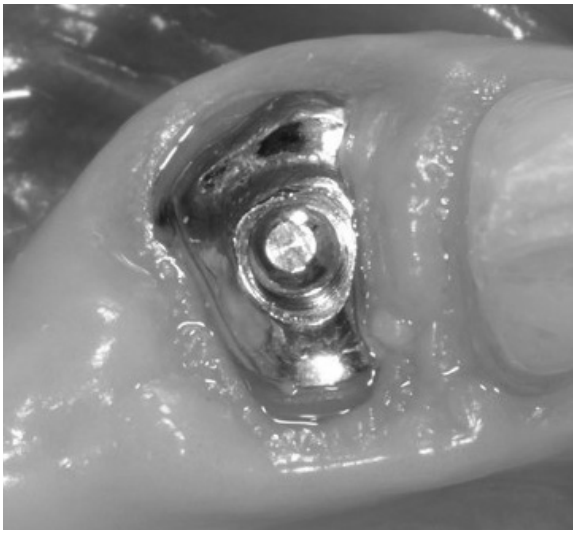

Fig. 6. *Metal post cemented.*

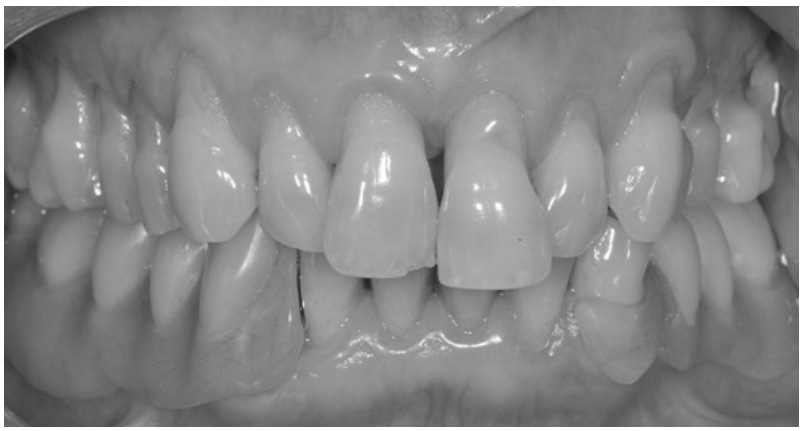

Fig. 7. *Post-operative intraoral view.*

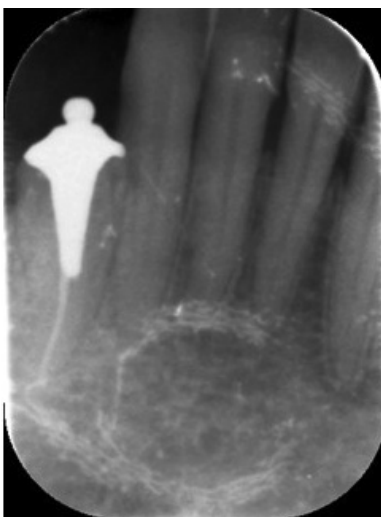

Fig. 8. *Post-operative intraoral X-ray.*

There is still need to us conventional impression materials to have a satisfying post space impression and subsequent digitalization. In any case, it must be considered that the use of conventional impression materials is dependent on the clinician's skills. The clinical result reached in this case seems to be acceptable even though further studies are necessary to validate clinically the production of cast post and core by means of an IOS.

Restoring an endodontic treated tooth is a challenging clinical situation that demands a detailed treatment plan. This clinical case presents a high loss of coronal structure and so the necessity to use cast metal post and core technique to obtain a sufficient retention. The clinical case was completed with a complete digital workflow.

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COMPARATIVE ANALYSIS OF CLEANING ABILITY OF TWO ROTARY INSTRUMENT SYSTEMS: MTWO AND PROTAPER UNIVERSAL. AN *IN VITRO* SCANNING ELECTRON MICROSCOPIC STUDY

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This study is to compare the cleaning effectiveness of two Ni-Ti files systems. Thirty single-rooted human teeth were selected and two NiTi rotary systems were used. Group A: canal shaping with ProTaper® Universal (Dentsply Tulsa Dental Specialties, Tulsa, OK) (PTU); Group B: (n=15) canal shaping with Mtwo Ni-Ti instruments (Sweden & Martina, Padova, Italy) and apical finishing with Mtwo Apical Ni-Ti instruments (Sweden & Martina, Padova, Italy). The amount of debris and smear layer were quantified on a basis of a numerical evaluation scale. The data established for scoring the debris and the smear layer was recorded separately and statistically analysed using the Kruskal-Wallis test. No significant differences were found for debris. Mtwo instruments resulted in significantly less smear layer ($P<0.05$) compared with ProTaper® Universal. Under the conditions of this study, Mtwo resulted in significantly less smear layer compared with canal preparation with ProTaper® Universal.

Root canal instrumentation create dentine debris and smear layer (1-3). Removal of this debris is necessary to decrease the risk of bacteria contamination and transportation in the apical area (4, 5), to increase the permeability of dentin (6), to allow a greater penetration of filling material into lateral canals and dentinal tubules (7, 8), to enhance the seal of the root canal filling (9-15) and to create an appropriate space for the post (16-20).

Currently, no instrument can predictably clean the entire root canal system (21-25). The superior cleaning ability in the coronal and middle parts of the root canal has been described for different rotary nickel-titanium instruments when compared with the apical part (26-35). In general, there are some clues that the flute design of a rotary nickel-titanium file may be a key factor for the cleaning efficiency of these instruments. Instruments with sharp edges seem to be superior to those having radial lands in cleaning the root canal (3, 31, 36, 37).

The aim of this study was to compare the cleaning efficacy (residual debris, quality of the smear layer) after preparation with Mtwo and ProTaper® Universal under normal clinical conditions using a combination of NaOCl and EDTA, in order to obtain more reliable clinical value.

The null hypothesis tested was that there is no difference between the two full-sequence rotary NiTi systems regarding their cleaning ability in root canals.

Key words: detersion, NiTi rotary instruments, SEM, smear layer

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MATERIALS AND METHODS

Selection of samples

Thirty mandibular premolars with single root canals, not treated with composite materials, (38-46) extracted for periodontal (47-51) or orthodontic reasons (52-57) were selected, before dental extraction a rinsing was performed with mouthwash to lower the bacterial load (58-63).

The teeth were stored in 1% sodium hypochlorite (NaOCl) for three days to eliminate organic debris. They were then removed, washed under tap water and stored in 1% T-chloramine solution for disinfection of the teeth. The crown of each tooth was removed at the level of the cementum enamel junction. Two longitudinal grooves were prepared on the palatal/lingual and buccal surfaces of each root with a diamond bur used with a high-speed water-cooled handpiece to facilitate vertical splitting with a chisel after root canal instrumentation. The roots were randomly divided into two experimental groups of 15 each.

The working length (WL) was determined by measuring the length of No.15 K-file (Dentsply, Malleifer) so that the tip was just visible. Individual WL was calculated 0,5mm short of this position.

Root canal instrumentation

Samples were divided in two groups: Group A (n=15) canal shaping with ProTaper® Universal Ni-Ti instruments (Dentsply Tulsa Dental Specialties, Tulsa, OK). Group B (n=15) canal shaping with Mtwo Ni-Ti instruments (Sweden & Martina, Padova, Italy) and apical finishing with Mtwo Apical Ni-Ti instruments (Sweden & Martina, Padova, Italy). A 16:1 reduction handpiece powered with a torque-controlled electric stepper motor (Tecnika Digital Torque Control Motor; ATR srl, Pistoia, Italy) was employed for both instrumentation groups.

Group A

The sequence used for canal shaping corresponded to the manufacturer's instructions: S1 and S2 ProTaper® Universal (speed 350rpm; torque 100) at WL; F1 and F2 ProTaper® Universal (speed 350rpm; torque 100) at WL.

Group B

The sequence used for canal shaping corresponded to the manufacturer's instructions (speed 250rpm; torque 100): Mtwo 10.04 taper at WL; Mtwo 15.05 taper at WL; Mtwo 20.05 taper at WL; Mtwo 25.06 taper at WL.). Apical gauging was verified using a #25 K – file. An additional step of shaping using a rotating apical 25/45 0.02 taper (A3) (Sweden & Martina, Italy) was performed. This instrument show taper 20 (A3) only on the first mm, taper 0.02 for the remaining part of their working area. Therefore, their shaping action includes only the 3mm apical.

Each sample was prepared using a new set of rotary instruments. The intracanal irrigant used after each file was three mL of 5% NaOCl (Niclor 5, OGNA, Milano, Italia). At the end of preparation, 4 mL of 17% EDTA (OGNA, Milano, Italia) were left in situ for 120 s. followed by 1 mL of 5.25 NaOCl for 30 s. as a final rinse. For irrigation, the solution was delivered using a plastic syringe and 30-gouge needle tip to reach the apical third. The canal was thoroughly dried with sterile paper points. All the samples were prepared by a single operator, who had 4-year experience of working with rotary NiTi instrument.

Evaluation

All root canal preparations were completed by one operator, whilst the scanning electron microscope (SEM) evaluations were carried out by a second examiner who was blind respect to all experimental groups and who underwent a training process with reference to the scoring system to the SEM evaluations (64, 65).

The teeth were split in half with a stainless-steel chisel. The section with the most visible part of the apex was chosen for examination. The root halves were dehydrated in graded concentration alcohol, dried in an oven and then gold sputtered (AGAR AUTO SPUTTER COATER). The samples were viewed with a SEM (LEIKA LEO 440) at different magnification (30X, 250X, 1000X) at each third (apical, middle, coronal)

The cleanliness of each root canal was evaluated in three areas (apical, middle and coronal third of the root). Separate evaluations were recorded for debris and smear layer by means of a numerical evaluation scale (27). The following scheme was used:

Debris (dentine chips, pulp remnants and particles cloosely attached to the canal wall)

- Score 1: clean canal wall, only very few debris particles.
- Score 2: few small conglomerations.
- Score 3: many conglomerations; less debris than 50 %

of the canal wall covered.

- Score 4: more than 50% of the canal wall covered.
- Score 5: complete or nearly complete covering of the canal wall by debris.
- Scoring of debris was performed using a 250X magnification.
- Scoring of smear layer was performed using a 1000X magnification, and the scores were the following (dentine particles, remnants of vital or necrotic pulp tissue, bacterial components and retained irrigant):
- Score 1: no smear layer, orifice of dentinal tubules patent.
- Score 2: small amount of smear layer, some open dentinal tubules.
- Score 3: homogenous smear layer along almost the entire canal wall, only very few open dentinal tubules.
- Score 4: the entire root canal wall covered with a homogenous smear layer, no open dentinal tubules.
- Score 5: a thick, homogenous smear layer covering the entire root canal wall.

Statistical analysis

Attributed scores were tabulated and submitted to statistical analysis. Owing to the ordinal nature of the scores, the data were subjected to the nonparametric Kruskall-Wallis test. P-values were computed and compared to the P=0.05 level.

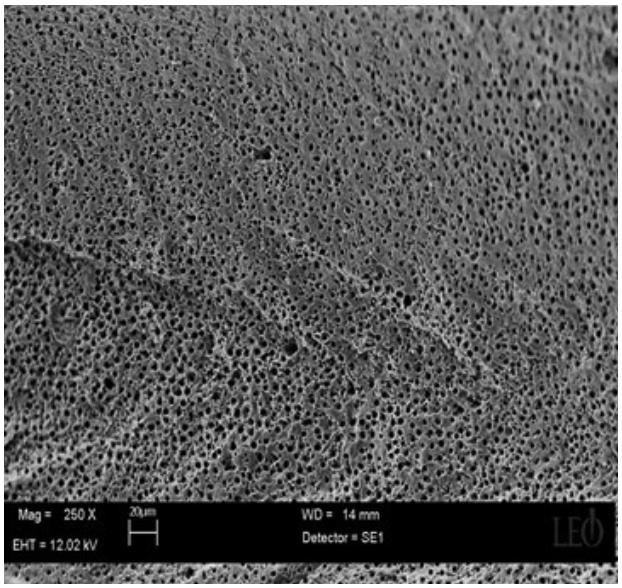

Fig. 1. *Micrograph of an apical third showing more than 50% of the dentinal tubules even in the anatomic depressions of the canal (**Group A**).*

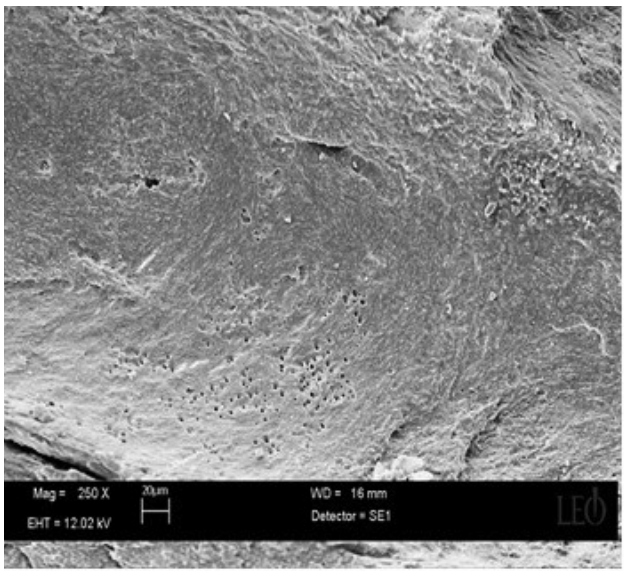

Fig. 2. *Micrograph of an apical third showing a thick and homogeneous smear layer completely covering the root canal wall (**Group A**).*

RESULTS

The scores for debris and smear layer are detailed in Tables 1-4. No significant differences were found for debris. Mtwo instruments resulted in significantly less smear layer ($P<0.05$) compared with ProTaper® Universal instruments.

DISCUSSION

The success of root canal treatment is dependent from the removal of the smear layer and the debris produced during either manual or rotary root canal instrumentation. An adequate cleansing of the root canal walls reduces bacteria contamination (4), enhances sealer penetration (7-9) and reduces the possibility of microinfiltration (66). The association of EDTA and NaOCl solutions has proved effective in removing smear layer formed during endodontic instrumentation (67-69). EDTA acts upon the inorganic components of the smear layer, causes the decalcification of peritubular and intertubular dentine, and leaves the collagen exposed. Subsequently, the use of NaOCl dissolves the collagen, leaving the entrances to the dentinal tubules more open and exposed (67, 68).

Another factor conditioning the removal of debris and smear layer from the root canal system is the cleaning efficiency of the instrumentation used.

The design of the cutting blade of rotary instruments and its rake angle can affect root canal cleanliness. A rake angle is positive when the blade is in front of the perpendicular. A rake angle is negative when the blade is behind the perpendicular (70). A file with a positive rake angle will actively engage dentine and cut the dentin surface in a curetting manner, producing dentin shavings that can be easily irrigated away (3, 30, 36, 71, 72). A file with a negative rake angle will not actively engage dentine, it removes dentine with a scraping action compressing smear layer along the root canal wall (3, 36, 73, 74). The Mtwo cross-sectional design resembles that of the S-file (75) with two blades and feature a large groove between them and a non-cutting tip. The blade angle is almost vertical, and the helical pitch increases from the tip to the handle. This design is claimed to eliminate threading and binding in continuous rotation, and to reduce transportation of debris towards the apex.

The ProTaper® Universal cross-sectional design resembles that of a reamer, the cross-section exhibits convex sides with a triangular core and the cutting blades do not exhibit a radial land or a positive rake angle (76).

In this study, the cleaning efficiency of these instruments was assessed using 2 criteria: debris and smear layer. Debris was defined as dentine chips, and residual vital or necrotic pulp tissue attached to the root canal wall, which in most cases is infected (27). Thus, debris might prevent the efficient removal of microorganisms from the root canal system. The smear layer is a surface film of a thickness of approximately 1–2 μm. Smear layer, which is mainly inorganic, is produced when a canal is instrumented, no smear layer is found on areas that are not instrumented (77). Although the influence of smear layer is still controversial on the outcome of

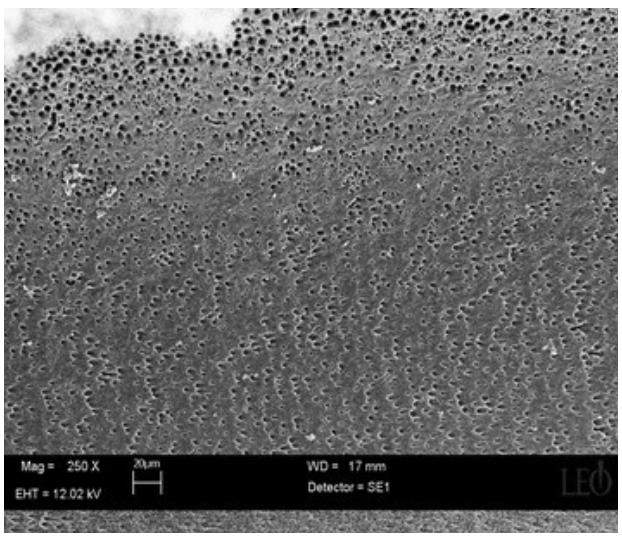

Fig. 3. *Samples of middle third of group A showed partial openings of the dentinal tubules and a thin homogeneous smear layer covering root canal wall*.

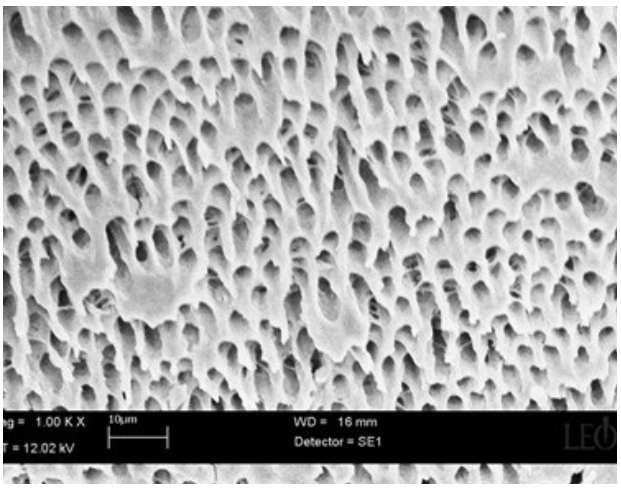

Fig. 4. *Cervical third with evident peritubular and intertubular erosions of dentinal tubules (**Group A**)*.

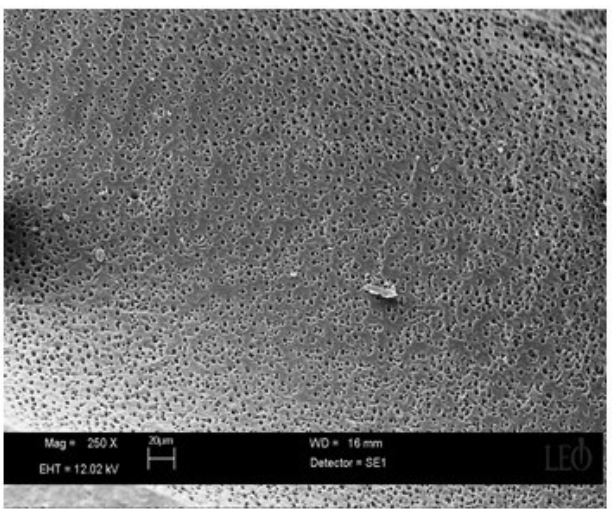

Fig. 5. *Several samples of group B showed at the apical third with more than 50% openings of dentinal tubules and a thin homogeneous smear layer covering the other half (**Group B**)*.

Table I. *Summary of score for debris.*

	Coronal third Scores					Middle third Scores					Apical third Scores					Total Scores				
Instrument	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
Mtwo/Mtwo Apical	11	3	1	0	0	6	7	2	0	0	3	7	3	4	1	20	17	6	4	1
ProTaper Universal	8	6	1	0	0	3	8	3	1	0	2	4	2	5	2	15	20	6	6	2
P values	0.304					0.183					0.065					P<0.05				

Table II. *Average score for debris for the coronal, middle and apical third of the canals*.

Instrument	Coronal	Middle	Apical	Overall
Mtwo	1.33±0.62[a]	1.73±0.70[a]	2.2±0.86[a]	1.75±1.80[a]
Protaper Universal	1.53±0.64[a]	2.13±0.83[a]	3.07±1.33[a]	2.24±1.15[b]

Values with the same superscript letters were not statistically different at P=0.05.

Table III. *Summary of score for smear layer.*

	Coronal third Scores					Middle third Scores					Apical third Scores					Total Scores				
Instrument	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
Mtwo/Mtwo Apical	12	3	0	0	0	12	3	0	0	0	6	6	3	0	0	30	12	3	0	0
ProTaper Universal	8	7	0	0	0	0	4	11	0	0	4	0	8	0	3	12	11	26	0	3
P values	P=0.128					P<0.05					P<0.05					P<0.05				

Table IV. *Average Score for smear layer for the coronal, middle and apical third of the canals*.

Instrument	Coronal	Middle	Apical	Overall
Mtwo	1.2±0.41[a]	1.2±0.41[a]	1.87±0.74[a]	1.42±0.62[a]
ProTaper Universal	1.47±0.51[a]	2.73±0.45[b]	2.87±1.40[b]	2.35±1.09[b]

Values with the same superscript letters were not statistically different at P=0.05.

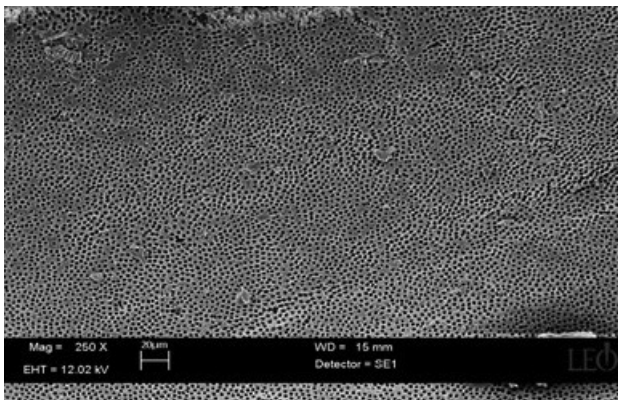

Fig. 6. *Micrograph of an apical third free of smear layer, complete openings of dentinal tubules and some debris (**Group B**).*

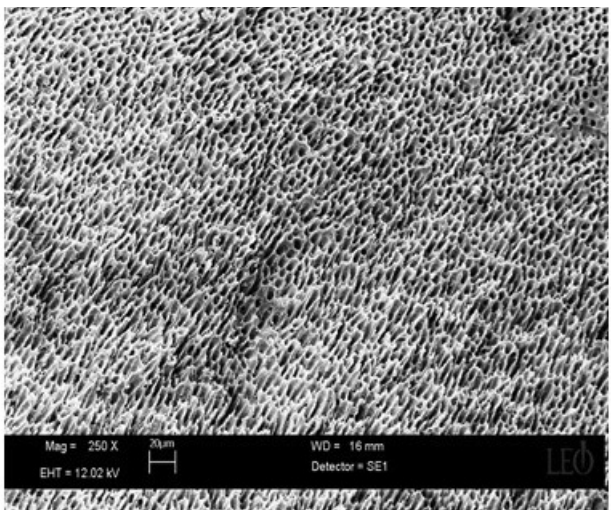

Fig. 7. *Some samples of Group B showed peritubular and intertubular erosions of tubules even in the middle third of the canals.*

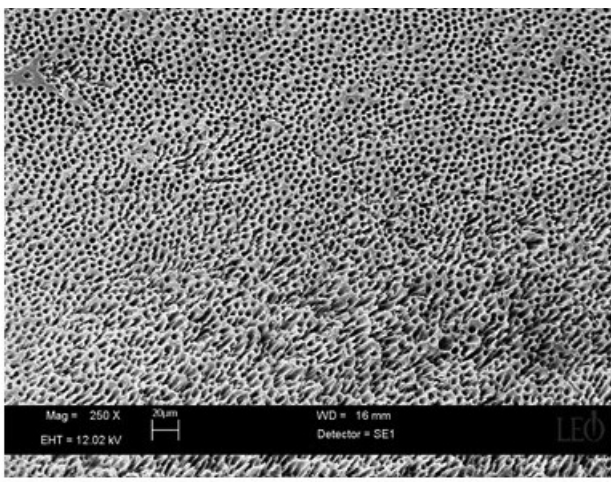

Fig. 8. *The cervical third of group B showed complete cleaning of the root canal walls, with opening of the dentinal tubules mostly accompanied by tubules erosions.*

endodontic treatment, it is considered desirable its removal for its potential deleterious effects (78).

Use of antibacterial irrigants are recommended in combination with chelating agents in order to remove debris as well as the inorganic/organic smear layer (27, 77, 78). Most investigations on the cleaning efficiency of root canal instruments do not use any irrigant or just NaOCl alone (64, 65). For this reason, in the present study both irrigant solutions were used, which, in association has proved effective in removing the smear layer formed during endodontic instrumentation (67).

According to the literature, instrumentation alone leaves debris in all canal sections, especially in uninstrumented areas (22, 24, 25, 27, 28, 64, 65) and the cleanliness decreases from the coronal to the apical part of the root canal (22, 24, 25, 28, 31-33, 64, 65, 79). Therefore, sufficient disinfection and copious irrigation are essential to improve root canal cleanliness (28, 32).

In the present study, the cleanliness decreased from the coronal to the apical part of the root canal. The combination of mechanical instruments with the association of NaOCl and EDTA irrigating solutions determined an improvement in canal cleanliness. Bürklein et al. (65) reported significant differences in debris between these two instruments and no significant differences in smear layer. In the present study, no significant differences in debris were found, while the smear layer significantly decreases in the Mtwo group when compared to the literature (64, 65).

Furthermore, the cervical third of both groups and the middle third of group B showed in some samples peritubular and intertubular erosions in many tubule orifices; dentinal tubules appeared larger and with a funnel entrance and in some cases excessive erosion often led to the conjunction of two or more tubular orifices (30).

Differences in debris and smear layer removal capacity of endodontic instruments are due to the cross-sectional design of the instruments used (65, 80-82). Mtwo have a positive cutting angle, ProTaper a neutral rake angle. While positive rake angles allow cutting dentine chips that curl away from the edge of the blade, negative angles scratch the dentine

surface. This different cutting ability might influence the efficacy of the irrigating solutions.

Within the parameters of this study, the use of Mtwo files resulted in significantly less smear layer compared with canal preparation with ProTaper® Universal instruments, whereas in terms of debris no statistically significant differences were apparent.

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MODIFIED BONDED ACRYLIC EXPANDER IN PATIENT WITH ANTERIOR CROSSBITE AND PSEUDO-CLASS III

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The open-bite treatment can be considered one of the most difficult malocclusions to treat in children as well as in adult patients. Several papers show that the traditional maxillary expander device contribute to increase the vertical face dimension and bite opening due to posterior rotation of the mandible, buccal tipping of lateral segments and cuspal interferences. Other more specific studies compared the effects of traditional maxillary expander to those of bonded acrylic expander and evidenced that the acrylic expander can better control the vertical effects of the maxillary expansion by the resin bite plane on which the heavy occlusal forces are exerted. We decided to use an acrylic expander in order to prevent worsening of anterior openbite after a careful assessment of nasal airflow by the otorhinolaryngologist.

The open-bite treatment can be considered one of the most difficult malocclusion to treat both in children as well as in adult patients. Several etiological conditions can be related to the anterior open-bite malocclusion such as: hyperdivergent growth pattern, oral breathing, sucking habits, orofacial and lingual activity factors, eruptive and occlusal forces. The complexity of factors involved in anterior open-bite and the difficulties encountered to achieve stable results have led to several research to determine the causes (1-4).

Ricketts stated that the main characteristics of respiration obstruction syndrome are the presence of hypertrophied tonsils or adenoids, mouth breathing, openbite, crossbite and narrow external nares (5).

Tomer indicated that children with mouth breathing develop a V-shape maxillary arch with consequent crowding in the upper arch, associated with nasal obstruction due to hypertrophied adenoid and tonsils, chronic and allergic rhinitis congenital nasal deformities (6).

Linder and Aronson stated that increased adenoids aggravate nose breathing, which disrupts the balance of lingual, labial and cheek muscles. Further studies showed that mouth breathing also influences the increase of the lower third of the face, mandibular rotation and the excessive mandibular angle (7).

More recently, Vig et al. found that patients with long vertical face height had higher nasal resistance when compared with normal patients. O'Ryan in a critical literature review, found that mouth breathing resulting from nasal obstruction is not always related to development of long face syndrome but only in maxillary constriction (1, 3, 6).

Further experimental studies have been carried out to assess the consequence of inducted nasal obstruction in animals, and the effects were a narrowing maxillary dental, with consequential crowding in the upper arch, a lowered posture of the mandible and increased vertical face height (2, 8).

According to Proffit, the ideal period to begin treatment is during the mixed dentition because every

Key words: open-bite, malocclusion, children, acrylic expander

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modification in deciduous dentition could recur due to continuous growth changes (4).

Diagnosis and treatment plan

The patient, an 8-year-old boy, presented with skeletal Class III and dental Class I malocclusion, maxillary constriction in mixed dentition and moderate crowding in upper and lower arches. No nasal airflow obstruction nor oral breathing have been detected. He showed anterior cross bite of both upper central incisors with inverted overbite and overjet and traumatic occlusion on anterior teeth with slight periodontal consequence (recession) on lower central incisors (Fig. 1). No posterior cross bite has been detected but an overall space deficiency in anterior area of maxillary arch is evident. The cephalometric analysis evidenced normal growth pattern with hyperdivergent tendency (SnGoGn=32°), skeletal Class III malocclusion (ANPg=-1) associated to lingual inclination of upper incisors (1/AnsPns= 99°) and slight vestibular inclination of lower incisors (1/GoGn=101°). Facial profile analysis shows high mandibular detection while poorly represented maxilla can be evidenced as well as negative torque of upper incisors at profile smiling.

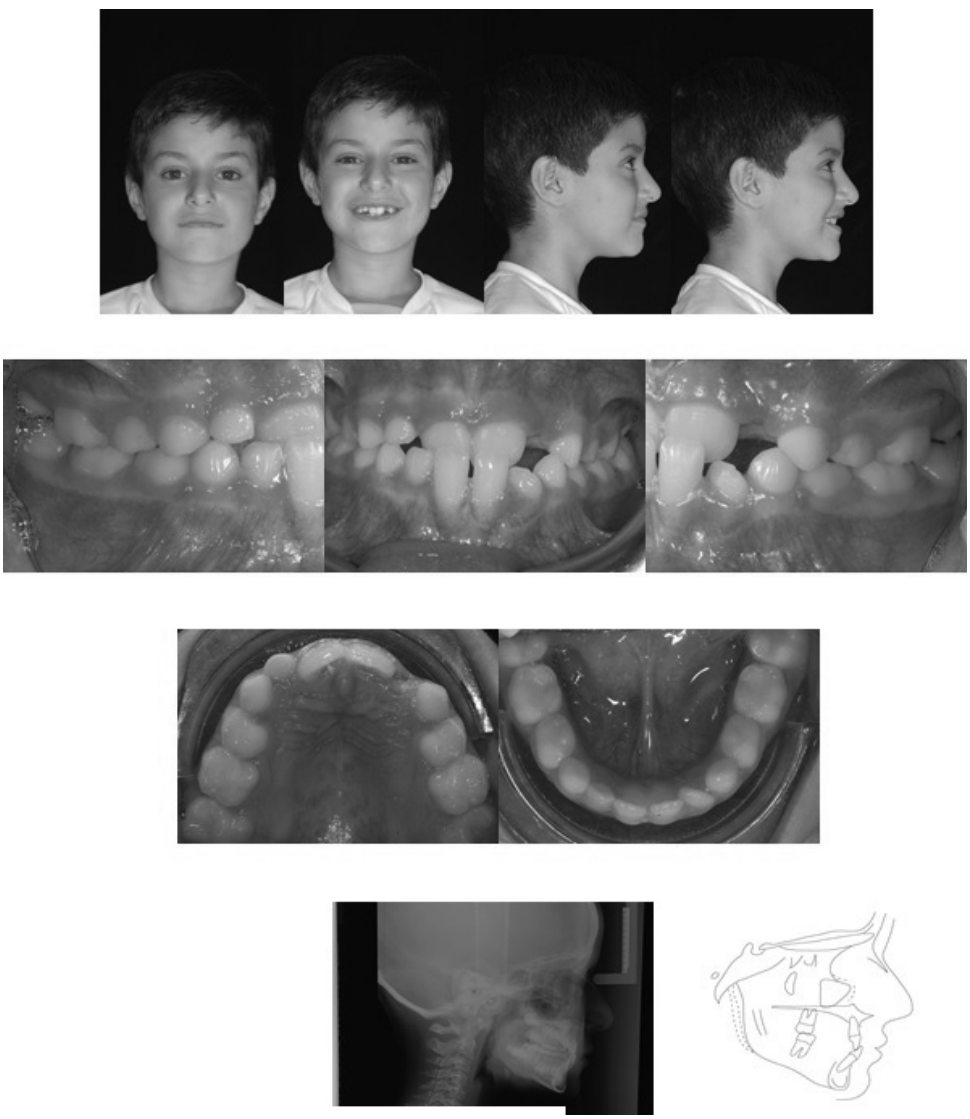

Fig. 1. *Pre-treatment records*

Treatment

The treatment plan was to correct the maxillary space deficiency and compensate Class III malocclusion by means of a bonded acrylic expander in order to prevent mandibular clockwise rotation, facilitate cross bite resolution and prevent dental inclusion. Moreover, to enhance anterior cross bite correction, two TMA springs have been incorporated in the expander, activated and bonded to palatal surface of upper central incisors in order to promote vestibular inclination. During the application of the acrylic expander only the vestibular and lingual surface of lateral teeth were acid etched and bonded, while occlusal surface was not treated to avoid difficulties or pain during removal. The expander activation was performed by patients' parents and lasted 10 days (Table I, Fig. 2, 3).

The acrylic expander remained bonded for 5 months but after cross bite resolution (2 months) the TMA springs have been cut out. Afterwards, a Hawley appliance with occlusal rests and two derotation Z-springs was placed to refine incisors position and rotation.

RESULTS

At the end of treatment, after 12 months, the anterior cross bite was completely corrected and transverse dimension of maxillary arch re-defined. The Class III malocclusion was well compensated by the improvement in upper incisors inclination, the light crowding in lower arch was not corrected while in upper arch the arch expansion and incisors vestibularization allowed laterals eruption nonetheless the complete crowding correction will be refined during second phase of treatment (Fig. 4).

The negative OVJ and inverted OVB was corrected immediately after expansion activation, but after 12 months, stable Class I occlusion was achieved on both side, proper incisors inclination was established with correct sagittal and vertical relationship. The facial esthetics showed more balanced profile with improvement in maxilla projection and improved upper and lower jaws relationship associated to better upper lip protrusion and smiling. The skeletal superimposition showed huge growth vertical change following patient

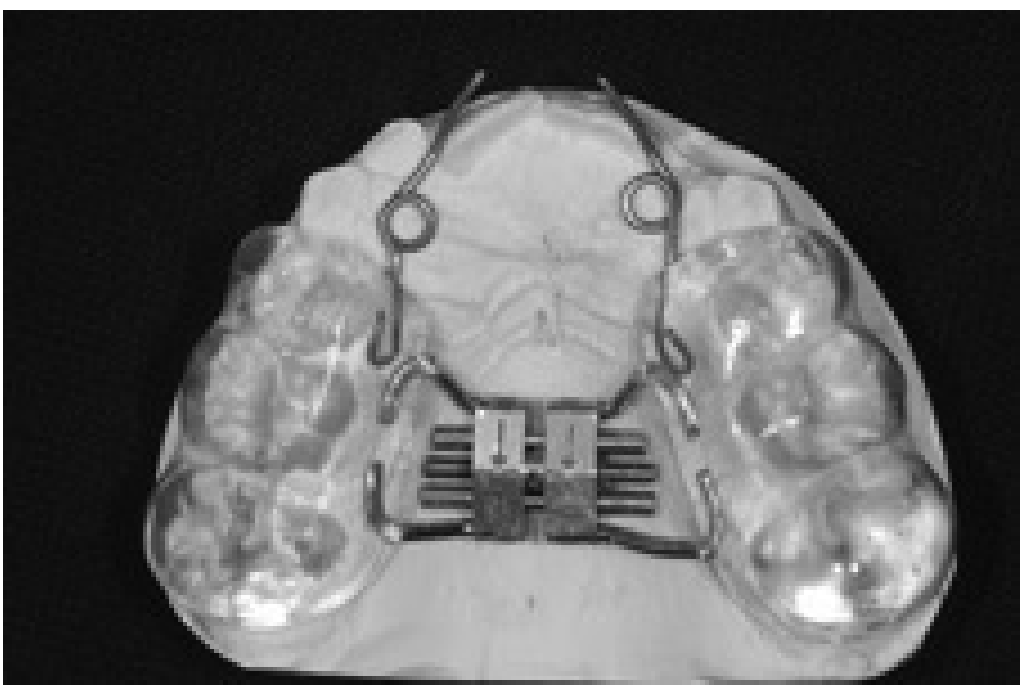

Fig. 2. *Modified acrylic expander with TMA springs.*

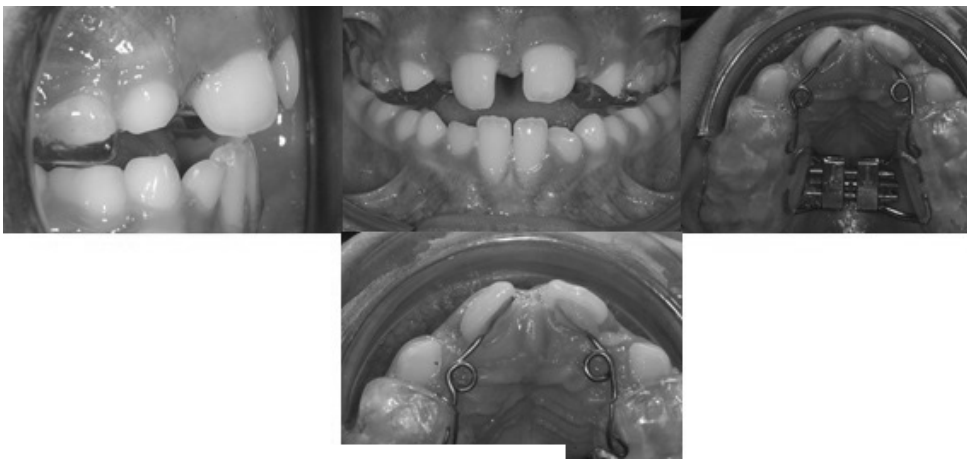

Fig. 3. *Post-expansion records.*

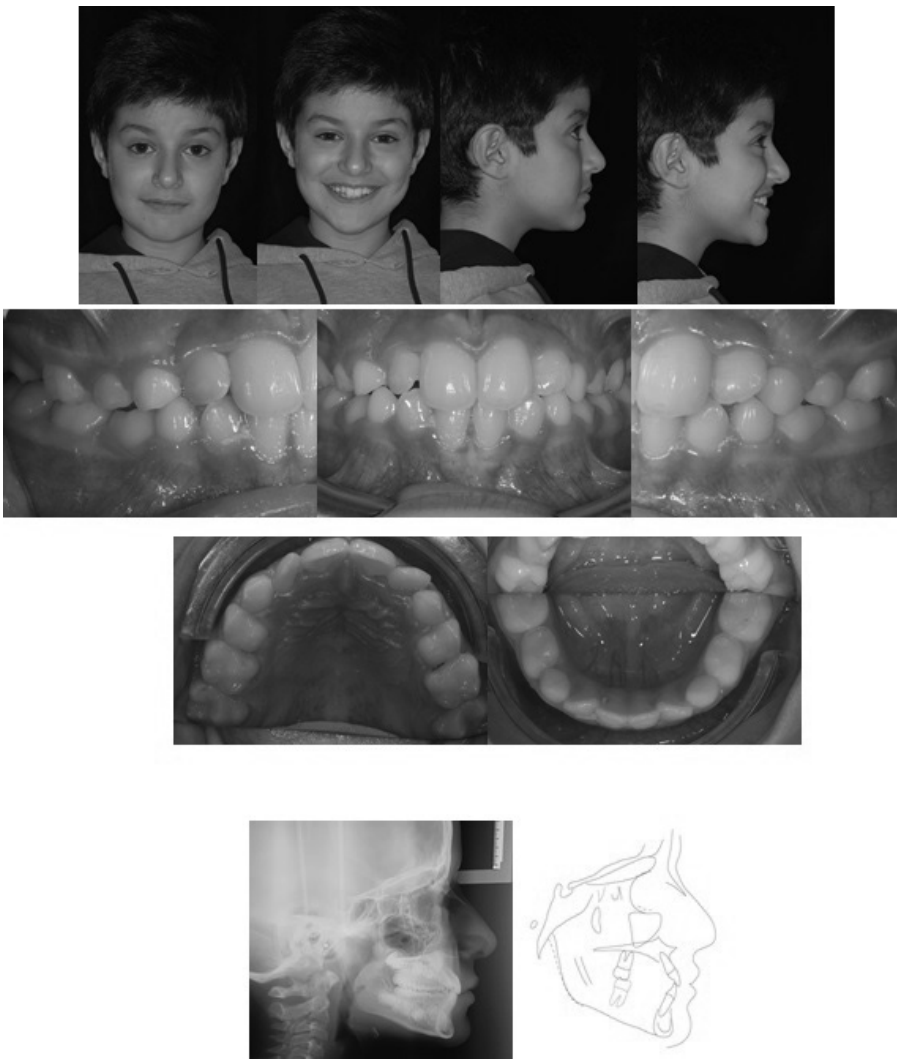

Fig. 4. *Post-treatment records.*

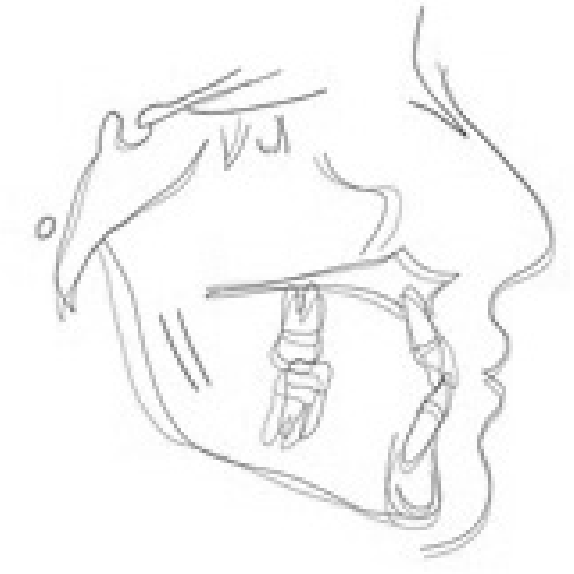

Fig. 5. *General superimposition.*

skeletal pattern, and poor sagittal growth as expected in hyperdivergent patient; sagittal relationship has been corrected obtaining Class I relationship probably due to mandibular clockwise rotation with improved modifications in incisors inclination both for upper and lower (Fig. 5).

DISCUSSION

Several papers showed that the traditional maxillary expander device contribute to increase the vertical face dimension and bite opening due to posterior rotation of the mandible, buccal tipping of lateral segments and cuspal interferences. Other more specific studies compared the effects of traditional maxillary expander to those of bonded acrylic expander and evidenced that the acrylic expander can better control the vertical effects of the maxillary expansion by the resin bite plane on which the heavy occlusal forces are exerted (8-10).

To treat the complexity of factors involved in openbite different approaches have been proposed such as functional appliances or exercises associated to RME (bionator, lingual spurs, molars extraction, clenching exercise) all requiring various degrees of patient compliance (11-13).

According to these literature findings, we decided to use an acrylic expander in order to prevent worsening of anterior openbite after a careful assessment of nasal airflow by the otorhinolaryngologist. Another treatment goal was to reduce the upper crowding by means of arch expansion allowing lateral incisors to erupt, and to improve the nasal breathing without any other functional or more invasive appliance, which requires high patient compliance.

The post-treatment dental analysis shows that transverse diameter was re-established (5 mm expansion), the crowding was corrected as well as the openbite (from -4mm to 3mm) using only the same appliance. The alignment of central incisors was improved and lateral incisors could take place. Obviously, in order to complete the treatment a second phase with fixed appliance will be planned in late mixed dentition.

The post-treatment cephalometric analysis shows 1 mm growth downwards and forward in 12 months of treatment and a favorable change of in occlusal plane. The posterior teeth were maintained in the same position while the incisors extruded (1/ANS-PNS varied from 114° to 104°) following the maxillary growth and the loss of occlusal contacts (Table II).

Table I. *Expander activation setup.*

	First day	**Day from 2nd to 10th**
Activation	1 mm	0.50 mm each day - 5 mm in total

Table II. *Cephalometric values before and after treatment.*

CEPHALOMETRIC MORPHOLOGICAL ASSESSMENT	MEAN SD	PRE-TREATMENT	POST TREATMENT
SAGITTAL SKELETAL RELATIONS			
MAXILLARY POSITION S-N-A	82° ± 3.5	79°	79°
MANDIBULAR POSITION S-N-PG	80° ± 3.5	80°	76°
SAGITTAL JAW RELATION A-N-PG	2° ± 2.5	-1°	3°
VERTICAL SKELETAL RELATIONS			
MAXILLARY INCLINATION S-N/ANS-PNS	8° ± 3.0	10°	16°
MANDIBULAR INCLINATION S-N/GO-GN	33° ± 2.5	32°	38°
VERTICAL JAW RELATION ANS-PNS/GO-GN	25° ± 6.0	22°	22°
DENTO-BASAL RELATIONS			
MAXILLARY INCISOR INCLINATION 1/ANS-PNS	110° ± 6.0	99°	120°
MANDIBULAR INCISOR INCLINATION 1 / GO-GN	94 ± 7.0	101°	96°
MANDIBULAR INCISOR COMPENSATION 1 / A-PG (MM)	2 ± 2.0	3	1
DENTAL RELATIONS			
OVERJET (MM)	3.5 ± 2.5	-2	1
OVERBITE (MM)	2 ± 2.5	3	2
INTERINCISAL ANGLE 1 / 1	132° ± 6.0	138°	126°

The esthetic improvement is evident on smiling due to the correction of incisors exposition and consequent well-balanced lip posture. In conclusion, oral health is an objective indicated by the WHO. The multidisciplinary collaboration of all the specializations in the dental field is fundamental for the well-being of the patient (14-69).

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PERIODONTAL DISEASE IN SUBJECTS SUFFERING FROM CORONARY HEART DISEASE

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The aim of present study is to evaluate the greater risk of periodontal disease in subjects affected by cardiovascular disease. The statistical study includes 200 patients equally divided into a test and a control group. The test group is made up of hospitalized patients from the cardiology division of the S. Salvatore hospital of L'Aquila and the second made up of subjects that frequented a dental clinic. All patients were subject to anamnesis and clinical evaluation for periodontal disease. The index used for this clinical examination were CPTIN index (Community Periodontal Index for Treatment Needs), pocket depth index (PPD), probing bleeding index (PBI), and plaque index (Silness, Loe). All data were collected and a comparative analysis was done of the results obtained from the two groups. Analysing the data concerning the average of lost and present teeth in the oral cavity and the damage of periodontal attachment we notice that cardiopathic subjects had a loss of periodontal attachment 2 times greater than in the control group and major condition of edentulism. A frequency test called the "chi-square test" showed that cardiopathic patients had a greater frequency of periodontal disease. The development of periodontal disease in subject suffering from coronary heart disease is faster and more aggressive than in healthy subjects. Therefore, the prevention of periodontal disease is simple and effective way to reduce the risk of cardiovascular disease.

Researchers have found many distinctive features in common between periodontal disease and coronary heart disease, including the fact that subjects with periodontal disease are at greater risk of developing cardiac problems (1, 2, 3).

The first studies published on the possible association between the two diseases was in the 60's and showed a greater loss of alveolar bone in atherosclerotic heart disease subjects than in diabetic subjects (4). Next, studies (5) showed a close connection between periodontal disease and myocardial infarction. In fact, patients hospitalized for acute myocardial infarction (AMI) showed a higher score on the pantomography index than the control group in regard to caries, periapical lesions, pericoronitis, periodontitis and furcation lesions. A complex analysis on a male population also demonstrated important mutual relations between dental infections and coronary atherosclerosis (6).

Other researchers have established that there is a greater risk of coronary heart disease (CDH) in patients affected by periodontal disease (25%) (7).

In recent studies that stressed the association between periodontal disease and CDH it was concluded

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that the bone loss found on periodontal examination had the greatest correlation to cardiovascular disease, with an increased incidence of CDH of 40% for every 20% increase in bone loss (3).

Researchers explained the possible biological mechanism between periodontal disease (29) and cardiovascular disease as being the result of Gram-negative bacteria and their metabolic products [Lipopolysaccharides (LPS)], entering the blood stream and causing either the formation of an inflammatory cell infiltration in the blood vessel, or an increase in the proliferation of the vessel muscular wall, finally inciting fatty degeneration of the vessel with consequent intravascular coagulation (9, 10, 29)

The possible association between periodontal disease and atherosclerosis is shown in the following observations:

a) Cells of the monocytic lineage and the inflammatory cytokines play a critical role in initiating and propagating both atheroma formation and periodontal disease (11). In fact, cytokines create detrimental effects on the periodontium causing vasodilation, an increase in vascular permeability and in inflammatory cells, the destruction of the connective tissue and the bone loss.

b) The monocytic lineage trait appears to be under both genetic and environmental influence.

c) Periodontal disease produces systemic vascular challenges, with bacterial LPS and host-derived inflammatory cytokines that are capable of initiating and promoting vasculitis and atheroma formation. In particular, an intravascular infusion of LPS upregulates the expression of endothelial molecule adhesion. In this way it is possible to cause platelet aggregation, acceleration of the formation of lipid-laden foam cells, the deposition of cholesterol in the intima and a delay of fibrinolytic process.

Researchers have also found that there are some dietary factors that can influence the monocytic response. For example, oxidization and the subsequent hydrolysis of arachidonic acid can result in the synthesis of several inflammatory and vasoactive products. On the contrary, a diet made up a large quantity of ω3 fatty acids (EPA), as found in some fish, can reduce the synthesis of proinflammatory and procoagulant molecules (12, 24).

Recent studies have shown that after a challenge caused by cytokines, there can be an increase of C reactive protein (CRP), a protein produced by liver that is considered as a non-specific marker of infection or of tissue injury (13). In particular, CRP levels are greatly increased in presence of periodontal microorganism in the gingival sulcus (14).

The mechanism by which CRP increase is related to the development of the cardiovascular diseaseis not very clear; probably it is involved in the activation of the complement and in the formation of the foam cells of the atheroma (15). In the future sCRP could be the most important predictor of increased risk for cardiovascular disease among the several plasma variables.

The difficulty in finding a direct relationship between periodontal diseases and myocardial infarction is caused by the problem of establishing a real temporal relationship between the two affections. Gingival bleeding and the pocket depth provide information on disease activity at the time of the examination, but they do not give information about the situation before the observation. Moreover, the status of the sites measured may not be representative of all the sites in the mouth. A large number of patients are excluded from the research for medical reasons, and it is in such patients that the prevalence of myocardial infarction is greater.

Recent studies have demonstrated that the microbial species of the oral cavity are extremely complex (23,28) and most of the pathology (including periodontal and endodontic disease) is associated with, and perhaps caused by a select group of pathogens (17, 30, 31, 32-56). The principals pathogens considered are P.gingivalis, the Bacteroides forsythus and the A. actinomycetemcomitans (18, 22, 26). The biological consequences of the oral infections caused by *P.gingivalis* and the risk of myocardial infarction or of other vascular diseases, are supported by the following evidence:

1) *P.gingivalis* can induce the aggregation of platelets and trigger cytokines that play an important rule in the atheroma formation through bacterial products such as LPS and fimbriae**.**

2) The organism can cause local inflammation, which then leads to gingival ulceration and to a

vascular challenge that increases the incidence and the severity of transient bacteremias when the gingiva is traumatized. For example, procedures such as dental extractions, periodontal surgery and scaling, can force bacteria in the blood stream.

3) *P. gingivalis* can invade endothelial cells in the vascular wall. Activated or injured endothelial cells resulting from such invasion show a great variety of atherogenic properties, including the procoagulant activity, the secretion of vasoactive and inflammatory mediators and the expression of adhesion molecules.

4) These substances may be released into the blood stream, which could have direct effects such as platelet aggregation triggered by *P. gingivalis* surface antigens and fimbriae leading to the increasing risk of thrombosis and indirect effects that may be mediated by LPS and other bacterial components that induce cytokine production by macrophages that are in the atheroma. This could lead to accumulation of lipids and foam cells in the atheroma and a greater production of IL-1ß by the endothelial cells, with a consequent increase in thrombus formation.

Other oral pathogens that have properties similar to *P.gingivalis,* for example *S. sanguis*, can also express on its surface a platelet aggregation protein (19, 22, 25, 27). In this way, those bacterial proteins show or feign the normal platelet receptor that starts the thrombus formation.

Among the problems in establishing a possible association between periodontal diseases and CDH, is the difficulty in distinguishing a *bacteremia* of exclusively oral origin from one of a different cause. Furthermore, no research on humans has ever studied the prevalence and severity of *bacteremias* over time and related these findings with the prevalence of atherosclerosis. The strict association between *bacteremias* and atheroslerosis in humans is based mainly on indirect documentation. Therefore, there is a need for greater cooperation between cardiologists and periodontists.

METHODS AND MATERIALS

This study was based on a sample of 200 patients equally divided into a test and a control group, the first made up of hospitalized patients from the cardiology division of the S. Salvatore hospital of L'Aquila and the second made up of subjects that frequented a dental clinic. The entire follow-up lasted about one year.

Patients, who previously gave permission, were subjected to an accurate anamnesis for periodontal diseases and a through clinic examination and the data was recorded. The periodontal evaluation, either from a diagnostic point of view or from a therapeutic one, was made based on the CPTIN index (Community Periodontal Index for Treatment Needs). The dental examination was divided into six sextants, one anterior and two posterior for each dental arch; if only one tooth remained in a sextant, it was included in the next sextant.

The probing was done around all teeth and the one with the worst measurements was considered be representative of that sextant. Probing carried out at the six radicular points: mesial-vestibular (mv), vestibular (v), disto-vestibular (dv), mesial-oral (mo), oral (o) and disto-oral (dv). All measurements were done by the same person for standardization. The different periodontal conditions were classified as follows: Code 1- there is absence of pockets, calculus, and overhanging restorations, but there is bleeding at many points after light probing. Code 2- the pockets are less than 3 mm deep, but deposits of subgingival plaque and calculus are present. Code 3- there are pocket depths of 4-5 mm. Code 4- there are pockets of 6 mm or more.

The necessity for eventual treatment was based on the worst code: TN0 in presence of healthy gingival; TN1 where there was the necessity of improving oral hygiene (Code1); TN2 when it was necessary to do scaling, remove overhanging restorations and improve oral hygiene (Codes 2 and 3); TN3 when the most complex treatment was necessary (Code 4).

Careful consideration in the case history was given to recording the number of teeth that were in the dental arch and the number of teeth lost from periodontal disease. The average loss of periodontal attachment (expressed by the average probing depth) or the maximum pocket depth found for every single teeth and the degree of tooth mobility were also noted.

The pocket depth index (PPD) (calculated in mm and defined as the distance between gingival margin and the top of periodontal probe inserted in the pocket) was measured using monoprobes (Vivacare TPS Probe), which had a changeable handle with a Teflon top calibrated to 3-2-

3-3 mm. Other parameters considered were the probing bleeding index (PBI), valued at a time of 15 seconds from when the periodontal probe passes in the gingival sulcus, and the plaque index (Silness, Loe) that values the presence of plaque in four degrees: 0= no visible plaque; 1= plaque visible only on the passage of the periodontal probe on the gingival margin; 2= visible plaque; 3= abundant plaque.

After having collected all the data, a comparative evaluation of the results obtained was done to determine if there were differences between the test and control groups. Then, the two group of patients were divided into five classes based on age to verify if there was a correlation between the increase of periodontal disease and coronary problems.

At the end of the evaluation for the prevalence of periodontal disease (the number of cases that were in a group at a specific time) has been made; the follow-up lasted two year, and the parameter used as index of periodontal disease is the average of probing, both in the test and the control group**.**

Using the data obtained, a frequency test called the "chi-square test" was done that allowed us to verify if the frequencies observed of a determined value on a sample were different from theoretical frequencies that we expected.

RESULTS

A total of 3200 teeth per group were examined. In the test group, more than 2160 teeth had been lost for periodontal disease, while in the control group the number of lost teeth was only of 602 (Fig. 1). In the test group, in fact, patients have on the average 10.4 teeth in the oral cavity despite of the control group where the average is of 25.8 teeth.

The most representative and indicative value was the average of lost teeth in patients suffering from coronary disease. In this group, in fact, the average was about 21.6 (Fig. 1); the damage of periodontal attachment was therefore very consistent and the loss of periodontal attachment was about 2 times greater than in the control group (Fig. 2). When the patients are divided into different ages, the valuation of the

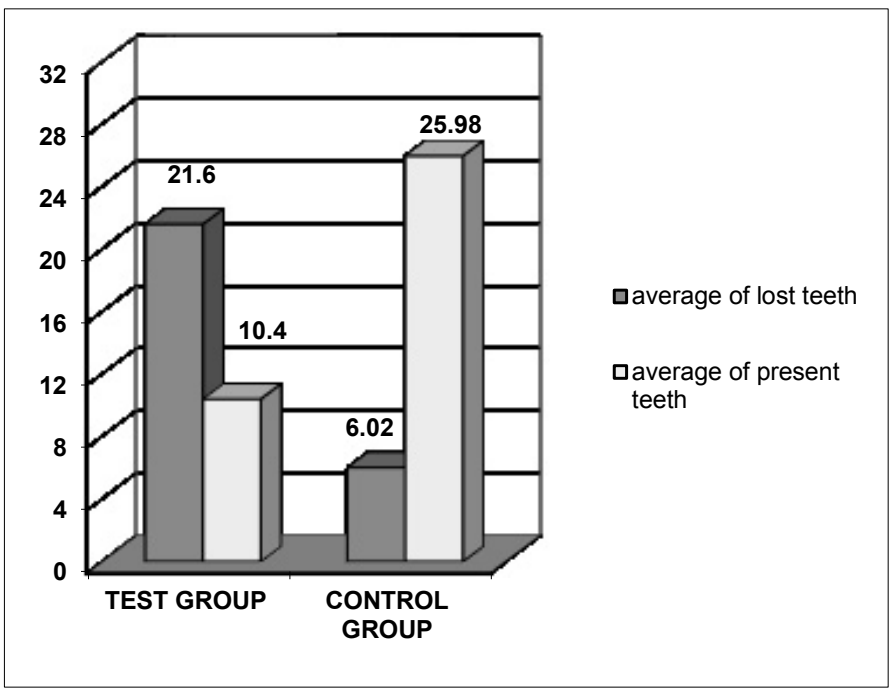

Fig. 1. *Average of lost and present teeth.*

risks become more specific.

Analysing, for example, the data concerning the teeth that were in the oral cavity in the test and the control groups, we can notice some differences, particularly after 45-years of age. Before 45 years, the values are not very discordant; in particular, until 35 years the results are similar and the difference between the two groups is minimum. However, in patients more than 45 years old the differences are greater; in the test group a great loss of teeth is seen in subjects 45-55 years old. Compared to subjects of 35 years, they have an average of about 25 teeth in the oral cavity (Fig. 3).

After 65-years-of-age there is a drastic diminution in the number of teeth present, especially among cardiopathic subjects; in healthy subjects the reduction (even if present) is of less importance. Results obtained in this age class could be influenced by the physiological aging of the periodontal attachment of the teeth, a condition that inevitably

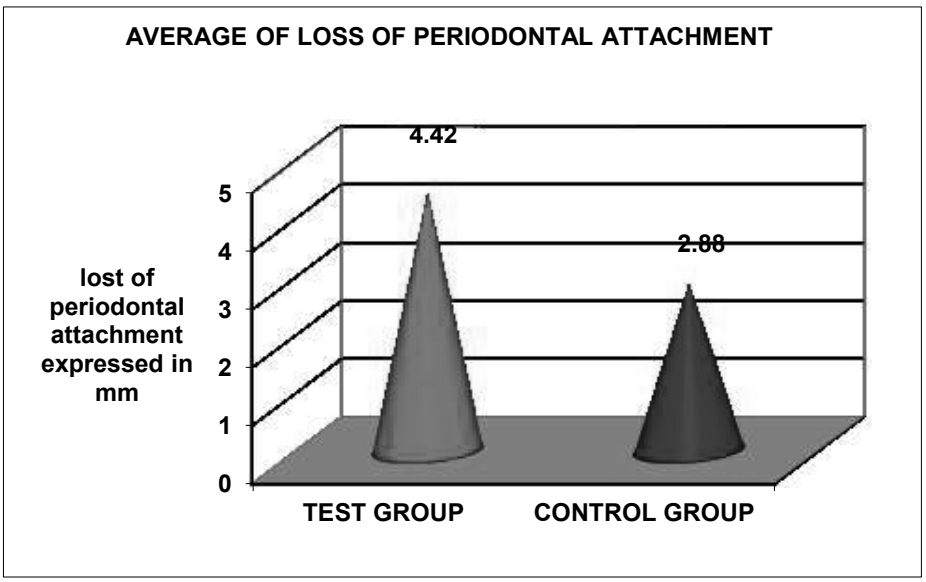

Fig. 2. *Average of loss of periodontal attachment.*

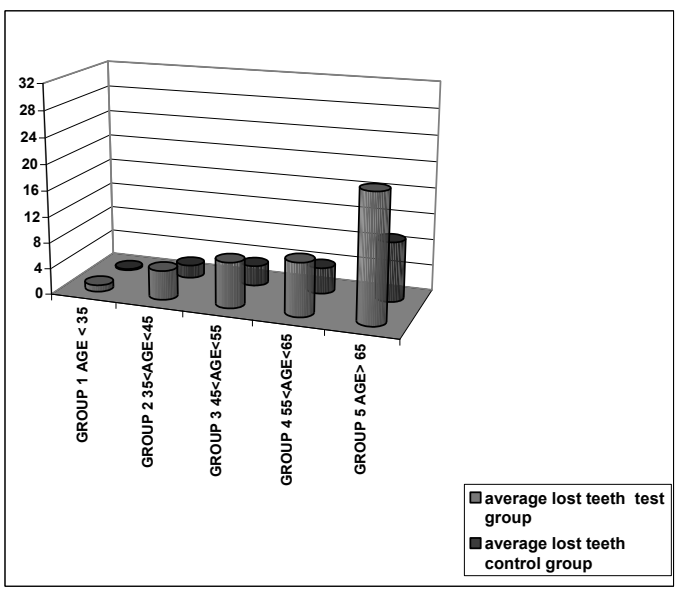

Fig. 3. *Average of lost teeth.*

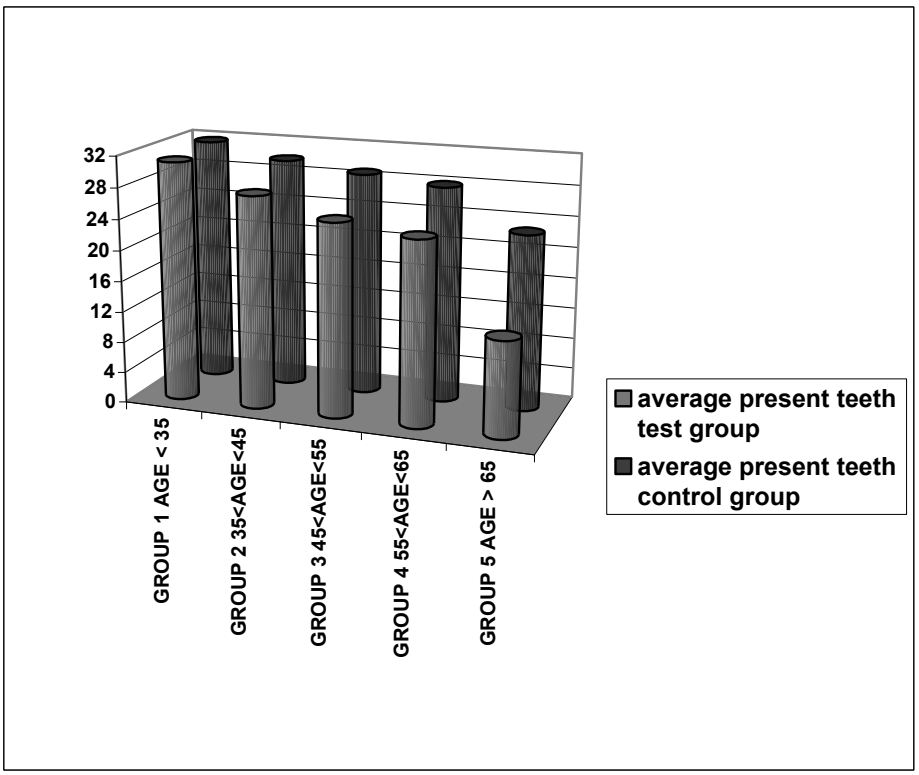

Fig. 4. *Average of present teeth.*

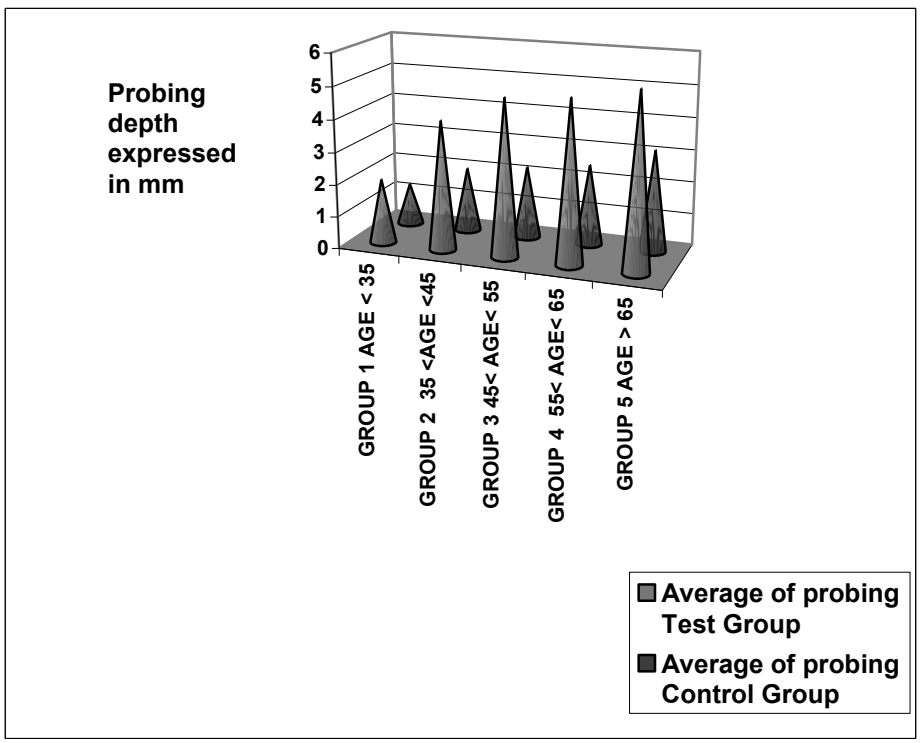

Fig. 5. *Average of probing.*

leads to alveolar bone resorption and to changes in the periodontal attachment, with a subsequent risk of tooth loss.

Analysing the data concerning the average tooth loss from periodontitis, we notice that the greatest difference between the two groups happens at an age between 45 and 55 years; the age group in which there are the most subjects suffering from coronary disease. (Fig. 4).

Considering only the test group, we notice how there is also a sudden increase in the number of teeth lost in patients from 35 to 45 years of age, a period in which predisposed subjects have the fastest advance of the periodontal disease. It is also very interesting analyse the situation of patients from 35 to 45 years of age in respect to probing depth (Fig. 5). Even in this case, is very important to realize that the measurements are greater among patients suffering from coronary heart disease, in particular after 35 years of age.

The results obtained from evaluation of the prevalence of periodontal disease in the two group are also very interesting; the prevalence of subjects suffering of periodontal disease in the test group was 82**,** whereas it was 30 in the control group. A similar difference was also found by the chi-square test (Table I), which showed that cardiopathic patients had a greater frequency of periodontal disease.

CONCLUSIONS

From all these data, we can notice how in subjects suffering from coronary heart disease the course of periodontitis is faster and more aggressive than in healthy subjects; in fact, in patients affected by cardiovascular disease we have a greater loss of periodontal attachment and more cases of edentulism. In this case the maximum destruction of periodontal attachment occurs between 35 and 45 years of age. Therefore we can suppose that subjects affected by periodontitis are more predisposed to the development of coronary heart diseases despite of subjects in which the loss of attachment and the destruction of periodontal tissues is of less entity**.**

Based on these findings, we believe that the prevention of periodontal disease could represent one of several ways to reduce the risk of cardiopathy. By reducing periodontal pathogens and consequently the products of bacterial metabolism, it is hoped that all the implications and possible complications of coronary heart diseases can be diminished (22).

In this study, we have found all the associations between periodontal disease and CDH that many researchers did. Our group is continuing to work toward confirming this hypothesis. This association should lead to more active cooperation between cardiologists and periodontologists with the common goal of preventing the devastation of coronary heart disease.

Table I. *Chi-Square Test.*

Board 1					
82	30	122		P di Chi2	
18	70	88		1.30E-24	
100	100	200			
Board 2					
14	14			Value of Chi2	
11	11			104.8701	

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CHLORHEXIDINE GEL USED AS ANTISEPTIC IN PERIODONTAL POCKETS

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The aim of this study was to evaluate the adjunctive benefit offers by the administration of a chlorhexidine based local drug deliver (Chlo-SITE) into periodontal socket after a full mouth disinfection session. The study design was a randomized, crossover, clinical trial conducted on 60 non-smokers subjects with chronic periodontitis. Each volunteer was subjected to a one-stage full mouth disinfection session and, immediately after that, test product (Chlo-SITE) was inserted in 1 pocket in 2 quadrant. The 1° and 4° quadrant were used for the study with the application of antiseptic (Test); the 2° and 3° as a control. Periodontal probe (PD), bleeding on probing (BOP) and plaque index (PI) was collected at baseline (T0), after 7 days (T1), after 4 weeks (T2). The results of this study suggest that the application of xanthan-based chlorhexidine gel (Xan-CHX) offers a great benefit in improving of the indices in chronic periodontitis.

Periodontal disease is now widespread and occurs in people of all ages. The main causes of the evolution of this disease are to be found both in the bacterial component present in the oral cavity (1-4), with direct damage, and in the host immune response, damage indirectly, and related to a process, slow but inexorable aging of the periodontal structures (5-7). This pathological phenomenon may be limited by a regular professional supervision and a good oral hygienic control (8,9). The clinician has many products, which in a more or less significant way and in combination with mechanical causal therapy, allow to obtain satisfactory results (10-12). The Chlo-SITE is xanthan gum gel, contains the 1.5% chlorhexidine, capable of ensuring, for at least two weeks, optimal conditions for disinfection active and passive, since it gives progressively into the liquid crevicular high amounts of chlorhexidine. The xanthan is a poysaccharide, which with water forms a three-dimensional pseudo-plastic network, able to suspend and hold various substances, that are released gradually in relation to their physical and chemical characteristics.

The study was evaluated the effects of a xanthan-based chlorhexidine (Xan-CHX) gel used as an adjunct to scaling and root planing (SRP) in patients with generalized periodontitis.

MATERIALS AND METHODS

Clinical procedures and subgingival plaque collection

Sixty non-smoking patients, 30 males and 30 females (mean age 54.1± 6.9 years) were enrolled in this study. This study was conducted on patients recruited from the department of Periodontology of

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the Dental Clinic at the University of L'Aquila. The subjects had to comply with the following criteria:
- positive for diagnosis of mild-to-severe chronic periodontitis,
- good general health according to medical history,
- negative for hypersensitivity to chlorhexidine,
- negative for the use of any antibiotic or anti-inflammatory drugs within the 3 months preceding the beginning of the study.

Patients were analyzed at baseline (T0, prior to any treatment), after 7 days (T1), after 4 weeks (T2). The 1° and 4° quadrant were used for the study with the application of antiseptic (Test); the 2° and 3° as a control; then each study group was composed of 120 test sites and 120 control sites.

At T0, a clinical monitoring was performed and at each patient the following parameters were recorded: gingival bleeding within 15 s after probing (BOP+) with a 20 g controlled-force probe, plaque index (PI-Plaque Control Record) assessed by visual criteria and probing depths (PD) measured as the distance from the bottom of the pocket to the most apical portion of the gingival margin. Moreover, the same operator always collected the clinical data.

During the first visit, each patient was given the rules for proper use of the toothbrush and toothpaste. Each patient has repeated the indications received so as to correct any inaccuracies. Repeated oral hygiene instructions (OHI), consisting of Bass' brushing technique and regarding the correct use of dental floss and an interdental brush, were given to all participants when they initially underwent SRP. The same OHI were further reinforced throughout the study. Finally, subjects were not allowed to take any antibiotics and anti-inflammatory drugs, or chlorhexidine-based mouth rinses, during the entire length of the study. After 7 days (T1) the patient was subjected to a one-stage full mouth disinfection under local anesthesia (13, 14).

At the end the pockets were dried with sterile absorbent paper cones, and in two of the four quadrants (Test sites) has been applied Xan-CHX gel containing a mixture of CHX digluconate and CHX dihydrochloride (ratio 1:2) to a total of 2.5% CHX (Chlo-SITE, Ghimas, Casalecchio di reno, BO, Italy). The gels were administrated by using a syringe with a non-traumatic needle. The needle was inserted into the periodontal pocket and the gel applied around the tooth in a gentle probing manner in an attempt to include the full extent of the pocket. Gel was applied until the pocket was overfilled, leaving a residue visible at the gingival margin of all affected sites of the tooth. Care was taken to avoid any tissue injury.

At the end of the procedure in quadrants 1 and 4 has been applied antiseptic (Test sites), while the quadrants 2 and 3 (Control sites), were treated with the only mechanical causal therapy without the use of chemical agents.

At T2 (after 3 weeks) the patients were subjected to a revaluation and BOP+, PI and PD were evaluated.

RESULTS

The clinical results obtained in the test sites treated with xanthan-based chlorhexidine (Xan-CHX) gel and the results relating to the control sites, treated with only FMD.

The baseline examination revealed that both study groups demonstrated similar values for PI, BOP, and PD. In all the treated sites was obtained a reduction of the depth of probing, a reduction of the bleeding in the short term and a reduction of the inflammatory state.

In particular, the results showed that the xanthan-based chlorhexidine (Xan-CHX) gel used as an adjunct to scaling and root planing (SRP) led to an improvement in the values of PI and BOP statistically significant against the therapy non-surgical periodontal treatment alone.

It is interesting to note that for all the study the subjects maintain a motivation to oral hygiene insufficient or moderate and no person arrives at a rated discrete or good (Table I).

DISCUSSION

The analysis of results showed that, in cases of therapy supported by locally delivered antibacterials containing chlorhexidine, a greater advantage was observed in reference to the PD; the use of such antiseptics especially, offers a substantial s

Table I. *Data relating to the assessment of oral hygiene.*

PZ	SEX	AGE	ORAL HYGIENE	POCKETS	ATTACHMENT RECOVERY (ANTISEPTICS)	ATTACHMENT RECOVERY (NO ANTISEPTICS)	PI	PI (antiseptic)	BOP	BOP (antiseptic)
1	F	51	-	Between 3-5 mm	Between1-3 mm	Between1-2 mm	3	1	+	+
2	M	59	+	Between 3-4 mm	2 mm	Between1-2 mm	1	1	-	-
3	F	53	+	Between3-5 mm	Between 1-2 mm	1 mm	1	0	+	-
4	M	50	+	Between1-4 mm	Between1-2 mm	1 mm	1	1	-	-
5	F	60	-	Between 1-5 mm	Between1-2 mm	1 mm	2	1	+	-
6	M	57	+	Between 2-5 mm	Between1-3 mm	Between1-2 mm	1	0	+	-
7	F	53	+	Between 5-2 mm	Between1-2 mm	1 mm	2	1	+	+
8	M	52	+	Between 5-2 mm	Between0-2 mm	Between0-2 mm	1	0	+	-
9	M	60	-	Between 1-5 mm	Between1-3 mm	Between1-2 mm	3	1	+	+
10	F	39	+	Between2-5 mm	Between1-2mm	Between1-2 mm	2	1	-	-
11	F	45	+	3-5 mm	Between 1-2 mm	Between0-1mm	1	1	-	-
12	M	57	+	2-5 mm	Between 1-2 mm	Between 0-1 mm	1	0	+	-
13	F	58	-	2-5 mm	Between0-2 mm	Between 0-2 mm	2	1	+	-
14	F	37	+	2-5 mm	Between0-2mm	Between 0-1 mm	1	0	+	-
15	M	59	-	2-5 mm	Between0-2 mm	Between 0-1 mm	2	1	+	+
16	F	60	-	3-5 mm	Between 0-2mm	Between 0-2 mm	3	1	+	+
17	M	60	+	2-5 mm	Between1-2 mm	Between 0-2 mm	1	0	+	-
18	F	59	+	2-5 mm	Between0-1 mm	Between 0-1 mm	1	1	+	-
19	M	60	+	2-5 mm	Between 1-2 mm	Between 0-1 mm	1	0	-	-
20	M	58	-	2-5 mm	Between0-2mm	Between 0-1 mm	2	1	+	-
21	F	50	+	2-5 mm	Between 0-2 mm	Between0-1 mm	1	0	+	-
22	F	57	-	2-5 mm	Between1-2 mm	Between 0-1 mm	2	1	+	+
23	F	38	-	2-5 mm	Between0-1 mm	Between0-1 mm	2	1	+	+
24	M	45	+	3-5 mm	Between1-2 mm	Between0-1 mm	1	0	-	-
25	F	60	+	3-5 mm	Between1-2 mm	1 mm	1	0	-	-
26	M	59	+	3-5 mm	Between0-1mm	Between0-1 mm	1	0	-	-
27	M	58	-	3-5 mm	1 mm	Between0-1 mm	3	1	+	+
28	M	60	-	3-5 mm	1 mm	Between0-1 mm	3	2	+	+
29	F	53	+	3-5 mm	Between0-1 mm	Between0-1 mm	2	1	+	-
30	M	56	-	2-4 mm	1 mm	Between0-1 mm	2	1	+	-
31	F	51	-	Between 3-5 mm	Between1-3 mm	Between1-2 mm	3	1	+	+

improvement in the plaque index with reduction of the periodontopathic bacteria and consequent improvement in bleeding index in regards to non-surgical therapy alone (15-17).

In both types of treatment there was obtained a reduction of inflammatory diseases in agreement with studies by numerous authors (19-23).

The causal therapy has no mechanical limits in the clinical application if not related to tolerance by the patient to undergo such treatment (operative time, awareness, trauma of teeth, possible pain symptoms) (20, 24).

From the results obtained, it was possible to note how the use of Chlo-SITE improved the disease in patients with generalized periodontitis (25-27). Although this study has been carried out for a rather short period, on a restricted number of patients, we can see the effectiveness of the Chlo-SITE, both in the improvement of the clinical and microbiological indices. Also, the subjects treated with Chlo-SITE showed soft tissues healthy, pink, devoid of the typical signs of inflammation. The improvement of the periodontal tissues depend largely on the reduction of pathogen loads, the Chlo-SITE, in fact, has an antimicrobial activity against periodontal pathogens such as to alter the biochemical function and metabolism, thus reducing virulence (8, 9, 28). These results were obtained both thanks to a good compliance by patients, that the application of the Chlo-SITE that interfere with the re-colonization of the microorganisms in the pocket for at least three weeks.

The results of this study underline that the application of xanthan-based chlorhexidine (Xan-CHX) gel offers a great benefit in improving of the indices of periodontal disease, proving to be essential as adjunctive therapy in patients with a serious or moderate high chronic adult periodontitis, and of course as part of a program of periodontal treatment (11,12, 29-50).

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APPLICATION OF THE RADIOSURGERY ASSISTED GINGIVAL DISPLACEMENT TECHNIQUE IN THE AESTHETIC AREA: A CASE REPORT

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The use of digital tools offers a new perspective to daily clinical activities even though sometimes different clinical approaches are necessary. This case report of a maxillary anterior rehabilitation demonstrates the application of a gingival displacement technique to enhance the recording of subgingival finish line by means of an intraoral optical scanner (IOS). The temporary restoration was used as a guide for the radiosurgery tip in order to displace the gingiva in a guided and mini-invasive approach. It was then possible to create the space between the tooth structure and the soft tissues for the light beam of the IOS to properly detect the finish lines of the dental preparation. Six single porcelain fused to zirconia crowns were delivered. This technique could be considered as a solution of complex cases with subgingival dental preparation to be detected by means of an IOS.

The factors that have been documented to influence the marginal fit of a dental restoration are the preparation design, location of the preparation finish line (subgingival or supragingival), restorative material, fabrication method, and impression material and technique (1).

The inflammation of periodontal tissues and the increased risk of recurrent caries, particularly for the crown margins located in the subgingival position, can be determined by the lack of margin precision and by vertical and horizontal overhangs (2, 3).

Although a supragingival margin is preferable to maintain a sound periodontal attachment (4), patients can refuse such approach particularly in the area of the mouth of esthetic concern. Moreover, in certain clinical conditions the finish line needs to be placed subgingivally to increase the retention form, refine the preexisting margins, treat cervical caries or abrasion and cover the root surface to eliminate the sensitivity (3, 5, 6).

Nowadays, an increasing number of dentists are using intraoral scanners (IOS) in their daily practice as an alternative to conventional impression taking (7-11).

Digital impression procedures may be an approach to improve the accuracy of dental restorations as by their nature these processes eliminate the error prone conventional impression and gypsum model casting and warrant a high degree of standardization (12). However, one of the main problems is related to the ability to scanning the subgingival anatomy of the preparation, usually very demanding due to the limited operative field and the presence of oral fluids (13). The need of a direct vision of the subgingival finish line as well as the absence of bleeding and cord artifacts requires a way for the clinician to clearly detect the emerging anatomy of subgingival dental preparations through IOS.

Key words: radiosurgery, Cad-Cam, intraoral scanner, digital impression, electrosurgery

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Opening of the gingival crevice is mandatory to ensure accurate recording of the finish line by means of the scanner light beam. The retraction cord may assist the clinician to displace the marginal gingiva, however later, discerning the subgingival finish line from the cord itself in the software can be challenging especially for deep dental preparations (14-16). Moreover, in more than 50 percent of situations, the use of the retraction cords is associated with gingival bleeding (14, 17, 18).

The need of a direct vision of the subgingival finish line as well as the absence of bleeding and cord artifacts requires a way for the clinician to clearly detect the emerging anatomy of subgingival dental preparations through IOS.

Arcuri et al. described a technique to enhance the digital impression of the prosthetic die, discerning the anatomy of the tooth from that of the gingival tissue at the finish line level (13). The radiosurgery assisted gingival displacement technique (RAGD) uses the contour of the interim prosthesis to guide the tip of the radiosurgery electrode along the tooth surface to open the gingival crevice selectively with a prosthetically driven and minimally invasive approach.

This clinical report describes the application of the RAGD technique to detect the dental preparation finish lines in the anterior maxilla.

MATERIALS AND METHODS

A 55-year-old male with no significant past medical history, presented to solve the aesthetics of his maxillary anterior teeth. On examination, the patient showed multiple carious lesions as well as recessions. Tooth 1.2 was a worn out porcelain fused to metal Implant cemented crown (Fig. 1).

The patient was not interested in any treatment of the posterior teeth. He was only interested in the anterior maxillary esthetic reconfiguration. A digital workflow was chosen to perform the prosthetic rehabilitation.

The crown on the tooth 1.2 was removed and the abutment underneath prepared. Teeth 1.3, 1.1, 2.1, 2.2, and 2.3 were also prepared. The prosthetic preparations were performed with a subgingival beveled shoulder in order to hide the finish line and to treat the cervical caries (Fig. 2).

A provisional Polymethylmetacrilate (PMMA) metal reinforced bridge was relined (Voco GmbH, Cuxhaven, Germany) on the preparations, and then refined and temporarily cemented (Tempbond, Kerrcorporation, KaVoKerr group, Danaher, Washington, USA).

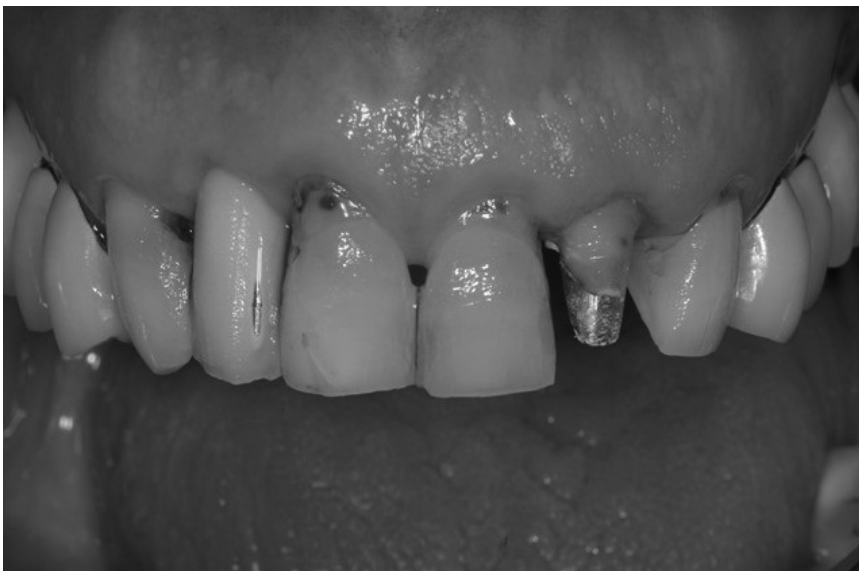

Fig. 1. *Intraoral preoperative view.*

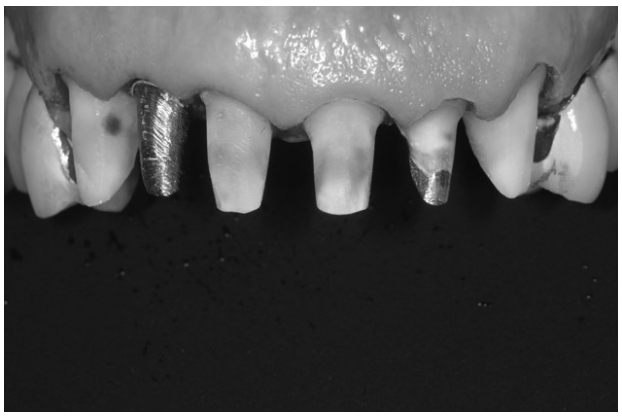

Fig. 2. *Subgingival prosthetic preparation, buccal view.*

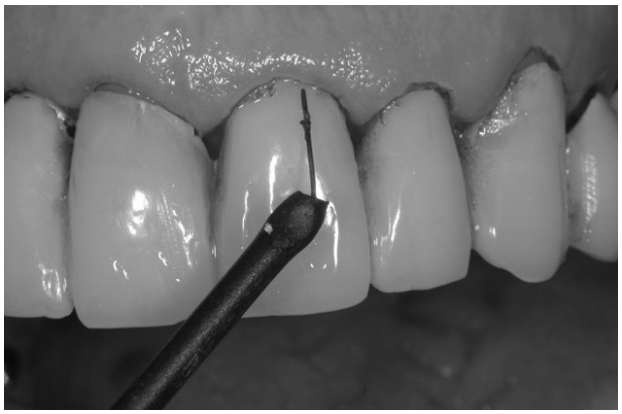

Fig. 3. *RAGD technique applied on the buccal side.*

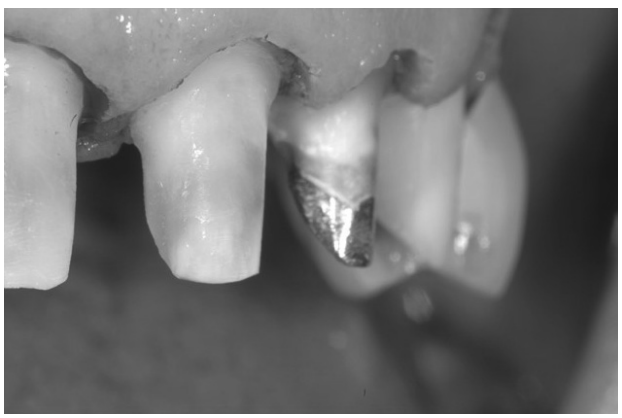

Fig. 4. *Space created through the RAGD technique.*

After three weeks of soft-tissue healing and maturation the definitive impression was carried out with a confocal microscopy IOS (Trios3, 3Shape A / S, Copenhagen, Denmark). Before taking the impression, the RAGD technique was adopted to displace the gingival tissue and allow the IOS to properly record the dental preparations finish lines. The provisional restoration contour was used to guide the tip of the radiosurgery device in order to create a controlled gingival displacement both on the palatal and vestibular aspect (Fig. 3).

The provisional restoration was then removed and the radiosurgery tip was passed in the interdental areas. The RAGD technique allowed for a clear vision of the dental preparations finish lines through the creation of a space between the teeth and the surrounding gingiva (Fig. 4).

Once the gingival sulcus was properly dried from oral fluids and bleeding, the dental preparations were detected by means of an IOS (Trios3, 3Shape A/S, Copenhagen, Denmark) as well as the other teeth of the upper jaw (Fig. 5).

By removing the color tool, it was possible to visualize and check the finish lines detection of all the dental dies better (Fig. 6). If some areas were not clear, they were selectively cancelled and scanned again. Antagonist, articulation relationship and color were also recorded with the IOS and sent to the dental technician.

Six single porcelain fused to zirconia crowns were produced. The zirconia structures were milled (Katana Zirconia HT, Kuraray Noritake Dental Inc, Higashiyama, Miyoshi, Aichi 470-0293, Japan) and seated over the three dimensional (3D) printed model. The porcelain was manually layered. The definitive crowns were finally cemented with a glass ionomer cement (Ketac, 3M, Minnesota, U.S.) (Fig. 7).

RESULTS

The RAGD technique allowed for a clear detection of the subgingival finish line by means of an IOS. The prosthetic rehabilitations delivered were properly integrated with the surrounding periodontal tissues. A satisfying aesthetic and functional result

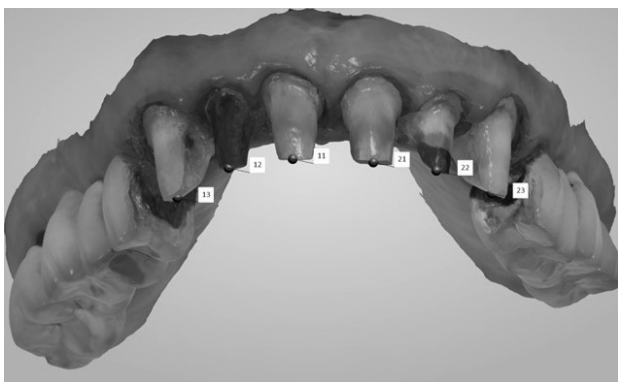

Fig. 5. *Intraoral digital impression.*

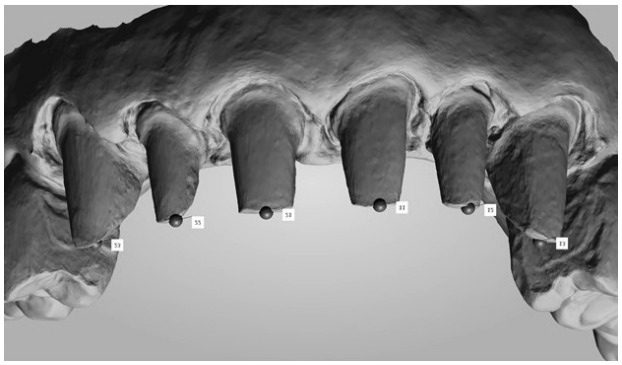

Fig. 6. *Intraoral digital impression without colors.*

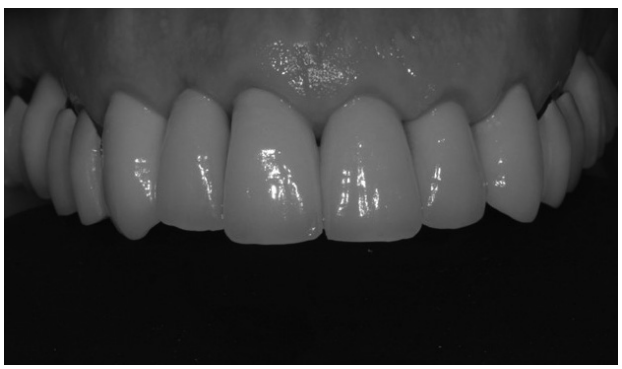

Fig. 7. *Definitive restorations.*

has been achieved and no biological and mechanical complications were recorded at 1 year follow up. Besides, the application of the direct digital workflow led to a reduction of the overall operative time.

DISCUSSION

In fixed prosthodontic treatment, deflection of gingival tissues before making an impression is one of the important phases. The glossary of prosthodontic terms ninth edition defines gingival displacement as "displacement of the marginal gingiva away from a tooth" (19).

In 1986, Benson described the significance of lateral and vertical gingival retraction. Lateral retraction displaces the tissues so that an adequate bulk of impression material can be interfaced with the prepared tooth. Vertical retraction exposes the uncut portion of the tooth apical to the finish line (20).

Several gingival displacement methods were described in the literature, classified as mechanic, mechano-chemical (chemicals embedded in cords or in an injectable matrix form), surgical (radiosurgery (RS), electrosurgery (ES), lasers, and rotary curettage), or combinations of these (21, 22).

Few and old articles have analyzed the effects of ES as a gingival displacement technique. Azzi et al. analyzed clinically and histologically the effects of ES, retraction cord, and the rotary gingival curettage technique in dogs. Postoperative periods analyzed ranged from 6 hours to 14 days (6). The results showed that all methods induce some type of damage of a transient nature and that tissues heal after their use without any permanent loss of attachment. Gingival recession was greater and of probable clinical significance with the rotary gingival curettage technique, minimal with ES, and nonexistent with the cord. Another study showed that although there is loss of tissue soon after ES, 70 to 100% of the lost tissue is regained over a period of months (23).

To the best of our knowledge, no studies were published describing the RS as a gingival displacement technique. However, in some studies there was no statistically significant difference between ES and RS in terms of lateral thermal damage and the proliferation of epithelial cells between ES and RS (24).

RS was recently suggested to promote the detection of the dental finish lines by means of an IOS (13).

Detecting the subgingival dental preparations through an intraoral scanner is considered the weak part of the digital impression (14, 25-46). The RAGD technique uses an anatomically-contoured provisional restoration to guide the radiosurgery electrode tip through the gingival crevice and dislocate the gingival margin horizontally, to enhance the optical reading of the finish line of the subgingival preparations. The final target of that technique is the creation of the sufficient space between the tooth and the gingiva for the light beam of the scanner to clearly detect the finish line of the dental preparations. Compared to the

traditional impression materials, the light beam of the IOS is not able to displace the gingiva through a mechanical action; for this reason, a greater time and meticulousness should be dedicated to the gingival displacement procedure when using an IOS to detect dental preparations. The operator's experience and ability in the use of RS are relevant to the success of the technique; moreover, it is important to remember that, when radio wave equipment is used in the presence of cardiac pacemakers or defibrillators, a cardiology consultation should be obtained.

Contraindications to its use are the presence of flammable anesthetics, liquids, or skin preparations and its use should be avoided in patients with severe periodontal disease and/or with metal restorations close to the interested area. After the application of RAGD, it should wait 3 weeks for the soft tissues healing. However, irreversible changes may occur because of the heat degeneration leading to possible gingival retraction.

Based on our clinical experience, the RAGD technique can allow a correct and predictable use of the IOS for the subgingival impression, but a detailed evaluation of the soft tissues response should be performed, considering different variables such as the periodontal biotype, systemic diseases and smoking habits. Long term studies over a greater specimen are necessary to better analyze the RAGD technique.

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THE EFFECTIVENESS OF ENDODONTIC SEALERS AND ENDODONTIC MEDICAMENTS ON THE ELIMINATION OF *ENTEROCOCCUS FAECALIS*: AN *IN VITRO* STUDY

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The aim of this study was to investigate the efficacy of endodontic sealers and endodontic medicaments: Aureoseal (OGNA), MTA (DENTSPLY), calcium hydroxide (CH) (Endoidrox OGNA) and iodoformic paste (OGNA) against *Enterococcus faecalis*. Thirty-six Biomeraux plates (18 MH and 18 DCO) were inoculated with the experimental suspensions. The *E. faecalis* broth culture suspensions were prepared and adjusted to no. 0.5-0.7 McFarland standard. In each agar plate, three cavities were created, each measuring 4mm in depth and 7mm in diameter, and then completely filled with the product to be tested. To investigate the root canal sealers' antimicrobial activity, the agar diffusion method is used. The diameters of the zones of microbial inhibition were measured in millimeters around the plate. The results showed that the antimicrobial activity of Aureoseal was superior to those of MTA, iodoformic paste and calcium hydroxide for the microorganisms tested. The study confirmed the resistance of *Enterococcus faecalis* to endodontic sealers. Aureoseal and Calcium hydroxide showed the best results in inhibition test suggesting to employ them in clinical cases.

Microorganisms and microbial products present in root canal systems, also in oral cavity (1-4) and saliva (5, 6, 7), are responsible for progression or maintenance of periradicular inflammatory lesions (8, 9).

The endodontic spaces are normally sterile and do not have any contact with bacteria from the oral cavity (*Streptococcus Mutans* and *Lactobacillus*) (5, 6), which instead is a normal condition for the exposed crowns of erupted teeth.

In the case of deep cavities, which are caused by a complex multifactorial etiology, the same bacteria responsible for dental caries are the first species to contaminate and infect the endodontic spaces where the particular environmental conditions progressively select for facultative or obligate anaerobe bacteria.

The purpose of endodontic therapy is to eliminate bacteria and their by-products from the infected root canal system and prevent subsequent reinfection (10). However, it is difficult to eliminate all the microrganism from the root canals with biomechanical preparation because of the complex anatomy of the pulp cavity (11, 12). *Enterococcus faecalis (E. faecalis)* have been associated with superinfecting organism in periodontitis and, most importantly, in persistent root canal infection (13, 14). This has been proved with its factor of survival and virulence, including the ability to invade dentinal tubules, resistance to deprived nutrition, and to compete with different microorganism (15).

Key words: endodontic sealers, aureoseal, MTA, calcium hydroxide, Enterococcus faecalis

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DISCLOSURE: ALL AUTHORS REPORT NO CONFLICTS OF INTEREST RELEVANT TO THIS ARTICLE.

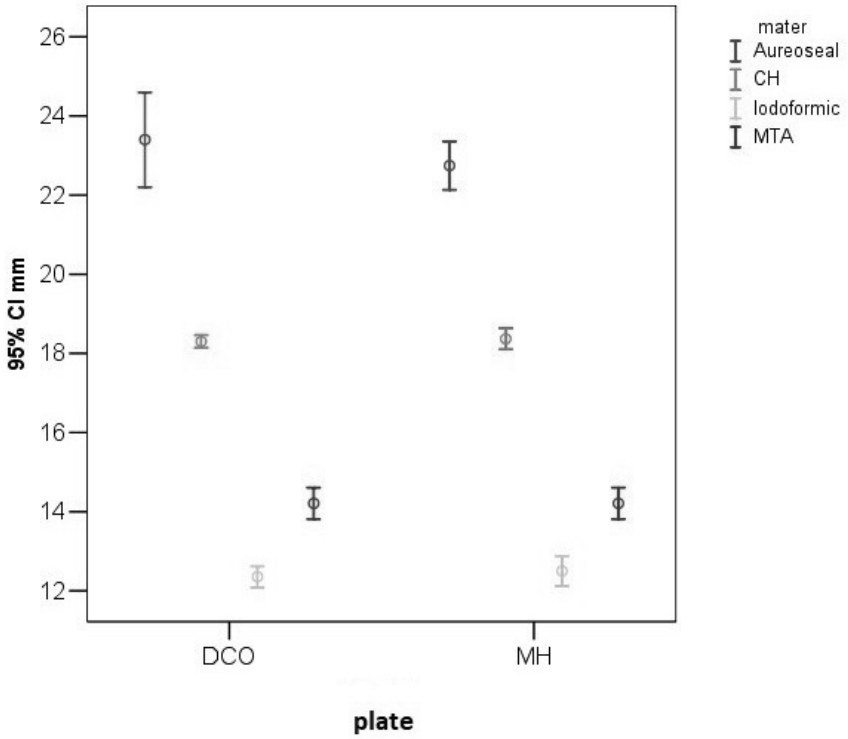

Fig. 1. *Inhibition zone in DCO plate a MH plate.*

In addition to several virulence factors displayed by this species, *E. faecalis* isolates expressed varied resistance patterns to some agents used as intracanal medications including calcium hydroxide pastes. Calcium hydroxide based intracanal medications are recommended for varied clinical situations including treatment of apical periodontitis (16). Although previous studies have established that enterococci are generally resistant to calcium hydroxide dressings (17).

The first Portland cement-based material used as a dental cement was mineral trioxide aggregate (MTA) which is a mixture of Portland cement and bismuth oxide added for radiopacity. When MTA is used as a root-end filling it is claimed to form a bacterial-resistant barrier (18) that has been attributed to the presence of calcium hydroxide in the set materials (19). Moreover, it has been suggested that the longer setting time needed by MTA contributes to its good sealing ability. Other independent studies have shown that MTA exhibits antimicrobial activity as well (20) specifically on some of the tested facultative bacteria. The presence of bacteria like *Enterococcus faecalis, Porphyromonas gingivalis, Prevotella intermedia, Tannerella forsythensis, Treponema denticola* in infected root canals is associated to clinical signs of endodontic disease. The purpose of this study was to investigate the efficacy of endodontic sealers and endodontic medicaments: Aureoseal (OGNA), MTA (DENTSPLY), calcium hydroxide (CH) (Endoidrox OGNA) and iodoformic paste (OGNA) against *Enterococcus Faecalis*.

Table I. *Results for DCO and MH plate.*

PLATE	Material	Mean	Std. Deviation	N
DCO	Aureoseal	23,400	1,5572	9
	CH	18,300	0,2121	9
	Iodoformic	12,356	0,3468	9
	MTA	14,211	0,5159	9
DCO	No material	negative	-	9
DCO	Vancomycin	positive	-	9
MH	Aureoseal	22,744	0,7955	9
	CH	18,367	0,3464	9
	Iodoformic	12,500	0,4924	9
	MTA	14,211	0,5159	9
MH	No material	negative	-	9
MH	Vancomycin	positive	-	9

MATERIALS AND METHODS

The endodontic sealer and medicaments tested in this study were Aureoseal (OGNA), MTA (DENTSPLY), calcium hydroxide (CH) (Endoidrox OGNA) and iodoformic paste (OGNA). Thirtysix Biomeraux plates (18 MH and 18 DCO) were inoculated with the experimental suspensions. The Mueller-Hinton and DCO agar medium were prepared and poured using 20 mL of sterilized pipettes into Petri dishes which are sterilized and on a flat horizontal surface up to a depth of 4 mm (20 mL). The Petri dishes were stored in a refrigerator under 4°C which is used within 1 week from preparation.

The *E. faecalis* broth culture suspensions were prepared and adjusted to no. 0.5-0.7 McFarland standard. In each agar plate were realised three cavities, each measuring 4mm in depth and 7mm in diameter, and then completely filled with the product to be tested. The mixing of the sealers is done as per the manufacturer's instruction. The plates were pre-incubated for 1h at environmental temperature for prediffusion of the material and then incubation at 37°C for 48h. To investigate the root canal sealers' antimicrobial activity, the agar diffusion method is used. In suitable culture media, the subculture of the microorganism was made to check their purity. Three wells of equal size were made by extracting the agar at the same distance and filled the root canal sealers. The diameters of the zones of microbial inhibition were measured in millimeters around the plate after incubation by a single investigator.

An antibiotic (Vancomicyn) was used as positive control and cavities alone as negative control. The data calculated were analyzed statistically. All data were initially entered into an Excel database (Microsoft, Redmond, Washington, United States) and the analysis was performed using the Statistical Package for the Social Sciences Windows, version 15.0 (SPSS, Chicago, Illinois, USA). Descriptive statistics consisted of the mean ± standard deviation (SD) for parameters with gaussian distributions (after confirmation with histograms and the Kolgomorov-Smirnov test). Comparison among groups was performed with the ANOVA univariate whit multiple comparisons by Bonferroni test. A p value of <0.05 was considered statistically significant.

RESULTS

The results showed that the antimicrobial activity of Aureoseal was superior to those of MTA, iodoformic paste and calcium hydroxide for the microorganisms tested, presenting inhibition zones of 22-23mm. The calcium hydroxide showed a good effectiveness with inhibition zone of 18-19mm similar to MTA. The iodoformic paste showed a scarce antimicrobical effect if compared to the other groups. The bacterial strains tested is inhibited by Vancomicyn (Fig. 1, Table I).

DISCUSSION

In this *in vitro* study, antimicrobial activity of root canal sealers was evaluated against *E. faecalis. E. faecalis* is strongly associated with persistent periapical infections and endodontic failures (13, 14). It has been reported to be resistant to several antimicrobial agents. It is not part of the common flora

of the root canal system. However, once inside it shows great resistance to instrumentation and intracanal medicaments and persists even after root canal filling (21). For in vitro preparations, the most common method for antimicrobial activity assessment is agar diffusion method, as also supported by previous studies (14). The zones of bacterial inhibition around each medicament was used as the criteria for comparison of the effectiveness of the medicament. Iodoform has a mild antiseptic property, as it releases iodine slowly when it is exposed to other body fluids. The iodine content makes it fungicidal and bactericidal. The mechanism of action of the iodoform is to release its oxidizing agent iodine, which can oxidize irreversibly and inactivate vital metabolic compounds, such as a protein by its antimicrobial action (22). Due to the importance of calcium hydroxide pastes in endodontic treatment, and its use as interappointment medication, the present investigation demonstrated that calcium hydroxide pastes were capable to act synergistically to shaping and chemomechanical preparation (23). Aureoseal consists mainly of PC type I, with a radiopacifier, accelerator and plasticizing agents that are not specified by the manufacturer (24-34). MTA sealer contains calcium oxide, which forms calcium hydroxide in contact with water and confers antibacterial property to MTA.

Because of physical and chemical properties of MTA, its use for perforation repair and root-end filling of failed root canal treatments has been advocated recently to seal pathways of communication between the root-canal system and the external surface of the teeth. This material has been investigated extensively by Torabinejad and other authors (26-49). Statistical analysis demonstrated the equivalence between the measurements performed on the two plate types and confirmed a statistically significant difference in behavior of all four materials tested. The study confirmed the resistance of *Enterococcus faecalis* to endodontic sealers. Aureoseal and Calcium hydroxide showed the best results in inhibition test suggesting to employ them in clinical cases.

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FIT EVALUATION OF CAD/CAM FABRICATED ALL-CERAMIC RESTORATIONS BASED ON DIRECT AND INDIRECT DIGITALIZATION IN VIVO: A SYSTEMATIC REVIEW

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The aim of this study was to compare the fit of all-ceramic restorations on natural teeth fabricated through a direct digital workflow or an indirect digital workflow. An electronic search of publications was established from three electronic databases: Cochrane, PubMed 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, are presented below. The authors used two filters to follow data for the research: papers written in English and published in the last 5 years. The search resulted in 3042 titles. Following the first stage of screening, after the records identification through database manual searching, 3047 potentially relevant studies were identified. After the second stage screening, 38 full text publications were obtained and analyzed and 17 were excluded. Afterwards, 22 articles resulted eligible after full text reading and a cross search of the articles references was accomplished and 5 articles were consequently added. At last, 6 articles were included in the quantitative analysis. This study was designed to compare the fit of restorations obtained by means of a direct or indirect digital workflow. The values reported on the maximum acceptable gap in scientific literature range from 50 to 200 µm, so there does not seem to be an objective limit based on scientific evidence. According to the most accepted marginal discrepancy in the literature, most of the values of the studies examined are in the 200 µm acceptability range. Within the limitations of this systematic review, computer-aided design and computer-aided manufacturing (CAD/CAM) fabricated restorations obtained by means of an intraoral scanner (IOS) showed better marginal and internal fit than restorations obtained through conventional impression and subsequent laboratory scanning. According to the results of this systematic review, the direct digital workflow resulted as a valid alternative to the indirect digital workflow to produce CAD/CAM all-ceramic restorations.

In the last decade, advancements in computer-aided design and computer-aided manufacturing (CAD-CAM) technologies and the improvement of intraoral scanners (IOS) allowed for a complete digital workflow, avoiding most of the steps of the conventional workflow (1). CAD/CAM was used to produce ceramic restorations including all-ceramic crowns and fixed dental prostheses (FDP) since 1980s (2). The first digital intraoral impression system commercially available was invented and brought to use in 1987 known as CEREC1 System. It worked on the principle of "triangulation of light" and needed an opaque powder coating on the surface of abutments before scanning to improve the

Key words: CAD/CAM, digital impression, all-ceramic crowns, intraoral scanner, marginal fit

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quality of scan. Since then, several digital intraoral impression devices were developed.

Other than CEREC (Dentsply Sirona, York, Pennsylvania, USA), LavaTM C.O.S (3M, Maplewood, Minnesota, USA), iTero (Straumann, Basilea, Switzerland), E4D (Planmeca, Helsinki, Finland) and TRIOS (3Shape, Copenhagen, Denmark) are some of the intraoral digital impression systems available in the dental field (3).

The accuracy of dental impressions is particularly crucial in restorative dentistry (4). Accuracy is a key aspect in function and aesthetics of indirect restorations (5). Accurate and precise replicas of the teeth are essential for producing FDPs with accurate internal and marginal adaptation. The impression technique is a significant factor in this context (6). The success of FDPs is determined by four main factors: biocompatibility, aesthetic value, resistance to fracture and marginal adaptation. Marginal and internal fit can be influenced by several factors, starting from the impression to the cementation (7). Good marginal and internal fit, along with high mechanical strength, good interfacial adhesion to veneering material and luting cement, are the most important factors in improving the prognosis of the FDPs (8). The size of the marginal discrepancy between restoration and tooth preparation is an important predictor of future ceramic fracture, periodontal health, plaque retention, caries, several pathology and bone resorption.

Precise marginal adaptation is essential to ensure long-term prosthetic success (9). The authors analyzed the influence of direct and indirect digital workflow on the fit of all-ceramic FDPs.

The considerable evolution of digital impression techniques and CAD/CAM technologies led to the use of new materials such as zirconia. However, there remain some difficulties and defects that need to be addressed regarding intraoral digital impression taking. Unlike the working process of extraoral scanner which has been proved to be steady and accurate, intraoral digital impression systems are facing a major problem of scanner displacement during the scanning process which may affect the accuracy of scanning (3). Numerous studies have compared the fit of FDPs made from both direct and indirect digitalization, but the results of most of the current studies are still inconsistent (10).

The objective of this systematic review was to evaluate and compare articles regarding marginal and internal fit of all-ceramic manufactured FDPs on natural teeth based on direct and indirect digitalization.

MATERIALS AND METHOD

Inclusion and exclusion criteria were defined by the authors, before the start of the study.

The inclusion criteria were as follows. All studies published in English language, based on human subjects. All studies analyzed are not older than five years, all studies analyzing the fit of CAD/CAM fabricated all-ceramic restorations based on direct and indirect digitalization in vivo.

The exclusion criteria were as follows. Publications that reported the same data as later publications by the same authors and systematic reviews, commentaries and letters to the editor, case report, in vitro studies, studies in animal models and case series were not considered.

Types of interventions

Replica technique on CAD/CAM fabricated all-ceramic restorations based on direct and indirect digitalization was considered. Replica technique allows to measure the space between the inner surface of the copings and the abutment.

To replicate the interface between the crown and the preparation, each crown was cemented on its corresponding clinical preparation with ultra-flow silicone. Each crown was embedded in acrylic resin to stabilize the registered interface and then cut in slices in a bucco-lingual orientation. The internal and marginal gap was determined as the vertical distance from the internal surface of the crown to the prepared tooth surface at different points.

Outcome measures

Each section of the replica technique was evaluated in different points, summarized in the records included in quantitative analyses in three points for internal gap: ca = chamfer area, aw = axial wall, aot = axio-occlusal transition area, oa = occlusal area and at one point for

marginal gap = mg.
Even though not all the studies adopted the same success criteria, they were always well specified in the publications included.

Research Question

We have formulated a specific question according to the Participants, Interventions, Control, and Outcomes (PICO) format. The question was: "how is (O) the precision of direct digitalization (I) compared to indirect digitalization (C) in *in vivo* studies in dentistry (P)"?

An electronic search of publications was established from three electronic databases: Cochrane, PubMed and Web of Science. The search strategy used a combination of controlled vocabulary and free-text words. The detailed search design and strategies 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. Two filters were used to follow data for the research: papers written in English and published in the last 5 years. The following combination of words was used: [Dental prosthesis retention(MeSH Terms)] 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 (dental implants) NOT (implants) NOT [Dental implants(MeSH Terms)] NOT Dental implants single tooth (MeSH Terms) NOT [Dental prosthesis, implant-supported(MeSH Terms)] NOT Dental Implantation[MeSH Terms]) NOT (Prostheses and Implants[MeSH Terms])) AND English[lang])) OR ((((((((((((digital vs conventional impression) OR (digital and conventional impression)) AND dentistry) OR dentistry[MeSH Terms]) AND dental prosthesis) OR Dental prosthesis[MeSH Terms]) NOT dental implants) NOT implants) NOT Dental implants[MeSH Terms]) NOT Dental implants single tooth[MeSH Terms]) NOT Dental prosthesis, implant-supported[MeSHTerms])NOTDentalImplantation[MeSH Terms]) NOT (Prostheses and Implants[MeSH Terms])) AND English[lang])) OR ((((((((((((dental impression) OR (dental AND impression)) AND digital dentistry) OR (digital AND dentistry)) AND accuracy) OR Dimensional Measurement Accuracy[MeSH Terms]) NOT Dental implants[MeSH Terms]) NOT Dental implants single tooth[MeSH Terms]) NOT Dental prosthesis, implant-supported[MeSH Terms]) NOT dental implants) NOT implants) NOT Dental Implantation[MeSH Terms]) NOT (Prostheses and Implants[MeSH Terms])) AND English[lang])) OR ((((((((((((dental impression materials[MeSH Terms]) AND digital dentistry).

Selection criteria and data extraction

The search resulted in a great number of published studies about 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 included 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 3042 titles. Following the first stage of screening, after the records identification through database manual searching, 3047 potentially relevant studies were identified. After the second stage screening, 38 full text publications were obtained and analyzed and 17 were excluded. Afterwards, 22 articles result eligible after full text reading and a cross search of the articles references was accomplished and 5 articles were consequently added. At the end, only 6 articles were included in the quantitative analysis (Table I).

DISCUSSION

Most of the clinical studies about accuracy of digital impression in vivo used the replica technique to evaluate the precision of restorations produced

through direct digital workflow compared to indirect digital workflow. Analysis of the literature revealed that there are not many studies fulfilling the inclusion criteria of the present systematic review because an *in vivo* measurement of the marginal and internal fitting accuracy is challenging, as a direct evaluation (requiring a removal of the cemented restoration from the oral cavity) is not possible. Therefore,

Table I. *Flow chart of search process and retrieval of publications.*

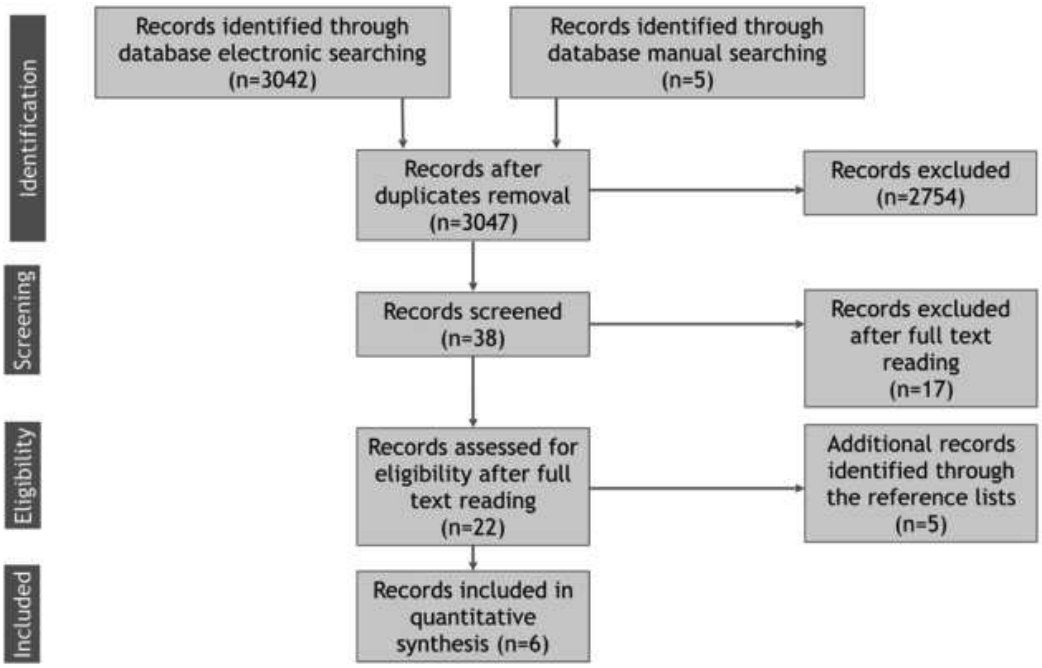

Table II. *Comparison of samples size, IOS, conventional impression method and laboratory scanner used and restoration materials.*

	N theet	Reference scanner	Conventional impression method	Restoration material	Laboratory scanner
Matthias Rödiger et al	20 molars	Trios	one-step putty-wash technique with a polyvinylsiloxane material	Zirconia	3shape D700
S. Berrendero et al	30 molars	Trios	two-step impression technique with a polyvinylsiloxane material	Zirconia manually veneered with a compatible feldspathic porcelain	3Shape D700
Guillermo Pradı´es et al	34 molars and premolars	Lava	two-step impression technique with a polyvinylsiloxane material	Zirconia	Lava Scan ST
Danush Ahrberg et al	25: 17 single crowns and 8 three-unit	Lava	monophase technique with a polyether material	Zirconia	Lava Scan ST
Moritz Boeddinghaus et al	49 teeth	CEREC, TRIOS, Lava	monophase with a vinyl polyether silicone	Zirconia	3Shape D700
Cristina Zarauz et al	26: 10 molars and 16 premolars	iTero	monofase with vinyl polysiloxane material	Zirconia-ceramic	Straumann Cares CS2

a validated and commonly accepted measuring technique is the prerequisite for a comparison of the findings with other studies.

This study was designed to compare the performance of two different digital workflows, a direct and an indirect one. CAD-CAM technology has been introduced in the dental field to improve conventional workflows like impression making procedures and design of FDPs. Furthermore, CAD-CAM allows for the fabrication of high strength ceramic restorations like partially stabilized zirconia frameworks, for which milling is the only approach available nowadays (11).

One major parameter for clinical success is the fit of a restoration. The larger the marginal discrepancy, the faster is the rate of cement dissolution and the higher is the risk of bacterial insult, causing pulpal inflammation and necrosis (12). The precision of direct and indirect digital workflow was evaluated in this in study measuring the marginal and internal fit of the fabricated all-ceramic frameworks (13).

There are many clinical factors, besides impression material and technique, which may influence the quality of an impression, including: location of the finish line, periodontal health, sulcus bleeding during impression taking or saliva flow rate, as well as patient compliance. In addition, if the impression is taken by means of an IOS, the accessibility of the preparation for the IOS wand becomes critical for the success of the impression. Accessibility can be limited especially in the retromolar region of patients (14). For the in vivo assessment of the clinical fit of the copings, the silicone replica technique is a reliable, non-invasive, well-established (15, 16), and commonly used method (13, 17-32).

The values reported on the maximum acceptable gap in scientific literature range from 50 to 200 μm, so there does not seem to be an objective limit based on scientific evidence (33-36). At present, many investigators still use the limit established by McLean and Von Fraunhofer of 120 μm (37). For the interpretation of the results of the present study, it is important to consider the limitation of in vivo measurements regarding fitting accuracy. An *in vivo* measurement of the marginal and internal fitting accuracy is challenging, as a direct evaluation (requiring a removal of the cemented restoration from the oral cavity) is not yet possible. Despite that, the *in vivo* evaluations based on the crowns fit are the only ones that allow for a real comparison between

Table III. *Comparison of marginal gap (μm) in restorations obtained through a direct (IOS) and indirect digital workflow (CI): MG, Axial wall gap (μm): AW, Axio-occlusal transition area gap (μm): AOT, Occlusal area gap (μm): OA.*

	MG (mean in μm)		AW (mean in μm)		AOT(mean in μm)		OA (mean in μm)	
	IOS	CI	IOS	CI	IOS	CI	IOS	CI
Matthias Rödiger et al	87.40± 91.21	82.17±73.17	74.68±42.43	73.88±40.00	187.17±77.35	182.93±66.07	164.22±73.17	207.60±69.99
S. Berrendero et al	106.6±69.6	119.9±59.9	82.8±40.3	105.2±49.8	275.4±95.6	287.6±93.8	275.1±114.0	279.5±91.6
Guillermo Pradı´es et al	76.33±65.32	91.46±72.17	128.96±62.80	146.35±66.45	195.21±67.44	200.40±72.42	198.96±67.81	224.89±67.52
Danush Ahrberg et al	61.08±24.77	70.40±28.87	88.27±41.49	92.13±49.87	144.78±46.23	155.60±55.77	155.57±49.85	171.51±60.98
Moritz Boeddinghaus et al	CEREC 149 TRIOS 112 Lava 88	113	No data	No data	No data	No data	No data	No data
Cristina Zarauz et al	80.29±26.24	133.51±48.78	81.62±26.91	120.93±53.22	152.81±149.80	214.73±73.31	178.04±56.85	314.31±93.24

different workflows without comparing impressions to each other and without using reference measures. The examined studies are summarized in Table II, with the description of the samples, of the IOS used, of the conventional impression technique and laboratory scanner used and restoration materials. In Table III the mean values in μm of the marginal fit calculated in different points for internal gap and marginal gap are summarized (7, 13, 24, 25, 38-63).

According to the most accepted marginal discrepancy in the literature, most of the values of the studies examined are in the 200 μm acceptability range. In the study of Berrendero et al (7) the values that exceeded this range were related to the gaps on AOT and OA; in both cases, there were no significant differences between direct and indirect digitalization. In the same study, all measurements on FDPs made through a direct digital workflow showed better results in terms of fit compared to the indirect digital workflow, although not significantly. In the studies of Guillermo Pradies et al and of Cristina Zarauz et al (13, 26) the values that exceeded the 200 μm range are related to the gaps on AOT and on OA but only for the indirect digitalization sample. The difference between the gap on the copings made by means of direct and indirect digitalization was not significant. The greater value of gap of 314.31 ± 93.4 was found in the coping milled after indirect digitization in the study of Cristina Zarauz et al (26). Summarily, the measurements made on caps constructed on the basis of a direct digital impression showed lower values of misfit, except in the work of Matthias Rödiger et al (38) in which for measurements on MG, AW and AOT, values slightly lower than those for the conventional one were noticed. The work of Moritz Boeddinghaus et al (25) had a marginal role because it analyzed only the gap on the marginal fit; three intraoral scanners were compared with a conventional imprint and the value of greatest discrepancy is the one relative to the Cerec IOS.

Within the imitations of this systematic review, CAD/CAM-fabricated restorations produced by an intraoral scanning system showed a comparable, or even better, precision in terms of marginal and internal fit than restorations based on conventional impressions in combination with the laboratory scanning technique. Therefore, the complete digital workflow can be rated as a suitable alternative to conventional impressions, followed by a lab-side digitization.

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WHY PATIENTS WITH CARDIOVASCULAR RISKS GO TO DENTISTS. IS THERE SUFFICIENT EVIDENCE OF INFLUENCE OF PERIODONTAL THERAPY ON CARDIOVASCULAR DISEASE?

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Cardiovascular disease (CVD) is a common cause of death, representing 29% of the mortality all over the word. Estimates for 2006 show that CVD is one of the world's main cause of death, with 17.1 million death per year. 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. There is strong evidence that periodontal disease (PD) is associated with an increased risk of CVD. In addiction 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. 34 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 relations between PD and CVD, however there is sufficient evidence to justify a periodontal treatment to prevent CVD, in fact PD is very prevalent in 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 many 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 etc. Therefore, there is reason to hope that the strong evidence identified by these studies, until now, could lead to significant improvements in treatment of periodontal infections and that, it may come to 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 (1). PD is caused by specific bacteria of the biofilm formed by plaque. The bacteria leak into the periodontal ligament space, causing anaerobic infection creating a cascade of events, which end with the production of inflammatory

Key words: chronic periodontis, periodontal therapy, cardiovascular disease, atheromas, inflammation

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mediators and bacterial metabolites (2). At least two potential metabolic pathways could develop from the periodontal pocket and the resulting from 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 bloodstream (2).

The pathological mechanisms by which PD may contribute to the pathogenesis of systemic inflammatory diseases are currently 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 etiopathogeneis of PD. The most frequently identified include three species called "red complex": *Porphyromonas gingivalis, Tannerella Forsythia, Treponema denticola* (3).

Periodontal disease and cardiovascular disease

Cardiovascular disease (CVD) is a common cause of death, representing 29% of the mortality all over the word. Estimates for 2006 show that CVD is one of the world's main cause of death, with 17.1 million death per year. 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 CVD is atherosclerosis, which is responsible for 50% of deaths in the United States, in Europe and Japan (4).

After eliminating other risk factors, some studies indicate that severe PD is associated with an increased risk of CVD ranging from 25% to 90%; The 8.91% of patients with CVD is also affected by PD, which can be mild or severe. While 66% of people who have CVD manifest PD also (5). This data must be explained also by the fact that CVD and PD share similar risk factors such as smoking, diabetes mellitus, obesity and hypertension (6). In literature some studies demonstrated a mild-moderate association between CVD and PD (7-9).

In a study of Ziegler et al. they 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 (10). In addiction 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 (11).

Another study affirmed that PD has a significant relation as a risk factor in peoples over 40 years, who had coronary artery disease proved by coronary angiography (12). 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 (13).

A recent randomised controlled trial concluded that the use of Triclosan-toothpaste have influence on biomarkers of cardiovascular risk. These studies suggested that triclosan-toothpaste might influence some inflammatory biomarkers of CVD. However, it is unclear if this influence is clinically significant. The reason why PD therapy could modify CVD management is still unknown (14).

On the contrary, a recent study found very low quality evidence that was insufficient 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 (15).

Likewise, the study previously cited, Thomopoulos stated that from the clinical point of view, advising periodontal prevention or treatment targeting on the prevention of CVD it is unjustified. By contrast, oral hygiene including periodontal health might contribute to the overall well-being and healthy lifestyle and hence as might at least partially contribute to cardiovascular prevention (16).

Hypothesis about mechanism of action

The presence of pathogenic bacteria correlated with periodontal disease may be localized in plasma

or ateromasic plaques (17-18). Always investigating these plaques Cairo et al. have identified various bacteria: *T. forsynthensis* (79%), *F. nucleatum* (63%), *P. intermedia* (53%), *P. gingivalis* (37%) and *A. actinomycetemcomitans* (5%) (19). We can also find these bacteria in the carotid, coronary and occluded arteries of patients who have Buerger disease (20). The pathogens induce the release of inflammatory cytokines; even the normal chewing contributes to the spread of pro-inflammatory cytokines from the mouth into the bloodstream (TNF, IL-1 and PGE2) (21). Animal studies have been able to demonstrate atherosclerosis induced by pathogenic bacteria of PD (22).

The presence of acute or chronic infections may play a significant role on CVD onset, causing increased vascular inflammation and thrombosis promotion (23-24). These infections may be considerated a secondary pathogenic pathway (25-27). PD might be the most common.

There are two main hypotized ways of pathogenesis:

1. direct invasion by the bacteria into endothelial vascular wall;
2. release, in response to infection, of inflammatory mediators with aterogenic 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 cells proliferation (28-29). In addition platelets could be activated and aggregated by periodontal pathogens present in the atheromas through collagen-like platelet aggregation-associated proteins expression. The activated and aggregated platelets could induce atheromas formation and thrombosis, and finally lead to CVD (30).

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 (31).

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 in the bloodstream, thus causing systemic disease in distant organs. Membrane microparticles are released by multiple cells in inflammatory environment. Recent data suggested the role of these microparticles in the pathogenic process of many systemic diseases, that can be also associated to periodontitis. So, periodontitis could be a chronic reservoir of microparticles, hence elucidating partially the interaction with systemic diseases initiation or progression (32).

Other studies confirm that periodontal therapy improves serological markers of CVD. In fact, when PD is active the levels of systemic inflammation markers will be increased and, when PD is treated, the level of systemic inflammatory mediators significantly decrease (33-34).

There is sufficient evidence to justify a periodontal treatment to prevent CVD (35-42). PD is one of the most widespread diseases in the world and the correlations with other systemic diseases must still be studied (43-47). There are many surgical and non-surgical therapeutic approaches of PD and perimplantitis that can affect both PD and CVD (48-76).

DISCUSSION

There are some lack of knowledge on relations between PD and 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 very prevalent in middle-aged population and can have a significant impact on the cardiovascular function.

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AN OVERVIEW ON PERIODONTAL CHANGES AND DENTAL MOVEMENTS

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Only in recent times has been enhanced the importance of gingival crevicular fluid in periodontal health and in particular in maintaining the integrity of periodontium during application of orthodontic forces. The aim of this short review is to evaluate the importance of substances as valid biomarkers of periodontal health during orthodontic movements. A search on PubMed and Cochrane database was performed considering the literature from 2003 to 2014, using the following key words: gingival crevicular fluid, biomarkers of periodontal tissue, orthodontic movements. After abstracts screening, the full-texts of selected papers were analyzed and the papers found from the reference lists were also considered. The search focused on clinical applications documented in studies in the English language: levels of evidence included in the literature analysis were I, II and III. Literature analysis showed 28 papers that fulfilled the inclusion criteria. The conclusion is that GCF is a powerful vehicle for clinical diagnostics, since it contains different biochemical and cellular arrays in relation to different clinical situations indicative of the state of periodontal health during orthodontic treatment.

Gingival crevicular fluid (GCF) is an important source of biomarkers related to the state of health of deeper-seated tissues of periodontium. These properties can be particularly useful in monitoring the effectiveness of orthodontic treatment and the response of the alveolar bone to orthodontic forces. In recent years, thanks to sophisticated biochemical analysis, several biomarkers of alveolar bone have been discovered. These biomarkers change in special clinical situations, such as severe periodontal disease, osseointegration, trauma due to orthodontic movements and are therefore indicative both of active pathology or healing. This review aims to assess the current knowledge about biomarkers of periodontal tissues during orthodontic treatment. In fact, the clinical parameters do not allow to assess the state of suffering of the periodontium, especially alveolar bone remodeling during orthodontic treatment. Gingival crevicular fluid allows very specific and non-invasive biochemical analysis, so the study of biomarkers of GCF is a very exciting prospect for future clinical applications. The biological mechanism that controls the passage from the stimulus, application of continuous orthodontic forces, to the reaction, displacement of the tooth in the periodontal space, can be evaluated in accordance with the theory of tension-pressure and cell differentiation, resulting in tooth movement or retention controlled by biochemical signals (1-2).

The aim of this short review is to evaluate the importance of substances as valid biomarkers of periodontal health during orthodontic movements.

MATERIALS AND METHODS

A search on PubMed and Cochrane database was

Key words: gingival crevicular fluid, biomarkers of periodontal tissues, orthodontic movements

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performed considering the literature from 2003 to 2014, using the following key words: gingival crevicular fluid, biomarkers of periodontal tissue, orthodontic movements. After abstracts screening, the full-texts of selected papers were analyzed and the papers found from the reference lists were also considered.

RESULTS

The search focused on clinical applications documented in studies in the English language: levels of evidence included in the literature analysis were I, II and III. Literature analysis showed 28 papers that fulfilled the inclusion criteria. These papers describe the main gingival crevicular fluid (GCF) biomarkers related to orthodontic movements:

Biomarkers of inflammation:

Interleukins (IL-1β, IL-6, IL-8), Tumor Necrosis factors (TNF-α), Colony-stimulating factors (M-CSF, G-CSF, GM-CSF), Prostaglandins (PGE), Vascular endothelial growth factors (VEGF), Calcitonin gene related peptide (CGRP), Substance P.

Interleukins (IL-1β, IL-6, IL-8)

Interleukins (IL-1β, IL-6, IL-8) are cytokines involved in the bone remodeling, bone resorption and neo-bone apposition during orthodontic movements (3-6).

IL-1β is the most potent cytokine stimulating osteoclast activity and attracting white blood cells and other cellular mediators in the process of bone remodeling. It is the first polypeptide regulating the processes of resorption and neo-bone apposition in relation to mechanical stress. Moreover, IL-1β is one of the mediators of inflammation which induces the secretion of substances causing pain. Beside, IL-1β is produced by the periodontal ligament in a quantity sufficient to diffuse into the gingival crevicular fluid and has been identified as a biomarker of orthodontic movement.

Interleukin-6 (IL-6) is a cytokine involved in the process of bone resorption. IL-6 promotes the differentiation of osteoclast cells and can activate mature osteoclasts (7-8). Moreover, IL-6 is involved in osteoclastogenesis through stromal or osteoblastic cells (9), in fact the level of IL-6 in gingival crevicular fluid (GCF) increases during orthodontic tooth movement (10). These findings suggest that IL-6 may play important roles in bone resorption during orthodontic tooth movement.

Several studies have demonstrated increased level of IL-8 at periodontal deep ligament (PDL) tension sites, so IL-8 is promoting factor for bone remodeling (11). Increased levels of these proinflammatory cytokines are demonstrated in GCF during orthodontic tooth movement.

Tumor Necrosis factors (TNF-α)

Tumor Necrosis factors (TNF-α) is another potent proinflammatory cytokine that stimulates acute or chronic inflammation and bone resorption, favoring the production of osteoclasts in the presence of Colony-stimulating factors (M-CSF, G-CSF, GM-CSF) (12).

Macrophages-Colony-Stimulating Factors (M-CSF)

M-CSF are glycoproteins promoting the activity of monocytes-macrophages and granulocytes. In mice they interact in bone remodeling during the movement of the teeth (13). The M-CSF are produced in the early phase of bone remodeling, stimulating osteoclasts maturation (13).

Prostaglandins (PGE)

Prostaglandins, produced by deformed osteoblasts and gingival fibroblasts, are cytokines implicated in inflammation provoked by orthodontic tooth movement. Among the subclasses of prostaglandins, prostaglandin E_2 (PGE_2) is strongly related to bone resorption (14).

Prostaglandin E_2 (PGE_2) is induced by interleukin-1β. IL-1β synergically up-regulate the formation of prostaglandins in the periodontal tissue subjected to stress orthodontic. Orthodontic and orthopedic forces evoke changes in the levels of inflammatory mediators in the periodontal tissues and can trigger the processes of bone resorption in tissue around the tooth root (15).

Vascular endothelial growth factors (VEGF)

Vascular endothelial growth factor (VEGF) is

a cytokine involved in tissue neoformation since it increases vascular permeability and promotes angiogenensis. During orthodontic movements compressive forces induce the formation of new capillaries by the activation of VEGF (16-17).

Calcitonin gene related peptide (CGRP), Substance P

During orthodontic therapy the increase of concentration of biologically active proteins contribute to increase neurogenic inflammation. Nociceptive somatosensory neurons transmit signals from the peripheral fibres of the periodontal tissues to the central nervous system. With the application of orthodontic forces the peripheral nervous system periodontium fibres release calcitonin gene related peptide (CGRP) and substance P also acting as vasodilators, increasing vascular flow and permeability (diapedesis) and stimulating plasma extravasation and leukocyte migration into tissues (transmigration). Furthermore, the substance P promotes the secretion of proinflammatory cytokines by immunocompetent system and stimulates the production of PGE_2 (18)

Biomarkers of bone metabolism: Osteoprogerin (OPG), Receptor activator of nuclear factor kappa-B (RANK), Receptor activator of nuclear factor kappa-B ligand (RANKL).

Osteoprotegerin (OPG), receptor activator of nuclear factor-(KB) ligand (RANKL), and its decoy receptor RANK, are protein ligands. They share similarities to the superfamily of receptors for tumor necrosis factors and function as paracrine regulators of bone metabolism and osteoclastogenesis. Osteoprotegerin lacks transmembrane and cytoplasmic domains and is secreted as a soluble protein, mainly by osteoblastic lineage cells**.** The primary biologic actions of OPG are inhibition of osteoclast differentiation, inhibition of osteoclast resorptive function, and stimulation of osteoclast apoptosis (19-20)**.**

Biomarkers of cell death: Caspase-1, B-glucuronidase (βG), Aspartate aminotransferase (AST), Lactate dehydrogenase (LDH).

The capsasi-1 is the main mediator of the apoptotic response triggered by changes in the intracellular fluid. The capsasi-1 has the function to process and activate the pro-inflammatory cytokine interleukin proIL-1β and other proinflammatory interleukins (21).

Biomarkers of bone deposition and mineralization: Osteoprotegerin (OPG), Bone alkaline phosphatase (ALP).

Osteoprotegerin (OPG) acts on bone cells in terminal stages of osteoclast differentiation, suppression of activation matrix of osteoclasts, and induction of osteoclast apoptosis (19).

Bone metabolism is linked with alkaline phosphatase (ALP) and acid phosphatase (ACP), expressed, respectively, by osteoblasts and osteoclasts. During orthodontic therapies these enzymes diffuse into GCF from periodontium. This process has been demonstrated in humans and animal models. (25). Oral health and a pleasant smile are one of the most successful goals in the general population, so GCF physiology is of great interest in dentistry (26-66).

DISCUSSION

GCF is a powerful vehicle for clinical diagnostics, since it contains different biochemical and cellular arrays in relation to different clinical situations indicative of the state of periodontal health during orthodontic treatment. GCF can be considered as an exudate or transudate. It's a fluid arising from the gingival margin, that can be collected in a non-invasive and site-specific way.

The results of several studies suggest that GCF is the product of vascular tissue in the gums, where the effect of trauma on the capillaries produces the gingival fluid. In periodontal disease there is an evident increase of GCF due to the loss of continuity of the basement membrane of the junctional epithelium. This process is accompanied by a widening of the intercellular spaces of the junctional epithelium and by a partial destruction of the basal membrane. These events lead to an increase of the osmotic gradient for the formation of a half-waterproof membrane. The different osmotic gradient drains fluid from the

capillaries of the periodontal tissue.

The initial exudate is usually serous, but later is contaminated by inflammatory molecules making it an exudate containing biomarkers that represent the metabolic state of deep periodontal tissues. The importance of GCF in the assessment of orthodontic movements could be influenced by different parameters that do not typically relate to inflammation and bacterial plaque. The trauma of the alveolar bone resorption at the level of the deep periodontal apparatus induces a pressure on the surrounding tissues which determines an increase of production of GCF, which can be used to test the factors influencing orthodontic movements.

This short review has focused attention on current knowledge about biomarkers of GCF in relation to orthodontic movements. In conclusion, the definition of a package of biomarkers could help us in making a better diagnosis and treatments more precise, thereby providing orthodontists additional information that cannot be deduced from clinical parameters.

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THE ROLE OF IMPLANT-ABUTMENT CONNECTION IN PREVENTING BACTERIAL LEAKAGE: A REVIEW

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Osseointegration can be affected by oral conditions; in particular, the micro gap at the implant-abutment-connection (IAC) represents a site for dental plaque aggregation favoring bacterial leakage that can increase inflammatory cells at the level of the IAC, causing peri-implantitis. This micro gap, once early colonized, may constitute a bacterial reservoir that could subsequently contaminate fixture's surroundings and interfere with peri-implant tissues health. The aim of this review is to describe, according to the most recent literature, the different kind of implant-abutment connection and their ability to reduce bacterial leakage and thus preventing peri-implantitis. The following database were consulted: Pubmed (n=26), Scopus (n=90), Research gate (n=7) and 123 articles were found. Duplicates were excluded and after reading abstract and titles, those articles that were off topic were also excluded. The remaining ones (n=24) were assessed for full-text eligibility. We excluded 5 articles because they were case reports, 2 because there was no clear reference to the relationship between IAC and bacterial leakage and 2 because they were not pertinent to the argument. Fifteen articles were included in the review. From the review, it is clear that a relationship between the IAC and bacterial leakage exists. All the connections presented an amount of micro-gap and bacterial micro-leakage but conical and mixed connection systems seem to behave better. Moreover, both connections seem to have a better load distribution and the mixed one has anti-rotational properties, very useful during the positioning of the prosthesis.

Implants are devices widely used to rehabilitate edentulous area. In order to say that the placement of an implant is successful it must be osseointergrated. Albrektsson proposed the following criteria for implant dentistry that today are still the most widely used (1), i.e.:

- The absence of clinical mobility of the implants;
- The absence of subjective sensitivity or pain;
- The absence of peri-implantitis;
- The absence of persistent radiolucency around the implants.

It is important to underline that an implant is completely successful only when is loaded and there is no bone resorption around it.

Implants are made of titanium alloy that had proven to have the following properties: corrosion resistance, strength, toughness, resilience, low density and low stiffness (2-4).

Osseointegration can be affected by oral conditions; in particular, the micro gap at the implant-abutment-connection (IAC) represents a site for dental plaque aggregation favoring bacterial leakage that can increase inflammatory cells at the level of the IAC, causing peri-implantitis. Two-piece

Key words: peri-implantitis, bacterial leakage, osseointegration, IAC

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implants unavoidably present a micro-gap between the implant and the abutment (5-11). These spaces, once colonized, may constitute a bacterial reservoir that could subsequently contaminate fixture surroundings and interfere with peri-implant tissues health. The presence of a micro-gap, and thus a reservoir of bacteria, when in close relation to bone, may have a role in bone loss (11).

The bacteria found at IAC level can be both anaerobic and facultative anaerobic, depending on the features of this microhabitat. In addition, patients at risk for periodontal disease have a higher risk of peri-implantitis (5, 6). In addition, it is widely known that oral health and a pleasant smile are one of the most successful goals in the general population (12-19). Therapies that allow the maintenance of oral health have been widely studied in the last years.

The inflammatory content could increase due to the adhesion and proliferation of bacteria on the biofilm around IAC during soft tissue manipulation for prosthetic component installation. The potential colonization of the implant-abutment micro-gap is probably related to multifactorial conditions, i.e. the precision fit between the implant components, which is associated with the implant system design, the torque used to connect the components and the repeated screw loosening and re-tightening (6). The structure of the IAC could have an impact on the amount of microbial leakage between the implant connection and the abutment. In addition, the success of implant rehabilitation is related to mechanical properties, such as correct loading.

Occlusal overloading after prosthetic rehabilitation can result in increased stress in both the implant and implant-abutment connection, as well as in the surrounding bone. The IAC design and fit influence the loading of the implant-abutment system during the physiological movements of the jaws. If this load is oversized, the implant system can fail due to screw fracture and loss, implant fracture or damage to the prosthesis. In order to avoid a fail in the implant system, fixture should be placed correctly and the prosthesis should be designed to minimize the length of lever. In addition, occlusion should be designed so that the loads are transferred along the implant axis in order to prevent an excessive concentration of stress at IAC (4, 6).

The aim of this review is to describe, according to the most recent literature, the different kind of implant-abutment connection and their ability to reduce bacterial leakage and thus preventing peri-implantitis.

MATERIALS AND METHODS

An electronic research was conducted using database to find articles published in the last five years regarding to IAC and bacterial leakage. In the review process were included only English articles and studies conducted on human. The following key words were used: implant-abutment connection or fixture-abutment connection or IAC and bacterial leakage or implant abutment interface. Inclusion criteria were:

- *In vivo* e *in vitro* studies
- Prospective e retrospective studies
- Systematic and narrative reviews

Exclusion criteria:

- There was no clear reference to the relationship between IAC and bacterial leakage
- Case report because of limitated clinical relevance

The following database were consulted: Pubmed (n=26), Scopus (n=90), Research gate (n=7) and 123 articles were found. Duplicates were excluded and after reading abstract and titles, those articles that were off topic were also excluded. The remaining 24 articles were assessed for full-text eligibility: we excluded 5 articles because they were case reports, 2 because there was no clear reference to the relationship between IAC and bacterial leakage and 2 because was they were not pertinent to the the argument. (Fig 1).

RESULTS

The presence of a microgap at IAC is well know and varies from 1 to 49 μm according to different implant system (5). The IAC is usually situated under the soft tissue of the gingiva, sometimes very near to the bone for this reason control of bacterial leakage through IAC can be a major aspect in preventing the infection of the peri-implant tissues. In patients with previous periodontal diseases, reduction of the

passage of pathogenic bacteria should be considered important specially beacause studies have shown that bacterial species of periodontal disease are very similar to those that cause perimplantitis.

It is known that peri-implantitis and loss of osseointegration are due to bacterial leakage through IAC, but also to other factors, i.e. different positions of the implant shoulder on the bone crest, implant diameter in order to better engage the cortical bone, implant design (conical or cylindrical) and its coil, implant length, surgical technique and bone characteristics such as density and thickness (20-44). The main implant connection and their most important characteristics will be listed below.

Internal hexagonal connection

Nowadays the internal hexagonal connection (i.e. a part of the abutment is inserted in the body of the implant) is still the most widely used in the two-piece implant system. This is most likely due to the fact that this connection ensures proper abutment seating, anti-rotational engagement, resistance to lateral forces and excellent esthetic results. This connection is reported as being less favorable to the infiltration of fluids than the external one. Higher stability under loading conditions has been reported for different internal connections compared to external ones that, under functional loading, promotes micro-movements of the abutment and so bacterial leakage. The performance of bridge rehabilitations was observed to be worse than that in single crown rehabilitations (4, 9, 10).

Mixed connection

Mixed connection is given by the union of a conic connection with a geometrically defined one, for example the conical plus octagonal morphology, which provides a cone that terminates in an octagon. The cone gives the abutment remarkable stability; the octagon has instead anti-rotational properties and precise positioning. With this connection can be adopted the principle of platform-switching (the prosthetic platform is smaller than the implant platform). This method enables the distance between the margin of closure of the prosthesis and the crestal bone to be increased, in this way, it is possible to avoid bacterial leakage coming into direct contact with the bone margin thus helping in prevention of bone resorption and peri-implantitis (6, 7).

Conical connection

Cone connection locks the implant/abutment system because of the friction between the external wall of the abutment and internal wall of the implant. A recent systematic review indicated that conical and non-conical abutments showed sufficient resistance to maximal bending forces and fatigue loading (11). However, conical abutments showed superiority in terms of seal performance, microgap formation, torque maintenance, and abutment stability.

Loading forces on the prosthetic components may induce implant-abutment system bending or micromovement. This has been associated with the increase in the microgap and a pump effect between the inside of the implant and the peri-implant tissues. Conical systems appear to be superior to internal and external connections in limiting this inconvenient.

Another factor for long-term implant–abutment stability is the maintenance of torque value between implant and abutment after tightening. Obviously, this can prevent abutment screw loosening or movement and microgap formation. All tested connection systems showed torque loss after initial tightening, particularly external and internal hexagonal connection systems were more susceptible to abutment micromovements, but conical systems showed higher resistance to torque loss and therefore is less likely to form a microgap and the consequent probability of occurrence of peri-implantitis.

We can say that the conical implant connection system is more favorable insofar as maintenance of marginal bone is concerned (4, 11).

CONCLUSION

To date, no implant system or connection design has been able to provide a perfect sealing at the IAC. All the connection presented an amount of micro-gap and bacterial micro-leakage but conical and mixed connection systems seem to behave better. Moreover, conical abutment seems to have less mechanical complications such as screw loosening

or fracture and higher torque preservation, mixed connection can provide the greater load distribution, anti-rotational properties and precise positioning.

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PERIODONTITIS AND CEREBROVASCULAR DISEASE: A NEW NOVEL IN MEDICINE

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The aim of this review is to determine if there is a relationship between periodontal disease and stroke. The included case-control and cohort studies mediate the incidence of stroke and periodontal disease by analyzing different parameters. A literature review was carried out in PubMed, Scopus and Embase databases using the key word "stroke" AND "periodontal disease". An amount of 932 articles came out from our research on these three databases. These articles were selected according to PRISMA criteria. The following inclusion criteria were established: studies conducted in humans, articles published in English and published in the last ten years. Exclusion criteria were: experimental studies on animals, articles published more than 10 years ago, non-English language articles, articles of non-indexed journals, and articles not directly related to the association between stroke and periodontitis. These criteria reduced the number of articles from 932 to 399. At the end, articles that appeared to be repeated in different databases have been eliminated: 254 articles remained. All these articles titles were reviewed by the authors, who decided whether or not to include them in the review. We selected an amount of 43 articles. These studies were reviewed by reading the titles and abstracts, and by finally selecting the ones with the same topic of this review. When titles or abstracts were not clear, the complete article was read. At the end 7 articles were selected. In addition, 2 systematic reviews and 1 article, cited in the discussion, and regarding the protocol used in patients suffering from cardiovascular diseases and periodontitis, were selected. The quality of these articles was evaluated through the JADAD system. In conclusion, patients with stroke have a higher prevalence of periodontitis.

Cerebrovascular accident (CVA) or stroke is a cerebral circulatory disorder that results in a transient or definitive alteration of the functioning of some area of the brain. It is the leading cause of death in women and the leading cause of disability in adults. They represent a recurring cause of hospitalization with a significant social-health expenditure and it is estimated that, in the future, it will increase due to the progressive aging of the population (1, 2).

According to its pathogenesis CVAs can be classified into two categories: ischemic and hemorrhagic. Depending on duration of symptom, ischemic stroke can be classified in transient ischemic attack (TIA), if it lasts less than 24 hours, and in ischemic infarction when it is longer than 24 hours and when tissue necrosis appears. There are two main type of hemorrhagic stroke: intracerebral hemorrhage (intraparenchymal or intraventricular) and subarachnoid hemorrhage. Other forms of intracranial hemorrhage exist: epidural and subdural hematoma (3).

The most important modifiable risk factor for

Key words: stroke, cerebrovascular disease, periodontal disease, periodontitis

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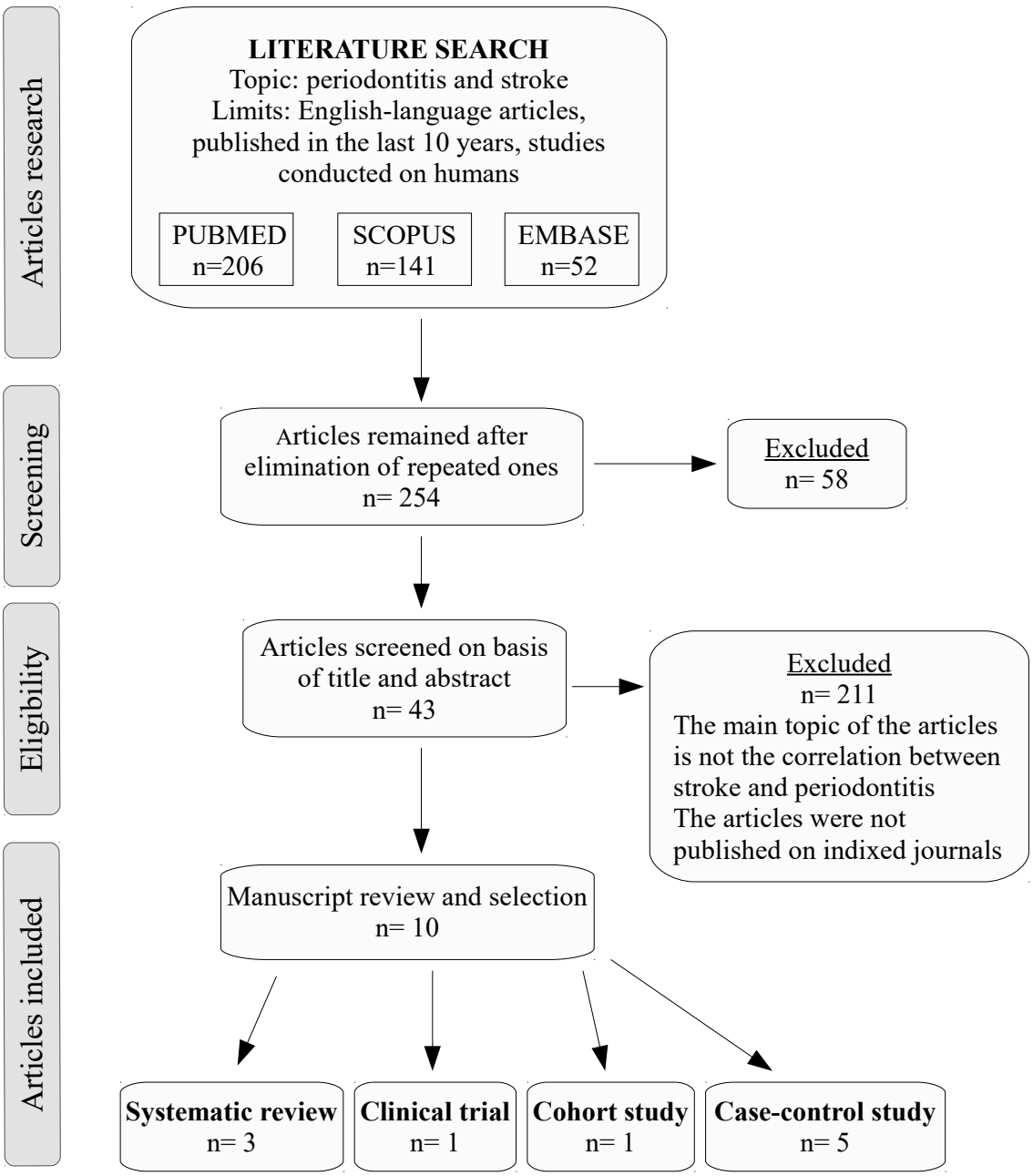
cerebrovascular disease is hypertension, although dyslipidemia, diabetes mellitus, and cigarettes smoking are also important. In young patients, one of the most relevant risk factors is the increased levels of lipoproteins (4). Hypertension is the main cause of cardioembolic strokes, as it is related to atherothrombotic strokes and atrial fibrillation (5).

The main symptoms of these patients are sudden weakness or numbness in the face, arm, or leg, confusion, trouble speaking or difficulty understanding speech, sudden trouble seeing, sudden trouble walking and dizziness (6,7).

In regards to oral health, CVAs can induce hemiparesis and hemiplegia of the muscles of mastication and of the muscles of pharynx, tongue and palate. This involves a loss of function, with

Fig. 1. *Flow chart showing selection of studies for the review.*



complications in eating and chewing food, and dysphagia that can lead to a reduction in nutrient intake: in this situation patients require caregivers to provide for their nutrition (8).

In addition, these patients have altered or decreased salivary clearance, with important difficulty of the tongue and saliva in self-cleaning oral cavity, which lead to a greater amount of bacterial load in the mouth. Consequently, there could be an increased risk in upper respiratory tract infection.

Medication treatment for cerebrovascular disease can induce a parasympathetic system alteration, which can results in salivary glands inhibition: reduced salivary secretion, xerostomia and increased risk of dental caries could be the consequences (9).

The loss of strength or hemiparesis of the arms cause difficulties in maintaining a good oral hygiene. This has a significant impact on the patient's autonomous oral hygiene and, unless they have access to effective assisted support, the risk of dental problems increases (8).

The aim of this study is to assess the possible association between periodontal disease and stroke, through a review of the most recent literature on the topic.

MATERIAL AND METHODS

A literature review was carried out in PubMed, Scopus and Embase databases using the key word "stroke" AND "periodontal disease". A total of 932 articles were identified through our research on these three databases. These articles were selected according to PRISMA criteria. The following inclusion criteria were established: studies conducted in humans, articles published in English and published in the last ten years. Exclusion criteria were: experimental studies on animals, articles published more than 10 years ago, non-English language articles, articles of non-indexed journals, and articles not directly related to the association between stroke and periodontitis.

These criteria reduced the number of articles from 932 to 399. At the end, articles that appeared to be repeated in different databases were eliminated and 254 articles remained. All these articles titles were reviewed by the authors, who decided whether or not to include them in the review. We selected an amount of 43 articles. These studies were reviewed by reading the titles and abstracts, and by finally selecting the ones with the same topic of this review. When titles or abstracts were not clear, the complete article was read. At the end 7 articles were selected. In addition, 2 systematic reviews and 1 article, cited in the discussion, and regarding the protocol used in patients suffering from CVA and periodontitis, were selected. The detailed procedures of the literature search and article screening are shown in Fig. 1.

RESULTS

Among the articles selected for the review, five are case-control studies, one is a clinical trial and one a cohort study.

Leira et al. (10) studied 62 patients with stroke and compared them with 60 controls. The following variables were studied: plaque index, bleeding on probe index (PoB), missing teeth index, clinical attachment loss (CAL), probing depth index and gingival recessions. They obtained significant differences between both groups in all the studied parameters.

Diouf et al. (11) saw 120 patient with CVD and 120 controls, by analyzing periodontal parameters such as plaque index, gingival index, clinical attachment loss (CAL), probing depth index, and community periodontal index of treatment needs (CPITN). All the parameters was statistically significant, so it has been possible to quantify the incidence of periodontitis: as a result the disease was found in 73,3% of the cases and in 40,8% of the controls.

Likewise, Söder et al. (12), followed-up 1676 randomly selected subjects for a period of 26 years: in relation to the topic, this is one of the studies with the largest sample and the longest follow-up period. 39 patients suffered from cerebrovascular disease: in these subjects parameters of plaque index, missing teeth index, gingival index and calculus index were measured. Only these last two parameters were statistically significant.

In the study of Budin et al. (13), parameters of gingival index, DMFT index, probing depth index and oral hygiene index were recorded in 80 subject affected by cerebrovascular disease and 80 controls:

all the studied variables resulted to be statistically significant.

Lafon et al. (13) studied a sample of 48 patients with stroke and 47 controls. The plaque index, gingival index, percentage of pockets with depth greater than 5 mm ($p = 0.04$), bleeding on probe index ($p = 0.003$), DMFT index, and bone loss were recorded. This study is the one with the major number of variables: all of them were statistically significant.

Even the study of Pradeep et al. (14), where the sample included 100 patient with cerebrovascular disorders and 100 controls, shows significant differences beetween cases and controls. The variables studied were plaque index, gingival index, clinical attachment loss (CAL), probing depth index.

Lastly, Slowik et al. (15) studied 230 patients: 169 were included in the study and 61 were excluded. The clinical attachment loss and the probing depth were measured. The presence of advanced periodontitis or edentulism was an independent risk factor for stroke and it was associated with greater neurological deficit on admission. The characteristics of the studies analyzed are shown in Table I.

Regarding the plaque index, which was obtained by Leira et al. (10), Diouf et al. (11), Söder et al. (12), Lafon et al. (13) and Pradeep et al. (14), if we consider all the cases and the controls together we obtain 369 and 1964 subjects, respectively. The resulting average Odds Ratio obtained from these studies is 13.88 for the cases and 8.56 for the controls. Therefore, there is almost a 62% chance of having periodontitis due to a higher plaque index, in patients who have suffered a stroke compared to those who did not suffer from this disease.

In relation to gingival index that was studied by Budin et al. (2), Diouf et al. (11), Söder et al. (12), Lafon et al. (13) and Pradeep et al. (14), we can consider a sample of 387 patients and 1984 controls. This shows that patients affected by cerebrovascular disease have an increased probability of developing gingivitis, which is twice that of healthy ones.

Clinical attachment loss is another of the most studied parameters, and it is present in the articles by Leira et al. (10), Diouf et al. (11) and Pradeep et al. (14). By taking all these articles into account, we can collect a total of 282 patients and 280 controls. As a result, the average OR is 4.06 in patients with stroke and 4.66 in controls.

The last parameter analyzed is the probing depth index, that was studied by Leira et al. (10), Diouf et al. (11), Budin et al. and Pradeep et al. (14). The total number of patients considered is 362 and 360 controls. In this variable, we obtain an average OR of 4.035 in the patients and 2.91 in the controls.

Table II shows the results of the measurements of the various periodontal parameters analyzed by all the studies and described above.

Table I. *Characteristics of the studies analysed.*

Author, year	Type of study	Population	N° Patients excluded	Type of stroke	Periodontal parameters (OE: oral exam, QNR: questionnaire)	Exclusion criteria	Confounding factors
Leira, 2016	Case-control	Santiago de Compostela	NR	Lacunar infarction	OE: CAL, PPD, REC, FMPS, FMPS, MT Neurological examin., IMR, mRS, Qst.	<15 teeth, aggressive periodontits, previous periodontal treatments, neurovascular disease, immunosuppressant therapy in the previous 3 months, use of NSAIDs	Physical activity, education, socio-economic status, BMI, CRP
Diouf, 2015	Prospective case-control	Senegal	NR	Ischemic or hemorrhagic	OE: PI, BoP, CAL, PPD, CPITN Qst.	NR	The joint study of two different type of stroke
Söder, 2015	Prospecrive	Sweden	NR	Unspecified	OE: PI, GI, Calculus index, MT Qst.	NR	NO
Budin, 2014	Case-control	HUSM (Malaysia)	NR	Unspecified	OE: CAL, GI, DMFT, OHI	Comatose patients	NO
Lafon, 2014	Prospective case-control	Dijon	Pts (n) 16 Ctrls (n) 11	Ischemic	OE: PI, GI, DMFT, BoP, PPD, PBL, MT	NR	NO
Slowik, 2010	Cohort	Stroke Unit in Poland	NR	Unspecified	OE: CAL, PPD Neurological examin. (NIHSS), mRS, IB	Previous stroke	NO
Pradeep, 2009	Prospective case-control	Bangalore (India)	NR	Ischemic	OE: CAL, PPD, GI, PI Qst.	Previous stroke, previous periodontal treatments, edentulism, suffering from hemorrhagic stroke	NO

DISCUSSION

All the studies included in this review affirm that the prevalence of periodontal disease, estimated by analyzing different periodontal parameters, is higher among the population with cerebrovascular disease. The studies agree that the majority of patients with stroke have a poor oral health status, and a greater progression of oral diseases, such as periodontitis, due to plaque accumulation (2, 12, 14). Diouf et al. (11) propose to sensitize physicians on the need to include an oral examination in the follow-up visits of these patients.

Many of the above-mentioned studies reported that two main parameters, the plaque index and the gingival index, were much higher in patients with cerebrovascular disease than in the control group (2, 10, 11, 13). The same result is had by considering the missing teeth index (10). It therefore appears evident that patients with cerebrovascular disease have a much worse oral status, compared to the control group.

According to the results analyzed above, patients with stroke show a high plaque index. This accumulation of plaque causes an inflammation in the gingival tissue (gingivitis), which is maintained over time; depending on age and other general health conditions, it can lead to the destruction of the supporting tissues of the tooth and consequently to the development of periodontitis. The clinical situation of these patients can be aggravated for two reasons: the physical disability, that makes it difficult to perform correct oral hygiene measures and the presence of additional risk factors due to the disease,

Table II. *Parameters of the studies analysed.*

Author, year	N° PTs	N° CTRs	Plaque index	Gingival index	Calculus index	% PP >5mm	FMBS	DMFT	Missing teeth	CAL	PPD	CPITN	% PD	OHI	REC	PBL
Leira, 2016	62	60	62,4±19,1 37,1±14,4				67 (52,5-78) 33 (26,2-38)		10 (8-12) 7 (5-9)	6,2±2,1 3,8±1,2	5,0±1,6 3,3±1,0				1,1±0,7 0,5±0,2	
Diouf, 2015	120	120	1,9±0,404 1,4±0,371	0,5±0,374 0,3±0,287						2,0±1,657 1,0±0,913	2,7±0,673 2,4±0,510	2,3±0,744 1,7±0,498	73,3 40,8			
Söder, 2015	39	1637	0,74±0,52* 0,71±0,49*	1,46±0,53 1,27±0,53	0,68±0,75 0,45±0,58				1,62±2,12* 1,25±2,12*							
Budin, 2014	80	80		51,31±20,01 29,18±19,75				18,06±7,45 12,88±7,57			3,94±1,78 2,29±0,91			76,60±21,27 41,58±20,94		
Lafon, 2014	48	47	2,63±1,01 2,12±1,27	2,68±0,94 2,12±1,27		p: 0,04	p: 0,003	18,1±9,2 13,7±6,4	13,7±10,4 8,6±6,5							18,1±9 13,7±6,4
Slowik, 2010	169												p: 0,025			
Pradeep, 2009	100	100	1,73±0,45 1,48±0,5	1,23±0,31 1,07±0,38						3,99±1,21 3,18±0,94	4,50±1,16 3,65±0,86					

***: non statistically significant (all the other parameters result statistically significant); **PP**: periodontal pocket; **FMBS**: full mouth bleeding score; **CAL**; clinical attachment loss, **PPD**: probing pocket depth; **CPITN**: community periodontal index of treatment needs; **PD**: periodontal disease; **OHI**: oral hygiene index; **REC**: gingival recessions; **PBL**: periodontal bone loss.*

Table III. *Results of the study by Kim et al.*

PERIODONTAL PARAMETERS	INITIAL EVALUATION	FINAL EVALUATION	p-VALUE
Plaque index			0,001
Patients	2,09±0,44	0,84±0,51	
Controls	2,10±0,67	1,85±0,68	
Gingival index			0,001
Patients	1,54±0,47	0,47±0,64	
Controls	1,30±0,53	1,60±0,61	
Clinical attachment loss			0,02
Patients	1,36±1,11	1,10±1,24	
Controls	1,53±0,88	1,63±0,85	

such as diabetes, smoking, hypertension, presence of inherited cardiovascular diseases or previous infarcts.

Chronic periodontitis is characterized by the maintenance of a chronic low level of tissue inflammation that produces high levels of inflammatory biomarkers in serum, saliva and crevicular fluid (15). This condition is related to a higher cardiovascular risk and therefore also to a possible cerebrovascular accident (16).

Periodontal disease contributes to the development of systemic inflammation: although in the initial stages of the disease the main pathogenetic mechanism is represented by the growth of periodontal pathogenic bacteria, during the progression of the disease the pathogenesis is immunologically mediated. In this connection, Roquer et al. (17) observed, in almost 10% of patients with stroke, the presence of an infection occurred in the month preceding the cerebrovascular attack.

Periodontitis can induce the maintenance of higher levels of cytokines in the gingival crevicular fluid and in the blood. Major bleeding during the exploration of a periodontal pocket may indicate an acute inflammatory process. Some periodontal pathogens, such as *Porphyromonas gingivalis*, *Prevotella intermedia* and *Aggregatibacter actinomycetemcomitans*, can release lipoproteins that, once entered the bloodstream, are able to induce the production of C-reactive protein in the liver. The chronic increase of C-reactive protein is associated with a higher incidence of acute thromboembolic events that can induce a cerebrovascular accidents (18, 19).

Some authors believe that the risk of stroke may be high in patients with severe periodontitis: it has been proved that periodontal disease may be an independent risk factor for cardiovascular and cerebrovascular disease in patients under 60 years of age. The possible association between periodontitis and cerebrovascular disorders may be in the formation of atheromatous plaques, which is the main responsible for vascular problems, and that can be induced, among other factors, also by periodontal bacteria (20).

In order to avoid the development or the aggravation of periodontitis among the population of patients with stroke, specific oral hygienic habits should be established. At this point, we should distinguish between hospitalized patients, with an oral hygiene protocol established by health personnel, and patients living in residences or at home, whose oral hygienic measures is entrusted to their caregivers or to their relatives.

As described in the study conducted in 2014 by Kim et al., patients must perform two brushes a day, of at least two minutes, with a high fluoride toothpaste and with an electric brush or long handle and small head brush. They also have to perform irrigation and cyclic rinses with chlorhexidine and to apply chlorhexidine gel using gauze on the mucosa and on dental surfaces. In addition, a lingual brushing should be performed to remove plaque accumulated on the tongue surface. In unconscious patients, we should use a mouth prop to perform dental hygiene in an assisted manner (21). Table III shows the effect of the oral hygienic care program for stroke patients proposed by Kim et al. and applied in a sample of stroke patients in the intensive care unit.

There is sufficient evidence to justify a periodontal treatment to prevent stroke (22-29). Periodontitis is one of the most widespread diseases in the world and the correlations with other systemic diseases must still be studied (30-34). There are many surgical and non-surgical therapeutic approaches of periodontitis and perimplantitis that can affect stroke also (35-63).

There are some lack of knowledge on relations between periodontitis and stroke. 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 very prevalent in middle-aged population and can have a significant impact on the cardiovascular function. In conclusion, patients with stroke have a higher prevalence of periodontitis.

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CURRENT CONCEPTS ON CLEFT LIP AND PALATE ETIOLOGY

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Nonsyndromic cleft lip with or without cleft palate is the most common craniofacial anomaly affecting around 1 in 700 live births worldwide. Clefts of the human face can be classified anatomically as cleft palate only (CPO), cleft lip only (CLO), cleft lip and palate (CLP) or a combined group of cleft lip with or without cleft palate (CL/P), based on different in embryologic development. These malformations have some genetic origin, in fact several association studies have been performed to obtain important information about the candidate genes; but more important are gene-environment interactions that play an increasing role in its etiology. Epidemiological studies have shown how environmental factors (alcohol, smoking, drugs), as well as possible gene-environment interactions, play an important role in the onset of the malformation. On the contrary, folic acid intake seems to have a protective effect. In this review we analyze the role of environmental factors related to onset of cleft.

Orofacial clefting (OFC) is the most common craniofacial anomaly affecting around 1 in 700 live births worldwide (1). Clefts of the human face can be classified anatomically as cleft palate only (CPO), cleft lip only (CLO), cleft lip and palate (CLP) or a combined group of cleft lip with or without cleft palate (CL/P), based on different in embryologic development (2).

Several association studies have been performed to obtain important information about the candidate genes involved in malformation (3). The higher incidence of CPO and CL±P observed in monozygotic twins (36%) compared with dizygotic twins (4.7%) further supports the hypothesis of a genetic component (4, 5). Epidemiological studies have highlighted the importance of gene-environment interactions as factors that play an increasing role in its etiology (6, 7). The most studied environmental factors are smoking, alcohol, drugs and folic acid.

In this review we analyze the well known environmental and genetic factors related to the onset of cleft.

Environmental factors: alcohol

Materno-fetal intoxication with ethanol during pregnancy causes a well-known syndrome - the so-called fetal alcohol syndrome – characterized by pre/postnatal growth retardation and facial dysmorphism; but the association between alcohol and NS CL/P is inconsistent. In fact, during the last two decades, a

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series of epidemiologic studies have also revealed the role of alcohol in determining non-syndromic OFC.

Munger et al. (8) showed that alcohol increases the risk of non-syndromic CL±P, whereas no significant association was found between alcohol and CPO, or in syndromic clefts. In 1999, Shaw and Lammer observed that the risk of delivering infants with orofacial cleft phenotypes for mothers who take alcohol during pregnancy is dose-related (9). In the same year, Romitti et al. examined the allelic variants of three genes - TGFA, TGFB3, and MSX1 - and their interaction with two exposures during pregnancy (cigarette smoking and alcohol consumption) (10). The authors demonstrated that the development of CL±P and CPO may be influenced by these risk factors, especially when they interact with specific allelic variants. The risk estimated for maternal smoking was significantly elevated in the case of CPO, and was higher among infants with allelic variants at the TGFB3 or MSX1 sites. By contrast, the risk estimated for maternal alcohol consumption was significantly more elevated in the case of CL±P, and was higher among infants with allelic variants at the MSX1 site.

Drugs

There are drugs whose effects on CPS development are demonstrated. Diazepam assumption has an important effect in developing CPS during embryogenesis (11,12). Diphenylhydantoin, a teratogen known to induce cleft palate in human newborns might be related to anomalous palate development (13,14). In fact the morphogenetic processes during palate development are related to extracellular matrix composition, which is important both into cell activities and in gene expression. This drug can modify cytoskeletal components and extracellular matrix-cell adhesion influencing the expression of genes involved in the development of the palate.

In the study of Baroni et al., has been shown that other factors, such as the transforming growth factor-β (TGF-β), retinoic acid (RA) and γ-aminobutyric acid (GABA)ergic are potentially involved in the malformation (15).

Cigarette smoking

The role of cigarette smoking has been analyzed in epidemiologic studies by several authors, sometimes with conflicting results; although its clefting effect is universally accepted, the type of cleft induced is less clear. Shaw et al. (16) investigated whether parental periconceptional cigarette smoking was associated with an increased risk of offspring with CL±P. They also investigated in which way the genetic variation of the TGFA locus could interact with smoking in causing cleft. The authors found that risks associated with maternal smoking were most elevated for isolated CL±P and for CPO when mothers smoked 20 cigarettes or more per day. Clefting risks were even greater for infants with the uncommon TGFA allele.

Subsequently, in a Danish case-control study of CL±P, Christensen et al. (17) studied the effects of smoking and TGFA alleles in an ethnically homogeneous setting. Unlike Shaw et al. (16) in this study they have shown that smoking was associated with a moderately increased risk of CL±P, but not of CPO. TGFA genotype was not associated with either CL±P or CPO, and no synergistic effect with smoking was observed. Instead, in a case-control study of non-syndromic oral clefts, Beaty et al., (18) could not confirm neither the association between oral clefts and TGFA genotype nor its interaction with maternal smoking.

Wyszynski et al., (19) performed a meta-analysis whereby they found a small, but statistically significant association between maternal cigarette smoking consumption in the first trimester of gestation and the risk of CL±P or CPO. However, in a further study (20), the same author employed large samples from the 1996 and 1997 U.S. Natality database, and obtained data indicating that smoking is only a minor risk factor. In a large case-control study conducted by Lieff et al., (21) the authors found a positive dose-response association between smoking and infants with syndromic CL±P.

The debate about folate interference began when it was demonstrated that women periconceptional taking multivitamins containing folic acid lowered their risk of having children with CL±P (22). Although Hayes et al., (23) did not find any evidence

to confirm a protective association between the periconceptional use of folic acid supplements and the risk of oral clefts, further evidence to support the role of folic acid was produced by Werler et al. (24).

Methylenetetrahydrofolate reductase (MTHFR) is a key enzyme in folic acid metabolism. The C677T mutation of the MTHFR gene produces a form of methylenetetrahydrofolate reductase thermolabile with reduced activity. This characteristic has been related to elevated plasma homocysteine levels and lowered plasma folate on account of reduced MTHFR activity (25). Mills et al., (26) in an investigation on Irish population highlighted that the homozygosity for the common folate-related polymorphism associated with the thermolabile form of MTHFR is more frequent both in CL±P patients and sporadic CPO.

Linkage disequilibrium was not found by Gaspar and colleagues (27) in their evaluation of parental allele transmissions. However, this study reported that the MTHFR polymorphic system was not in Hardy-Weinberg equilibrium amongst mothers of CL±P patients. The authors suggested that homozygosity of either the T or C allele of C677T polymorphism in females constitutes an important susceptibility factor for CL±P onset; they postulated that the CT heterozygotes would have an advantage over the homozygotes in relation to this trait.

Wong and colleagues (28) observed that maternal hyperhomocysteinemia may be a risk factor for having CL±P offspring: this is interesting if we consider that one effect of reduced MTHFR activity is hyperhomocysteinemia.

In 2001, a significantly higher mutation frequency for C677T polymorphism at MTHFR was demonstrated in the mothers of CL±P patients as compared to controls. The results support the involvement of the folate pathway in the etiology of CL±P, and sustain the hypothesis of an effect due to the maternal genotype, rather than an influence of the embryo genotype (29). These findings have been borne out by a subsequent independent study (30).

In a subsequent investigation, Pezzetti et al., have investigated c.665C>T (commonly known as 677C>T; p.Ala222Val) and c.1286A>C (known as 1298A>C; p.Glu429Ala) polymorphisms in the MTHFR gene in 110 non-familial patient/parents triads and 289 unrelated controls. The results of these study highlight that mutations in the MTHFR gene in pregnant woman are responsible for a higher risk of having CL±P affected children.

Folate receptors (FOLRs) mediate the delivery of 5-methyltetrahydrofolate to the interior of cells or between cells in a process known as potocytosis.

In order to verify whether FOLRs could be responsible for the onset of non-syndromic CL±P, were analyzed, using linkage and linkage disequilibrium, a study sample consisted of patients and their mothers from 71 families CL / P pedigrees and 75 sporadic cases of the Italian population. The results of this study have shown only a silent mutation in FOLR1 present in a mother and her child. But this does not allow supporting FOLR1 and FOLR2 genes in the onset of CL/P.

In addition, the involvement in CL/P etiology of four genes belonging to the folate pathway was verified: transcobalamins (TCN1 and TCN2), methionine synthase (MTR) and MTR reductase (MTRR). The results suggest that TCN2 is involved in causing CL±P, through a reduction of homocysteine remethylation efficiency. These data are highly interesting and require further investigation of different sample collection.

In an Italian study, genetic variants of folate and homocysteine metabolism were evaluated in influencing the risk of orofacial clefts; but did not find significant level of association between betaine-homocysteine methyltransferase (BHMT and BHMT2) and cystathionine beta-synthase (CBS) variants with CL+/-P.

The common MTHFR 677T variant lead to a reduced folate availability in mother and then for the embryo, who uses maternal reserves. In fact, this genetic variant is an important factor associated to increased risk of CL/P.

Lack of association could mean that the sample size was not sufficient to detect a very low effect. A sample selection criteria including periconceptional drugs or medication assumption may help to increase the power of the study, thus identifying the possible feto-maternal interaction between ABCB1 and MTHFR genotypes. Oral health and a pleasant smile

are one of the most successful goals in the general population, so CL/P therapy are of great interest in dentistry (31-71)

DISCUSSION

It is well-established that non-syndromic OFC is a congenital disease that may affect the lip with or without the involvement of the palate (CL+/-P) or just the palate. Both have a genetic origin, but also environmental factors play an important role in the onset of these malformations.

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TWO-WAY RELATIONSHIP BETWEEN DIABETES AND PERIODONTAL DISEASE: A REALITY OR A PARADIGM?

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Diabetes mellitus (DM) and periodontal disease (PD) are both chronic diseases. From one side, DM have an adverse effect on PD, and on the other side PD may influence DM. Systemic therapy of DM with glycaemic control, affects the progress of PD. Reversely treatment of PD combined with the administration of systemic antibiotics seems to have a double effect on diabetic patients reducing the periodontal infection and improving the glycaemic control. Inflammation, altered host responses, altered tissue homeostasis are common characteristic of both DM and PD. The potential common pathophysiologic pathways of direct or reverse relationship of DM and PD are still unknown and further in vitro and in vivo studies are needed to explore this relationship.

Diabetes Mellitus (DM)

Diabetes mellitus (DM) is a metabolic disorder characterized by hyperglycaemia. DM is a chronic, lifelong condition that affects your body's ability to use the energy found in food. There are three major types of diabetes: type 1 diabetes; type 2 diabetes, and gestational diabetes. All the forms of DM are associated with hyperglycaemia, hyperlipidaemia, and major complications: microangiopathy, nephropathy, neuropathy, macrovascular disease, and delayed wound healing. Periodontal disease (PD) is another important complication associated with DM (1).

DM affects more than 16 million people in the United States. The number of adults who will be diagnosed with type 2 diabetes in the world will grow from 346 million to 439 million in 2030 and people with diabetes type 2 up 90% of the diabetic population[2], with an increase of 54% in 20 years to encompass almost one tenth of adults 20 years and older. DM is a major cause of disability estimated at 20.8 million people. DM is the ninth most common disorder in the world, with an increase of 67,2% over the 20 years from 1990 to 2010 (2).

Periodontal disease (PD)

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[3]. Specific bacteria of the biofilm formed by plaque cause PD. The bacteria leak into the periodontal ligament space, causing anaerobic infection creating a cascade of events, which end with the production of inflammatory mediators and bacterial metabolites (3). PD affects about the 50% of adults, or more, while the severe form periodontitis affects around 10% (range: 5–20%) of adults and moderate periodontitis around

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30%. In elderly people the prevalence of PD is estimated about 70–90% of individual's aged 60–74 years (3).

Correlation between diabetes mellitus (DM) and Periodontal disease (PD)

The direct correlation between diabetes and periodontal disease is supported by different etiopathogenetic mechanisms. Patients suffering from DM have an exaggerated response to inflammation, for which the chronic presence of pathogenic bacteria of PD increases inflammation and destruction of periodontal tissue. In diabetic patients, proteins become glycated to form advanced glycation end products (AGE) (4). The AGE accumulated in the periodontal tissue may change the structure of the cell and extracellular components. Collagen degradation is easier provoked by the activity of matrix metalloproteinase (MMP), which production is higher in DM patients. AGE acts on bone collagen also, resulting in in alterations in bone metabolism. Glycation of collagen proteins may affect bone turnover, leading to reduced bone formation, delaying the formation of new bone (5,6). This, phenomena reduces osteoblastic differentiation and extracellular collagen matrix (ECM) production (7-9). Some studies have reported significant increased osteoclast activity in diabetic patients.

Patients with DM have high levels of receptor of AGE. The cell surface receptor for AGE and its ligands are expressed in the periodontium of individuals with DM and seem to mediate these processes. Even in PD high levels of AGE are present, activating their receptors with increased production of proinflammatory cytokines such as IL6 and TNF-α, worsening progression of PD (10). Furthermore DM slows the healing of tissues. This phenomenon can be explained by increased apoptosis of fibroblasts (8). The periodontal tissue is mainly formed by fibroblasts, whereby the PD is particularly aggressive in these patients (9). Patients with DM have an impaired immune response. Alteration of immune functions such as phagocytosis and chemotaxis of neutrophils may predispose to the most severe manifestations of PD (11).

The mechanism by which diabetes influence the progress of PD is well known. Many studies have shown that patients with DM have a higher incidence and more aggressive forms of PD. DM causes vascular changes in periodontal tissue (microangiopathy), hypofunction granulocyte, changes of the oral microflora and, as mentioned before, changes in collagen of periodontal tissue and alveolar bone (12). All these studies demonstrate changes at periodontal tissue level, but do not give information about the relationships between DM and PD in general. Latest researches suggest important changes at the cellular and molecular level with changes in systemic physiology. These changes provide alterations of immune cell phenotype and increase of serum proinflammatory cytokine/lipid (13-16).

Recent studies have demonstrated that PD can produce these same alterations and, if in association with DM, they are exacerbated. As previously mentioned the receptors of AGE (RAGE) are over-expressed on the surface of smooth muscle cells, endothelial cells, neurons, macrophages, and monocytes in DM. The binding between AGE and RAGE, may lead to alterations including endothelial surface changes, vascular permeability, adhesion molecules, and migration and activation of monocytes. Till now we have described a glucocentric view of DM. Recently, new knowledge has shifted the spotlight on a lipocentric vision. Obesity is related to insulin resistance and worsens clinical manifestations of DM and PD. It was discovered that adipocytes are not only fat storage cells, but also produce enzymes called adopokines. These adipokines modulate levels of insulin and insulin sensitivity of DM patients (17-20).

The role of reactive oxygen species (ROS) and reactive nitrogen species (RNS)

Both ROS and RNS are involved in the etiopathogenesis of DM. Likewise in DM, ROS and RNS are important factor responsible for local tissue damage in PD. In DM patients oxidation of low-density lipoproteins (LDL) favours cellular adhesion, cytokines and growth factors production, proliferation of smooth muscle cell proliferation and vascular thickness (21-24).

Reverse correlation between PD and DM

Less knowledge we have with regard to the treatment of PD on the glycaemic control. The systemic inflammatory response typical of PD may contribute to insulin resistance and hyperglycaemia.

PD is a major complication of DM. A number of studies have found a high prevalence of PD among diabetic patients. There is a significant evidence of a relationship between DM and PD (25).

In general, complications of DM in the long term are represented by the high concentration of glucose in the blood (hyperglycaemia). As previously mentioned hyperglycaemia leads to the formation of AGE (26). AGE act in a way so that endothelial cells and monocytes become more susceptible to stimuli that induce cells to produce inflammatory mediators.

AGE accumulation in the gingival tissue leads to increase vascular permeability, to a substantial decrease of the collagen fibres, and to an accelerated destruction of non-mineralized tissues and bone. Pathophysiology of diabetes is closely similar to PD, both producing an inflammatory response, in DM caused by accumulation of AGE, and in PD by the accumulation of bacteria stimulating an immune response. Hyperglycaemia leads to the production of numerous inflammatory cytokines including IL-6 and TNF-α (tumour necrosis factor-α), which arise partly from adipose tissue; IL-6 damages insulin release from β cells of TNF-α while pancreas is implicated in the development of insulin resistence (27).

The iperinflammatory state caused by adipocytes that secrete proinflammatory cytokines, proves the relationship between type 2 diabetes, obesity and periodontal disease. This also supports the idea that obesity is an important predictor of PD and insulin resistance (28). There is a strong two-way relationship between PD and DM. In fact patients with uncontrolled diabetes show a greater susceptibility to the development of PD, and PD can lead to a worsening of diabetes control. Treatment of PD combined with the administration of systemic antibiotics seems to have a double effect on diabetic patients reducing the periodontal infection and improving the glycaemic control. Dentists should check blood sugar of the patient to provide adequate health prevention. It is well documented that PD therapy is effective on glycaemic-control in diabetic patients. In fact, the results of all meta-analysis demonstrate a significant decrease in HbA1c of 0.36–0.65% in patients with a follow-up of 3–6 months (29). This reduction allows avoiding the addition of a second drug to metformin and therefore presents important clinical and therapeutic implications (30). On the contrary a recent randomized clinical trial concluded that PD therapy is uninfluent on HbA1c (31). Although this was a large study of over 500 patients, the scaling and root planning therapy apparently was only partially effective in resolving the periodontal disease. For example, reduction in bleeding on probing was only 19% as compared to the approximately 45% reduction previously seen in numerous studies, and 42% of sites bled after treatment and there were 10% of sites with 5 mm or greater pocket depth after treatment.

Furthermore, in this study 72% of the sites had residual plaque suggesting poor compliance with oral hygiene. This clinical trial failed to achieve clinical results comparable to what has been found in previous studies both on those with diabetes as well as those without diabetes, and is not clear from this study that one can conclude that non-surgical periodontal therapy does not alter HbA1c. Further research where periodontitis is successfully treated is needed to clarify this issue. However reduction of HbA1c should be an important goal to reach, in fact it improves micro-vascular complications such as retinopathy and nephropathy (32) and reduces mortality of 10% of DM patients (33).

DM and PD are among the most prevalent worldwide diseases and its relationship is demonstrated by numerous studies (34-41). Periodontal disease is one of the most widespread diseases in the world and the correlations with other systemic diseases must still be studied (42-46). There are many surgical and non-surgical therapeutic approaches of PD (47-53). Just as diabetes affects periodontal disease, diabetes also has effects on the development of peri-implantitis (53-73).

DISCUSSION

Many studies have reported that DM increases the incidence and severity of PD. The mechanisms of direct or reverse relationship of the two diseases are still unknown and further in vitro and in vivo studies are needed to explore this relationship.

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EFFICACY OF A NEW CHEMICAL DEVICE TO MINIMIZE MICROBIAL CONTAMINATION ALONG IMPLANT-ABUTMENT CONNECTION: AN IN VITRO STUDY

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Osseointegrated dental implants showed elevated success rates on the long-term treatment in the last ten years. However, the risk of peri-implantitis and implant failure is the main complication of implantology. The presence of a micro gap at the implant-abutment connection (IAC) allows microorganisms to penetrate and colonize the inner part of the implant leading to biofilm accumulation and consequently to peri-implantitis development. Some chemical devices (CD) has been studied to reduce bacterial penetration at IAC level but no one have been demonstrated to be effective for this purpose. Aim of the present study is to evaluate the effectiveness of a new chemical formulation STCX-1, placed in the internal part of dental implants for killing bacteria present in the IAC. To identify the antibacterial power of SXTC-1 at interface between implant-abutment connection, the passage of genetically modified Escherichia coli across IAC was evaluated. A total of eight implants were used (Edierre Implant System, Edierre SpA, Genova, Italy). The inner side of four out of the eight implants were firstly contaminated with few microliters of pure bacteria, subsequently were treated with SXTC-1 for few second and finally, the antibacterial was replaced with Lysogeny Broth (LB) and antibiotics without bacteria. The remaining four implants were not treated with SXTC-1 and just filled with LB with antibiotics. Bacteria viability was determined by measuring their Optical Density (OD) at 600nm. The analysis revealed that, in untreated implants, bacteria grew (internally and externally) for the first 48 hours, but subsequently they started to dye. In treated implants, instead, bacteria grew just in the space surrounding the device suggesting that, even if bacteria were able to get into, they immediately died thanks to the presence of SXTC-1. The STCX-1 liquid formulation have been demonstrated to be an adjuvant CD effective for prevention of of bacterial colonization at IAC level.

Osseointegrated dental implants showed elevated success rates on the long-term treatment in the last ten years (1). These rates are referred to primary stability, in turn, related to the quantity and quality of the receiving bone (1). However, despite of the high success rates in the long-term, the risk of peri-implantitis and implant failure is the main complication of implantology (2). There are many hypothesized causes of perimplantitis, but bacterial colonization at the implant-abutment connection (IAC) is the most accreditate one (3). The presence of a micro-gap at the IAC allows microorganisms to penetrate and colonize the inner part of the implant leading to biofilm accumulation and consequently to

Key words: Chemical devices, implant-abutment connection, microbiological leakage, peri-implantitis.

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peri-implantitis development (4).

The bacterial colonization at IAC level has an important role in the onset of peri-implantitis (5). The presence of a gap in IAC is associated with a significantly higher inflammatory cell infiltration and bone loss (6).

In fact, some minutes after implant placement, bacterial colonization of implant surfaces and peri-implant tissues, immediately starts (6). The connection between abutment and implant creates a gap resulting in bacterial leakage and in an area of inflamed soft tissue around the IAC (6). Prevention of microbial leakage at the level of IAC is the main aim for the construction of two-piece implant systems to avoid inflammation in peri-implant tissues. This assumption is confirmed by the fact that the percentage of peri-implantitis is minimized in one piece implants, where there is no IAC and no bacteria leakage (7).

Several in vitro studies have demonstrated that, even if different internal connection designs were proposed, no one prevented the passage of bacteria along IAC in static or dynamic loading conditions (8-16). Micro-leakage has been confirmed bidirectional, from the inner parts of the implants to the external environment and vice versa (8-16). Some reports have been demonstrated that the use of sealing materials, decontamination of the inner-implant cavity, use of shape memory alloy and different connection geometries, have been unsuccessful to prevent bacterial leakage (17-20). Many studies tried to quantify microbiological penetration between micro-gaps at IAC level, all concluding that no one IAC has been demonstrated to perfectly close the gap between implant and abutment, favoring the one set and maintenance of peri-implantitis (21-24). Limited success has been achieved in eliminating the implant–abutment gap or simply avoiding its effects. Some different solutions, such as inclusion of polymeric washers between the parts of different implant systems, only decreased, but did not eliminate, bacterial contamination (25).

Aim of the present study is to evaluate the effectiveness of a new chemical formulation, STCX-1, placed in the internal part of dental implants for reducing bacteria leakage al level of IAC.

MATERIALS AND METHODS

STCX-1

The antimicrobial STCX-1 formulation consists of a solution of alkyl-polysiloxane oligomers, linked to titanate units bearing hydroxyl functions. These functionalities allow a stable binding to Ti-OH active sites of titanium implants and the same time the alkyl chains of the syloxane units allow trapping of molecular level antimicrobial species, which are thus stabilized on the titanium surface and can display

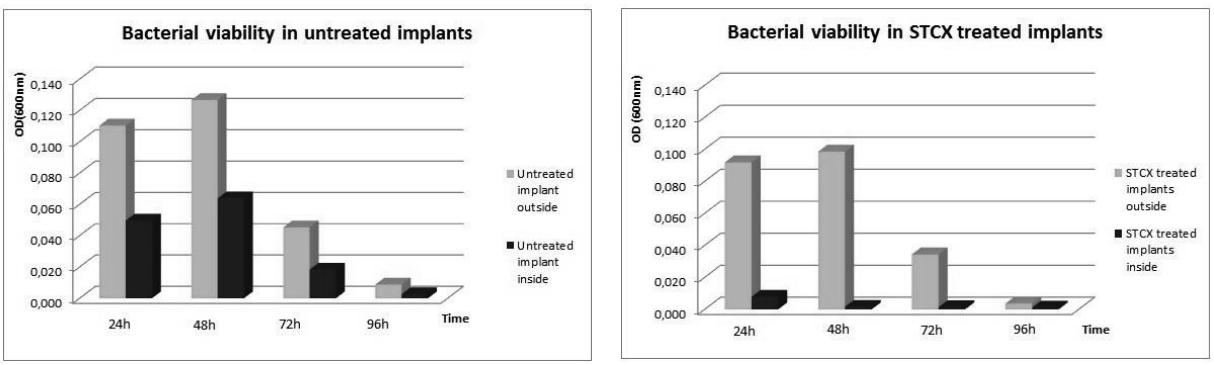

Fig. 1. *Optical density (OD) measurement inside and outside untreated and treated implants, at four different time points.*

a long term antimicrobial activity. The STCX-1 formulation contains chlorhexidine. Analogous formulations containing polyhexamethylene biguanide (P), didecyldimethylammonium (DDA) and benzalkonium (B) cations were analyzed.

Implants preparation

With the aim to confirm the antibacterial power of STCX1 we evaluated its effect on bacteria able to get into dental implants. To this goal we used modified E. coli containing synthetic DNA target sequences in their plasmid. In detail, the plasmid contains two sequences specific for two bacterial species (*P. gingivalis and T. forsythia*) as well as two genes for antibiotic resistance (Kanamycin and Ampicillin).

Bacteria were cultured in Lysogeny Broth (LB) with both Kanamycin and Ampicillin (at a final concentration of 50ug/ml), in order to avoid the growth of non-specific bacteria. Cultures were then let at 37°C for 12-18hr in a shacking incubator.
Subsequently, few microliters of this culture were used to "contaminate" fresh LB with antibiotics contained in a microcentrifuge tube where we put the implant. A total of eight implants were used.

The inner side of four out of the eight implants were firstly treated with STCX1 for few second and subsequently, the antibacterial was replaced with LB and antibiotics without bacteria. The remaining four implants were not treated with STCX1 and just filled with LB with antibiotics. Tubes were then let at 37°C for 48h in a heater, in order to allow bacterial growth.

To check for the presence of any bacterial contamination, we included in the study a negative control containing both inside and outside the implant just LB with antibiotics. Twenty-four hours later, implants were opened and samples were collected by dipping a paper probe both outside and inside the implants, for each implant, and for the negative control too. Four paper probes for implant were collected. Two out of these four probes were used to measure the bacteria optical density outside the implant, and the remaining were employed for the internal measurement.

optical density (od) measurement

Paper probes were put, in a pair, in a microcentrifuge tube with 200ul of LB with selective antibiotics, and let at 37°C in a heater for optical density measurement at four time point: 24, 48, 72 and 96 h.

Bacterial OD600 was measured by using NanoDrop 2000c spectrophotometer (Thermo scientific, Wilmington, USA). For each sample 1uL of LB with or without bacteria was measured. Sterile LB broth with antibiotic, instead, was used to blank the spectrophotometer.

Statistical analysis

To evaluate if the different bacterial growth among STCX1 treated and untreated implant was statistically significant, we applied Student's t-test on average OD both for external and internal measurement, at each time point.

RESULTS

Figure 1 shows the bacterial growth by measuring optical density at 600nm (OD600). We reported the behavior on untreated implants on the top of the image, and the bacterial OD in STCX1 treated implants at the bottom.

To evaluate the effectiveness of the treatment, we compared the average OD between treated and untreated implants, both externally and internally at each time point. The analysis revealed that, in untreated implants, bacteria grew (internally and externally) for the first 48 h but subsequently they started to dye, probably, because of nutrient consumption. In treated implants, instead, bacteria grew just in the space surrounding the device suggesting that, even if bacteria were able to get into, they immediately died thanks to the presence of STCX1.

Comparing treated and untreated implants, the difference in bacterial growth was statistically significant just when considering the inner side at 24, 48 and 72h. After 96h, in fact, bacteria died both in treated and in untreated implants (Table I). Externally, no differences were pointed out (Table II).

DISCUSSION

It is clear that peri-implantitis occurs due to the

Table I. *Outer average OD values of STCX1 treated and untreated implants, and their comparison.*

Time Point	OD600 Untreated Implants	OD600 Treated Implants	*P-value*[(a)]
24h	0.092	0.110	*0.1820*
48h	0.099	0.110	*0.9257*
72h	0.034	0.045	*0.4090*
96h	0.004	0.009	*0.0599*

[(a)]: data were considered statistically significant for P-value <0.05.

Table II. *Inner average OD values of STCX1 treated and untreated implants. and their comparison.*

Time Point	OD600 Untreated Implants	OD600 Treated Implants	*P-value* [(a)]
24h	0.008	0.050	***0.0282***
48h	0.002	0.064	***0.0208***
72h	0.001	0.018	***0.0214***
96h	0.001	0.003	*0.3001*

[(a)]: data were considered statistically significant for P-value <0.05.

presence of pathogenic micro-organisms colonizing the surrounding implant area and the suppression or eradication of these microbes result in prevention of peri-implantitis (26-48). The main cause of peri-implantitis consists in the passage of pathogenic bacteria in the abutment-implant gap. The inner spaces were easily colonized, and bacteria may leak out from these spaces through the IAC into the peri-implant area. The formation of a biofilm around the implant plays a fundamental role in the onset of peri-implantitis. In any case, the peri-implantitis is associated with gram-negative bacteria similar to those that cause periodontal disease.

The peri-implantitis, such as periodontal disease is the result of the bacterial insult and the subsequent host response. Many studies have shown that bacterial species of periodontal disease are very similar to those that cause peri-implantitis. For which it is clear that blocking the passage of bacteria in the peri-implant space is essential to prevent peri-implantitis. The use of a chemical devices (CD) in the gap between implant and abutment can represent a valid solution to prevent the development of peri-implantitis. The advantages of topical liquid involve the use of antimicrobical agents directly into the peri-implant area. The potential benefits of local CD include improved patient compliance and an easier access to implant-abutment space.

The topical use of CD have been demonstrated to dramatically reduce the bacterial leakage. Local delivery of CD into the implant-abutment gap achieves a greater concentration of drug locally, shows bactericidal effect for most perio-pathogens, and at the same time, exhibits negligible impact on the microflora residing in other parts of the body.

Our study evaluated the efficacy of STCX-1 in killing bacteria infiltrated between implant and abutment, considered the primary etiological factors for peri-implantitis.

Microbiological testing was thought appropriate

to evaluate the effect of STCX-1 on subgingival microbial population. It is well known that both P. gingivalis and T. forsythia occur concomitantly with the clinical signs of bone resorption surrounding implant. They appear closely 'linked' topologically in the developing biofilm, exhibiting 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 peri-implant bone and in the local development of peri-implantitis. Our results demonstrated that STCX-1 along IAC performs very well in preventing bacteria proliferation and is effective in killing the microbial species which comes in contact with it.

The STCX-1 liquid formulation have been demonstrated to be an adjuvant CD effective for prevention of bacterial colonization at IAC level.

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COMPLICATION IN THIRD MOLAR EXTRACTIONS

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Mandibular third molars (MM3s) are responsible for pericoronitis, primary and/or secondary crowding of the dentition, odontogenic tumors and cysts, periodontal defects associated with the posterior part of mandibular second molars. Tooth extraction is indicated for prophylactic and therapeutic purpose in patients with problems caused by impacted teeth. Common postoperative complications associated with third molar extraction are alveolitis (0.5e32.5%), infection (0.9e4.2%), postoperative bleeding (0.2e1.5%), transient dysfunction of the inferior alveolar nerve (0.6e5.5%), and permanent dysfunction of the inferior alveolar nerve (0.1e0.9%). A literature review reveals number of individual case reports of accidental displacement to various anatomical locations, namely, the infratemporal fossa, pterygomandibular space, lateral pharyngeal space, submandibular space, and sublingual space.

Mandibular third molars (MM3s) are responsible for pericoronitis, primary and/or secondary crowding of the dentition, odontogenic tumors and cysts, periodontal defects associated with the posterior part of mandibular second molars (MM2s), caries between MM2s and MM3s, and myofascial and neurogenic pain (1).

Tooth extraction is indicated for prophylactic and therapeutic purpose in patients with problems caused by impacted teeth. Therefore, the impacted third molar surgery is one of the most common procedures in oral surgery (1). Inflammatory response of tissue caused by trauma can cause complications such as pain, edema, and trismus after impacted tooth surgery in patients (2-4). Postoperative edema reaches the highest level between 12-48 h after surgery and its complete alleviation takes 5-7 days (4).

Trismus, or limited mouth opening, is another undesirable effect commonly reported after oral surgeries. Trismus prevents eating and talking and impairs the quality of life of patients; thus, decreased trismus translates to patients' reduced discomfort and increased quality of life (5).

Currently, the most important objective is to increase the patient's comfort by minimizing all complications in the postoperative period. The surgical removal of third molars is the most frequent operation performed by oral and maxillofacial surgeons (6).

Considerations of the surgical aspects

Although it is generally considered a safe procedure, some complications can occur during surgery or in the postoperative period. Common

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postoperative complications associated with third molar extraction are: alveolitis (0.5e32.5%), infection (0.9e4.2%), postoperative bleeding (0.2e1.5%), transient dysfunction of the inferior alveolar nerve (0.6e5.5%), and permanent dysfunction of the inferior alveolar nerve (0.1e0.9%) (7).

Luxation injuries are the most common group of dental injuries, with reported incidences ranging from 30% to 44%.10 However, oral surgical complications such as concussion and subluxation injuries to the neighboring second molar during the removal of the impacted third molar are very rare and have never been reported in literature (8).

The updated guidelines for the third molar are based on evidence, with an extensive review of 180 references. Like most surgical procedures, third molar surgery bears its own risks of intraoperative and postoperative complications. Pain, swelling, and trismus are among the most common complications after third molar removal. Of more concern is the risk of nerve damage, particularly the inferior alveolar nerve and the lingual nerve damage after mandibular third molar surgery, leading to paresthesia of the chin, lower lip, and/or the tongue (9). Local anesthesia may be categorized in a number of ways according to the extent of the area to be anesthetized. To anaesthetize the mandible, there are many local anesthesia methods that target the inferior alveolar nerve, which runs along the mandibular canal. The conventional inferior alveolar nerve block has been used frequently in various procedures for many years. However, the success rate of the inferior alveolar nerve block is, in fact, only modest and associated complications, such as aspiration and nerve injury, are fairly common. Thus, various anesthesia methods have been continuously studied to try to address this issue (9-10).

The opinions of all relevant dental specialists, such as orthodontists, periodontists, radiologists, and oral and maxillofacial surgeons, are considered. One of the 3 primary aims of the guidelines is to prevent health problems related to third molars. A prospective clinical study in Hong Kong has shown an incidence of 0.35% for permanent inferior alveolar nerve deficit and 0.69% for permanent lingual nerve deficit after mandibular third molar surgeries (9).

At present, oral and maxillofacial surgeons commonly use panoramic radiography or orthopantomography (OPG) to view MM3s and estimate possible damage of the inferior (IAN) alveolar nerve (11). However, OPG is not an accurate or precise estimation of the risk assessment of damage to the IAN canal during surgery (6). Computed tomography (CT) scans provide high-resolution images with the exact position of the MM3 in all three planes (7).

DISCUSSION

The surgical extraction of impacted third molars is one of the most common operations, which is performed by oral and maxillofacial surgeons and dentists as well. Indications for the removal of impacted third molars include chronic pericoronitis, presence of cysts or a tumor, periodontal problems, and presence of a carious lesion on the second or third molar.

A literature review reveals number of individual case reports of accidental displacement to various anatomical locations, namely, the infratemporal fossa (10), pterygomandibular space (8), lateral pharyngeal space (11), submandibular space (12), and sublingual space (12).

The timing of the surgical retrieval of the displaced tooth or root segment may be controversial. Some authors advocate the late intervention (3 or 4 weeks following the accident) to allow the encapsulation of the tooth with fibrous tissue, thus stabilizing its position (13). This approach, however, necessitates close monitoring with frequent clinical follow-up and radiographic examination. In favor of this concept, Xavier et al (14) presented an accidentally displaced mandibular third molar to the pterygomandibular fossa which migrated spontaneously to the oral cavity. Osunde et al. (15), observed that age, sex, and surgical difficulty level had no effect on the limitation of mouth opening.

The application of excessive and uncontrolled force results in damage to the attachment apparatus (periodontal ligament and cemental layer) of the adjacent tooth. The apical neurovascular supply to the pulp is also affected to varying degrees, resulting

in an altered or non-vital tooth and leading to pulpal inflammation (16).

Studies have recorded the force employed by operators during tooth extractions on different jaws and have found that the strength needed to extract lower and higher teeth was not significantly different (17, 18). This result differs from ours because in the present study all of the root canal treatment requirements were on the lower jaw. This could be the result of the density of the lower jaw or the complexity of the root angulations. Cicciu` et al. (19) also observed the force applied for teeth extraction and concluded that factors such as strange tooth anomalies, large root angles, or strange root forms are the cause of the complications. However, the same authors concluded that bone structure and density do not influence the strength (force applied) values (20). Some recent studies have demonstrated increased numbers of intra- and postsurgical complications with the removal of impacted third molars in older patients. Surgical removal of impacted mandibular third molars should be carried out well before the age of 24 years, especially for female patients (21). The highest risk of complication is in persons aged 25 years to 34 years. Sisk et al (22) reported that the age and experience of the surgeons were significant factors for complications such as alveolar osteitis and nerve dysesthesia. Oral health and a pleasant smile are a goal of every oral surgery (23-32). Therefore, all dental disciplines must contribute to this goal and all dentists must guarantee their patients the best possible care.

The results of the present study can be compared to previously published articles, and further clinical investigations are needed.

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