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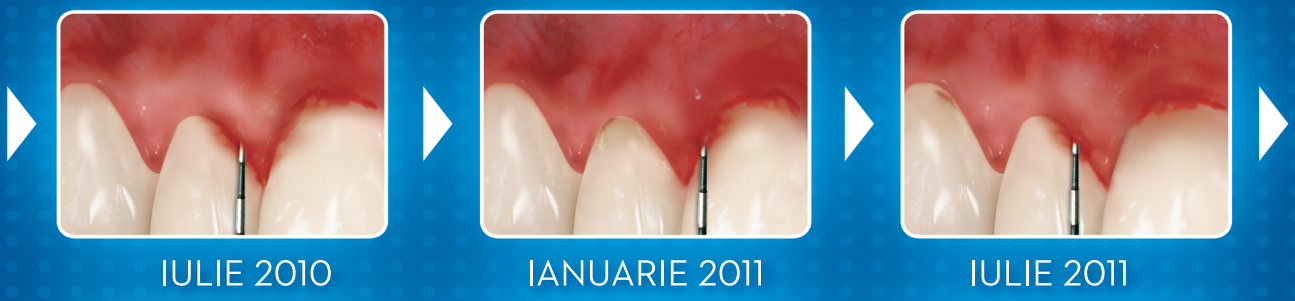
# REDUCE ŞI AJUTĂ LA PREVENIREA PROBLEMELOR GINGIVALE ÎN 4 SĂPTĂMÂNI PENTRU A ÎNTRERUPE CICLUL GINGIVITEI



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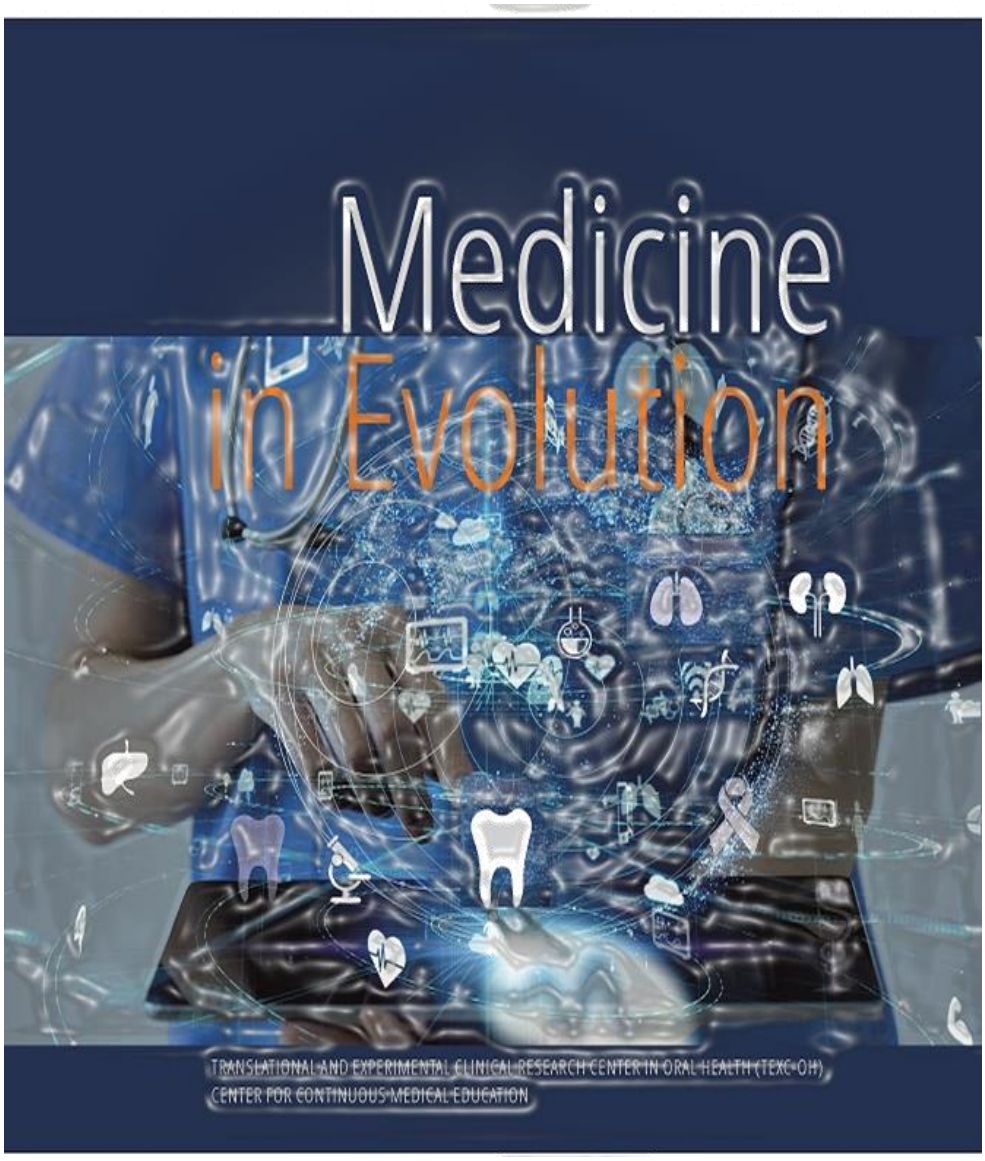
# MEDICINE IN EVOLUTION

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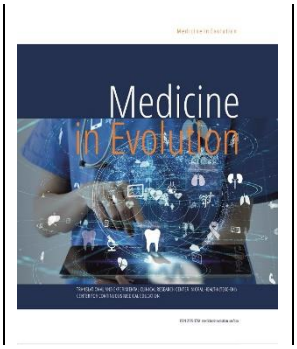

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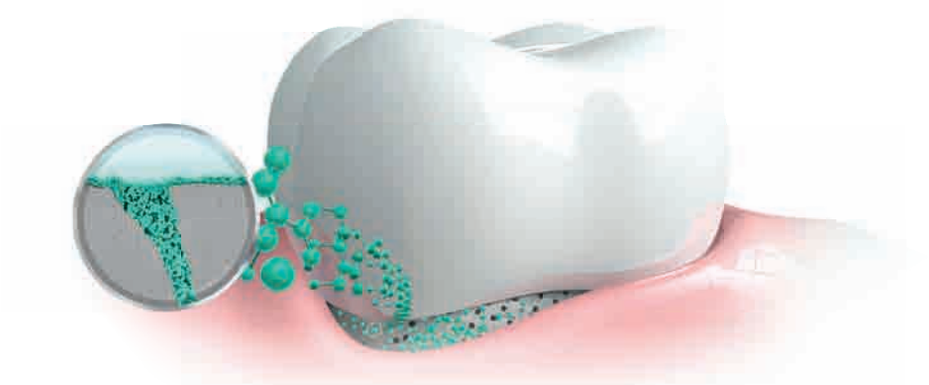
## Calmarea **imediată* și de durată** a durerii din sensibilitatea dentară[1,2]



- În contact cu saliva, se formează un strat bogat în calciu, care obturează instant[1,*] tubulii dentinari deschiși
- Stratul rămâne intact în timp, chiar după expunerea la acizi, asigurând calmarea de durată a durerii din sensibilitatea dentară[2,3]

## 93% dintre pacienți confirmă calmarea durerii din sensibilitatea dentară[4]

**93%** dintre pacienți — confirmă reducerea hipersensibilității dentinare[4]

**91%** dintre pacienți — apreciază recomandarea medicului stomatolog[4]

Calmarea imediată* a durerii din sensibilitatea dentară[1]


**Calmarea imediată* și de durată începe cu recomandarea dumneavoastră****

*Pentru calmare imediată, aplicați direct cu degetul pe dintele sensibil și masați ușor pentru 1 minut;
**Doar în legătură cu pasta de dinți

**Referințe:** 1. Nathoo S, et al. J Clin Dent. 2009;20(Spec Iss):123 –130; 2. Docimo R, et al. J Clin Dent. 2009;20(Spec Iss): 17– 22.; 3. Report Deon Hines-0003, 2016; 4. Studiu Ipsos cu privire la utilizarea produsului elmex® SENSITIVE PROFESSIONAL Repair & Prevent, efectuat în Polonia, rezultate după 2 săptămâni de utilizare, cu 325 de participanți (2017).

# elmex® SENSITIVE PROFESSIONAL realizează obturarea superioară a tubulilor dentinari în comparație cu tehnologiile concurente[1,2,*]

## Studiul 1[1,*]

Tehnologia cu fluorură de staniu/ fluorură de sodiu

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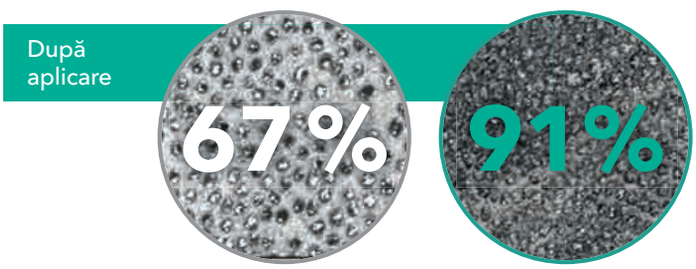
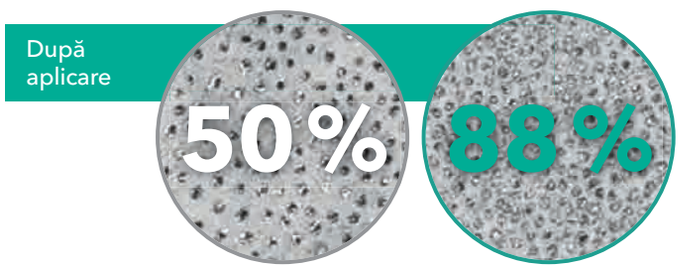



## Studiul 2[2,*]

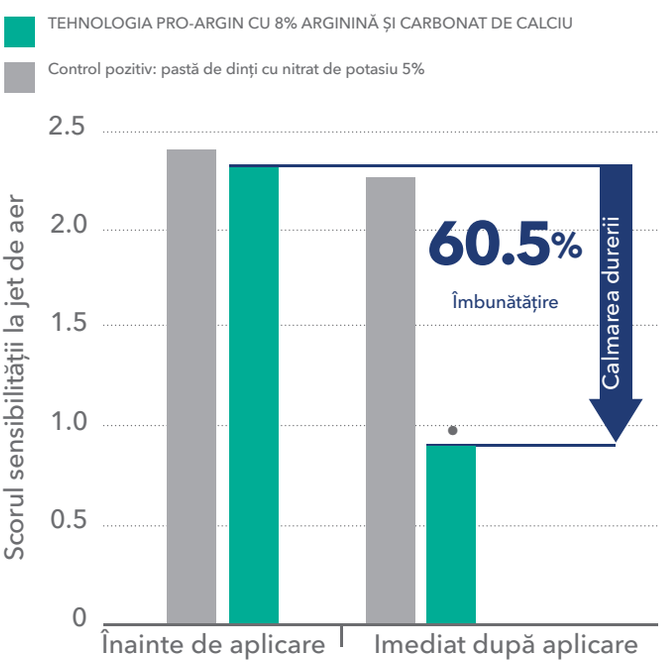
Tehnologia Novamin/ fluorură de sodiu

Tehnologia PRO-ARGIN



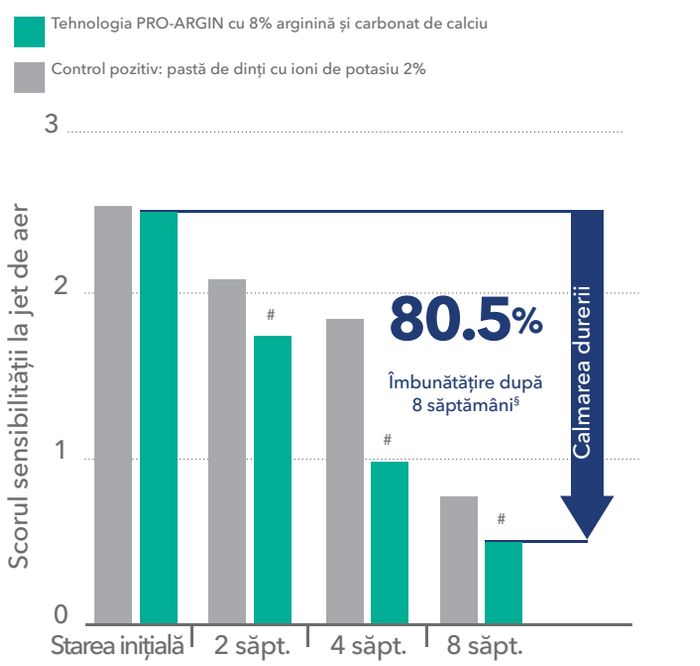

# elmex® SENSITIVE PROFESSIONAL oferă calmare semnificativă imediată** și de durată a durerii din sensibilitatea dentară[3,4]

## Calmarea semnificativă a durerii din sensibilitatea dentară instant[3,‡,**]




‡ În comparație cu starea inițială (sunt prezentate doar datele relevante)
• Semnificativ statistic (p<0,001)

## Calmarea semnificativă de lungă durată a durerii din sensibilitatea dentară după 2, 4, și 8 săptămâni de utilizare[4,§,&]




§ În comparație cu starea inițială
& În comparație cu o pastă de dinți comercială desensibilizantă, ce conține 2% ioni de potasiu și 1450 ppm de fluor (NaF)
# Semnificativ statistic (p<0,05)

*Studiu in vitro, imagini reale de microscopie confocală după 5 aplicări (p<0,05%);
**Pentru calmarea imediată aplicați direct pe suprafața sensibilă și masați ușor cu vârful degetului timp de 1 minut.

Referințe: 1. Hines D, et al. Poster acceptat, July 2018 IADR. Colgate- Palmolive Company 2018.; 2. Hines D, et al. Poster #0742, March 2018 AADR. Colgate-Palmolive Company 2018.; 3. Nathoo S, et al. J Clin Dent. 2009;20(Spec Iss):123 –130; 4. Docimo R, et al. J Clin Dent. 2009; 20(Spec Iss): 17– 22.

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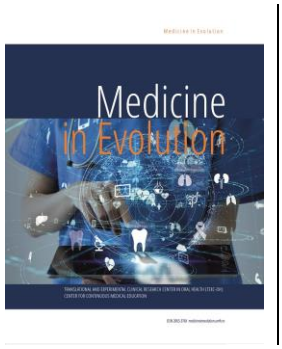
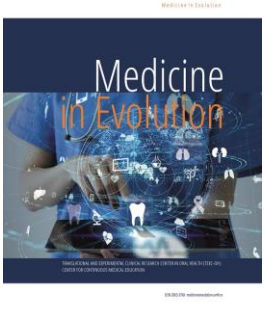
# CONTENTS

## *ARTICLES*



# Clinical management of inverted mesiodens – case report

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## Abstract

Mesiodens is a common developmental anomaly characterized by the presence of one or more supernumerary teeth in the maxillary midline region. However, inverted mesiodens, where the tooth crown points towards the nasal cavity, is a rare variation of this condition. This paper aims to present a case study of a 7 years old male patient with inverted mesiodens and aspects of treatment, successful management and aetiology of this unique dental anomaly.

**Keywords:** diagnostic methods, clinical features, mesiodens, supernumerary

## INTRODUCTION

Mesiodens is a type of dental anomaly where an extra tooth, called a supernumerary tooth, develops in the midline of the upper or lower jaw, usually between the two central incisors. It is the most common type of supernumerary tooth and is found in about 0.15% to 1.9% of the general population [1].

Mesiodens can be either fully formed or rudimentary, meaning it may not erupt or be fully developed. When it does erupt, it can cause a variety of dental problems, including crowding, misalignment, and malocclusion. In some cases, mesiodens may also cause damage to the adjacent teeth or affect the development of permanent teeth [1,2].

An inverted mesiodens, also known as an inverted impacted mesiodens, is a rare variation of a mesiodens where the tooth is upside down, with the crown facing downwards and the root facing upwards. This dental anomaly can occur in both the upper and lower jaw, but it is more commonly found in the upper jaw [2,3].

Diagnosis of mesiodens is typically done through a dental examination, X-rays, and other imaging tests. Treatment options may vary depending on the size, shape, and location of the tooth. In some cases, it may be recommended to extract the mesiodens to prevent further dental problems or to create space for the normal teeth to grow properly. In other cases, orthodontic treatment may be necessary to correct any misalignment or crowding caused by the mesiodens [4].

Inverted mesiodens can cause several dental problems, such as pain, swelling, infection, and damage to adjacent teeth or structures. In some cases, the inverted mesiodens can also cause obstruction of the nasal cavity or sinus [2,4].

### *Aim and objectives*

This paper aims to present the clinical-surgical approach of a case of inverted mesiodens present in a 7-year-old child patient with mixed dentition. This rare situation requires good preoperative planning due to the degree of difficulty of the surgical intervention but also from the point of view of the specific behavioral management.

## CASE REPORT

The 7-year-old patient P.N. presented in the Pediatric Dentistry discipline for a specialist consultation regarding the persistence of the temporary central incisor 5.1 and the presence of the permanent incisor 2.1 as well as multiple carious lesions (Fig.1).

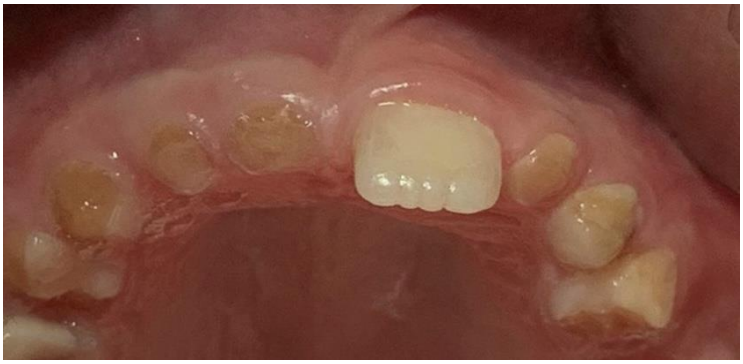

Figure 1. Clinical view

The anamnesis was performed which did not present special events or pathological aspects, the patient having a good state of health, being followed by the clinical and

radiographic examination. Radiographic analysis such as orthopantomography (Fig.2) and CBCT (Fig.3) were performed and following their completion, the presence of a supernumerary mesiodens type tooth on the midline, with an inverted position obstructing the eruption of the permanent central incisor, was observed, necessitating its extraction.

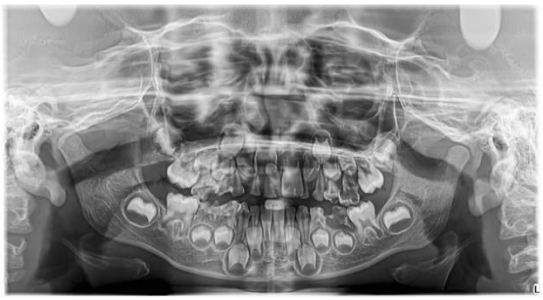

Figure 2. Orthopntomography analysis

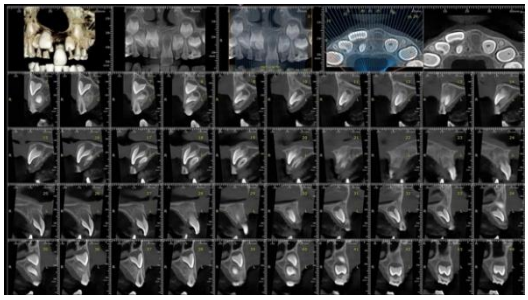

Figure 3. CBCT analysis

Parents were informed and explained about and the surgical procedure steps and the written informed consent was obtained. The inverted mesiodens removal was carried out under local anaesthesia with the use of topical 10% lidocaine and labial and palatal infiltration with 4% articaine and 1:100.000 epinephrine with a 30G needle. Using a primary anterior forceps, the decayed primary central and lateral incisors were extracted (Fig.4).

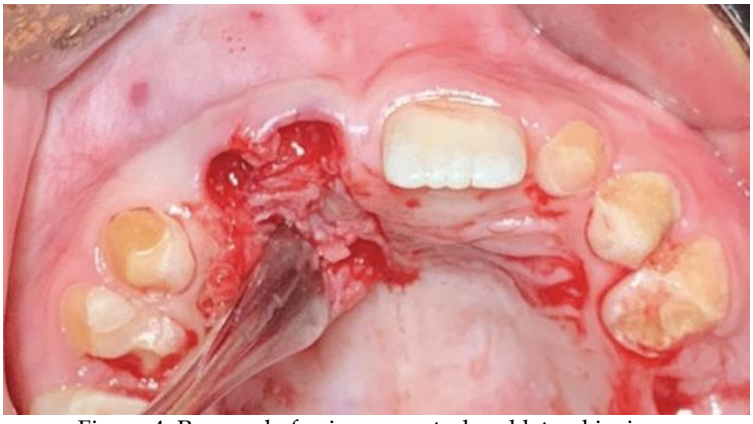

Figure 4. Removal of primary central and lateral incisors

With a #15C blade, an incision was given at the alveolar crest in the midline without a releasing incisión (Fig.5).

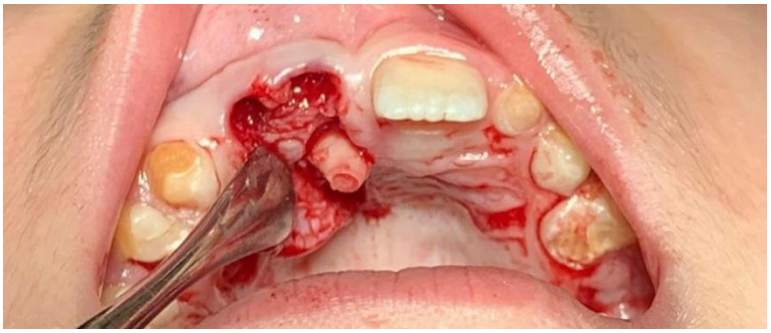

Figure 5. Visualization of the inverted mesiodens

The flap was slightly lifted to visualize the mesiodens and the tooth was luxated with an elevator, taking care not to damage the permanent teeth, and carefully extracted (Fig. 6).

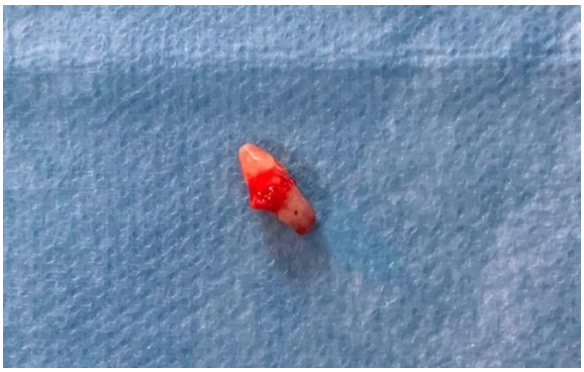

Figure 6. Supernumeray tooth

The extraction site was gently curetted, irrigated with saline, and the wound closed with a simple 4-0 silk suture (Fig. 7). Home care instructions, including oral hygiene measures and diet counseling, were given to the parents.

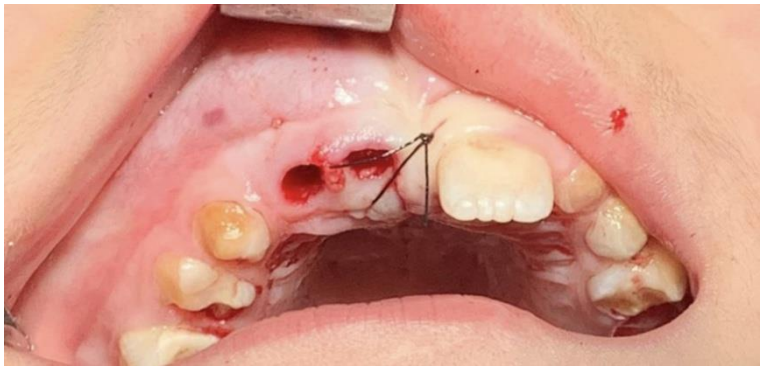

Figure 7. Aspect after the extraction

**DISCUSSIONS**

Mesiodens is a relatively rare dental anomaly, but its prevalence can vary widely depending on the population studied and the diagnostic criteria used [1,2].

In the general population, mesiodens is estimated to occur in about 0.15% to 1.9% of individuals. However, in certain high-risk populations, such as those with cleft lip and palate or other genetic disorders, the frequency of mesiodens can be much higher, up to 25% or more [2,3].

In addition, mesiodens appears to be more common in males than females, with a male-to-female ratio ranging from 2:1 to 4:1. The condition also tends to be more prevalent in people of Asian and African descent compared to those of European descent [2,3].

The exact etiology of mesiodens is not fully understood, but several factors have been proposed as potential contributors to the development of this dental anomaly. Some of the commonly suggested etiological factors of mesiodens include:

1. Genetic factors: Mesiodens has been reported to have a genetic component, with studies showing that there may be a higher prevalence of this anomaly in certain families. Some genetic syndromes have also been associated with an increased risk of developing mesiodens, such as cleidocranial dysplasia.
2. Environmental factors: Environmental factors such as maternal illnesses, exposure to certain chemicals or toxins, and infections during pregnancy have been suggested as possible causes of mesiodens.

3. Disturbances in dental development: Mesiodens can also occur due to disturbances in dental development during the formation of teeth. These disturbances can lead to the development of extra teeth, or the teeth may develop in abnormal positions.

4. Idiopathic causes: In some cases, the cause of mesiodens may be unknown, and it is classified as idiopathic [4,5,6].

Overall, the etiology of mesiodens is likely multifactorial, and the exact causes may vary among individuals. Understanding the potential etiological factors of mesiodens can help with the diagnosis, management, and prevention of this dental anomaly [7].

In a study published in the Journal of Oral and Maxillofacial Surgery, the authors reported that among 222 cases of mesiodens, only 3 cases (1.3%) were inverted mesiodens. Another study published in the Journal of Clinical and Diagnostic Research reported that among 181 cases of mesiodens, only 1 case (0.55%) was an inverted mesiodens [4,5,8].

Overall, inverted mesiodens appears to be a rare dental anomaly, and its occurrence may vary depending on the population studied and the diagnostic criteria used. Nonetheless, it is important to be aware of its potential occurrence and associated dental complications, especially in individuals who are at higher risk due to genetic or other underlying factors. Early detection and appropriate management of inverted mesiodens can help prevent complications and improve dental outcomes [4,5].

The diagnosis of an inverted mesiodens is usually made through a combination of clinical examination, dental radiographs, and other imaging techniques such as CT scans. The treatment of an inverted mesiodens typically involves surgical removal of the tooth, either through a traditional surgical approach or a minimally invasive technique such as endoscopic sinus surgery [1,2].

The extraction of a mesiodens, or supernumerary tooth, is typically recommended when it causes dental problems such as crowding, malocclusion, or damage to adjacent teeth. The extraction process may involve a simple surgical technique, where the tooth is loosened and removed from its socket using dental instruments such as forceps or elevators. In some cases, however, the tooth may be impacted or difficult to access, requiring a more complex surgical approach [9].

After the extraction, the dental professional may provide post-operative instructions such as biting down on gauze to control bleeding, applying ice packs to reduce swelling, and avoiding certain foods and activities that may interfere with healing. Pain medication and antibiotics may also be prescribed to manage pain and prevent infection [10,11].

It is important to follow the dental professional's post-operative instructions carefully to ensure proper healing and to prevent any complications such as infection or dry socket. In addition, regular follow-up visits may be necessary to monitor the healing process and to address any concerns or issues that may arise [12].

## CONCLUSIONS

Inverted mesiodens is a rare variation of the mesiodens anomaly, characterized by an inverted orientation of the tooth crown towards the nasal cavity. Understanding the etiology, clinical features, and appropriate treatment considerations is essential for the successful management of this unique dental anomaly. Although rare, inverted mesiodens should be considered in the differential diagnosis of patients presenting with nasal obstruction and other associated symptoms. Further research is warranted to explore the underlying causes of inverted mesiodens and optimize treatment strategies for improved patient outcomes.

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# Digital photography in endodontics


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## Abstract

The current article presents itself as an experimental study on non-biological material, as it will describe, explain and interpret photographic documentation in endodontic practice using the dental microscope.

The purpose of the descriptive research is to develop an updated protocol with practical applicability, easy to use in endodontic treatments and retreatments, on a series of clinical cases.

The material taken in the study is represented by the complementary examinations (digital photographs) of a number of patients undergoing endodontic treatment/retreatment in a dental office where endodontic treatments are carried out under the direct view of the dental microscope.

**Keywords:** endodontics, dental photodraphy, digital, microscope

## INTRODUCTION

Digital photography is an additional method used in case documentation, diagnosis and treatment planning. This technique is based on the use of semi-professional Single Lens Reflex (SLR) digital cameras, which allow the attachment of an auxiliary flash or removable Through the Lens (TTL) lenses (1).

The word dental photography or photography in the context of dental medicine refers to the set of photographic techniques and frames that can be applied in various fields of dental medicine, both in clinical practice and in the fields of research or medical events. Clinical dental photography represents the most effective way of visual communication between doctor and patient, between doctor and dental technician, as well as in the interdisciplinary context. This offers the possibility of self-assessment and control, allowing the highlighting of clinical manifestations (2).

Technological advances in digital photography have had a significant impact on the concept of photography as a powerful means of expression and communication, offering a wide range of perception, interpretation and execution. Photography and dentistry are two interconnected fields, revealing dental and oral cavity defects that would otherwise be overlooked (3).

Post-treatment photos are equally important for critically evaluating treatment results, encouraging professionals to improve their performance. Magnifying the image allows for details that might be overlooked, helping specialists identify potential errors and decide on appropriate solutions. These images can also be used to demonstrate correct procedures, thus representing a valuable tool in the pursuit of excellence in the practice of dental medicine (5).

In terms of benefits, digital technology has changed dentists' perspective on data collection, academics and treatment aspects. Intraoral cameras are available to capture the image of a tooth or oral lesions from different angles in just a few seconds. Therefore, it is recommended to take a complete set of intraoral and extraoral photographs for each patient before and after treatment, together with video recordings of the procedures performed (4).

### *Aim and objectives*

The scientific objectives to be solved within the scientific research are: standardization of an updated work protocol in endodontic practice; familiarizing doctors with digital photographic documentation in endodontic practice; the importance of endodontic doctors' ergonomics in specialized practice, (in cases where modern means of magnification (the dental microscope) are used/helped; listing the advantages of digital photography in endodontic practice: the meaning of documentation and more effective communication between doctor and patient, for self-evaluation to the doctor/tracking the evolution of the treatment, promotion and marketing, medico-legal document); listing the disadvantages of digital photography in endodontic practice: creating, during treatment, additional times for photography.

## MATERIAL AND METHODS

The actual experiment will include the photographic documentation of a series of clinical cases of endodontic treatments and retreatments, explaining the conditions, frameworks and norms from which a digital photograph is executed.

The material taken in the study is represented by the complementary examinations (digital photographs) of a number of patients undergoing endodontic treatment/retreatment.

The material taken into account consisted of a series of patients: 93 in number, of which 46 were clinically healthy, 17 had periodontal problems, 19 had heart and respiratory conditions and 11 wore orthodontic appliances.

The experimental study was made up of 58 patients from western Romania, Timişoara, who came to our clinic.

The criteria for the inclusion of patients in the study being the following: clinically healthy, aged between 25-40 years, non-smokers, periodontally healthy, without edentulous teeth

The exclusion criteria of the patients taken into account were: patients with cardiac and respiratory diseases, had periodontal diseases, wearers of orthodontic appliances.

The material used in this research includes a Sony A6000 digital camera and a CJ Optic dental optical microscope.

The CJ Optik dental optical microscope is equipped with the Flexion Advanced system, offering a complete and comprehensive solution that includes a wide range of accessories. This system, available at an economical price, features an integrated, fanless LED source, ensuring pure and bright light for optimal working and shooting conditions. The microscope also features a 5-step apochromatic magnifier (0.4x/0.6x/1x/1.6x/2.5x), ensuring accurate and detailed magnification.

The CJ Optik dental optical microscope has a 30-degree tilt beam splitter and retrograde photo port, offering optimal ergonomics and nearly symmetrical balance. It also features an HD camera adapter compatible with all major manufacturers. The focal lengths and optics have been designed to be perfectly compatible with the most advanced photo-video cameras on the market.

The microscope's VarioFocus system offers a working distance between 210 and 470 mm, and its plano-apochromatic optics ensure superior image quality. The microscope is equipped with an integrated and removable lens protector, providing additional protection and facilitating cleaning and maintenance.

The binocular tube of the microscope can be tilted between 0 and 200 degrees, providing a large range of vertical flexibility for comfortable user positioning.

With the uniquely designed MonoBall coupling, the user can make smooth and easy repositioning at any angle without having to unscrew or re-screw the fastening systems.

The CJ Optik dental optical microscope has an integrated orange filter, which is useful when working with composites. Thanks to the best optical coatings and optimal alignment, the light transmission between the user and the camera is efficient and provides a clear and detailed image.

The microscope rotation plate allows the user to maintain an upright and ergonomic working position while the microscope is angled to the left or right.

Ergonomically placed control handles provide immediate access to all functions, enabling quick inter-procedural changes.

The CJ Optik dental optical microscope benefits from the integration of HDMI, USB, camera AC/DC power, power cable and monitor cables into its arm, providing efficient cable management and superior ergonomics.

The long and stable suspension arm is identical for all wall, ceiling or floor mounting systems.

The Sony A6000 digital camera used in the research offers the advantage of a larger sensor, which translates into high-quality images. The camera's APS-C sensor is 1.6 times larger than that of 4/3 sensors and 13 times larger than that of 1/2.3 sensors, helping to achieve a remarkable level of quality in every image.

The A6000's BIONZ X™ image processor ensures fast data processing, accurately capturing textures, reducing blurred details and eliminating visual noise in certain areas,

resulting in clear, high-fidelity images. These features benefit both still images and video clips recorded with the camera.

The A6000's ultra-fast autofocus makes it one of the most versatile interchangeable lens cameras around. With a focus speed of just 0.06 seconds, you can capture perfect images in any situation, from family events to sporting events or natural landscapes.

The A6000 camera can shoot at a rate of 11 frames per second, allowing you to capture desired moments or expressions with precision and speed.

Full HD 1080 recording at 60p or 24p ensures cinematic quality motion capture in your videos.

The A6000 camera benefits from features such as Auto Eye Focus and Auto Focus Hold, which make it easy to get sharp, well-focused images.

Compared to DSLR cameras, the A6000 is smaller and lighter, offering superior portability and maneuverability. With advanced features and manual control, the A6000 makes no compromises in terms of creativity and photographic performance.

The A6000's Tru-Finder™ OLED electronic viewfinder allows you to plan and preview images. Exposure control ensures proper compensation of brightly lit or dimly lit scenes, and focus zoom allows details to be magnified for more accurate assessment. The display mode presents useful information such as histograms.

## RESULTS

In all photographs, the most important features in the stages of photographic documentation are, first of all, the achievement of a professional sanitation by removing tartar, staining and food debris in order to achieve a correct treatment plan and improve the visibility of the remaining hard tissues.

Then, the intraoral focus will always be on the remaining tooth tissue left by the coronal destruction.

Framing will also be achieved by exposing hard tissues and fixed gingiva.

The position of the patient must be horizontal on the dental chair, to facilitate the direct visibility of the microscope, positioned at an angle of 90 degrees with the patient's horizontal.

The magnification of the microscope in the current endodontic photographic documentation was 7.5.

## DISCUSSIONS

Therefore, according to the article "Endodontics and the aging patient", written by M Johnstone, P Parashos, the initial digital photograph in the form of preoperative retroalveolar radiographs and the postoperative radiograph in the form of control retroalveolar radiographs are imperative in the realization of a photographic protocol in endodontics (6).

Also, the authors of the article "Endodontic treatment of maxillary lateral incisors with anatomical variations" Moon-Hwan Lee, Jung-Hong Ha, Myoung-Uk Jin, Young-Kyung Kim, Sung-Kyo Kim strongly support taking an intraoral photograph of the access cavity.

Other authors, Hiroshi Kato and Takashi Kamio, find according to the article "Diagnosis and Endodontic Management of Fused Mandibular Second Molar and Paramolar with Concrescent Supernumerary Tooth Using Cone-beam CT and 3-D Printing Technology: A Case Report", that a successful treatment endodontic cannot be done without intraoral photography in the oral cavity with the respective lesion (7).

As in the study "Guided endodontic treatment of multiple teeth with dentin dysplasia: a case report" written by the authors Ralf Krug, Julian Volland, Sebastian Reich et al, in order

to achieve a correct photographic protocol in endodontic practice, digital photography must not be missing in the stage of instrumentation and preparation of the tooth as well as in the stage of definitive obturation of the root canals.

## CONCLUSIONS

In conclusion, according to what was presented and documented above, an updated digital protocol in endodontic practice would look according to the scheme below:

Initial digital photograph through an initial x-ray of the studied case (Figure 1); Initial digital photograph of the tooth in the oral cavity with the respective lesion (Figure 2); Digital photograph of the tooth in the stage of making the access road; Digital photography in the stage of instrumentation and preparation of the tooth; Digital photography in the obturation stage of the root canals (Figure 3); Digital photograph in the stage of making the definitive coronal restoration (Figure 4); Final digital photograph of the tooth in the oral cavity after endodontic treatment and adhesive coronal restoration; Final digital photograph by a case control retroalveolar radiograph.

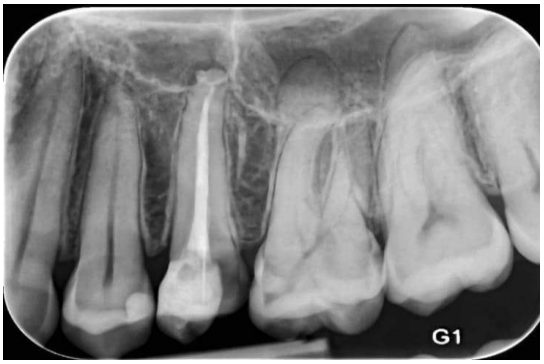


Figure 1. Initial digital photograph through an initial x-ray

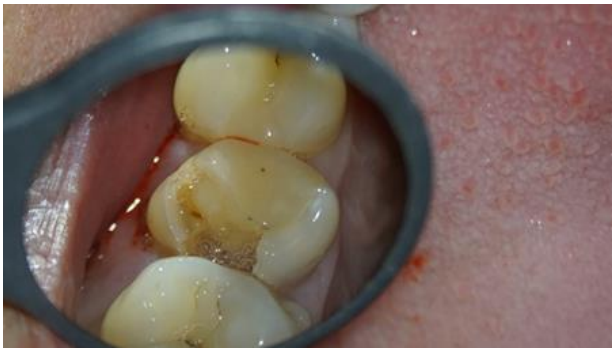

Figure 2. Initial digital photograph of the tooth in the oral cavity with the respective lesion

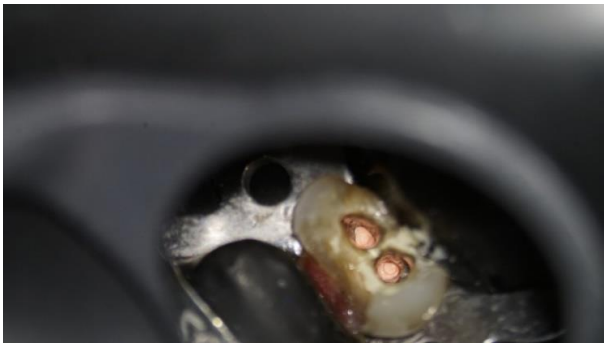

Figure 3. Digital photography in the obturation stage of the root canals

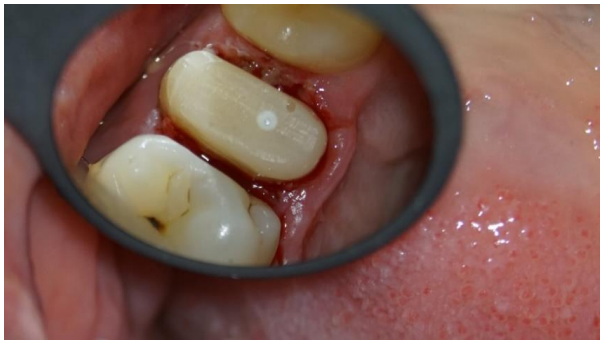

Figure 4. Digital photograph in the stage of making the definitive coronal restoration

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# Patients' perception regarding the effects of smoking on periodontal health


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## Abstract

R Smoking is one of the major risk factors for periodontal health, leading to higher progression rate of periodontitis and less predictable response to periodontal therapy. The aim of the present study was the assessment of patients' perception and level of knowledge regarding the effects of cigarette smoking over periodontal health. Material and method: the cross-sectional study was conducted in 2022 on a sample of 100 smokers using an on-line self-administered questionnaire. Results showed that 52% of participants were heavy smokers and only 57% would consider quitting if they were diagnosed with periodontitis. Signs and symptoms of periodontitis in smokers of which there were most frequently aware are halitosis (75%) and gingival recession (43%) rather than masked gingival bleeding (20%), tooth mobility (31%), bone resorption (20%) or periodontal abscess (23%). Regarding the risks for periodontal treatments in smokers, only reduced percentages of subjects were aware of impaired periodontal healing (24%), infection as complication (32%) and relapse (31%). Conclusion: Participants in the present showed a reduced level of awareness regarding the periodontitis signs and symptoms, a low level of knowledge regarding the potential risks of smoking over periodontal treatment and a low interest in quitting smoking because of periodontal issues.

**Keywords:** oral health, periodontal health, smoking, tobacco use, periodontal health perception

## INTRODUCTION

Smoking is one of the common risk factors for both general and oral health [1], leading to reduced lifespan because of its negative effects on health [2]. In spite of the globally-applied programs and policies for tobacco control initiated and supported by the World Health Organization Framework Convention for Tobacco Control in 2003 [3], at the global level is still about one quarter of the population aged 15 years and older who smokes, with the prevalence of tobacco users decreasing from 32.7% in 2020 to 22,3% in 2020 [4].

Its negative effects on oral health are a consequence of both direct and indirect mechanisms because of the content of nicotine and other compounds, as well as the increase in the temperature it produces on the tissues in the oral cavity [5]. The decrease in oral mucosa vascularization [6], the suboptimal immunologic response [5-7], the potential for malignant transformation [5,8], impaired quality of mucosa [5], as well as the discoloration of the teeth surfaces and mucosa [9], as well as halitosis [10] are some of the important negative effects seen in smokers. Cigarette smoking is responsible for the increased risk for tooth loss twice higher compared to non-smokers [11]. Moreover, leukoplakia, which is the most frequently met premalignant lesion of oral mucosa, is seen 6 times most frequently in smokers [12].

When it comes to the association between smoking and periodontal disease, there is strong evidence to support the fact that cigarette smoking is one of the major risk factors for periodontal inflammation [13] and currently it is one of the criteria taken into consideration for the diagnosis of periodontitis, namely used to establish the grade of periodontitis [14]. There is a dose-dependent effect of cigarette smoking on periodontal disease [14-16] with the highest risk exposure among patients smoking at least 10 cigarettes per day, these being considered as heavy smokers, in comparison to light smokers who smoke <10 cigarettes per day [14,16]. Quitting smoking is of utmost importance in periodontal vulnerable or compromised patients [17] but formal smokers are still at risk compared to never smokers [16]. Exposure to smoking does not only influence the initiation and progression of periodontal inflammation but also the response to periodontal treatment, both initial therapy and surgical therapy [14,15,18]. Because of the bad prognosis of the surgical procedures in smokers because of the risk of poor wound healing [15,18], frequently heavy smokers are not good candidates for periodontal surgical corrective phase, thus the treatment is limited to pre-surgical, antimicrobial phase as active therapy and kept under supportive periodontal therapy phase, with a shorter follow-up intervals [18].

### *Aim and objectives*

The aim of the present study was the assessment of patients' perception and level of knowledge regarding the effects of cigarette smoking over periodontal health.

## MATERIALS AND METHODS

The Oral Health and Community Dentistry Department from the Faculty of Dental Medicine of the "Carol Davila" Medicine and Pharmacy University (Bucharest, Romania) conducted this cross-sectional study between March and June 2022. There were included 100 participants in the study, Romanian adults with an age varying between 20 and 63 years. The inclusion criteria were the current smoker status and a history of cigarette smoking of minimum 1 year. Exclusion criteria were professional field of dentistry, either dentist or dental student. The assessment was performed using an on-line questionnaire with 18 items, both open and close-ended questions. Participants in the study were informed that the forms

were anonymous, that no sensitive personal data were collected and were offered details about the aim of the study and their rights as participants in a study in accordance with the Declaration of Helsinki. All invited participants agreed to participate and proceeded to fill-in the forms.

## RESULTS

Participants had a mean age of 31.9 ± 11.6 years and 42% (42 subjects) were females.

In the studied group, all the included participants were smokers with a history of cigarette smoking of 12.3 ± 9.1 years, on average. Heavy smokers (≥ 10 cigarettes/day) were 52% of subjects, light smokers (<10 cigarettes/day) were 26%, while 22% were occasionally smokers (Figure 1).

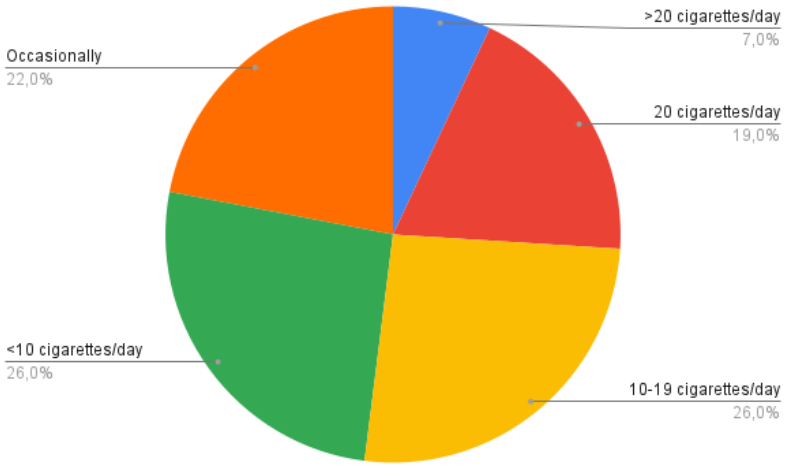


Figure 1. History of smoking among respondents

Among subjects included in the study, 63% declared having a previous attempt to quit smoking. Asked about medical reasons taken into consideration to quit smoking in the future in case of being diagnosed with different general and oral health issues, only 48% stated they would contemplate about quitting if they had gingivitis (as the first phase of periodontal tissues inflammation) and 57% if they had periodontitis (the deep and irreversible phase of periodontal inflammation) (Table 1). Higher percentages were observed for oral cancer and peri-implantitis as oral health-related reasons to give up cigarette smoking (Table 1). For general health conditions, 66% of subjects stated they would quit and 29% would contemplate giving up this habit only in the case of being diagnosed with a severe systemic disease (Table 1).

Table I. Attitude toward the contemplation of quitting smoking

| **Contemplating quitting smoking in case of developing different health condition** | **% (N)** | | |
|---|---|---|---|
| | **Yes** | **No** | **I don't know** |
| **Frequency** | | | |
| *Gingivitis* | 48% (48) | 17% (17) | 35% (35) |
| *Periodontitis* | 57% (57) | 15% (15) | 28% (28) |
| *Dental caries* | 27% (27) | 57% (57) | 16% (16) |

| Contemplating quitting smoking in case of developing different health condition | % (N) | | |
|---|---|---|---|
| | **Yes** | **No** | **I don't know** |
| **Frequency** | | | |
| *Oral cancer* | 95% (95) | 3% (3) | 2% (2) |
| *Peri-implantitis* | 66% (66) | 17% (17) | 17% (17) |
| *Dental esthetic issues* | 52% (52) | 24% (24) | 24% (24) |
| | **Yes** | **No** | **Yes, only in severe systemic diseases** |
| *Systemic diseases* | 66% (66) | 5% (5) | 29% (29) |

When it comes to the clinical signs and symptoms specific for periodontitis to which they, as smokers, are prone, the most frequently mentioned answer was the halitosis (Figure 2). Basic and easy-to-be-observed by the patients symptoms, such as increased gingival bleeding (as the first signs of periodontal tissues inflammation) and reduced gingival bleeding (specific modification in smokers diagnosed with periodontal disease due to the microvascular dysfunction induced by tobacco use) are recognized by only 27% and 20% of the subjects (Figure 2). A low percent of participants were aware of the potential to develop more severe signs, such as bone resorption, periodontal abscess, tooth mobility and pathological tooth migration, which are characteristic for advanced forms of periodontitis (Figure 2).

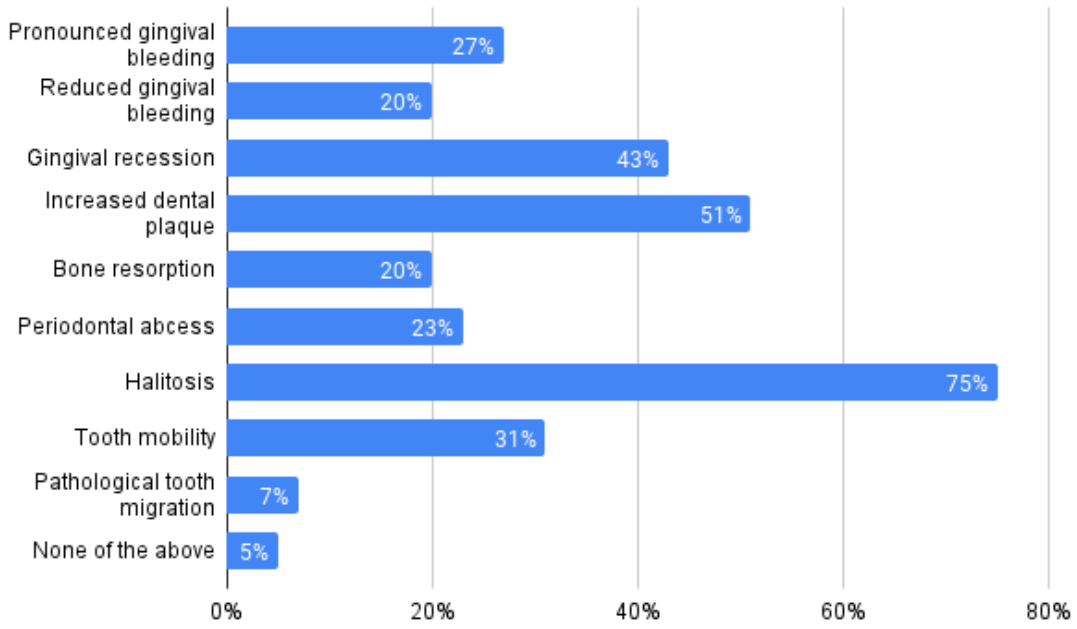


Figure 2. Acknowledgement of the periodontitis signs and symptoms among smokers

Regarding the risk to which these subjects, as current smokers, are exposed in case of developing periodontal disease and after active periodontal therapy, only 24% were aware of the compromised, partial response to periodontal treatment while 64% believed that the healing response is rather delayed (Figure 3). Infection, as the main complication after

periodontal surgical treatment, is declared as potential risk by only 32% of participants (Figure 3).

Unfortunately, even though smoking has a significant negative impact on maintenance phase after active periodontal treatment, only 31% of subjects were aware of this potential risk (Figure 3).

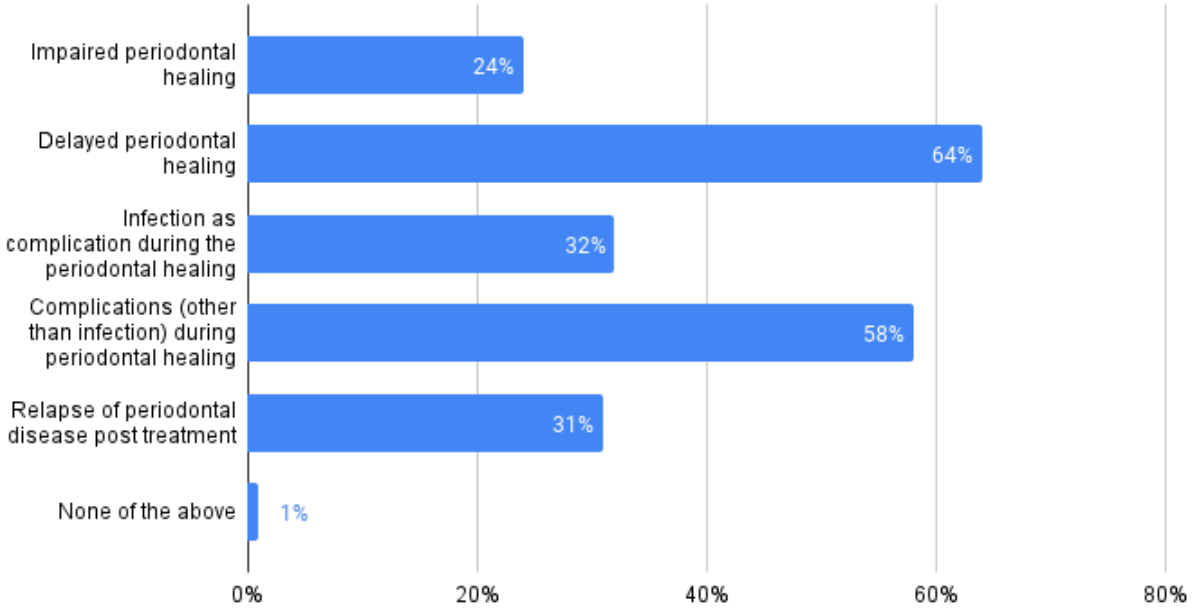


Figure 3. Acknowledgement regarding the potential effects of smoking after periodontal treatment

## DISCUSSIONS

According to the most recently published Consensus over the Classification System of Periodontal and Peri-implant Diseases and Conditions [13], smoking status is one of the parameters used for periodontal diagnosis and it contributes to the setting of the stage of periodontitis, which is relevant to the progression of the periodontitis, namely the likelihood of greater rate of progression and a less predictably of response to periodontal treatment [19]. Thus, heavy smokers, which are current smokers of ≥ 10 cigarettes/day, are considered by default in Grade C of periodontitis, irrespective of the other risk factors, thus the greatest rate of progression and the least predictable outcome of the treatment. In our studied group, half of the participants were considered heavy smokers. However, the present study did not include clinical examination to identify the presence of periodontitis among the participants and the questionnaire did not include items regarding the personal history periodontal conditions or previous periodontal treatment.

Gingival bleeding is both a symptom often reported by periodontal patients and a sign known as bleeding on probing, which is one of the main clinical parameters and diagnostic criteria for gingivitis and periodontitis [13,14,19]. In our study, one quarter of the participants selected pronounced gingival bleeding as an answer for signs of periodontitis to which they, as smokers, are at risk. However, what is characteristic to cigarette smokers is the masked clinical signs such as gingival probing because of the microvascular constriction and fibrosis in spite of the presence of inflammatory infiltrate [13]. Thus, gingival bleeding is rarely observed in smokers, leaving the false impression of reduced inflammation. In our study, only one fifth of participants were aware that smokers could have reduced gingival bleeding in spite of the presence of periodontitis. And because of the absence or rarely seen bleeding,

smokers are at risk to be diagnosed belated, developing more advanced signs and symptoms such as tooth mobility and pathological tooth migration, bone resorption and periodontal abscesses (acute form of inflammation) but of which participants in our study are rarely aware of but are more likely to recognize gingival recession as potential symptom of periodontitis in smokers.

Because combusted tobacco use induces dysbiosis, reduced gingival perfusion, increased inflammatory response because of suppressed immune response, decreased expression of angiogenic factors, impaired morphological and functional recovery of periodontal tissues [20], smokers are at a higher risk of early debut of periodontitis, higher rate of progression, improper response to either non-surgical or surgical periodontal therapy and relapse after the active treatment [15,19]. However, among the assessed participants, these risks are acknowledged by low percentages.

Quitting smoking is recommended in case of periodontal patients who smoke and counseling the patients by the dental professional is encouraged as part of periodontal therapy [17,19] although previous research showed that the rate of quitting varies between 4% and 30% among patients affected by periodontitis, after various behavioral change intervention [17]. In addition, the benefits of quitting smoking with the purpose of reduction of risk over periodontal health at a level of non-smokers could be seen in 10 up to 20 years after giving up this habit [11].

The fact that only about one half of the subjects in the present study would consider quitting smoking in case they get to be affected by gingivitis and periodontitis could be explained by these reduced levels of knowledge and awareness regarding the symptoms and risk over periodontal health and therapy.

## CONCLUSIONS

In the present study, participants, predominantly heavy smokers, showed a reduced level of awareness regarding the periodontitis signs and symptoms, a reduced level of knowledge regarding the potential risks of smoking over periodontal treatment and a low interest in quitting smoking because of periodontal issues.

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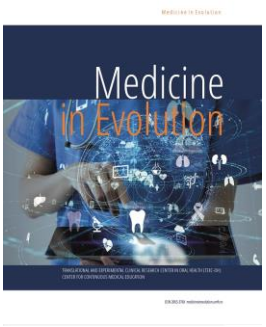
# Recurrent aphthous ulcers (RAU) and recurrent aphthous stomatitis (RAS) enigmatic etiopathology

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## Abstract

Oral aphthous ulcer is a well-known condition that significantly affects the quality of life for patients by producing intense pain and difficulties in speaking and chewing. When recurrence is present we refer to them as recurrent aphthous ulcers (RAU) and recurrent aphthous stomatitis (RAS). Mouth ulcers are typically uncomfortable sores that can occur as a result of a variety of local and general disorders. It is one of the most prevalent oral ulcerative conditions.

**Keywords:** aphthous ulcers, aphthous stomatitis, etiopathology

## INTRODUCTION

A frequent condition known as recurrent aphthous ulcers (RAU) or recurrent aphthous stomatitis (RAS) causes repeated, excruciating sores on the non-keratinized oral mucous membranes [1, 2, 3]. Recurrent aphthous ulceration (RAU) and recurrent aphthous stomatitis (RAS) are terms used when recurrence is frequent [4]. This pathologies manifested as oral ulcers are commonly known as aphthae or canker sores [1, 2, 3].

The term "aphthous" is derived from a Greek word "aphtha" which means ulceration, syntagma which was initially used by Hippocrates to describe disorders of the mouth [5, 6, 7]. Aphthous ulcer can be described as a painful inflammatory condition of the mouth mucosa, developed on its own or as a result of various different disease conditions [4].

Believed to be one of the most prevalent oral lesions in the general population, aphthous ulcers affect 20–25% of the population. It is considered that the prevalence is higher in developed nations and women are slightly more susceptible [8]. Even though it is the most prevalent oral mucosa disorder, the etiopathology is still unclear [1, 2, 3].

It can reoccur any time, with a frequency that varies up to 3 months. These type of ulcers can be extremely painful and may make it difficult to chew, speak or swallow, affecting people's daily lives. Although the majority are benign and self-healing, a tiny fraction of ulcers are malignant [9].

Clinically, this lessions are characterized by circular or oval ulcers with circumscribed margins and floors that are slightly below the level of the surrounding mucosa [10]. They are usually found on the buccal and labial mucosa as well as the tongue. A white fibrous layer protects the ulcers, which contrasts with the reddish edge [11]. According to Stanley's 1972 classification of RAS, three distinct clinical variations have been recognized: minor RAS, major RAS and herpetiform ulceration [12].

### *Aim and objectives*

Considering the most recent papers in the specialized literature, the aim of this research is to attempt to elucidate the etiopathology of recurrent aphthous ulcers (RAU) and recurrent aphthous stomatitis (RAS) in the oral cavity.

## ETIOPATHOLOGY

The origin of this conditions occurrence has not been defined precisely. Aphthous ulceration of the oral mucosa has an idiopathic, complex etiology that also involves the activity of the cell-mediated immune system. Being unrelated to acute infections, recurrent aphthous ulcers (RAU) and recurrent aphthous stomatitis (RAS) are non-contagious illness [13, 14, 15].

Although the exact causes of aphthous ulcers remain unknown, it is believed that one or more extrinsic triggers are responsible for sores. Local trauma, emotional or physiological stress can all cause aphthae [16].

Minor mouth injuries, such as those caused by cuts, burns or bites while eating, incorrect dental work, improperly fitting dentures or vigorous brushing can also be responsible for the appearance of ulcers [16].

Mouth ulcers frequently develop because of stress. While stress does not directly cause ulcers, it can raise the likelihood of them happening and can influence how quickly they heal. By limiting what and how a person can eat and drink, mouth ulcers can also lead to stress creating what is called a never-ending vicious circle [17, 18].

It is well acknowledged that both local and systemic immunological, genetic and environmental variables are responsible [10, 19]. Given that 40% of those who develop ulcers have a family history of the disease, it may potentially be partially genetic [16].

In some patients, general associated causes, such as malabsorption, enteropathy or celiac disease, have been identified. About 20% of cases are related to hematinic or other deficiencies (iron, zinc, thiamine – vitamin B1, folic acid - vitamin B9, vitamin B6, vitamin B12, vitamin D) [13, 14, 15].

Rarely, recurrent aphthous ulceration (RAU) may be a symptom of a number of serious illnesses, such as Crohn's disease, Celiac disease, Behcet disease or AIDS [16]. To determine if a condition is caused by a systemic disease process or is truly idiopathic, a complete history and systemic examination are necessary [4]. Menstruation and hormonal disorders associated with pregnancy were also mentioned as a possible cause of mouth ulceration [13, 14, 15, 16].

One of the crucial environmental factors has been identified as heterogeneity in microbiota composition [20]. It is considerd that oral aphthous ulcers microbiology is made up of a variety of bacteria, authors even incriminating changes in the oral microbiome [4].

Another cause of aphthous ulcers can be an allergic reaction or sensitivity to certain excipients present in toothpastes or mouthwashes (sodium lauryl sulfate), in food (cinnamon, cheese, citrus fruits, figs, pineapple) or exposure to toxins (nitrates in water) [13, 14, 15].

Aphthous ulcers are more common in non-smokers and smokers who quit smoking, and less common in people with good oral hygiene practices [13, 14, 15].

## DISCUSSIONS

Recent studies have linked bacterial and fungal dysbiosis to recurrent aphthous stomatitis (RAS). In RAS patients and healthy controls, Stehlikova et al. looked at microbial shifts during RAS manifestation at an ulcer site, in its environs and at an unaffected site in comparison with healed mucosa. The area with the most obvious variations in microbial alpha and beta diversity between RAS patients and healthy controls was found to be the lower labial mucosa. This author shows how active RAS ulcers alter the types of bacteria and fungi that colonize healthy oral mucosa and how this transformation continues in some form even after the ulcer has healed [21].

In RAS patients, microbiological analyses showed that active ulcers are associated with *Fusobacterium*, *Leptotrichia*, *Cardiobacterium*, *Lachnoanaerobaculum*, *Clostridia*, *Malassezia*, *Streptococcus*, *Haemophilus* and *Porphyromonas*, while healed zones revealed strict association with *Selenomonas*. Moreover, compared to healthy controls, RAS patients had higher serum levels of IgG against *Mogibacterium timidum*, emphasizing the immunological component of this disease [21].

Yun-ji et al. also demonstrated that RAS is linked to dysbiosis of the mucosal and salivary microbiota, *Streptococcus salivarius* and *Acinetobacter johnsonii* being two of the RAS associated species. A mechanism that could explain why ulcers take longer to heal is the fact that *Acinetobacter johnsonii* significantly decreased gingival epithelial cell growth and displayed increased cytotoxicity against the cells [22]. Yang et. al proposed *Escherichia coli* and *Alloprevotella* colonisation as a cause of RAS and concluded that limiting this bacteria growth promotes healing [23].

Slebioda et. al indicated that although no unique gene has been found, twin studies have provided some indication of a familial component with early onset and increased severity. The study illustrates a higher prevalence of aphthae among family members, pointing the condition's hereditary origins. Family members may be more susceptible to RAS

if they inherit certain gene variants, particularly those that produce pro-inflammatory cytokines, which are involved in the development of aphthous ulcers [24].

Thus, immunologically mediated processes is significantly contributing to the pathogenesis of oral aphthous ulcers. A theory that could explain why ulceration gets worse after local injury, after the cessation of smoking or both, suggests that it may be caused by an unchecked or excessive production of interleukin-1 (IL-1) or interleukin-6 (IL-6), crucial factors for its development [25].

Some toothpastes and mouthwashes contain sodium lauryl sulfate, a chemical that has not been confirmed to be a trigger for ulcers, but is known to delay their healing [16].

It is considered that diet has a significant impact on the appearance of mouth ulcers, which is why the relationship between dietary habits and RAS appearance has been intensively investigated. Several foods and beverages, including acidic or spicy dishes, coffee, chocolate, eggs, cheese, cow's milk, almonds and gluten are accused [16]. Researchers have shown a link between the consumption of this specific foods and the development of RAS, but no substantial correlation between RAS and three particular incriminated foods - tomatoes, strawberries, walnuts - was discovered by Eversole et al [26].

According to Du et al. regular consumption of carbonated beverages or frequent thirst will raise the probability of this condition, whereas a preference for nuts offers protection [27]. The lack of fluid consumption causes an imbalance between free water and bound water in oral mucosa, with consequences such as changes in local energy metabolism, explaining the appearance of local heat and burning symptoms specific to aphthous ulcers [28]. Nuts rich content in vitamin A, vitamin B, vitamin E and proteins explain the protective effect on RAS [29, 30]. Fruit consumption is believed to have no good effects [27]. Consuming sugary foods and citrus fruits alters the pH of the oral cavity, encouraging the development of stomatitis and explaining why ulcers appear [31]. Because to its glycosides, which stimulate the oral mucosa, and its protease, which can cause allergic reactions in some people, pineapple may cause or worsen mouth ulcers [32]. However, according to Xu et al. eating fruits and drinking water may be useful as daily preventive strategies against RAS [33].

Although diet has a minimal part in the pathophysiology of RAS, it can have a function in the development of RAS by inducing hypersensitivity or by depleting the body of certain vitamins and minerals [34].

When a person stops smoking, their risk of developing RAS rises, presumably as a result of the loss of nicotine's protective effects or the mucosal keratinization that tobacco smoke promotes [35]. Furthermore, Hill et al. demonstrated that nicotine replacement treatment is effective in healing ulcers brought on by quitting tobacco use [36]. Mohamed et al. considered that cigarette smokers experience aphthous ulceration less frequently, while other authors suggested that this effect of smoking on RAS is time-dependent and dose-dependent [37, 38].

## CONCLUSIONS

Within the limitation of this research and literature provided, it is safe to say that recurrent aphthous ulcers (RAU) and recurrent aphthous stomatitis (RAS) etiopathology is multifactorial and a single etiological factor cannot be specified.

A number of factors, including genetic, immunological, traumatic and environmental ones, contribute to the development of oral aphthous ulcers.

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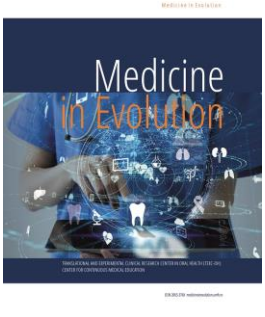
# Inflammation and its relationship to oral cavity

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## Abstract

The inflammation process was described in science and literature a long time ago, but the understanding of the process took a long time. Inflammation is a complex reaction to harmful agents and includes vascular responses, migration and activation of leukocytes. Inflammation begins with an acute reaction, which evolves into a chronic phase if left to persist. Acute inflammation is a rapid process characterized by fluid exudation and leukocyte migration and primarily neutrophils. Chronic inflammation, on the other hand, extends over a longer period of time and is associated with the infiltration of lymphocytes and macrophages, the proliferation of blood vessels and fibrosis. Inflammation ceases when the invader is eliminated and secret mediators are removed. However, many factors change the course and morphological appearance, as well as the pattern of termination and duration of inflammation. Chronic inflammatory diseases are now seen as problems that could have an impact on the periodontium. The reciprocal effects of periodontal diseases are potential factors that change the severity of the progression of systemic inflammatory diseases.

This review aims to review studies in the literature on the processes, interactions, classification and morphological, and clinical characteristics of inflammation, relating it to the oral cavity and describing the main cell types and chemical mediators used for its occurrence. As a research source we used the PubMed database.

**Keywords:** Innate immune system, macrophage, mast, cells, oral disease, inflammation

## INTRODUCTION

The oldest known description of the inflammation comes from the Edwin Smith papyrus, one of the Egyptian papyri that was found in a tomb near Thebes. Papyrus dates approximately from 1550 BC. This document is undoubtedly a copy of the ancient tests of the archaic period of Egyptian history (3200-2780 BC.). Documents from Egyptian civilizations and other early civilizations leave no doubt that the features of inflammation were recognized from those periods, but the understanding of the process took a long time.

Inflammation is didactically characterized by the following quintet: redness (rubor), heat (calor), swelling (tumor), pain (dolor) and dysfunction of the organs involved (functionio laesa). The first four characteristics were described by Celsus almost 2000 years ago. Functio laesa was added to the definition of inflammation by Rudolf Virchow in 1858, considered the predecessor of modern pathology and social medicine. The latter was the one who, in the field of inflammation, critically analyzed the significance of the four key symptoms and postulated that inflammation could not be represented as a single process, but rather as consisting of various inflammatory processes.

Inflammation is a protective, inherent response that is evolutionarily preserved in all multicellular organisms. As a crucial function of the innate immune system, it cleanses infectious agents and degenerate cells and repairs damaged tissue [1]. Acute inflammation is an auto-limitative, transient response that facilitates tissue repair and is beneficial for the body. However, chronic inflammation could lead to the development of various pathologies, including degenerative diseases associated with aging, fibrosis and cancer [2,3]. Inflammation involves the activation and chemotactic migration of leukocytes (neutrophils, monocytes and eosinophils) and mast cells to the site of the lesion. These cells secrete growth factors, cytokines and other inflammatory mediators, ie histamine, heparin, metalloproteases and serum proteases, which profoundly affect endothelial and mesenchymal cells, stimulating proliferation, differentiation and migration.

In acute inflammation, platelet aggregation and activation occurs immediately after tissue damage and helps to accelerate coagulation by forming a thrombus followed by a fibrin matrix to prevent bleeding and infection with microorganisms pathogenic. Fibrin clot also acts as a reservoir of growth factors released by platelets, such as platelet-derived growth factor (PDGF) and transformer-β growth factor (TGF-β), which are essential to attract neutrophils, monocytes, fibroblasts and myofibroblasts. These cells, together with the formation of a new extracellular matrix and the induction of angiogenesis, facilitate the appearance of granulation tissue. In tissues, monocytes are differentiated into macrophages and, once activated, macrophages are the main source of growth factors and cytokines that modulate tissue repair. The final phase of healing consists of re-epithelizing the wound by proliferating and migrating epithelial cells to its edge, a process that requires the dissolution of the fibrin clot and the degradation of the underlying collagen by serum proteases and metalloproteases. Persistence of causal factors or failure to resolve the inflammatory response could lead to chronic inflammation and a large number of clinical and experimental studies have linked inflammation and cancer. In fact, many malignancies occur in places of persistent infection and inflammation [4].

The oral cavity is one of the most complex ecological microenvironments in the human body, where the interactions between the host and the microbes define health and disease. The teeth are the only functional hard tissues that extend from the inside to the outside of the human body, crossing a series of other hard tissues (meaning bones) and soft (meaning connective tissue and epithelium), surrounded by a tight biofilm consisting of the richest collection of bacteria outside the colon. Such an architecture creates several areas, which

operate in concert during inflammatory responses in the oral cavity. The regulation of immuno-inflammatory mechanisms in oral diseases is partly governed by the patient's susceptibility and environmental factors [5].

***Aim and objectives***

This article presents a review of studies in the literature that report the processes, interactions, classification and morphological and clinical characteristics of inflammation, relating it to the oral cavity and describing the main cell types and chemical mediators used for its occurrence.

**Vascular and cellular component of inflammation**

A definition of inflammation is complicated because local vascular and tissue reactions are often accompanied by systemic effects. These effects include fever, leukocytosis, malaise, metabolic disorders and shock. The inflammatory response consists of a vascular and a cellular component.

The vascular component of the inflammatory response is characterized by vasodilation and, consequently, increased blood flow and vascular permeability. Increased blood flow causes, clinically, redness and heat in the inflamed tissue, and increased vascular permeability results in plasma loss and the formation of inflammatory exudate. Exudate contains many proteins (fibrin, immunoglobulin) and is responsible for edema. They can compress nerve endings and thus cause pain.

The cellular component involves the migration of leukocytes from the blood vessels to the inflamed tissue. They are extravasated from capillaries and reach the inflamed tissue where they phagocytic bacteria and cellular debris. Neutrophil influx is one of the first stages of the inflammatory response. These cells generate a fast and nonspecific phagocytic response. Later, macrophages and lymphocytes (specific subsets of T and B lymphocyte) appear at the site of inflammation.

Lymphocytes are the primary cells of the immune system and have developed one of the most sophisticated defense mechanisms in the biological system. T lymphocytes play a major role in organizing the immune response, eliminating intracellular pathogens (viruses and bacteria) by generating cytotoxic T lymphocytes. B lymphocytes protect the body against extracellular pathogens by producing antibodies. Natural killer cells (NK) are an important component of innate immunity. Dendritic B cells begin the immune response by presenting T lymphocyte antigens. The interaction between T lymphocytes, B lymphocytes, dendritic cells and natural killer cells (NK) is the fundamental defense mechanism of the host [6].

The mechanism against pathogens requires different responses depending on the characteristic of the pathogen and the attacked tissue. Chaplin's study and collaborators claim that the host body has developed innate and adaptive immune defense mechanisms. The first mechanism is non-specific, attacking any structure or antigen non-self, and the second mechanism is extremely specific [7]. Both types of immune response work together to eliminate pathogenic antigens.

The sequelae of acute inflammation depend on the type of tissue involved and the size of the destroyed area, which in turn depend on the nature of the harmful agent. Possible results of acute inflammation are either healing or evolution towards chronic inflammation. Chronic inflammation is characterized by the predominant presence of macrophages in the injured area. These cells provide a strong defensive mechanism in the body, and the mediators they release are harmful to both the body's tissues and invading agents. This is why chronic inflammation is almost always accompanied by tissue destruction [8]. In addition to macrophages, inflamed tissue is infiltrated with lymphocytes and plasmocytic. In

addition, in chronic inflammation there is a proliferation of fibroblasts that form collagen fibers.

**The role of cytokines in inflammation**

The cytokine family includes interleukins (IL), chemokines (CKs), interferon (IFN), growth factors (GF), tumor necrosis factor (TNF) and colony stimulating factor (CSF colonization stimulating factor) [7].

Interleukins (IL1 - IL32) are different from each other, have different functions and are secreted by different cells. Chemokines are very important in controlling the migration of cells between and inside tissues. Interferon has several subunits: IFNα (leukocyte IFN, a viral replication inhibitor), IFNβ (IFN fibroblast, a viral replication inhibitor) and IFNγ (lymphocyte secret, with immune control functions). Growth factors (TGF, IGF and many others) were initially identified due to functions that are not related to the immune system but can have effects on immune cells.

Tumor necrosis factor includes TNFα (more frequently secreted by monocytes) and TNFβ (secreted by T cells). Colony stimulating factor (G-CSF, M-CSF, GM-CSF and others) is able to differentiate bone marrow cells into different types of specific cells, such as monocytes, macrophages and neutrophils. Interleukin-8 (IL-8) was the first cytokine identified as having chemotactic activity. It has been shown to be a selective chemo-attractor for neutrophils [9].

The study by Essayan et al, (1998) showed the role of the family of IL-1 cytokines, which are a group of proteins that possess synergistic and contrasting biological responses. According to their study, IL-1 and its precursor forms are strongly involved in determining the inflammation and defense of the host [10].

The production of cytokines at the site of inflammation in the oral tissues is part of the host's response, which is essentially protective in nature. Unrestricted production of cytokines can lead to the destruction of oral tissues. It is traditionally believed that immune functions are regulated by signals from the immune system. It is now obvious that the immune system is partially regulated by the central nervous system, acting mainly through the hypothalamic-pituitary-suprarenal axis and through the sympathetic nervous system [11,12]. The pathways between the immune system and the brain seem to be two-way, and the goal is to maintain homeostasis. The sympathetic nervous system provides a major integrative and regulatory path for this communication. Sympathetic lymphoid tissue innervation, the presence of adrenergic receptors on immune cells (B and T lymphocytes, macrophages) and studies of catecholamine interactions with the immune system [13], provides substantial evidence for the role of the sympathetic nervous system in immune regulation. In addition, the cellular products of an activated immune system, namely cytokines, can send signals to the brain. Cytokines, such as IL-6 and TNF-α, appear to be involved in the dialogue between the brain and the immune system through the secretion of corticotropin-releasing hormone (CRH) and therefore, activates both the hypothalamic-pituitary-suprarenal axis, but also the sympathetic nervous system [14,15].

**Inflammation in the oral cavity**

The oral cavity is an open cavity, which is thus exposed to various potential microbial agents. In addition to these factors, some treatments applied to the teeth may favor the deposition of the dental plaque associated with additional resistance applied to the teeth, such as orthodontics. Moreover, inflammation in orthodontics also comes from the forces applied to the teeth.

Pulpitis and periodontitis, the most common infections in dentistry, are common in daily practice in surgery offices. Periodontitis shares many pathological features with other inflammatory diseases with concomitant bone resorption, such as rheumatoid arthritis, with evidence that both conditions are manifested as a result of an imbalance between proinflammatory and anti-inflammatory cytokines [16]. In both forms of inflammation, the pathological consequences are associated with the accumulation of bacteria leading to a host response that generates the infiltration of inflammatory cells [17]. Because the soft and hard tissues of the oral cavity are part of the same functional and physiological organ, the separation of the host's response to several components is artificial and does not recognize the dynamic relationship between cells, bacteria and extracellular structures. Also, although practical and instructive, the assumption of a linear change in lesions from acute to chronic is unclear. Recent discoveries that define resolution pathways in inflammatory processes challenge the concepts of compartmentalization and linearity in acute and chronic responses [18,19].

Once oral inflammation, such as pulpitis, gingivitis or periodontitis, an inflammatory infiltrate of various cell types, such as neutrophils, lymphocytes, macrophages, is formed, mast cells that will produce different subtypes of cytokines responsible for the immunopathology of diseases.

**The role of leukocytes in inflammation of the oral cavity**

To mediate an effective response, leukocytes must find their way to places of infection or inflammation. Leukocyte invasion of tissues can be induced by the chemotactic activity of several substances, such as interleukin-1 (IL-1), tumor necrosis factor (TNF-α) and bacterial lipopolysaccharide (LPS), which causes leukocyte migration when injected in vivo. All such compounds induce the production of chemo-attractors, which in turn cause leukocyte migration. Therefore, chemotactic activity includes receptor-mediated gradient perception and should be measured by a chemo-attractor's ability to induce targeted leukocyte migration in vitro [20].

Th lymphocytes are divided into two subclasses: Th1 and Th2. Th1 cells secrete mainly IFN-γ and IL-2 that increase cellular immunity. Th2 cells secrete a different set of cytokines, mainly IL-4, IL-10, IL-13 and IL-9, which increase the humoral immunity [21]. CD4 cells + can be differentiated in either Th1 or Th2, and the differentiation is strongly dependent on cytokines produced by cells of the innate immune system. IL-12 produced by activated monocytes / macrophages is a major inducer of Th1 differentiation and therefore of cellular immunity. IL-12 together with TNF-α and IFN-γ act synergistically in inflammation and further promote Th1 responses and are therefore considered major proinflammatory cytokines [22]. Th1 and Th2 responses are mutually inhibitory. Thus, IL-12 and IFN-y inhibit Th2 and vice versa, IL-4 and IL-10 inhibit Th1 responses and the production of proinflammatory cytokines. IL-4 and IL-10 are major anti-inflammatory cytokines and an increasing number of evidence suggest that catecholamines selectively inhibit Th1 functions and proinflammatory cytokines and promote Th2 responses and anti-inflammatory cytokines [23].

**The role of macrophages in inflammation of the oral cavity**

Macrophages are essential for coordinated resolution of oral inflammation and return to tissue homeostasis. In the first stage of the oral inflammatory process directed against microorganisms, bacteria and their virulence factors trigger the receptor-mediated production of cytokines by epithelial cells with the simultaneous release of neuropeptides, which causes

vasodilation of local blood vessels. The generation of chemo-attractant proteins (chemokine) at this stage results in the attraction of the first line of defense, neutrophils, which leave the vessels and migrate to the site of the microbial invasion. This step is critical and plays a key role in generating an effective defense system. Neutrophils are followed by macrophages. This is usually the stage where clinical signs of oral inflammation are detectable, including bleeding, swelling and redness of the gums. Inflection can be either limited and eliminated by neutrophil and macrophage function at this early stage, or it can be extended to include other cells and structures. Being myeloid cells of hematopoietic origin, the overall role of macrophages is to limit the pathological changes of soft tissues or to raise the inflammatory response to the next level. Major macrophage functions include the elimination of invasive bacteria, the recruitment of other cells at the site of infection, the elimination of excess neutrophils, the production of cytokines and chemokines, and the activation of the lymphocyte-mediated adaptive immune response. The net result of these functions can be either complete healing, limiting the resulting fibrosis infection and healing with the formation of scar tissue, or failure to eliminate infection by establishing a chronic inflammatory lesion. If the oral inflammatory process is prolonged and chronic, the destruction of soft and hard tissues, including alveolar bone, is observed due to direct destruction of inflammatory-mediated tissues [24].

Macrophages along with neutrophils are responsible for the phagocytosis and digestion of microorganisms and foreign substances through surface receptors that recognize and bind certain surface molecules of bacteria, such as lipopolysaccharides. These receptors are the key components to distinguish between host and invader and are defined as recognition receptors, called TLR receptors, which mediate the elimination of pathogens by phagocytosis. TLR receptors regulate apoptosis, inflammation, and immune responses. Evidence is accumulated that supports a role of TLR-mediated macrophages in the resolution of oral inflammation [25].

It now becomes obvious that the cells of the innate immune system are the determining factors of tissue and organ destiny and are more than transient, and their role is not limited to the phagocytosing of microbes. Neutrophils and macrophages are the key cells of the host's response where their role exceeds „defense" and are involved in tissue homeostasis, where protection, healing-repair and regeneration are encoded.

**The role of mast cells in inflammation of the oral cavity**

Numerous studies have investigated the participation of mast cells in inflammation and other pathologies. Such studies have been useful, for example, in documenting changes in the number of mast cells in different anatomical areas that also have a specific pathology.

Once activated, the mast cells secret many vasoactive and proinflammatory mediators [26-29]. These include preformed molecules such as histamine, serotonin, TNF, kinins and proteases stored in secretory granules. Leukotrienes (LT), prostaglandins and PAF (activated flat facto) are synthesized during the activation of mast cells from arachidonic acid released by the action of phospholipases. In addition, a number of cytokines (for example, IL-1, 2, 5, 6, 8, 9, 13 and TNF) and vascular endothelial growth factor (VEGF) are synthesized de novo and released a few hours after stimulation [30].

Mature mast cells vary considerably in their cytokine content (Bradding, 1995) and proteolytic enzymes. Mastocytes in the presence of SCF produce predominantly proinflammatory cytokines, while in the presence of SCF and IL-4, they mainly produce Th2 cytokines [31].

More and more evidence indicate that mast cells are critical to the pathogenesis of inflammatory diseases. Genetical analysis of IgE-activated human mast cells showed

overexpression of many genes, mainly related to inflammation [32]. Prostheses released from mast cells could act on plasma albumin to generate histamine-releasing peptides [33] which would further propagate mast cell activation and inflammation. Proteases could also stimulate protease-activated receptors (PAR) inducing widespread inflammation [34,35]. However, unlike allergic reactions, mast cells are rarely seen as degranulating during inflammatory processes. The only way to explain the involvement of mast cells in non-allergic processes would be through the „ differential" or „selective" secretion of non-granular mediators [36,37].

Mastocytes are present in oral tissues and appear to be involved in the initiation of a number of inflammatory conditions in the oral cavity. In inflammatory processes in the oral sphere, cytokines activate and stimulate mast cells to secrete proinflammatory molecules. These molecules play a crucial role in inducing inflammation [38]. Mastocytes are responsible for releasing elevated levels of proinflammatory cytokines, such as IL-1, IL-6 or TNF. In acute oral inflammation, mast cells release proinflammatory cytokines, such as histamine, proteoglycans, metabolites of arachidonic acid, TNF and tri-phase that promote the inflammatory process.

After activation, MC induces T lymphocyte migration, either directly by releasing chemokines (lymphactin, IL-16 and MIP-1), or indirectly by inducing the expression of the adhesion molecule on endothelial cells [39]. Histamine increases vascular permeability through structural changes that include endothelial contraction and the formation of intercellular spaces. In addition, histamine promotes leukocyte adhesion to endothelial by transient mobilization of the selectin P-adhesion molecule to the surface of endothelial cells. This functional relationship between mast cells and T lymphocytes has proven to be two-way, fulfilling mutual regulatory and / or modulatory roles, including influences on cellular processes such as growth, proliferation, antigen activation and presentation. In addition, mediators derived from T lymphocytes, such as β-chemokines, directly induce mast cell degranulation. These findings led to the proposal of a functional relationship between these two cell populations that could facilitate the emergence of an immune response that contributes to the onset of pathogenesis of periapical inflammatory lesions [40].

Studies in the literature show an increase in the number of mast cells in the inflamed place compared to healthy places and have suggested important dynamic changes in the migration and location of mast cells in the evolution of periodontal disease. The significance of mast cell distribution in tissue compartments refers to their influence on nearby cells, with stimulating, inhibitory or toxic effects. The participation of mast cells in the defense mechanism and destructive events both as effect cells and receptive to chronic inflammation, as well as possible functional populations in periodontal lesions are still debatable. Thus, it was concluded that periodontitis is not unidirectional, but rather interactive. Mastocytes that produce destructive proinflammatory cytokines can also produce mediators that activate the healing process [41].

**Future prospects**

Oral inflammatory processes involve microbial etiological factors, induce a number of host responses that mediate an inflammatory cascade of events in an attempt to protect and / or heal tissues. It is becoming increasingly clear that the macrophage phenotype is very important for the evolution of the lesion for resolution or chronicity. Because the response of the macrophage is essential for health and disease, it is important to achieve a more complete understanding of the molecular events in this complex system.

Mastocytes play a critical role in the development of inflammation in the oral cavity, both in the early stages and during the transition from acute to chronic inflammation.

Mastocytes in these inflammatory lesions are associated with increased vascular permeability, angiogenic response, collagen synthesis, inflammation regulation, bone resorption and destruction of the extracellular matrix. Based on the concept that mast cells play an important role in chronic inflammation, it is possible to use drugs therapeutically to influence the release of cytokines by mast cells and therefore, to counteract the inflammation. In the future, it may be possible to develop new approaches that influence the release of proinflammatory or neuropeptide molecules to relieve inflammation caused by mast cells. Because mast cells play a key role in inflammation, therapies aimed at mast cell function may have value in managing oral inflammation.

The classification of inflammation, cell types, cytokines and chemokines involved gives us an indication of its nature and importance, although much remains to be discovered, especially in terms of the oral cavity.

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# The impact of epileptic disorder in oral pathology


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## Abstract

Epilepsy alongside mentally depreciation and other neurological disorders might develop physical, social and mental concerns, particularly, when they start during adolescence. In addition, the seizures scenes along mental deterioration may reduce oral and dental attention bringing about various periodontal and prosthodontics issues.

Epilepsy has direct negative consequences for patient's general dental condition and oral health, the two of which are additionally influenced by lacking oral hygiene; weak oral hygiene itself is regularly likewise brought about by epilepsy-related unforeseen disadvantage. Therefore, missing teeth, caries and periodontal conditions happen progressively regularly in epilepsy patients and they need increasingly dental treatment options and beforehand planning. In any case, in certainty the epileptic patients can get less and more straightforward treatment modalities. The point of this thesis was to survey and integrate examinations on dental treatment in epilepsy patients and to make reference to possible triggers and seizures management in dental practice.

**Keywords:** Epilepsy, mental deterioration, anti-epileptic medication

## INTRODUCTION

Epilepsy is an illness that is often faced by oral and maxillofacial medical practices [1]. It is thought to influence a large number of individuals worldwide and has a commonness of 0.5% - 0.9% in general population [2]. Chapman et al. [3] have proclaimed that epileptic seizures are the second most rudimentary clinical episode during dental medical procedures. Studies have stated the fact that every dental specialist sees in his/her expert life 1.5 occasions summed up as tonic-clonic seizures from the patients [4]. It has been accounted for that the epilepsy happens autonomous of race, age and sexual orientation [1,2]. It has likewise been accounted for those instances of epilepsy that create in adolescence are hereditary in starting point, while those that show up in adulthood are identified with cerebrovascular maladies [2, 5].

In 70% of epilepsy cases, the particular etiology isn't known and there is still beyond a shadow of a doubt. These cases are characterized as idiopathic or essential primary epilepsy. At the point when the etiology of seizures is referred to, the condition is known as optional or obtained epilepsy [5,6]. Auxiliary epilepsies are the after-effect of metabolic, hereditary, basic or practical oddities as electrolyte awkwardness, acidosis, hyperglycemia, hypoglycemia, hypoxia, lack of hydration, water inebriation [7, 8].

Epilepsy is characterized by the World Health Organization (WHO) as an affection of numerous etiologies, described by repeating scenes of paroxysmal cerebrum fists brought about by an unexpected disordered and over the top neuronal release [4].

According to the World Health Organization, epilepsy represents about 1% of the worldwide diseases, as projected by the incapacity to live a balanced life, positioning it soon after some mental issues, for example, alcohol dependence [8]. The dental treatment planning and management of the patients suffering of epilepsy that manifest seizures ought to be done only by dental specialists who have learned about these issues.

The elective treatment generally speaking incorporates the association of the best possible adversary of epileptic prescriptions (AEDs like carbamazepine, phenytoin, phenobarbital, primidone, valproic acid and many others) for the sort of seizure. In any case, different medications prescribed to control the seizure mechanism might have very serious side effects on the oral care and also can massively affect the dental treatment planning and management [9, 10].

### *Aim and objectives*

The aim of the present study was to identify the existent side effects of anti-epileptic medication on dental and oral structures, also observing and analyzing the type of seizures that occurred during dental treatment.

The purpose of this paper is to identify the challenges that healthcare providers encounter during the management of patients suffering of epilepsy that experience frequent seizures. Also, the aim of the research is to focus on identifying the oral hygiene status by evaluating the decayed teeth status and occlusion issues that could be connected to the recurrent epileptic incidents. By evaluating these alterations, specific recommendations can be stated, that could be followed during the dental treatment and further management of the patients.

## MATERIAL AND METHODS

The present study is designed as a retrospective observational research that was conducted in the Department of Maxillofacial Rehabilitation of the Oral Medicine and

Hospital Dentistry Clinic (Rambam Medical Center) – Haifa, Israel. A number of 71 diagnosed epileptic patients were included in the study, patients that were admitted into the facility and underwent a dental treatment, during March 2019 – January 2020.

The applied inclusion criteria:

- Patients admitted into the facility who suffered of Epilepsy
- Patients that had a treatment with anti-epileptic drugs
- Patients who underwent a dental treatment under anesthesia

The applied exclusion criteria:

- Patients that were admitted into the facility only for consultation and didn't have a dental treatment into the clinic.

The included information was collected form pre-existing computer archives of the admission into the hospital consisting of case files that enclosed all the medical information needed. The numerical parameters looked up in the study were processed, analyzed and classified using the functions available in 2015 Microsoft Excel. All patients were informed, and a written consent was signed for taking part in the research.

## RESULTS

Out of the 71 patients we included in our study, we have found that 11 are under 18 years old, 18 are aged between 18-30 years old, while the majority of patients who underwent a dental treatment in the facility were aged 30 to 50 years old. The lowest age group consisted of 10 patients aged 50 to 70 years old (Figure 1).

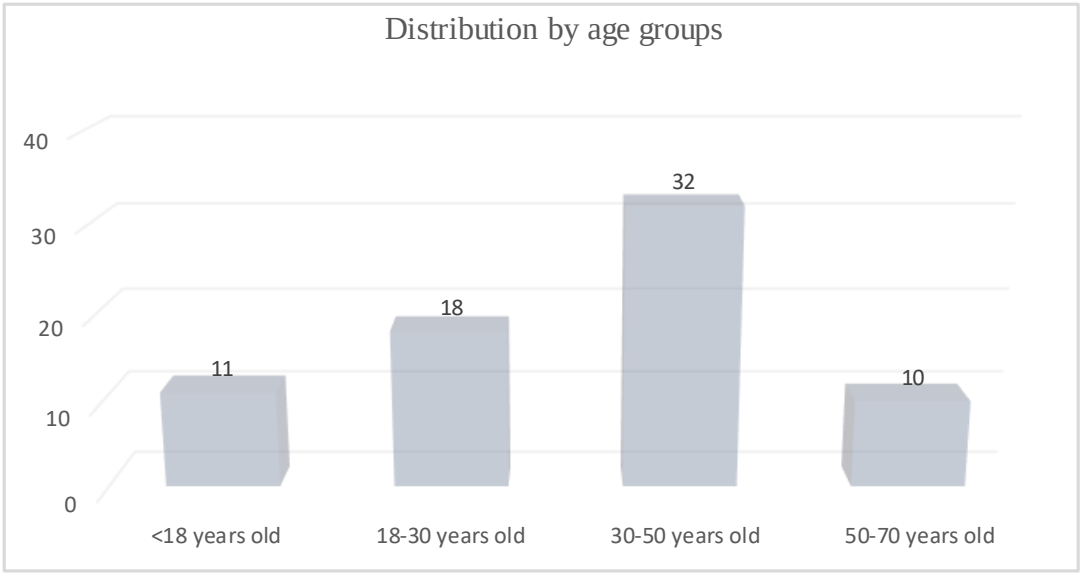


Figure 1. Patients distribution by age groups

As observed in Figure 2, from the total of 71 patients, 39 which represent 54,9% are males, while the rest of 45,1% consisted of 32 epileptic female patients.

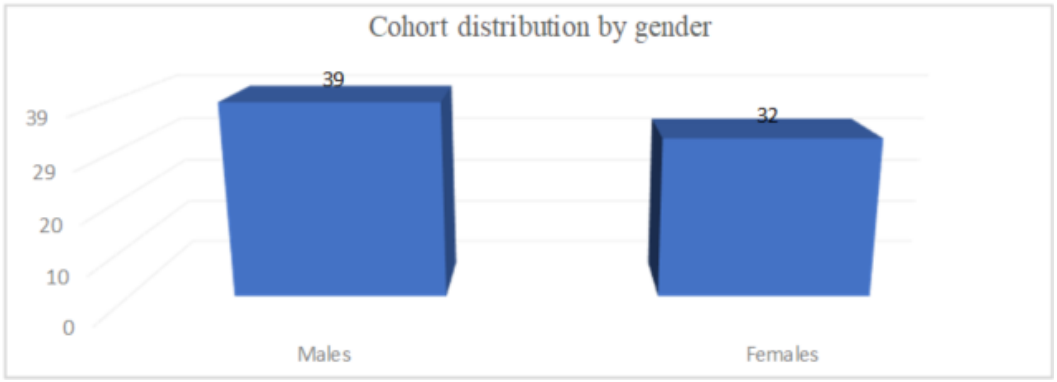


Figure 2. Cohort distribution by gender

Findings showed that a number of 36 patients (50,7%) were addressing the clinic from rural areas and the rest of 35 (49,3%) were located in urban areas (*Figure 3).*

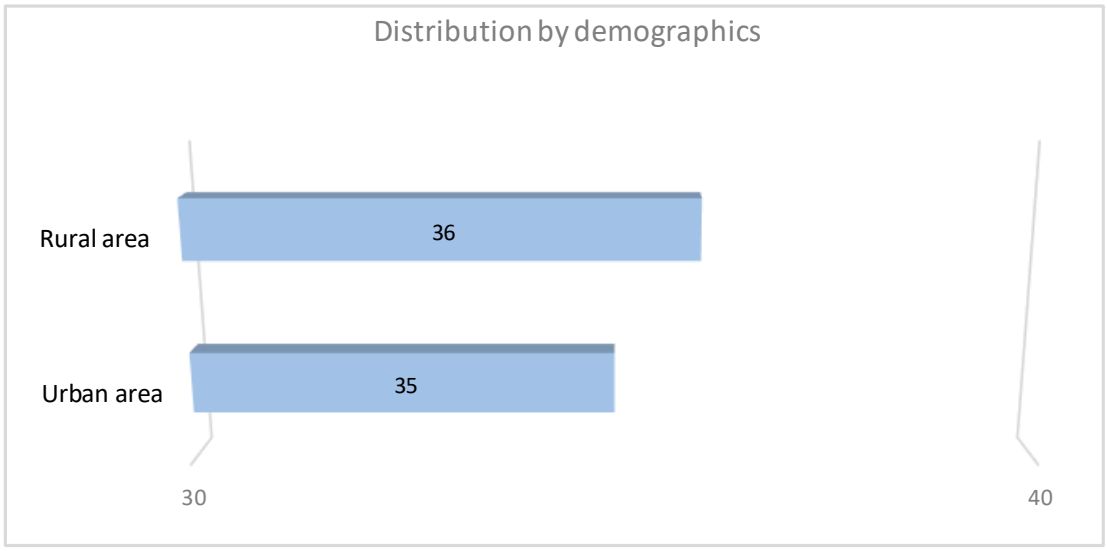


Figure 3. The patients distribution by demographics

Going through the cohort health history and treatment plan, we identified that 57,7% (41) of the patients included in the study had Carbamazepine in their treatment; 47,8% (34pacients) were treated with Phenobarbital, while 33,8% (24 patients) had as anti-epileptic drug Phenytoin. Another 33 patients representing 46,4% out of the total, were keeping seizures under control using Diazepam, while only 8,45% which represented 6 patients were being treated with Ethosuximide. A total of 31 patients (43,6%) are using Valproic acid as a anti-epileptic drug, 16,9% (12 patients) Gabapentin; 19,7% (14 patients) Felbamate; 5,63% (4 patients) Levetiracetam; 12,6% (9 patients) Oxcarbazepine; 7,04% (5 patients) Tiagabine and 9,85% (7 patients) Zonisamide (*Figure 4*).

| **Antiepileptic drug name** | **Number of patients using the AED** |
|---|---|
| Carbamazepine | 41 |
| Phenobarbital | 34 |
| Phenytoin | 24 |
| Diazepam | 33 |

| | |
|---|---|
| Ethosuximide | 6 |
| Valproic Acid | 31 |
| Gabapentin | 12 |
| Felbamate | 14 |
| Levetiracetam | 4 |
| Oxcarbazepine | 9 |
| Tiagabine | 5 |
| Zonisamide | 7 |

Figure 4. Patients' medication history

Regarding the encountered side effects in the oral cavity or related to the dental structures, 57,7% (41) of the patients included in the study that had Carbamazepine in their treatment suffered of drowsiness, xerostomia, stomatitis, gingival bleeding, rash, osteopenia and osteocalcin. A total of 47,8% (34 patients) that were treated with Phenobarbital were identified with side effects such as osteopenia, steomalacia and drowsiness at times. The 33,8% (24 patients) that had as anti-epileptic drug Phenytoin had associated side effects like gingival hyperplasia, osteopenia, osteomalacia and gingival bleeding. Another 33 patients representing 46,4% out of the cohort that were keeping seizures under control using Diazepam, experienced drowsiness/sedation, same as the 8,45% which represented 6 patients that were being treated with Ethosuximide and the one taking Gabapentin 16,9% (12 patients). The 19,7% (14 patients) that had included Felmabate in their treatment experienced mild cognitive side effects. Patients taking Levetiracetam, Oxcarbazepine, Tiagabine and Zonisamide mentioned unknown side effects of the drugs (*Figure 5*).

| **Antiepileptic drug name** | **Common side effects on oral cavity/ Dental Considerations** |
|---|---|
| Carbamazepine | Gingival bleeding<br>Xerostomia<br>Osteopenia/Osteomalacia<br>Drowsiness/Sedation<br>Stomatitis<br>Rash |
| Phenobarbital | Drowsiness/Sedation<br>Osteopenia/Osteomalacia |
| Phenytoin | Gingival hyperplasia<br>Osteopenia/Osteomalacia<br>Gingival bleeding |
| Diazepam | Drowsiness/Sedation |
| Ethosuximide | Drowsiness/Sedation |
| Valproic Acid | Gingival bleeding<br>Petechiae<br>Decreased platelet count |
| Gabapentin | Drowsiness/Sedation |
| Felbamate | Mild cognitive side effects |

| | |
|---|---|
| Levetiracetam<br>Oxcarbazepine<br>Tiagabine<br>Zonisamide | Unknown side effects |

Figure 5. Medication side effects of anti-epileptic medication on dental/oral structures

Related to the occurrence of epileptic seizures during the dental treatment procedures, as observed above, out of the 71 patients that we have included in the study, 11 of them representing 7,81%, experienced a seizure during the dental treatment (*Figure 6*).

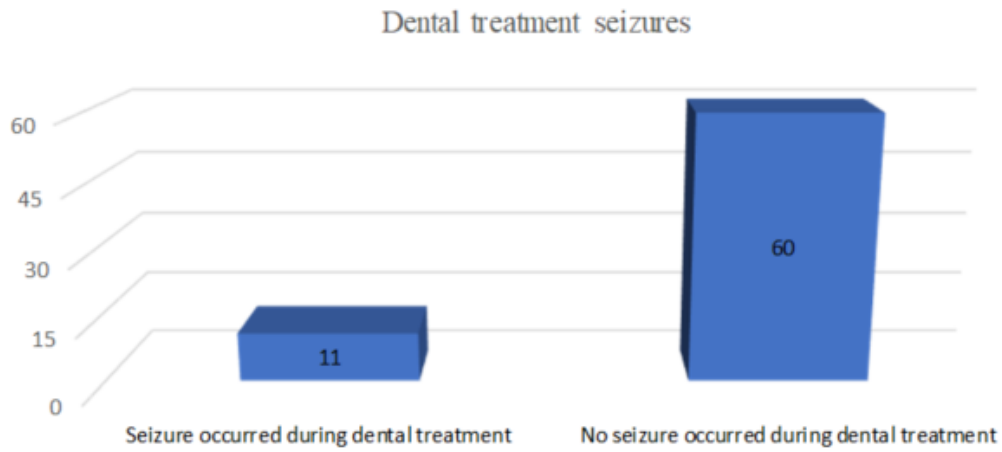


Figure 6. Classification by the presence/absence of the seizure during treatment

Out of the total of 11 patients that had suffered a seizure during the dental procedure, 5 patients representing 46%, went through a generalized tonic-clonic seizure, while 2 patients representing 18% had a first-time seizure and last but not least, 1 patient went through a status epilepticus that required emergency medical attention.

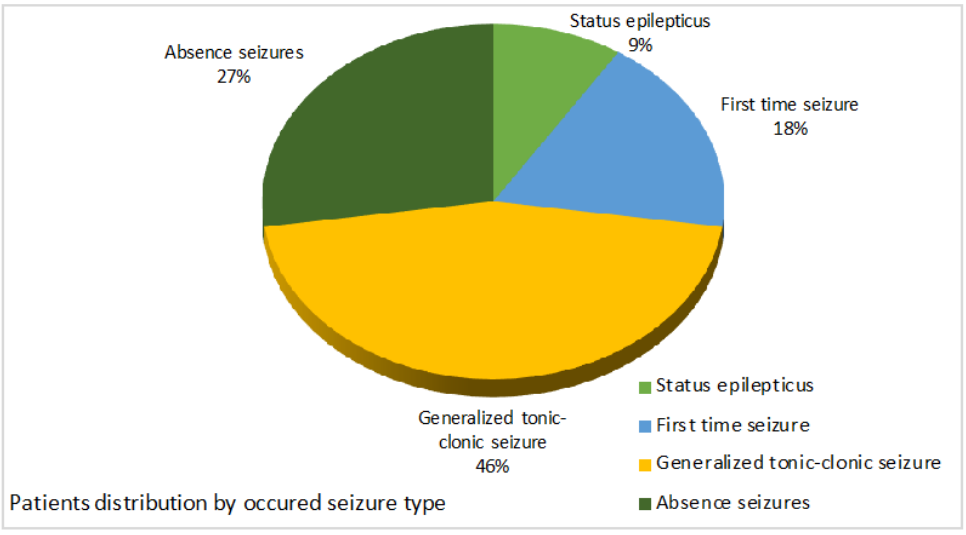


Figure 7. Patient distribution by occurred seizure type during dental treatment

## DISCUSSIONS

Epilepsy is an incessant disorder portrayed by the hazard of manifesting repetitive seizures. In Canada, the prevalence is 5.6 per 1,000 people, while in United Stated, the rate looks to be around 43 patients for every 1,000 personas. According to the World Health Organization, epilepsy represents about 1% of the worldwide, as projected by the incapacity to live a balanced life, positioning it soon after some mental issues, for example, alcohol dependence. Understanding epilepsy and seizures brings issues to light of the confusion effect it has on a patient's overall clinical state and mental wellbeing. The dental treatment planning and management of the patients suffering of epilepsy that manifest seizures ought to be done only by dental specialists who have learned about these issues. It is in like manner portrayed subject to the explanation, and it might be characteristic (achieved by a developmental transformation), idiopathic (when an innate condition is careful) or cryptogenic (when the justification is obscure) [8].

The elective treatment generally speaking incorporates the association of the best possible adversary of epileptic prescriptions (AEDs like carbamazepine, phenytoin, phenobarbital, primidone, valproic acid and many others) for the sort of seizure. In any case, different medications prescribed to control, the seizure mechanism might have very serious side effects on the oral care and also can massively affect the dental treatment planning and management [9].

In most undeveloped nations, for example in India epilepsy is mistakenly accepted to be irresistible or potentially brought about by detestable spirits. Subsequently, patients with epilepsy are derided and segregated [10]. This demeanor unfavorably influences the relational connections, instruction, profession/business openings, and the general strength of patients [11]. Self-care is fundamental to one's oral health. be that as it may, because of the unsupportive condition of numerous people with epilepsy, oral care and health is regularly disregarded [12, 13].

Looking at numerous studies worldwide, we have found that about 80% of the people suffering of epilepsy are keeping this disorder under control using anti-epileptic medication. This medication resembling anti-epileptic drugs are also called AEDs and most of them are being used in order to prevent or treat different types of seizures [14, 15]. Anti-epileptic drugs are prescribed by physicians assessing the patients kind of previous seizure, age, possible side effects and drug interaction and also there is considered the cost of the specific medication contrasting financial situation of the patient. There are multiple cases when the seizure episodes cannot be kept under control using only one prescribed medication and, in this situation an alternative AED is added creating a polytherapy against future seizures [16, 17, 18]. Certainly, mono-therapies are most desirable by the physicians because poly-therapy containing various drugs raise the development of multiple side effects. The most well-known unfriendly impacts of the treatment with AEDs are tiredness, drowsiness, unsteadiness, ataxia, and gastrointestinal issues. [19, 20] Anticonvulsants can likewise be the reason of many pathological modifications in the oral cavity. Most of the time, the patient might have the accompanying symptoms like soreness, dry mouth, red, sore or bleeding gums followed by swallowed lips, tongue or face. Other conceivable reactions of anticonvulsant medicine may incorporate bone abnormalities, which can prompt osteoporosis over the long haul of utilization. On the other side, the anti-epileptic drugs can cause augmentation of the gums since there is present gingival hyperplasia [21]. Preceding 1993 the selection of anticonvulsants was restricted to prescribing common drugs like carbamazepine, phenobarbital, phenytoin, primidone or valproic acid [14]. In the course of recent years, a few innovative different prescription drugs have been endorsed and approved after numerous studies by FDA also known as “Food and Drug Administration of United Stated.”

Medical surgery procedure is another treatment alternative for patients who seem to be refractory to the use of AEDs or have seizures or reactions that fundamentally debilitate their personal satisfaction [22]. They should likewise be somewhere in the range of 12 and 50 years of age. Past investigations have demonstrated 75% of patients become seizure free inside the main postoperative year. A few examinations archive the more drawn out the patient has epilepsy preceding medical procedure the more noteworthy there slip by hazard and they are bound to have post-careful airs. There are four generally acknowledged surgeries: corpus colostomy, hemispherectomy, central resection and the last is the different subpair exchange [23, 24, 25].

Factors like toothache and oral contamination, which cause torment, pain and make the patient awkward, may incite epileptic seizures. It is conceivable to treat and dispose of these elements during intermittent dental specialist arrangements, before intricacies emerge [16]. The level of gingival hyperplasia brought about by phenytoin ought to be constrained by forestalling the arrangement of plaque [26]. Be that as it may, plaque expulsion would be incapable except if hyper-plastic tissue is appropriately evacuated during gingivectomy [14]. On the off chance that hyperplasia repeat, the patient should change his prescription after a discussion with his primary care physician [5]. Examination shows that epileptic patients have seriously lacking mouth hygiene, oral wellbeing and poor dental condition, as contrasted and non-epileptic patients.

This is clarified by the way that these patients get inadequate dental consideration since they spend just a brief timeframe in the dental specialist's seat because of the danger of seizure [27]. Besides, their dental condition is compounded by wounds and harm caused to both hard and delicate tissues in the maxillofacial area during seizures. In this manner, defensive strategies, for example, the utilization of chlorohexidine and fluoride and having a routine dental check-up [28].

In the case where a patient suffering of epilepsy needs prosthetic treatment, the dental specialist ought to reflect the manufacture of the prosthetic rebuilding efforts impervious harm or rearrangement throughout an epileptic assault [16]. The removal of prosthesis conceivable dangers yearning of the prosthesis stacking in the respiratory tract. Fixed scaffolds of cast gold or embed reclamations can be unadulterated. They offer minimal possibility of relocation or break. All porcelain materials can present a very high danger of crack or might run a more serious danger by removal [21]. Thus, the patient ought to be educated regarding their remedial choices and the advantages and dangers.

## CONCLUSIONS

A careful approach with the patient's health history is the principle essential for effective treatment and can forestall numerous inconveniences. This data should be evoked during their underlying visit when the health history is revised. An important aspect related to the prescription of these patients should make the dental specialist aware of a potential seizure issue. The goal of such inquiries is to determine a total image of the patient's wellbeing. This incorporates assessing the effect of epilepsy in their lives, recognizing any oral issues, and limiting the danger of having an epileptic seizure during a dental visit. The data likewise helps with overseeing and treatment planning and limit any oral or general consequences later on.

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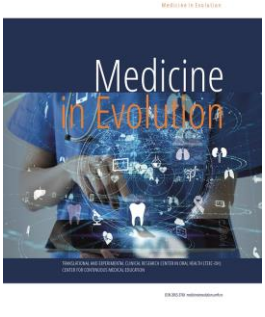
# Oral distribution of dental calculus in schoolchildren in Bucharest, Romania

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## Abstract

**Aim and objectives**: The main role of this paper is to find a pattern of oral distribution of dental calculus in children. **Material and methods**: The data presented in this paper are part of the PAROGYM study: 1595 of Bucharest schoolchildren aged 11 to 14 years were examined. Dental calculus was measured using Silness and Löe calculus index (CI). **Results**: The prevalence of dental calculus (CI score > 0) was 44%. Calculus was observed more on buccal and lateral surfaces of the first upper molars and on oral and lateral surfaces of the lower incisors. **Conclusions**: The oral distribution of dental calculus respects the general pattern but is quite different from the pattern of oral distribution of gingivitis.

**Keywords:** dental calculus, distribution, children

## INTRODUCTION

Caries and plaque-induced gingivitis are common oral diseases and frequently observed in children [1]. They are prevalent among schoolchildren from Bucharest, Romania aged 11-12 years [2,3].

Dental calculus is a complete mineralized bacterial deposit which covers some teeth surfaces or dental restorations and is one of the main risk factors for gingivitis and periodontal diseases. However, the correlation between calculus and gingivitis/periodontitis is not strong as correlation between dental plaque and prevalence of gingivitis/periodontitis, but still it is also difficult to compare because dental calculus is frequently covered with dental plaque [4]. Fortunately, dental calculus is not observed very often in children. It is very rare in preschool children and up to 34% between 14-17 years [5]. The calculus score was under 0.1 in 11-14 years old Bucharest schoolchildren population [3]. Green, orange and black stain can also be observed on dental check-ups in children [5].

### *Aim and objectives*

The main scope of this paper is to detect a pattern of oral distribution of dental calculus in children, including the teeth and surfaces frequently covered by calculus.

## MATERIAL AND METHODS

The data from this paper are part of the PAROGYM cross-sectional study and its design was described in previously papers. Briefly, 1595 children aged 11 to 14 years were examined for caries, teeth eruption pattern, periodontal diseases including gingivitis, and their risk factors such as calculus, caries and restorations in relation with periodontal tissues. Some data about caries, gingivitis and teeth eruption were previously published [2,3,6,7].

The classes ($5^{th}$ to $8^{th}$) were used like clusters and the sample was stratified on geographic areas of Bucharest (downtown, middle, outskirts and surrounding areas), grades, and the presence of a dental unit in schools.

Dental calculus was measured using Silness and Löe calculus index (CI) [8]:

- "0" = no calculus
- "1" = supragingival calculus
- "2" = subgingival calculus
- "3" = abundance of calculus

The analysis, including mean values of CI scores, was performed using the EpiInfo (Centers for Disease Control and Prevention, Atlanta, GA, USA) and SPSS software, version 16 (SPSS Inc., Chicago, IL, USA). At least one parent for every child examined in this study signed the inform consent. The study was approved by the Ethical Committee of the "Carol Davila" University of Medicine and Pharmacy.

## RESULTS

The prevalence of dental calculus (CI score > 0) was 43.7%.

Different forms of dental calculus were found: black stains (figures 1-3), supragingival calculus (figure 4), abundance of calculus (figure 5).

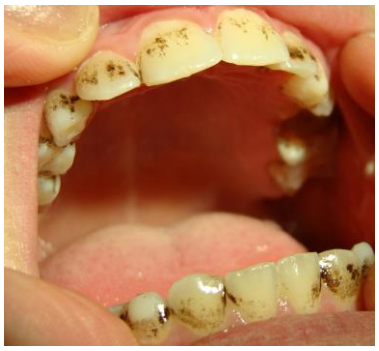
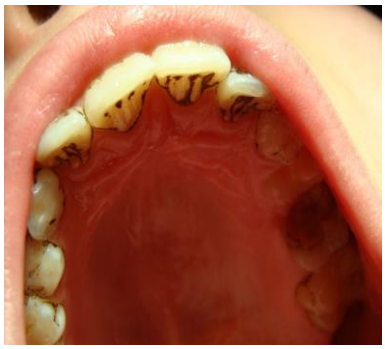
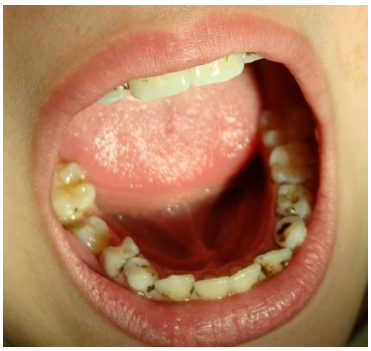

Figure 1, 2, 3. Black stains

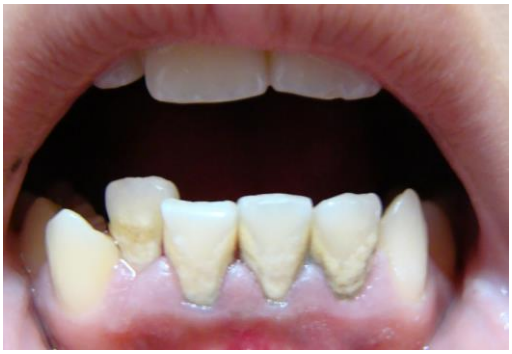

Figure 4. Supragingival calculus

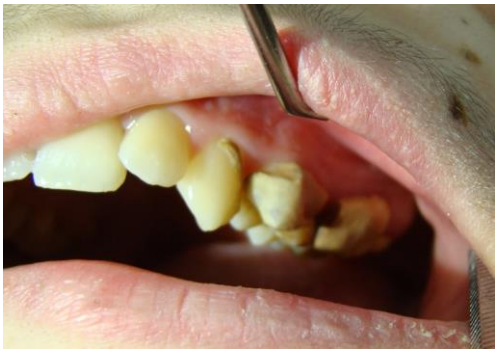

Figure 5. Abundance of calculus

The mean values for the CI scores for every tooth surface are exposed in tables no. I and II.

Table I. CI scores for the surfaces of the upper teeth

| Upper teeth | | | | | |
|---|---|---|---|---|---|
| **Tooth** | **Surface** | **CI score** | **Tooth** | **Surface** | **CI score** |
| 17 | buccal | 0.03 | 27 | buccal | 0.02 |
| 17 | mesial | 0.04 | 27 | mesial | 0.03 |
| 17 | distal | 0.04 | 27 | distal | 0.03 |
| 17 | oral | 0.02 | 27 | oral | 0.02 |
| 16 | buccal | 0.17 | 26 | buccal | 0.19 |
| 16 | mesial | 0.20 | 26 | mesial | 0.21 |
| 16 | distal | 0.18 | 26 | distal | 0.20 |
| 16 | oral | 0.04 | 26 | oral | 0.05 |
| 15 | buccal | 0.03 | 25 | buccal | 0.05 |
| 15 | mesial | 0.05 | 25 | mesial | 0.08 |
| 15 | distal | 0.06 | 25 | distal | 0.08 |
| 15 | oral | 0.02 | 25 | oral | 0.03 |
| 14 | buccal | 0.02 | 24 | buccal | 0.03 |
| 14 | mesial | 0.04 | 24 | mesial | 0.05 |
| 14 | distal | 0.04 | 24 | distal | 0.05 |
| 14 | oral | 0.02 | 24 | oral | 0.02 |
| 13 | buccal | 0.01 | 23 | buccal | 0.01 |
| 13 | mesial | 0.03 | 23 | mesial | 0.03 |
| 13 | distal | 0.03 | 23 | distal | 0.03 |
| 13 | oral | 0.02 | 23 | oral | 0.02 |
| 12 | buccal | 0.01 | 22 | buccal | 0.01 |
| 12 | mesial | 0.04 | 22 | mesial | 0.04 |
| 12 | distal | 0.03 | 22 | distal | 0.04 |
| 12 | oral | 0.03 | 22 | oral | 0.03 |
| 11 | buccal | 0.01 | 21 | buccal | 0.01 |

| | | | | | |
|---|---|---|---|---|---|
| 11 | mesial | 0.04 | 21 | mesial | 0.04 |
| 11 | distal | 0.04 | 21 | distal | 0.04 |
| 11 | oral | 0.03 | 21 | oral | 0.03 |

Table II. CI scores for the surfaces of the lower teeth

| Lower teeth | | | | | |
|---|---|---|---|---|---|
| **Tooth** | **Surface** | **CI score** | **Tooth** | **Surface** | **CI score** |
| 37 | buccal | 0.01 | 47 | buccal | 0.01 |
| 37 | mesial | 0.02 | 47 | mesial | 0.02 |
| 37 | distal | 0.02 | 47 | distal | 0.02 |
| 37 | oral | 0.03 | 47 | oral | 0.02 |
| 36 | buccal | 0.02 | 46 | buccal | 0.03 |
| 36 | mesial | 0.05 | 46 | mesial | 0.04 |
| 36 | distal | 0.04 | 46 | distal | 0.04 |
| 36 | oral | 0.07 | 46 | oral | 0.06 |
| 35 | buccal | 0.01 | 45 | buccal | 0.01 |
| 35 | mesial | 0.04 | 45 | mesial | 0.03 |
| 35 | distal | 0.03 | 45 | distal | 0.03 |
| 35 | oral | 0.03 | 45 | oral | 0.03 |
| 34 | buccal | 0.01 | 44 | buccal | 0.01 |
| 34 | mesial | 0.03 | 44 | mesial | 0.04 |
| 34 | distal | 0.03 | 44 | distal | 0.03 |
| 34 | oral | 0.03 | 44 | oral | 0.03 |
| 33 | buccal | 0.01 | 43 | buccal | 0.01 |
| 33 | mesial | 0.08 | 43 | mesial | 0.07 |
| 33 | distal | 0.08 | 43 | distal | 0.06 |
| 33 | oral | 0.06 | 43 | oral | 0.05 |
| 32 | buccal | 0.04 | 42 | buccal | 0.04 |
| 32 | mesial | 0.32 | 42 | mesial | 0.33 |
| 32 | distal | 0.28 | 42 | distal | 0.29 |
| 32 | oral | 0.19 | 42 | oral | 0.19 |
| 31 | buccal | 0.05 | 41 | buccal | 0.06 |
| 31 | mesial | 0.54 | 41 | mesial | 0.56 |
| 31 | distal | 0.50 | 41 | distal | 0.52 |
| 31 | oral | 0.32 | 41 | oral | 0.32 |

## DISCUSSIONS

This paper shows that at least 2 from 5 children have dental calculus. Similar results on similar age interval were discovered by Goel at all [9]. This is a very important item since presence of dental calculus influences gingival condition [10]. However, prevalence of gingivitis in Bucharest schoolchildren is much higher (slightly over 90%) [3], the mechanism of gingival inflammation being more complex than a simple presence of dental calculus. From the analyses of the previous papers, we discovered that both, gingivitis and dental calculus are related to economic and educational level and the presence of a dental unit in school [3].

Higher values of calculus scores were on oral and lateral surfaces of lower incisors, and on buccal and lateral surfaces of upper first molars. This observation is absolutely normal since dental calculus is a mineralized dental plaque deposit present where is a constant supply of saliva [11]. However, gingivitis is more intense around the maxillary lateral incisors. First upper molars and lower incisors being are not affected by gingival inflammation as much as lateral upper incisors [3]. So, the oral distribution of gingivitis is quite different from the oral distribution of dental calculus. There are many risk factors that influence gingival inflammation in addition to dental calculus, such as dental plaque and oral hygiene, hormonal background, dental crowding, dental restorations and crowns. So, the oral distribution of gingivitis is more complex and is related to all risk factors involved.

## CONCLUSIONS

The oral distribution of dental calculus respects the main pattern, first upper molars (buccal and lateral surfaces) and the lower incisors (oral and lateral surfaces) have deposits of calculus more often. On the other hand, the oral distribution of gingivitis is different, the mechanism of gingival inflammation being more complex than a simple presence of dental calculus.

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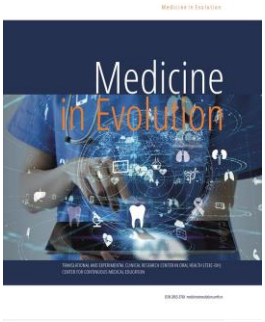
# Salivary interleukin-6, interleukin-8, and Tumor Necrosis Factor-alpha as a potential biomarker panel for early detection of oral squamous cell carcinoma

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## Abstract

Aim: Salivary components are now receiving more attention as potential biomarkers for various diseases. This study aims to investigate the possibility of using IL -6, IL -8 and TNF-α as salivary biomarkers for early detection of OSCC

Methods: This study followed the PRISMA protocol for reviews and meta-analyses and used PubMed as a database to identify articles that examined salivary concentrations of these mediators as potential biomarkers for early detection of oral squamous cell carcinoma.

Results: We found 14 studies that examined salivary markers in oral pathology and met the requirements for review.

Conclusion: Most studies showed increased concentrations of these mediators in the saliva of patients with OSCC. However, the values of salivary concentrations of IL -6, IL -8, and TNF-α in both OSCC and healthy patients remain controversial. Moreover, salivary concentrations of these mediators are influenced by the presence of oral comorbidities or the use of mouthwashes.

**Keywords:** Interleukin, tumor necrosis factor, oral squamous cell carcinomas

## INTRODUCTION

Saliva, along with blood, is one of the body fluids that contains a number of components whose properties can be helpful in diagnosing and evaluating the effectiveness of treatments for various diseases (1). Saliva contains proteins, metabolites, hormones, mRNA and enzymes (2). Previous studies have shown that these are present in saliva in much higher concentrations than in blood (3). The simple and non-invasive way of collection makes saliva a suitable tool for the detection of specific biomarkers (4). Analysing variations in their concentrations or changes in their structure/function in saliva is a simple way to diagnose and monitor certain diseases, with the detection of various diseases in their early stages or the identification of patients at risk of developing chronic diseases or even cancer being essential (5).The extent to which these goals are achieved can have a critical impact on the treatment, progression, and prognosis of these diseases, which in turn can affect the well-being, quality of life, and even lifespan of patients suffering from these diseases. The discovery of specific salivary biomarkers that provide information contributing to these goals is critical to the dynamics of this process.

Considering the multifactorial elements associated with the etiopathogenesis of oral cancers, the most recent studies focused on assessing a panel of salivary biomarkers targeting proteomic and transcriptomic targets (2, 5, 6, 7). Although most recent studies use modern techniques for detecting a multi-target panel with implications in oral squamous cell carcinoma (OSCC) pathogenesis, there is no uniformity in the results. Moreover, the same salivary biomarkers are also found in other cancers, such as ovarian, lung, and pancreatic cancer (8, 9, 10). In addition, the concentration of salivary biomarkers varies with salivary flow, viscosity, and dietary intake (11).

In recent years, mounting evidence has suggested that cytokines play a significant role in carcinogenesis (12). In most tumor processes, there is an abundant cytokine palette of pro-inflammatory cytokines, chemokine, and growth factors associated with tumor induction and development (13). Most neoplastic processes show a perturbance in the balance between cell survival and apoptosis (14). Among the principal cytokines involved in this process are the pro-inflammatory cytokines interleukin-6 (IL-6) and Tumor Necrosis Factor-alpha (TNF-α) and chemokine interleukin-8 (IL-8) (15). Data from studies so far suggest that IL-6, IL-8, and TNF-α contribute to the initiation of the neoplastic process by protecting it from apoptosis and favoring cell growth and angiogenesis (16). These inflammatory mediators have been detected in tumor cells and stroma but also in the plasma and saliva of patients with oral and general diseases, including cancer (14,15, 19).

### *Aim and objectives*

In this study, I aim to analyze the potential usefulness of a combination of IL- 6, IL- 8, and TNF-α as a reliable salivary panel biomarker for early detection of oral cancer.

## METHODOLOGY

An electronic literature search for research articles published between 2003 and 2023 was conducted using PubMed. For the search engine, the following keywords were used:

OSCC, oral premalignant lesion, oral leukoplakia, dental caries, gingivitis, periodontitis combined with salivary interleukin, or salivary cytokine.

**Study selection**

Study selection was done using the PRISMA statement. Study selection was conducted using the following criteria.

Inclusion criteria

1. Original research articles including interleukin 6, 8, and TNF-alfa as a salivary biomarker panel in oral diseases and disorders.
2. Original research articles including over ten subjects.
3. Full text is available.

Exclusion criteria

1. Reviews, meta-analysis, case series.
2. Studies that did not analyze this biomarker panel in saliva.
3. Studies that analyze this biomarker panel implication in cancer progression, evolution, metastasis, and survival rate.
4. Studies including subjects less than eighteen years old.

Abstracts were exported to Sci-Hub for full text.

**Data extraction**

From the included articles, we extract only data referring to the interleukin 6, interleukin 8, and TNF-alpha.

## RESULTS

The initial search of the PubMed database identified 597 articles. After evaluating the articles, we included 14 articles in this analysis (Figure. 1).

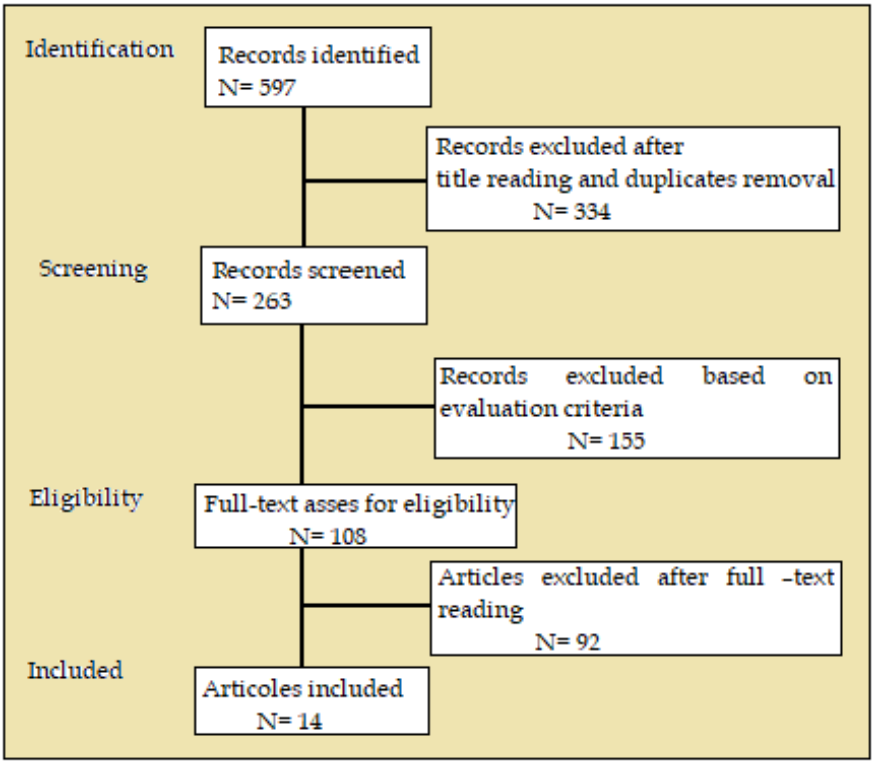


Figure 1. PRISMA flowchart of study selection

## DISCUSSIONS

Squamous cell carcinoma is the most common oral tumor (17), and diagnosis at advanced stages challenges treatment efficacy, prognosis, and survival. Despite all efforts, there are currently no biomarkers for early diagnosis of OSCC or for identifying patients at increased risk of developing OSCC. However, there are a number of indications of the

possibility of using inflammatory mediators for the detection of early OSCC (16). Because of its accessibility, the noninvasive nature of saliva collection (18), and also because of its direct contact with the tumor process, it is considered a suitable tool for the detection of these mediators (16). Among the inflammatory mediators, IL -6, IL -8, and TNF-α, are among the most studied salivary compounds in OSCC and other oral diseases and disorders.

Previous data from studies examining this salivary panel suggest that these mediators are significantly elevated in the saliva of OSCC patients in most cases. Lee et al. (2017), Csősz et al. (2017), Dikova et al. (2021), and Rai et al. (2021) found increased levels of IL -6, IL -8, TNF-α in the saliva of OSCC patients compared to patients without cancer using the same study method (Luminex-based multiplex kit) (Table 1) (3,19,20, 21). Moreover, in the analysis performed by Dikova et al. a progressive increase in the levels of these cytokines in the saliva of patients with early-stage OSCC was detected compared to patients with oral leukoplakia (OL) and healthy patients (Table 2), indicating the pre-neoplastic character of OL (21).

Table 1. Salivary cytokine in OSCC

| Study | Citokine | p-value | Diseases evaluated |
|---|---|---|---|
| Lee at al. 2017 (3) | **IL-6 ↑**<br>**IL-8 ↑**<br>**TNF-α ↑** | **< 0.001**<br>**0.001**<br>**0.001** | **OSCC > HC** |
| Csősz et al. 2017 (19) | **IL-6 ↑**<br>**IL-8 ↑**<br>**TNF-α ↑** | **0.0002**<br>0.1087<br>**0.0157** | **OSCC > HC** |
| Dikova et al. 2021 (21) | **IL-6 ↑**<br>**IL-8 ↑**<br>**TNF-α ↑** | **<0.001**<br>**<0.001**<br>**<0.001** | **OSCC > HC**<br>**OSCC > HC**<br>**OSCC > HC** |
| Rai et al. 2020 (20) | **IL-6 ↑**<br>**IL-8 ↑**<br>**TNF-α↑** | **0.0259**<br>**0.0228**<br>**0.0321** | **OSCC > HC** |

Abbreviation: OSCC, oral squamous cell carcinoma; HC, Healthy control; ↑, Increased level

Similar results were previously reported by Rhodus et al (2005), who used ELISA analysis to detect elevated levels of these mediators in the saliva of patients with OSCC and premalignant oral lesions compared with controls (Table 2) (22).

Table 2. Salivary cytokine in OSCC versus oral precancerous lesions

| Study | Citokine | p-value | Diseases evaluated |
|---|---|---|---|
| Dikova et al. 2021 (21) | **IL-6 ↑**<br>**IL-8 ↑**<br>**TNF-α ↑** | **a) <0.001; b) 0.001**<br>**a)≤0.05; b)0.004**<br>**a)<0.001; b)0.001** | **a)OSCC> OL**<br>**b)OL>HC** |
| Rhodus et al. 2005 (22) | **IL-6 ↑**<br>**IL-8 ↑**<br>**TNF-α↑** | **<0.001**<br>**<0.001**<br>**<0.01** | **OSCC> OPML>HC** |

Abbreviation: OSCC, oral squamous cell carcinoma; OL, oral leucoplakia: HC, Healthy control; OPML, oral premalignant lesion; ↑, Increased level

Salivary levels of this group of mediators were also examined independently of OSCC. Using an ELISA assay, Kaur et al. (2015) found significantly elevated levels of these cytokines in the saliva of patients with premalignant oral lesions compared with controls (23). In this study, subgingival fibrosis had the highest salivary levels of IL -6, IL -8, and TNF-alpha (Table 3). In contrast, in a 2022 study examining these three mediators, only IL -6 and TNF-α were found to be significantly elevated in the saliva of patients with oral lichen planus (OLP) (24).

Table 3. Salivary cytokine in oral precancerous lesio versus healthy control

| Study | Citokine | p-value | Diseases evaluated |
|---|---|---|---|
| Kaur et al. 2015 (23) | **IL-6 ↑**<br>**IL-8 ↑**<br>**TNF-α ↑** | **<0.05**<br>**<0.05**<br>**<0.05** | **SB>OL>OLP>HC**<br>**SB>OLP>OL>HC**<br>**SB>OLP>OL>HC** |
| Zhu et al. 2022 (24) | **IL-6 ↑**<br>**IL-8 ↑**<br>**TNF-α ↑** | **0.022**<br>0.172<br>**0.012** | **OLP > HC** |

Abbreviation: OLP, Oral lichen planus; OL, oral leucoplakia; HC, Healthy control; SB, submucous fibrosis; ↑, Increased level

Aside from OSCC and salivary premalignant lesions, IL-6, IL-8, and TNF-α, have been considered salivary biomarkers for inflammatory processes in the oral cavity. Evaluation of the salivary concentration of these mediators showed a significant increase of these mediators in the saliva of patients with dental caries (25, 26). Salivary levels of IL -6, IL -8 and TNF-α were also significantly increased in patients with chronic periodontitis (Table 4) (27).

Table 4. Salivary cytokine in oral inflammatory condition

| Study | Citokine | p-value | Diseases evaluated |
|---|---|---|---|
| Gornowicz et al. 2012 (25) | **IL-6 ↑**<br>**IL-8 ↑**<br>**TNF-α ↑** | **<0.005**<br>**<0.008**<br>**<0.002** | **DC>HC** |
| Hussein et al. 2020 (26) | **IL-6 ↑**<br>**IL-8 ↑**<br>**TNF-α ↑** | **0.005**<br>**0.008**<br>0.063 | **DC>HC** |
| Kaczyński et al. 2019 (27) | **IL-6 ↑**<br>**IL-8 ↑**<br>**TNF-α ↑** | **< 0.0001**<br>**< 0.0001**<br>**< 0.0001** | **PG>HC** |

Abbreviation: DC, dental caries; HC, Healthy control; PG, periodontitis group; ↑, Increased level

Following the salivary values of IL-6, IL-8, and TNF- α detected in the studies analyzed, we observed significant differences between the salivary concentrations of these mediators in healthy patients. Salivary concentrations can vary up to 2.5-fold for TNF- α, 5-fold for IL-6 and 6-fold for IL-8 (Table 5).

In addition, Rhodus et al. determined a TNF-α level in the saliva of patients with OSCC (28.9 ± 14.6 pg/ml) that was lower than the level found by Gornowicz et al. in patients with dental caries (36.50 ± 41.46 pg/ml), using the same examination method (22, 25). Controversy regarding salivary concentrations in OSCC was also noted for Il-8. Korrostof determined values of 1242 ± 408 pg/ml in exophytic lingual SCC, 1585 ± 348 pg/ml in healthy smoking patients, and 1672 ± 310 pg/ml in healthy patients consuming alcohol and smoking (28).

Table 5. Saliva concentrations of IL-6, IL-8, and TNF- α in healthy people were measured using the ELISA technique. (pg/ml)

| Study | IL-6 | IL-8 | TNF-α |
|---|---|---|---|
| Laliberte at al. 2021 (29) | 5.21 ± 1.14 | 256.50 ± 86.21 | 3.06 ± 0.66 |
| Dikova et al. 2021 (21) | 7.95 ± 0.95 | 526.17± 59.03 | 7.62 ± 0.84 |
| Gornowicz et al. 2012 (25) | 2.68 ± 5.51 | 619.19 ± 311.79 | 7.32 ± 6.98 |
| Korrostof et al. 2011 ( 28) | 3.4 ± 1 | 932 ± 262 | 3.9 ± 2.6 |
| Rhodus et al. 2005 ( 30 | 2.18 ± 0.71 | 1507.2 ± 398.5 | 3.36 ± 2.07 |
| Rhodus et al. 2005 (22) | 1.4 ± 0.9 | 1580 ± 789.0 | 3.0 ± 1.9 |

In addition, the oral localization of OSCC and the associated periodontal inflammation caused by poor oral hygiene in these patients lead to changes in salivary concentrations of these cytokines (3). In addition, the use of mouthwash for a prolonged period of time was found to affect salivary concentrations of IL -6, IL -8, and TNF-α. OLP patients who used

mouthwash for one month showed a decrease in the concentration of these mediators according to Rhodus et al. (2006) (31).

## CONCLUSIONS

Despite promising results showing remarkable differences of these mediators in OSCC compared with premalignant lesions or healthy patients, there are still conflicting results regarding the concentrations of these mediators in saliva. To date, there is no clear consensus on the concentration of these mediators in saliva of healthy individuals. In addition, the concentration of these mediators in saliva may be influenced by the presence of local comorbidities or the use of mouth rinses.

Although these salivary biomarkers have been detected in OSCC, the evidence is not convincing enough to consider the combination of IL -6, IL -8, and TNF-α as a valid salivary biomarker panel for early detection of OSCC or for screening high-risk patients.

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# An insight into the effect of ultraviolet radiation: from promotion of skin malignancies to use in dentistry


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## Abstract

Exposure to ultraviolet radiation (UV) can occur either naturally - through exposure to solar radiation, or synthetically - through exposure to various devices that emit ultraviolet radiation. As far as the oral cavity is concerned, the oral cells meet solar ultraviolet radiation once the mouth is opened or with ultraviolet radiation applied with the help of devices used for diagnostic or treatment purposes in dental procedures.

Ultraviolet radiation is on the list of recognized carcinogens (WHO, 2009) and specialist studies have highlighted the fact that oral cells are much more sensitive to these radiations than skin cells. Considering these premises, the current study proposes to highlight the effects produced by ultraviolet radiation, through the prism of its use for diagnostic and therapeutic purposes in the field of dental medicine, but without neglecting the negative effects produced on the skin, the first defense barrier of the human body against harmful factors in the environment. At the same time, the connection between the damage of oral tissues due to exposure to environmental ultraviolet radiation and the state of knowledge in the field regarding the effectiveness of the use of UV in the newest techniques in dentistry is analyzed.

**Keywords:** oral cavity, ultraviolet rays, tumor cells, carcinogenic effect

## INTRODUCTION

The general health implications of exposure to ultraviolet radiation (UV) are dependent on the type of radiation, dose, and exposure time. Of clinical importance are type B ultraviolet radiation (range 280-315 nm) and type A ultraviolet radiation (315-400 nm) [1]. Solar UV type B radiation is mainly responsible for inducing erythema and increasing melanin content (producing tanning) [2]. These clinical, acute effects can also be produced by UV type A radiation if the physical doses (expressed in J/cm$^2$) administered are approximately a thousand times higher [3].

Ultraviolet radiation produces immunosuppression, the consequences of which are not fully known, but which play a central role in the initiation of malignant diseases [2,4]. Overexposure to ultraviolet radiation can weaken the immune system, an effect that simultaneously weakens the skin's role as a barrier against harmful agents [2]. Regarding the differences between ultraviolet radiation of natural origin and those of artificial origin, it should be specified that there are no intrinsic differences in terms of physical properties, but there may be differences in the spectral profile which, in turn, may produce biological effects. The comparison between the acute effects produced by natural radiation and artificial radiation is relatively easy to achieve, while the comparison between the chronic effects represents a challenge for specialists in the field [2,5]. Tanning devices for cosmetic purposes have sparked a series of controversies since both positive and negative effects have been recorded following their use [6]. Regarding the positive effects, besides obtaining the desired cosmetic effect (tanning of the skin), achieving a state of well-being, even improving the status of vitamin D (although the data in this regard are quite limited). Regarding the negative effects, these devices are associated with the development of malignant melanoma, respectively ocular melanoma [2,6].

The biological effects exerted by a certain emission spectrum have a relevant importance compared to the specific irradiation (the wave bands that represent physical parameters) [7]. The effective dose in terms of the biological effect is achieved after weighting a given emission spectrum with the relevant action spectrum. This relevant spectrum of action should be the spectrum of action of human erythema, known to be like the spectrum of action that produces the tanning effect but also to the spectrum of action that leads to the development of squamous cell carcinoma [2].

Therefore, the irradiation should not exceed a value of 0.3 W/m$^2$, the value associated with the erythematous weighted maximum irradiation, which is equivalent to the tropical sun, called extreme by the World Health Organization (with the specification that it is valid only for the known acute effects) [2,8].

Following the evaluation of the risk of malignant diseases, especially those of the skin, it is not possible to establish some limits regarding the dose due to the lack of dose response data in humans [9]. The main factors that lead to the appearance of malignant transformations are of two types: biological factors (age, sex, skin phenotype, family history, moles) and environmental factors (mode of exposure to ultraviolet radiation) [2,10].

### *Aim and objectives*

The main purpose of this study is to highlight the effects produced by ultraviolet radiation, through the prism of its use for diagnostic and therapeutic purposes in the field of dental medicine, but without neglecting the negative effects produced on the skin, the first defense barrier of the human body against harmful factors in the environment. At the same time, the connection between the damage to oral quality due to exposure to environmental

ultraviolet radiation and the state of knowledge in the field regarding the effectiveness of the use of ultraviolet radiation in the newest techniques in dentistry is analyzed.

## MATERIAL AND METHODS

Six specialized databases were used for the original systematic study: PubMed, Science Direct, de Gruyter, Wiley Online Library, Springer, and Google Scholar. The databases were searched for research and analyzes that describe the involvement of ultraviolet radiation in the field of dentistry, but also their connection with malignant skin diseases. The search was focused on the six databases in English and later the bibliographic references of the research considered relevant. The selection criteria were based on the importance of using ultraviolet radiation for diagnostic and treatment purposes in the field of dentistry, and following the analysis of the studies considered relevant, a quantification and interpretation of the results was carried out.

Further, a questionnaire was proposed to evaluate the specialized knowledge among the specialists in the field of dentistry. The selected studies were evaluated by a scientific committee whose members come from the disciplines involved in the study, together with specialists, and the following steps were taken: 1) reducing the number of studies to design a simple and concise questionnaire, 2) making decisions definitive questions related to the development of the questionnaire, 3) establishing the questions so that the answers can be scored based on a Likert-type scale from 1 to 4.

## RESULTS AND DISCUSSIONS

Ultraviolet radiation is one of the oldest methods used for sterilization/decontamination and is still successfully used to inactivate various microorganisms. In dentistry, ultraviolet radiation is used for both diagnostic and therapeutic purposes. Ultraviolet light is an invisible light, with few exceptions, the cases where fluorescence occurs, one of the properties for which it is used intensively in modern medicine, as can be observed in figure 1 [11].

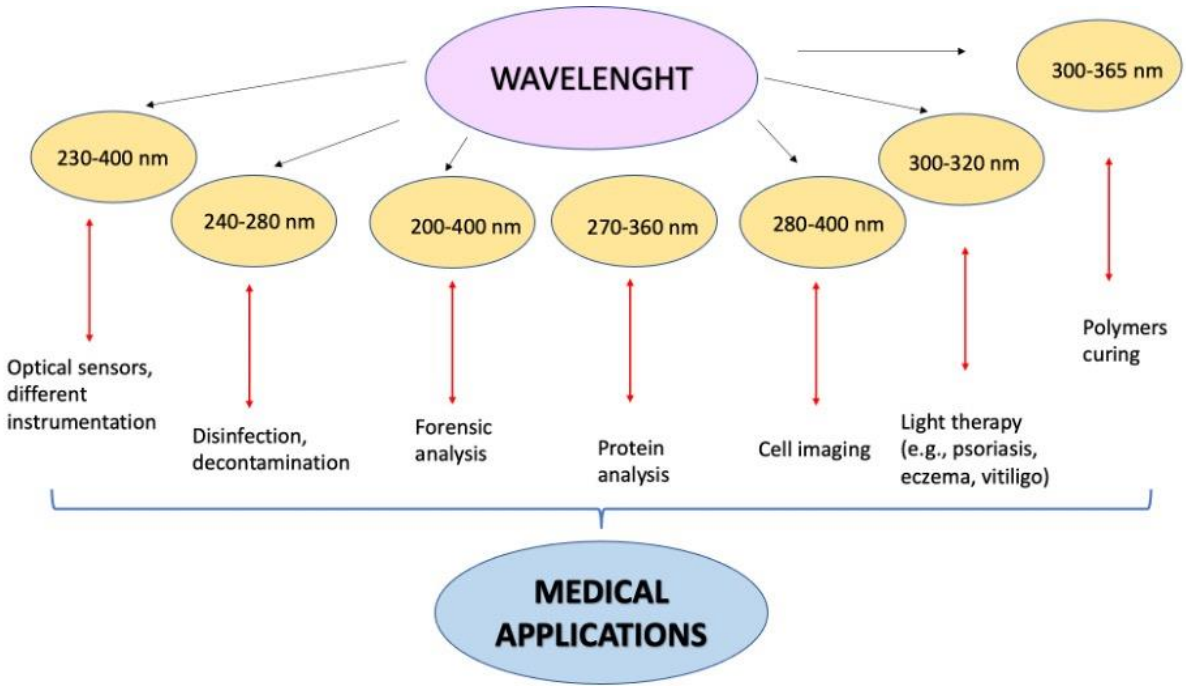


Figure 1. Ultraviolet radiation related to medical applications

At an initial search, in six different databases (PubMed, Science Direct, Wiley Online Library, de Gruyter, Springer and Google Scholar), after the keywords "ultraviolet radiation in dental medicine", the results were extremely varied, as can be seen in the figure below (figure 2).

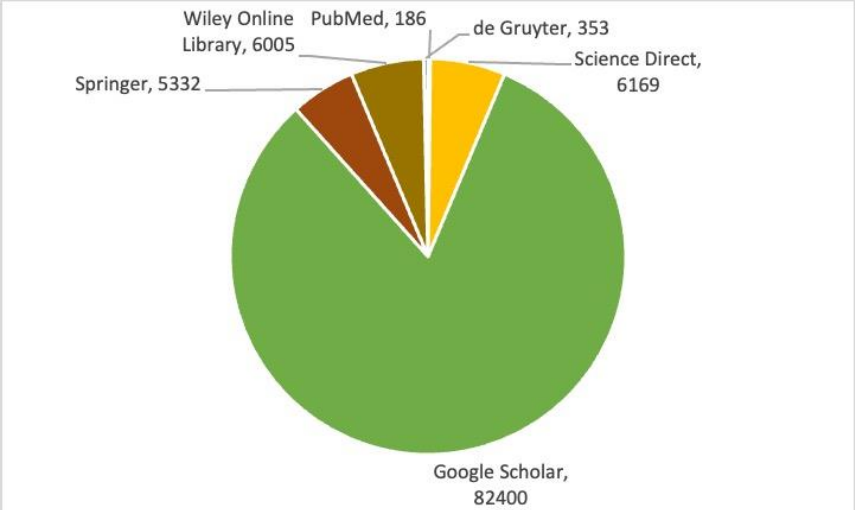


Figure 2. Ultraviolet radiation in dental medicine – results from databases

Several key searches were performed in the mentioned databases, and the results of the searches are presented in figure 3.

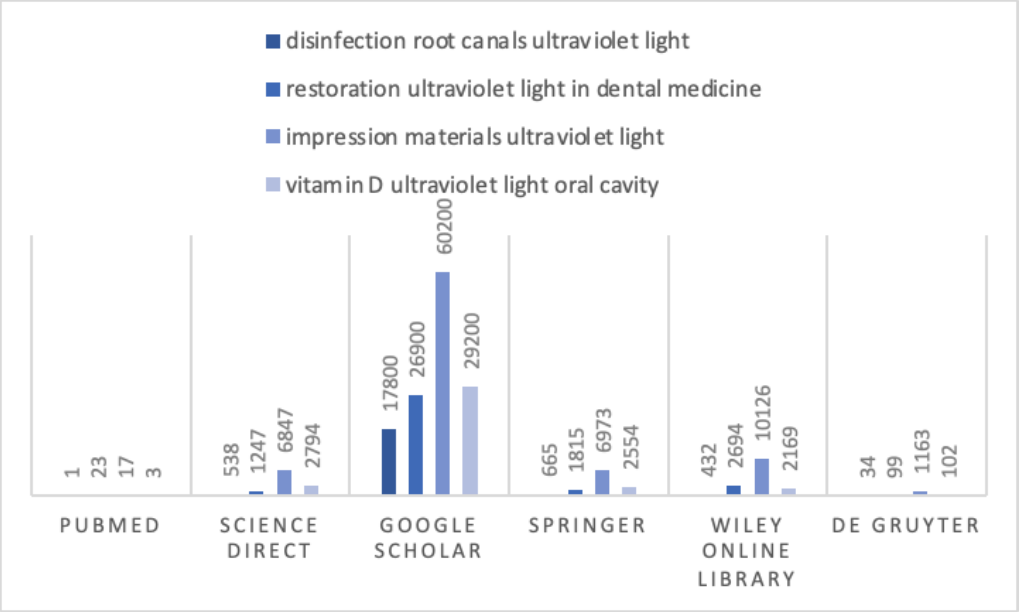


Figure 3. Ultraviolet radiation related to disinfection of root canals, restoration, impression materials and vitamin D – results from databases

Based on the literature study and following the analysis of the search results in the six databases, a questionnaire was proposed to be used by health specialists. The role of this questionnaire is to increase the knowledge related to the applicability of ultraviolet light in dental medicine, to deepen the knowledge related to the latest techniques that use ultraviolet light in dental medicine and to be aware of both the beneficial effects and the risks deriving from the use of ultraviolet light in the oral cavity.

Table 1. Questionnaire model for specialists in the field of dentistry

| Questions | Responses / Observations |
|---|---|
| Are you concerned with the use of techniques that involve ultraviolet radiation in the diagnosis of dental conditions? | |
| Specify if you have in-depth knowledge about the use of ultraviolet radiation in the medical field | |
| How often do you use ultraviolet light in the specialized techniques you practice? | |
| Do you use ultraviolet radiation for root canal disinfection? | |
| Do you use ultraviolet radiation for restoration? | |
| Do you use ultraviolet radiation for impression materials? | |
| What do you know about the role of vitamin D production after exposure to ultraviolet radiation and the effects in the oral cavity? | |
| Describe the products you use most frequently that involve ultraviolet radiation | |
| Describe the products (which involve ultraviolet light) that present risks for specialized personnel | |
| What do you think about the use of ultraviolet radiation in combination with classic treatments against microbial agents in the oral cavity? | |
| Do you know if there is any connection between exposure to ultraviolet radiation and the occurrence of oral cancer? | |

One of the frequent uses in dental technique is related to working with resins in the oral cavity. A series of composite resins have the property of emitting fluorescence in contact with ultraviolet light (optimal excitation length 385-395 nm), being divided into intensely fluorescent composite resins, moderately fluorescent composite resins and weakly fluorescent composite resins [12]. The application of ultraviolet light is a particularly useful technique that helps the specialized medical staff to evaluate the complete removal of this in each individual case. Data related to the fluorescent properties of composite resins, in the presence of ultraviolet light, are detailed and reliable. Therefore, following the development of technology in recent years, reliable tools have been developed that can be easily manipulated by dentists (e.g., flashlights with ultraviolet light emitting diodes) and more. Even forensic doctors can use these tools in special conditions, in which victims are identified by dental impressions, because currently amalgams are no longer used in dental procedures, but composite resins [13]. For example, it should be mentioned that resin brands emit fluorescence at certain varying wavelengths and at the same time, with intensities [14]. Therefore, the use of flashlight-type devices revealed that the most useful excitation wavelengths, for the detection of composite resin, are in the range of type A ultraviolet radiation (365 and 380 nm) [12,15]. Thus, restorations (for example those made of porcelain) and fillings (based on composite resin) can show different responses to the mentioned wavelengths, thus the use of both is recommended, especially in the forensic field [11].

Another application of ultraviolet light in dentistry is based on its disinfecting properties. In the case of using ultraviolet light (254 nm, 300 mJ/cm$^2$) as a disinfection technique on root canals, immediately after treatment with 5% sodium hypochlorite, it led to spectacular results regarding the eradication of *Enterococcus faecaliss* [16,17]. Therefore, the application of ultraviolet radiation to the root canals can be a successful complementary technique in combating harmful microorganisms found in the oral cavity.

And in the case of bacteria that produce tooth decay, ultraviolet light finds its applicability. These bacteria, following metabolic processes, generate porphyrins which, following ultraviolet light irradiation, emit a specific red fluorescence [11].

The impression materials are also sterilized with the help of ultraviolet light, with different results [18]. Specialized research has highlighted the effectiveness of using ultraviolet light, specifying that silicone-based impressions can be sterilized with ultraviolet light (sterilization period 20 minutes) [11,19].

Regarding the processes that take place in the skin following exposure to ultraviolet radiation, the mechanisms involved are multiple, some of them understood, some of them incompletely elucidated. What is known for sure is that exposure to ultraviolet radiation stimulates the natural production of vitamin D. Therefore, the beneficial effects exerted are closely related to this process, taking into account the fact that vitamin D has multiple roles in the body, among which regulates calcium metabolism, increases immunity, stimulates cell proliferation, keeps blood pressure within normal parameters, etc. [11]. As for the beneficial effects on the oral cavity, they are related to the beneficial action on calcium metabolism and the induction of cathelicidin (antimicrobial peptide) liable for fighting the bacteria responsible to produce dental caries [20]. Despite these known data, it is not yet known exactly whether the additional administration of vitamin D has a significant contribution in combating dental caries or in reducing the risk of their occurrence [21].

It is well known that UV radiation causes cell damage that can lead to malignant cell transformation. These radiations act directly on cellular molecules, causing DNA damage, through energy absorption, and induce the generation of intracellular reactive species. Instead, the use of ultraviolet radiation in an innovative cold plasma treatment device in the dental field has proven to be safe and a real success, taking into account the fact that no damage was recorded, and it was not proven to favor carcinogenesis [22]. Cold atmospheric pressure plasma is mainly used to promote re-osseointegration, decrease the risk of antimicrobial infections and contribute to wound healing. The long-term treatment of this method compared to the irradiation of cells was studied on an animal model and the results of the study indicated that the technique is safe, as no damage to the cells was recorded. In contrast, exposure of the cells to irradiation revealed non-invasive lesions and squamous cell carcinoma. Therefore, the repeated exposure of the cells to this treatment is well tolerated, being devoid of carcinogenic effects at the same time [22].

## CONCLUSIONS

Studies reveal a connection between natural ultraviolet radiation (UV) and the occurrence of malignant processes. Oral cells are significantly more sensitive to ultraviolet radiation than skin cells. At the same time, the use of ultraviolet radiation in the dental field, both for diagnostic and therapeutic purposes, is devoid of significant adverse effects. The antimicrobial activity in the dental sphere of ultraviolet radiation has been proven to be pronounced, especially when they are used in combination with classic disinfection treatments. However, there is a need to deepen the mechanisms involved in the damage to oral cells by the two types of ultraviolet radiation of clinical interest, UV type B and UV type A, considering their origin (natural/synthetic), exposure time and the dose used.

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# Efficacy of Goccles medical device in the screening of potentially malignant oral lesions- an experimental study


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## Abstract

Oral cancer is characterized by the abnormal growth of cells that can invade any part of the mouth, including the gums, tongue, hard palate, and soft palate. GOCCLES® uses autofluorescence technology to aid in the early detection of oral cancer lesions. This non-invasive screening method is more effective than visual inspections and can detect even the slightest tissue changes. While traditional white lights decrease the dentist's ability to identify affected areas, GOCCLES® allows them to see subtle changes in intense fluorescent green light. With such advanced technology, dentists can more efficiently diagnose oral lesions at the first signs, thereby increasing the chances of successful treatment. Additionally, GOCCLES® can be used as a monitoring tool to ensure that patients have ongoing oral health.

**Keywords:** GOCCLES, oral cancer, fluorescent green light

## INTRODUCTION

Oral cancer or malignant oral tumour, presents as a swelling or lesion of the oral mucosa that does not heal, caused by the uncontrolled growth and division of cells. The early symptoms of oral cancer are not visible to the naked eye. [1] The spread of oral cancer is rarely associated with severe pain. More than 2/3 of all oral cancers are diagnosed only in advanced stages. Early detection of oral cancer increases the survival rate to approximately 80% [1].

The screening procedure of oral cancer is painless, free of radiation risk, non-invasive, and quick to perform during routine visits to the dentist [2]. It is a test that uses clinically proven technology to detect oral lesions and is recommended at least once a year.

The screening requires a special effort to ensure the quality of services, as only some people invited to participate in cancer screening have the disease or incipient conditions that could lead to cancer [3].

In recent years, the number of cases of oral cancer have increased significantly, especially among men. This has led researchers to conduct studies to find out the etiological factor. The largest increases have been reported in throat and tongue cancer. [3] This has been attributed to the human papillomavirus (HPV) [4],[5].

The American Cancer Society estimates that approximately 50,000 people will be infected with this virus, and 9,500 of them will die of this, only this year [6].

Invasive squamous cell carcinoma [7] is often preceded by clinically recognizable premalignant changes [8], [9] of the oral mucosa. These lesions are often present as white or red patches, known as leucoplakia and erythroplakia. As the cancer develops, the patient may notice the presence of a persistent ulcer. [10] Symptoms in later stages include bleeding, tooth mobility, difficulty wearing dentures, dysphagia, and the development of a mass in the posterior region of the oral cavity [9].

Efforts should be made to establish a definitive diagnosis and maximize time for treatment of patients with more severe lesions, using a therapeutic protocol [11],[12].

GOCCLES® (Glasses for Oral Cancer - Curing Light Exposed - Screening) (PIERREL PHARMA, ITALY) is a medical device created to provide comfortable, easy, and low-cost direct visualization of abnormal tissue in the oral cavity [13].

GOCCLES® Medical Glasses (Glasses for Oral Cancer - Curing Light Exposed - Screening) (PIERREL PHARMA, ITALY) is a pair of glasses with a filter that highlights the autofluorescence of the mucosa when illuminated with a photopolymerizable lamp or the light from Oral ID that emits blue light (435-460nm) [18],[19].

The device was created to provide direct fluorescence with low costs, visualizing oral tissue abnormalities, and was introduced to the consumer market in 2015. Other fluorescence-based devices available on the market, such as VELscope Vx® (Visual Enhanced Lesion Scope, by LED Dental Inc, BC, Canada, do not require the use of dyes or rinses during the procedure [16].

The GOCCLES device represents a modern pair of glasses, reported to be less heavy and noisy than a device such as VELscope Vx [14], [21-31]. VELscope Vx has its own halogen tungsten light source over which current passes and heats very quickly. This design means that it needs to be charged frequently and has a cooling fan inside the device. The latter is responsible for the noise of the device [15], [20].

By comparison, GOCCLES® works efficiently with any light curing unit, which mostly are LED and, therefore, do not create heat, making them easier to use. These glasses are packaged in a special protective box, which makes them easy to transport if working in

multiple locations. VELscope Vx constantly requires single-use green filters, while the glasses have an incorporated filter, meaning that post-purchase costs are nil [14],[15].

Another aspect related to VELscope Vx is the device's design being too large, which requires the clinician to get too close to the patient's face. By using the special glasses, the clinician can stand in front of the patient and orient themselves well through peripheral and spatial awareness [17-23]. The only advantage of the VELscope Vx over Goccles is that the halogen has a higher light intensity than LED and is not as sensitive to ambient light [16-24].

However, a factor to consider for both devices is the possibility of reducing ambient light. The darker environment, reflect the better fluorescent tissues.

The research method involving analysis of the oral mucosa during a routine consult increases the early detection rate of oral cancer. Including screening in the daily routine of a physician treating their patients is perhaps the most important thing after pain relief.

### *Aim and objectives*

The objective of this clinical study was to evidence the ability/ efficacy of the GOCCLES® medical device examining the autofluorescence of the oral mucosa, inpatients with different potentially malignant lesions.

The major objectives of oral screening include: detecting and recognizing lesions with malignant potential in the oral cavity, examining, collecting, and analysing samples for confirmation and differentiation, developing a presumptive, differential, and definitive diagnosis, and establishing treatment plan in accordance with the diagnosis.

## MATERIAL AND METHOD

A clinical experimental study was conducted at the Discipline of Oral Pathology of the Faculty of Dental Medicine in Timisoara, respecting the regulations of the Declaration of Helsinki regarding the studies on humans. Different patients, referred to the department for an adjuvant screening for oral cancer were randomly tested, and those who presented lesions were prioritized. A number of 9 patients, aged 18-29, were taken in consideration for this experimental study. All participants signed inform consent upon their inclusion in the study.

Most of the patients presented oral lesions that were visible to the naked eye, but there were also patients who wanted to be screened even if they did not present lesions.

The research method is clinically non-randomized. Patients were placed in the dental chair, with protective glasses, and the dental assistant positioned the light from the lightcuring unit perpendicular to the lesion.

To capture the autofluorescence of the oral mucosa, we used the camera of the phone, and the GOCCLES® glasses were placed as a filter in front of the camera. The light was held at a distance of 20-40 cm from the mucosa. Some practitioners used the blue light from Oral ID.

We examined the entire oral mucosa as well as the dorsal and ventral surface of the tongue. Several pictures were taken from different incidences of the lesions. Some of the patients were recalled after two weeks for a follow-up in order to observe the evolution of the lesions.

## RESULTS

The first case was that of a patient who did not present visible lesions with the naked eye. The patient wanted to benefit from an oral cancer screening in the absence of obvious lesions. We took pictures of the ventral and dorsal surface of the tongue, the left and right jugal mucosa using GOCCLES glasses (PIERREL PHARMA, ITALY). After examination, no

abnormal images were present, tissue autofluorescence was normal. The second patient examined was a young man with night-time bruxism who often bit his cheek. We took pictures of the upper and lower lip mucosa, the ventral and dorsal surface of the tongue, the hard and soft palate, and the left (Figure 1) and right jugal mucosa.

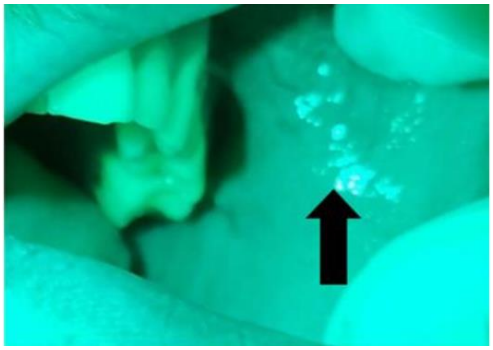

Figure 1. Left buccal mucosa visualized with GOOCLES (PIERREL PHARMA, ITALY), where the afferent vascularization can be highlighted

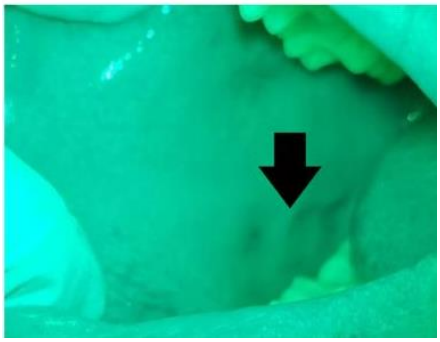

Figure 2. The right jugal mucosa with lesions on the surface. By viewing the lesions with the help of glasses, darker areas were highlighted indicating their presence

Upon visualization with the glasses, the lesions appeared with low fluorescence. Since the patient presented dark-colored lesions at the time of examination with the glasses, we asked the patient to come back for a follow-up in two weeks.

Upon his return, we only took pictures of the left and right jugal mucosa. Upon examination, we realized that the patient's lesions in the right jugal mucosa had healed but presented smaller ones in the left and right jugal mucosa. GOCCLES glasses (PIERREL PHARMA, ITALY) helped us confirm the presence of lesions. These are dark green to black. The patient still presented night-time bruxism.

The third patient did not present any visible pathology at the level of the oral mucosa that he knew of and that would be visible upon routine inspection. He requested an oral screening. Upon examination with the filter glasses, two areas were highlighted at the level of the left and right jugal mucosa that showed colour closures typical of a lesion. The patient recalled biting his cheek during a meal a few days after the examination, but the lesions appeared completely healed upon inspection without glasses.

After examination with the filter of the glasses, two areas were highlighted at the level of the left and right jugal mucosa that presented specific colour closures of a lesion. The patient remembered that a few days prior to the examination he bit his cheek during a meal, but on inspection without glasses, the injuries seemed completely healed.

Following this examination, in which lesions were detected that were not visible without GOCCLES glasses (PIERREL PHARMA, ITALY), we can conclude that it is a routine clinical stage that provides beneficial information for the patient as well as for the treatment plan.

After examining with the filter of the GOCCLES glasses, two areas were highlighted on the left and right jugal mucosa that showed color closures specific to a lesion. The patient remembered biting his cheek during a meal a few days before the examination, but upon inspection without the GOCCLES glasses, the lesions appeared completely healed.

As a result of this examination, which revealed present lesions that were not visible without the GOCCLES glasses, we can conclude that it is a routine clinical stage that provides beneficial information for the patient and the treatment plan.

The fourth examined patient is a young man who presents a jugal white line due to a deep bite. He wanted to check the degree of hyperkeratinisation of the oral mucosa and also if he had pathological lesions. At the level of the left jugal mucosa, the normal layer of

hyperkeratosis can be seen normally, slightly whiter than the surrounding mucosa. When we examined the dorsal surface of the tongue, the depapillated areas had low autofluorescence.

The fifth patient did not present obvious lesions upon inspection or palpation, but oral cancer screening was also performed with the GOCCLES glasses. In this patient, some darker areas were often evident, but this was due to intense vascularization. No pathological lesions were detected in the oral mucosa. Everything that appeared dark was due to rich vascularization.

Pictures were taken for oral screening at the level of the lip mucosa, the tongue Figure 3) the hard palate, and the jugal mucosa in the sixth patient. Depapillated areas were observed on the dorsal surface of the tongue. A lesion appeared on the right jugal mucosa due to the cheek bite, and the right jugal mucosa also had areas of hyperkeratosis next to the bite lesions.

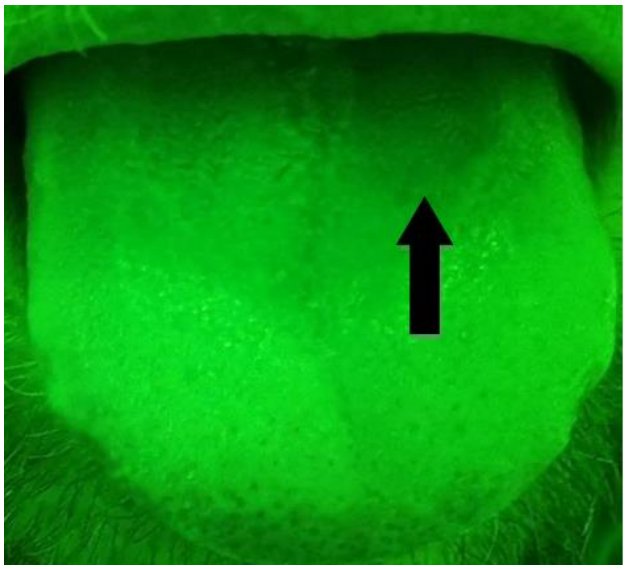

Figure 3. Dorsal view of the tongue with depapillated areas on the left side

The seventh patient had a history of a tongue piercing. Afterward, only a scar remained on the dorsal surface of the tongue, which was highlighted with the GOCCLES glasses as a darker area with hyperkeratinized margins. The eighth patient did not present visible lesions upon inspection or with the glasses. Tissue autofluorescence was normal with rich vascularization.

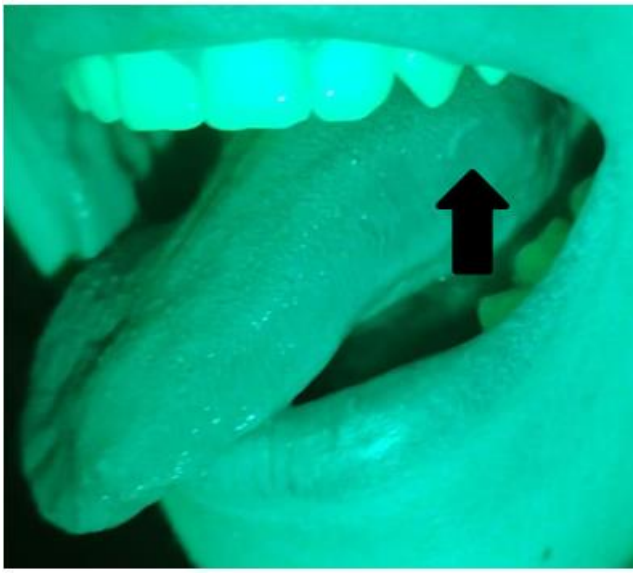

Figure 4. Following visualization with GOCCLES glasses (PIERREL PHARMA, ITALY), a lesion was detected on the lateral side of the tongue, which was the cause of the patient's pain

The last examined patient came for a consultation because she had painful sensitivity on her tongue when consuming different foods. Pictures were taken on the dorsal and lateral surfaces of the tongue Figure 4). Slightly darker and depapilated areas were observed. The patient had geographic tongue, and in certain periods of the Following the examination, slightly darker and depapilated areas were observed. The patient had a geographic tongue and it reappeared during certain periods of the mouth.

Following the analyses performed on the 9 patients, we have gathered a clinical picture with various signs after visualizing them with GOCCLES® glasses (Glasses for Oral Cancer – Curing Light Exposed – Screening) (PIERREL PHARMA, ITALY).

The first case was that of a patient who did not present visible lesions with the naked eye. The patient wanted to benefit from an oral cancer screening in the absence of evident lesions. We took pictures of the ventral and dorsal sides of the tongue left and right jugal mucosa using GOCCLES glasses (PIERREL PHARMA, ITALY). After examination, there were no abnormal images, and the tissue autofluorescence was normal.

The second patient examined was a young man who had night-time bruxism and often bit his cheek. We took pictures of the upper and lower lip mucosa, ventral and dorsal sides of the tongue, hard and soft palate, and left and right jugal mucosa.

When visualized with the glasses, the lesions appeared with low fluorescence. Since the patient presented dark-coloured lesions at the time of visualization with the glasses, we asked the patient to return for a check-up in two weeks.

## DISCUSSIONS

It has been demonstrated internationally that these medical glasses, GOCCLES® (Glasses for Oral Cancer - Curing Light Exposed - Screening) (PIERREL PHARMA, ITALY), are used to detect the loss of tissue autofluorescence when affected.[22],[23] The glasses can detect moderate and severe dysplasia as well as oral cancer. This device can be used with the dental unit lamp but has also been tested with a halogen lamp. Another study was conducted with patients sitting in the dental chair and trying to reproduce the situation as closely as possible to the dental office [24]. According to the scientific literature, examination of autofluorescence determined characteristics of the mucosa that appeared invisible to simple inspection [27]. International studies are trying to encourage the acquisition of this type of

material, especially GOCCLES® (Glasses for Oral Cancer - Curing Light Exposed - Screening) (PIERREL PHARMA, ITALY), to increase the chances of detecting oral cancer and to increase the rate of oral screening. The glasses are a much more accessible device than other apparatus that act on the same principle [26]. Additionally, Nichola Tong in her articles [25] presents the advantages of the glasses and makes a comparison with Oral ID, arguing that a device such as glasses is much easier to use in day-to-day dental practice. Huang et al in one of their publications supported the fact that there is a very high rate of detection of oral cancer and premalignant lesions due to tissue autofluorescence [28], [29]. The new technology for early detection of oral cancer and malignant lesions is now accessible to all through various devices that use tissue autofluorescence and could save many patients [30], [31].

## CONCLUSIONS

The lesions present in the oral cavity appear dark in color due to the loss of mucosal fluorescence;

Even post-piercing or other scar tissue appears with lost fluorescence;

Hyperkeratosis areas appear with increased fluorescence compared to surrounding unaffected tissue;

Incomplete healing lesions appear with lower fluorescence even if they are not visible on visual inspection;

GOCCLES® glasses (with a filter for visualizing fluorescence of affected and unaffected tissue proved to be a highly effective device for oral cancer screening.

The use of oral screening devices such as GOCCLES® glasses is essential for preventing and early detection of premalignant lesions and oral cancer.

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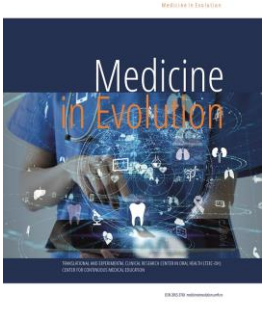
# Testing the efficiency and versatility of Helbo photodynamic therapy in periodontal disease

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## Abstract

Helbo photodynamic therapy is a treatment method that combines light and photosensitizers to eliminate specific cells. This therapy has gained interest as a possible treatment for various medical conditions, such as cancer, infections, and periodontal diseases. This study aims to demonstrate the effectiveness of Helbo photodynamic therapy in treating and reducing periodontal pockets, after the scaling and root planing procedure, if there is a noticeable difference in comparison to periodontal pockets treated solely by scaling and root planing.

**Keywords:** Helbo photodynamic therapy, periodontal disease, periodontal pocket

## INTRODUCTION

Every year, multiple new technologies are being discovered and invented in the world of medical and dental science. Also, these technologies continue to be improved and evolve each year in order to treat patients more efficiently in the specific field. Either the technology is improved by being able to apply quicker and efficiently treatment for a specific disease and/or increase in overall versatility, being utilized in supplementary various situations. One such example can be viewed in the use of Helbo Photodynamic Therapy.

This approach has different applications in dentistry, designed as an alternative treatment for bacterial, fungal, and viral infections, as well as oral cancer [1]. Originally, photodynamic therapy (PDT) was used as an early method to aid in the elimination of cancer cells although it can be seen more frequently in disinfecting root canals, periodontal pockets and fungal infections like Candida [2]. In endodontics, both Candida albicans and Enterococcus faecalis are some of the most recurrent microbes that are able to cause an assortment of post treatment diseases [2]. Being a non-invasive and non thermal option, antimicrobial photodynamic therapy (aPDT) can be described as a local antibacterial procedure used to reduce bacterial contamination in oral infections. It is a two step process that firstly involves the application of a photosensitizer. Then It is followed by illumination with laser light on the sensitized tissues [1]. The photosensitizer is activated by an appropriate wavelength from the laser light [3]. This causes a toxic photochemistry reaction within targeted cells, resulting in the formation of reactive oxygen species, which can include hydroxyl radicals, singlet oxygen, and superoxide [4]. The resulting consequence is the apoptosis of these microorganisms. Depending on the target area that is being treated, such as root canals or periodontal pockets, aPDT is usually applied in combination with either other chemical antimicrobials or mechanical subgingival instrumentation [3]. Helbo photodynamic therapy appears to be an interesting alternative to the usage of antibiotics in treatments involving oral cavity diseases. Due to the nature of biofilms, bacteria and other microbes are able to evolve and become more resistant to antibiotic therapy [5]. Therefore, for the purpose of this study, the applications and mechanisms of Helbo photodynamic therapy will be explored in order to determine its efficiency and versatility when applied in periodontal disease therapy.

### *Aim and objectives*

The aim of this study is to see the efficacy of Helbo photodynamic therapy application in of periodontal pockets reduction, after the scaling and root planing (SRP) procedure has been performed, in comparison to periodontal pockets that have been treated by scaling and root planing alone.

## MATERIAL AND METHODS

A controlled clinical study was conducted at the Department of Oral Pathology of the University of Medicine and Pharmacy betwee 2019-2020. The study was conducted according to the guidelines provided by the Declaration of Helsinki regarding patient privacy and data protection and after receiving ethical approval from the Ethics Committees.

All participants were informed regarding the entire protocol of the study and prior to the study onset they completed consent forms as well as a medical questionnaire regarding the patient's general health. Thirty patient were included in the study, and randomly assigned in one of two groups: group (A): 15 patients received SRP plus Helbo photodynamic therapy, group (B); 15 patients that received SRP alone.

The inclusion criteria were: individuals, both male and female, age ranging between 18-70, with diagnosis of periodontitis, that at least 1 periodontal pocket with a probing depth  5mm. The exclusion criteria included individuals under the age of 18 or above the age of 70, as well as individuals that presented with sites of probing depth 3 mm. Other exclusion criteria were: pregnant women, autoimmune disease and/or tumors

After the inclusion in one of the groups, an initial periodontal clincial examination was performed using UNC-15 periodontal probe (Hu-Friedy, Chicago, IL, USA). Six sites on each tooth were evaluated and periodontal parameters were recorded on an online chart from available at http://www.periodontalchart-online.com/uk/[6].

The following parameters were recorded: probing depth, distance between gingival margin and cemento-enamel junction, clinical attachment level, the plaque index, bleeding on probing, and tooth mobility following the standard clinical definitions[7]. Once all the aforementioned information was entered, values for the mean probing depth, mean attachment loss and total percentages of the plaque and bleeding on probing were calculated for each case. Each patient received a treatment plan following the present guidelines [8].

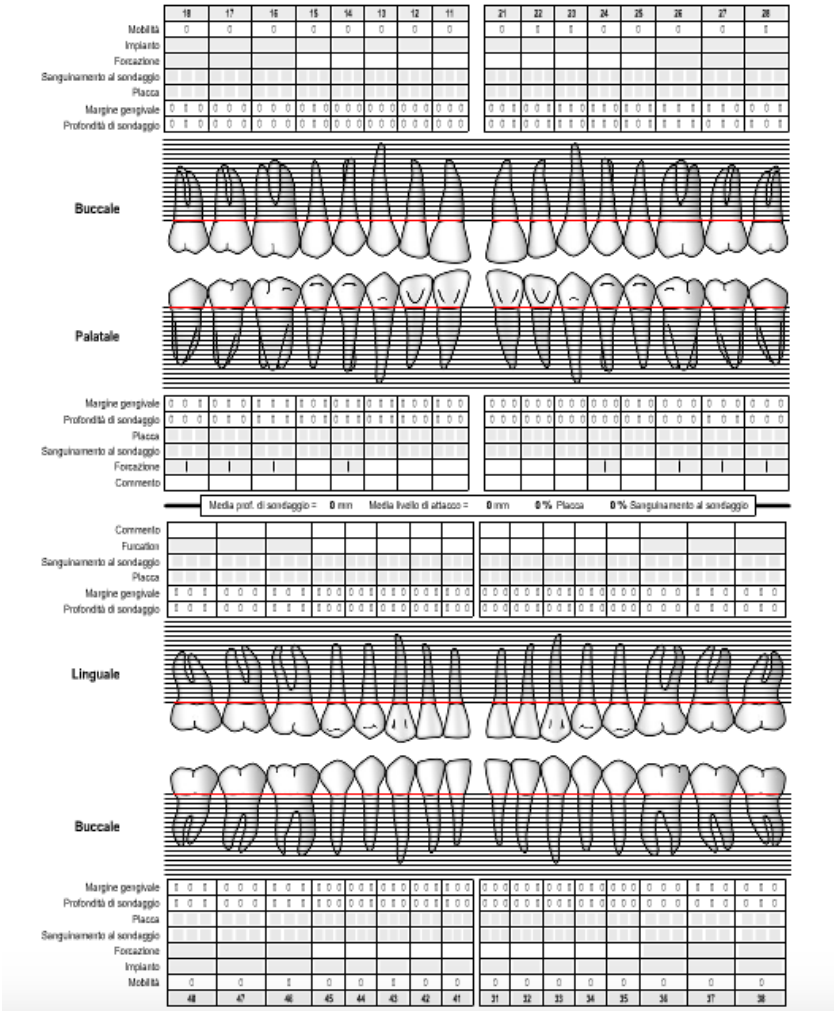



Figure 1. Copy of periodontal chart used for to record all periodontal parameters of each patient [6]

Patient underwent periodontal treatment. The first step of therapy consisted in a full mouth supragingival mechanical instrumentation using an ultrasonic dental scaler (Unit-P5 Booster Suprason - Satelec, Acteon, Mount Laurel, NJ, United States) followed by a professional brushing.

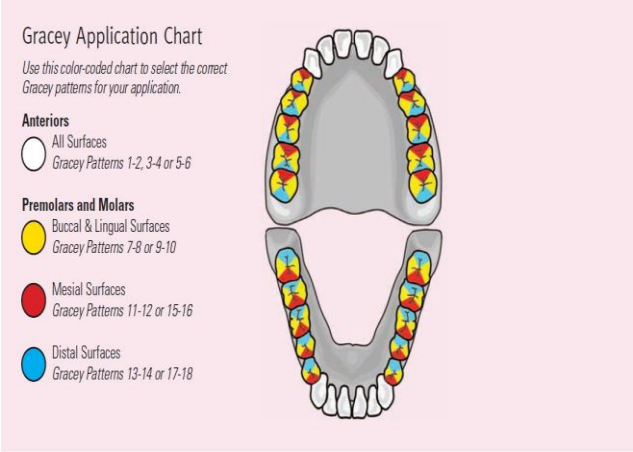



Figure 2. Gracey chart used to determine which type of curette is used for each particular area of each tooth during the root scaling procedure [9]

One week after, the subgingival mechanical instrumentation was performed.

Patients received a full mouth scaling and root planing. Each tooth that presented PD sites greater than 5 millimeters were anesthetized, and SRP was performed, using color-coded Gracey curettes (Hu-Friedy, Chicago, IL, United States and the following chart was implemented in order to target specific areas of each tooth.

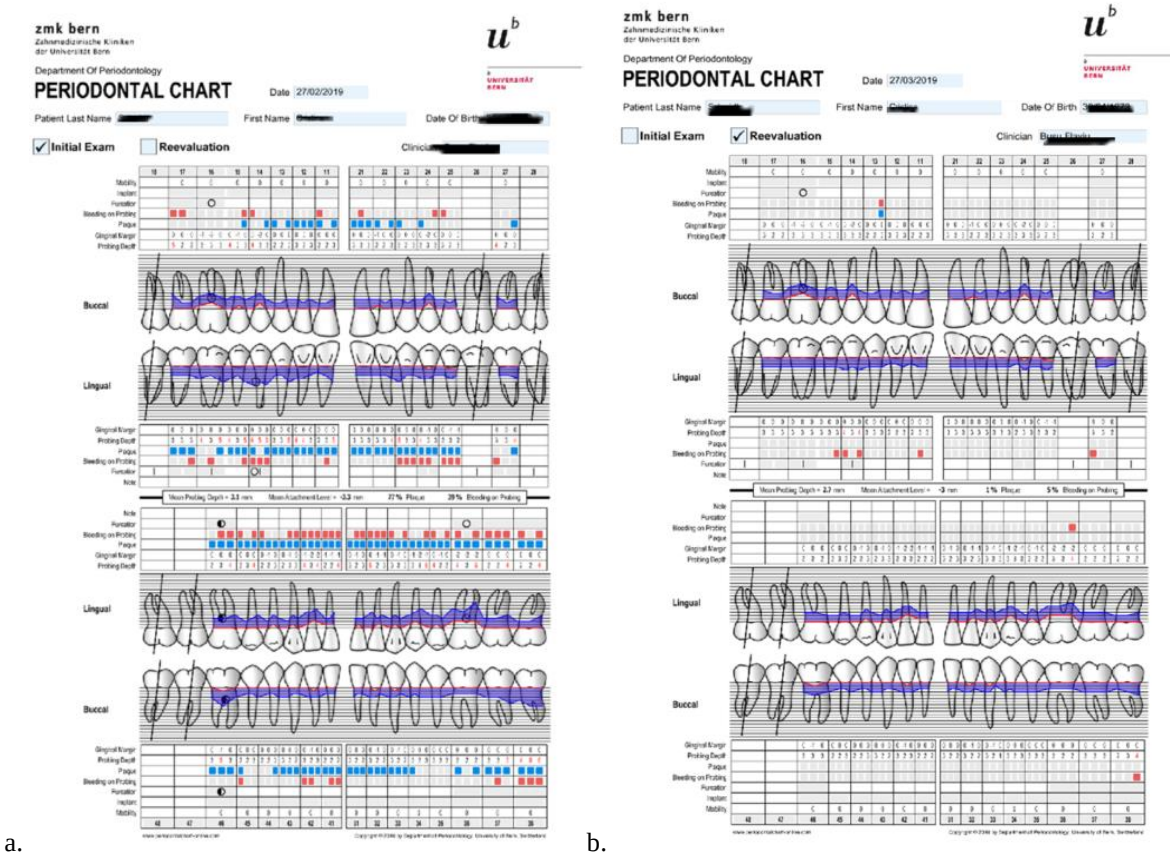
Within a period of 24 hours following SRP and oral irrigation, patients in group A underwent the application of the Helbo photodynamic therapy consisting in the following protocol

- Step 1: Helbo Blue photosensitizer [10], containing Methylene Blue (MB) was applied to the periodontal pockets with an endodontic needle, starting at the pocket fundus, as it is required to be applied from an apical to coronal direction [11]. In addition, MB was the chosen photosensitizer for this study due to its cationic nature, low molecular weight, and its ability to target both gram-positive and gram-negative bacteria [12].
- Step 2: The Helbo Blue photosensitizer was left for approximately 3 minutes to react within each periodontal pocket, after which the stained areas were rinsed with distilled water in order to remove excess MB. An excess layer of photosensitizer will not allow efficient light penetration from the accompanying laser [12].
- Step 3: Once dried, a handheld, battery operated, diode laser, (Theralite laser) [10] was used to illuminate the zones originally stained by the MB. By channeling the light through a fiber optic tip (3D Pocket Probe) [10], the tip of the laser was placed within each of the six sites of every affected tooth, for approximately one minute per site.

The recall phase was performed one month later and consisted in afull mouth reevaluation chart, recording again all the periodontal parameters.

## RESULTS

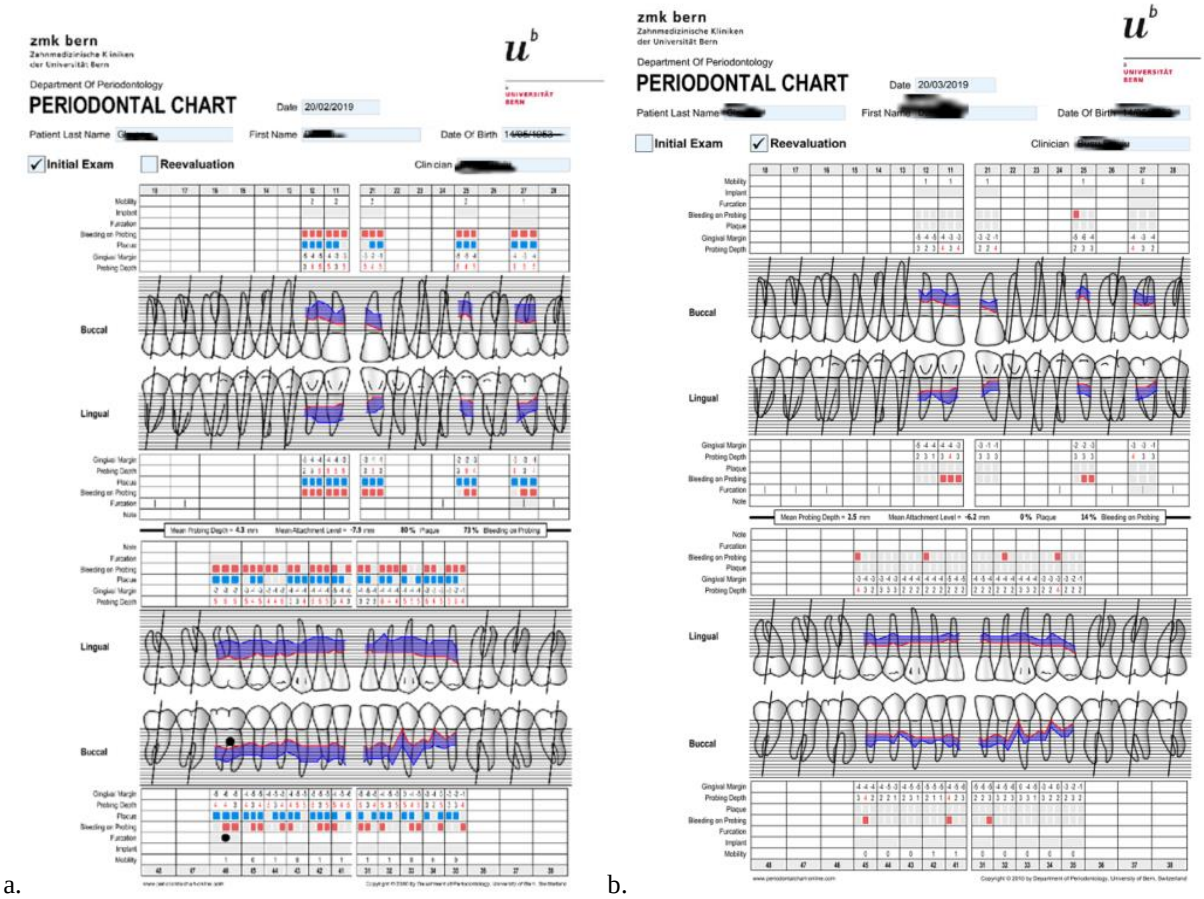
Case 1: Male patient of 65 years old with hypertension and rhinitis, former smoker, presented with 47 probing sites that were greater than or equal to 5 millimeters and 48 probing sites that were less than equal to 4 millimeters during the initial examination. For the reevaluation exam, the patient presented 0 probing sites that were greater than or equal to 5 millimeters and 90 probing sites that were less than or equal to 4 millimeters (Figure 3).




a. b.

Figure 3. (A) Initial panoramic image of patient's teeth; (B) Re-evaluation exam results of case 1

Case 2: Female patient of 45 years old with no general diseases, smoker, presented with 16 probing sites that were greater than or equal to 5 millimeters and 146 probing sites that were less than or equal to 4 millimeters during the initial examination. For the reevaluation exam, the patient presented 0 probing sites that were greater than or equal to 5 millimeters and 162 probing sites that were less than or equal to 4 millimeters (Figure 4).




a. b.

Figure 4. (A) Initial panoramic image of patient's teeth (B)Re-evaluation exam results of case 2

The minimum values of the mean probing depth, mean attachment level, plaque percentage and percentage of bleeding on probing for the initial exam of Group A are 2.1mm, 2.1mm. 0%, and 1% respectively. Correspondingly, the maximum values are 4.5mm, 7.9mm, 100%, and 73%. The average values of each parameter are 3.15mm, 3.95mm, 38.1% and 42.5%.

The minimum values of the mean probing depth, mean attachment level, plaque percentage and percentage of bleeding on probing for the reevaluation exam of Group A are 1.9mm, 1.9mm, 0%, and 0% respectively. Correspondingly, the maximum values are 2.8mm, 6.2mm, 4%, and 21%. The average values of each parameter are 2.47mm, 3.31mm, 1.27% and 6%.

The minimum values of the mean probing depth, mean attachment level, plaque percentage and percentage of bleeding on probing for the initial exam of Group B are 2.5mm, 2.5mm. 9%, and 13% respectively. Correspondingly, the maximum values are 4.8mm, 5.5mm, 76%, and 85%. The average values of each parameter are 3.59mm, 4.05mm, 32.3% and 50.9%.

The minimum values of the mean probing depth, mean attachment level, plaque percentage and percentage of bleeding on probing for the reevaluation exam of Group B are 2.2mm, -2.4mm, 0%, and 3% respectively. Correspondingly, the maximum values are 4.7mm, 5.3mm, 31%, and 39%. The average values of each parameter are 2.93mm, 3.77mm, 7.6% and 16.9%.

Shows the mean values of the recorded periodontal parameters at the the initial and reevaluation exams for Group A and Group B

Table 1. Average values of both Initial and Reevaluation exams of both groups

| Group(Exam) | Mean Probing Depth Value (mm) | Mean Attachment Level Value (mm) | Mean Plaque Value | Mean Bleeding on Probing Value |
|---|---|---|---|---|
| **Group A (Initial Exam)** | 3.15 | 3.95 | 38.1% | 42.5% |
| **Group A (Reevaluation Exam)** | 2.47 | 3.31 | 1.27% | 6% |
| **Group B (Initial Exam)** | 3.59 | 4.05 | 32.3% | 50.9% |
| **Group B (Reevaluation Exam)** | 2.93 | 3.77 | 7.6% | 16.9% |

## DISCUSSIONS

After the initial therapy of patients with periodontitis, an improvement of periodontal parameters in terms of PD reduction, bleeding on probing is observed [8], photodynamic therapy used as an adjunctive therapy, could bring some supplementary benefits [8]. By analyzing the results obtained for this study, it could be noted that patients in Group A had an average reduction value of 0.68 mm for the reduction in mean probing depth and 0.64mm for the reduction of mean attachment loss, over a one month period. Furthermore, an overall reduction in the average value of plaque percentage and bleeding on probing were calculated at 36.83% and 36.5% respectively. Within Group B, the average reduction values of mean probing depth and mean attachment loss were 0.66 mm and 0.28 mm, whereas the average reduction values in plaque and bleeding on probing were 24.7% and 34%.

When comparing the two groups, it could be established that Group A had only a slightly greater reduction of 0.02mm in mean of probing depth. However, Group A had an average for attachment lossvalues reduction with 0.36 mm more than Group B. Better results were observed for the average plaque and bleeding on probing index: 12.13% and 2.5% more.

This study investigated the efficacy of photodynamic therapy as adjunctive therapy to SRP in terms of improvement of periodontal parameters. Our results showed the reduction of the mean values when comparing the test group with the control group, proving some clinical additional benefits of the Helbo therapy. The limitations of this study were the decreased number of participants, the constraints of patients in order to respect the date and time of each procedure executed during the study, and the patients oral hygiene habits that were diffcult to control during the study as instructed. One of the most important steps while obtaining the results for this study was the aspect of good reproducibility. A lack in this element could result in an overall inaccurate treatment procedure. Therefore, it is important to note that calibration is a key attribute when conducting clinical research, as well as having a trained supervisor overlooking all investigations and procedures.

Research on photodynamic therapy was conducted in a variety of other studies, focused on the periodontal disease and endodontic pathology treatment or even Candida and halitosis therapeutical approaches [13] [14]. One study that was done by Hokari T. et al, analyzed the effects of antimicrobial photodynamic therapy and minocycline ointment in patients with chronic periodontitis. Using 30 patients in their study, their results showed that the aPDT group only had significant improvements within clinical parameters [15].

Ahad A. et al analyzed the effect of aPDT in deep periodontal pockets, with a total of 30 patients diagnosed with chronic periodontitis. The patients were split into two groups and were checked at 1 month and 3 month intervals after the treatment procedures were carried out. They found that at the 1 month interval, there were significant differences in the parameters of each group, whereas at the 3 month interval, the difference in average values of all parameters were much smaller [16].

Furthermore, Grzech-Leśniak K et al performed a similar study, incorporating 84 patients into 3 groups, where a comparison was made between 3 different methods: scaling and root planing on its own, SRP in combination with PDT, and the use of an erbium-doped yttrium aluminum garnet laser (ERL). The results later showed that the values of bleeding on probing and the reduced percentage of certain bacteria after 3 months were greater when the ERL and PDT methods were implemented [17].

In a split mouth design study of ten patients diagnosed with aggressive periodontitis, the average values of probing depth, gingival margins, bleeding on probing, and plaque index obtained from both groups appeared to show similar clinical results over a 3 month period [18].

Other studies that utilized photodynamic therapy can be seen in the treatment research of candida and herpes simplex virus [19]. One study aimed to show the possibility of exploiting photodynamic inactivation as a possible option to Candida infections. In combination with caspofungin, the results showed a complete eradication of biofilms within the infections [20]. Within a similar study, there was evidence presented that PDT can be used as an effective and alternative therapy method to treat Candida tropicalis, which is known to be highly drug-resistant [21].

## CONCLUSIONS

Given the limitations of this study, it could be concluded that although scaling and root planing alone can help to reduce certain parameters when it comes to the treatment of periodontal pockets, the supplementary use of Helbo photodynamic therapy brings supplementary clinical benefits in overall improving the periodontal parameters values.

Helbo photodynamic therapy is an alternative approach to antibiotic treatment and can provide effective elimination of bacteria and other microbes withoutserious side effects. The simplicity and efficiency of this additive treatment option can help to aid in the overall healing of patients with periodontal disease and, in turn, maybe even promote more motivation in the maintenance of good oral hygiene. because Further research on Helbo PDT in randomized clinical trials with a larger number of participants should be performed in order to clinically prove its efficacy as adjunctive treatment in periodontal therapy, and also to prove the utility in the treatment of other oral diseases.

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# Digitalizing ceramic inlays – a dental lab view


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## Abstract

Case presentation: Ceramic dental inlays are known as very high-quality prosthetic works, similar to physiognomic composite fillings, made in the laboratory based on impressions. This gives them a special aesthetic, similar to the natural tooth.

Material and method: In this study we realized two types of ceramic inlays in the dental laboratory: on the second upper right bicuspid – a mesio-occluso-distal type, and on the first upper left molar – an occluso-mesio-palatal type for the same patient. We used Empress Multi CAD® ceramics (Ivoclar, Lichtenstein) together with the DentalCAD software (Exocad, Germany).

Discussions/Conclusions: Ceramic inlays are laborious micro prostheses that require a lot of involvement, attention and precision, any imperfection could compromise the adaptability of the final piece. They ideally restore the coronary morphology from both a functional and aesthetic point of view, and thanks to this fact, they are ideal for patients where there are special aesthetic imperatives.

**Keywords:** ceramics, CAD-CAM, aesthetics, inlays

## INTRODUCTION

Inlay and onlay systems can be made using sintered, cast, pressed or mechanically milled ceramics. Ceramic inlays are classified into two categories: all-ceramic and metal-ceramic. The development of the all-ceramic concept in the last ten years has led to the opening of new ways in fixed prosthodontics, namely adhesive dentistry, through the biocompatibility of materials. These have gained place thanks to double-acid etching techniques (ceramics with HF and dental hard tissues with H3PO4), the development of CD and dentinal adhesives that have changed the concept of aggregating these single-dental prostheses) [1].

The introduction of CAD-CAM systems represented a real revolution in dentistry. With this, a ceramic inlay can be designed and manufactured in a single session. The reconstruction of the three-dimensional image is based on the accumulated information and consists in calculating the three coordinates in space (X, Y, Z) with the help of mathematical algorithms for each point of the prosthetic field. After the optical impression is made, the computer later reproduces the image of the imprinted structures on a monitor to allow the user to control the design of the restoration. After designing the morphology of the restoration, the data about its shape are transformed into a set of instructions that will be transmitted to the milling device [2].

### *Aim and objectives*

The aim of this case report was to emphasize that digitally techniques in dental labs, such DentalCAD (Exocad, Germany), could maximize productivity. They are safe and robust, could lead to satisfying results even when used in complex cases, and are easy to use, flexible and fast [3,4].

## CASE REPORT

At clinical examination we found two old incorrect composite restorations on 1.5 and 2.6, in a young female patient with a good oral hygiene. The first proposal was to replace these restorations with new composite ones, but the patient refused. Finally we decided to realize two types of ceramic inlays in the dental laboratory: on 1.5 – a mesio-occluso-distal type, and on 2.6 – an occluso-mesio-palatal type (Figure 1).

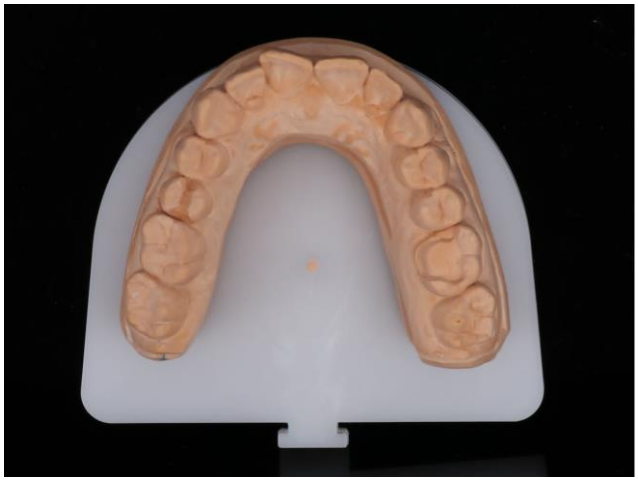


Figure 1. Working model with the two types of inlays (1.5 and 2.6)

**Model scanning**

Before scanning the model, we must enter the patient's data and select the type of work we want to do (Figures 2, 3) into the DentalCAD software.

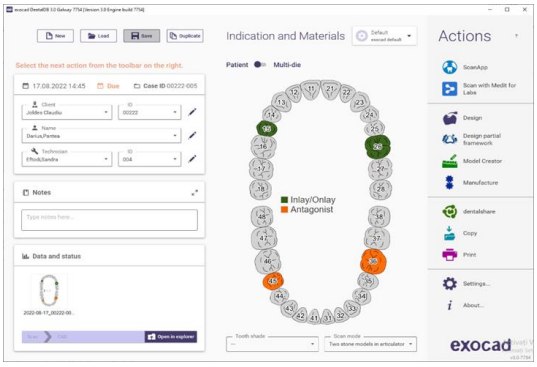



Figure 2. Entering case data into ExoCad

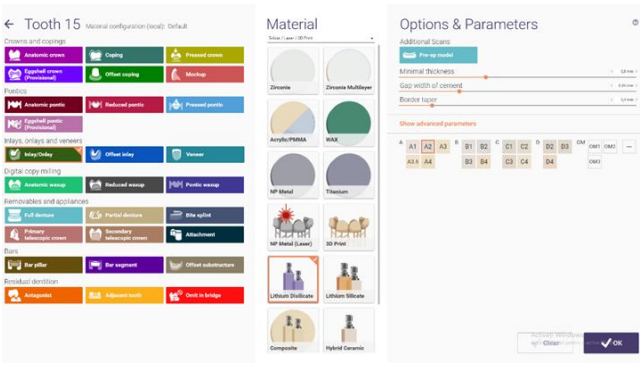



Figure 3. The choice of material

After entering the data and selecting the desired job type, we will open the scanning software and select the scanning strategy we will use. In this case we opted for scanning the model with movable abutments (Figure 4).

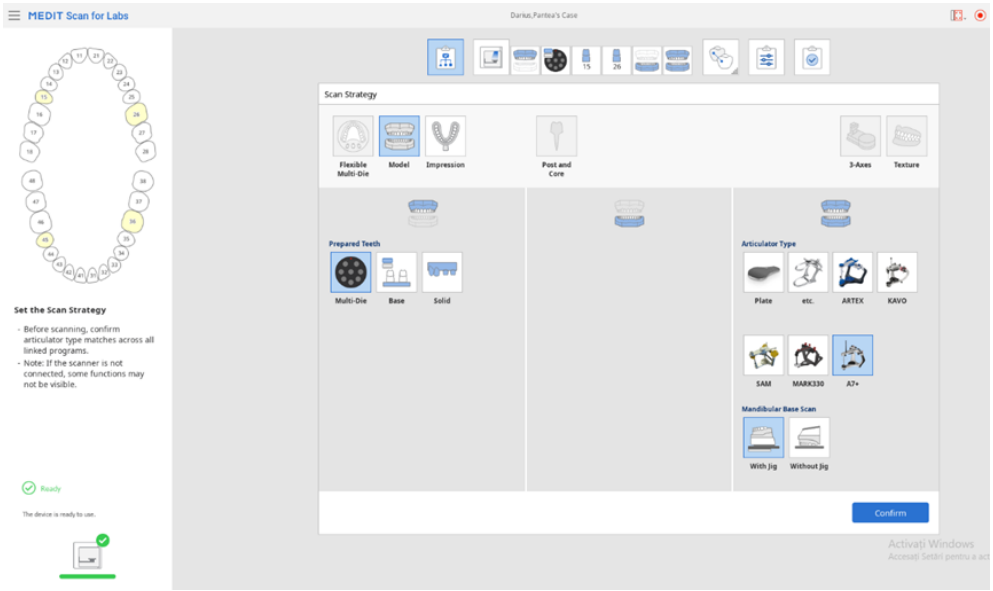



Figure 4. Choosing the scanning strategy

We started with scanning the maxillary model, after which we did the separate scanning of the movable abutments (Figures 5,6). Next was the antagonist model scan (Figure 7) and the occlusion scan (Figure 8).


Figure 5. Selecting the scan area

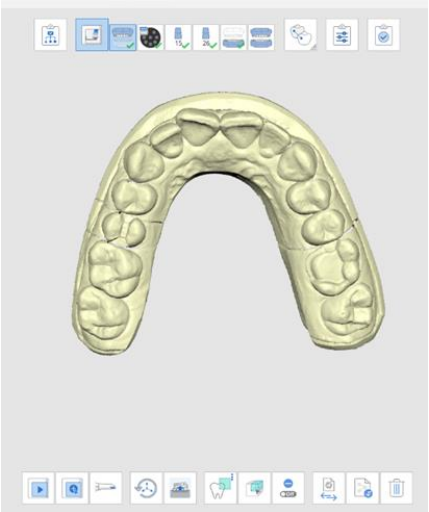


Figure 6. The scanned working model

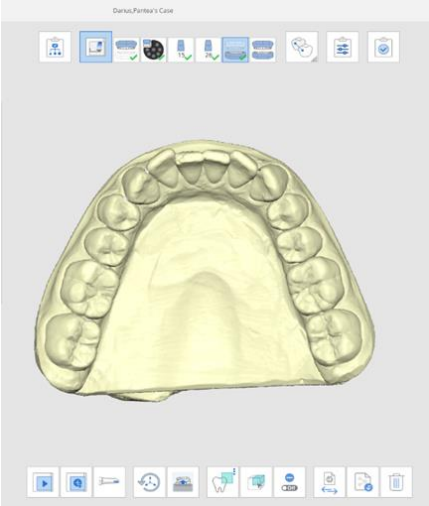


Figure 7. The scanned antagonist model

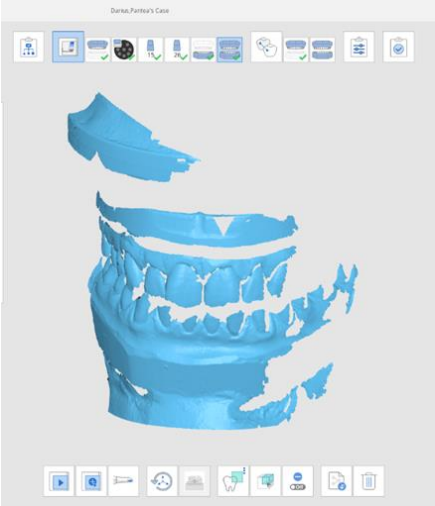


Figure 8. The scanned occlusion

After both models and the occlusion have been scanned, their overlay follows. Initially, the scans of the abutments and the working model are superimposed, followed by superposition of the occlusion with the two models (Figure 9).


Figure 9. The models after superpositioning

**Designing the inlays**

The first step of the design creation was drawing the edges of the preparations. Initially we selected "*Detect*", where we positioned points for automatic detection of the edges of the preparation, and then we selected "*Correct/Draw*" to adjust the positioning of the edges of the preparation (Figures 10,11).

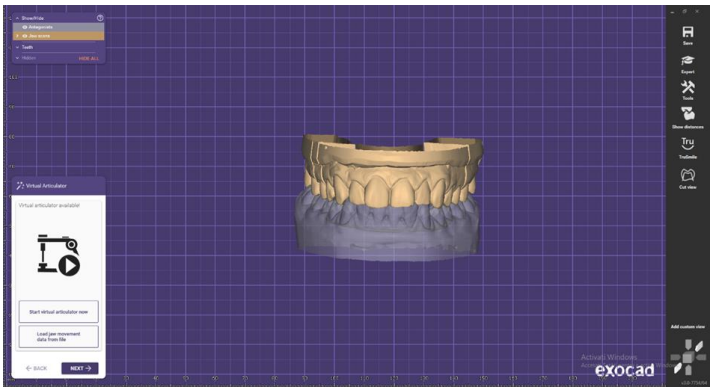
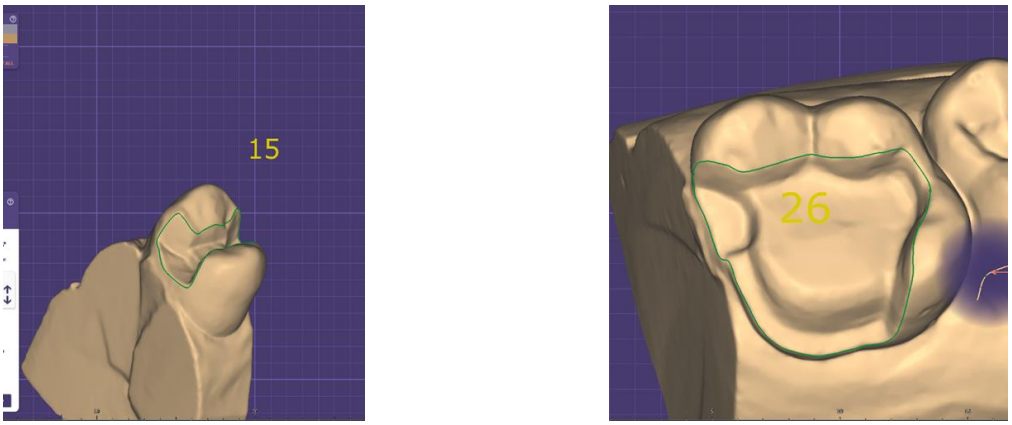


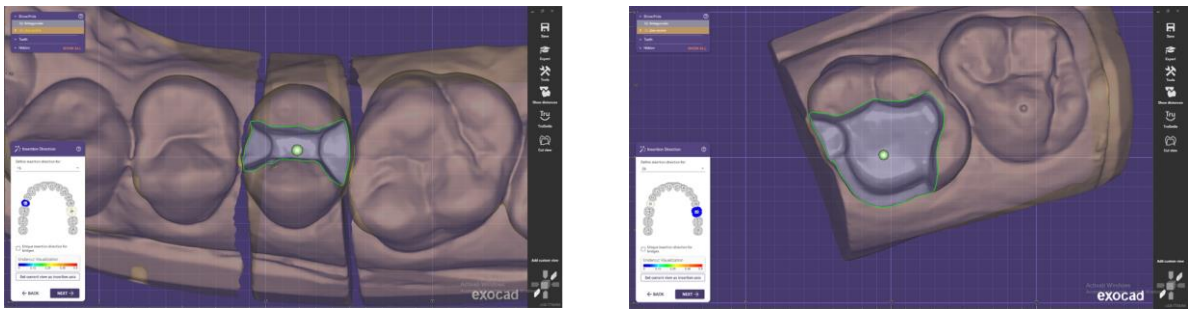

Figure 10. The ExoCAD model




a b

Figure 11. Selection of preparation limit (a – for 1.5 and b – for 2.6)

The next step was the selection of the insertion axis. It was selected by rotating the model until it reached the axis we wanted for the future restoration, then we selected "*Set current view as insertion axis*" when the model was in the desired position (Figure 12).




a b

Figure 12. Insertion axis selection (a – for 1.5 and b – for 2.6)

The next step was to make the inside of the prosthetic work: to select the thickness and the angle we want for the edges of the preparation; the thickness of the cementation space, as

well as the shape and minimum number of cuttings with which the inlay will be milled. Here we paid attention to have enough thickness so that cracks do not appear during milling (Figure 13).

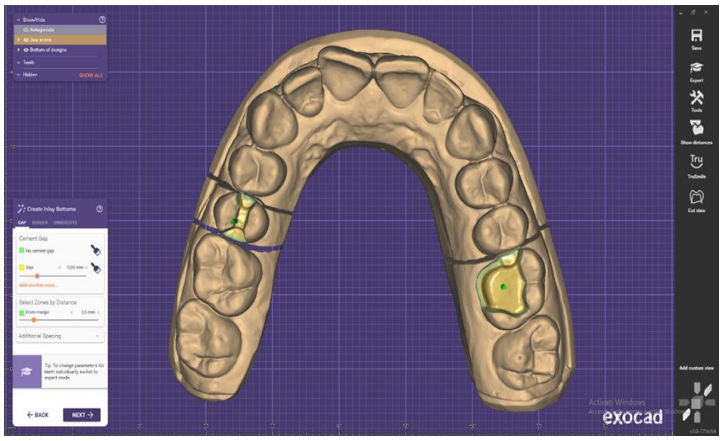



Figure 13. Selection of parameters

In the next step, we chose and positioned the tooth from the library, adapting it to the given case (Figure 14). We had different tools that helped us adjust the dimensions and position of the chosen tooth (Figure 15).

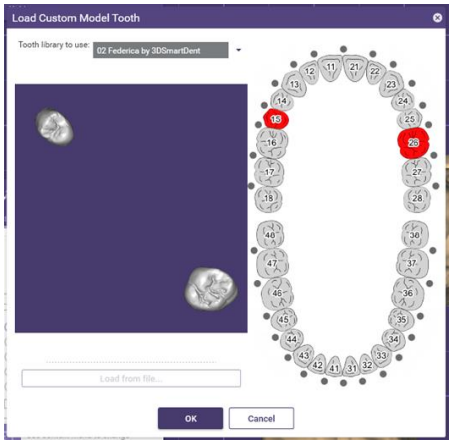



Figure 14. Selecting teeth from the software library

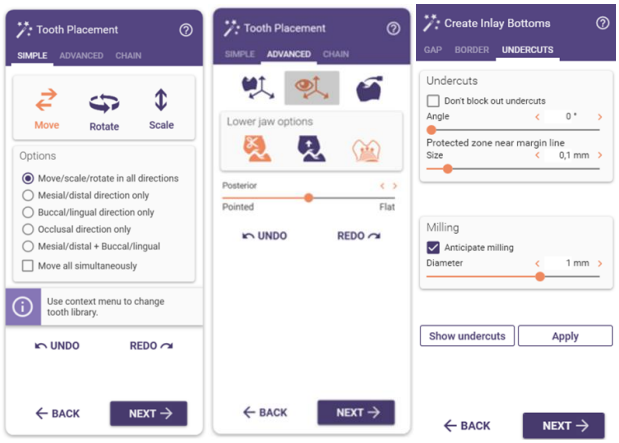
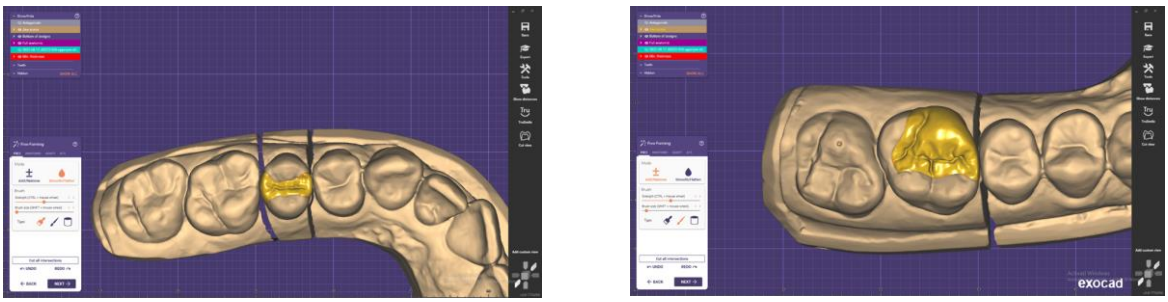



Figure 15. Software tools used to adapt the teeth to the model

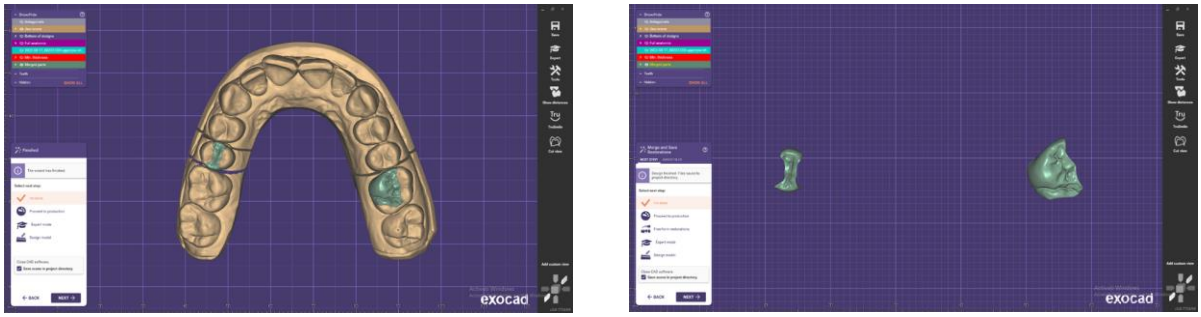
After adapting the teeth to the model, we changed their morphology, so that it was correctly registered in the arch. After this we checked and made the contact points with the opposing teeth and the proximal contacts with the adjacent teeth (Figure 16).




a b

Figure 16. Inlay modeling and adaptation (a – for 1.5 and b – for 2.6)

In the end, we finished with the tools from the soft design of the future inlays, and then we saved them. So we were able to move on to the next stage: milling the inlays (Figure 17).




a b

Figure 17. The final aspect of the inlays (a – full arch and b – only inlays)

**The milling**

We started by choosing the ceramic blocks that we will mill. We used blocks of IPS Empress CAD Multi in this case [5-9]. Next was positioning the design into the ceramic block we have chosen, making sure it was positioned entirely within the ceramic block (Figure 18).

a b

Figure 18. The ceramic blocks Empress®CAD Multi (a) and the positioning of the prosthetic piece within ceramic block (b)

We checked if the cutter of the milling machine can touch all the surfaces of the future prosthetic work, in order not to have extra ceramic amount compared to the design. We positioned the ceramic block in the machine and started milling (Figure 19). We used a PrograMill PM7 (Ivoclar, Liechtenstein) milling machine. After cutting the rods we verified the adaptation of the inlays on the cast model (Figure 20).

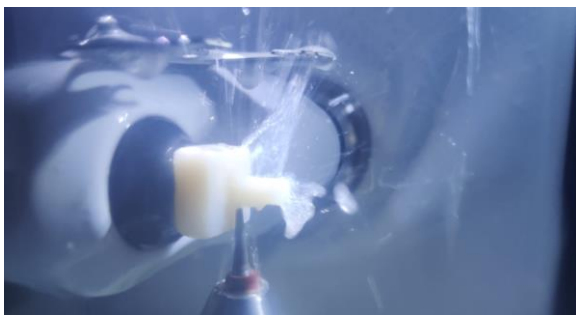


Figure 19. Inlay milling

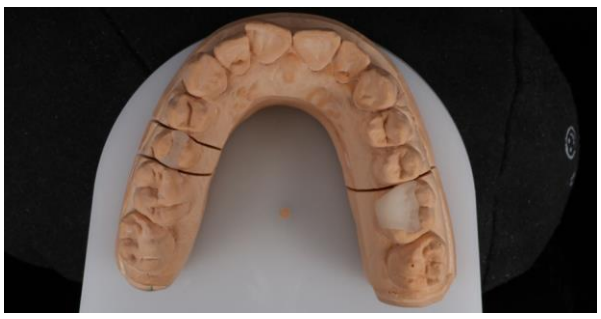


Figure 20. The inlays on the model (after milling and rods ablation)

**Stain burning**

After adapting and processing the prosthetic piece, we cleaned it of impurities with the steamer, and then we proceeded for the application of stains and shades.

For a good adhesion, we applied IPS IVOCOLOR liquid on the occlusal face, thus obtaining more intense shadows from several burns and highlighting the occlusal morphology. To highlight the grooves and pits, we used A2 color and essence mahogany stains (Figure 21). We mixed stains white with glaze and applied them to the occlusal ridges to highlight them. For firing we selected the program IPS e.max CAD staining Technique => Stain firing e.max Ceram and entered the inlays into the oven.

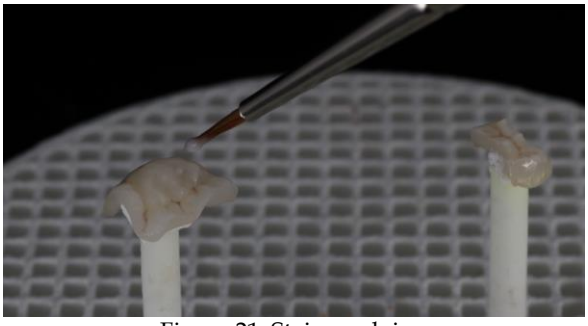


Figure 21. Stain applying

**Glaze firing**

We mixed IPS e.max® Ceram Paste Glaze with glaze liquid and applied the glaze to the occlusal face of the work in an even layer so that it does not pool. We selected the program and put the work in the oven (Programat EP3010 G2 Ivoclar Vivadent), at a temperature of 770°C with vacuum. After glazing we checked the contact points again. The final result was that one from Figure 22 and 23.

a b

Figure 22. The inlays after glazing (a – on 1.5 and b – on 2.6)

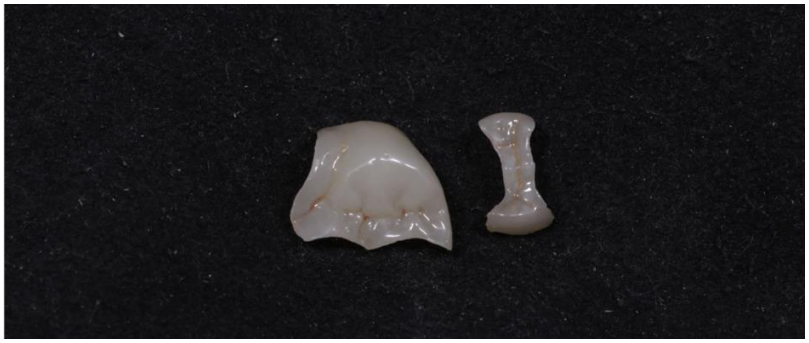


Figure 23. Inlays final aspect

## DISCUSSIONS

The objective of making ceramic inlays is to offer the patients a prosthetic work that meets all functional and aesthetic requirements, preserves the vitality of the teeth, integrates perfectly into their physiognomy and is in harmony with the entire arch. Manufacturing technology requires a longer time, compared to restorations made directly in the office.

As we can clearly see, the path to "excellence" is not easy, especially when the aesthetic treatment must be performed using a minimally invasive approach. This certainly requires an understanding of the patient's expectations and a great team effort between the general dentist, specialists and the dental laboratory involved. Only then can we really talk about a successfully completed aesthetic.

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# Application of Chitosan in Dentistry


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## Abstract

Natural polymers such as chitosan, have been shown to be optimal materials for drug delivery due to their intrinsic biocompatibility. Chitosan has been extensively studied in the development of controlled release drug delivery systems because it facilitates the transmucosal absorption of drugs because the electrostatic interaction with the negatively charged mucosal surface is due to its positive charges. The present review aims to describe the role of chitosan in different fields of dentistry such as endodontics, periodontology, prevention, and surgery.

**Keywords:** chitosan, technology, biological features, tissue regeneration

## INTRODUCTION

Chitosan is a biopolymer derived from the 70% deacetylation of chitin in a basic solution. Chitin is a naturally occurring complex carbohydrate that is present in the exoskeleton of shrimps, crustaceans and insects.

Chitosan has been used as both a direct and indirect pulp coating agent, an antimicrobial agent against E. faecalis bacteria. It is one of the ingredients of the intracanal triple antibiotic drug. It is used to remove smear layer during biomechanical root canal preparations, guided tissue regeneration, guided bone regeneration but also to promote healing after periodontal surgery.

Restorative materials, such as glass ionomer cements, composites and dental adhesives, have been modified using chitosan to improve their antimicrobial properties and enhance their adhesion to tooth structure. Another area where it has found application is in oral surgery, where it has been used to perform haemostasis, oral reconstruction, bone replacement and temporomandibular joint disc repair. The most studied property of chitosan is its property to remineralize and regenerate enamel and dentin.

As far as enamel remineralization is concerned, it is different from dentin remineralization, this difference is that in enamel remineralization the demineralized tissue is remineralized again and is more resistant to acid attacks compared to the natural enamel that existed before, whereas dentin remineralization involves the regeneration of a new mineralized collagen matrix and the formation of hydroxyapatite crystals that block dentin, tubules and protect the pulp-dentin complex. In terms of dentin remineralisation, this is more difficult compared to enamel remineralisation, either in a clinical or laboratory setting. Remineralisation systems are classified into fluoride-based and fluoride-free remineralisation.

### *Implications of chitosan in the treatment of incipient lesions*

Different chitosan formulations characterized by different pH and different materials are presented on the market. There are chitosan-based gels containing lactic acid, some contain distilled water, and others contain chlorhexidine. The antibacterial activity of chitosan is strongly influenced by its formulation.

It has been associated with antibacterial effects on Streptococcus mutans, Actinomyces actinomycetemcomitans and Porphyromonas gingivalis. Bacteria contained in plaque are the primary risk factor in the development of primary and secondary caries, peri-implant and periodontal diseases or other systemic diseases such as neurodegenerative disease, following recent findings. These species are able to penetrate the micro-grooves that are created between the restorative material and dental tissue. Therefore, by reducing the number of bacteria at the resin-tooth interface, the incidence of secondary caries can also be reduced. Therefore, the incorporation of antimicrobial agents into dental resin materials can be effective in preventing secondary caries. Although fluoride and chlorhexidine are the antimicrobial agents most commonly incorporated into resin materials, their release does not continue for long. In addition, the mechanical properties of resinous materials change and significantly reduce their bond strength. Research is currently aimed at increasing the durability of the resin-dentin bond, in other cases between resinous materials and other dental cements. Therefore, by introducing chitosan methacrylate into the primer of a three-step "etch and rinse" adhesive system, we can achieve good adhesion values and good stability of the hybrid layer when subjected to the mechanical simulation of mastication and thermal stress. It would also appear to improve the adhesion characteristics of the mucus to the enamel, producing better remineralization.

Unlike other tissues of the human body, enamel and dentin alone do not undergo repair because there are no cells within them that can be activated to begin a repair process. The maximum effect of this material is on gram-positive bacteria such as Streptococcus sanguis, S. mutans, Streptococco mitus, Streptococcus salivarius and yeasts.

It has some other favourable characteristics and applications such as prevention of demineralization, prevention of plaque and biofilm formation, stimulation of salivary secretion, antitumor activity, hemostatic properties, improvement of wound recovery, antihypertensive properties, reduction of serum cholesterol, drug for release system, implant lining, bone tissue engineering and bone regeneration, blood vessel repair and nerve repair.

***Implications of chitosan in endodontic treatment***

Chitosan has also found its application in endodontics, following studies by Ballal et al., it has been shown that in root canals, prolonged release of calcium hydroxide ions occurs through the addition of chitosan to calcium hydroxide paste. Furthermore, Silva et al. indicated that a 0.2% chitosan solution was as effective as EDTA and CA at higher concentrations (15% EDTA and 10% CA) at removing the smear layer.

Several chelating solutions, including organic acids such as citric acid (CA), maleic acid and inorganic acids such as ethylenediaminetetraacetic acid (EDTA), phosphoric acid, were used to remove the smear layer. Although EDTA is one of the most widely used chelating molecules, it has some limitations and drawbacks as a duct irrigant. Studies showed that EDTA was not effective in removing smear layer in the apical third of root canals. In addition, the longer contact time with EDTA may cause loss of dentinal surface and reduced microhardness of dentinal walls. Therefore, researchers are looking for an alternative to EDTA solution because of its erosive and toxic side effects on dentinal and periapical tissues.

Various chelating agents have been recommended by researchers for effective smear layer removal. In a previous study, 0.2% chitosan removed the smear layer as effectively as 15% EDTA and 10%CA from the middle and apical thirds of the canal. Studies have proven the positive biological features of chitosan, such as biocompatibility, biodegradability, bioadherence and lack of toxicity. Chitosan is used in both medicine and pharmacy and has numerous benefits including antibacterial and antitumor properties.

***Applications of chitosan in periodontology***

Chitosan as mentioned in the previous sections shows antibacterial effect, promotes guided tissue regeneration, has antioxidant and antimicrobial properties. It also intervenes in the gain of periodontal epithelial attachment loss. Chitosan gels can be used in non-surgical periodontal therapy and in the treatment of periodontitis.

The antimicrobial activity of chitosan prevents possible infections. The functional groups present in chitosan derivatives are quaternary ammonium, guanidinyl, carboxyalkyl, hydroxyalkyl, thiol and hydrophobic groups such as long alkyl chains and phenyl rings and substituted bands. The amino groups of chitosan in contact with physiological fluids are thought to be protonated. Chitosan binds to the anionic groups of microorganisms and causes microbial cells to clump together and inhibit their growth. Some researchers argue that the antimicrobial activity of chitosan is directly related to the uptake of polysaccharide by the bacteria and this causes changes in the cell wall structure and increases the permeability of the cell membrane, causing cell death. It also interferes with bacterial coagulation [19].

Chitosan is found as a gel and hydrogel base used in conjunction with toothpastes, mouthwash and chewing gums, they exhibit antimicrobial properties in fighting microorganisms in the oral cavity. According to a study by Subbiah et al,[20] the antiplasmid effect of chitosan nanoparticles inhibits Cyperus rotundus and Anacyclus pyrethrum. Another study by Costa et al[21] reported that chitosan inhibits violacein production in

Chromobacterium violaceum. Abedian et al[22] demonstrated that, chitosan has a significant antibacterial effect on common oral bacteria such as Streptococcus mutans and Streptococcus sobrinus and further inhibits biofilm formation. Chitosan also exhibits anti-plaque activity against several oral pathogens such as Porphyromonas gingivalis, Prevotella intermedia and Aggregatibacter actinomycetemcomitans.

Chitosan also exhibits anti-inflammatory activity which it exerts by inhibiting the production of inflammatory cytokine interleukin (IL)-6 in human keratinocytes and the production of IL-12 in human monocyte and prostaglandin E2 levels. Expression of tumor necrosis factor-alpha and IL-6 at mRNA levels are down-regulated. The lipopolysaccharide-activated c-Jun NH terminal kinase and p38 mitogen-activated protein kinase signal pathways are attenuated by chitosan. Studies have concluded that the anti-inflammatory effect of chitosan particles in periodontal and gingival fibroblasts reduces inflammation in periodontal diseases.

Chitosan's involvement in periodontal tissue regeneration and haemostatic properties eliminate any need for additional material such as barrier membranes and bone grafts in regenerative therapies. Chitosan also exhibits osteoconductivity and induction of neovascularization, leading to accelerated bone growth. In a study by Park et al. Chitosan incorporated with platelet-derived growth factor BB and hydroxyapatite in the treatment of intraosseous defects resulted in increased bone formation. Mukherjee et al. evaluated a chitosan-hydroxyapatite glutamate paste as a synthetic bone graft material in rats and concluded that the paste exhibited osteoinductive factors such as bone morphogenetic protein-2. Chitosan gel can be effectively used in combination with demineralized bone grafts [19].

In terms of bone repair in periodontology, the biodegradability and biocompatibility properties of chitosan make it suitable for application as a biomaterial and scaffold to induce hard tissue regeneration. Chitosan with its chemical chains of H-bonds, cross-links and NH2+ with negative tissues in the human body thus provides good stability to produce new bone cell formation at an early stage of bone healing. Klokkevold's study demonstrated that spongy chitosan supports osteoblast proliferation, can increase osteogenesis and aids guided bone regeneration. Chitosan-filled alveoli have also been shown to result in higher bone density than untreated dental sockets.

Wound healing and haemostasis are some of the main goals of clinicians, in which chitosan stimulates macrophages to release IL-1 which in turn stimulates fibroblast proliferation. Chitosan also releases acetylglucosaminidase N and increases biosynthesis of hyaluronic acid and extracellular components related to scar formation and wound healing. Treated wounds showed increased collagen, more active fibroblasts and osteopontin with a strong infiltration of polymorphonuclear leukocytes.

Antibiotics such as metronidazole, chlorhexidine and nystatin, can be delivered to periodontal tissues by chitosan nanoparticles. When chitosan gel incorporated with or without 15% metronidazole was applied as an adjunct to scaling and root planing in chronic periodontal patients, they showed significant improvements in bleeding indices, probing depth, and clinical attachment levels. One study revealed that a chitosan concentration of 3% g/g could provide a basis for optimal drug dose modulation and make them effective to use as a local drug delivery agent. According to researchers Jothi et al, the local drug delivery system using chitosan-based polymer chlorhexidine reported a reduction in probing depth and a gain in clinical attachment levels and concluded that chitosan-loaded drugs may be an alternative treatment modality for patients with chronic periodontitis [19].

***Applications of chitosan in surgery***

Chitosan-based bone reconstructions may be a potential candidate in the areas of regenerative tissue due to its low immunogenicity, biodegradability, bioresorbable characteristics, low cost. Bone repair include autografts, allografts and surgical reconstructions, but they may carry a potential risk of donor site morbidity, rejection, risk of disease transmission and repetitive surgery. Bone tissue engineering is a multidisciplinary field that offers promising substitutes in biopharmaceutical applications. Thermo/pH-responsive chitosan-based injectable hydrogel formulations are advantageous in terms of their high water absorption capacity, minimal invasiveness, porous networks and ability to seamlessly transform into an irregular defect. In addition, chitosan combined with other naturally or synthetically derived polymers and bioactive agents has proven to be an effective alternative to autologous bone and dental grafts. Bone, composed of collagen apatite and calcium phosphate crystals, is a known internal support system, providing rigidity, strength and a degree of elasticity to the living body. In recent years, amidst increasing population ageing, accidental injury, disease, trauma, obesity and poor physical activity in internal and external mediators, bone disorders and diseases are on the rise worldwide. Although natural healing is a stable and reliable process, patients with bone trauma always experience impaired healing and rehabilitation. Traditional healing strategies include autografts, allografts and xenografts which are used as bone substitutes to help repair bone. However, these grafts have many disadvantages in the repetitive handling process, high cost, immune rejection and potential infectious diseases. Bone Tissue Endothelialization (BTE) leads to advanced development of bone regeneration at the defective host site without postoperative complications (e.g. morbidity and immunogenicity) structured around four key components: osteoblasts generate a matrix of bone tissue, the biocompatible spine mimics the extracellular matrix, the vascularization process provides nutrient and waste transport, and morphogenesis signals guide cell activation. Therefore, bone tissue engineering material requires favourable properties (e.g. osteoinduction and osseointegration), which can promote the differentiation of progenitor cells to osteoblasts, support bone growth and facilitate bone fusion to form new bone tissue. In addition, these materials should have chemical and mechanical stability, non-thrombosis, easy sterilisation and easy manufacturability in the host environment. For example, alveolar bone defects are urgently needed to be regenerated by relying on advanced materials to have a positive impact on dental tissue engineering for periodontal therapy. Natural polymers with good biocompatibility and biodegradability have a variety of beneficial characteristics and properties for living tissues and cells. As a representative, chitosan, the deacetylated form of chitin, is a natural linear cationic heteropolymer extracted from shrimp or crab shells. It has compositions and structures analogous to glycosaminoglycans and offers high biocompatibility, good biodegradability and minimal immune response to tissues and cells. Its physical properties are mainly based on molecular weight, degree of deacetylation and purity. For example, due to its cationic attribute, chitosan possesses outstanding antimicrobial activity against Gram-positive and Gram-negative bacteria, which is based on the type and degree of deacetylation of chitosan as well as other extrinsic environmental conditions. Due to the presence of protonated amino groups of D-glucosamine residues, chitosan can form a non-Newtonian fluid, which shear thinning in most dilute acidic solutions at pH below 6.5 (pKa value ~ 6.3) and further contributes to complexes with metal ions, polymers, lipids, proteins. In addition, chitosan-based hydrogels can be chemically cross-linked by glutaraldehyde, oxidized dextran or other carbohydrates and genipin due to reductive amination between amino and aldehyde groups under mild conditions. Although chitosan-based hydrogels have many advantages, their

mechanical properties are poor. Thus, it should be combined with other functional materials to promote osteogenic differentiation and tissue regeneration.

Chitosan is usually combined with other natural or synthetic biomaterials via covalent and non-covalent bonds, producing a variety of multifunctional hydrogels. In which, physical gelation is a typical approach for manufacturing chitosan-based hydrogels with good biocompatibility and gradual degradability to promote cell-material interactions and stimulate osteoprogenitor cell proliferation and differentiation. Therefore, the development of injectable chitosan-based hydrogels would enable an effective therapy for bone regeneration, especially for areas with irregular bone tissue defects. Based on this physical gelation of chitosan-based injectable hydrogel, injectable hydrogels sensitive to environment such as pH, light and temperature are widely used for repairing large bone defects, because an externally applied trigger for gelation can easily adapt the sol-gel transition with easy penetration into defect areas and rapid in situ gelation to completely seal the lesion.

***Analgesic and anti-inflammatory properties of chitosan***

These have been heavily debated, with several studies being conducted in this direction, one of which is the combination of ibuprofen and chitosan properties. The study evaluated the analgesic and anti-inflammatory properties of ibuprofen when administered through two different drug delivery systems after mandibular third molar extraction surgery. The study was conducted on 100 patients requiring surgical removal of impacted mandibular third molars under local anaesthesia. Subjects were divided into two groups of 50 patients each. Patients in the study group were administered chitosan-based microspheres embedded in ibuprofen, which were inserted into the molar alveoli after extraction of impacted teeth. Patients in the control group were prescribed 400 mg ibuprofen tablets to be administered orally after extraction of the impacted third mandibular molars. All patients were assessed for pain, swelling and trismus on the second, fourth and seventh postoperative days, and wound healing was assessed on the seventh postoperative day. Patients in the study group had significantly less pain and comparatively better mouth opening on the second, fourth and seventh postoperative day, which showed clinically and statistically significant results of $p<0.05$, while swelling assessment for the study group did not show statistically significant results on any of the three postoperative days. Of the 50 patients in the study group, two experienced delayed healing, and of the 50 patients in the control group, four experienced poor healing and three patients developed dry socket. Chitosan-based microspheres embedded in ibuprofen (study group) had comparatively better analgesic and anti-inflammatory properties, with a drastic reduction in pain, swelling, trismus and also had better lesion healing compared to orally administered ibuprofen (control group) after mandibular third molar extraction surgery [24].

## CONCLUSIONS

Medical developments in recent years have led to the availability of bioactive compounds for damaged tissues. Such compounds should have a regenerative effect and promote wound repair with the lowest possible morbidity and high biocompatibility. Chitosan polymers have been shown to serve as scaffolds that induce tissue regeneration, and not only they are considered to be an ideal polymer for the manufacture of bioactive compounds. This is possible because there is a potential synergy in their by-products when combined with growth factors and stem cells, either of mesenchymal or neural origin.

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# Marginal closure of ceramic-based restorations feldspatic fixed on unprepared teeth


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## Abstract

Introduction: In recent years, the demand for treatments designed to improve dental aesthetics has increased. Objectives: The aim of this study was to fix adhesive on fixed prosthetic restorations made of feldspathic ceramics directly on the enamel surface of the unprepared tooth. Materials and Methods: The study was performed on a number of 10 extracted teeth. They were divided into 2 groups according to the fixing technique used. Results: The results obtained on the group in which the marginal closure was not finished was significantly more deficient from an aesthetic and functional point of view. Discussions: To improve the aesthetics of the anterior teeth through all-ceramic veneers, two types of materials are indicated for translucency and their potential to be used in small thicknesses. Conclusion: When using minimally invasive techniques without tooth preparation, it is mandatory that the marginal closure be finished with rotary tools. Recommendations for original studies

**Keywords:** minimally invasive, no prep, feldspar pottery, marginal closure

## INTRODUCTION

In recent years, the demand for treatments designed to improve dental aesthetics has increased. In this context, both patients and dentists prefer to preserve the dental structures as much as possible. Thanks to technological advances, especially in adhesive dentistry, new materials, and minimally invasive techniques such as "no-prep" veneers have made this a reality.

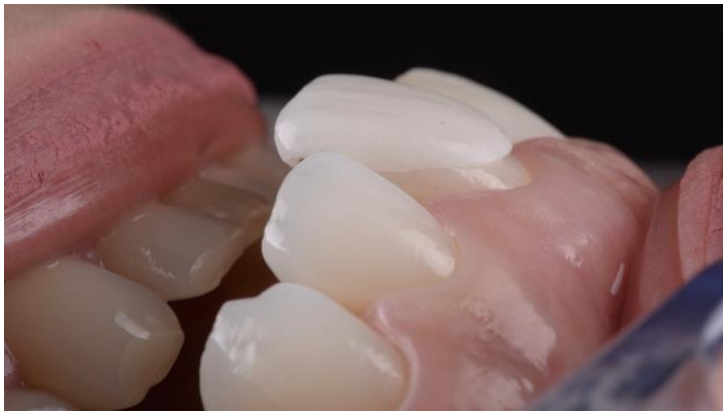

Figure 1. Non-prep veneers

### *Aim and objectives*

The aim of this study is to demonstrate that in the case of adhesive bonding of a feldspathic ceramic veneer to an unprepared tooth, it is mandatory that the marginal closure be finished with rotary instruments.

## MATERIAL AND METHODS

Advances in dentistry and dental materials have helped dentists sacrifice much less tooth structure, materials are increasingly resistant for minimal thickness, dental aesthetics and biocompatibility help the teeth be kept vital much longer.

The study was conducted on 10 extracted teeth. They were divided into 2 groups according to the fixation technique used.

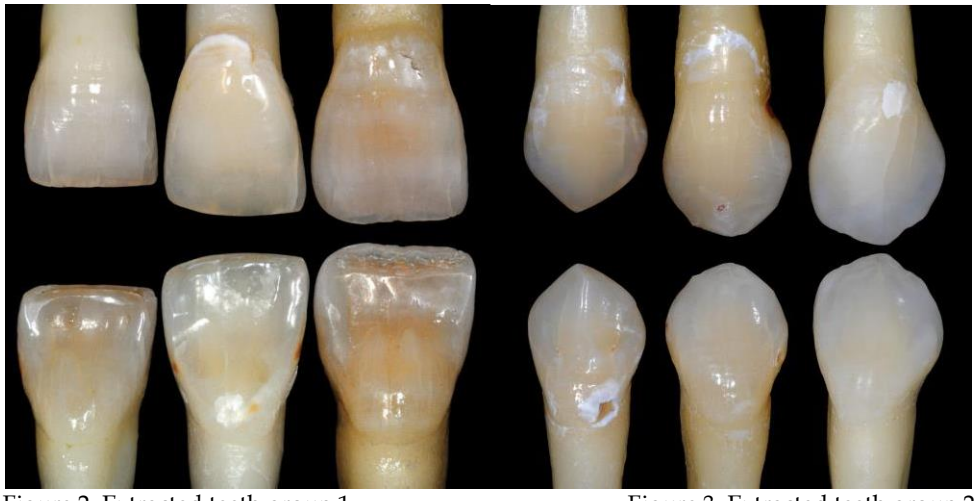

Figure 2. Extracted teeth group 1

Figure 3. Extracted teeth group 2

On one group the restorations made of feldspathic ceramics were fixed without finishing the marginal closure.

Figure 4. Unfinished veneer fixed on the tooth

Figure 5. Unfinished veneer fixed on the tooth

On the other batch, feldspathic ceramic restorations were fixed, and after fixing, the marginal closure was finished using rotary tools.

Figure 6. The veneer is finished and fixed on the tooth

Figure 7. The veneer is finished and fixed

Analysis of the marginal closure areas between the veneer and the tooth was done by optical microscopy and the results of the two groups were compared.

Figure 8. Unfinished marginal closure under the microscope

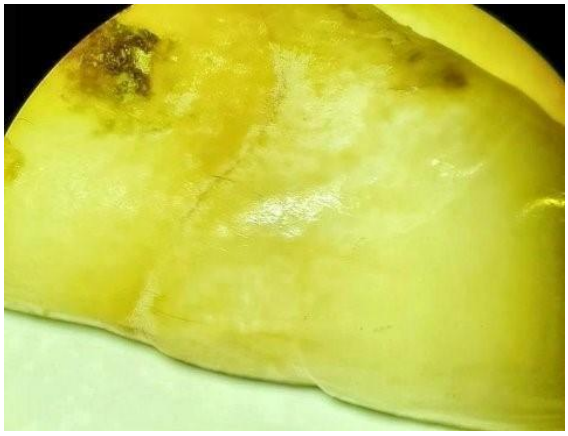

Figure 9. Finished marginal closure

The impression was made with a silicone with addition reaction in 2 consistencies - increased and fluid consistency.

Figure 10. Imprinting

The refractory pattern was destroyed when we created the facet Casting the extradur model-ghips.

Figure 11. Extra hard gypsum

Fixing the feldspathic veneer to the tooth structure is done in the following steps:

1. Conditioning the tooth- etch the tooth with orthophosphoric acid for 45s-1min, wash and dry.

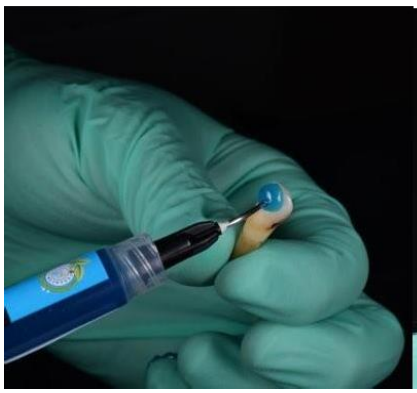

Figure 12. Application of acid

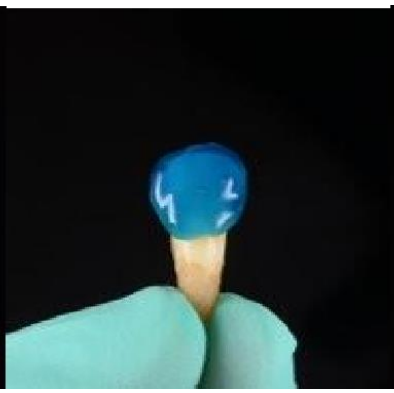

Figure 13. I left it for 45s-1min

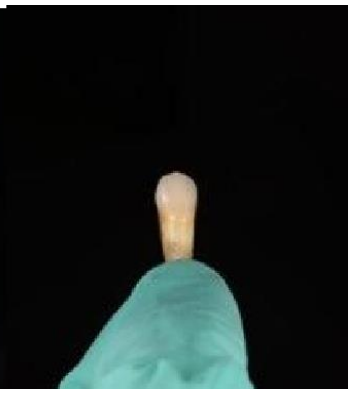

Figure 14. Washed and dried


Figure 15. Orthophosphoric Acid 37% -Cerkamed

Apply bonding and remove excess by blowing with air, then light cure for 20s.

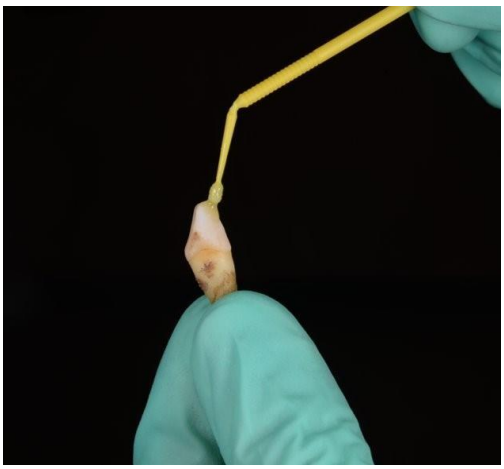

Figure 16. Application of bonding

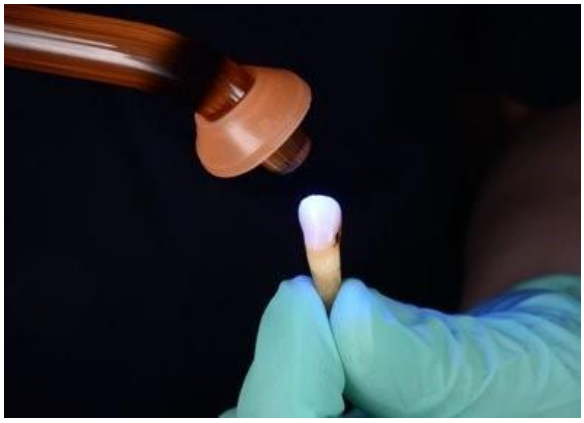

Figure 17. Photopolymerization of the tooth

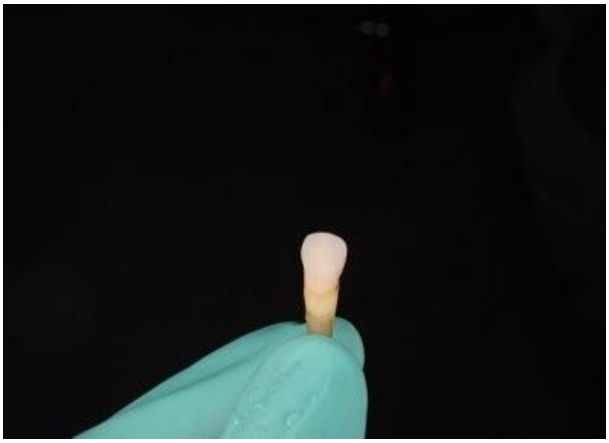

Figure 18. The tooth prepared for the following procedures


Figure 19. Bonding, Vivapen Ivoclar

2. Conditioning the feldspathic ceramic veneer- we take the veneer from the duplicate model with an Optrastik (Ivoclar), use hydrofluoric acid first for 1min after which we use orthophosphoric acid to remove salts from the acid etching and for degreasing at the same time.

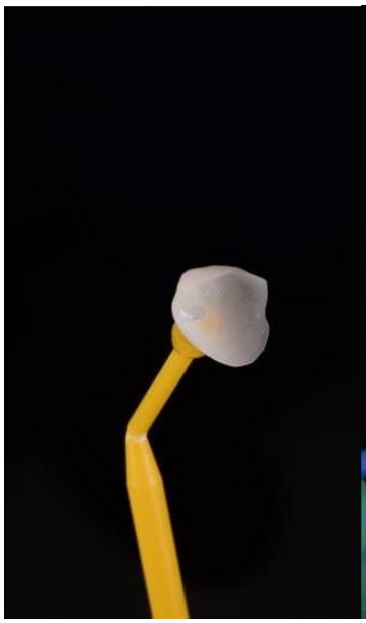

Figure 20. Veneer on an Oprastik

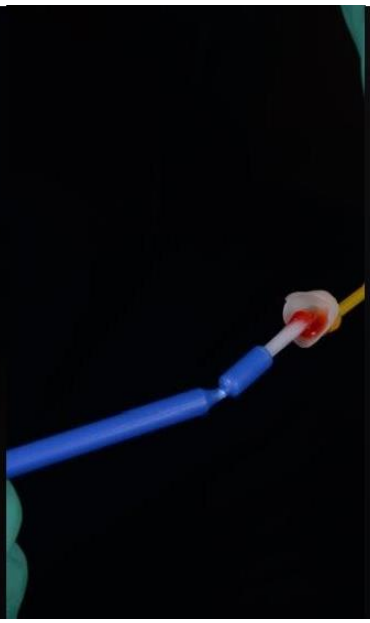

Figure 21. Application of hydrofluoric acid

Figure 22. Cleaning


Figure 23. Optrastik Ivoclar


Figure 24. IPS Ceramic Etching gel Ivoclar

Wash and dry, then apply primer (silane), dry and apply primer again.

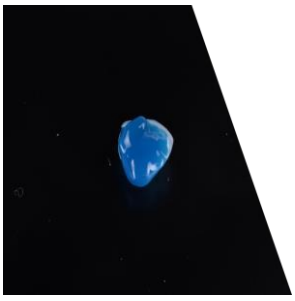

Figure 25. Degreasing the fase

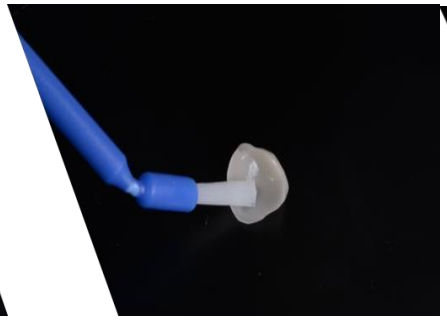

Figure 26. Primer application

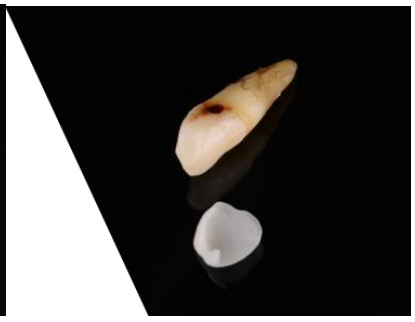

Figure 27. The prepared veneer


Figure 28. Universal ceramic primer

After these steps we apply Variolink cement on the veneer for fixation, clean the excess and light cure it.

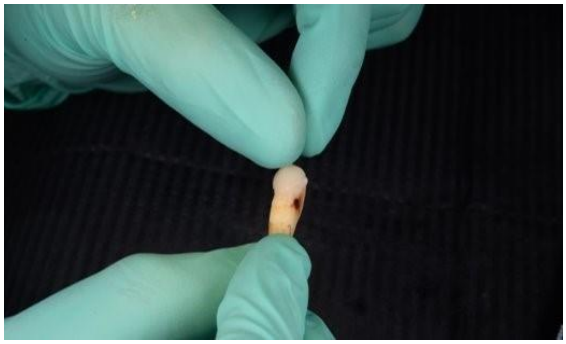

Figure 29. Fixing the veneer on the tooth

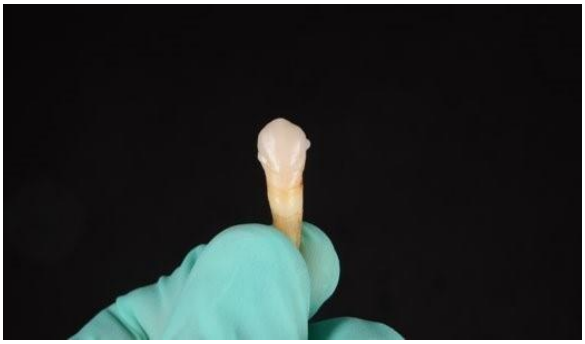

Figure 30. Fixing the veneer on the tooth

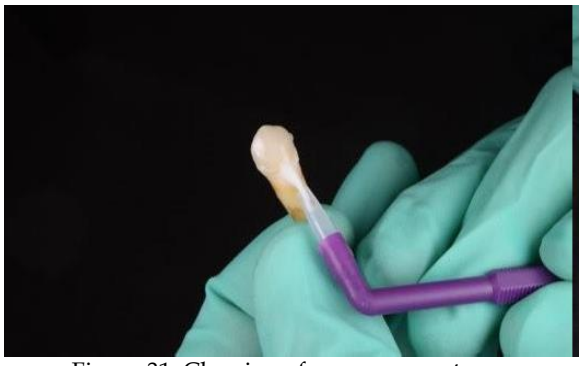

Figure 31. Cleaning of excess cement

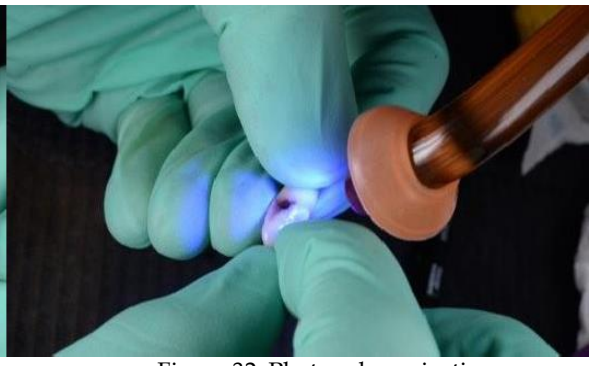

Figure 32. Photopolymerization


Figure 33. Variolink Esthetic LC Ivoclar

We finish with an Arkansas stone and a gum.

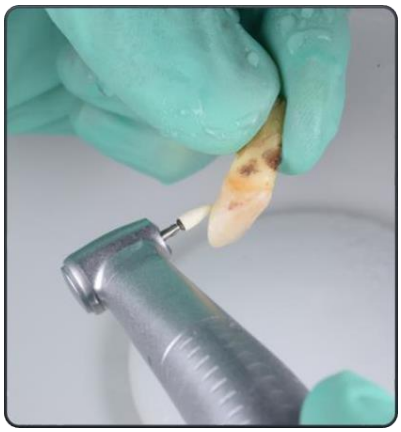

Figure 34. Finishing the veneer with arkansas Stone

Figure 35. Finishing the veneer with gum

We have demonstrated that in all clinical cases that we can fix the veneer on the non-prep teeth is ideal to be able to keep the teeth vital and intact for as long as possible, until we reach other interventions in the future such as crowns or advanced prosthetic work.

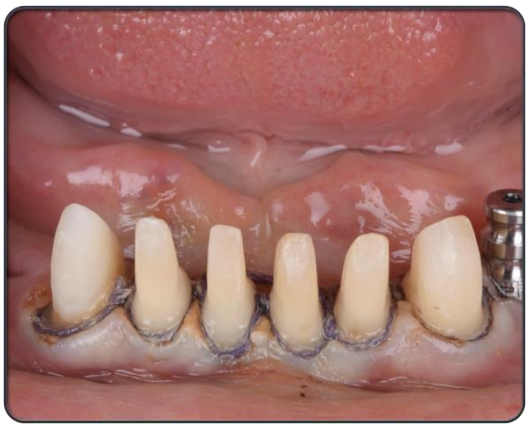

Figure 36. Grinded teeth for crowns

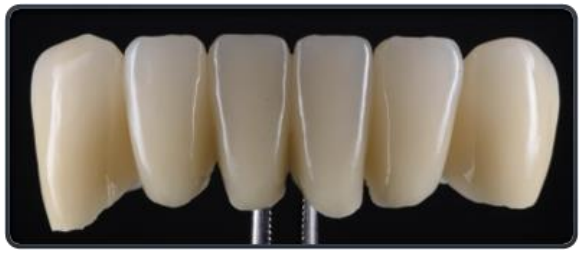

Figure 37. Dental crowns

## RESULTS

The results obtained on the lot where the marginal closure was not finished was significantly more deficient from an aesthetic and functional point of view, observing a step of overconturation. The results for the group in which the marginal closure was finished were similar to the situation in which the tooth was intact. No transition was observed at the level of the marginal closure between the veneer and the tooth, resulting at the level of the closure only in a conglomerate of ceramic, enamel and luting cement.

## DISCUSSIONS

To improve the aesthetics of front teeth with all-ceramic veneers, two types of materials are indicated for their translucency and potential to be used in small layers: sintered feldspathic ceramics and pressed ceramics that can be used and milled using computer-aided CAD/CAM techniques.

Practitioners must choose the material on the requirements of the tooth to be restored, such as the indication and the need for tooth preparation to improve aesthetics and functionality. Feldspathic veneers have undergone a significant evolution. Nowadays, their use has expanded beyond a simple covering for anterior teeth to include the covering of coronal tooth structures. Feldspathic veneers are created by layering with powder-based (silicon dioxide) and liquid materials. The mechanical properties of feldspathic ceramics are low, with flexural strength typically 60 to 70 MPa. However due to adhesive bonding to the glaze the success rate is increased. Due to the low strength of feldspathic ceramics the finishing protocol was easy at the marginal closure of the facets.

## CONCLUSIONS

When using minimally invasive techniques without tooth preparation it is mandatory that the marginal closure is finished with rotary instruments. An important aspect to note is the ease of finishing feldspathic facets.

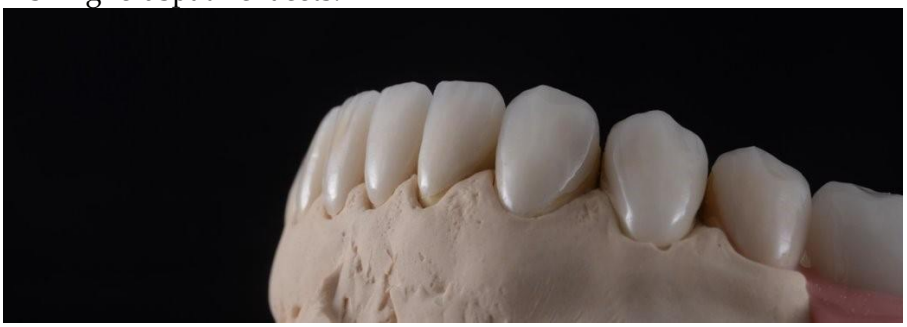

Figure 38. Veneers

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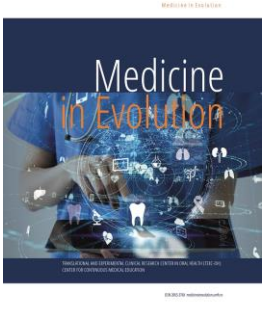
# Obtaining the bicomponent tissue adhesive from blood collected preoperatively

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## Abstract

The difference between primary and secondary hemostasis is that primary hemostasis is defined by the formation of the primary platelet plug whereas secondary hemostasis (coagulation) is defined by the formation of insoluble, cross-linked fibrin. Activated platelets are responsible for primary hemostasis and activated clotting factors are responsible for secondary hemostasis.

Platelets play a crucial role in hemostasis and wound healing, with platelet-derived growth factors as a source of healing cytokines. Platelet concentrates for surgical use are innovative tools of regenerative medicine and have also been tested in oral and maxillofacial surgery. These products are extracts of the blood tissue, they are tissues themselves, and not pharmaceutical preparations. Fibrin tissue adhesive has applications in several fields of medicine, can be prepared by various methods.

**Keywords:** Hemostasis, platelet, growth factors, tissue adhesive

## INTRODUCTION

Primary and secondary hemostasis relates to clot formation. The elements of hemostasis are vascular response, platelet number and function, von Willebrand's factor (vWF) level and clotting factor levels. Primary hemostasis is a procoagulation clot-forming process associated with the initiation and formation of the platelet plug. Secondary hemostasis is associated with the propagation of the coagulation process through the intrinsic and extrinsic coagulation cascades. Secondary hemostasis depends on appropriate interactions of coagulation factors leading to fibrin clot formation.

In all surgical branches the search for adjuvant techniques is unceasing. Current research aims to develop materials capable of enhancing the healing process and regulating inflammation, focusing on solving the limitations of conventional treatments [1].

Edwin J. Cohn and his team developed in 1946 a process to extract albumin from blood plasma. The process is based on the differential solubility of albumin and other plasma proteins based on pH, ethanol concentration, temperature, ionic strength and protein concentration (Figure 1) [2].

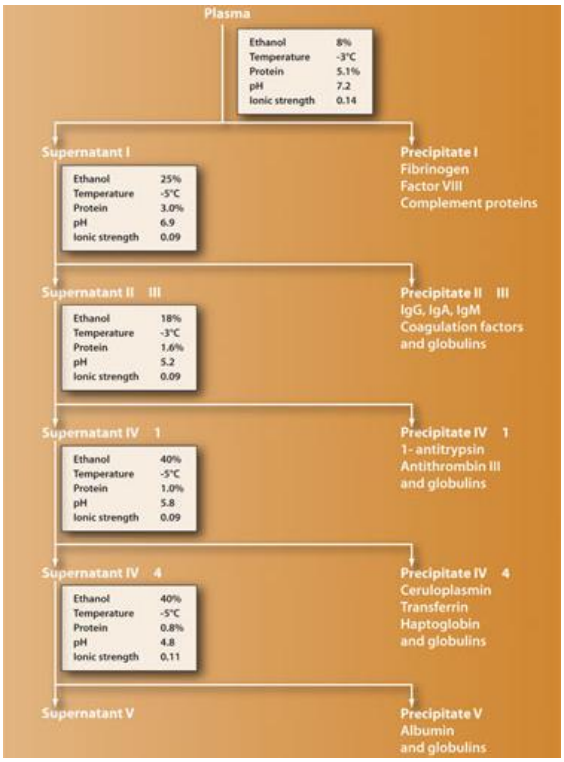


Figure 1. Basic plasma fractionation scheme using the Cohn process [3]

Studies of interactions between fibrinogen/fibrin and plasma proteins and receptors on platelets, leukocytes, and other cells have demonstrated complex functions in hemostasis, thrombosis, inflammation, infection, cancer and other pathologies [4,5].

Autologous or commercial fibrin sealants and platelet concentrates have been used alone or in association with bone substitutes to promote bone healing in oral surgery [6-8].

The autologous platelet concentrates, Platelet-Rich Plasma (PRP) and Platelet-Rich Fibrin (PRF), are used in various medical fields, for local and infiltrative use in orthopedic and sports medicine and also in oral and maxillofacial surgery [9,10]. They are obtained after different processing of a blood sample, mostly by centrifugation [11]. The purpose of the processing is to separate the blood components, remove the elements considered unusable

(red blood cells) and concentrate the elements that can be used in therapeutic applications (fibrinogen/fibrin, platelets, growth factors, leukocytes) [12].

### *Aim and objectives*

The study is a combination of the technique of concentrating fibrinogen with ethanol originally described by Cohn and more recently by Kjaegaard and the separation of thrombin from the patient's blood with a technique derived from the studies of Thorn, Kumar and Ghassab.

## MATERIAL AND METHODS

### *Blood collection and coarse separation*

Collect 50 ml of blood in vacuum tubes with 1.4 ml of citrate phosphate-dextrose anticoagulant (Figures 2,3). Centrifuge gently at 330X g for 15 minutes. In this way most platelets remain in the superficial layer.

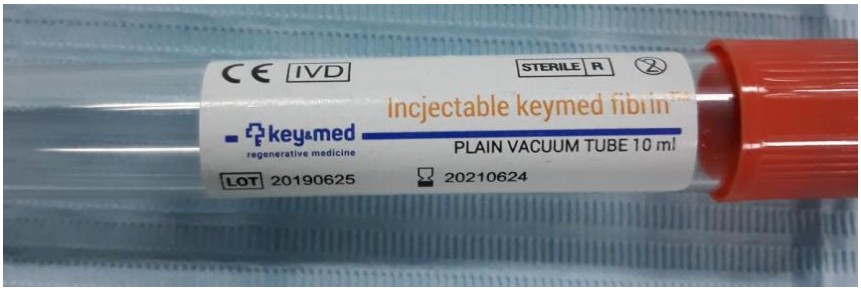


Figure 2. Vacuum tube made of polyethylene, without additives

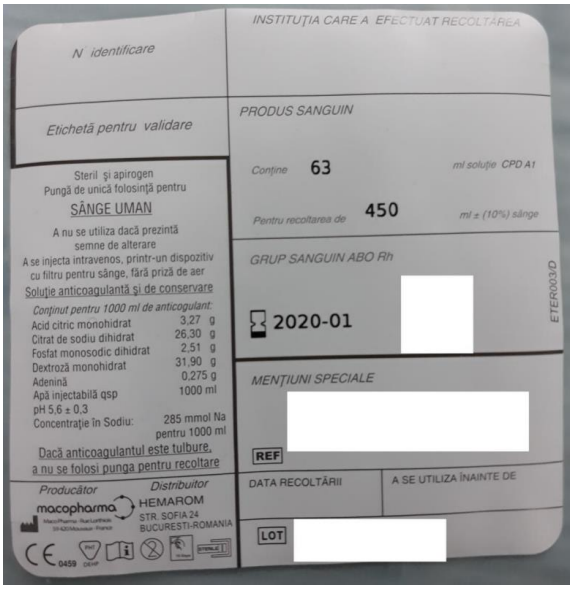


Figure 3. Transfusion bag containing 63 ml of anticoagulant citrate-phosphate-dextrose


Figure 4. Programmable centrifuge, Rotor radius 100mm

The centrifuge used is programmable and has a rotor with spaces for test tubes inclined at 45 degrees (Figure 4). So, the maximum distance between the tip of the test tube and the center of the rotor is 10 cm. To calculate the speed to which the centrifuge must be programmed, the formula was used $G=(1.118 \times 10^{-5})RS^2$, where "R" is the radius of the rotor, "S" is the speed and "G" is the relative centrifugal force.

After centrifugation for 15 min at 330xG, a separation of blood components in 3 layers is obtained, as follows: the superficial layer obtained is plasma rich in proteins plasma and the inorganic fraction of blood; the white intermediate layer contains most platelets and leukocytes; and the densest layer consists of erythrocytes (Figure 5).

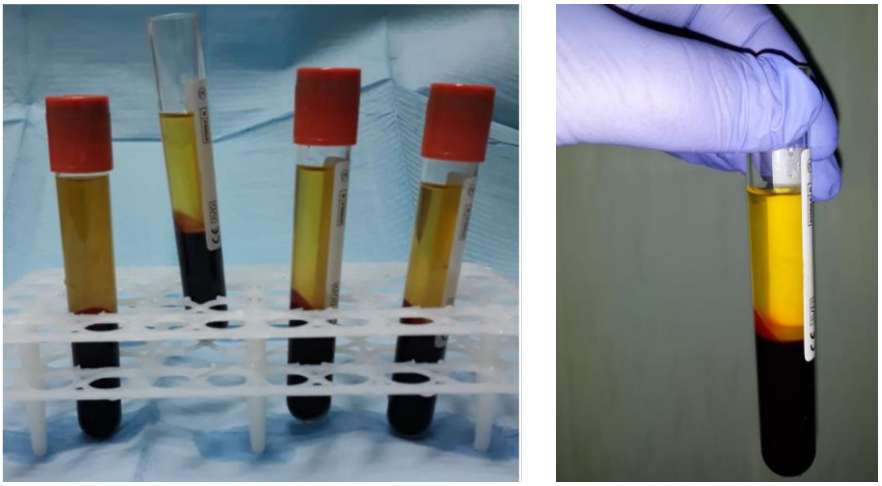

Figure 5. The blood collected in tubes with anticoagulant is centrifuged for 15 minutes at 330xG. Separation of blood elements occurs according to mass; the superficial layer is rich in platelets because the centrifugation was gentle

***Separation of the euglobinic fraction and obtaining one product concentrated in thrombin***

2.5ml of the plasma solution is extracted and diluted with approximately 22 ml of citric acid of concentration 2.84mM (Figure 6). The obtained solution is centrifuged for 5 minutes at 3000xG at 4°C. The supernatant is removed (Figure 7) and the precipitated fraction is dissolved in approx. 0.2 ml of calcium Ca gluconate (0.1M) (Figure 8). The pH is neutralized by adding of 0.1 ml of sodium bicarbonate of 75mM concentration (Figure 8) and thus the activation is initiated prothrombin. In the next 3-10 minutes, a fibrin clot forms. The clot is lightly pressed during formation for fibrin aggregation. After 20-30 minutes, the liquid containing thrombin is removed in a syringe. Prothrombin activation continues for another 2-4 hours, but it is important to remove the clot from the rest of the liquid because fibrin has the property of fixing thrombin.

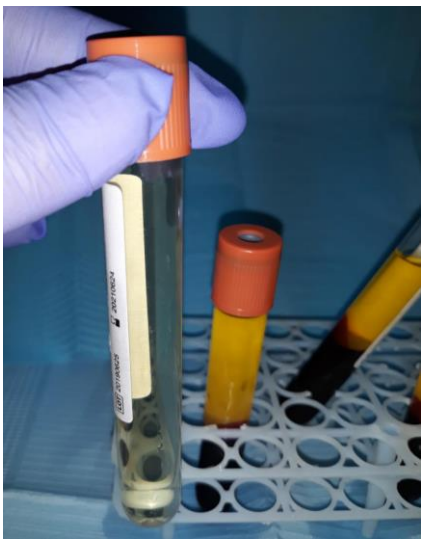

Figure 6. 1 ml of plasma diluted in 9 ml of citric acid

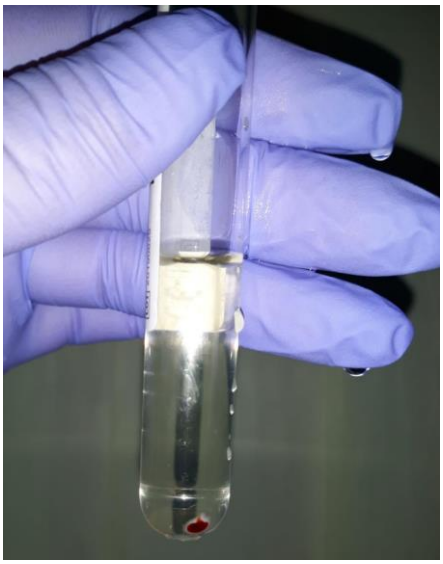

Figure 7. After centrifugation at 3000xG for 5 minutes, the supernatant is removed

Figure 8. After precipitation of the euglobulin faction, coagulation is initiated by adding calcium chloride and bicarbonate

***Preparation of concentrated fibrinogen***

The rest of the platelet-rich plasma is mixed with approximately 1 ml of acid tranexamic to prevent simultaneous precipitation of plasminogen with fibrinogen. Ethanol is added until a concentration of approximately 10% ethanol is obtained and the mixture is cooled in an ice water bath for 20-30 minutes to obtain a temperature close to 0°C (Figure 9). The product is centrifuged for 8 minutes at 3000xG at 0-4°C (Figure 10). The supernatant is removed and the precipitated fibrinogen is redissolved by heating to 37°C (Figure 11). Heating to 37°C this arrangement and produces a cloudy, stable liquid that coagulates very slowly when Ca is added (because it lacks the other factors present in the plasma), but it coagulates immediately in contact with thrombin.

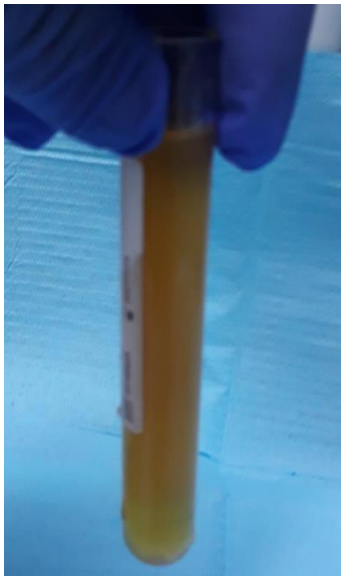

Figure 9. Plasma mixed with ethanol up to a concentration of about 10% and cooling the mixture in a bath of ice water for 20 minutes

Figure 10. Centrifuge the cooled plasma at 3000xG for 4 min and discard the supernatant

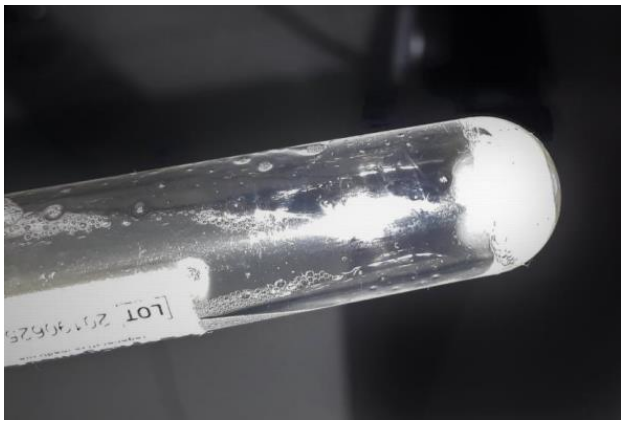

Figure 11. After centrifugation, the supernatant is separated and concentrated fibrinogen is obtained

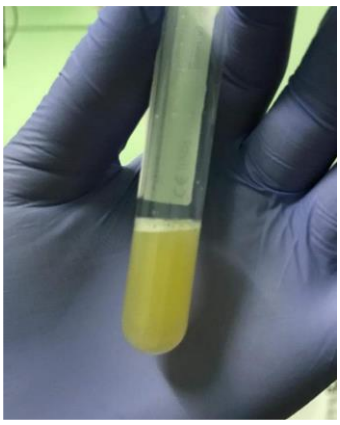

Figure 12. The precipitated fibrinogen

The precipitated fibrinogen in the 5 test tubes is mixed with 1ml of physiological serum, and after heating and mixing becomes a cloudy liquid (Figure 12).

***Thrombin concentration adjustment***

Concentrations range from 50 to 500 NIH u/ml, values where coagulation occurs immediately, thus diluting with calcium chloride solution 0.05M is important for a slower and more efficient polymerization of the fibrin network - three-dimensional polymerization with the inclusion of cytokines in the formed network (Figure 13).

Figure 13. Coagulated fibrinogen in contact with thrombinase product and Ca gluconate

**RESULTS**

Immediately before using the adhesive, the thrombin concentration is adjusted in depending on the required working time. Values of 10%, 5% or 2% are the concentrations used and should always be tried on plasma. The mixture of 150 µL of plasma with 50 µL of thrombin should coagulate in an interval of 30-60 seconds.

## DISCUSSIONS

Thrombin used in surgical procedures as a hemostasis adjuvant [13-16], derived mainly from bovine sources, is associated with adverse reactions, for example, the formation of antibodies against human factor V leading to bleeding episodes [17,18], and the transmission of bovine prions that could cause a variant of Creutzfeldt-Jacob disease (vCJD) [19]. The use of autologous thrombin is an alternative in surgery because it avoids the risk of infectious diseases and immunogenicity problems.

Fibrin sealants are hemostatic agents and possess pattern characteristics for cell migration, supporting the growth of keratinocytes and fibroblasts. Research has confirmed that fibrin adhesives can be used safely at sites of infection [20].

Thorn *et al.* conducted a study with the aim of preparing autologous fibrin glue with platelet growth factors and using it together with cancellous bone particles in maxillofacial reconstructive surgery. The glue they obtained had a concentration of fibrinogen about 12 times higher, and the concentration of growth factors was about eight times higher than their value in platelet-rich plasma [4].

Kumar *et al.* conducted a study to investigate the stability of thrombin produced using the thrombin processing device (TPD; Thermogenesis Corporation) and the addition of plasma (11 ml) and reagent ($CaCl_2$ and ethanol, 3.75 ml). Their study showed that the active thrombin produced by TPD depends on both the production temperature and the storage temperature [21].

Ghassab *et al.* conducted a study to compare the coagulation efficiency of platelet-rich plasma (PRP) and platelet-poor concentrated plasma (cPPP) with citrated whole blood after activation by autologous thrombin, bovine thrombin, or calcium chloride ($CaCl_2$). Their study showed that PRP provided the best combinations for clinical use when combined with either bovine thrombin or $CaCl_2$. Autologous thrombin was suboptimal but could be an autologous alternative for clinical application, and cPPP had ineffective coagulation [22].

Fibrin glues generally contain fibrinogen and thrombin with a small amount of calcium chloride to create a clot that can be administered. Their advantage is that they do not require active bleeding and can work independently of the patient's own fibrinogen [23].

Valbonesi *et al.* confirmed the utility of fibrin-platelet glue in reducing infection and length of hospital stay in patients with skin and soft tissue loss from recent trauma or chronic pathology. The authors believe that the preparation of the glue is very easy, cheap and creates excellent and stable hemostasis [24].

## CONCLUSIONS

Compared to traditional wound therapy, biologic adhesives are an attractive choice due to their ease of operation, rapid hemostasis, and wound recovery. Currently, research on adhesives is focused on improving their mechanical properties to achieve completely sutureless medical procedures.

Bicomponent tissue adhesive (platelet-rich plasma in combination with fibrin adhesive) constitutes the basic mixture for intraoral bone grafting techniques.

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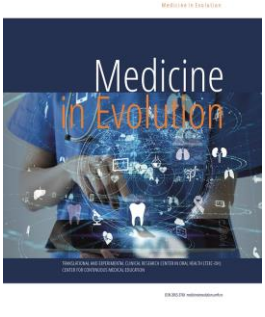
# Patient's rights to dental treatment - the influence of the Covid-19 pandemic

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## Abstract

Aim and objectives: The present study aims is to observe and analyse the impact that the COVID-19 pandemic has on patients' right to have access to dental medical services. Both the national quarantine by decreeing the state of national emergency in Romania and the state of alert instituted due to the pandemic had an important impact on patients' appointments for dental procedures.

Material and methods: We analyse the statistical data starting from the two groups of patients and follow the main differences that appear due to the COVID-19 pandemic. Group 1-non-pandemic included patients who presented themselves in the office between March 14, 2019 and March 14, 2020 and group 2-pandemic included patients who presented themselves in the office between March 15, 2020 and March 15, 2021.

Conclusions: Following this study, we can say that the COVID-19 pandemic has a major impact on patients' right to dental treatment, affecting both access to services medical as well as unjustifiably delaying the treatment of dental pathology.

**Keywords:** dental treatment; Covid-19, pandemic, patient rights

## INTRODUCTION

In December 2019, a case of unknown pneumonia was detected in the city of Wuhan (China). It was first reported to the WHO China Office on 31 December 2019. This pneumonic infection spread rapidly from Wuhan to most other Chinese provinces and in 24 other countries. On January 30, 2020, the outbreak was declared a Public Health Emergency with International concern. Chinese researchers quickly discovered and isolated a new coronavirus, (2019-nCoV), responsible for the onset of pneumonia. On February 11, 2020, the WHO announced a name for the new disease caused by the coronavirus, COVID-19, and increased the risk assessment of spread to "very high" on 28 February 2020. On 11 March 2020, the WHO Director-General stated that the spread of COVID-19 is no longer limited to certain geographical areas, but a pandemic spread throughout the world. The first two cases of COVID-19 in Europe appear in Italy, a couple by Chinese tourists, being confirmed positive on January 30, 2020 by the Spallanzani Institute (Rome) where they were hospitalized and isolated until their recovery on February 26, 2020 [1,2].

Transmission routes of SARS-CoV-2 include Flügge droplets (particles with diameter ≥ 5 μm) generated from coughs and sneezes of infected patients, as well as direct contact with oral, nasal and ocular mucosa. In addition, studies have shown that SARS-CoV-2 can be transmitted through saliva [12]. Some studies suggest that the salivary glands may represent a reservoir for asymptomatic COVID-19 infection. In fact, the expression of the angiotensin-converting enzyme 2-ECA, a key receptor for COVID-19, is higher in minor salivary glands than in the lungs. This could explain the occasional lack of symptoms in infected subjects. More than both, the positive rate of COVID-19 in patients' saliva can reach up to 100%, and the samples of saliva is a culture medium for the virus. These considerations are of major importance in dental treatment, due to the contagious potential of saliva [3,4].

The oral manifestations of COVID-19 are polymorphic and represented by ulcerations of oral mucosa, erosions, bubbles, vesicles, pustules, fissured, tongue, macule, papule, placard, pigmented areas, halitosis, whitish areas, haemorrhagic crusts, areas of necrosis oral mucosa, petechiae, oedema, erythema and spontaneous bleeding. The most common affected sites, in descending order, they are: tongue (38%), labial mucosa (26%), palate (22%), gum (8%), oral mucosa (5%), oropharynx (4%) and tonsil (1%). These lesions are classified as stomatitis foot and mouth, herpetiform lesions, candidiasis, vasculitis, mucositis, eruptions, ulcero-necrotic stomatitis, angular cheilitis. Oral lesions are symptomatic, with patients complaining of pain, itching or paresthesias. The latency time between the appearance of systemic symptoms and oral lesions can be between 4 days before the appearance of general manifestations of the disease and up to 12 weeks after the appearance general symptoms. Oral lesions heal with complete remission between 3 and 28 days after initial appearance. Various types of therapy, including mouthwash with hydrogen peroxide and chlorhexidine, nystatin, oral fluconazole, topical or systemic corticosteroids, systemic antibiotics, acyclovir systemically, artificial saliva and photobiomodulatory therapy were prescribed for oral lesions [5].

Dental procedures, by their nature, present a high risk of infection with COVID-19 due to face-to-face contact between patients and the dentist and nurse. Furthermore, frequent contamination with saliva, blood and other biological fluids such as pus, but also use sharp and high-speed rotating instruments increase the risk of transmission of infection in dental offices. A published report suggests that the transmission of the pathogen SARS-CoV-2 it can also occur by inhaling remnants of the virus that can survive on surfaces for some time hours. Clinical studies indicate that most dental procedures involving the use rotating handpieces

generate a considerable amount of aerosols, contaminated droplets and potentially infectious [6,7].

In Romania, the first measures taken to limit the COVID-19 pandemic have started by issuing a DECREE by the President of Romania, decree no. 195 of 16 March 2020 regarding the establishment of the state of emergency on the territory of Romania, published in the GAZETTE OFFICIAL of Romania no. 212 of March 16, 2020. He took into account the evolution of the situation epidemiological on the territory of Romania and public health risk assessment for the period immediately following, which indicated a massive increase in the number of people infected with the SARS-CoV-2 coronavirus, taking into account the fact that the failure to take urgent, characterful measures exceptionally, in the social and economic field, for limiting infection with the SARSCoV-2 among the population would have a particularly serious impact, mainly on the right to life and, subsidiarily, on the right to health of individuals, the state of emergency was established on he territory of Romania for 30 days[8].

Subsequently, the Ministry of Internal Affairs issues MILITARY ORDINANCE no. 2 of March 21, 2020 on measures to prevent the spread of COVID-19, which in article 1 stipulates that: (1) Activity in dental offices is temporarily suspended. (2) By exception, they are emergency dental interventions allowed. (3) The measure applies starting from March 22 2020, 22:00, Romanian time [9].

After this military ordinance, by Decision of the National Executive Office no. 16/3BExN/2020 of the CMSR, clarifications were made regarding the performance by the offices of public and private dentistry of emergency dental interventions and was approved the plan of measures regarding the general framework for performing emergency dental interventions. It is the situation in which the patient needs medical care is considered a dental emergency immediate for the control of pain, infection or bleeding, according to the provisions of art. 12 para. (3) from the ethical code adopted by the Decision of the National General Assembly by Decision no. 15/2010. On throughout the territory of Romania, according to the CMSR website, around 150 emergency rooms were operating in dentistry, both public and private.

Thus, the activity of dental offices was limited exclusively to ensuring the therapeutic solution of dental emergencies:

- postextractional haemorrhage;
- pain due to acute pulpitis;
- pain due to acute apical periodontitis;
- pericoronitis of the impacted teeth;
- postextractional alveolitis;
- odontogenic cellulitis/abscesses;
- jaw/mandible fractures (emergency immobilization);
- dislocation of the temporomandibular joint;
- dento-alveolar traumas (dislocations, avulsions, dental fractures);
- ulcer necrotic gingivostomatitis.

On 14.05.2020 the National Committee for Emergency Situations adopts DECISION no. 24 regarding the approval of the establishment of the state of alert at the national level and the measures of infection prevention and control, in the context of the epidemiological situation generated by the virus SARS-CoV-2. This stipulates in article 1 that starting from 15.05.2020, it is declaredState of alert at national level, for a period of 30 days [10]. The state of alert was extended repeatedly.

***Aim and objectives***

The purpose of this study is to observe and analyse the impact that the COVID-19 has on the right of patients to have access to dental medical services. so much national quarantine by declaring the state of national emergency in Romania as well as the state of alert instituted due to the pandemic had an important impact on patients' appointments for dental procedures. Both ongoing treatments and emergency procedures were affected.

## MATERIAL AND METHODS

In this study, we included patients of our private practice dental office located in Timişoara, Romania.

The observational study focuses on the comparison of two groups of patients who meet the inclusion and exclusion criteria:

o *group 1-non-pandemic* included patients who presented themselves in the office, during March 14 2019 – March 14, 2020.

o *group 2-pandemic* included patients who presented themselves in the office, period March 15, 2020– March 15, 2021.

Patients included in the observational study were informed of a possible medical research and have given their consent by signing the informed consent form, according to Ministry of Health Order 1411 of 12.12.2016, annex no. 1 to the rules methodological-Informed patient consent expression form. It states that: "According to articles 19 and 20 of Law no. 46/2003 regarding patient rights, I express myself consent to participate as a patient in clinical medical education and scientific research, as well as regarding my pre-, intra- and post-op photography/filming, all of these information that can be used for didactic, medical and scientific purposes".

The aim is to analyze the statistical data starting from the two groups of patients and tracking the main differences arising from the COVID-19 pandemic in programming patients, addressability, emergency treatment, curative treatment, medium-term impact and long, but also the influence on diagnosis. For this purpose, we have classified dental treatments into 3 large classes:

A. Dental emergency requiring immediate dental treatment that cannot be delayed

B. Emergency dental care that can be scheduled within 24-48 hours

C. Routine dental treatment

From category A, of dental emergencies that require immediate dental treatment which cannot be postponed include: postextractional haemorrhage; pain due to acute pulpitis; pain due to acute apical periodontitis; pericoronitis of the impacted teeth; postextractional alveolitis; odontogenic cellulitis/abscesses; jaw/mandible fractures (emergency immobilization); dislocation of the temporomandibular joint; dento-alveolar traumas (dislocations, avulsions, dental fractures); ulcer necrotic gingivostomatitis.

From category B, of urgent dental medical assistance, which can be scheduled in 24-48 hours, include: patients with painful pathology due to chronic acute pulpitis that succumb to pharmaceutical treatment; patients with painful pathology due to chronic acute periodontitis that succumb to pharmaceutical treatment; patients who require tooth extractions; patients with loose prosthetic restorations.

Category C, of routine dental treatment, includes: patients with old, painless decays; patients with various edentulous that require implantologic treatment; patients presenting for prosthetic treatment; patients presenting for professional dental cleaning; patients who want aesthetic dental treatment; patients who want dental fillings or want their replacement; patients who want dental treatment planning.

## RESULTS

*Group 1, non-pandemic*, included 985 patients who presented to the dental office. *Group 2, pandemic*, included 642 patients. From the point of view of the distribution of patients by gender and age: in group 1, non-pandemic, we included 545 male patients representing 55.3% and 440 female patients, representing 44.7%. Group 2, pandemic, included 642 patients - 358 males, representing 55.7% and 284 female patients, representing 44.3%.

Regarding age categories:

- 18-30 years: group 1- 252 patients, group 2- 185 patients
- 31-60 years: group 1- 380 patients, group 2- 258 patients
- >60 years: group 1- 353 patients, group 2- 199 patients

Regarding the dental operations performed and classified in groups A, B and C, the distribution was as follows: A. Dental emergency requiring immediate dental treatment that cannot be delayed: group 1-218 patients (22.1%), group 2-301 patients (46.9%).

B. Urgent dental care, which can be scheduled in 24-48 hours: group 1- 342 patients (34.7%), group 2-165 patients (25.7%).

C. Routine dental treatment: group 1-425 patients (43.2%), group 2-176 patients (27.4%).

## DISCUSSIONS

Patient access to dental services has suffered since 15.05.2020, with the establishment of the state of emergency at national level. Patients' right to treatments dentistry was affected by decree no. 195 of March 16, 2020 regarding the establishment of the state of emergency on the territory of Romania, published in the OFFICIAL MONITOR of Romania no. 212 of March 16 2020. Thus, starting from this date, the accessibility of dental services becomes almost nil due to the limitation or prohibition of the movement of vehicles or people to/from certain areas or between certain hours, as well as exiting those areas. Even if the activity of our office has not been officially suspended by normative acts, most patients have theirs cancelled the appointments they had or wanted to reschedule them without specifying a specific date. Remained for treatment only patients in category A, of dental emergencies that require immediate dental treatment that cannot be postponed.

The impact of the closure of dental offices was important, by the reason of not treating at the time the carious pathology and its complications as pulpitis and periodontitis, by accentuating the pain of odontogenic cause, through odontogenic infections of the cervico-facial fascial spaces. Starting from May 15, 2020, dental activity resumes, respecting all the laws, norms and recommendations issued by the legislator, including CMSR. In the first 6 weeks, the flow of patients is almost double, both for initial consultations, emergencies and completing dental treatments stopped due to the state of emergency, then following a trend downward. As can be seen from the results of our study, the number of pre-pandemic patients it was much higher, with 343 more patients compared to the group analysed from the time the pandemic. The explanations for this massive decrease found in the number of patients is on the one hand the state of emergency with the closing of dental offices, but also the reluctance of patients for services represented by the fear of contacting the virus from medical facilities. This trend it is also confirmed by specialized literature, which shows similar data all over the globe [11-14].

In terms of performing dental procedures, the number of dental emergencies that required immediate dental treatment that cannot be postponed, it almost doubled during the pandemic compared to the pre-pandemic period. As an explanation, and in close correlation, may be the massive decrease in the number of routine dental treatments observed today study. Patients postpone the presentation to the dentist until the dental pathology is acute.

## CONCLUSIONS

We can state that the COVID-19 pandemic has a major impact on patients' right to dental treatment, affecting both access to medical services as well as unjustifiably delaying the treatment of dental pathology. If during the pre-pandemic period the number of dental pathologies that required emergency treatment was at one acceptable level, during the pandemic it increased substantially.

Although most of the public attention and medical forums focus on the prevention of infection with the SARS-CoV-2 virus and the possible aspects of the pathology of COVID-19 consequences on the health of the oral cavity caused by the fear of infection with the coronavirus must be investigated. Understanding the impact of the pandemic on dental services should have a defining role on the management of dental prophylaxis measures and the education of the population regarding dental health.

We believe that this study should be continued on a larger group of patients, multicentric, underlying the reconsideration of the role of preventive dentistry and taken into account for rethinking health policies at the national and global level.

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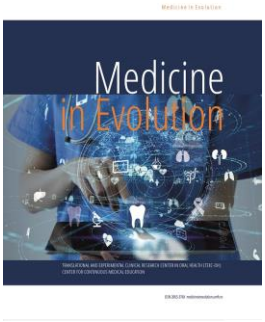
# Three filters intravital fluorescence microscopy evaluation of tissue loss progression induced by ligatures in experimental peri-implantitis in a dog model

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## Abstract

Objectives: Tissue breakdown was obtained by placing ligatures around dental implants on the same edentulous dog hemimandible. Evaluation of hard tissue loss progression was performed using intravital fluorochrome labeling, as an alternative to standard light microscopy, on thin sections of non-demineralized hybrid bone-implant specimens. Material and Methods: Intravital fluorochrome labeling was administered by intraperitoneal injection of oxytetracycline and alizarin red S. Sections were cut, stained and evaluated by light microscopy and under the epifluorescent microscope. Bone loss architecture was analyzed under three filters: UV, green and red. Results: Epifluorescence microscopy demonstrated continuous bone activity in the surrounding area of the implants during the healing period. LM confirmed the intense cellular activity in the hard tissues around the implants. Conclusion: Within the limits of the present study, epifluorescence can be seen as a complementary approach to the LM "golden standard", providing both reliable and sufficient information with regard to bone resorption/apposition kinetics.

**Keywords:** animal study, dental implant, ligature induced, peri-implantitis, intravital fluorochrome labeling, light-microscopy, epifluorescence

## INTRODUCTION

Extensive research into the peri-implantitis field has led to the conclusion that it is the most common biological complication in implant treatment [1-5]. Peri-implantitis is defined in the latest Classification for Periodontal and Peri-implant Diseases and Conditions (2018) as a plaque-associated pathologic condition occurring in the tissue surrounding dental implants, characterized by inflammation in the peri-implant mucosa and subsequent progressive loss of supporting bone [6]. While imagistic data during bone support loss in humans may be easily collected [7], additional histopathological data concerning peri-implant modifications can only be obtained through soft and hard tissue biopsies, which are governed by technical and ethical guidelines [8]. So far, the animal model, especially with regard to dogs, was thought to be a reliable source of details on tissue reactions in experimentally induced peri-implantitis [8-11].

The most frequently employed fluorescence microscopy method in life sciences is epifluorescence microscopy, also known as wide-field fluorescence microscopy (WFM). Fluorescence microscopy enables the detection of cell morphology, cellular/subcellular compartments, and disease or phenotypic indicators [12]. Fluorescence is described as the ability of some substances to transform short wavelengths of light into longer visible wavelength radiation [13]. The intrinsic ability of substances to fluoresce when exposed to an exciting UV light source is referred to as primary fluorescence (autofluorescence). The fluorescence created in substances by the application of fluorescent chemicals or dyes (fluorochromes) is known as secondary fluorescence [14, 15]. The administration of fluorochromes in animal models allows researchers to identify bone and cartilage development, remodeling dynamics and regeneration, which are critical criteria in bone tissue engineering investigations [16-18].

Fluorochromes have been used in bone research since the 1950s and are widely accepted substances. Several pioneers, for example Harold Frost, have carefully examined the possibilities of fluorochrome usage in bone formation and bone remodeling dynamics research, also in human studies, where he investigated several diseases [19-21]. Since the introduction of bone tissue engineering, there has been an increasing interest in the benefits of fluorochrome usage.

Fluorochrome labeling is based on the idea that some stains can bind directly to hydroxyapatite, the major component of bone, via calcium chelation. This occurs in all areas where new bone is formed [16, 20, 22]. Fluorochrome staining is highly effective for learning more about the beginning of the bone formation process (at the implant surface or another region of the implant) and bone remodeling activities surrounding implants. The mineralization process of newly produced bone may be entirely observed when the labels employ variation in fluorescent color and are supplied at different time-points. The labels can be administered to experimental animals subcutaneously, intraperitoneally, intramuscularly, or intravenously [16, 23].

Different intravital labeling protocols have been suggested in the processing of the bone specimens, some of which also contained dental implants. As indicators of new bone development, fluorochromes such as alizarin red S [24, 25]/complexone [26-28], calcein green [28, 29]/blue [27, 30], tetracycline [33-33] /oxytetracycline [16, 28, 34, 35], or xylenol orange [27, 35, 36] and more recently BAPTA labeling may be administered [27]. Tetracycline meets the characteristics of Frost's ideal bone label, as established in 1983. It represents the fluorochrome of choice in clinical studies, due to their non-toxic, cheap, easy to give, generally accessible, stable nature. Also, they present high affinity for calcium and are

incorporated at the site of active mineralization of hydroxyapatite and may be used as a tissue time-point marker [16, 23, 37-39].

Oxytetracycline can aid in determining the quantity of new bone development. It is exclusively visible in UV light, excites around 365-490 nm, emits at 520-570 nm, and fades fast. According to some authors [14, 23, 40, 41], it bounds to apatite and fluoresced yellow, yellowish-green, or leaf green; however, others claim that labelled new and old bone fluoresced bright golden yellow and dark sea green, respectively [42 43]. Continuous bone labeling allows for the assessment of total new bone formation and is often performed by administering the substance at doses that do not exceed 50 mg/kg to minimize adverse effects on osteogenesis [44].

Alizarin red S label provides strong contrast between cells, soft tissues, and calcified tissues. It excites at 530-580 nm and emits at 600-645 nm [16]. Green light is optimal for excitation of alizarin to fluoresced red light. At a dosage of 25-30 mg/kg, this chemical appears to be well tolerated in all species [14, 23, 28, 40, 45]. Because fluorochromes are bound in skeletal tissues as calcium complexes, proper histological processing is required. Optimum fluorochrome label preservation is attained when the fixed specimens are treated without interruption until they are embedded in plastic, and non-decalcified bone histology is performed before the microscopical examination [23]. It is also not required to utilize thicker sections for brighter fluorescence when using appropriate microscopic equipment, as opposed to thinner sections. However, multiple labels are more easily observed in thinner sections [23, 46, 47]. Additional filters are also useful in obtaining supplementary data, such as the red filter, that allows a label to be visible when it is excited at 510-560 nm and has the emission point of 573-648 nm [48]. In our study, this range covers both alizarin red S label and oxytetracycline.

Fluorescence microscopy is performed utilizing a microscope that has a fluorescence illuminator. Essentially, unstained sections are exposed to a light of a particular length (or wavelengths), which is absorbed by fluorophores and causes them to emit light of longer wavelengths (i.e., a different color than the absorbed light). A spectrum emission filter separates the transmitted light from the considerably weaker emitted fluorescence. In studies evaluating treatment strategies for peri-implantitis lesions, this method enhances the evaluation of both the direction and topographic localization of new bone growth [22, 40].

Multiple intravital fluorochrome labeling has been occasionally used for the study of bone formation following surgical intervention in the same animal over a certain period of time [49], and it is a common technnique to track the osseointegration of implants over time [50-52]. However, the literature does not mention studies using together green, red and UV filters to visualise intravital fluorochrome labeling to evaluate primarily the tissue loss around experimentally-induced peri-implantitis on non-decalcified sections. As data obtained under fluorochrome microscope are not influenced by the filter used, this method may be of interest in the study of lesions with hybrid consistency, like peri-implantitis.

### *Aim and objectives*

The aim of this pilot study was to determine whether the tissue loss progression during experimental peri-implantitis in ligatured implants placed on the same edentulous dog hemimandible, using intravital fluorochrome labeling can be successfully observed using green, red and UV filters, as an alternative to standard light microscopy, on thin sections of non-demineralized hybrid bone-implant specimens.

## MATERIAL AND METHODS

This pilot study was conducted in a single animal to reduce the number of experimental animals to be sacrificed, so that most of the information could be obtained.

The experiment was performed on one ten-years old adult half-breed dog, with a fully erupted permanent dentition (male, body weight 20 kg). The dog was housed under good conditions (in a single kennel with indoor and outdoor areas, room temperature range was approximately 18°C, with humidity above 30%) and fed once a day using granulated dog food and water *ad libitum*. Clinical examination determined that the dog was in good general health, with no systemic diseases [34].

Dental implants consisted of 5 titanium cylinders (3.3 mm, two 3.8 mm and two 4.1 mm in diameter, all 8 mm in length). All implants were fabricated by OT medical GmbH, Germany.

*Operative procedures*

All surgical procedures were carried out under general anesthesia (intravenous Diazepam 0,5%, 0,4mg/kg I.V. and Ketamine 10%, 10mg/kg I.V., endo-tracheal intubation 2–5% isoflurane gas). To maintain hydration, the animal received a constant-rate infusion of Ringer's solution while anesthetized. The protocol of the whole experiment was approved by the Ethical Commission of Scientific Research of UMFVBT (approval Nr. 06-16/09.01.2019). The timeline of the experiment is presented in Figure 1.

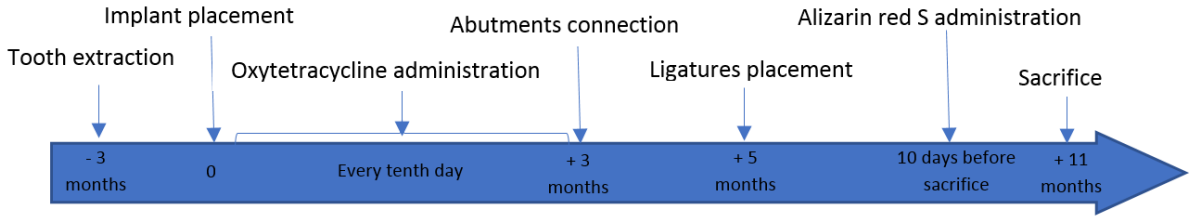


Figure 1. Timeline of the experiment

*Postoperative procedures*

Implants were exposed 3 months post-implantation and their healing abutments were connected (Figure 2). No oral hygiene regimen was administrated during this period, so that the peri-implant inflammation could initiate spontaneously. Five months post-implantation (two months post implant exposure), to accelerate the progression of the initial lesions, cotton ligatures were placed according to the method described by Lindhe et al. [53] in a submarginal position around the neck of implants. (Figure 3). The ligatures were not replaced or removed during this experiment. The animal was then fed a soft diet to induce plaque accumulation and to provoke peri-implant inflammation and bone resorption.

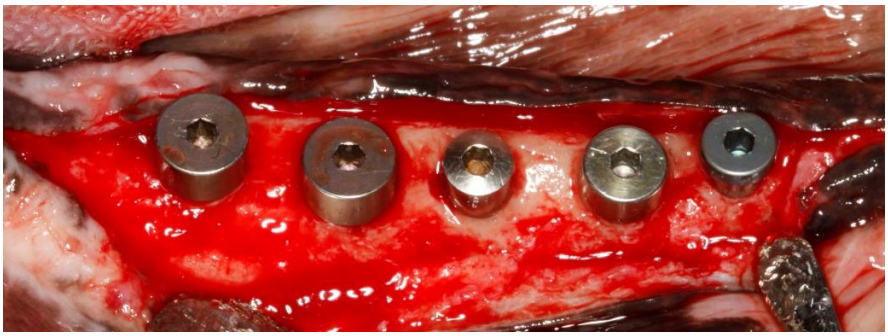

Figure 2. Intra-operative view of the implants at the exposure time, 3 months post-implantation, with the healing abutments in place. Note the integrity of the buccal and proximal bone

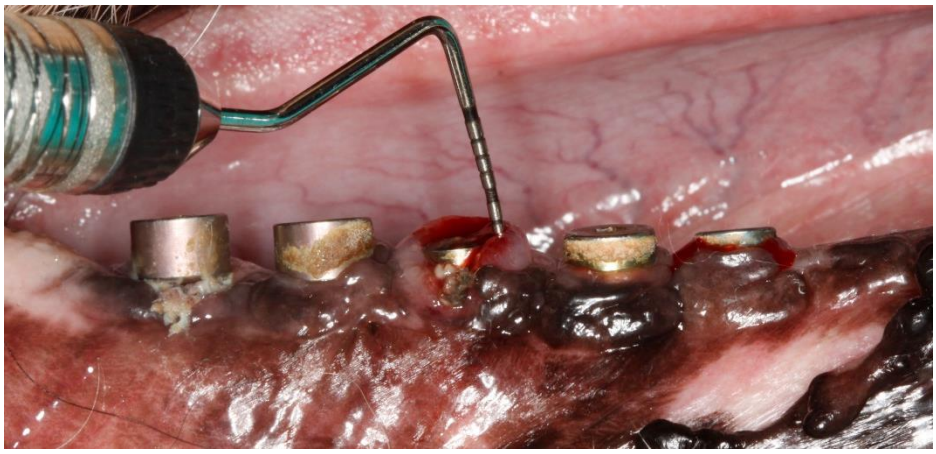

Figure 3. Post-operative image at 9-month post-implantation. Note the local inflammation induced by the ligatures

Intravital polyfluorochrome labeling was carried out for the histological evaluation of the bone reactions by intraperitoneal injection of oxytetracycline (OXY L.A. INJ, Dopharma BV, Raamsdonksveer, Holland), 50 mg/kg body weight, every tenth day, from implant placement until abutment installation for assessing the total new bone formation and alizarin red S 1% (Merck KGaA, Darmstadt, Germany), 40 mg/kg body weight, 10 days before sacrifice, to evidence bone remodeling around implants.

*Histological preparation of the specimens*

At the end of the experiment, 8 months after abutments were installed, the dog was sacrificed under general anesthesia (sodium pentobarbital: 200 mg/kg I.V.). The part of the jaw including the implants was sectioned and fixated in 10% neutral buffered formalin until laboratory processing.

The detailed histological protocol of the experiment was presented in a previously published paper (Boldeanu et al. 2022) [54]. In brief, Technovit 9100 (Kulzer GmbH, Hanau) embedding resin was used for the infiltration and embedding steps. Sections were prepared using the cutting/grinding method described by Donath & Breuner [55] on a cutting/grinding system (Exakt, Norderstedt, Germany). The polymerized resin blocks containing the embedded sample were trimmed and transferred to glass slides. A cut was performed in buccal-lingual direction through the middle of each implant, in the long axis. For each implant, one section was carried out distally from the initial mid-section, while another section was carried out mesially, both in the long axis, so that two relatively equal central sections of 30 microns resulted. One section of each implant sample was stained with MOVAT Pentachrome (after Verhöff) (Morphisto, Frankfurt am Main, Germany) (MOV). After the staining of the sections, deplastination of the thin-sections was performed by incubation in two baths of acetone and twice in methoxyethyl-acetate (MEA). Finally, the stained slides were dehydrated and cover-slipped with a mounting medium. These stained sections were used in another research (Boldeanu 2022) [54].

The slides were analyzed and photographed using an epifluorescence Olympus® EX51 microscope (Olympus, USA) fitted with Canon E600 digital camera at x 4, x 10, x 40 and x 100 magnification. Three filters were used to analyze the slides: green filter for the alizarin red S stain, UV filter for the oxytetracycline stain and red filter for both of the labels. The images obtained were recombined using and the program ImageJ/FIJI (https://fiji.sc/) and examined with Aperio ImageScope software (version 12.4.6.5003, Leica Biosystems GmbH, Nussloch, Germany). For comparison, the stained thin sections were also scanned using the microscope slide scanner Leica Aperio® AT2 (Leica Biosystems, Wetzlar, Germany), under the 40x objective, that resulted in LM scanned slides.

The sections containing the fluorochrome-labeled tissues were kept in appropriate storage conditions, protected from intense light during processing, and were stored in the dark.

*Histomorphologic analysis*

On the alveolar crest and periosteal surfaces, plaque development and gingival inflammation, junctional epithelium-to-implant contacts, active or previous bone resorption, active bone formation, and the presence of fluorochrome labels were evaluated.

**RESULTS**

During the experimental peri-implantitis, none of the 5 implants failed. All implants presented heavy plaque accumulation, inflammation and bleeding, while implant no. 3 exhibited suppuration.

*Histological observations*

Both oxytetracycline and alizarin red S labels were observed when examinating the sections under UV filter (for the oxytetracycline label), green filter (for the alizarin red S filter) and red filter (for both labels). Figure 4 presents both of the labels, under the three filters, under various magnfications for better observation of their signal, knowing that longer exposure time allows the short-lived autofluorescent signal to fade out. Due to the long period of time of administration the substance during the osseointegration phase of the implants, oxytetracycline was evident under the UV filter as a wide yellow blurry line that was frequently found extending into the marrow space of the surrounding bone tissue (Figure 4A). As opposite, under the green filter, alizarin red S label formed a narrow intense red line extending outside the bone marrow, towards the osteons (Figure 4B). Toghether, both filters seemed to fuse under the red filter, however they remain destinguishable due to their variation in intensity (Figure 4C, x 100 magnification, 20 µm measure bar).

Epifluorescence microscopy demonstrated that there was continuous bone activity in the surrounding area of the implants during the healing period, as evidenced by the presence of high levels of oxytetracycline in this location. Fluorochrome labeling indicated an uneven pattern around the neck of the implants, as well as in the periosteal and endosteal areas. Cavities of bone growth, bone resorption, and bone remodeling were found around the implants in both green and UV filters. The oxytetracycline label was most prominent in the apical part of the implant, below the area where the ligatures provoked plaque-accumulation and subsequent bone resorption, whereas more alizarin red S than oxytetracycline could be detected surrounding the coronal part of the implants (Figure 5B), as bright red lines that are heavily disrupted. This observation was more evident under the red filter (Figure 5C). The kinetic data on bone turnover was observed using oxytetracycline sequential labeling of bone. From the time the implants were inserted until the abutments was placed, the substance was administered every 10 days. However, where bone resorption was experimentally initiated, subsequently irregular interrupted endosteal surface with scattered oxytetracycline label was observed. This can be interpreted as evidence of bone remodeling following the induced peri-implantitis. Implants were observed in close contact with the bone, as seen in the light microscopy section (Figure 6A), and fluorochromes apeared in close proximity to the implants. Other observations revealed significantly stained oxytetracycline rims at the implant interfaces, particularly in the apical threads (Figure 6B), more visible than the alizarin red S label (Figure 6C). This is confirmed by examining the sections under the red filter (Figure 6D).

The fluorescently labeled bone areas surrounding the implants showed a trend for intense alizarin red S label in the surrounding coronal tissue. When all threads in the cortical

area were labeled, the surrounding bone exhibited considerably higher oxytetracycline concentration.

*Comparison between fluorochrome filters and LM mirror sections*

In brief, the processed specimens showed partial osseointegration due to the induced peri-implantitis and subsequent bone resorption, deep peri-implant sulcus with damaged epithelial lining and extensive infiltrated connective tissue (ICT) difusion into the bone marrow (mainly constituted of inflammatory cells: lymphocytes and plasma cells with scattered neutrophils) separated by hyperemic blood vessels and a network of sporadic collagen fibers) (Figure 5A). There was a "subjective correlation" between new bone area and fluorocrome incidence. In areas between the threads of the fluorescent labeled sections and in the mirror LM ones, the distribution of new bone and fluorochrome occurrence followed the same pattern (Figure 6).

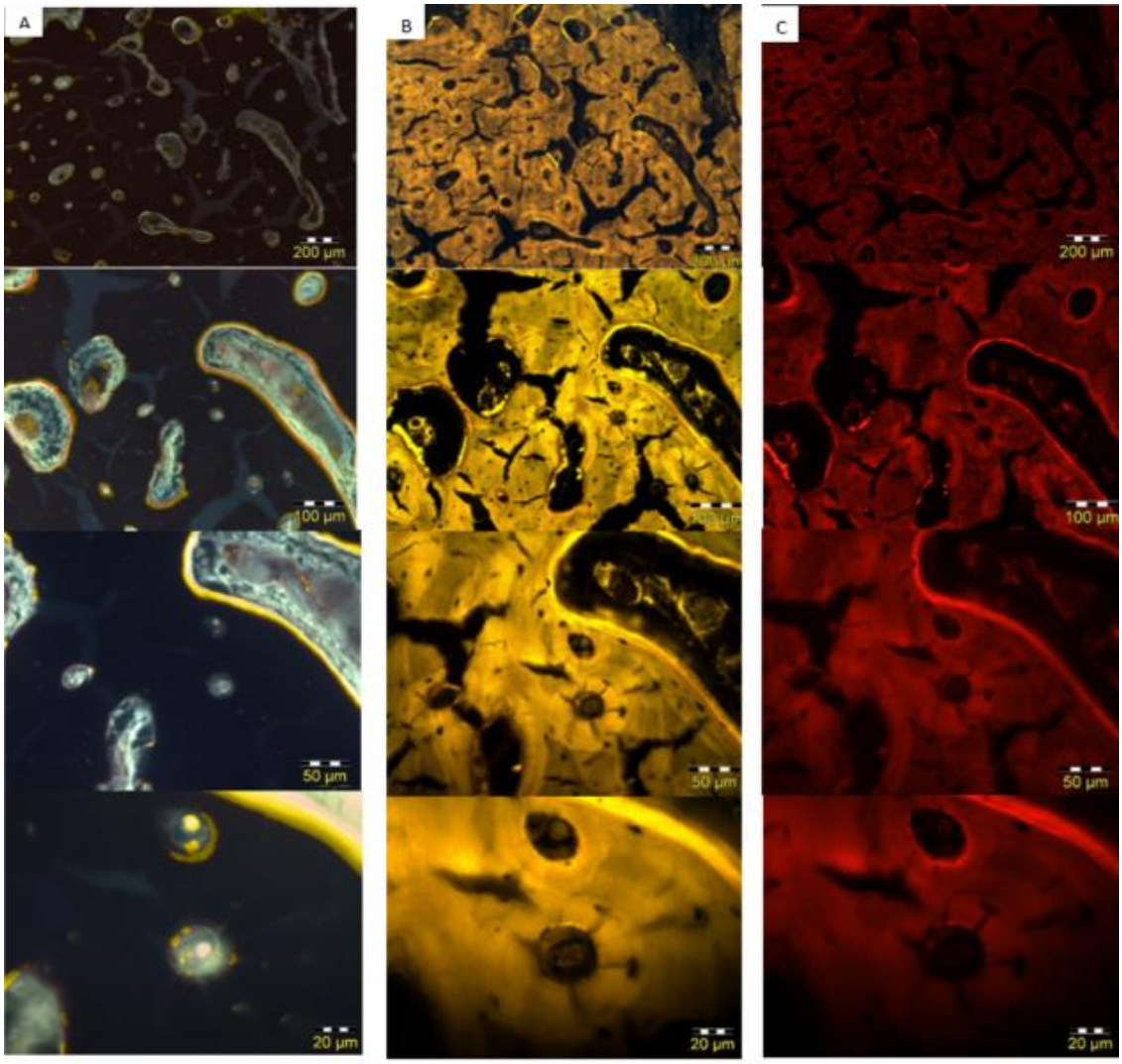


Figure 4. Detailed investigation of the surrounding hard tissues on the oral aspect of implant no. 3 under fluorochrome microscope UV filter (A), green filter (B) and red filter (C) (implant 3, scale on the images, x 4, x 10, x 40, x 100 magnification). Note the presence of the oxytetracycline lines deposited at the limit with the bone marrow under the UV filter (A, x 100 magnification). Alizarin red S is more evident at x 40 and x 100 magnification under the green filter, as a very narrow darker line towards an osteon (B, x 40). Under the red filter, both substances can be distinguished: oxytetracycline as a wide red blurry line and Alizarin red S as a narrow intense red line (C, x 100 magnification)

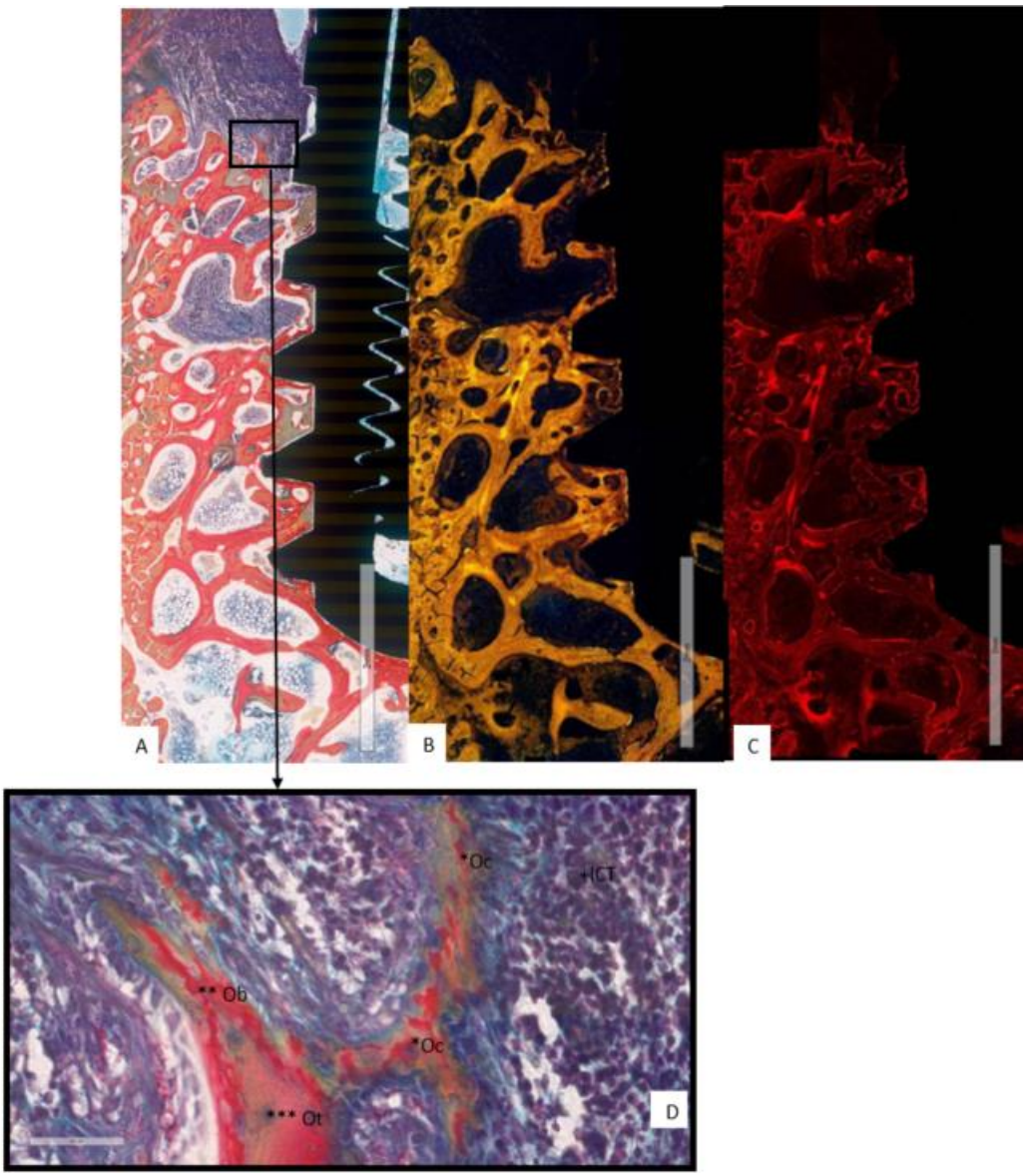


Figure 5. Scanned images of the implant and surrounding hard and soft tissue on the buccal aspect of implant no. 3. Observe the focal bone loss achieved by osteoclasts when activated by adjacent inflammation. Uneven endosteal surface is seen under light microscopy (A), fluorochrome microscope green filter (B), red filter (C) (implant 3, MOV staining, 2mm bar measure). In the detailed image (D), note the active alveolar bone remodeling involving giant multinucleated osteoclasts (*Oc), along with a osteoblast rim (**Ob) observed on the bone surface; osteocyte (***Ot) surrounding bone matrix is also observed along with a great amount of infiltrated connective tissue (+ICT) (LM image, bar measure 60 μm).

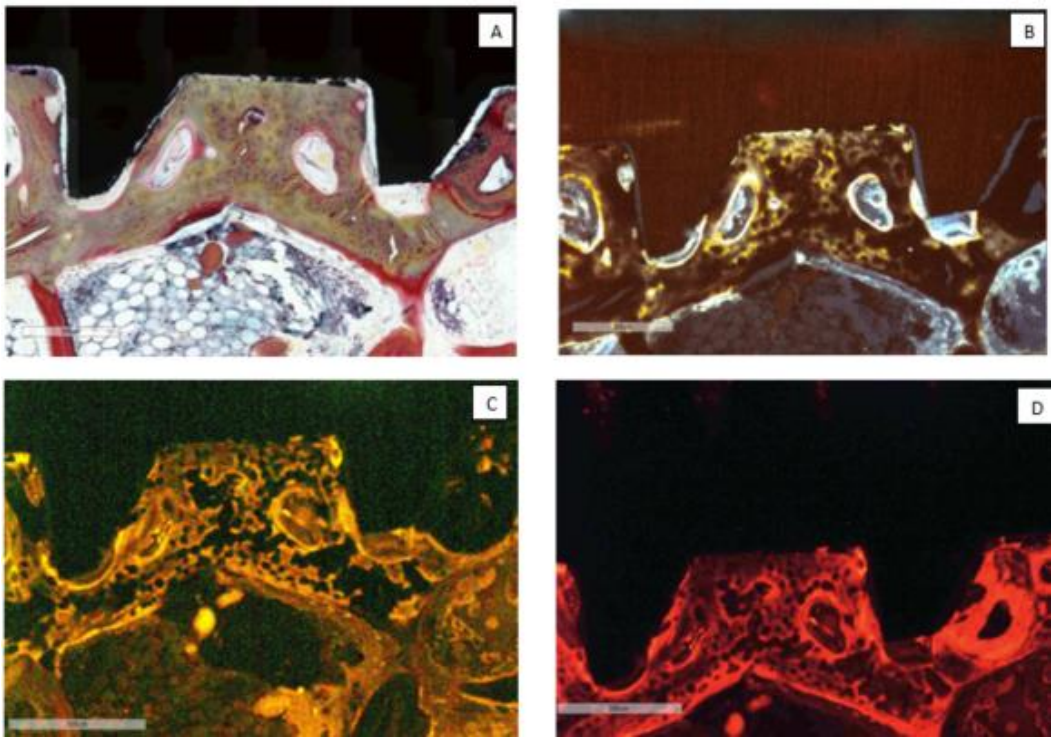


Figure 6. Detailed images of the implant and surrounding bone on the oral aspect of implant no. 1 under light microscopy (A), fluorochrome microscope UV filter (B), green filter (C), and red filter (D). Note the close contact between the bone and the implant, the new bone formation around the implants apically from the ligatures and the absence of histological signs of inflammation. Merging under the red filter, the two fluorochromes reveal that there was continuous bone activity in the vicinity of the implants during the healing period (D) (implant 1, MOV staining, x 4 magnification, bar measure 300µm)

## DISCUSSIONS

In the present study we have explored the utilization of epi-fluorescence under three filters to observe the bone remodeling that occurs in an experimentally induced peri-implantitis. Bone labeling and microscope analysis appeared to be governed by the authors' unique selections, based on specific study aims, the quality of previous results and personal preferences, rather than a clear association between the label and the desired histological findings, as some authors have also noticed [16]. In general, the researchers give no explanation for selecting a specific intravital fluorochrome. Furthermore, there is a limited amount of histological literature comparing multiple intravital labels observed under varying filters.

In the majority of previous studies, the most commonly utilized approach for studying bone loss progression after experimentally induced peri-implantitis is light microscopy. Although conventional histology has shown to be a reliable method of evaluating the ossoeintegration of a dental implant, it is expensive, time consuming, damaging, and restricted to one or a few sections [56]. In comparison, epi-fluorescence allows the visualisation of the modifications of the bone architecture and the bone formation in time, by demarking the mineralization front at the time of administration without any further staining [16].

Utilizing fluorochrome labeling methods for *in vivo* bone research is not a novel development. Hunter documented alizarin, a madder dye, in bone remodeling research as early as the 1770s [57]. It shares calcium chelating and fluorescence capabilities with oxytetracycline, discovered in 1950 [58], and many other fluorochromes [16].

Only the emitted fluorescent light is permitted to pass through to the eye-piece or detector by using the appropriate light filters and mirror. Although in our study, both labels emitted in red color and the differences between them were small, each of them exhibited a characteristic fluorescence spectrum. This observation has also been noted by Pautke et al. when they experimented injecting 8 different substances into experimental models to assess the feasibility of sequential *in vivo* bone labeling using distinguishable fluorchromes [39].

Using multiple fluorochrome labeling and particular filter combinations offers many applications in experimental bone biology. In our study, we have used the UV filter to observe the oxytetracycline label (excited around 365-490 nm, and emitting at 520-570 nm), green filter for the alizarin red S label (excited at 530-580 nm and emitting at 600-645 nm) and red filter for both of them (for labels that were excited at 510-560 nm and with the emission point of 573-648 nm [48]. Rahn et al, have also applied a method that used intravital fluorochrome labeling of two substances and a special filter combination for allowing data gathering by computer-compatible systems, that resulted in improved image contrast in the fluorescence microscope [59].

While experimentally injected fluorescent substances are commonly used for the study of hard tissue mineralization and bone aposition/regeneration, fluorescence seems useful also for the study of bone resorptive processes, like in the present study of experimentally induced peri-implantitis. In addition to the informations obtained by conventional histology, in our study fluorescence has reflected the kinetics on bone turnover, with marked uneven endosteal surface and dispersed fluorochromic labels at the coronal part of the implants and with intense fluorochromic activity at the apical part of the implants. This is evidence of bone remodeling following the induced peri-implantitis in the coronal part and of osseointegration in the apical region of the implants. Similar observations have been noted by Carlsson et al. when investigating implant integration in both nondecalcified rutinely stained and fluorochrome labeled sections to determine if there was a resemblance between the methods. Analyzing the very same samples, like in our case, they have also noted that fluorescence microscopy can provide pertinent data as a complementary analysis [28]. With regard to mapping bone destructive processes, Weinlaender et al. initiated a radiation therapy to observe how the bone healing is influenced around three types of endoosseous dental implants in dogs. Similar to our observations, they had subjective estimations of parameters, though not yielding exact measurements like computer-assisted histomorphometric data, rendering supplimentary data about new bone formation and remodeing dynamics at different time intervals before and after onset of the radiation therapy, in their case, of the induced peri-implantitis, in our case [61].

Regarding the intravital fluorochrome administration sequence, with regard to oxytetracycline being administered after the implant placement and alizarin red S label few days before sacrifice, Marcaccini et al. obtained great results in determining when mineralisation occurs. In their study, the markers demonstrated different colors and provided sequential data, and their contrast made is possible to evaluate the changes during the experimental period. Similar observations are present also in our study, when observing where bone resorption was initiated, and generating subsequently uneven endosteal surface with scattered oxytetracycline label was observed.

After induction of peri-implantitis, clinical signs of inflammation were obviously more pronounced in the vicinity of the ligatures. These findings agreed well with the histological findings (Figure 5A) where a deep peri-implant sulcus with damaged epithelial lining and profound ICT infiltration of bone marrow was found. The onset of these changes around the ligatured implants can be evoked by analyzing the fluorescentely labelled sections, where the oxytetracycline infiltrated hard tissue has been lost. The remaining hard tissues appear to be uneven with scattered signs of label (Figure 5B). These finding were similar to those of

Zechner et al. [34], when the authors have investigated two types of implants, subjected to experimental peri-implantitis in dogs. In their study, various resorption of old and new peri-implant alveolar bone was present, and losses of alveolar bone-to-implant contact levels were seen. This applies also to our findings (Figure 5C).

## CONCLUSIONS

Within the limits of this investigation, it can be concluded that properly fluorescence-labeled and processed sections of non-demineralized hybrid specimens of experimentally induced peri-implantitis in the dog model deliver quickly accessible additional information regarding the bone resorption mechanism. When compared to light microscopy, they can be analyzed promptly under an epi-fluorescence microscope to reveal significant data regarding bone remodeling. While conventional light microscopy is the "gold standard" in evaluating soft and hard tissues around dental implants, providing both reliable and sufficient information, fluorescence microscopy is a complementary approach, allowing for the measurement of bone activity around dental implants in both time and space, from the insertion point until the complete integration of the implants, and later, until complete tissue loss during peri-implantitis progression. Future studies may focus on refining and simplifying the examination protocol of the labeled hybrid specimens.

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# Correlations between the age of dental implants and color changes of the peri-implant mucosa


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## Abstract

Aim and objectives: The aim of the present study is to detect the presence of changes in the color of the peri-implant mucous membranes in a group of patients with implants supported dentures and to correlate these changes with the age of the dentures. Our study group consisted of 62 patients with one or more dental implants placed between 6 months and 5 years prior to the study. Data were collected by dental examinations and were photographically documented. Later, the data were introduced and analyzed in SPSS software, version 14.0. The correlations revealed that all patients with peri-implant mucosal discoloration had prosthesis placed in the previous 2 years. If these color changes are not a sign of peri-implant inflammation, we recommend evaluating them regularly to follow their evolution.

**Keywords:** implant, mucosa, color modifications

## INTRODUCTION

The high frequency of implant supported dentures prosthetic treatments demonstrated over time an increase in the patient's life quality, but also led to an increased number of peri-implant diseases. Studies revealed a frequency of peri-implant mucositis up to 63.4% reported to patients and up to 30.7% reported to dental implants [1]. On the other hand, the frequency of peri-implantitis can reach up to 18.8% in relation to the carriers of dental implants and to 9.6% in relation to the number of implants. It is necessary to establish preventive protocols for the prosthetic treatments performed on dental implants in order to obtain good results.

### *Aim and objectives*

The main purpose of this study is to establish a correlation between the age of implant-supported dental prosthesis and the color changes in the peri-implant mucosa in a group of romanian patients.

## MATERIAL AND METHODS

The study group consisted of 62 patients recruited from 5 dental offices, 2 from the city of Bucharest and 3 from the city of Râmnicu Vâlcea. Every patient enrolled in this study signed the informed consent form.

A single examiner performed all dental check-ups for the 62 patients in order to avoid distortions (bias) that may occur when more clinicians must record similar data in a single file type [2].

Various data were collected, including the clinical aspect of the peri-implant mucous membranes, respectively color and volume. During this session, a series of photographs were also taken for each patient, focused especially on the level of implant-supported prostheses. The photographs were recorded on electronic media.

The color changes of the peri-implant mucosa were recorded by the operator during the dental check-ups and reviewed later based on the photographs.

The collected data were coded into the Microsoft Excel program. Later, they were imported and analyzed in SPSS 14.0. Apart from the simple statistical analysis, correlations were also calculated between the data recorded in the file in this study and the data collected through the clinical evaluation.

## RESULTS

The analysis of the age of on implants-supported prostheses according to the recorded data is presented in Table 1.

Table 1. Age of dental implant prostheses

| | | Frequency | Percent | Valid percent | Cumulative percent |
|---|---|---|---|---|---|
| Valid | 0-6 months | 17 | 27.4 | 27.4 | 27.4 |
| | 6-12 months | 14 | 22.6 | 22.6 | 50.0 |
| | 1-2 years | 21 | 33.9 | 33.9 | 83.9 |
| | 2-5 years | 9 | 14.5 | 14.5 | 98.4 |
| | N/A (< 5 years) | 1 | 1.6 | 1.6 | 100.0 |
| | Total | 62 | 100.0 | 100.0 | |

The analysis of color changes of the peri-implant mucosa is presented in Table 2.

Table 2. Clinical status of peri-implant tissues - color changes

| | | Frequency | Percent | Valid percent | Cumulative percent |
|---|---|---|---|---|---|
| Valid | No | 42 | 67.7 | 67.7 | 67.7 |
| | Yes | 20 | 32.3 | 32.3 | 100.0 |
| | Total | 62 | 100.0 | 100.0 | |

The correlations between the age of dental implants-supported dentures in patients from the study group and the appearance of color changes in the peri-implant mucosa are presented in Table 3.

Table 3. Age of implant prosthesis * Clinical status of peri-implant tissues - color changes

| | | | Color changes | | Total |
|---|---|---|---|---|---|
| | | | No | Yes | |
| Age of prosthesis | 0-6 months | Numerical value | 11 | 6 | 17 |
| | | % from Age of prosthesis | 64.7% | 35.3% | 100.0% |
| | | % from Color changes | 26.2% | 30.0% | 27.4% |
| | 6-12 months | Numerical value | 11 | 3 | 14 |
| | | % from Age of prosthesis | 78.6% | 21.4% | 100.0% |
| | | % from Color changes | 26.2% | 15.0% | 22.6% |
| | 1-2 years | Numerical value | 11 | 10 | 21 |
| | | % from Age of prosthesis | 52.4% | 47.6% | 100.0% |
| | | % from Color changes | 26.2% | 50.0% | 33.9% |
| | 2-5 years | Numerical value | 9 | 0 | 9 |
| | | % from Age of prosthesis | 100.0% | .0% | 100.0% |
| | | % from Color changes | 21.4% | .0% | 14.5% |
| | N/A (< 5 years) | Numerical value | 0 | 1 | 1 |
| | | % from Age of prosthesis? | .0% | 100.0% | 100.0% |
| | | % from Color changes | .0% | 5.0% | 1.6% |
| Total | | Numerical value | 42 | 20 | 62 |
| | | % from Age of prosthesis | 67.7% | 32.3% | 100.0% |
| | | % from Color changes | 100.0% | 100.0% | 100.0% |

**Chi-Square Tests**

| | Value | df | Asymp. Sig. (2-sided) |
|---|---|---|---|
| Pearson Chi-Square | 9.476[a] | 4 | .050 |
| Likelihood Ratio | 12.284 | 4 | .015 |
| N of Valid Cases | 62 | | |

## DISCUSSIONS

The highest percentage of dental implants with color changes of the peri-implant mucosa (result of the analysis) was in patients with an age of implant prostheses of one to two years. The lowest percentage with color changes, i.e., 0%, was found in patients with implant prostheses from 2 to 5 years old (Table 3).

The analysis of clinical signs specific to peri-implantation pathology, revealed that over 30% of patients showed color changes in the peri-implantation mucosa.

Most patients with color changes at the level of peri-implant mucosa (50%) have implant prostheses from 1-2 years old, followed (in order of frequency) by those 0-6 months old and those 6-12 months old. No patient with implant prostheses older than 2 years showed peri-implant mucosal color modifications.

These results showed us that the lifespan of implants for an extended period can be linked to the lack of peri-implant mucosal diseases [3] which is also dependent on the maintenance of good oral hygiene [4].

On the other hand, we must also consider the fact that in some cases the color changes of the peri-implant mucosa are not due to inflammatory causes, but to factors related to the type of implant, prosthetic abutment, prosthetic restoration, tissue augmentation, etc. [5]. Therefore, the presence of tissue inflammation cannot be confirmed (but it can be indicated) only by visual inspection of the tissues, but it must also be correlated with other clinical aspects such as values of microbial plaque and calculus scores [6], peri-implant probing depth and probing bleeding [7].

## CONCLUSIONS

The high percentage (30%) of peri-implantation mucosal color changes makes us believe this is a problem which must be carefully and thoroughly investigated from two points of view: design and construction of implant prostheses and from the perspective of early detection of peri-implant inflammations to prevent their progression and complications.

The presence of color changes in the peri-implant mucosa should be a warning signal for every dentist.

If these changes do not represent a sign of peri-implant inflammation, we recommend an accurate recording of data and a strong photographic documentation in order to be able to follow them correctly.

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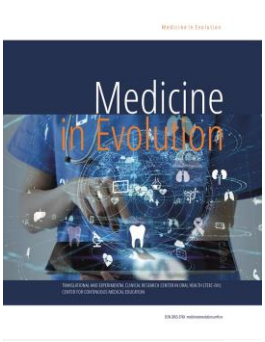
# Apical resection in oral surgery: current data

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## Abstract

Apical surgery is considered a standard oral surgical procedure. It is often a last resort to maintain a tooth with a periapical lesion that cannot be managed with conventional endodontic (re)treatment. The main goal of apical surgery is to prevent bacterial leakage from the endodontic system into the periradicular tissues by placing a filling at the root end after its resection.

The microscope and the endoscope in dentistry have enabled a significant evolution in apical surgery techniques. Indeed, the microscopic enlargement with the addition of light optimizes the visibility of the operating field and improve the results.

**Keywords:** Apical surgery, periapical lesion, resection

## INTRODUCTION

Over time, many authors tried to define and understand the history of apical surgery. The increase in endodontic surgical methods before 1900 was combined with a few clinicians and scientists who were lucid enough to document and register their work.

Indeed, the current apical resection would have been identified by Saville in 1720 following the discovery of a skull with a pierced tooth, but this theory was refuted by Fastlicht [1].

In literature, Hullihen is also credited with surgical trephination (1845). The "Hullihen operation" consisted of "making a hole through the gum, the outer edge of the alveolar process, and the root of the tooth into the nerve cavity, and then opening the blood vessels of the nerve." Unfortunately, this technique aimed at allowing the conservation of a tooth, it was very rarely used because of its difficulty. However, existing studies show Smith's (1871) technique was the first apical resection used on a tooth with necrotic pulp. Claude Martin was also the inventor of apical resection in 1881. The latter describe the utility and use of this method to treat teeth with a sinus draining path.

John Farrar in 1884 recommended radical removal by amputation of parts of the roots that were no longer needed. In that same article, he wrote that root surgery was “a bold act, which removes the entire cause and which will lead to a permanent cure, may not only be the best in the end, but the most human”. Since then, endodontic surgeries have become inevitable in the choice of root canal treatment and periapical disease [1].

At the beginning of the 20th century, the development of endodontic surgery was progressive and regressive. Indeed, while major progress has been made in Europe and the United States in improving techniques and even in all aspects of endodontics and oral surgery, the medical profession was reluctant to these advances. However, surgical advances and diverse applications highlighted the entry of endodontics into this century.

Between 1915 to 1920, root resection took an important part in the dental science. The crescent or semilunar incision were standard, as was the sealing of the gutta-percha with a hot burnisher after resection. Zinc oxyphosphate was frequently used as a sealant with gutta-percha. In the early 1930s, extractions were often the first choice of treatment. But a handful of practitioners have persisted in promoting periapical surgery [1].

Karl Peter published in 1936 his text “Die Wurzelspitzenresektion der Molaren” which will be the foundation of contemporary endodontic surgery [1]. He gives a classification of the position of the inferior alveolar canal in relation to the molar roots and indicates the connections with the maxillary sinus and its position in relation to the roots of the maxillary teeth. After the Second World War, Louis Grossman also gave details on the apical resection technique. Indeed, he recommended surgical curettage followed by a through-canal obturation technique. He used eucalyptol with the gutta-percha and cut off the excess at the apex with a hot instrument.

The period 1960-2000 is essential in the history of surgical endodontics, it represents the development of new procedures for the 21st century. While many authors around the world have written textbooks consecrated exclusively to endodontic surgery.

After the 1990s and the introduction of microsurgical principles, apical surgery's technique was significantly improved. Microsurgical instruments for root cavity preparation and the development of magnification tools such as the surgical microscope or endoscope are the most significant acquisitions. Those two innovations have considerably facilitated the apical surgical technique and improved its result. By several studies, successful healing is more frequent with the microsurgical technique than with the conventional technique [2].

Interest in using this treatment in addition to apical surgery after performing tissue regeneration techniques in periodontics and implant dentistry has increased. A rising number of practitioners are recommending the use of regenerative techniques (RT) in apical surgery [2].

*Aim and objectives*

The advent of new root filling materials has provoked debate about the long-term outcome of endodontic microsurgery performed on teeth with post-treatment apical periodontitis. The purpose of this study is to evaluate the results of endodontic microsurgery in teeth diagnosed, by radiographic examination, with secondary apical periodontitis.

## MATERIAL AND METHODS

Study is a systematic review, and the question in the center of the research was: ”What is the long-term clinical and radiographic outcome of endodontic microsurgery in teeth diagnosed with secondary apical periodontitis?”.

Two websites have been consulted: Pubmed and The Cochrane Library. The studies included were meta-analyses and systematic reviews, critical reviews, longitudinal studies and case reports. The review of research articles followed the PRISMA guidelines (Preferred Reporting Items for Systematic Reviews and Meta-Analyses).

The following medical terms have been used for the selection of articles: ”root canal treatment”, ”endodontic microsurgery”, ”apical root resection”, ”apicoectomy”, ”root canal filling”, ”retreatment”, ”periapical surgery”, ”endodontic surgery”, ”root resection”, ”radiographic outcome”, ”root cavity preparation”.

A specific selection of clinical studies that examined clinical and radiographic outcomes after endodontic microsurgery was made through the searching criteria.

**Inclusion criteria:**

- Studies from 1990 to 2020.
- Studies evaluating the long-term clinical and radiographic outcome after endodontic microsurgery.
- Clinical studies on endodontic microsurgery (using microscope, endoscope, ultrasonic ultrasonic root-end preparation).
- Clinical and radiographic results according to the criteria given by Rud & al. [3] and Molven & al. [4].
- The given success rate of endodontic microsurgery.

**Exclusion criteria:**

- Studies including patient under 18 years old.
- Studies using perforated or fractured tooth samples.
- Studies that do not use microsurgery.
- Studies without periapical or clinical radiographic evaluation.
- The lack of evaluation of the success rate of endodontic microsurgery.

**Data collection:**

An initial selection was based on the article titles. Then, the abstracts were analyzed to retain meta-analyses and systematic reviews, critical reviews, longitudinal studies and case reports. Finally, a manual search was performed using the sources contained in the selected reviews and not detected by the search equation.

## RESULTS

An extraction, data analysis and methodology evaluation in a total of 10 articles corresponding to the previously explained inclusion criteria was performed.

Table 1 summarises the information from the systematic review. Thus, 6 prospective clinical studies and 4 randomized clinical trials constitute the 10 selected articles. The smallest sample size examined was 87 teeth (Truschnegg & al. 2020) [5] and the largest sample size was 339 teeth (Von Arx & al. 2014) [10] over a period of 2 to 13 years. These studies used filling materials such as MTA, IRM, dentine-bonded resin composite and SuperEBA. The recall rate of the studies ranged from 59% (Chong & al. 2003) [14] to 89% (Taschieri & al. 2008) [13].

Table 1. Studies included in the clinical review and success rates

| Study | Type | Number of teeth | Follow-up | Obturation material | Recall Rate | Success rate of obturation material | Overall success rate |
|---|---|---|---|---|---|---|---|
| Truschnegg & al. [5] | Prospective clinical study | 87 | 10 to 13 years | IRM | 71 % | not available | 76 % |
| Von Arx & al. 2019 [6] | Prospective clinical study | 119 | 10 years | MTA grey *44 teeth*<br>MTA white *75 teeth* | 61 % | 84 %<br>80 % | 82 % |
| Kim & al.[7] | Randomized clinical trial | 260 | 4 years | MTA *83 teeth*<br>SuperEBA *99 teeth* | 70 % | 92 %<br>90 % | 91 % |
| Caliskan & al, [8] | Prospective clinical study | 103 | 2 to 6 years | MTA | 87 % | not available | 80 % |
| Tawil & al. [9] | Prospective clinical study | 155 | 3 years | MTA grey and SuperEBA | 82 % | not available | 69 % |
| Von Arx & al. 2014 [10] | Prospective clinical study | 339 | 5 years | MTA *134 teeth*<br>Dentine-bonded resin composite *137 teeth* | 80 % | 93 %<br>77 % | 85 % |
| Song & al. [11] | Randomized clinical trial | 172 | 6 to 10 years | IRM, MTA grey and SuperEBA | 61 % | not available | 93 % |
| Von Arx & al. 2012 [12] | Prospective clinical study | 191 | 5 years | MTA *44 teeth*<br>SuperEBA *49 teeth*<br>Dentine-bonded resin composite *77 teeth* | 88 % | 86 %<br>67 %<br>75 % | 76 % |
| Taschieri & al. [13] | Randomized clinical trial | 113 | 2 years | SuperEBA | 89 % | not available | 92 % |
| Chong & al. [14] | Randomized clinical trial | 183 | 2 years | MTA *61 teeth*<br>IRM *47 teeth* | 59 % | 92 %<br>87 % | 90 % |

The overall success rate is between 69% (Tawil & al. 2015) [9] and 93% (Song & al. 2012) [11]. Nevertheless, in order to evaluate the influence on the outcome of endodontic microsurgery of each clinical trial, a statistical analysis of potential prognostic factors is presented in the following paragraphs.

Truschnegg & al. 2020 [5], produced a prospective clinical study through assessment parameters such as: age, gender, smoking and drinking habits, tooth location, previous endodontic surgery, pre- and postoperative lesion size and perioperative antibiotic use. During a 10 to 13 years follow-up on 73 patients and 87 teeth, the radiographic success rate was 76% healing. Prognostic factors described a lower success rate in smokers (33.3%) than in non-smokers (80%), but no significant differences for other parameters evaluated (age, sex, alcohol habits, tooth location, previous endodontic surgery, size of the pre and postoperative lesion, or perioperative antibiotics).

Von Arx & al. 2019 [6], produced a prospective clinical study through assessment parameters such as: sex, age, tooth type, type of MTA used (grey or white), surgery (first-time or repeat surgery). During a 10 years follow-up on 119 teeth, the radiographic success rate was 82% healing (gray MTA group 84% and white MTA group 80%). The prognostic factors describe a significant difference in success rate according to tooth type (higher for maxillary molars: 95,2%, compared to maxillary premolars: 66,7%). No significant differences for other parameters evaluated (age, sex, type of MTA, or first-time versus repeat surgery).

Kim & al., 2016 [7], produces a randomized clinical trial through a type of material used (MTA, Super EBA). During a 4 years follow-up on 260 teeth, the radiographic success rate was 91% healing (MTA group 92% and Super EBA group 92%). Thus, prognostic factors describe no significant difference in success rate by material type used.

Çalışkan & al., 2016 [8], produced a prospective clinical study through assessment parameters such as: sex, age, tooth type and location, quality of the root canal filling, presence/absence of a post, previous endodontic treatment/retreatment, previous nonsurgical or surgical endodontic treatment, size and histopathology of periapical lesions, antibiotic therapy, postoperative healing. During a 2 to 6 years follow-up on 108 patients and 108 teeth, the radiographic success rate was 80% healing. Thus, the prognostic factors describe no significant difference in success rate according to the parameters assessed.

Tawil & al., 2015 [9], produced a prospective clinical study through assessment parameters such as: sex, age, tooth location, presence/absence of dentinal defect, root-end filling material (Super EBA/MTA). During a 3 years follow-up on 155 teeth, the radiographic success rate was 69% healing (dentinal defect group 32% and intact dentinal group 97%). Prognostic factors described a lower success rate in the dentinal defect, but no significant differences for other parameters evaluated.

Von Arx & al., 2014 [10], produced a prospective clinical study through assessment parameters such as: type of material (MTA or dentine-bonded resin composite, age, sex, tooth type (maxillary anterior, premolar, and molar or mandibular anterior, premolar, and molar), presence or absence of post, type of surgery (first-time surgery or repeat surgery). During a 5 years follow-up on 339 patients and 339 teeth, the radiographic success rate was 85% healing (MTA group 93% and dentine-bonded resin composite group 77%). The prognostic factors describe a significant difference in type of material used (higher for MTA treated teeth). No significant differences for other parameters evaluated (age, sex, type of tooth treated, presence of post, or type of surgery).

Song & al., 2012 [11], produced a randomized clinical trial through assessment parameters such as: age, sex, tooth type, tooth location, type of lesion, type of material (IRM, MTA, SuperEBA). During a 6 to 10 years follow-up on 172 teeth, the radiographic success rate was 93% healing. Thus, the prognostic factors describe no significant difference in success rate according to the parameters assessed.

Von Arx & al., 2012 [12], produced a prospective clinical study through assessment parameters such as: patient-related (age, sex, smoking), tooth related (type, pain, clinical signs/symptoms, size of periapical lesion, interproximal bone level, apical extent of root canal filling, post, and previous apical surgery), treatment related (antibiotic prescription, root-end filling material, and initial postoperative healing). During a 5 years follow-up on 194 patients and 194 teeth, the radiographic success rate was 76% healing (MTA group 88%, SuperEBA group 67% and dentine-bonded resin composite group 75%). The prognostic factors describe a significant difference in success rate based on interproximal bone level (higher success rate when the mesial and distal interproximal bone level was less than or equal to 3 mm from the cementoenamel junction, there is also a significant difference in success rate based on material type (higher success rate for MTA compared to SuperEBA). No significant differences for other parameters evaluated.

Taschieri & al., 2008 [13], produced a randomized clinical trial through assessment parameters such as: type of magnification device (microscope/endoscope) and tooth location. During a 2 years follow-up on 70 patients and 113 teeth, the radiographic success rate was 92% healing. Thus, the prognostic factors describe no significant difference in success rate according to the parameters assessed.

Chong & al., 2003 [14], produces a randomized clinical trial through a type of material used (MTA, IRM). During a 2 years follow-up on 183 patients and 183 teeth, the radiographic success rate was 90% healing (MTA group 92% and white IRM group 87%). Thus, the prognostic factors describe no significant difference in success rate according to the parameters assessed.

Table 2 summarises the information from the ten selected articles whose data are explained above and compares the success rates according to the obturation material used.

Table 2. Data summary

| Study | Obturation material | Follow-up | Sample size | | Previous Treatments | | Success rate |
|---|---|---|---|---|---|---|---|
| | | | Nb patients | Nb teeth | Nb re-surgery | Nb nonsurgical endodontic retreatment | |
| Truschnegg & al. [5] | IRM | 10 to 13 years | 73 | 87 | 19 | 0 | 76 % |
| Von Arx & al. 2019 [6] | MTA grey or white | 10 years | not available | 119 | 12 | not available | Overall rate: 82 %<br>*gray MTA: 84 %*<br>*white MTA: 80 %* |
| Kim & al.[7] | MTA grey and SuperEBA | 4 years | not available | 260 | not available | not available | Overall rate: 91 %<br>*MTA : 92 %*<br>*SuperEBA: 90 %* |
| Caliskan & al, [8] | MTA | 2 to 6 years | 108 | 108 | 18 | 42 | 80 |
| Tawil & al. [9] | MTA grey and SuperEBA | 3 years | not available | 155 | not available | not available | Overall rate: 69 %<br>*dentinal defect: 32 %*<br>*intact dentina : 97 %* |
| Von Arx & al. 2014 [10] | MTA and Dentine-bonded resin composite | 5 years | 339 | 339 | 31 | not available | Overall rate: 85 %<br>*MTA: 93 %*<br>*Dentine-bonded resin composit: 77 %* |
| Song & al. [11] | IRM, MTA grey and SuperEBA | 6 to 10 years | not available | 172 | not available | not available | 94 % |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Von Arx & al. 2012 [12] | MTA, SuperEBA and Dentine-bonded resin composite | 5 years | 194 | 194 | 16 | not available | Overall rate: 76 %<br>*MTA: 88 %*<br>*SuperEBA: 67 %*<br>*Dentine-bonded resin composite: 75 %* |
| Taschieri & al. [13] | SuperEBA | 2 years | 70 | 113 | not available | 113 | 92 % |
| Chong & al. [14] | MTA and IRM | 2 years | 183 | 183 | not available | not available | Overall rate: 90 %<br>*MTA: 92 %*<br>*IRM: 87 %* |
| **AVERAGE :** | | **6 YEARS** | **–** | **339 TEETH** | **–** | **–** | **83,4 %** |

Table 3 compares the success rates of the different studies according to the obturation material used. The studies by Tawil & al [9] and Song & al [11] have been excluded from this comparison because their work do not detail the number of teeth studied according to the type of obturation material used.

Table 3. Success rates by comparison of obturation material

| **Obturation material** | **Study** | **Number of teeth** | **Success rate** |
|---|---|---|---|
| IRM | Truschnegg & al. [5]<br>Chong & al. [14] | 134 | 81,5 % |
| MTA | Von Arx & al. 2019 [6]<br>Kim & al.[7]<br>Caliskan & al, [8]<br>Von Arx & al. 2014 [10]<br>Von Arx & al. 2012 [12]<br>Chong & al. [14] | 544 | 87,5 % |
| SuperEBA | Kim & al.[7]<br>Von Arx & al. 2012 [12]<br>Taschieri & al. [13] | 539 | 83 % |
| Dentine-bonded resin composite | Von Arx & al. 2014 [10]<br>Von Arx & al. 2012 [12] | 331 | 76 % |

The quality the assessment of the risk of bias of randomized clinical trials, were done according to the Cochrane recommendations. For Kim & al, 2016 [7] and Taschieri & al, 2008 [13], the randomization process, deviations from planned interventions, missing outcome data, outcome measurement, and selection of the reported outcome presents a low risk. For Song & al, 2012 [11], the randomization process has some concerns, deviations from planned interventions and missing outcome data have a high risk of bias while the outcome measurement and selection of reported outcome has a low risk. Chong & al, 2003 [14], the randomization process, deviations from planned interventions, outcome measurement, and selection of the reported outcome has a low risk while missing outcome data has a high risk of bias.

The quality risk of bias assessment of the included prospective clinical studies, were done according to the Cochrane recommendations. For Von Arx & al, 2019 [6] and Von Arx & al, 2012 [12], has a low risk of bias for confounding, selection of participants, classification of interventions, deviations from planned intervention, missing data, outcome measures and selection of reported outcomes. Truschnegg et al, 2020 [5], had a moderate risk of bias for

confounding factors and a low risk for participant selection, intervention classification, deviations from planned intervention, missing data, outcome measures, and selection of reported outcomes. In the study by Çalışkan & al. 2016 [8], moderate risk of bias for confounding and outcome measures and low risk for participant selection, intervention classification, deviations from planned intervention, missing data, and selection of reported outcomes. Tawil & al, 2015 [9], has a low risk for confounding, participant selection, intervention classification, deviations from planned intervention, missing data, but a moderate risk for outcome measurement and selection of reported outcomes. Von Arx et al, 2014 [10], presents in the study a low risk for confounding, participant selection, classification of interventions, deviations from planned intervention, missing data, selection of reported outcomes, but a moderate risk for the outcome measure.

The risk of bias in the included randomized clinical trials and prospective clinical studies was found to be low except for the study by Song & al, 2012 [11] which presents some concerns.

## DISCUSSIONS

According to the results of these studies, 2 to 13 years after the procedure, the overall success rate of endodontic microsurgery varies from 78% for prospective clinical studies to 91% for randomized clinical trials. This success rate varies from 69% for the study of Tawil & al. [9] to 93% for the study of Song & al. [11], a difference of 24%. This difference could be explained by the methodology of these studies. Indeed, Tawil & al, 2015 [9], using transillumination, were to evaluate the post-surgical periapical healing of teeth with dentinal defects compared to healthy teeth. This work concluded that the success rate was significantly lower for the group of teeth with root dentinal defects compared to the group of teeth evaluated. Moreover, Tawil & al. considered cases with incomplete healing as unhealed. For these reasons, there is a significant decrease in the overall success rate. And the work of Song & al, 2012 [11], only resulted in the outcome of healed teeth at the less than one year to five years follow-up. Thus, the actual recall rate is 39% instead of 61%, which probably leads to biased results.

Among the evaluation of potential prognostic factors, only five showed statistically significant differences in the outcome of endodontic microsurgery: smoking, location and type of tooth, presence/absence of a dentinal defect, interproximal bone level and type of obturation material. The effect of the type of obturation material was the most frequently analyzed factor in the selected studies.

The use of gutta-percha alone or glass ionomer cement (GIC) in endodontic microsurgery was not specified in these studies, nor was the use of amalgam as a type of obturation material [15.] The materials used for the evaluation of the results were: intermediate restorative material (IRM) [5, 11, 14], athoxy benzoic acid (SuperEBA) [7, 9, 11-13], resin-based cements [12], mineral trioxide aggregate (MTA) [6-12,14]. Chong & al, 2003 [14], Song & al, 2012 [13], and Truschnegg & al. 2020 [5] have used IRM as a root filling material in their studies of endodontic microsurgery. Chong et al. 2003 is the only study with comparative results between IRM and MTA, and no significant difference was found. The study by Von Arx & al, 2012 [12], was the only one that found significant differences between the MTA group (86%) and the SuperEBA group (67%).

In the present study, two articles (Von Arx & al., 2014 and Von Arx & al., 2012) [10,12] using a dentin bonding agent (MTA, Super EBA, dentin-bonded resin composite) estimated the outcome of endodontic microsurgery. Both studies concluded that the success rate was higher when using MTA. These results can be explained by the need for a dry field during the etching/priming/bonding process [16] and the moisture control of the filling material [15].

Due to their high biocompatibility, the materials of the generation of hydraulic calcium-silicate cements (MTA and Biodentine) have caused great interest. MTA has been used as an obturation material by many authors selected for this study [6-12,14]. Indeed, this material has shown higher success properties than SuperEBA and dentin-bonded resin composite. These results can be explained according to Torabinejad M, Higa RK, McKendry DJ, and other, by good tissue tolerance, fibrous formation on contact with MTA, excellent sealing, wet setting, antibacterial activity, antifungal activity (alkaline pH), a non-absorbable and radiopaque material [17]. But MTA causes some clinical concerns due to the fact that its mechanical properties are only maximal after 24 hours and the difficulty of handling due to its sandy consistency after mixing with sterile water [18].

Recently, new obturation materials such as bioceramic-based root canal sealants have been developed to improve the setting time [19]. However, as scientific evidence remains rare and due to the short follow-up period, studies using this type of obturation material were excluded from this work.

The risk of bias was assessed for all randomized clinical trials and prospective clinical studies. A low risk was recorded with the exception of the study by Song & al., 2012 [11] due to the lack of data on recall rates. However, several selected authors considered teeth extracted from follow-up as a dropout because the rationale for extraction was unknown or not related to endodontic microsurgery (fractures, prostheses) [7, 10, 12]. Another concern is related to the risk of bias due to the results. The selected studies classified their radiographic results according to Rud & al [3] and Molven & al [4]. However, Tawil & al, classified cases with incomplete healing as non-healed. This classification therefore compromised the assessment of the risk of bias. Indeed, an underestimation of the outcome of endodontic microsurgery may have occurred.

The European Society of Endodontology (ESE) and the American Association of Endodontists (AAE) recommend regular clinical and radiographic follow-up for a minimum of one year after endodontic microsurgery. The ESE also recommends to increase the follow-up period at 5 years when a radiolucent area defined as "surgical defect" persists 1 year after surgery [20].

However, this follow-up time is still debated. Indeed, some studies show a relapse 4 years after traditional endodontic surgery, which confirms that a short follow-up period might be insufficient to identify a recurrence of apical periodontitis [19]. But in studies using a modern microsurgical approach, these results were not recorded [7, 9, 14].

Recently, studies with long-term follow-up have looked for significant differences in outcome compared to outcomes assessed over a short-term follow-up period. The study by von Arx & al., 2019 [6] presented a lower success rate after 10 years (82%) compared with success rates following a 1-year (91.6%) and 5-year (91.4%) recall. In the study by Kim & al, 2016 [7], the overall success rate after 4 years (89.5%) was lower than the 1-year follow-up (94.3%), thus a reduction in success rate of 4.8%. This cause of decrease can be explained by a lower recall rate at the 1-year follow-up. Von Arx & al, 2014 [10] confirmed that cases recorded as healed after 1 year were still healed after 5 years in 93.9% of cases.

For all the above reasons, a 1-year follow-up may be not sufficient to assess the success of endodontic microsurgery. It is necessary to continue the follow-up after 1 year and to take in consideration the obturation material in cases of uncertain healing. Furthermore, long-term follow-up gives a more reliable result and increases knowledge of the risk factors involved in long-term failures: root fracture [6], prosthesis [12], endodontic or periodontal reasons [5], caries and crown fractures [7].

In order to obtain the most reliable results, strict inclusion and exclusion criteria were selected for this systematic review. Articles in which the surgical procedure was not

performed under endoscope or microscope were excluded and each article used the same classification of radiographic findings (Rud & al [3] and Molven & al [4]).

However, this work has some limitations. Firstly, only studies with a long-term follow-up period were included. This risks the quality of some studies, since the longer the follow-up, the higher the drop-out rate. This may result in a loss of scientific validity of some conclusions [11]. Secondly, the inclusion of comparative studies between two-dimensional and three-dimensional outcome measures was not chosen due to short follow-up time [21] or the lack of classification of clinical and radiographic criteria according to Rud & al. and Molven & al. Although some studies show that two-dimensional assessment overestimates healing compared to three-dimensional assessment [22, 23], the study by Kruse & al [24] aims to determine the periapical lesions diagnosed by these two different radiographic methods. In this study, it is reported that after histopathological examination, 40% of the unsuccessful cases diagnosed by CBCT did not show signs of periapical inflammation. It is therefore concluded that CBCT may underestimate the healing outcome as it may diagnose incomplete healing as the presence of pathology (uncertain healing). According to the European Society of Endodontology, CBCT should only be used when its benefits exceed those of conventional imaging [25].

There are some concerns about the validity of the results obtained. Firstly, it is known that the outcome is influenced by the operator [26]. However, the 10 studies selected for this work were performed in a hospital or university environment, which may overestimate the results compared to when the procedure is performed in a private environment. It is therefore important to develop multicentric studies under conditions similar to daily clinical practice to evaluate the results of endodontic microsurgery. In addition, the study by Chong & al, 2003 [14] established strict exclusion criteria, such as a probing depth of 4 mm or more. The study of Taschieri & al, 2008 [13], established exclusion criteria for teeth that have not undergone non-surgical endodontic retreatment or for teeth with traumatic injuries. These criteria serve to increase the effectiveness of the procedure and therefore contribute to the overestimation of the results of endodontic microsurgery.

Finally, none of the selected studies reported the cost-benefit ratio of the obturation material. For the dentist and the patient, this information should be taken into account in the treatment decision.

## CONCLUSIONS

This work has shown that the application of a strict surgical protocol, in accordance with the latest scientific data, leads to excellent results. This therapeutic approach is highly reliable when performed with modern surgical techniques such as the use of magnification instruments (microscope or endoscope) and biocompatible obturation materials.

This surgical technique, which was very uncertain at the beginning, has benefited from extensive studies, the development of new obturation materials (such as MTA, SuperEBA and IRM) and micro-instruments (such as ultrasonic tips), making it a safe and approved alternative nowadays.

The chance of preserving a tooth averages 83.4% in patients with a mean follow-up of 6 years after endodontic microsurgery. However, relapse is observed 10 years after surgery, confirming that a short follow-up period may not be sufficient to identify a recurrence of apical infection. A 1-year follow-up may not be sufficient to evaluate the success of endodontic microsurgery. It is necessary to continue the follow-up after 1 year and to consider the filling material if healing is uncertain. In addition, long-term follow-up gives a more reliable result and provides insight into the risk factors involved in long-term failures such as root fractures, prosthesis, endodontic or periodontal reasons, caries and crown

fractures. Indeed, the long-term success rate of endodontic microsurgery is influenced by various significant factors such as smoking, the presence or absence of a dentin defect, the level of interproximal bone, the use of magnifying instruments (microscope or endoscope) and the type of material used for the root obturation.

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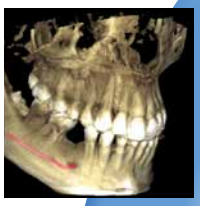

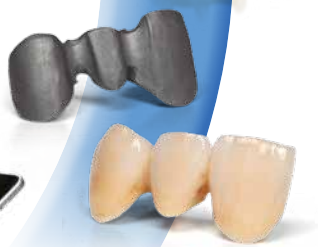




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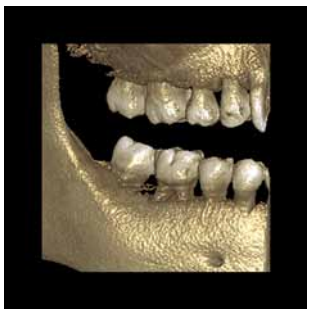

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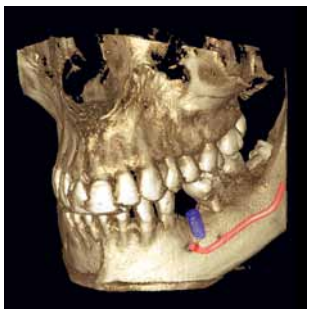

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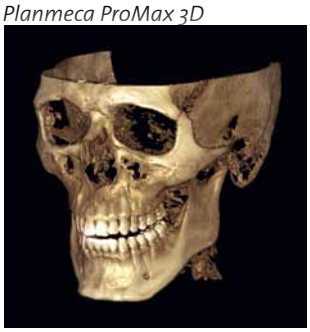

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The journal publishes general reviews, studies and clinical, epidemiological, experimental and laboratory research, clinical case presentation, papers from the history of medicine, reviews, scientific and technical state-of-the-art articles, medical informations and opinions. Only papers which have not been published or sent for publishing in other journals are accepted. The authors are responsable for the opinions expressed in the papers. ***The paper must be edited both in Romanian and in English; the English version will be supervised by our collaborator Dana Brehar-Cioflec, MD, PhD; typed on white $A_4$ paper and on CD, DVD or Memory Stick***.

Manuscripts will not exceed:

- general reviews: 6-8 pages
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The paper will be edited according to international editing rules for manuscripts. The title will be written in capital characters and it will be followed by the name and surname of the author (authors), followed by their place of work (place where the paper has been elaborated). Studies and researches will be followed by a brief abstract, followed by 3-4 key-words.

The body of the paper will be structured on the following chapters: introduction, aim, objectives, material and method, results and discussions, conclusions. The references will be presented alphabetically and in conformity to the Vancouver Convention, including:

- for articles: name of the authors and surname initials, title of the article in the original language, title of the journal according to the international abreviation system, year of issue, volume, number, pages;
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Citation of references inside the body of the paper will be put between brackets, Harward style (author, year) or Vancouver style (number in square brackets or superscript). Cited reference titles will be selected, maximum 6 for studies and case presentations and 12 for general reviews. Acceptance, rejection or the need of alterations in sent materials, or in inconography, will be comunicated to the authors in due time. For this, the authors will indicate the person and address for corespondence (phone number, e-mail address). Given the less pleasant experience of the editorial board with some articles being rejected because they did not meet publishing criteria, we decided to support those who intend to publish in this journal by detailing the way such a paper should be elaborated, as well as our requirements.

Except some particular aspects concerning this journal, the following details are general requirements asked or imposed by other journals as well. Conditions to be met in order to propose a paper for publishing. The main author has the responsability to make sure the article has been approved by all the other authors. The journal will have copyright

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Do not use name of patients, initials or hospital observation charts numbers. If a photograph of a body part which could allow direct or deductive recognition of the patient needs publishing, then the paper must be accompanied by the written consent of the patient and clinician, as well.

## 6. PRESENTING THE MANUSCRIPT

### 6.1. CONTENT OF THE PAPER - INDICATIONS FOR ORIGINAL ARTICLES

# Paper title [Book Antiqua 20, bold, left alignment]


## Surname N.[1], Surname N.[2] [Book Antiqua, 14, bold]

[1] *Author Affiliation (DEPARTMENT, FACULTY, UNIVERSITY, CITY/COMPANY) [10, italic]*
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## Abstract [Book Antiqua, 12, bold, justify alignment]

Recommendations for original studies

Original studies must include a structured abstarct of maximum 150 words, containing the following titles and informations: Aim and objectives; Material and methods; Results; Conclusions; Key words: give 3-5 key words; The abstract will be translated into an international circulation language.

**Keywords:** Innovation, technology, research projects, etc. [Book Antiqua 9].

## INTRODUCTION [Book Antiqua, 11, bold, left alignment]

Introduction presentation of general aspects, in the context of the approached theme.

Introduction include **Aim and objectives –** Define the aim of the article. Briefly expose the rationale of the presented study or observation. Make strictly pertinent referals and do not exhaustively review the subject. Do not include data or conclusions from the paper.

There is a limitation of 4/6 pages. All pages size should be A4 (21 x 29,7cm). The top margins should be 2 cm, the bottom, right, margins should be 2cm and left margins should be 2,85 cm. All the text must be in one column and Book Antiqua font, including figures and tables, with single-spaced 10-point interline spacing.

### *Aim and objectives* [Book Antiqua 11, bold italic, left alignment]

The text included in the sections or subsections must begin one line after the section or subsection title. Do not use hard tabs and limit the use of hard returns to one return at the end of a paragraph. Please, do not number manually the sections and subsections; the template will do it automatically.

[Book Antiqua, 11 point, normal, justified alignment].

## MATERIAL AND METHODS [Book Antiqua, 11, bold, left alignment]

Describe the selection of observations or subjects for the experiment (including controls). Identify methods, equipments (with the name and address of the manufacturer in brackets) and give sufficient details on procedures. Give references for the selected methods, including statistical methods; offer details and brief descriptions for previously published methods which are not well known; describe new or substantially modified methods, justify their use and assess their limitations. Precisely identify all used drugs and chemicals, including generic names, dosage and administration ways. Describe statistical methods with sufficient details for reported results to be verified. Whenever possible, quantify discovered aspects and present them with appropriate measurement indicators for the uncertainty or error of measurement (such as confidence intervals). [Book Antiqua, 11 point, normal, justified alignment].

## RESULTS [Book Antiqua, 11, bold, left alignment]

Present results in a logical succession as text, tables and illustrations. Emphasize or briefly describe only important observations. [Book Antiqua, 11 point, normal, justified alignment].

## DISCUSSIONS [Book Antiqua, 11, bold, left alignment]

Underline new, important aspects of the study. Do not repeat in detail data which have been presented in previous sections. Include implications of revealed aspects and their limitations, including implications for future studies. Connect your observations to other relevant studies. Relate the results to the aim proposed for the study. [Book Antiqua, 11 point, normal, justified alignment].

## CONCLUSIONS [Book Antiqua, 11, bold, left alignment]

Organize conclusions which emerge from the study. In the end state: a) contributions to be acknowledged but which do not justify paternity right; b) thanks for technical support;

c) thanks for financial or material support. [Book Antiqua, 11 point, normal, justified alignment].

**REFERENCES [Book Antiqua, 11, bold, left alignment]**

A numbered list of references must be provided at the end of the paper. The list should be arranged in the order of citation in the text of the publication, assignment or essay, not in alphabetical order(according to the Vancouver rules). List only one reference per reference number. It is very important that you use the correct punctuation and that the order of details in the references is also correct.

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[1] _____
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Content of the paper for case report will respect indications for original articles.

Themes may be selected from all medical fields. Manuscripts which offer a special gain for daily activity will have priority. The title must be clearly, precisely stated. It may be completed by a subtitle. It is advisable to include in the key words of the title the main message, the special element which may be observed from the case evolution. The content of a case report must be divided into three parts:

Introduction – It must include a maximum of 15 typed rows (half page). Here, the main medical problem is summarized in order to place the case in a specific domain.

Case report – It contains essential specific information on the case. In order to make a logical, chronological and didactical case report the following 5 chapters are needed:

I. Anamnesis;
II. Clinical examination data;
III. Laboratory data;
IV. Additional paraclinical investigations;
V. Treatment and evolution.

Discussions – The reason for the case report must be stated. The report must be patient-centered. Occasional deviations from typical (characteristic) evolutions, nosologically important facts must be presented in such a manner to expose the clinical picture as completely as possible. The case report must not appear as an appendix of a general review. Dimensions of a case report: maximum 6-8 typed pages, 30 rows of 60 characters/page.

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All measurements must be expressed in International System (IS) units. Abreviations must be fully explained when first used.

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