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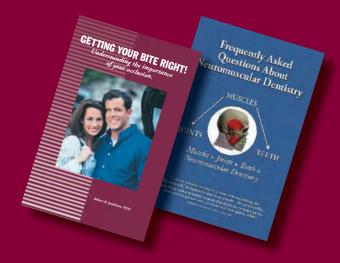
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Global prevention

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€ 5 https://doi.org/10.25241/stomaeduj.2021.8(1).edit.1



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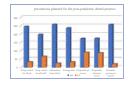
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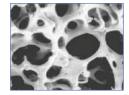
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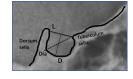
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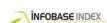
















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# Global prevention



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# Dear readers,

The year 2020 is over and it was completely dominated by the COVID-19 pandemic, which as we all know started on December 30<sup>st</sup> 2019 in Wuhan [1]. In our minds we look back at a very bad year with more than 1.8 million people that passed away due to the infection that may end up being utterly deadly, especially if individuals are exposed to additional risks. We were all faced with severe restrictions which have profoundly changed our lifestyles, from the obligation to wear masks and keep social distancing to skipping social gatherings, large events, and travelling. Altogether a bad year and a big crisis. However, in every crisis there is a chance as well. The positive thing is that the world population is now talking about prevention and is sensitized to everything that is prevention. Furthermore, we can widely see how people may react to preventive measures by either accepting them or rejecting them by hiding behind abstruse theories like claiming the pandemic is an illusion created by the mighty and powerful.

This brought me to the idea that we dentists that (I claim it for myself) have grown up with prevention should jump on the bandwagon of general prevention and based on our experience help to move general health up to a higher level. Wherever I look I see prevention and potential to increase its level with the corresponding knowledge. Prevention works best when it can be consumed without a special effort; examples here are the water fluoridation to reduce the incidence of caries or the reduction of saturated fats or the reduction of sugar in processed food to fight obesity. Looking at cars, ABS or airbags work according to the same principle. Prevention that requires the recipient's activity mainly boils down to a change in lifestyle, which is the most difficult task to accomplish. Here the input of psychologists is welcome as well as the one of legal experts when preventive measures should be backed with laws and enforced. We have seen during the corona year that this may be a big challenge. There were episodes where people were afraid to ask other people to wear a mask where it was mandatory, because they were afraid of violent reactions. Another example I remember were party goers that violated all requirements to wear masks and social distancing for a big new year's party that became so violent that the police guit dispersing the masses, or simply people attending discos that instead of giving their address just wrote down a bogus address to avoid contact tracing. A way of enforcement is controlling and fining violators. But it may be tricky to find the right way. I remember that many years ago, when wearing a seat belt in a car became mandatory, you could buy t-shirts with a seatbelt printed on them to avoid being pulled out of traffic and getting a ticket!

Today some cars offer the seatbelt once the person is seated or simply start with an ugly buzz a few seconds after the passenger is seated. To reduce the level of fatalities because of car accidents by defensive driving is much more difficult to enforce.

The overall conclusion is that we need to bundle all our expertise to improve prevention, since now the moment seems to be favorable. We must come out of our silos and think outside the box. In dentistry we train our students in the techniques of motivation, which is the very first step in changing people's behavior, and we have established simple measuring tools to monitor the progress of oral hygiene. In fighting infectious diseases vaccination has become the favorite tool, but we notice more and more people that do not let themselves or their kids to be vaccinated, with putting themselves and others at risk. Remember how many

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# Guest Editoria

years it took to eradicate small pox or polio? It took a lot of measures to accomplish what required bundled knowledge in immunology, as well as information transfer, persuasion and logistics. Law makers may be required to do that when it comes to regulations. I know that in Denmark a "fat tax" was introduced with the idea to fight obesity. In Switzerland for the same reason, students were required to take more physical education classes.

To help with this process I have decided to create a webpage (<a href="www.globalprevention.ch">www.globalprevention.ch</a>) where every specialty interested in prevention can share their ideas and find partners for the common goal: make our lives and health better. Dear experts, make your knowledge, your success, but also your problems and challenges known to the others by sharing them on this website.

Furthermore, develop crazy ideas! What about stimulating the use of facemasks by design: incorporating pheromones makes the wearer feel good; or incentivize the use of tracing applications and social distancing by a reduction of health care insurance premiums (this may work for many preventive measures that require behavioral changes).

Or create immunization by aerosols at events that attract big masses, like concerts, festivals, sports competitions, or political rallies.

Take the chances and help to make this a better world!

Sincerely yours

J-F Roulet <sup>©</sup> Stomatology Edu Journal Founding Editor

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# Editoria

# Value of randomized controlled trials



Marco FERRARI MD, DMD, PhD, FADM, Professor, Chairperson, Dean University of Siena, Siena, Italy Stomatology Edu Journal Editor-in-Chief

Dear readers,

In the globalization era technology is rapidly advancing in all the fields of dentistry and there is an urgent need to collect longitudinal clinical data to be shared in the world dental community. Hundreds of laboratory studies performed following different techniques are continuously published in international peer-reviewed journals (with Impact Factor) and they are useful to provide comparative data among several products within the same category. Such investigations can potentially predict to some extent the clinical performance of new materials and techniques. However, in vivo trials based on predictable and reproducible protocols should always precede the large-scale clinical use of recently introduced products. Clinical validation is indeed a cornerstone of 'Evidence-Based Dentistry'. Such requirement is understandably strict with new adhesive materials, but it should be even more cogent when dealing with implant surgery techniques. Based on the Helsinki Declaration on the ethical principles for medical research involving human subjects, a clinical research protocol should preliminarily receive the written approval of the pertinent Ethical Committee, and should clearly state the study's inclusion and exclusion criteria. Patients should be fully informed on the objectives of the research, as well as on the methods and possible related risks. The patients' written informed consent to the study should be obtained. All the researchers performing clinical studies should conform to this policy and the editorial boards of scientific journals should verify that all the requirements are met. However, by reading some of the internationally published literature, the impression can be gained that ethical issues are not always given the due attention by the authors and then also overlooked by the journals reviewers. It is not uncommon that published papers are found to miss relevant details on the ethical aspects of the clinical study, such as whether or not the protocol was approved by a pertinent Ethical Committee, what were the contents of the patient informed consent, who was the principal investigator. Such defective information can indeed limit the scientific value of the research. Moreover, some published clinical studies have been conducted in countries where the regulation on research in humans is more permissive than that of the Helsinki Declaration. Not to mention that false declarations, although disreputable, are always possible.

Those we agree that credibility is the highest quality of a researcher cannot escape the feeling that in some studies patients are used as experimental animals or even worse, if one considers that in some advanced countries animal research is actually strictly ruled.

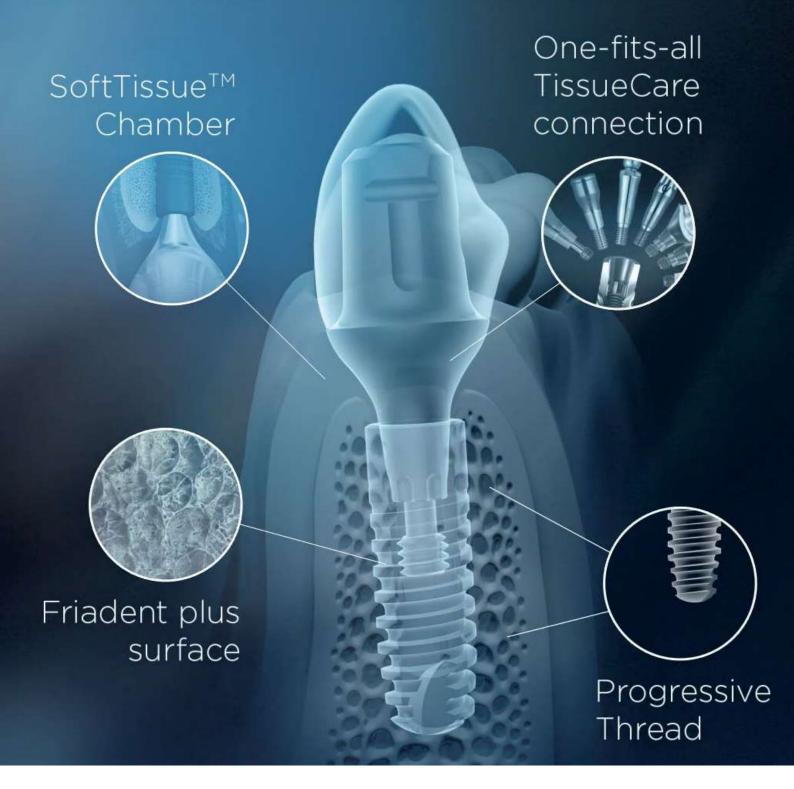
Certainly, imposing a more ethical approach to clinical research will not be an easy task. Nevertheless, against this perspective, it would be advisable if peer-review journals could request, as a condition for publication of clinical studies, that the authors provide evidence of the Ethical Committee approval and if reviewers could verify with the authors a proper list of required ethical issues related to the study have been properly addressed. Although such a policy would predictably, initially affect the submission rate of clinical studies, however it would also limit the dissemination of research that does not have a solid, ethical foundation.

Sincerely yours,

M. Ferrari Stomatology Edu Journal Editor-in-Chief

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IMPACT OF HYPERGLYCEMIA ON THE RATE OF IMPLANT FAILURE AND PERI-IMPLANT PARAMETERS IN PATIENTS WITH TYPE 2 DIABETES MELLITUS
SYSTEMATIC REVIEW AND META-ANALYS

J Am Dent Assoc. 2021 March 01, 152 (3): 189-201–667. Doi: https://doi.org/10.1016/j.adaj.2020.11.015 https://jada.ada.org/article/S0002-8177(20)30819-9/fulltext

This article has an accompanying online continuing education activity available at: <a href="http://jada.ada.org/ce/home">http://jada.ada.org/ce/home</a>.

DOI: https://doi.org/10.1016/j.adaj.2020.11.015

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# SRI LANKAN DENTAL PROFESSIONALS' KNOWLEDGE OF THE CORONA VIRUS DISEASE-19 (COVID-19): A QUESTIONNAIRE SURVEY

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# **ABSTRACT**



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Background Dental practitioners are some of the most vulnerable professionals exposed to the risk of contracting the Corona Virus Disease-19 (COVID-19), by virtue of the aerosol generating procedures (AGPs). Hence, an evidence based and detailed knowledge of the disease is important in order to mitigate the effects of the COVID-19 transmission.

Aim To identify the perspective, knowledge, and attitudes of Sri Lankan dental surgeons on the COVID-19 pandemic.

Methods An online web-based, self-administered questionnaire survey (Google) was conducted among Sri Lankan dental surgeons. All questions, in the pre-tested questionnaire were close-ended, and formulated to elicit data on the views, knowledge, attitudes, and infection control practices related to the COVID-19

Results One quarter of the cohort responded and the majority (44.7%) were 30-40 years old with a marginal female preponderance (52.3%). In general, females were significantly more knowledgeable than males on COVID-19, and the post-pandemic preparedness for dental practice (p<0.05). One sixth (18.2 %) incorrectly surmised that, i) pet animals were a source of infection, ii) COVID-19 is not transmitted via surface contact and iii) hand hygiene is not important in preventing infection transmission. All respondents correctly identified AGPs as a high-risk procedure for infection transmission, but approximately two thirds failed to identify specific AGPs in dentistry.

Conclusion Taken together, the knowledge, attitudes and practices of Sri Lankan dental surgeons on the COVID-19 pandemic appear satisfactory, but there are knowledge gaps that need to be fulfilled through further continuous education courses.

# **KEYWORDS**

COVID 19; Dental Practice; Knowledge; Dental Surgeons.

# 1. INTRODUCTION

The first case of Corona Virus Disease-19 (COVID-19) caused by the severe acute respiratory syndrome corona virus 2 (SARS-CoV-2) was reported in Wuhan, China in December 2019 [1]. Even though scant attention was paid to the disease at the time, within

weeks it became a serious health concern leading to an epidemic spread in China, prior to the subsequent pandemic spread the world over. COVID-19 was declared a public health emergency of international concern by the World Health Organization on 30th of January 2020 [2]. As of this writing, in December 2020, the number of COVID-19 cases worldwide

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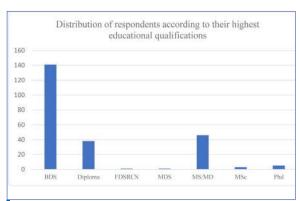


Figure 1. Distribution of respondents according to the highest educational qualifications.

COVID-19 cases worldwide has surpassed 75 million, with over 1.6 million deaths [3]. In Sri Lanka, the first case of COVID-19, was reported in a Chinese tourist, in January 2020, and the first, communally transmitted patient reported two months later; at the time of writing (December 2020), Sri Lanka has over 36,000 confirmed cases, with 165 reported deaths. SARS-CoV-2 primarily spreads via droplets produced by coughing, sneezing, and talking. When droplets are large and heavy, they usually fall to the ground or onto surfaces, but the smaller particles, called aerosols, are entrained in the air for prolonged periods of approximately 3 hours if the ambient circulation is stagnant [4,5]. A substantial proportion of dental procedures entail aerosol generation due to the high speed instrumentation accompanied by air/water coolants, and hence dentistry is considered a high risk profession in terms of contracting air borne diseases such as COVID-19 [6]. Furthermore, experience from previous epidemics such as the severe acute respiratory syndrome (SARS) has shown the susceptibility of health care providers, including dental health care workers to the risk of possible infection [7]. In order to protect dental professionals, and their patients from COVID-19, many national bodies, including the Sri Lanka Dental Association, have issued guidelines on infection control in dental settings to the profession at large [6,7]. Nevertheless, there has been little follow up after such promulgations and, no feedback elicited from dentists on the adherence to, and implementation of these guidelines in clinical practice. Clearly, such feedback is helpful in rectifying deficiencies, and preparing for future emergencies. Hence the main aim of the current questionnaire survey was to identify the views, knowledge and attitudes of Sri Lankan dental surgeons on the current COVID-19 pandemic through a web-based questionnaire survey. A particular focus of the survey was to evaluate the adherence to the COVID- 19 protective measures and awareness of methods of SARS-CoV-2 transmission, infection control precautions that need to be implemented during the pandemic, and the challenges associated with their implementation.

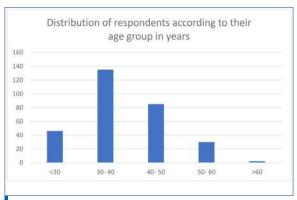
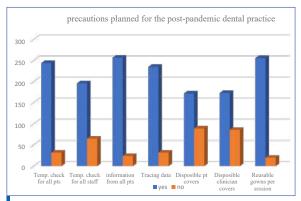


Figure 2. Distribution of respondents according to the age group.



**Figure 3.** Details of precautions planned by the respondents for the post-pandemic dental practice.

# 2. METHODS

An online web-based questionnaire survey was conducted among dental specialists and dental surgeons in Sri Lanka. Those with access to the World Wide Web, both in the government service and in private practice, were included in the study. A self-administered pre-tested Google form (Appendix 1) was used as the study instrument.

The questionnaire, formulated by the authors (LPS, RJ), was first pre-tested among a group of 10 dental surgeons to ensure clarity of interpretation, and ease of completion by the participants. The questionnaire, of all close-ended questions, comprised demographic data and specific questions on the views, knowledge and attitudes of Sri Lankan dental surgeons on the current COVID-19 pandemic. The pre-tested questionnaire was then converted into a Google form and the survey link was disseminated as a Uniform Resource Locator (URL) web address among the cohort, via social media and email. A cover letter was included with the questionnaire, which described the purpose of the study and its outline, and instructions were given on completing the questionnaire; the confidentiality and anonymity of the data provided were assured. The data management and statistical analyses were performed using the statistical software SPSS version 21.0. Frequencies and percentages were obtained for categorical data, and Chi-square test was used to determine the association between variables.



**Table 1.** Comparison of P values highlighting statistical significance between respondents' gender, age groups and highest educational qualifications with knowledge of COVID-19 and its impact on dental practice.

Question	Sex (P)	Age group (p)	Education (p)
Incubation period of COVID 19 infection?	0.000	0.412	0.249
Attendance to webinars or continuing education programs on maintaining dental practices during COVID-19 pandemic	0.000	0.361	0.305
Believe patients with chronic diseases run a higher risk of contracting COVID 19?	0.000	0.643	0.409
Believe older patients are at a higher risk for COVID 19 infections?	0.000	0.566	0.401
Believe mortality rate for the young population is zero?  Covid-19 transmission		0.074	0.315
		0.476	0.000
Prevented by good hygiene practices?	0.000	0.655	0.369
Minimum social distance advised to prevent COVID-19 spread?	0.000	0.691	0.780
Loss of taste and loss of smell as early symptoms of COVID-19	0.000	0.243	0.420

P level <0.05 was considered significant). Ethical clearance for the study was obtained from the Ethics Review Committee of the Faculty of Dental Sciences, University of Peradeniya, Sri Lanka.

# 3. RESULTS

# 3.1. Demographic and personal information

The survey was transmitted to a cohort of 1598 dental surgeons, of which 302 (25%) responded. Most respondents were aged 30-40 years (44.7%), with a marginally female preponderance (52.3%). Two thirds of the respondents were dental surgeons (n=120) working in the dental clinics of the Ministry of Health, Sri Lanka, with the rest, though employed by the Ministry of Health, were concurrently engaged in private practice. Most of the respondents had only the primary qualification of BDS (n=177) while 45 (15%) also had a postgraduate diploma; 60 were specialists with anMD/ MS degree (Fig. 1). Questioned on the attendance and participation in continuing education programs during the COVID-19 pandemic, a majority (60%) responded in the affirmative. Of these, 74% were respondents younger than 30 (p<0.05).

# 3.2. Knowledge and belief related to COVID-19 patient identification

The vast majority of the respondents (91%) was aware of the incubation period for SARS-CoV-2 infection as 2-3days, but that it may take up to 21 days. Almost all of the respondents (96%) under 30 provided an accurate response to this question. All participants identified at least one symptom of the infection, while 18% identified all the stated symptoms of the disease. Similarly, 96% of the respondents believed that patients with chronic diseases run a higher risk of contracting COVID-19. Awareness that older populace run a higher risk of contracting the COVID-19 than the younger individuals was also very high (97%) and only 4% (12/ 302) respondents

incorrectly believed that the mortality rate for young population is zero (Table 1).

# 3.3. Knowledge regarding transmission of COVID-19

All respondents were aware that the aerosol generating procedures (AGPs) were a high risk activity for the infection transmission, but only one third (39%), accurately identified that implants placement, scaling and restorative procedures as high risk interventions that may transmit the infection (Table 1).

Surprisingly, a sixth of the respondents (55/302; 18.2%) incorrectly surmised that, i) pet animals were a source of infection, ii) COVID-19 is not transmitted via surface contact and iii) hand hygiene is not important in preventing infection transmission. Nevertheless, 82% were aware that the infection spreads through droplets or nasal discharge (p<0.05). Further, 93% of the respondents believed that COVID-19 infection can be transmitted through the fluid of an infected person (p<0.05). Nearly 85% have correctly identified 1 meter as the minimum social distance to be kept in order to minimize transmission of infection.

# 3.4. Preparedness in treating dental patients during and post pandemic period

Most of the participants (92.4%) had purchased personal protective equipment, but two-fifths (40.4%) did not possess N95 masks. Almost two thirds of the respondents (62.3%) had treated emergency patients during the peak month from 15th March to 15th April 2020 when Sri Lanka was under the lockdown. One half of the respondents (52.2%) were confident of starting dental practice once the pandemic subsided, whereas 25.2% were hesitant, and 6% were not confident in doing so. Although over 92% have been provided with personal protective equipment (PPE), only 57% had access to N 95 masks during the initial period of the COVID-19 pandemic. All respondents were planning to implement precautions during the post-pandemic

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Table 2. Percentage of responses regarding knowledge and plan of the respondents to start dental practice during and post COVID-19 pandemic.

Questions in the questionnaire	%
Can disease be prevented by good hygiene practices? Yes No	95 3
What is minimum social distance advised to prevent COVID-19 spread?  1m  1.5m  2m  2.5m	84.4 4.3 8.6 1.3
Are you aware that loss of taste and loss of smell are early symptoms of COVID-19 Yes no	86.8 12.6
How important is it to know the patient's residential area when taking the history? Highly important Not important May be important	91.4 6.6 0.7
Will you consider patient's travel history before treating him? Yes No May be	97.7 1 1.3
How confident are you about starting your dental practice after the pandemic? Highly confident Confident Hesitant Not confident	12.6 52.3 25.2 6.0
What is your biggest fear on starting your post-pandemic dental practice? (Select ALL that apply) Increased cost of care delivery Risk of contracting disease from patients Limited resources including PPE	5.41 36.2 58.6
Will you ask your patient to get tested for Covid-19 before treatment?  Yes, all the patients for aerosol generating procedures  No  May be, only if patient is symptomatic	10.9 18.2 70.9
How worried are you about Medico Legal issues once you open up your dental practice? (1 not worried, 5 extremely worried) Rate from 1 to 5  1  2  3  4  5	11.3 11.3 38.4 17.9 19.9

practice. The majority were to introduce temperature checks for all patients (88.4%), and the staff (75%), but planned use of reusable gowns per each session and of disposable over wear for both patients and clinicians were low (Fig. 3). Ninety one percent of the respondents felt it is highly important to know the residential area of the patients, while 97.7% thought knowing the patient's travel history is important before treating them. The highest fear among the respondents to start dental practice was the limited availability of resources for protection such as PPE (Table 2). The answers covering the respondents' knowledge on the pandemic and the plans in relation to the maintenance of post-pandemic dental practice were analyzed according to their gender, and females almost always responded better than males (p<0.05). In terms of the age group, the plans for triage of the clinic attendees, was highest for the 36-45 years age group (p<0.05) (Table 3).

# 4. DISCUSSION

The unprecedented COVID-19 pandemic caused by SARS-CoV-2 has taken the world by surprise. It is now clear that aerosols are the main mode of COVID-19 transmission, and dentistry, being an important branch of health care has come under intense scrutiny due to the many AGPs that entail clinical dental practice. Hence, infection control procedures in dentistry have been freshly reviewed with revised guidelines promulgated by various bodies including US Centers for Disease Control [8]. A good awareness and knowledge of the mode of transmission of COVID-19 among dental professionals is essential to implement these guidelines. Although a number of reports are available on the dentists' awareness of the pandemic and attitudes towards the implementation of clinical guidelines [9,10] no such data are available from Sri Lanka. Hence, we conducted



**Table 3.** Comparison of P values highlighting statistical significance between respondents' gender, age groups and highest educational qualifications with their plan for patient handling at the dental practice during COVID-19 pandemic and post pandemic period.

	Sex p	Age group (p)	Education (p)
Do you triage your patients?	0.000	0.013	0.071
Will you consider patient's travel history before treating him?	0.000	0.206	0.846
How important is it to know the patient's residential area when taking the history?	0.000	0.257	0.009
Have you purchased / provided with Personal Protection Equipment (PPE) kits?	0.000	0.795	0.668
Do you have access to purchase / provided with N95 masks?	0.000	0.154	0.415
Have you treated emergency patients during the month from 15th March to 15th April?	0.000	0.974	0.821
Have you treated emergency patients during the month from 16th April to 15th May?	0.000	0.910	0.192
Have you treated emergency patients after 16th of May?	0.000	0.368	0.297
How confident are you about starting your dental practice after the pandemic?	0.000	0.151	0.327
Will you ask your patient to get tested for Covid-19 before treatment?	0.000	0.202	0.202
US centers for disease control (CDC), and many dental associations including SLDA have proposed guidelines for COVID-19 prevention in dental clinics.	0.000	0.946	0.750
Precautions planning at the post-pandemic practice 1.Temperature checking of all patients	0.054	0.179	0.324
2.Temperature checking of all staff before they start work	0.066	0.552	0.430
3. Information form on COVID-19 to all patients	0.002	0.894	0.894
4. Tracing data	0.013	0.805	0.519
5. Disposable Patient covers	0.000	0.687	0.513
6. Disposable clinician covers changed for each patient	0.000	0.497	0.577
7. Reusable gown worn for session/ day	0.052	0.194	0.730
8. Will you edit the patient history questionnaire	0.000	0.599	0.816
9. Will you question the patient on recent loss of taste or smell?	0.005	0.073	0.05

the current electronic, online survey to elicit the knowledge and practices of 1598 dental surgeons and dental specialists working in the public and private sector in Sri Lanka [11]. A pre-tested questionnaire was sent via email to the cohort and open invitations for response were posted in the social media platforms, including Facebook and specific groups created among dental surgeons. Our study demonstrated a relatively poor response rate with only a quarter (25%) of the dental surgeons and specialists responding to the questionnaire. Low response rates are common in electronic questionnaire surveys and non-respondent bias may have affected the outcome of our results. Given this caveat, the findings of our survey shed light on the knowledge of, and attitudes to infection control in Sri Lankan dental surgeons during the pandemic period. Overall, a large majority of respondents had envisioned and anticipated the impact of COVID-19 on their clinical practices, as they demonstrated a very satisfactory knowledge of the disease symptomatology and preventive measures. It was encouraging to note that most of them attended webinars to improve their knowledge of COVID-19, in contrasts to a recent similar study from Turkey that has shown low attendance rates at continuing education courses by dentists [12].

Prevention of COVID-19 is mainly achieved by proper hand washing, social distancing and by respiratory protective measures such as the use of face masks/ face shields [13]. In contrast to the findings of other similar surveys [10,12], almost a fifth of our respondents incorrectly mentioned that hand hygiene is not important in preventing infection and pet animals are a major source of infection. Personal protective equipment (PPE) is an essential prerequisite for the safe delivery of dental care, as well as for the protection of the dental surgeon and the dental team. A number of organizations including the US Centers for Disease Control [8] and the Sri Lanka Dental Association [6] have emphasized the importance of PPE in dentistry. PPE include gloves, respirators or face masks, face shields or goggles and protective clothing [14]. Most of the respondents (92.4%) in our survey had purchased PPE, but only 60% had N95 masks. This is better than the figures reported in similar studies on Indian and Turkish dentists, respectively [12,15]. As fever is one of the demonstrable early symptoms of COVID-19, dental health care providers must as a routine,

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measure the patients' body temperature as well as those of his staff and him/herself, prior to entering the clinic premises [14]. The majority were aware of this recommendation as 80% were planning to measure the temperature of the patients, and 65% in both the patients as well as the staff.

As a health care worker, it is the solemn duty of a dental surgeon to provide essential and emergency care to patients, irrespective of the pandemic circumstances. Hence it was heartening to note that some two thirds of the respondents (62.3%) managed patients with dental emergencies during the island-wide, lock-down period in Sri Lanka (from 15th March to 15th April 2020). This is in contrast to only one fifth of the dentists (22.8%) in India who rendered such emergency services during a similar lock-down period in India [15].

It is the general consensus that COVID-19 pandemic is unlikely to subside in the immediate term, and is likely to smolder, and remain as an endemic disease in most regions of the world for the foreseeable future. Yet, dental professionals, like all other similar care providers, need to maintain their services to the public while taking the necessary precautions to minimize infection spread. Due to the high levels of morbidity and mortality associated with COVID-19, there is nevertheless a reluctance, fear and anxiety amongst dentists to return to work, as shown in some recent studies [9,15]. This was clearly expressed in our survey, as one fourth of responding dental surgeons were hesitant to start their postpandemic dental practice in the short term, and 6% were not confident in doing so at all. However, it was heartening to note that approximately, one half of the respondents (52.4%) were very confident in returning to work after the lock-down period. This number is significantly higher than the figure reported by Kinariwala et al., where 54.3% of respondents in their study were not confident, and 35.7% were hesitant to commence their postpandemic dental practices [15]. In addition, the availability of COVID-19 vaccines in the near future,

for countries such as Sri Lanka, should help improve the prospects of dentists returning to work without much ambivalence.

# 5. CONCLUSIONS

In general, the knowledge, attitudes and practices of Sri Lankan dental surgeons on the COVID-19 pandemic appear satisfactory but there are significant gaps in their knowledge that need to be addressed such as the knowledge on the method of transmission. Highest fear among the respondents was limited availability of resources to practice dentistry. Health authorities and professional associations need to consider these factors when preparing guidelines for the management of patients in the dental clinics during the pandemic. Gender as well as educational qualifications-related differences were noted in some responses. Our survey highlights the importance of continuing education and further educational programs on COVID-19 for dental professionals in Sri Lanka.

# **CONFLICT OF INTEREST**

The authors declare no conflict of interest.

# **ACKNOWLEDGMENTS**

We like to thank all the dental surgeons who helped disseminate and respond to the questionnaire during a difficult period.

# **AUTHOR CONTRIBUTIONS**

RDJ: plan the study, plan the questionnaire and assisted in data collection and did the first draft. RMJ: was involved with the data collection, preparation of questionnaire, statistical analysis and finalizing the manuscript. PKH: was involved with the data collection, preparation of questionnaire and finalizing the manuscript. LPS: came up with the idea, assisted in preparation of questionnaire, supervise the project and assisted in finalizing the manuscript.



# Annex 1 Knowledge and Practices among Sri Lanka Dental Surgeons on Professional Dental Practice during the Corona virus Disease-2019 (COVID-19) Pandemic

Serial No				
Demographic and personal information				
1.	Gender- Male Female			
2.	Nationality Sri Lankan Any other (PI specify)			
3.	Your specialty			
	General practitioner			
	Consultant in Restorative Dentistry			
	Consultant in Orthodontics			
	Consultant in Oral and Maxillofacial Surgery			
	Consultant in Community Dentistry			
	Trainee in Restorative Dentistry Trainee in Orthodontics			
	Trainee in Oral and Maxillofacial Surgery			
	Trainee in Community Dentistry			
	Any other (PL specify)			
4.	Your education B.D.S Diploma MS/MD any other (pl specify)			
5.	Age (in years) < 30 30-40 40-50 50-60 >60			
6.	What best describes the type of practice you are in? (Select ALL that apply)			
	Ministry of Health (MOH) Academic Private Practice			
7.	Did you attend webinars or continuing education programs on maintain/ conducting dental practices during COVID-19 pandemic? Yes No			
Knowledg	e and belief related to COVID-19 patient identification			
8.	What is incubation period of COVID 19 infection?			
	1 day			
	2-3 days but may take up to 21 days			
	28 days			

# Original Articles

1.	Which are the symptoms of COVID 19 infection? (Select ALL that apply)
	Fever Dry cough
	Tiredness Nasal congestion
	Diarrhea Aches and pains
	None of the above All of the above
2.	Do you believe that patients with chronic diseases run a higher risk of contracting COVID 19? Yes No
3.	Do you believe that older patients are at a higher risk for COVID 19 infections?
	Yes No No
4.	Do you believe mortality rate for the young population is zero? Yes No
Knowledge	e regarding transmission of COVID-19
5.	Which dental procedures do you consider as high risk to transmit COVID 19
	History taking and Examination
	Dental Extraction
	Scaling
	Restorative procedures
	Providing dentures
	Implant placement
	Military Called Called Toward Annual Carter TDUE 2 (Called All all and a call )
6.	Which of the following statement is TRUE? (Select ALL that apply)
	Pet animals are the biggest source of infection.
	Covid-19 spreads through droplets of saliva or nasal discharge.
	Covid-19 is not transmitted via surface contact.
	Hand hygiene is not important to prevent transmission of Covid-19.
7.	Do you believe Covid-19 infection can spread through fluid of infected person?
	Yes No No
8.	Can the disease be prevented by good hygiene practices? Yes No
9.	What is the minimum social distance advised to prevent COVID-19 spread?
1 n	neter 1.5m 2m 2.5m



10. Are you aware that loss of taste and loss of smell are early symptoms of COVID-19:
Yes No No
History taking practice How important is it to know the patient's residential area when taking the history?
Highly important  Not important may be important
11. Will you consider the patient's travel history before treating him?
Yes No May be
12. Do you triage your patients?"Yes No
Fears associated with the best management practices  13. Have you purchased / provided with Personal Protection Equipment kits?  Yes No
ies ino
14. Do you have access to purchase / provided with N95 masks? Yes No
15. Have you treated emergency patients during the month from 15 <sup>th</sup> March to 15 <sup>th</sup> April?
Yes No
16. Have you treated emergency patients during the month from 16th April to 15th May?
Yes No No
17. Have you treated emergency patients after 16 <sup>th</sup> of May? Yes No
18. How confident are you about starting your dental practice after the pandemic?
Highly confident Confident Hesitant Not confident at all
19. What is your highest fear on starting your post-pandemic dental practice? (Select ALL that apply)
Limited availability of personal protection kits
Limited resources to sterilize/disinfect the equipment and the clinic premises
Risk of contacting infection from the patient
Increased operating cost and unaffordable cost of care delivery
20. Will you ask your patient to get tested for Covid-19 before treatment?
Yes, all the patients for aerosol generating procedures should get themselves tested.
□ No
May be, only if patient is symptomatic

# Original Articles

1.	How worried are you about Medico-Legal issues once you open up your dental practice? (1 not worried, 5 extremely worried)
	Rate from 1 to 5
2.	US centers for disease control (CDC), and many dental associations including SLDA have proposed guidelines for COVID-19 prevention in dental clinics.
	a. Are you aware of these guidelines Yes No
	b. Do you follow all the guidelines so proposed?
	Always Sometimes Rarely Never
3.	What precautions are you planning at the post-pandemic practice
٥.	Temperature checking of all patients: Yes  No  No  No  No  No  No  No  No  No  N
	2. Temperature checking of all staff before they start work: Yes No
	3. Information form on COVID-19 to all patients: Yes No
	4. Tracing data: Yes No
	5. Disposable Patient covers: Yes No
	6. Disposable clinician covers changed for each patient: Yes No
	7. Reusable gown worn for session/ day: Yes No No
28.	Will you edit the patient history questionnaire? Yes No
29.	If yes to question 28 above:
	a) Will you insert an immediate travel history question (2 weeks before dental endance): Yes No
	b) Will you question the patient on recent loss of taste or smell? Yes No
	**************Thank You. End of questionnaire***************

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# Questions

# 1. What is incubation period of COVID-19 infection?

□a. 1-day;

□b. 2-3 days but may take up to 21 days;

□c. 28 days;

□d. 48 days.

# 2. Which of the following is not a symptom of COVID-19 infection?

□a. Fever;

□b. Dry cough;

□c. Aches and pains;

■d. Sneezing.

# 3. What will be the best method to control COVID-19 infection?

☐a. Social distancing;

□b. Hand hygiene;

□c. Wearing face masks;

□d. All of above.

# 4. Which of the following groups are at a higher risk of getting COVID-19?

□a. Infants;

□b.Yong;

□c. Middle aged;

□d. Elderly.



# BIOSURFACE PROCESSING WITH ROLE IN IMPROVING THE OSSEOINTEGRATION OF THE ORAL IMPLANT

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# **ABSTRACT**



65 https://doi.org/10.25241/stomaeduj.2021.8(1).art. 2

Introduction The osseointegration of the oral implant involves a close contact between the bone and the implant, an important feature that allows the optimal transfer of stresses from the implant to the bone. Achieving and maintaining tissue integration is ensured by a biosurface design with a role in reducing the effect of shear forces on the interface, which can stimulate osteogenesis and facilitate tissue remodelling. Numerous specialized studies describe the method of modifying the biosurface area generated by a certain topography, among them are those that attest to the role of roughness in increasing the number of cells that will adhere to a biosurface with a larger area.

Methodology Research was performed in order to establish the influence of mechanical processing on the micro-roughness of the surface of the samples from the experimental bioalloy Ti10Zr. Atomic force microscopy (AFM), scanning electron microscopy (SEM) and fluorescence microscopy were used to evaluate the experimental results.

Results The study presents the results on the micro-roughness profile and the values of the parameters that characterize the micro-roughness profile groups (2D and 3D) of the biosurfaces processed by grinding and very fine polishing. Aspects regarding the modification of biosurface morphology, compared to mechanical processing, acid corrosion and anodic oxidation, as well as the results regarding cellular behaviour (e.g. adhesion of osteoblasts) to experimentally processed biosurfaces are also presented.

Conclusions The results of this experimental study together with those previously presented in "Controlled Changing of Implantable Bioinert Materials Biosurface "scientific paper compile a synthesis of information on the ability to modify the microtopography of the biosurface of the Ti10Zr alloy, by different ways in order to improve implant osseointegration.

# **KEYWORDS**

Bioalloy; Experimentally Processed Surface; Micro-roughness; Atomic Force Microscopy; Cell Adhesion.

# 1. INTRODUCTION

The direct and lasting connection between living and reshaped bone that defines the osseointegration of the implant [1,2,3] is determined by the tissue compatibility that influences the healing process of recovery and remodelling, immediately after implantation. Bone healing in the post-implantation period involves a series of cellular and extracellular biological processes at the bone-implant interface, completed with the formation of new bone [4,5,6]. The first reactions result in the formation of a clot interface, the biological processes being further controlled by growth and differentiation

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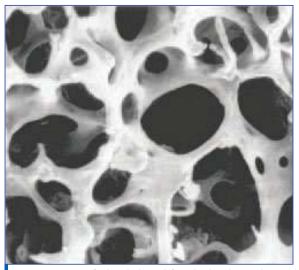
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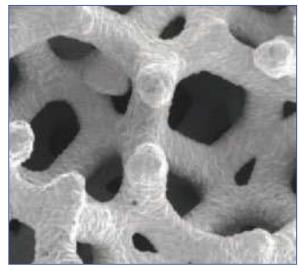


Figure 1. SEM view of trabecular bone (left) and Trabecular Metal Material (right) [18]

factors released by blood cells. They undergo morphological and biochemical changes as an effect of contact with the biosurface [7,8,9]. Achieving implant osseointegration, but also maintaining tissue integration are ensured by the presence of a high biocompatible material [10] and a design that stimulates osteogenesis and helps reduce the effect of shear forces on the interface, such as surface roughness and shape characteristics [11,12]. The role of implant surface roughness in stimulating and improving bone growth to implant surface in low bone density structures is highlighted in many specialized studies, which indicated higher post-load failure rates for implants with relatively smooth surfaces compared to implants with low bone density rough surface [13]; Also relevant are the results of comparative studies on the roughness of biosurfaces, obtained by different processing methods, those oxidized with increased roughness have shown a shorter post-implantation healing period due to improved cellular interaction at the bone-implant interface [14]. In summary, the conclusions of the studies on the effect of microrough biosurfaces on the osseointegration process clearly show the indication for the use of titanium "microrough" implants obtained by titanium plasma spraying, or by techniques such as Al<sub>2</sub>O<sub>3</sub> particle blasting, TiO, blasting and acid etching. The effects of microrough surfaces, such as faster integration, a larger bone-to-implant contact area compared to titanium implants with a polished or machined surface, have been confirmed by in vitro cell response studies, demonstrating that osteoblasts are sensitive to changes in the roughness of biosurfaces. The clinical benefits mentioned by the authors in recent clinical situations are related to the shortening of the healing period for these implants to 6-8 weeks instead of 12 weeks. The treatment of the implant surface by mechanical, physical or chemical methods but also the differentiated treatment led to a faster healing and a better stability of the

implant [15,16,17]; It has been shown, especially in low-density bone structures, that implant stability is influenced by implant design and that a combination of microscopic and macroscopic surface topography modification techniques can create a stable bone-implant interface.

In the case of titanium alloys, research on the application of techniques to improve cell interaction and cell development at the interface by intensifying protein adsorption processes has been carried out in order to determine whether bone apposition could be enhanced by a microrough surface obtained by processing techniques such as blasting, acid attack, or combinations thereof. In vitro research of the titanium implant with different surface microtopographies has shown the differentiation of bone and mineralization cells, dependent on roughness. Rough surfaces favour osteointegration of the implant through the attachment and subsequent proliferation of osteoblasts and the size of the implant-bone contact area; Notable results regarding the modification of the biosurface parameters of the titanium implant and titanium alloys were obtained by surface treatment with hydroxyapatite (HA), achieved by various physical, chemical, electrochemical methods such as: Plasma spray, Pulsed Laser Deposition, Chemical Vapor Deposition, Physical Vapor Deposition, HA Blast Coating, etc., noting that regardless of the process applied, metal surfaces are prepared by sandblasting, abrasion or chemical corrosion.

The diversification of the biosurface processing methods but also the advances registered in the design of the oral implant resulted in implants with differentially processed surfaces and well adapted clinically by the positioning mode and the placement area; The implant can have a portion obtained by mechanical surface finishing operations (e.g. by grinding), an engraved portion and a sandblasted portion at high temperatures with an ideal roughness of osseointegration, which ensures the

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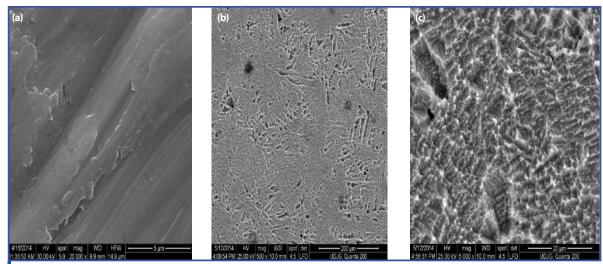


Figure 2. Microscopic aspects of the surface of the Ti10Zr samples experimentally processed through: casting + machining + grinding (a), acid corrosion (b), anodic oxidation (c)

optimal anchoring of the implant. Also, the control of the properties of the tissue-implant interface is well possible in the case of the titanium implant obtained by sintering (LST-Laser Sintered Titanium, 2009) which has a rough surface with micropores of 2-200 µm and a prescribed geometry from the design stage. The healing process begins with the insertion of the implant as this surface stimulates and accelerates the healing of bone tissue, which makes it possible to immediately load the implant into bone of density I, II, III, in much safer conditions. Research in recent years in interdisciplinary fields such as engineering and medicine has led to remarkable results for obtaining biomaterials with high biocompatibility and the promotion of advanced surface processing technologies. Some of these resulted in a porous biomaterial made of TiZrTa alloy with structure and elasticity close to those of bone.

The modern design introduced by the "Trabecular Metal" implant (Fig.1) conceptually revolutionized the theory of osseointegration and introduced the notion of osseoincorporation (growth of bone tissue including in the structure of the implant). Considered the newest discovery in the field of dental implantology, it is the only implant with three-dimensional structure (3D) that mimics bone cell architecture (80% porosity) and systematic nanotextured topography of superficial areas. The trabecular structure of the implant causes the bone to form inside it, resulting in a common body between the implant and the human bone. The implants have a treated surface of SLA type (Sandblasting with Large grit followed by Acid etching), chemically modified and moderately rough which increases the bone-implant contact surface ensuring a period of osseointegration twice less than other implant systems [18].

The paper presents the experimental research conducted in order to study the influence of changes in the microtopography of biosurfaces processed by casting + mechanical processing and casting +

mechanical processing + polishing / polishing to mirror gloss, of some samples from the experimental bioalloy TI10Zr.

The research represents the continuation of the study and the completion of the information previously presented in the works "Controlled Changing of Implantable Bioinert Materials Biosurface" [19] and "In Vitro Testing of Materials Biocompatibility with Controlled Chemical Composition" [20], in order to establish the optimal and efficient way to modify biosurface area, and in this way of improving the osseointegration of the oral implant from Ti10Zr, between the mechanical, chemical (acid attack) and electrochemical (anodic oxidation) processing processes.

# 2. METHODOLOGY

In the experiments, samples from the experimental bioalloy with titanium base were used (Ti10Zr/Patent no. 132079/2019). The samples were taken from the molded semi-finished product subsequently subjected to mechanical processing (casting process + mechanical processing by grinding and casting + mirror gloss polishing).

The analysis of the sample surface was performed by scanning electron microscopy (SEM), atomic force microscopy (AFM / EasyScan2 Model), and the investigation and evaluation of the interactions at the interface was performed by in vitro analysis by exposing G292 osteoblasts to these surfaces, under the same conditions with samples from the same bioalloy but treated on the surface by acid attack and anodic oxidation (previously published results) [19,20].

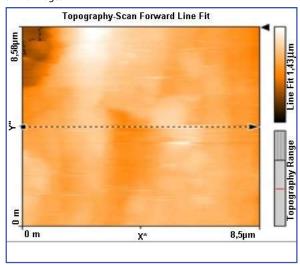
# 3. RESULTS

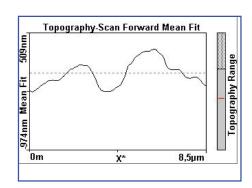
The results of the scanning electron microscopy analysis (Fig. 2) highlighted the changes in the morphology of the experimental biosurface modified by



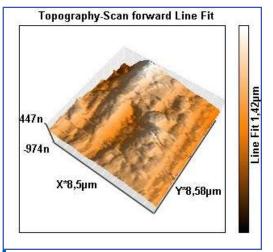
# A. Samples taken from cast semi-finished products and subsequently subjected to mechanical processing (grinding).

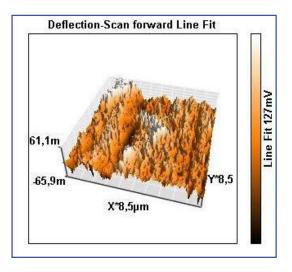
# a.1.2D images





a.2.3D images





**Figure 3.** Parameters that characterize the roughness profile.

 $R_a = 125,62$ nm,  $R_q = 147,75$ nm,  $R_y = 525,97$ nm,  $R_p = 266,18$ nm,  $R_v = -259,79$ nm,  $R_m = -3,628$ fm.

mechanical processing, compared to those obtained by corrosion (acid attack) and anodic oxidation, and the results of atomic force microscopy analysis (Fig. 3 and Fig. 4) illustrates the roughness profile (2D and 3D images) and shows the measured values of the parameters that characterize the roughness profile groups by mechanical sample processing. Figure 5 shows the aspects regarding the in vitro evaluation of the adhesion of osteoblasts on the surface of the investigated samples.

# Remarks:

The anodic oxidation method allows the development of an oxide layer on the surface of the material with a role in improving the adhesion and fixation properties.

The Ti10Zr alloy samples thus processed provide a special surface configuration, as shown in electron scanning microscopy images (Fig. 2c). The oxide film is a basis for the formation of the osteoinductive matrix. Micro-topographic analysis of the sample

surface through atomic force microscopy analysis (AFM) provided useful information on the roughness profile and the values of the parameters that characterize the roughness profile groups. The roughness corresponds in value to surfaces with very fine processing. There are isolated peaks of high roughness, with a rounded shape, characteristic of the surface obtained from mechanical processing (Fig. 4).

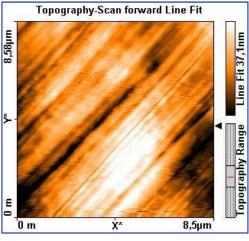
The investigation and evaluation of the response of experimentally processed biosurfaces to in vitro cellular behaviour were carried out by exposing G292 osteoblasts to Ti10Zr samples, with surface morphological characteristics conferred by mechanical processing described above, under the same conditions as samples processed by casting, acid corrosion and anodizing.

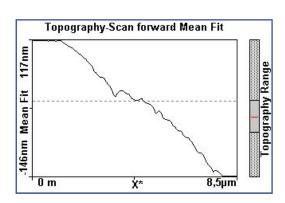
G292 osteoblasts were seeded in 6-well plates at a density of  $5 \times 104$  cells/cm<sup>2</sup> in the presence of Ti10Zr samples with differently processed surfaces; At the same time, cells were cultured in vessels but in

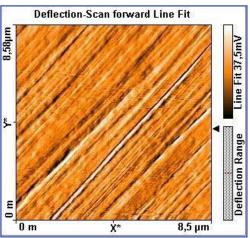
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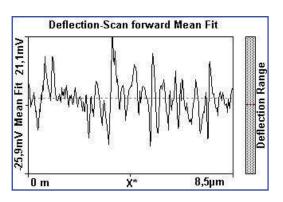
B. Samples subjected to mechanical processing (grinding + polishing to mirror gloss).

# b.1.2D images

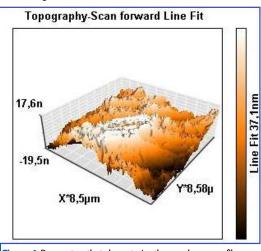








b.2.3D images



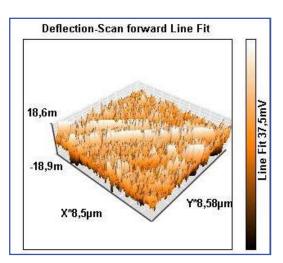


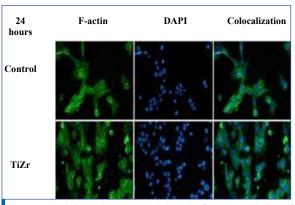
Figure 4. Parameters that characterize the roughness profile.  $R_a = 7,9282 \text{ nm}, R_a = 9,1452 \text{nm}, R_v = 43,537 \text{nm}, R_v = 16,564 \text{nm}, R_v = -26,973 \text{nm}, R^m = -3,4725 \text{fm}.$ 

the absence of any material (control). At 24 and 48 hours after incubation, medium was harvested from each well and fluorescent labelling of cytoskeletal actin filaments and intracellular glutathione was performed (Fig. 5).

Examination by fluorescence microscopy of the architecture of actin filaments revealed that the cells grew in a single layer, showed an osteoblast – like phenotype and there were no differences from the

control. The experimental results demonstrate, for all ways of processing the biosurface of Ti10Zr samples (acid attack, anodizing, mechanical processing), a good adhesion of osteoblasts, especially to oxidized ones, which have cells with a well-organized actin cytoskeleton, interconnected and with cell densities comparable to those of the control (in the absence of sample material).





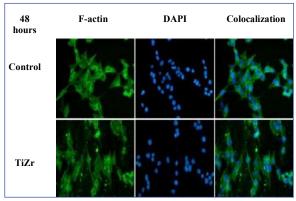


Figure 5. Highlighting the actin cytoskeleton by fluorescent labelling of F-actin with phalloidin-FITC (DAPI nucleus counter colouring) in osteoblasts grown for 24 and 48 hours on the surface of the culture vessel/Control and Ti10Zr alloy [21].

# 4. DISCUSSIONS

The study of the influence of the mechanical processing method on the micro-roughness profile and the values of the micro-roughness parameters determines the cellular behaviour, tested by evaluating the adhesion and the distribution of osteoblasts on surfaces thus processed.

In vitro testing of cellular behaviour, which provides the information needed to understand the mechanism by which surface micro-roughness controls the cellular response, proves that different changes in topography lead to differentiated responses, at least in cell distribution mode.

Corroborating the results obtained in the mechanical processing of the surface with those obtained in the chemical processing (corrosion or anodic oxidation) of the Ti10Zr bioalloy, differences were observed both in terms of the micro-roughness profile and the values of the micro-roughness parameters, but also in terms of the mode of cell spread on these surfaces. Mechanically machined surfaces contribute to the obtaining of microrough biosurfaces. The values characterizing the parameters of the microroughness profile are not the same for differently machined surfaces (with different micro-roughness profile) and are significantly lower for surfaces with fine machining (samples with almost smooth surface, polished).

Compared to the surfaces processed by corrosion or anodic oxidation, those obtained by mechanical processing (regardless of the processing method) in addition to a good adhesion of osteoblasts, presented an orientation of adherent cells depending on the direction of mechanical processing of samples. It has been observed that any further processing plays a decisive role in how the cells are oriented and adhered to these surfaces and annihilate the influence of previous processing.

# 5. CONCLUSIONS

The research highlights the possibilities of processing the biosurface of the implant with a role in improving

the biological processes at the implant-tissue interface in the immediate post-implantation period. The results of this experimental study together with those previously presented in "Controlled Changing of Implantable Bioinert Materials Biosurface" provides useful information on the ability of the Ti10Zr bioalloy to modify its microtopography of the biosurface, by different ways to improve implant osseointegration. The results of the research that aimed to establish the optimal way to modify the biosurface area through more ways of processing are presented. The modification of the biosurface parameters (microroughness) was evaluated, as well as their influence on the adhesion of osteoblasts and the cell proliferation capacity on the experimentally processed surfaces.

The conclusions of the research are that the increase of the biosurface area by modifying the morphology and/or micro-roughness either by corrosion, by oxidation or coarse either high-precision mechanical processing, has been demonstrated and denotes the ability of Ti10Zr bioalloy to improve biosurface characteristics. However, some observations of the experimental study should be noted, which may be of interest in selecting one or another of the experienced processing methods, as follows:

- a. considering any of the processes applied in coatings to increase the bioactivity of the metal surface, they require prior preparation by blasting, abrasion or chemical corrosion.
- b. the increase of the biosurface area by creating rough or microporous surfaces facilitates the cell adhesion processes and the growth of the trabecular bone directly on the surface of the titanium implant, shortening the post-implantation healing period.
- c. the anodic oxidation method creates surfaces covered with a uniform, continuous, adherent oxide film with a special morphological configuration with a decisive role in stimulating the processes at the interface.
- d. mechanically processed surfaces contribute to obtaining biosurfaces with a profile of microroughness and values of roughness parameters depending on the degree of processing and

influence the orientation of the adhered cells according to the direction of processing.

e. however, it was not noticed for corroded or anodized Ti10Zr, suggesting that additional processes have changed the properties of these surfaces, and thus have decisively influenced cell orientation and adherence:

f. these differences detected in surfaces with different processing could have a major influence on how osteoblasts managed to adapt and be the best option for dental implant.

# **CONFLICT OF INTEREST**

The authors declare no conflict of interest.

# **ACKNOWLEDGMENTS**

None.

# **AUTHOR CONTRIBUTIONS**

VGV: participated in the elaboration, writing and translation of the paper and contributed to the introductory part (synthesis of specialized information on the topic), to establishing the experimental conditions, interpreting the results and formulating the research conclusions. EV: contributed as follows: characterization of the materials researched and the interpretation of the results obtained in the investigation by advanced methods (SEM Microscopy, EDS Analysis). VS: participated in the writing and translation of the paper and contributed to structuring the bibliographical references. LTC: participated in the research of the documentary sources (bibliographical references), in the structuring of the research conditions and methodology and in the elaboration of the abstract.

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# Questions

# 1. Improving the osseointegration of the implant is possible by:

□a. The modification of the microtopography that determines the increase of the biosurface area;

□b. Use of implants with smooth (unprocessed) surfaces;

□c. Early implant loading;

☐d. The use of materials with high fatigue resistance.

# 2. Changes in biosurface morphology by anodic oxidation have the following effect:

□a. Increase of the oxide layer on the implant surface;

□b. Improve osteoinductive properties;

☐c. Decrease biological processes at the tissue-implant interface;

□d. Decreased cell adhesion.

# 3. Maintaining tissue integration is improved:

□a. By an implant biosurface design that increases the effect of shear forces;

□b. By factors that diminish the primary bone-implant stability;

□c. By a design of the implant biosurface that reduces the effect of shear forces;

☐d. In structures with low bone density.

# 4. The role of the microroughness of the implant surface is:

□a. To inhibit the growth of bone tissue to the surface of the implant;

□b. To improve protein adsorption in the cellular interaction at the interface between tissue and biomaterial;

□c. To reduce the bone-implant contact surface;

□d. To prevent the interfacial reaction in the post-implantation period.

# riginal Article

# AN ASSOCIATION BETWEEN DIMENSIONS AND BRIDGING OF THE SELLA TURCICA AND DENTAL ANOMALIES

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# **ABSTRACT**



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Introduction The aim of this study was to determine the incidence of bridging of the sella turcica and the dimensions of the Sella in subjects with dental anomalies (transpositions, hypodontia, and supernumerary teeth) and to compare them to controls.

Methodology Lateral cephalograms from 25 patients with dental transposition, 88 with hypodontia, and 26 with supernumerary teeth were evaluated. The shape, length, depth, diameter, and bridging of the Sella turcica were determined from radiographs and compared to those of control group (n=52). For statistical analysis, one-way ANOVA, Tukey post-hoc test, chi-squared test and T-test (to evaluate the influence of craniofacial growth) were used.

Results The frequency of complete calcification of the Sella was greater in the group with supernumerary teeth (23%) and in the group with hypodontia (14.7%), while partial calcification of the Sella was more frequent in the control group (77%) and in the group with supernumerary teeth (73%)(p<0.05). The depth of the Sella was greater in the group with dental transposition. Oval and round Sella shapes were more frequent in all groups, and a flat Sella was rarely seen. In terms of the influence of growth on the dimensions of the Sella, there was no statistically significant difference between pre- and post-treatment radiographs. **Conclusion** Significant relationships were found between dental anomalies and bridging and shape of the Sella. The Sella was also significantly deeper in patients with dental transposition. The bridging and shape of the Sella may be useful in the diagnosis of dental anomalies in early childhood.

# **KEYWORDS**

Sella Turcica Bridging; Hypodontia; Transposition; Supernumerary Tooth; Digital Radiography.

# 1. INTRODUCTION

The sella turcica is a depression in the middle line of the upper surface of the sphenoid bone. For orthodontists, the sella is a well-known anatomical structure on the scalp, because it is the central reference landmark in the evaluation of craniofacial morphology and the maxillomandibular relationship. The name sella turcica, Latin for "Turkish saddle," derives from the structure's similarity in shape to a saddle used by the Turks [1]. The anterior border of the sella turcica is marked by the tuberculum sella and the posterior border is marked by the dorsum sella [2]. The pituitary gland is located in the sella turcica, and two anterior and posterior clinoid processes project over the pituitary fossa [2].

During the embryological period, the sella turcica

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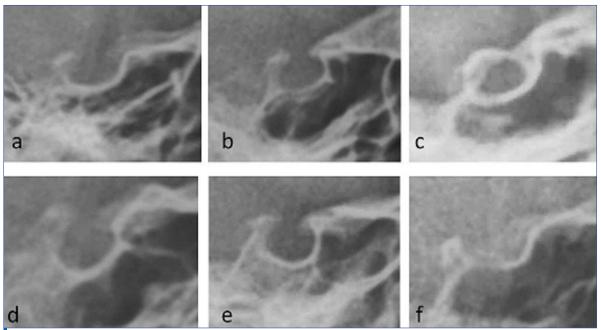


Figure 1. Classification of the bridging and shapes of the sella turcica: a) no calcification, b) partially calcified, and c) completely calcified; d) oval, e) round, and f) flat

Table 1. Descriptive statistics and gender distribution of study groups.

	Group				
	Transposition	Transposition Hypodontia Supernumerary Control		Total	
Gender Female/Male	21/4	55/33	13/13	33/19	122/69
Age (Mean±SD)	15.81±3.51	16.54±4.60	16.60±4.30	17.96±4.05	16.84±4.31

P value for age was 0.143

is the key point for the migration of the neural crest cells to the frontonasal and maxillary developmental fields [3]. The neural crest cells are involved in the formation and development of the sella turcica and the teeth [4]. Given this relationship between the sella turcica and the teeth, it has been argued that anatomic deviations in the sella turcica may be related to dental alterations. Thus, the morphology of the sella has been studied in relation to skeletal malocclusions [5,6], Down syndrome [7], cleft subjects [8], and dental anomalies such as dental transposition [9], absence of the second premolar [10], and palatally displaced canines [10].

In lateral cephalometry, the sella turcica is U-shaped. The size of the sella turcica has been evaluated widely in the literature and ranges from 4 to 12 mm in depth and from 5 to 16 mm for the anteroposterior diameter [11]. Three different shapes of the sella have been identified in the literature: oval, round, and flat. Of the three, the oval and round types are more common. The sella turcica is divided into three segments: an anterior wall, a floor, and a posterior wall [4]. Bridging of the sella turcica has been described as a bony union of the anterior and posterior clinoid processes. Bridging is regarded as an anatomical abnormality, and it is particularly prevalent in craniofacial deviations

and developmental conditions such as basal cell carcinoma, Williams syndrome, Rieger syndrome, and other disorders [6]. The current study was therefore performed to evaluate the dimensions of the sella turcica and the incidence of bridging of the sella turcica in patients with dental anomalies (transpositions, hypodontia, and supernumerary teeth), to compare them with the controls, and to assess whether any significant changes occurred in the dimensions of the sella during craniofacial growth in patients with dental anomalies.

# 2. MATERIALS AND METHODS

# 2.1. Study Population

This retrospective radiographic study, approved by the institutional ethical board (ref. 10840098-604), was carried out on the cephalometric radiographs of 25 patients (21 females and four males) with dental transposition, 88 patients (55 females and 33 males) with hypodontia, and 26 patients (13 females and 13 males) with supernumerary teeth. Only patients with good-quality lateral cephalometric and panoramic radiographs were included in the study. The exclusion criteria were as follows: presence of any syndrome or systemic disease, previous orthodontic treatment, excess craniofacial deviations, and history of trauma.

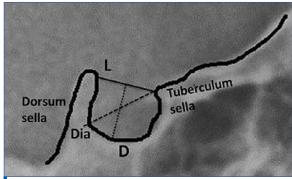


Figure 1. Reference lines and measurements of the sella turcica: L = length of the sella, D = depth of the sella, and Dia = greatest diameter of the sella

The control group was created according to power analysis ( $\alpha=0.05$ , f=0.25, power = 0.80) and consisted of 52 subjects (33 females and 19 males). The same inclusion and exclusion criteria were used as for the dental anomaly groups. The study groups are described in Table I.

# 2.2. Cephalometric Analysis

Pre-treatment lateral cephalometric and panoramic radiographs of all the patient groups and the control group were taken according to the standard technique for evaluating cranial structures, using a Kodak Extraoral Imaging System machine (Kodak 9000, Carestream Health, Inc., Rochester, NY, USA) and a cephalostat with a fixed magnification factor. Only radiographs with good-quality images of the Sella turcica area were selected for analysis. The region around the contour of the pituitary fossa from the tip of the dorsum sellae to the tuberculum sellae was traced on the lateral cephalometric images using acetate paper and a 0.5 mm lead pencil. The tracing and measurements were performed manually by one observer (SA) under ideal lighting conditions. Bridging of the sella turcica was evaluated in accordance with the standardized scoring scale method of Leonardi et al. [10] A sella was scored as having "no calcification" when its length was greater than or equal to three-fourths of its diameter, as "partially calcified" when its length was less than three-fourths of its diameter, and as "completely calcified" when only the diaphragma sellae could be seen radiographically (Fig. 1). The linear characteristics of the sella turcica were measured in accordance with Silverman's method [12]. The length of the sella turcica was measured from the tuberculum sellae to the tip of the dorsum sellae. Its depth was measured perpendicularly to this line passing through the deepest point of the pituitary fossa. Its diameter was measured in the sagittal direction by drawing a line from the tuberculum sellae to the most distant point on the posterior inner wall of the fossa (Fig. 2). Morphologically, each sella turcica was classified as one of the three basic types (round, oval, or flat), in accordance with the

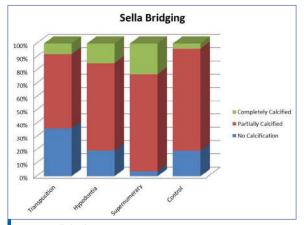


Figure 3. Sella bridging.

method of Jones et al. (Fig. 1) [13].

# 2.3. Evaluation of Influence of Craniofacial Growth

To assess the possible effects of craniofacial growth on the size, shape, and bridging of the sella, post-treatment cephalometric radiographs of 15 patients selected at random from the study groups were compared to their pre-treatment radiographs. The mean time period between pre- and post-treatment radiographs was  $3.21 \pm 1.19$  years.

# 2.4. Statistical Analysis

Analysis of the data was performed in the SPSS 16 package (SPSS for Windows, SPSS Inc., Chicago, IL, USA). Means, standard deviations, and minimum and maximum values were calculated, and the Shapiro-Wilks normality test was applied to the data. Oneway analysis of variance (ANOVA) and a Tukey post hoc test were used to assess differences between the linear dimensions of the groups, while nominal data were tested using a chi-squared test. A T-test was used to evaluate the influence of craniofacial growth on the measurements. To determine intra-rater reliability, 20 cephalometric radiographs selected at random were retraced after two weeks, and Pearson correlation coefficients were calculated (ranging from 0.810 to 0.862). The statistical significance was set at p<0.05.

# 3. RESULTS

Table I shows the descriptive statistics and p-values for the study groups and the control group. Table II shows the distribution of the degree of calcification of the sella turcica in the study and control groups. Complete calcification of the Sellawas more frequent in the group with supernumerary teeth (23%) and in the group with hypodontia (14.7%), while the partial calcification of the Sella was more frequent in the control group (77%) and in the group with supernumerary teeth (73%) (p=0.025).

The average linear dimensions of the sella turcica in the study groups and the control group are shown in Table II. The depth of the sella was greatest in the



Table 2. Distributions of the bridging types and the shapes of the sella, and average linear dimensions of the sella turcica in the study groups.

		Group					
		Trasposition	Hypodontia	Supernumerary	Control	P	
	No Calcification	9 (36)	17 (19.3)	1 (3.8)	10 (19.2)		
Bridging Type n(%)	Partially Calcified	14 (56)	58 (66)	19 (73.1)	40 (77)	0.025*	
(x)	Completelly Calcified	2 (8)	13 (14.7)	6 (23.1)	2 (3.8)		
Linear	Length	5.04±2.75	4.48±2.78	3.88±2.37	4.71±2.34	0.421	
Dimensions (Mean±SD)	Depth	7.48±1.36	6.93±1.26	6.42±1.10	6.86±0.99	0.018*	
(à)	Diamater	9.12±1.83	8.40±2.13	7.92±1.63	8.33±1.63	0.154	
	Oval	5 (20)	10 (11.4)	13 (50)	19 (36.5)		
Shape of Sella n(%) (x)	Round	18 (72)	69 (78.4)	13 (50)	27 (52)	0.001**	
	Flat	2 (8)	9 (10.2)	0	6 (11.3)		

(x) means Chi square statistics, \* P < 0.05. (à) means ANOVA test \* P < 0.05. According to Tukey Post hoc test; a statistically significant difference was found only between transposition and supernumerary groups P = 0.009\*

group with dental transposition, and the difference from the group with supernumerary teeth was statistically significant (p<0.05). The distribution of the shape of the sella turcica in the study and control groups is also shown in Table II. Oval and round shapes were more frequent in all groups, and a flat sella was rarely seen (p=0.001). According to the results of the T-test, there was no statistically significant difference between the pre- and post-treatment radiographs in terms of the influence of the growth on the dimensions and bridging of the sella.

### 4. DISCUSSION

In this retrospective study, certain characteristics and dimensions of the sella turcica were analyzed on pre-treatment standardized lateral cephalometric radiographs. The aim was to assess the shapes, dimensions, and the incidence of bridging of the sella turcica in subjects with dental anomalies (transpositions, hypodontia, and supernumerary teeth) compared to control subjects. These parameters have not previously been studied in Turkish orthodontic patients. The literature reports sella bridging, defined as ossification between the anterior and posterior clinoid processes in the dura mater, as a determinant factor for dental anomalies. Although bridging is seen in healthy subjects [14], it occurs more frequently in subjects with craniofacial deviations (i.e. carcinomas [15], syndromes [16], and skeletal malocclusions [17,18]). From an embryologic point of view, the anterior wall of the sella turcica develops from neural crest cells, as teeth do; thus, deviations in the anterior wall are

believed to be related to dental anomalies [14]. In the present study, bridging of the sella turcica at the completely calcified level was seen in 23.1% of the patients with supernumerary teeth, in 14.7% of the patients with hypodontia, in 8% of the patients with transposition, and in 3.8% of the control patients (total frequency = 12%). Bridging at the partially calcified level was seen in 73.1% of the patients with supernumerary teeth, in 66% of the patients with hypodontia, in 56% of the patients with transposition, and in 77% of the control patients (total frequency = 68.6%). The findings of the present study are different from those of comparable studies. For example, in patients with supernumerary teeth, bridging of the sella turcica has previously been reported at the partially calcified level in 53% of the patients and at completely calcified level in 6% of the patients; in that study, the levels for the control patients were 30% and 13%, respectively [19]. In a study of patients with transposition, 42.9% were at the partially calcified level and 23.8% at the completely calcified level; the levels for the control patients were 68.6% and 5.7%, respectively [9]. In a study of patients with supernumerary teeth, 21.7% were at the partially calcified level and 21.7% were at the completely calcified level; the levels for the control patients were 19.4% and 5.6%, respectively [20]. These differences between previous studies and the present findings may be due to the number of patients included in each study and the nature of their existing malformations, as bridging of the sella turcica has been related in the literature to craniofacial deviations. Many studies have reported patients with Class III malocclusion as having a greater proportion of bridging of the sella than Class I patients [17,18,21]. The size of the sella turcica has

been studied in detail in the literature, with the linear measurements ranging from 4 to 12 mm in depth and 5 to 16 mm in length [11,12]. In the present study, there were no statistically significant differences between the patient groups; lengths ranged from 3.88 to 5.04 mm and diameters from 7.92 to 9.12 mm. There was a statistically significant difference in the depth of the sella between the patients with transposition (7.48 mm) and those with supernumerary teeth (6.42 mm) (p=0.018). The difference between the present findings and the measurements in the literature may be due to the use of different landmarks and levels of radiographic magnification. Some studies have revealed a correlation between the linear dimensions of the Sella and age and growth as factors associated with bridging of the sella [22], whereas others have denied any association [23]. In the present study, the effects of growth on the characteristics of the sella were evaluated by measuring the size, shape, and bridging of the sella on the pre- and postorthodontic cephalometric radiographs of 15 patients. No statistically significant difference (p>0.05) was found in either the pre-treatment or the posttreatment data; however, the correlation between the sella characteristics and age should be analyzed on a larger sample of cephalograms. The shape of the sella turcica is affected by anatomical structures: the pituitary gland, the extent of the anterior and posterior intercavernous venous sinuses [24], and the internal carotid artery [25]. The shape of the sella turcica is also determined genetically [26]. In the present study, the shape of the Sella turcica was classified as oval, round, or flat, in line with the approach taken by Jones et al [13]. Although Jones et al. [20] did not provide any data about the prevalence of the different shapes, other studies have shown the oval shape to be the most common among Nigerian subjects [27]. In the present study, consistent with the literature, the round shape was the most common in all groups, followed by the oval shape and then the flat type. This study was limited by a number of factors, including a lack of gender discrimination between the groups. Some studies have suggested that there is no gender correlation in terms of the linear dimensions of the sella [6,28,29]. In the present study, gender data were taken into account, and thus the ratio of female to male patients in the study groups was balanced. A further limitation is that this study used two-dimensional lateral cephalometric radiographs instead of threedimensional cone beam computerized tomography images, which limited the capacity to determine the characteristics of the sella turcica. Three-dimensional

are no routine diagnostic tools in orthodontics for the dental anomalies under study here.

The clinical relevance of the present study relates to early diagnosis of dental anomalies. The findings may allow clinicians to provide preventive measures for specific patient groups, especially with a diagnosis of transposition and the possibility of supernumerary teeth in the early period.

### 5. CONCLUSION

The findings of the present study indicate that bridging of the sella turcica was more frequent in patients with supernumerary teeth and hypodontia. The sella was deeper in patients with transposition, and in terms of shape, oval and round sellae were the most frequent in all groups. Although the study groups were not divided according to age, the results of the growth evaluation showed no significant change in the dimensions of the sella during craniofacial growth in patients with dental anomalies.

### **CONFLICT OF INTEREST**

The authors declare no conflict of interest.

### **ACKNOWLEDGMENTS**

None.

### **AUTHOR CONTRIBUTIONS**

SA: contributed to the concept and design of the study, the acquisition, analysis and interpretation of the data and drafting the article. NKA: participated by adding her patient records and revising the article critically. All authors read and approved the final manuscript.

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The authors declare that they have not received any funding.

### **COMPETING INTERESTS**

The authors declare that they have no competing interests.

### **ETHICS APPROVAL AND CONSENT TO PARTICIPATE**

A written informed consent containing all the information about records and usage for study was obtained from all patients. All procedures in this study were approved by the Ethical Board of the Istanbul Medipol University (10840098-604) and complied with the Code of Ethics of the World Medical Association (Declaration of Helsinki).

images are more informative and reliable, but they



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# Original Articles

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### CV

Seden Akan graduated from the Hacettepe University, Faculty of Dentistry, Ankara, Turkey in 2003. She enrolled for her PhD degree in 2004 and she was awarded her PhD degree by the Hacettepe University in 2010.

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## Questions

# 1. What is the key point for seeking a relationship between the Sella turcica and dental malocclusions?

- □a. The anterior wall of the Sella turcica and teeth share in common the involvement of neural crest cells;
- □b. Their close neighborhood;
- ☐c. The effect of pituitary gland secretion;
- □d. The shape of the sphenoid bone.

### 2. In the present study, the shapes of the Sella turcica identified in:

- □a. Round;
- □b. Oval;
- □c. Flat;
- □d. Answers a-d are correct.

### 3. Sella turcica bridging is especially seen in the following cases:

- □a. Craniofacial deviations;
- □b. Developmental conditions;
- □c. Skeletal malocclusions;
- □d. Answers a-d are correct.

# 4. According to the present study results, the complete calcification of the Sella was shown in the...

- □a. Transposition group;
- □b. Hypodontia group;
- □c. Supernumerary teeth group;
- □d. Impacted teeth group.

## **EFFECT OF DIGITAL WORKFLOW ON THE MARGINAL FIT** OF LONG-SPAN IMPLANT-SUPPORTED BARS FOR KENNEDY II CLASS REMOVABLE PROSTHESES IN VITRO

Aristeidis Villias¹a\* 👨 , Triantafillos Papadopoulos²b 👨 , Nick Polychronakis¹c 👨 , Hercules Karkazis¹d 🧖 , Gregory Polyzois¹e 👨

### **ABSTRACT**



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Introduction The production procedures, including impressions, introduce errors affecting the passivity of fit. A completely digital workflow is possible nowadays because of the intraoral scanners (IOS). This study aimed to evaluate the effect of the impression technique (conventional versus digital) and the screw tightening sequence on the marginal discrepancy (MD) of implant-supported bars.

Methodology This laboratory study was conducted on a simulated Kennedy class II edentulous maxilla with three parallel implants in the edentulous quartile. The closed tray technique with a-silicon (CTM) and the intraoral scanning with the I-Tero™ system (IOS) were compared and three bars were manufactured from each technique. Depending on the screw tightening sequence (A11 and A17) 4 groups were created with 6 samples each. The MD was examined implementing 24 negative replicas, which were sectioned and studied under a stereomicroscope. The Horizontal Discrepancy (BHD), Vertical Discrepancy (BVD) and Conical Discrepancy (BCD) of the bar were calculated on the means of the measurements of the horizontal, the vertical and the conical MD respectively. The descriptive statistics, normality tests, one-way ANOVA (a=.05) and post-hoc Tukey's tests were run and the graphs were draw with SPSS.

Results There was a significant effect (P<.05) of the impression technique combined with the screw tightening sequence on all variables. The post-hoc Tukey's tests revealed significant differences between all groups except from those of the same impression technique only for the BHD (P<.05).

Conclusion In this study all groups resulted in marginal discrepancies. The closed tray impression technique gave better results.

### **KEYWORDS**

CAD/CAM; Digital Image Analysis; Implant-Supported Bar; Intraoral Scanner; Marginal Fit.

### 1. INTRODUCTION

The preservation of natural teeth is one of the goals of modern dentistry, resulting in a progressively increasing demand for partial dentures [1]. With the propagation of age, replacement of missing teeth is a common patient need [2]. Elderly patients are usually accompanied by general health issues, which also affect the dental treatment plan [3]. Therefore, the suggested dental treatment plans should be realistic, straightforward, versatile, aiming to restore the lost functionality and cover the esthetic needs of the patient. Removable partial dentures, either traditional or implant-supported are prostheses that

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# Original Articles



**Figure 1.** The pseudo-realistic model (PRM). A simulated partially edentulous, Kennedy class II maxilla, including three parallel  $(\pm 1^\circ)$ , standard, internal-hex implant analogs embedded in dental stone under 3-4 mm of simulated mucosa.

meet these requirements. The prosthodontic treatment of a Kennedy class II partially edentulous maxilla is demanding in terms of biomechanics and esthetics. A removable partial denture attached on an implant-supported bar might be an alternative treatment plan to a traditional partial denture [4]. For implant supported prostheses, passive fit is considered an ideal goal, preventing biological and mechanical complication in the future. The clinically accepted marginal fit (MF) might even surpass 200µm [5-7]. Furthermore, the shape of the internal connection implant features might affect the retention and the quality of the connection [8]. Ideally, discrepancies at the margin ought to be kept to a minimum. However, a passive fit and MF with undetectable discrepancies are technically almost impossible to achieve. Additionally, the correlation between the degree of MF and the incidence of clinical implications is yet to be defined. Nonetheless, it is clear that the various clinical and laboratory procedures introduce errors affecting the passivity of the fit.

The MF can be evaluated clinically with digital dental X-Rays, or with direct view if the margin can be directly observed [9]. Assessment of MF with an explorer can be unreliable [7]. Additionally, regarding implant supported prostheses, the explorer tip could scratch the delicate implant surface [10]. In vitro studies have a much wider armamentarium of methods to examine the MF [11]. The horizontal or the vertical marginal discrepancy at the restoration margin is reported in previous studies as an indication of the MF [6]. Destructive and non-destructive methods can be implemented [12-15]. The quality of the margin can be assessed with direct observation or through indirect procedures [11,16,17]. MF has been evaluated with image superimposition methods in combination with digital techniques as well [12,18]. Until recently impressions were only taken with impression materials placed in trays and inserted in the patient mouth. The procedure required a number of expendables, devices, skills and experience [19,20]. Introduction of intraoral scanners



**Figure 2.** The pseudo-realistic model (PRM) with the seated tray placed under a constant axial load of 30N at an axial loading device (ALD) for 12min until the impression material was fully set.

(IOS) for digital impression facilitated the direct digitization of the oral environment without impression materials. Furthermore, the already established implementation of laboratory scanners to digitize the definitive casts affected multiple areas of dentistry including implantology [21].

The main advantages of digital impressions are the capability to immediately evaluate the virtual model chair-side, to evaluate the preparation depth, to modify the virtual model, they are producing less waste and they are time efficient. However, the IOS systems require a large investment and are associated with low quality of evidence when the resulting prostheses are compared for the marginal and internal fit with those from traditional impressions [22]. Furthermore, their accuracy can be affected by several parameters, such as the distance between implants, their inclination, their depth, the lighting conditions and the user experience [23-32]. A fully digital approach in prosthetic dentistry is nowadays possible, given the fact that Computer Aided Design and Computer Added Manufacturing (CAD/CAM) procedures are widely acceptable over dental laboratories [33].

The introduction of IOS has offered a new way to register the implant location. In this direction implant manufacturers have introduced impression copings for digital procedures, the scan posts [34]. These scan posts are components of standard geometry accompanied with their corresponding digital design that facilitates the component recognition from the IOS software or the CAD software [23,34]. However, the implant system might affect the impression accuracy [23].

A challenge regarding edentulous areas is the lack of stable anatomical features on the mucosa that hinder accurate stitching of acquired images from the intraoral scanners [29,35-39]. Several studies have compared the accuracy of intraoral digital systems for crowns and short-span prostheses [20,22]. Scenarios implementing larger prosthesis have been run in simplified plaster models as well [39,40]. Furthermore, the introduction of digital

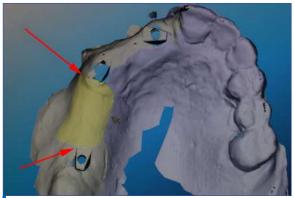


Figure 3. Simultaneous capture (red arrows) of the two most distant consecutive scanning posts attached to the underling implant analogs at positions 14 and 17 by the field of view of the scanner sensor (yellow highlighted area).

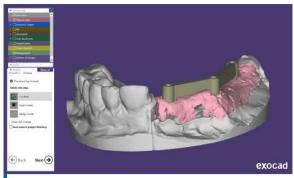


Figure 4. A screen image of a computer aided design (CAD) of a longspan, implant-supported, screw-retained at implant level, parallel bar on the digitized PRM created by an experienced dental technician with the exocad<sup>™</sup> software.



Figure 5. The parallel screw-retained Co-Cr bars manufactured on one plate by a computer aided manufacturing (CAM) - Selective Laser Melting (SLM) technique utilizing a 3D printer seen after the appropriate stress relieving procedure.



Figure 6. A customized Replica Production Device (RPD) utilized for the non-destructive negative replica technique implemented in this study. The RPD with three incorporated parallel implant analogs, identical to those utilized in the PRM, placed at the same relevant positions as the PRM and verified by two interchangeable verification jigs, one produced on the PRM and the other on the RPD.

techniques offer new ways in which the prostheses can be produced. However, there is not enough evidence regarding the resulting fit between implantsupported bars extending over a quadrant that are manufactured through a fully digital workflow and bars manufactured with a partially digital workflow. The purpose of this in vitro study was to compare the effect of a conventional impression technique and a technique implementing an intraoral scanner for digital impression on the marginal fit of implantsupported, long-span, parallel bars, manufactured with laser sintering technique. The effect of the screw tightening sequence was also examined. The null hypothesis was that the marginal fit of implant-supported bars would not be affected by the impression technique or the screw tightening sequence.

### 2. MATERIALS AND METHODS

This laboratory study was conducted on a simulated partially edentulous, Kennedy class II maxilla, which functioned as a pseudo-realistic model (PRM). The illustrated PRM (Fig. 1), included three, parallel (±1°) standard, internal-hex implant analogs (Implant analog Internal Hex. Seven, MIS Implants Technologies Ltd, Lot: W17007917) embedded in

the dental stone. The analogs were located under 3-4mm of simulated mucosa, at the locations of the lost maxillary right central incisor (11), first right premolar (14) and second right molar (17). The simulated teeth were acrylic and the maxillary mucosa was simulated with red pigmented acrylic resin.

### 2.1. Impression techniques

Two impression techniques were compared: the closed tray technique with monophase vinyl polysiloxane impression material (CTM) and the intraoral digital impression technique (IOS).

### 2.1.1. Closed Tray Technique

Initially three impression copings for the closed tray technique (Direct impression coping for closed tray, internal hex, MD-IT300-SP, MIS Implants Technologies Ltd, Dentsply Sirona, York, PA, USA-LOT#: W16002796) were tightened with 10Ncm utilizing a torque ratchet on the implant analogs of the PRM. The fit of the impression copings was verified with digital X-rays (Belmond Phot-X II, Takara Belmont Corp, Osaka, Japan) captured with the parallel technique utilizing a sensor (Schick CDR USB Remote HS, Schick Technologies Inc, NY, USA). Next, the provided plastic rings were firmly seated on the impression copings.

Table 1. Summary of experimental groups.

Table 1. Experimental groups		
Group I	CTM-A11	
Group II	CTM-A17	
Group III	IOS-A11	
Group IV	IOS-A17	

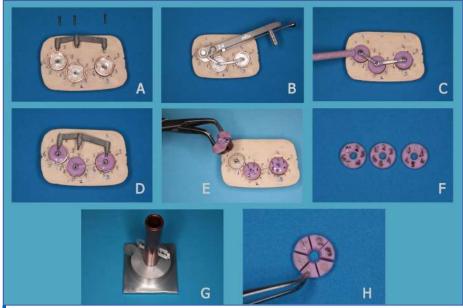


Figure 7. The production of negative replicas with a customized Replica Production Device (RPD) through a non-destructive method: A. A parallel bar before replication on the RPD with the implant analogs placed at the same relevant positions as the PRM. The three specialized copper trays seen in place surrounding the implant analogs. B. The bar seated and secured with three prosthetic screws on the RPD analogs following a preselected screw tightening sequence. C. Three negative replicas made simultaneously for each implant-bar connection from low viscosity addition silicone in the specialized cylindrical copper trays. D. The removed bar after polymerization of the A-silicone. E. The three specialized copper trays carefully detached from the RPD utilizing a modified crown forceps. F. The three removed-from-the-trays, trimmed and marked negative replicas corresponding to each implant-bar connection. G. The sample intersection device (SID) facilitating the standardized sectioning of each cylindrical negative replica in six 60° sectors. H. One sectioned negative replica in six standardized marked slices, corresponding to six oriented observations for each of the three implant-bar connections of an implant-supported bar.

Monophase vinyl polysiloxane (Variotime Dynamix Monophase, Heraeus Kulzer GmbH, Germany, Lot: K010109) from an automix device (Variotime Dynamix Monophase, Heraeus Kulzer GmbH, Germany) was used for the closed tray technique. 6±0.5ml of mixed material was loaded in each of the two syringes (Impression Jet, Heraeus Kulzer GmbH, Hanau, Germany) and the impression copings were covered with the material. Next, 25±1ml of mixed material was loaded on the perforated commercially available metal tray within 14±1s. The tray was appropriately seated on the PRM initially with a finger compression force (15-30N) for 30s. This procedure took place within 2min and 30s, which was the material working time. Next, the PRM with the seated tray was placed under a constant axial load of 30N for 12min at an axial loading device (ALD) until the impression material was fully set (Fig. 2). Finally, the impression was removed from the PRM. Each impression coping was unscrewed from the PRM, placed on a new implant analog, tightened with 10Ncm with the Torque ratchet and the assembly was snapped on the corresponding plastic ring, which was embedded in the set impression material. In this manner three impressions were taken at room

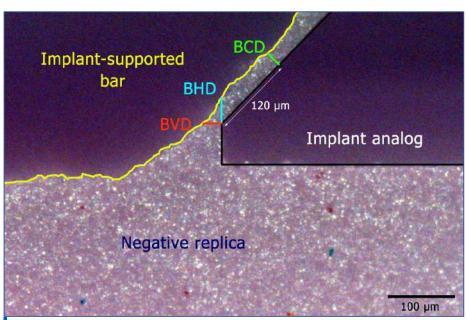
temperature 23±1°C, 50±10% Relative Humidity (RH) with suitable light conditions (1100 Lux, 5500K). After 24h, each impression was used to produce a dental stone model. 48h later, three scan posts were placed on the implant analogs of each model. Then, the three dental stone models were digitized with a laboratory optical scanner (Identica Blue ColLab scan v.2.003, Medit corp, Seoul, Korea) and the Standard Tessellation Language (STL) files were saved with a code name for later use. The PRM was scanned with the same scanner as well.

### 2.1.2. Intra-Oral Scanning

The Intra-Oral Scanner (IOS) I-tero (I-tero Model HDU-E Intra-oral Scanner Optical Impression Device, CADENT® Ltd., Or Yehuda, IL-60212 Israel) was utilized for a direct digitization of the PRM. Three scan posts (Scan Post, int.hex. connection, SP, MIS Implants Technologies Ltd, Dentsply Sirona, York, PA, USA- LOT#: W18002193) were placed on the implant analogs of the PRM. The scan posts on analogs 11-14 and 14-17 were simultaneously captured with the IOS (Fig. 3). Their proper seating was checked with digital X-rays. Next, the PRM was scanned without powdering. The scanning took place in standardized light conditions within a light chamber with artificial

Table 2. Summary of descriptive statistics and normality tests.

Group	Variable	N	Mean	SE	SD	Variance	Skewness	Kurtosis	Shapiro- Wilk (Sig.)
CTM-A11	BHD	6	57.91	4.048	9.915	98.308	.446	-1.452	.656
	BVD	6	186.82	53.411	130.831	17116.719	.110	-2.954	.068
	BCD	6	26.39	2.207	5.406	29.225	611	792	.486
CTM-A17	BHD	6	55.58	4.067	9.962	99.238	034	-3.068	.070
	BVD	6	180.52	51.932	127.208	16181.761	.081	-3.032	.055
	BCD	6	27.72	3.832	9.386	88.099	.982	1.156	.682
IOS-A11	BHD	6	313.59	89.160	218.396	47696.865	428	-2.007	.242
	BVD	6	469.63	119.117	291.777	85133.759	585	-1.895	.111
	BCD	6	423.99	147.872	362.212	131197.519	.121	-1.670	.401
IOS-A17	BHD	6	315.90	89.370	218.912	47922.460	534	-2.049	.180
	BVD	6	484.44	122.869	300.967	90581.294	685	-1.998	.061
	BCD	6	407.97	136.074	333.313	111097.375	099	-1.595	.511



**Figure 8.** Demonstration of the analysis of the acquired digital photomicrograph. The standardized image shows a negative replica slice presenting the marginal fit at a sixth of the periphery of one of the three implant-bar connections of an implant-supported bar of this study. The empty space left by the RPD implant-analog on the right and the implant-supported bar on the left shown as dark areas in the upper part of the photomicrograph. The space surrounding the analog-bar assembly presented as the purple illuminated area. The gap between the connected components presented as a characteristic elongated light purple protrusion towards the dark area of the image. The section of the bar surface traced in yellow and the implant analog surface section traced in black. The dependent variables Bar Horizontal Discrepancy (BHD), Bar Vertical Discrepancy (BVD), Bar Conical Discrepancy (BCD) calculated on the means of the 18 measurements from each bar corresponding to the blue, orange and green lines shown in the figure respectively.

light (~500 lux). Three consecutive scans were taken, each within 20±2 min. Those scans were used to create 3 STL files which were code-named and sent to the lab by e-mail.

### 2.2. Design of parallel screw-retained bars

A CAD of a long-span, implant-supported, screw-retained at implant level, parallel bar on the digitized PRM was created by an experienced dental technician with the exoCAD software (ExoCAD-DentalCAD v6136, 2016. Exocad GmbH, Darmstadt Germany) (Fig. 4).

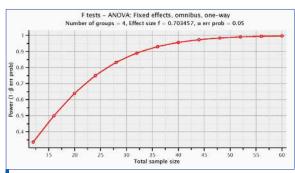
The designed bar was adjusted so that it would best fit the supporting implants at each of the six acquired digital models and a unique code-name was given to each design. In this way six similar designs were created, three for the CTM group and three for the IOS group.

### 2.2.1. Manufacturing of the bars

Six parallel screw-retained bars were manufactured with the CAM - Selective Laser Melting (SLM) technique utilizing a three dimensions (3D) printer certified for dental use (TruPrint 1000 Multilaser, TRUMPF GmbH, Ditzingen, Germany). The bars were produced from a Co-Cr dental alloy powder (Mediloy S-Co™, Bego®, Bremer Goldschlägerei Wilh. Herbst GmbH, Bremen, Germany, LOT# P180709B). The bars were manufactured on one plate with the designated printing sequence, followed by the appropriate stress relieving process (Fig. 5). Finally, the bars were removed from the base plate, supporting pins were trimmed and finishing procedures were applied.

### 2.2.2. Marginal fit assessment

The marginal fit of the bars was evaluated with two methods. Initially each bar was examined radiographically on the PRM, implementing the



**Figure 9.** Power-Sample size plot created with the G\*Power software, indicated for the a-priori calculation of adequate sample size for F-tests for a certain power level given the  $\alpha$ -error probability and the effect size.

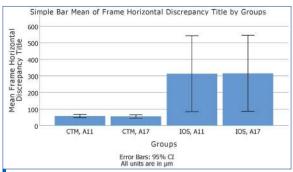
parallel cone technique. The parallel bars were placed on the PRM and the prosthetic screws were tightened in the sequence 11,17,14 with a standardized minimal torque 10Ncm.

Next, the MF was measured through a non-destructive method implementing the negative replica (NR) technique and a modification of the digital image analysis sequence (DIAS), which was previously described and validated [15]. The parallel bars were seated directly on the three implant analogs and secured with the designated prosthetic screws. For the production of NR, a customized Replica Production Device (RPD) was utilized. The RPD incorporated three parallel implant analogs identical to those utilized in the PRM. The analogs were placed at the same relevant positions as the PRM, verified by two interchangeable verification jigs, one produced on the PRM and one on the RPD (Fig. 6).

The RPD facilitated the simultaneous production of three NRs, one for each implant-bar connection. The NRs were made from light viscosity addition silicone (Image PVS Super light body fast, Dental Line Ltd, Piraeus, Greece) in specialized cylindrical copper trays. After polymerization of the A-silicon, the bar was removed and the three specialized copper trays were detached from the RPD. The NRs were removed from the trays, trimmed, marked and sectioned in a sample intersection device (SID). SID allowed the reproducible section of the cylindrical NR in six 60° sectors (Fig. 7). One NR was created for each of the three implant-bar connections and the aforementioned procedure facilitated the assessment of the MF at 18 points on every bar.

### 2.3. Digital Image Acquisition and Analysis

Following a standardized procedure the NR slices were examined under an optical microscope (Digital Microscope Leica DM 4000 B, Leica Microsystems, Mannheim, Germany) with a mounted camera at 320x final magnification. Photomicrographs were taken and stored in an external hard disc. The microscope settings were adjusted for maximum field of view, minimal depth of field and highest resolution for the selected magnification. External additional lighting



**Figure 10.** Bar charts of the mean BHD in  $\mu$ m for every experimental group separately. The confidence interval i-bars are drawn in black lines. All differences are statistically significant (p<0.05) except from those between groups of the same impression technique.

sources were utilized, increasing the illumination at the stage at a mean value of 57000 lux. In the acquired images one pixel corresponded to 0.556µm.

A DIAS was applied for the analysis of the captured images [15]. Each image showed the marginal fit at a sixth of the periphery of every implant-bar connection. The dark area in the photomicrograph of the NR slice corresponded to the empty space left by the analog and the bar assembly and the purple illuminated area corresponded to the space surrounding the assembly. If there was a gap between the connected components, it was presented as a characteristic elongated light purple protrusion towards the dark area of the image (Fig. 8). The image processing software Photoshop (Adobe Photoshop CS 5 V12.0.4 x64, Adobe Systems Inc.) was utilized for each image. The outline of the bar was traced and highlighted utilizing a sensitive digitizer (Bamboo CTH-470/S, Wacom, Toyonodai, Japan). Furthermore, the implant analog outline was superimposed at the best perceived fit position on the acquired image. One-pixel thick lines were drawn and the processed images saved without compression.

The standard geometry of the implant analogs in this experiment facilitated the a-priori drawing of the component outline. The analog outline included a marking point at the conical part of the component and two-line extensions from the inner edge of the shallow platform at the margin of the analog. These additions would aid the measurements on a next step.

### 2.3.1. Measurements

The marginal fit was evaluated on each photomicrograph through three dependent variables: Horizontal discrepancy (HD), Vertical discrepancy (VD) and Conical discrepancy (CD), measured in µm. VD and HD were defined as the length of the line segment from the internal edge of the shallow platform of the utilized implant analog extending perpendicularly or in parallel towards the outline of the bar, respectively. The conical discrepancy was geometrically defined at the conical part of the internal hexagon of the utilized implants. Following a standardized procedure, measurements were taken

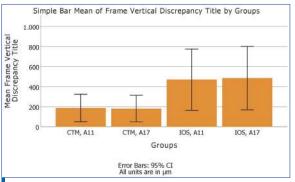


Figure 11. Bar charts of the mean BVD in μm for every experimental group separately. The confidence interval i-bars are drawn in black lines. None of the differences is statistically significant (ns).

with the open-source software ImageJ (ImageJ 1.52p, National Institutes of Health, Bethesda, MD, 20892 USA) on the processed image. The measurements were automatically stored, transferred and organized for statistical analysis utilizing programs. The overall marginal fit of each bar was quantitatively evaluated with 3 indices: Bar Horizontal Discrepancy (BHD), Bar Vertical Discrepancy (BVD), Bar Conical Discrepancy (BCD). These were calculated on the means of the measurements of the HD, VD and CD respectively. 2.3.2. Sample size

The marginal fit of the six bars (3 from the CTM and 3 from IOS) was also evaluated depending on the screw tightening sequence, forming two subgroups: Group A11 and Group A17. In group A11 the first tightened screw was at the most proximal implant analog (11), followed by the one at the most distal implant analog (17) and finally at the one in the middle (14). In group A17 the screws were tightened on the sequence 17, 11, 14. Deriving from the combination of the factors, impression technique and first tightened screw, 4 groups were formed and compared in this experiment. The NR technique was run twice for the samples in these groups, doubling the data. One researcher ran the experiment. The groups are summarized in Table 1.

### 2.3.3. Statistical analysis

The data were statistically analyzed and graphs were drawn with the SPSS software (SPSS for Windows 64-bit edition, V25. IBM Corp.). Descriptive statistics were computed. The normality of the data was examined by Shapiro-Wilk tests, Q-Q plots and corresponding histograms. A one-way Analysis of Variance (ANOVA) was conducted for each of the depended variables and Tukey HSD post hoc tests were utilized to locate the differences ( $\alpha$ =0.05). The effect sizes were computed and a power analysis was conducted post hoc with the open-source software GPower [41,42].

### 3. RESULTS

Four groups with 24 samples each were formed. 432 digital images were analyzed in total and 1296 measurements were taken from those images.

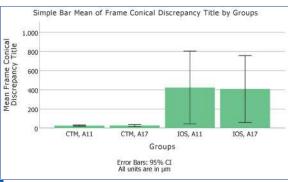


Figure 12. Bar charts of the mean BCD in μm for every experimental group separately. The confidence interval i-bars are drawn in black lines. None of the differences is statistically significant (ns).

The descriptive statistics per group are summarized in Table 2 together with the results of the Shapiro-Wilk test of normality for relatively small sample sizes. The latter examined if the distribution of data differed significantly from the normal distribution. No significant differences were found (P>.05ns), hence parametric tests were run.

The one-way ANOVA showed a statistically significant difference between groups for the BHD (F(3,20)=5,558, P<,01,  $\omega$ =,60,  $\eta^2$ =0,45, Power (1- $\beta$  err prob)=94%). A significant difference was also found between groups for the BVD (F(3,20)=3,299, P<,05,  $\omega$ =,15,  $\eta^2$ =0.33, Power (1- $\beta$  err prob)=75%), and between groups for the BCD differences were also statistically significant (F(3,20)=4,996, P<,05,  $\omega$ =,58,  $\eta^2$ =0.43, Power (1- $\beta$  err prob)=91%). The Sample Size -Power analysis diagram regarding the variable BVD is presented (Fig. 9).

Tukey's post hoc tests were run to reveal the differences by comparing the means of the different groups. The results of these tests are briefly summarized next. BHD was significantly different between groups [CTM-A11] (M= 57,91, SE=4.05)–[IOS-A11] (M=313,59, SE=89,16) (P<.05), [CTM-A11] (M=57,91, SE=4.05)–[IOS-A17] (M=315,90, SE=89,37) (P<.05), [CTM-A17] (M=55,58, SE=4.07)–[IOS-A11] (M=313,59, SE=89,16) (P<.05) and [CTM-A17] (M=55,58, SE=4.07)-[IOS-A17] (M=315,90, SE=89,37) (P<.05).

The implant-supported bars produced after a closed tray technique with Monophase PVS impression material either with their most mesial screw tightened first or the most distal one had a mean horizontal discrepancy between the bar and the implant, which was significantly smaller from that of bars produced after an intraoral scan independedly of the screw tightening sequence.

The post hoc tests could not detect the significant differences for the BVD vertical discrepancies at the marginal fit (P>.05). Furthermore, the post hoc tests could not reveal the significant differences between groups for the variable BCD. None the less, the [CTM-A11] (M=26,39, SE=2,21)– [IOS-A11] (M=423,99, SE=147,872) comparison of BCD means

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although not significant (ns) had P=0.50 (ns) and the [CTM-A17] (M=27.72, SE=3,83) – [IOS-A11] (M=423,99, SE=147,872) comparison had P=0.51 (ns). These results were graphically drawn as bar charts with the SPSS software. The mean BHD, BVD and BCD were drawn as bar charts with the 95% Confidence interval shown for every group (Fig. 10, Fig. 11, Fig. 12).

### 3.1. Radiological results

The analysis of the digital X-Rays taken directly at the PRM facilitated vertical and horizontal discrepancy measurements at the mesial and distal sides of every implant-bar connection. The mean values of these measurements CTM HD=67 $\mu$ m, IOS HD=322 $\mu$ m and CTM VD=67 $\mu$ m, IOS VD=689 $\mu$ m are comparable with the BHD and BVD of the [CTM-A11] and [IOS-A11] groups, shown in the descriptive statistics table.

### 4. DISCUSSION

This study compared the marginal fit of implantsupported, long span, parallel bars, produced after a fully digital versus a partially digital workflow. Within the limitation of this study the null hypothesis that there will be no differences among groups in all three marginal fit indices was rejected. Additionally, within the limitations of this study, the partially digital workflow combining conventional impressions resulted in prostheses with better marginal fit. The fully digital workflow implementing an intraoral scanning system for the direct digitization of the maxillar esultedin implant-supported bars that exhibited marginal discrepancies, which could not be considered as clinically acceptable. Digital impression is a key step in the outcome of a completely digital workflow. Complete arch scanning is associated with larger deviations as compared to partial arch scanning [22-24]. It has been found that the first scanned quadrant is recorded more accurately in comparison to the one that follows [27]. This laboratory study included complete arch scanning of a simulated partially edentulous maxilla under controlled lighting conditions. It has been found that artificial lighting can affect the scanning procedure [25]. The scanning initiated from the dentate quadrant, which provided orientation points for the stitching of acquired data. The quadrant with the implants was scanned later. Therefore, both deteriorating factors that have been associated with lower accuracy have been combined in this study, which could explain the unacceptable outcomes of the completely digital workflow. It has been found that by increasing the distance between implants the intraoral scanning precision is decreased [29]. Flügge et al. found that the implant system might also affect the impression accuracy [23]. The MIS°-Seven<sup>™</sup> implant system, however, was accompanied with scan-bodies with non-reflecting surface and high enough to notably protrude from the mucosa even when the implants were placed

relatively deep. Additionally, the scan bodies had flattened shape and elongated at the proximal-distal dimension, which facilitated the partial capture in a single frame with the IOS wand of the two most distant successive implant analogs 14 and 17 at the PRM. Nevertheless, the marginal discrepancies at the IOS group were not acceptable. Probably there might not have been enough orientation points to accurately stitch the frames on the edentulous area of the PRM.

Huang et al. found in their study that when a scan body includes an extensional structure, the scanning accuracy is significantly improved [35]. Although several methods are applied for the assessment of the margin, in this study the NR technique was selected [11,17]. The MF could be assessed with direct in-situ observation as well [18]. However, direct observation relies only on one-dimensional measurements at the observed gap between examined components. Alternatively, samples could be embedded in resin material and following a destructive method, their sections could be examined under a microscope [11]. Nevertheless, such a technique would have included several steps and in case an electron microscope would have been utilized, it would have been also a time-consuming approach and costs would have been increased as well [16]. In a previous study DIAS, a recently developed and reliable stepwise procedure, has been implemented for the assessment of the crown margin on cemented implant-supported crowns [15]. Because of its non-destructive approach, the NR technique could be applied in clinical studies, given appropriate modifications are introduced and in ex-vivo studies. It is a feasible tool for the quality assessment at the numerous stages of the prostheses production [13,14]. The strict and clear criteria applied in this study for the evaluation of MF, minimized observer subjectivity, facilitated reliable measurements and eliminated loss of data.

In this study, the VD found for the CTM group were smaller as compared to the IOS group. This trend was also reported by Lo Russo et al [6]. The implementation of the NR technique in this study also facilitated the evaluation of HD. Such measurements are indicative of the quality of the produced prosthesis. In this study the parameters BHD and BCD were assessed as overall indication of the mean HD and the mean VD at the restoration margin respectively. The shape of the internal connection implants like the ones that were implemented in this study presents complex features like conical internal walls. Such features are common in newer designs with steeper conical walls featuring Morse taper connections. Such features might affect the retention and the quality of the mechanical connection [8]. Therefore, an additional parameter was evaluated in this study, the BCD, as an evaluation of the near margin quality of the connection at the conical part of the internal connection implants that were used. BCD was the



mean discrepancy between the implant analog and the prosthesis observed  $120\mu m$  within the inclined internal walls of the implant analog.

There is no consensus about the clinically acceptable marginal fit. However, most studies report a range between 50-200µm. In this study the groups that included the parallel bars that were manufactured after a traditional impression with the closed tray technique presented discrepancies within that range. The observed discrepancies might be overestimated since the prosthesis was fixed on the implant analogs with prosthetic screws tightened with a minimal torque (10Ncm). An increased torque on the other hand might also amplify the tension and therefore compromise the passivity of fit. In this study, the marginal fit of the implant-supported bar produced after an impression with the traditional closed tray technique with VPS impression material was comparable with the results found by Lin WS et al [30]. In this study the marginal fit of bars created after an intraoral scanning was not acceptable, exceeding 1000µm gaps in some cases. Andersen FS et al., in their study using the iTero system for implant supported mandible complete dentures, found similarly unacceptable marginal fit levels [36]. Their explanation for those results was the lack of anatomical structures which could facilitate the orientation of the images by the scanner software. Earlier, Patzelt SB et al. found comparable results regarding intraoral scanners used in edentulous patients, concluding that such systems should be avoided for similar cases [37]. On the contrary Kim SY et al. found the iTero system had similar accuracy as the impression with addition silicone [38]. In their study however they utilized a plaster model of a partially edentulous case, which facilitated the orientation of the images by the scanner software. Keul et al. (2020) found that the iTero IOS had comparable results as the traditional impression technique. However, as compared to this study they utilized a more recent model with updated software [39]. It seems that differences in the connection type in combination with the implant inclination might affect the accuracy of the impressions especially for the internal connection type. That might be explained by the increased contact surface of the impression coping with the implant making the removal of the impression harder after the polymerization of the impression material. Additionally, the number and the relative positions of implants in the mesial-distal orientation might also affect the removal of the impression after setting [20]. The intraoral scanning systems seem to have adequate accuracy for single crowns and short bridges [34,40]. The complete digital workflow for constructing implant supported prostheses might raise additional limitations. In this study, it was found that the digital libraries provided by MIS only included antirotation design for the implant-level connection abutments. They suggest the use of prefabricated components without

antirotation design, which would later be connected with the rest of the metallic frame. The antirotation connection type might also be a reason for the unacceptable marginal gaps encountered in the intraoral scanner groups in this study. Furthermore, the older software version of the utilized scanning system might also be a reason.

In this study, the effect of the impression technique on the marginal fit of implant-supported parallel bars was evaluated at implant level. The field of view of the scanner sensor facilitated the simultaneous capture of two consecutive scanning posts attached to the three implant analogs in that region. The posts at positions 11 and 14 were simultaneously covered in a larger part of their surface than the posts at positions 14 and 17. This might have contributed to the insertion of errors during scanning, due to lack of adequate orientation points. Flügge TV et al, in their study, measured the distances and the inclinations of scan posts on the digital models and underlined the negative correlation between accuracy of intraoral scanning systems and the distance between scan posts [29]. In this study, although the PRM simulated the color of the tissues of the oral cavity and it was accordingly polished, clinically saliva, blood and humidity might be present, posing additional challenges for an accurate scan. Additionally, the scanner wand was bulky which probably poses an extra limitation for accessing distal areas clinically. Moreover, in this study implant analogs were placed parallel to each other, which might have favored the results. On the contrary, Gracis S et al. and Lee HJ et al., found that inclined implants could hinder an accurate scan [31,32]. Additionally, in this study implant analogs were used and not actual implants. No data could be found for the accuracy of these components. Furthermore, the analogs were located under 3-4mm of simulated mucosa, which is relatively deep. This might have a negative effect on the accuracy of the impression as well as the intraoral scan [28]. Especially for the latter, the scan posts were deeply submerged under the mucosa obscuring a large portion of their surface. Hence, there were less points of orientation available for the software to recognize for an accurate placement of the implant in the CAD software.

The bars in this experiment were manufactured with a 3D printing technique using Co-Cr alloy powder. Although the SLM frameworks are produced with acceptable accuracy, the surface of the components is rough. Such quality is not favorable when the components are intended for precision connections such as telescopic crowns and bars. Additionally, the coarse surface posed a challenge for the edge recognition on the negative replica. The marginal fit was examined in this study with two techniques. One was a direct technique, applicable clinically, by taking digital radiographs with the parallel cone technique of the analog-bar assembly. Yet, the radiographs revealed the quality of the implant-bar

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connection was inadequate for performing accurate measurements, due to a relatively low resolution. Furthermore, only two points at the margin could be examined, one distally and one mesially of every analog-prosthesis connection. Wahle et al. concluded that the marginal fit is not adequately evaluated only with radiographs [9]. The other technique was the negative replica technique in combination with a DIAS [15].

### 4.1. Clinical relevance

The intraoral scanning systems have undoubtedly advantages; however, regarding the implementation of such systems in implant dentistry, one should proceed with caution as not acceptable fit levels might result.

Furthermore, the availability of ready-to-use digital designs of components needs to be assessed in advance since proprietary rights might impose limitations leading to different treatment approaches.

### 5. CONCLUSION

The implementation of the negative replica technique in combination with the modified DIAS was a viable, non-destructive method to simultaneously assess the horizontal, vertical and conical fit of the implant-supported bars on three implants in this study.

Within the limitations of this study, it was concluded that all the examined combinations of impression techniques and screw tightening sequences resulted in marginal discrepancies, detectable with the applied method. The simulated intraoral scanning with the iTero system in the setup of the present study resulted in unacceptable marginal gaps, visualized on replica segments under the optical microscope. In this study, the closed tray impression technique with addition silicon resulted in better marginal fit levels, when examined with the applied method, while the screw tightening sequence does not seem to affect the prosthesis adaptation on the implants.

### **CONFLICT OF INTEREST**

The authors declare no conflict of interest.

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### **AUTHOR CONTRIBUTIONS**

TP and AV: the conception and design of the study. AV: acquisition of data. AV, TP, NP, HK and GP: analysis and interpretation of data. AV, NP, HK: drafting the article. TP, HK and GP: revising it critically for important intellectual content. AV, TP, NP, HK and GP: final approval of the version to be submitted.

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# Original Articles

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### CV

Aristeidis Villias graduated from the School of Dentistry of the National and Kapodistrian University of Athens, Greece in 2008. In 2015 he obtained his doctorate degree from the University of Cologne, Cologne (NRW), Germany after a scholarship for postgraduate studies abroad issued (2010) by the State Scholarships Foundation of the Hellenic Republic (IKY). In 2015 he also started his private practice in Piraeus, Greece. In 2019 he obtained his master's degree in Dental Biomaterials from the School of Dentistry of the National and Kapodistrian University of Athens, Greece. Since 2020 he has been Clinical Instructor in Removable Prosthodontics, School of Dentistry, National and Kapodistrian University of Athens, Greece and in the Section of Dental Technology, Department of Biomedical Sciences School of Health and Care Sciences, University of West Attica, Athens, Greece.

### Questions

# 1. According to the article, the implant-supported bars manufactured after the traditional impression technique as compared to those manufactured after implementation of the intraoral scanner:

- □a. Have significantly better horizontal discrepancy at the implant-bar interface;
- □b. Present undetectable discrepancies at the implant-bar interface;
- □c. Have inferior marginal fit at the implant-bar interface;
- □d. Present marginal discrepancies which are not clinically acceptable.

## 2. The screw tightening sequence when seating the implant supported bar in this study seems to:

- □a. Have no effect on the marginal fit of the bar at implant level;
- □b. Affect significantly the marginal fit of the bar at implant level;
- □c. Have a significant effect when the most mesial screw is tightened first;
- □d. Have a significant effect when the most distal screw is tightened first.

### 3. In this study the marginal fit of the computer aided manufactured bars

- ☐a. Was better when a fully digital workflow was followed;
- □b. Was worse when a fully digital workflow was followed;
- □c. Was similar either with a fully digital workflow or with a partially digital workflow;
- □d. Was worse when a partially digital workflow was followed.

### 4. The computer aided design of implant supported bars

- □a. Was versatile allowing smooth implementation of the conceived design;
- □b. Was versatile allowing implementation of the conceived design after certain auxiliaries had been purchased;
- □c. Was limited by proprietary design concepts;
- □d. Was not applicable in this study.

## A STANDARDIZED METHOD TO DETERMINE THE PROPER **WORKING DISTANCE FOR DENTAL MAGNIFICATION** UTILIZING NEUTRAL ERGONOMICS POSITIONING

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### **ABSTRACT**



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Introduction Evaluate and compare the relationship between anatomic measurements of the dental clinician in a neutral ergonomics position with the proper working distance and test the hypothesis that working distance can be estimated using specific anatomical measurements of the operator.

Materials and Methods Specific measurements were obtained from 134 participants utilizing a neutral ergonomics position. Values were applied using the Pythagorean theorem to calculate a hypothesized working distance (HWD) from lateral epicanthus (E) to antecubital fossa (F) and antecubital fossa to the tip of the thumb (T). The actual working distance (ET) was measured from lateral epicanthus to tip of the thumb in a neutral ergonomics position in a simulated setting.

Results The results showed a significant positive correlation between (ET) and (HWD) and a positive correlation for all of the anatomic measurements taken in simulation: ET to height and HWD to height; (EF) and ET, EF and HWD; antecubital fossa to the thumb (FT) and ET, FT and HWD (p<0.00). There was a marginally significant difference when comparing vision types, corrective and non- corrective (p<0.058), with noncorrective vision having a higher actual working distance. There was a statistical difference when comparing gender and HWD with male participants ranking higher hypothesized and actual working distance.

**Conclusion** When applying the Pythagorean formula using anatomic landmark measurements, the HWD is repeatable for most operators and may contribute to a more standardized method to measure the accurate working distance that fits the ergonomics.

### **KEYWORDS**

Ergonomics; Dental Education; Musculoskeletal Disorders; Dentist; Dental Hygienists.

### 1. INTRODUCTION

Proper ergonomics in dentistry have been viewed as contributory aspects in the prevention of musculoskeletal injury and the working distance with magnification should allow the operator to maintain optimum posture [1]. Many dental students

and clinicians may not be aware of what constitutes the correct ergonomics posture. The importance of an early introduction to proper ergonomics may be beneficial to a clinician's career, for both comfort and longevity. The cause of musculoskeletal disorders (MSD) is multifactorial and can develop from as little as a single event, or more likely through repetitive

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**Figure 1.** Clinical position based on Module 1 Section 3 of *Fundamentals of periodontal instrumentation and advanced root instrumentation.* 8th edition revised by Gehrig JS, Sroda R, Saccuzzo D. Philadelphia, PA: Wolters Kluwer; 2019.

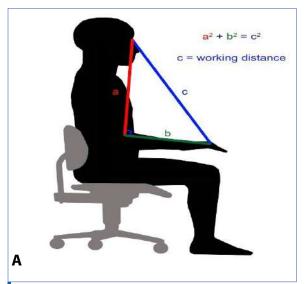




Figure 2. The theorem application: a) The Pythagorean theorem is the formula for calculating the length of one side of a right-angled triangle (c) when the length of the other two sides is known:  $a^2 + b^2 = c^2$ . In this formula, c represents the hypothesized working distance, a represents the distance from "Eye to Elbow" and b represents the distance from Elbow to Tooth; b) Investigational theorem application performed on the researcher.

occupational movements over time. However, risks for the development of MSD have been identified among dental students with reports of pain in the back and neck regions during training [2]. In an earlier study on the prevalence of body pain in a dental student population, 46-71% of students reported pain with the percentage increasing with the increase of years in school [3]. The prevalence of musculoskeletal disorders in the dentist's necks, backs, shoulders, and arms were higher than other areas of the body; intervention with magnification helped to improve the working conditions reducing discomfort for the clinicians [4]. Those who reported the regular use of some type of magnification were significantly less likely to report MSDs compared to those who used magnification occasionally or never [5]. There is a general acceptance of the use of magnification by dental students and practitioners and it is widely perceived as an adjunct, if not a standard, for dental care in the profession. Recommendations by The

Council on Dental Practice's (CDP) Dental Wellness Advisory Committee (DWAC), in conjunction with the ADA Health Policy Institute, stress proper diligence when choosing loupes and the importance of the magnification level, working length, field of view, and the angle of declination in order to maintain good head and neck posture [6]. Measurements are individualized and unique to the clinician.

Magnification can allow the clinician to better visualize the oral cavity and to reinforce the maintenance of neutral ergonomics. The use of dental magnification in dentistry is widely utilized and continued efforts to support its role in maintaining work posture remains an area of study. Employing magnification loupes has led to a reduction in the intensity of discomfort felt by dentists in areas of the "neck, shoulders and arms, back, elbow, forearm and the whole body" [4]. Magnification increases a clinician's visual acuity within the oral cavity and allows the clinician to see detail without compensating with improper bending or positioning. If magnification is to be considered



### Table 1. Data Collection Criteria.

Participant's Age:					
Participant's Gender:	Male	Male Female		Do not wish to answer	
Participant's Educational Status:	D1	D2	D3	Faculty	
Handedness	Right		Left		
Standing Height (inch)					
Do you wear glasses/contacts? Lasik or related procedure?	Yes		No		
EF lateral epicanthus of the eye to mid-elbow fold (inch)					
FT The mid-elbow fold of the cubital fossa to the tip of thumb and or to occlusal surface #19 or #30 (inch)					
<b>ET</b> Working distance without loupes lateral epicanthus eye to typodont mandibular molar (inch)					

Table 2. Correlation significance test.

Pearson Correlation	r	Sig. (2-tailed)	statistic
ET vs HWD	0.878	0.000	Significant
ET vs Height	0 .741	0.000	Significant
HWD vs Height	0.847	0.000	Significant
EF vs ET	0.854	0.000	Significant
EF vs HWD	0.956	0.000	Significant
FT vs ET	0.732	0.000	Significant
FT vs HWD	0.862	0.000	Significant

<sup>\*</sup>Correlation is significant at the 0.01 level (2-tailed).

an element in maintaining proper ergonomics, the ideal working distance with magnification cannot be ignored. The necessary working distance needed with magnification was credited with contributing to the positive effect and improvement among dental students by reinforcing the maintenance of proper ergonomics for the head, neck, and trunk [7].

As only one adjunct to achieving this goal, dental magnification has its many intricacies within itself. Proper alignment of declination angle and working distance can be viewed as essential in affirming this contribution. Working distance measurements may be more variable, depending on how the measurements are taken and obtained. Although the ideal working distance needed can fall within a range dependent on the magnitude of magnification (ie: 2.5x, 3.0x, 3.5x.etc), the working distance measurement/ focus point in this range may vary. A standard to obtain the most optimal measurement to help minimize the variation may be applicable and helpful in determining an ideal working distance specific for an individual.

The working distance is measured from the clinician's eyes to the operating site. It is of critical importance

since it reflects an individual length impacted by an operator's characteristics and the ability to maintain proper ergonomics while working. Maintaining proper ergonomics can be found in the curriculum of dental education as provided in a guide to neutral ergonomics [8]. Improper measurements may allow for awkward static positions and forward bending. Studies have indicated that participants expressed that loupes resulted in better vision, more comfort, a positive change in work posture [7], and improved working conditions that facilitated their work [4].

Of the current research available, emphasis on achieving the proper individualized working distance is scarce and further research is warranted to determine how an individual's characteristics may influence the necessary working distance for proper ergonomics positions in the selection of magnification loupes.

The following null hypotheses were tested:

- 1) There is a direct relationship between the specific anatomical measurements of a dental operator while in neutral ergonomics position and a mathematical determination of proper working distance which can be obtained
- 2) No difference in working distance between the corrective and non-corrective vision groups, and
- 3) No difference in working distance between genders.

### 2. MATERIALS AND METHODS

Participants were recruited through LECOM School of Dental Medicine by email invitation after getting the IRB approval for the study protocol. All D1, D2, and D3 students were asked to participate in the study. In addition, the dental faculty were invited to participate. Invitation to participate was not extended to the D4 students since their curriculum places them in outreach clinics of distant locations.

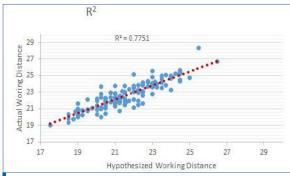


Figure 3. Strong linear correlation between HWD and ET. Distance in inches.

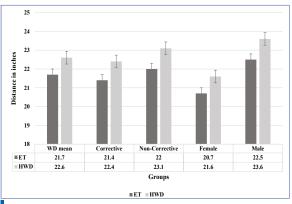


Figure 4. Group descriptive statistics.

contacts, glasses, or has had any corrected vision operation. The measurements were collected with a right-angle square and measuring tape in inches which can measure up to one eighth of the inch. Following demographic data collection, the participant was seated at a simulation station and instructed to assume the 9 o'clock position for righthanded participants and 3 o'clock for left-handed participants. The participant was given safety goggles to wear for the duration of the experimental session. The participant was asked by the student investigator and principal investigator to adjust the mannequin's head to be at waist level. The participant was instructed into neutral ergonomics based on Module 1 Section 3 of Fundamentals of periodontal instrumentation and advanced root instrumentation. 8th edition revised by Gehrig JS, Sroda R, Saccuzzo D. Philadelphia, PA: Wolters Kluwer; 2019. (Fig. 1).

- Step 1: Place buttock back in the chair with even distribution on the seat.
- Step 2: Adjust chair height so that feet rest flat on the floor. Spread feet to shoulders width apart and directly in front of the hips.
- Step 3: Tilt the seat until the back edge is one inch higher than the front edge or utilize a wedge-shaped ergonomics cushion.
- Step 4: Adjust the lumbar rest so the lower back is supported when the buttock is back in the chair.
- Step 5: Adjust lumbar rest so that the height supports your natural curve.
- Step 6: Raise the tail bone to establish correct spinal curvature.

- Step 7: Stabilize the lower back by pulling in the stomach muscles to the spine.
- Step 8: Relax shoulders down and back.
- Step 9: Position arms along the long axis of the torso and hold elbows near the body.

Student investigators and the principal investigator observed and verbally instructed changes in ergonomics keeping in mind the following recommendations:

- Head tilt of 0 to 20 degrees
- Trunk flexion of 0 to 20 degrees
- Torso in line with the long axis of the body
- Shoulders in a horizontal line
- Elbows at waist level held slightly away from the body, no greater than 20 degrees from the body
- Forearm position held parallel to the floor, the angle between 100 degrees and 60 degrees
- Wrist aligned with a forearm; little finger-side of the palm is slightly lower than the thumb side of the palm.

Once neutral ergonomics were achieved and confirmed by the investigators, the participant was asked to maintain this position while focusing on the occlusal surface of tooth 46 within the mannequin for right-handed participants and the occlusal surface of 36 for left-handed participants. For every experimental session, a minimum of three investigators were present to assist in measuring and recording the data. Three measurements were recorded:

- 1) EF: From the lateral epicanthus of the eye to the mid-elbow fold of the cubital fossa.
- 2) FT: From the mid-elbow fold of the cubital fossa to the tip of the thumb and or to the occlusal surface of #46 for right-handed participants and #36 for left-handed participants.
- 3) ET: From the lateral epicanthus of the eye to the typodont lower molar. The right-angle square was used to help maintain an angle consistent with our proposed hypotheses in the equation and record measurements from EF and FT. A measuring tape was used to measure the observed working distance while the participant maintained an ergonomics position. The investigator notified the participant when the working distance measurement was initiated and completed. A student investigator recorded the participant's demographics on a password-protected computer within a password protected flash drive.

Using the Pythagorean theorem (Fig. 2) EF measurements were used to represent (a), FT measurements were used to represent (b), and (c) was solved as our HWD in the formula and recorded.

## 2.1. The participant was informed of the following risks:

According to the Institutional Review Board (IRB) protocol, the participant was asked to maintain a neutral ergonomics position for approximately up to five minutes. The participant was informed that they may experience minimal muscle soreness during



or after the measurement session. To minimize this risk the participant was informed of their right to stop the session at any time. We asked participants to verbally announce muscle soreness during the session to both student researchers and verify that the position we measured from was comfortable. If the participant could not continue the session the previously recorded information would be discarded. The ruler edges were protected with a plastic tube to protect each participant and the student researchers. The ruler, measuring tape, and the participant's eyewear was disinfected with CaviWipes (Metrex™ Research, Orange, CA, USA) surface disinfectant premoistened wipe before and after each use.

### 2.2. Analysis

Both demographic and recorded measurements were analyzed to determine statistical significance based on individual characteristics with the application of anatomic measurements in neutral ergonomics and working distance. Levene's Test for Equality, Variances and t-test for equality of means was used for this study with Pearson's correlation sig. (2-tailed).

### 3. RESULTS

Levene's Test for Equality showed a significant difference when sig. (2-tailed) is ≤0.05. There was a marginally significant difference in vision type by "ET" to the vision; where the non-corrective vision had higher "ET" values compared to corrective vision values Sig.(2-tailed)= 0.058.

Significant differences were found (Sig. (2-tailed) =0.022) when comparing "HWD" between the corrective and non-corrective vision, where the non-corrective vision had higher HWD.

There was a highly significant difference in "ET" values by gender with males having significantly higher "ET" values than females. Sig.(2-tailed)=0.000 There was a highly significant difference in "HWD" by gender with males having a significantly higher "Hypothesized Working Distance" than females. Sig.(2-tailed)=0.000. There was a strong correlation between HWD "Hypothesized Working Distance" and ET "Actual Working Distance" (Linear regression= 0.775) see Fig. 3.

### 4. DISCUSSION

Our study involved obtaining measurements of subjects in anatomic position with proper ergonomics and using those values in the Pythagorean theorem to hypothesize an individual's working distance as the hypotenuse. The primary purpose of this investigation was to analyze the relationship between measurements in an anatomic ergonomics position and the individual working distance using the geometric principles of the right triangle (Fig. 2). The core of the objectives centers on neutral

ergonomics and the use of magnification. Our study does not attempt to explain the comparative effectiveness of different styles of magnification [9], but to provide an emphasis on the proper working distance in conjunction with magnification. Neutral ergonomics is described as having the shoulders parallel with the floor, elbows close to the sides, and the patient's mouth at elbow height of the clinician [8]. This picture of neutral ergonomics as viewed in (Fig. 1) is observed to follow the geometry of a right triangle with the working distance projecting to be the hypotenuse. Our investigation aimed to place a participant in a simulated position in neutral ergonomics prior to measuring working distance. This was based on the support of studies that attribute MSD to improper ergonomics positioning and allowed us to view a working distance that would be most conducive to a participant in neutral ergonomics [1]. While in this position, the working distance was visualized and measured directly. The results of our study provided data to show that our hypothesized measurement derived indirectly from measurements of anatomical relationships had a significant positive correlation with our observed actual working distance measurement in the same position. Within that anatomic measurement, (ET) and (HWD) were also both positively correlated to an individual's height. When viewed separately each component of our equation EF, ET, and HWD also individually showed a significant positive correlation between itself and the observed actual working distance. Our results showed a significant difference in Eye to Tooth (ET) measurements by gender with males having significantly higher ET measurements than females. This appears to be explained by the higher average ET measurement in males of 22.49 inches versus females with 20.73 inches. Similarly, there was a significant difference in the Hypothesized Working Distance (HWD) with males having a significantly higher HWD than females. Again, the average measurements for males was slightly higher at 23.61 inches compared to females at 21.62 inches. The results also showed a positive correlation of HWD and ET to height in both the female and male participants giving more support that collectively an individual's anatomic proportions can help to determine a proper working distance. It is unclear if the differences in male to female anatomic measurements is contributory to the development of poor posture or MSDs; however, previous studies have cited an increase in reported MSDs in females compared to males. In a study on ergonomics in preclinical dental students, Kamal et al [10] reported differences in the postures of male and female participants without magnification, where female students had worse ergonomic ratings than males. They also observed a positive correlation between posture and reported pain for both female and male students with 89.1% of female students and 65% of male students reporting MS pain after

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starting preclinical training. A marginally significant difference in vision type by ET was found where noncorrective vision had a higher ET than corrective vision. This finding cannot be definitively explained since our measurements were derived while the participant was sitting in neutral ergonomics and hypothesized from anatomic measurements. Further studies may be necessary to investigate the significance of corrective and non-corrective vision differences. Our interpretation of the results provides that our hypothesis was correct, and our working distance value obtained through anatomic measurements is similar to a measured working distance in neutral ergonomics. An individualized working distance correlating to a person's body proportions can accurately provide their expected working distance. This allows us to propose that ideal working distance can be reproduced consistently through the Pythagorean formula with measured values of the eye to the cubital fossa and the cubital fossa to the tooth. In addition, by using identifiable landmarks (ie: the outer canthus and antecubital fossa), one may be able to obtain these measurements to calculate the desired working distance assuming ergonomics position without the aid of simulation in a dental chair or with a mannequin. Furthermore, future applications of this study may assist in the development of standardized measuring methods, thus promoting bio ergonomic head and neck posture.

### 5. CONCLUSIONS

The results allow us to conclude that we can accept the hypothesis that there is a direct relationship to the specific anatomical measurements of a dental operator while in neutral ergonomics position and a mathematical determination of proper working distance can be obtained. When applying the principles of the Pythagorean theorem with specified anatomic measurements the hypothesized working distance has a significant positive correlation to a participant's actual measured working distance. This finding may contribute to a more standardized method to measure the working distance that fits the proper neutral ergonomics for the operator. The first null hypothesis was accepted, while the second and third hypotheses were rejected. Through this study, we are able to provide applicable information for students to reinforce proper ergonomics through the analysis of individual working distance values and the relationship to individual anatomic measurements in order to utilize adjuncts like magnification in dentistry.

### **CONFLICT OF INTEREST**

The authors declare no conflict of interest.

### **ACKNOWLEDGMENTS**

None.

### **AUTHOR CONTRIBUTIONS**

HH: Take the existing design. Come up with some modifications which improve the sensitivity, and reduce the error percentage of the previous design. Writing the manuscript; Data collection and data analysis. SA, MM, AG: Data collection and data analysis; Writing the manuscript. MZ: Methodology, calculation of modifications, writing and reviewing the manuscript. NA: Methodology, calculation of modifications, writing and reviewing the manuscript, data analysis and data interpretation.

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## Questions

1. In an earlier study on the prevalence of body pain in a dental student population, \_\_\_\_\_ of students reported pain with the percentage increasing with the increase of years in school.

□a. 25-30 %:

□b. 10-15%;

□c. 46-71%;

□d. 32-55 %.

# 2. All below are the null hypotheses tested in the study except one, which one is the exception?

□a. There is a direct relationship to the specific anatomical measurements of a dental operator while in neutral ergonomics position, and a mathematical determination of proper working distance can be obtained;

□b. No difference in working distance between the corrective and non-corrective vision groups;

□c. No difference in working distance between genders;

□d. No difference in the working distance when using different light.

# 3. In the study, the investigators observed and verbally instructed changes in ergonomics keeping in mind the following recommendation:

□a. Head tilt of 0 to 20 degrees;

□b. Head tilt 0-30 degrees;

□c. Head tilt 0-10 degrees;

□d. No head tilt.

# 4. In the conclusion, when applying the principles of the Pythagorean theorem with specified anatomic measurements, the hypothesized working distance has\_\_\_\_\_ to the actual working distance.

□a. Non- significant correlation;

□b. Significant positive correlation;

□c. Significant negative correlation;

□d. Borderline significance.

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## CONDYLOGRAPHIC RECORDING OF MASTICATORY **FUNCTION: EXPLORATIVE STUDY ON OCCLUSAL** PARAMETERS AND CHEWING PERFORMANCE WITH NATURAL FOOD AND A STANDARD FOOD MODEL

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### **ABSTRACT**



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Introduction Loss of teeth defines oral health status and chewing abilities. Caries and periodontal disease have been associated with systemic diseases, however they may contribute to cognitive impairment too. Mastication assessment appears to possess broad significance, and is needed to create background knowledge for chewing harmony. The aim of this study was to evaluate chewing performance and the characteristics of condylographic recordings, during mastication of Natural Food (NF) and a Standard Food Model (SFM), in subjects with different occlusal parameters.

 ${\bf Methodology} Twenty-three \ adult \ subjects' mastication \ was \ assessed \ with \ a \ standardized \ recording \ protocol,$ when chewing onto SFM and NF in three different textures. Detailed occlusal characteristics, condylographic recording data and condylographic patterns during mastication were all analyzed and compared.

Results Bilateral Crossbites, Missing Molars and Asymmetric Molar Class were related to higher disharmony and transversal displacement during chewing. Missing and unreplaced molars showed dysfunctional patterns and so did worn out occlusal surfaces. Molar Class alone did not prove to be a useful predictor in chewing test results.

**Conclusion** Increasing evidence indicates that oral health has an impact on Individual and Public Health. It is important to understand that oral health and its functional status are to be maintained during one's lifetime, and that preventive, therapeutic measures at all ages should have a common functional target to grant neurobiological health as well as nutritional goals of Mastication. Further studies are needed to better understand the relevance of additional parameters such as Occlusal Plane Inclination, Curve of Spee, and three-dimensional asymmetries.

### **KEYWORDS**

Mastication; Condylography; Axiography; Chewing Test; Occlusion.

### 1. INTRODUCTION

Throughout the history of medicine Mastication has triggered the interest of researchers due to its complexity, its functions and behavioral significance. Mastication is the process by which food is broken down by teeth into smaller pieces, mixed with saliva into a bolus and made suitable for swallowing. The quality of mastication and its efficiency are crucial even beyond the individual ability to perform chewing strokes and to swallow. Oral health has been held responsible for, or related to, a number of systemic conditions. For instance, poor oral health has been investigated in the past as an independent risk factor and association for systemic diseases such as myocardial infarction, coronary disease and

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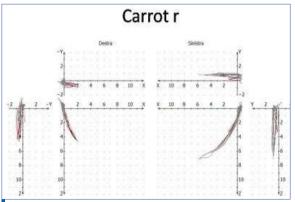


Figure 1. Expected chewing pattern - example of right side chewing of NF Carrot: ws displays shorter and steeper tracings as opposed to nws. Movement is harmonic and superimposable, transversal aspects of movement are contained.

### Table 1. Inclusion criteria.

### Inclusion criteria

Subjects were considered for the study if the following conditions were met:

- any Gender
- good overall health status (no prescription drugs taken routinely, asymptomatic)
- no previous diagnosis of Temporomandibular Disorder
- willingness to understand the procedure and give informed

diabetes [1-3]. This whole issue has reached a higher and wider awareness only in recent times, although earlier studies concerning these associations had already highlighted the importance of maintaining a satisfactory oral and dental status for the good of systemic health and for the consequences on Public Health [4]. Among the above-mentioned conditions, Dementia has more recently been related to oral health and chewing impairment. Because of its vigorous onset in the elderly populations, and because the world population is more and more represented by the elder age group, Dementia has become an increasingly important health and socioeconomic issue [5].

It is fair to understand that, among other factors, the mere number of lost teeth defines not only one's oral health status, but also his/her chewing abilities, with subsequent forceful changes in diet, malnutrition, and an inflammatory response which have all been related to an increased risk of Dementia and Alzheimer's Disease (AD). These emerging findings, besides harmony and good functioning of the stomatognathic system, call for further studies on mastication and on the significance of lost teeth, both for what concerns the number of missing teeth and what dental element in particular [6]. Teeth possess discrimination for direction of forces, texture and hardness of foods, thanks to the interaction between pulp-dentine-enamel units and periodontal ligaments [7,8]. Studying mastication and the stomatognathic system has shown to be a



Figure 2. Quality of occlusal surfaces, from left to right: poor, average,

hard and demanding guest which proceeded along with the development of ad hoc apparatuses and recording devices. Studies by Lundeen and Gibbs from the 1980s showed that there are differences in chewing in relation to growth, and that they are related to changes in form and function which take place during transition from infancy to adulthood (increase in the steepness of the articular eminence, development of the anterior tooth guidance, and change from deciduous to permanent teeth). Adults' chewing pattern has a midsagittal opening and wide lateral closing movements when observed from a dental perspective [9,10]. The quality of the occlusion, conditions such as Temporomandibular Disorders (TMJ) and interferences in occlusion all affect mastication: the more the interferences the longer the chewing time and the more atypical the pattern looks [11]. Regarding patterns, Lundeen and Gibbs studied mastication with the Replicator System [9,10]. The Replicator System was an electronic jawmovement recorder that allowed to replicate all border and chewing movements while also being able to examine 'chewing casts' from all views. The authors' evaluations of the chewing patterns were made on occlusal points (first molars and interincisal), on both sides, as well as by tracking the joints.

Studies concerning the masticatory patterns have been described in previous works, the most notable being the ones with the *Sirognathograph* first, and the *Kinesiograph* later on. This latter method can only identify movement of a single anterior point with a magnet positioned on the lower arch, but it has the possibility to study muscular activity through Electromyography at the same time [12,13].

Researchers have also recognized a repetition and reproducibility of chewing patterns, with harmony also being a characteristic of a healthy chewing function and, if harmony is missing, along with repetition and a scheme, then a dysfunctional mastication is observed. Malocclusions such as unilateral crossbite have shown a peculiar subversion of opening and closing direction which are substantially different to the expected chewing pattern: in these

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### Table 2. Exclusion criteria.

### **Exclusion criteria**

Subjects were excluded if the following conditions were met:

- age below 18
- age above 70
- food allergy (nuts)
- vegetarian/vegan subjects
- hyperglycemia/diabetes
- permanence of deciduous teeth
- removable total/partial prostheses
- ongoing orthodontic treatment
- any dental extraction/dental surgery in the previous 6 months
- composite/filling on occlusal surfaces in the previous 6 months
- acute oral health issue (caries, odontogenic abscess, odontogenic phlegmon)
- acute craniofacial issue (trauma with/without fractures)
- acute/chronic pain of masticatory and facial muscles
- primary headaches
- chronic neck, shoulders or back pain (pain >3 months)
- previous brain injuries
- cleft palate/lips
- facial clefts
- impaired saliva production due to drugs or systemic disease
- mental illness
- visual impairments (sightless subjects)
- neurological disorder with impaired motor capabilities with/without medication
- neurological conditions affecting cranial nerves
- any condition influencing proper informed consent to participation in the study
- any conditioning affecting the sitting position in a dental chair



Figure 3. Standard Food Model jellies, from left to right: soft, medium and hard to the

cases, the mandible will first move medially during opening and then laterally, so as to confront with the 'crossed' surfaces of antagonist teeth. Moreover, this altered pattern has a narrower width and a more vertical closing angle. If the 'Functional Matrix' of Moss is not to be forgotten, this supposedly has some effect on growing children, both structurally and with respect to motor control, and it is the reason why unilateral crossbite can be looked at as a 'neuromuscular syndrome' [12,14].

Some studies have explored human mastication through chewing tests and their outcome. Kikui and colleagues for instance assessed comminution of a jelly, claiming that it was a valid method for gathering relevant information from the number of pieces and their surface area.

This better replicates in their opinion what actually occurs during effective chewing: crushing and an increase in the surface area of food, so as to facilitate reaction with digestive enzymes, decomposition, and the absorption of nutrients [15]. Some other tests use natural foods as a material test, however physical properties of natural foods vary and are difficult to standardize. There are natural variations in size and texture which produce differences in the final outcomes. Besides, hardness is not constant, due to the various degrees of water incorporation

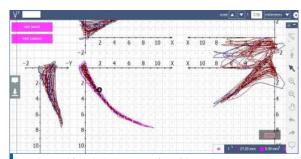


Figure 4. Tool for calculating areas of condylographic tracings in sagittal, cranial and frontal view. In this figure, the sagittal tracing was digitally outlined and in the bottom right-hand corner are the perimeter and the area of the right sagittal condylographic tracing. The same procedure was carried out for all planes (sagittal, cranial and frontal) on both sides for all chewing.

thus making the standardization for the masticatory tests difficult. Natural foods that are mostly described by authors are: peanuts, almonds, cocoa, carrots, hazelnuts, decaffeinated coffee beans and nuts [16-21]. Previous experience with the limitations of natural foods chewing tests brought about the need for an ideal food model for experimental, research and clinical testing. With this respect a Standard Food Model (SFM) was first developed, described and proposed by Slavicek in 2009 [22-26]. Such food model has been produced in a short cylindrical shape and three different hardnesses (A. Egger' Sohn, Süsswaren und Naturmittel GmbH., Mellergasse 4, A-1230 Vienna, Austria).

Condylography as a method for evaluating mandibular movements has its predecessors dating as back as the last decades of the 1800s. The first recordings were reported by Ulrich and Walker, and invaluable contributions to the study of hinge axis, Bennett movement, jaw tracking, articulator programming



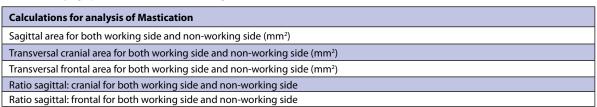
Table 3. Condylographic data collected directly from the recording software.

### Software-retrieved data for analysis of Mastication

Maximum displacement (s) of the hinge axis (mm) for both the working side and the non-working side (ws and nws) assessed on the sagittal plane

Sagittal Condylar Inclination SCI° (inclination of the hinge axis excursion) for both the working side and the non-working side (ws and nws)

Table 4. Condylographic data calculated from tracings with the use of an additional tool.



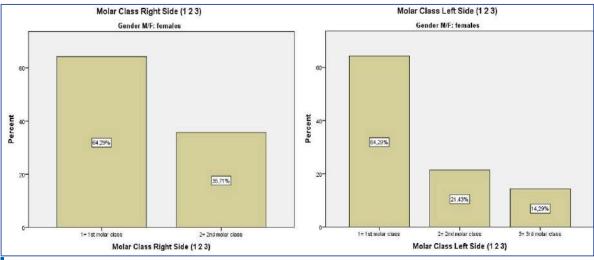


Figure 5. Molar Class Right and Left – Distribution Females.

and for diagnostic purposes have been made by Bennett, Eltner, Gysi, McCollum, Posselt, Messerman, Bewersdorff, Jankelson, Lundeen, Alsawaf, Missert and Slavicek [27].

Caries, periodontal disease and systemic conditions that can affect the periodontium, can all potentially lead to tooth loss. Tooth loss can somewhat be prevented and has therefore been the target for Public Health measures, but nevertheless it is still common, especially within the ageing population and in the lower socio-economic groups [28,29].

What is mostly unforeseen or prevented, but is just as common, is Dementia, the most frequent being Alzheimer's Disease (AD).

A World Health Organization report from 2013 stated that the total number of people with Dementia worldwide in 2010 was estimated at 35.6 million and is deemed to almost double every 20 years, to 65.7 million in 2030 and 115.4 million in 2050 [30]. AD occurs because of environmental, genetic and vascular risk factors, yet nearly half of ADs do not have a clear mutation or cause [6]. A relationship between AD and tooth loss has been sought for at least twenty years now, with publications focusing on the transition from memory impairment to AD, on oral health in nursing homes, cohort studies and community homes for the elderly [31-34]. This

correlation had been hypothesized even earlier, when basic findings were retrieved highlighting the fact that Dementia patients were more likely to have a greater tooth loss [35,36]. However different studies and authors have aspired to study this relationship as a predisposing condition, similarly to what has been done with the correlation between cardiovascular disease and periodontitis. A 5-year prospective cohort study investigated the effect of tooth loss on the development of Mild Memory Impairment (MMI) among the elderly [31]. A study on identical twins revealed that major tooth loss before the age of 35 was a significant risk factor for AD [32]. A study on a population of nuns, found that 9 or fewer remaining teeth were associated with an increased risk of Dementia [33].

Type 2 Diabetes patients were the target of a prospective cohort study that showed that Dementia and cognitive decline are associated with having no teeth [37]. Conversely, the presence of teeth, or better, the preservation of natural teeth and cognitive functioning have shown a significant positive relationship [38]. Worse episodic memory has been assessed in other studies and it has been correlated with tooth number, too [39]. Okamoto and colleagues also reported about a survey study that claimed that patients with MMI will more

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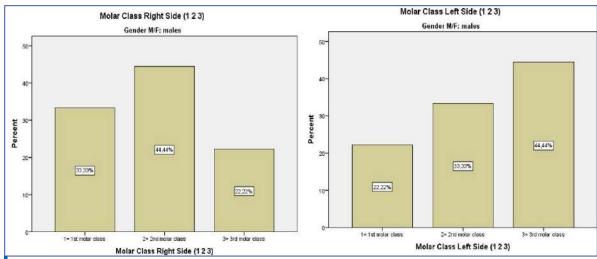


Figure 6. Molar Class Right and Left – Distribution Males.

likely progress into AD as opposed to other types of dementia, thus making MMI (examined through the Mini-Mental State Examination - MMSE) a good time-frame for evaluations of this kind [40]. In the Fujiwara-kyo study a population of 3696 healthy residents over the age of 65, was observed from 2007 to 2012. Among these, 2486 underwent follow-up examination and 2335 were included in the final analysis (only cognitively intact or MMI subjects). This very powerful and well-designed study showed that tooth loss and worse cognitive performance are indeed correlated, and that absence of tooth loss and preservation of the patients' baseline status meant no increase in risk of MMI [31].

Possible reasons for the association between memory disorder and tooth loss have been highlighted not only in the Fujiwara-kyo paper: the existence of an inflammatory status related to periodontal disease which caused tooth loss; genetic factors related to both MMI and periodontal disease; and a decrease in sensory receptors function. An observational study published in 2015 aimed at drawing some associations between tooth loss and Dementia from the late 1960s onwards [41].

The hypothesis behind this retrospective analysis of a cohort of women was that tooth loss was an expression of some proinflammatory activity and that there was a correlation between the number of teeth and Dementia. Mastication influences the activity in the cerebral cortex, stimulating cognitive function and preventing brain degradation. Rhythmic movements, and this may be one reason why sports have a similar beneficial effect, increase blood flow especially in the prefrontal cortex and the hippocampus, where learning and memory performance reside [31,38].

Thorough dissertation on the role of chewing, hippocampal, reticular formation and somatosensory associations is not the scope of this paper, however impoverishment of chewing abilities and function through alteration in number or quality of occlusal determinants (extraction or milling of molars) and a long-term soft-diet feeding, can inhibit learning

and memory [8,42-46]. A pilot study by Elsig allowed to point out a difference between the number of remaining teeth and chewing efficacy in Dementia patients, thus further encouraging the performance of routine chewing tests besides tooth count in geriatric evaluations [47]. Mastication and its study appear to possess significance both individually, in terms of prediction of disease or its risk, for its educational value, and in order to create a wider database for chewing patterns and unfavorable occlusal features, which will enable to fulfill the aforementioned purposes.

The aim of this study was to assess the characteristics of condylographic tracings, during mastication of natural food (NF) and a standard food model (SFM), in subjects with different occlusal parameters. Based on the existing literature, the purpose was to evaluate whether condylography was suitable for tracking mastication at the temporomandibular joint (TMJ), to evaluate whether condylography could reliably provide a clear tracing aspect working (ws) versus non-working side (nws) with the ws being steeper, straighter and shorter as opposed to the nws (see Fig. 1), if disharmony or subversion of the abovementioned aspect of ws and nws for some occlusal features (flat occlusal surfaces, missing teeth,...) and food properties.

### 2. MATERIALS AND METHODS

Twenty-three adult subjects' mastication was assessed by means of condylography with a standar-dized recording protocol, when chewing onto SFM and NF in three different textures (for SFM: hard, medium, soft; for NF: salad leaf, carrot slice, nut). The study protocol followed the ethical guidelines of the Declaration of Helsinki, and informed consent prior to recording was acquired from all subjects (see Tabs. 1 and 2). Sagittal first molar relationship (Class 1, 2 and 3) was recorded for each subject and each side (right and left). Asymmetric molar classes were included in the data collection. The presence of a Clear Buccal Intercuspation was checked according to the clinical



7	The distribution of SFM soft right: ratio xz/xy right is the same across categories of Asymmetric Molar Class (Y=1 N=2).	Independent- Samples Mann- Whitney U Test	,0321	Reject the null hypothesis.	77	The distributio ratio xz/xy rigi categories of / Class (Y=1 N=
11	The distribution of SFM soft left: sagittal area mm2 xz right is the same across categories of Asymmetric Molar Class (Y=1 N=2).	Independent- Samples Mann- Whitney U Test	,0321	Reject the null hypothesis	79	The distribution ratio xz/yz right categories of A
21	The distribution of SFM soft bilateral: sagittal area mm2 xz right is the same across categories of Asymmetric Molar Class (Y=1 N=2).	Independent- Samples Mann- Whitney U Test	,0301	Reject the null hypothesis.	81	The distribution bilateral: sagitt is the same ac Asymmetric M N=2).
38	The distribution of SFM medium right; ratio xz/xy left is the same across categories of Asymmetric Molar Class (Y=1 N=2).	Independent- Samples Mann- Whitney U Test	,032 <sup>1</sup>	Reject the null hypothesis.	86	The distribution bilateral: transiteft is the same of Asymmetric N=2).
17	The distribution of SFM medium left: ratio xz/xy right is the same across categories of Asymmetric Molar Class (Y=1 N=2).	Independent- Samples Mann- Whitney U Test	,0391	Reject the null hypothesis.	87	The distribution bilateral: ratio is same across of Asymmetric M N=2).
18	The distribution of SFM medium left: ratio xz/xy left is the same across categories of Asymmetric Molar Class (Y=1 N=2).	Independent- Samples Mann- Whitney U Test	,014 <sup>1</sup>	Reject the null hypothesis.	88	The distribution bilateral: ratio sacross categor Molar Class (Y
19	The distribution of SFM medium left: ratio xz/yz right is the same across categories of Asymmetric Molar Class (Y=1 N=2).	Independent- Samples Mann- Whitney U Test	,025 <sup>1</sup>	Reject the null hypothesis.	89	The distribution bilateral: ratio same across of Asymmetric M N=2).
57	The distribution of SFM medium bilateral: ratio xz/xy right is the same across categories of Asymmetric Molar Class (Y=1 N=2).	Independent- Samples Mann- Whitney U Test	,0041	Reject the null hypothesis.	117	The distribution bilateral: ratio same across of Asymmetric M N=2).
58	The distribution of SFM medium bilateral: ratio xz/xy left is the same across categories of Asymmetric Molar Class (Y=1 N=2).	Independent- Samples Mann- Whitney U Test	,004 <sup>1</sup>	Reject the null hypothesis.	118	The distribution bilateral: ratio across catego Molar Class (Y
59	The distribution of SFM medium bilateral: ratio xz/yz right is the same across categories of Asymmetric Molar Class (Y=1 N=2).	Independent- Samples Mann- Whitney U Test	,0041	Reject the null hypothesis.	177	The distribution ratio xz/xy right categories of A Class (Y=1 N=
60	The distribution of SFM medium bilateral: ratio xz/yz left is the same across categories of Asymmetric Molar Class (Y=1 N=2).	Independent- Samples Mann- Whitney U Test	,048 <sup>1</sup>	Reject the null hypothesis.	178	The distribution ratio xz/xy left categories of A Class (Y=1 N=
57	The distribution of SFM hard right: ratio xz/xy right is the same across categories of Asymmetric Molar Class (Y=1 N=2).	Independent- Samples Mann- Whitney U Test	,0391	Reject the null hypothesis.	179	The distribution ratio xz/yz right categories of A Class (Y=1 N=
68	The distribution of SFM hard right: ratio xz/xy left is the same across categories of Asymmetric Molar Class (Y=1 N=2).	Independent- Samples Mann- Whitney U Test	,005 <sup>1</sup>	Reject the null hypothesis.	180	The distributio ratio xz/yz left categories of A Class (Y=1 N=
	1000					

**Figure 7.** Mann-Whitney U Test results for differences in sagittal (xz plane of condylography) to transversal (yz and xy planes) ratios of chewing tracings for SFM and NF in Asymmetric Molar Class (Y/N).

assessment of a distinct 1:2 vestibular relationship between upper and lower arches. Other sagittal and transversal intermaxillary dental relationships were recorded as well: open bite (when overbite<0 mm), deep bite (when overbite>4 mm), bilateral crossbite, monolateral crossbite, scissor bite. The presence and location of missing teeth and prosthodontic/conservative restorations were recorded too. Occlusal surface characteristics were evaluated as Good, Average or Poor based on the investigators' assessment on overall tooth wear (Fig. 2). All subjects

77	The distribution of SFM hard left: ratio xz/xy right is the same across categories of Asymmetric Molar Class (Y=1 N=2).	Independent- Samples Mann- Whitney U Test	,0031	Reject the null hypothesis
79	The distribution of SFM hard left: ratio xz/yz right is the same across categories of Asymmetric Molar Class (Y=1 N=2).	Independent- Samples Mann- Whitney U Test	,021 <sup>1</sup>	Reject the null hypothesis
81	The distribution of SFM hard bilateral: sagittal area mm2 xz right is the same across categories of Asymmetric Molar Class (Y=1 N=2).	Independent- Samples Mann- Whitney U Test	,0311	Reject the null hypothesis
86	The distribution of SFM hard bilateral; transversal area mm2 xy left is the same across categories of Asymmetric Molar Class (Y=1 N=2).	Independent- Samples Mann- Whitney U Test	,0481	Reject the null hypothesis
87	The distribution of SFM hard bilateral: ratio xz/xy right is the same across categories of Asymmetric Molar Class (Y=1 N=2).	Independent- Samples Mann- Whitney U Test	,031	Reject the null hypothesis
88	The distribution of SFM hard bilateral: ratio xz/xy left is the same across categories of Asymmetric Molar Class (Y=1 N=2).	Independent- Samples Mann- Whitney U Test	,0091	Reject the null hypothesis
89	The distribution of SFM hard bilateral: ratio xz/yz right is the same across categories of Asymmetric Molar Class (Y=1 N=2).	Independent- Samples Mann- Whitney U Test	,018 <sup>1</sup>	Reject the null hypothesis
117	The distribution of NF salad bilateral: ratio xz/xy right is the same across categories of Asymmetric Molar Class (Y=1 N=2).	Independent- Samples Mann- Whitney U Test	,0221	Reject the null hypothesis
118	The distribution of NF salad bilateral: ratio xz/xy left is the same across categories of Asymmetric Molar Class (Y=1 N=2).	Independent- Samples Mann- Whitney U Test	,0111	Reject the null hypothesis
177	The distribution of NF nut bilateral: ratio xz/xy right is the same across categories of Asymmetric Molar Class (Y=1 N=2).	Independent- Samples Mann- Whitney U Test	,011 <sup>1</sup>	Reject the null hypothesis
178	The distribution of NF nut bilateral: ratio xz/xy left is the same across categories of Asymmetric Molar Class (Y=1 N=2).	Independent- Samples Mann- Whitney U Test	,005 <sup>1</sup>	Reject the null hypothesis
179	The distribution of NF nut bilateral: ratio xz/yz right is the same across categories of Asymmetric Molar Class (Y=1 N=2).	Independent- Samples Mann- Whitney U Test	,011 <sup>1</sup>	Reject the null hypothesis
180	The distribution of NF nut bilateral: ratio xz/yz left is the same across categories of Asymmetric Molar Class (Y=1 N=2).	Independent- Samples Mann- Whitney U Test	,0221	Reject the null hypothesis

**Figure 8.** Mann-Whitney U Test results for differences in sagittal (xz plane of condylography) to transversal (yz and xy planes) ratios of chewing tracings for SFM and NF in Asymmetric Molar Class (Y/N).

underwent a condylographic recording session during which a standard recording and a chewing recording were performed. The condylography was carried out by using an electronic recording system for jaw movements (Cadiax Diagnostic GAMMA Dental, \*GAMMA med. wiss. Fortbildungs GmbH, Josef Brennerstr. 10, A-3400 Klosterneuburg, Austria). One trained investigator performed all recordings. A paraocclusal clutch was applied, the hinge axis was localized and classic condylographic recording of movements would take place before the chewing

Table 5. Quality of occlusal surfaces (right side).

	# of subjects
Poor	9
Average	6
Good	8

recording session. A previously developed and described Standard Food was used in three different textures (soft, medium and hard) (A. Egger´ Sohn, Süsswaren und Naturmittel GmbH, Mellergasse 4, A-1230 Vienna, Austria). The shape of these jellies was cylindrical and the size was 10x20 mm (Fig. 3). The recordings were strictly performed for 18 seconds, on each side (right, left and bilateral chewing), for each texture of jellies and for each type of natural food. Eighteen seconds were mandatory because of the maximum recording time the software could host.

The participants were instructed to chew each food on the right side, on the left side and bilaterally, and break the food in as many pieces as possible without swallowing, since the food particles had to be collected at the end of each chewing cycle for separate evaluation.

A resting time of at least 30 seconds was allowed between recordings. The recording hardware was then removed, and the proband was asked to identify and score discomforts so that they could be collected and stored within the database.

For each proband the maximum 's' displacement of the Temporomandibular Joint Hinge Axis during the 4 baseline movements and during the chewing sequences was taken from the software analysis for both right and left condyle in millimeters (s, mm). For each chewing sequence, for SFM and NF, the data in Table 3 were collected from the appropriate tool in the software and stored. For each chewing sequence, SFM and NF, the data in Table 4 were retrieved with the aid of the online tool SketchAndCalc™ (iCalc Inc, 2017 Elliott Dobbs, www.sketchandcalc.com) and used for further calculations (Fig. 4).

All Descriptive Statistics and Tests were carried out with IBM SPSS Statistics for Windows, Version 24.0 AND 25.0 Armonk, NY IBM Corp. (Released from 2016).

### 3. RESULTS

### 3.1. Descriptive Analysis of Subjects

There were 14 females (60.87%) and 9 males (39.13%). The subjects' age ranged from 18 to 64 years of age (mean age  $37.74 \pm 12.53$ ).

The Molar Class for the right side was found to be Class 1 in 52.17% of subjects, Class 2 in 39.13% and Class 3 in 8.70% of probands. For the left side, the percentages were 47.83%, 26.09% and 26.09% respectively. For both sides Molar Class 1 was nearly half of the total and an Asymmetric Molar Occlusion

Table 6. Quality of occlusal surfaces (left side).

	# of subjects
Poor	8
Average	8
Good	7

was detected in 5 subjects (21.7%). See Gender distribution in Figs. 5 and 6. The Open Bite was present in 3 subjects overall (13%), whereas Deep Bite was found in 30.4% of subjects (7 probands). The Open bite was present in 2 cases of Class 1 molar relationships (16.67% of all class 1) and in 1 case (11.11%) with molar class 2, both for the right side. For the left side, the open bite was present in 2 cases (18.2%) in Molar Class 1 and in 1 case (16.7%) for class 2. No open bite was related to Molar Class 3 on either side. The Deep Bite was mostly present in Class 2 right side where it represented 44.44% of all class 2 subjects (25% for Class 1 right side, 0% for Class 3 right side).

For the left Molar Class 1, 2 and 3 the presence of the Deep Bite was present in 27.27%, 33.33% and 33.33% of cases.

A bilateral Crossbite was detected in 17.39% of probands (4 cases) and a monolateral crossbite was present in 2 cases only (8.7%). No proband had scissor bite.

The quality of the Occlusal Surfaces (Tabs. 5 and 6) was rated as poor for the right side on 9 counts (39.1%), and on 8 counts for the left (34.8%).

A Clear Buccal Intercuspation was found for 14 subjects (60.9%). Missing Premolars were found in one proband only (4.3%) with no edentulous gap between tooth 1.4 and tooth 1.6. Missing Molars were observed in 6 cases (26.1%). Distal Restorations were present in 7 subjects (30.43%).

### 3.2. Statistical Analyses

3.2.1. Condylographic Tracings for Working and Non-Working Sides with SFM and NF

For both food types, the working sides' tracings (s, mm) were shorter than the non-working sides (SFM ws mean 4.9 mm nws 6.8 mm; NF ws mean 3.6 mm nws 5.3 mm; paired t test set at 95% CI p=0.000 for SFM and p=0.000 for NF). The difference by hardness on both ws and nws is not detectable. Nevertheless, all SFM compared to the NF s traits were significantly different, with SFM having larger displacements in mm (SFM s mean 5.75 mm; NF 4.56; paired t test set at 95% CI p=0.000).

3.2.2. Condylographic Tracings' Distinctive Path Inclination SCI° for Working and Non-Working Sides with SFM and NF

For the SCI° (path inclination) SFM ws and nws were compared, with the nws showing a flatter inclination as expected (mean ws=61.12°, mean nws=53.31°), which was statistically different from the steeper inclination of the ws as tested with a paired t test



Figure 9. Male 31 yo, Asymmetric Molar Class (Right side Class 1, Left side Class 2).

set at 95% CI (p=0.000). Since the distribution of these data was not normal for SCI° SFM medium Left chewing and SFM hard Left chewing, a non-parametric test was also carried out (Wilcoxon Signed Rank Test with significance level at 0.05, p=0.01 and 0.003 respectively). The NF chewing SCI° were also compared for ws and nws by means of a parametric and a non-parametric test, since even here the normal distribution of data was not the case at all times. Both the paired t-test and the Wilcoxon Signed Rank Test allowed to highlight a significant difference in steepness of condylar path inclination. 3.2.3. Condylographic Distinctive Chewing Pattern for Working and Non-Working Sides with SFM and NF

The presence of a Distinctive Chewing Pattern was tested against the Quality of Occlusion Surfaces Right and Left but no significant correlation was observed ( $\chi^2$  p=0.343 right side p=0.577 left side). A significant correlation was observed instead for the variable Missing Molars and the absence of a Distinctive Pattern (Fisher Exact Test p= 0.006). The variable Gender and Presence of Distal Restorations did not yield a statistically significant result (Fisher Exact Test p= 0.064).

3.2.4. Condylographic Chewing Tracings' Areas and Ratios with SFM and NF

The mere assessment of areas in mm<sup>2</sup> showed few statistically significant results, but an observed tendency to have nws with bigger areas than ws for Molar Class 1 and 2.

However, assessment through ratios allowed the Authors to expressly identify the predominance of transversal movement during chewing over the sagittal one across all SFM and NF chewing (right side chewing, left and bilateral). Statistically significant results were retrieved for the variables Quality of Occlusion (poor, average, good Kruskal-Wallis max p=0.42), Bilateral Crossbite (Mann-Whitney U max p=0.048), Missing Molars (Mann-Whitney U max p=0.041), Clear Buccal Intercuspation (Mann-Whitney U max p=0.040), Distal Restorations (Mann-Whitney U max p=0.041) and most importantly Asymmetric Molar Class.

This latter variable in particular, gave significant results for all SFM hardness and for the NF too (for space reasons only these results are displayed in Figs. 7 and 8).

### 4. DISCUSSION

As there is increasing evidence indicating that oral health has an impact on Public Health issues, and not solely on Individual Health, it is important to understand and maintain the mastication tool properly fitted for its neurobiological objectives as well as nutritional ones.

What is expected from an 'ideal' mastication is, in this respect, of the utmost importance. If teeth are to be seen as refined, precise and sophisticated proprioceptors, then the scientific community's task is to test, assess and restore them accordingly. This is the assumption behind this work, which is an offspring of previous ones which aimed at studying human mastication quantitatively and qualitatively [22-26]. The Reader might want to know that other recorded condylographic parameters and chewing scores had to be kept out of this publication for space reasons (number of particles chewed, scores, number of chewing strokes, rotational angle GAMMA° according to the different food hardness). The population of the present study encompassed diverse occlusal features, but it was a small one, nevertheless it allowed the Investigators to set some ground for new hypotheses for future studies, and make this an explorative study on mastication and its recording. Females showed more absence of a clear intercuspation as opposed to males, and they had a poorer occlusion, more occlusal restorations and, most importantly, less presence of a distinctive chewing pattern. Yet, the females here were the group with the most frequent 'ideal' Class 1 occlusion (81%), and all Class 1s overall have 72% of clear buccal intercuspation. As elsewhere highlighted, this combination of findings suggests that the Molar Class alone is not a sufficient indicator at all for an ideal or functional mastication [48]. Monolateral Crossbite cases were too few to infer correlations. Condylography really fulfilled the task of showing geometrical features of working and non-working sides for SFM and NF: for both food types the working sides were in fact shorter than the non-working sides. This comes helpful in research settings in that SFM, which is not a vegetable but is still food, is generally chewed with the expected pattern that NF normally shows, but it has the potential to stress and highlight characteristics which small, easily chewed NFs may

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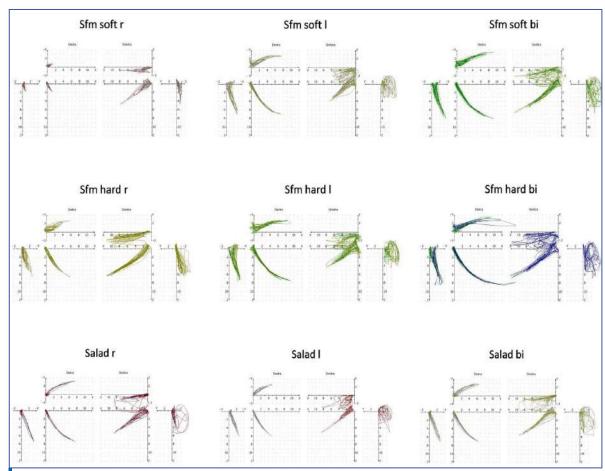


Figure 10. Condylographic Recording during chewing of SFM and NF (only soft and hard SFM, and salad leaf chewing are displayed for space reasons) by proband in Figure 9. Non-harmonic left side tracings with a very wide transversal component can be observed for SFM and NF chewing.

not have the power to exhibit. Much like many other clinical tests which can be performed at rest, but are sometimes required to be performed under physical or drug-induced stress to really recreate a condition or provoke one, the SFM chewing test might prove the treadmill test for the masticatory system. The results with nuts and carrots have shown in fact that every proband can chew on them with no trouble regardless of the occlusal quality, missing teeth and so forth. One single salad leaf is instead more demanding, since it can be properly torn only when the occlusal quality is maintained, for it requires closeness of upper and lower teeth which get to slide on their buccal and lingual slopes. If these are lost then a grinding movement between flatter surfaces occurs and this sets the basis for a more horizontal/transversal movement rather than a vertical/sagittal one. All lengths 's' of the SFM sagittal tracings were numerically bigger than all lengths of NF sagittal tracings and this might be the effect of the properties of SFM (standard size for instance). For SCI° (condylar path inclination), SFM ws and nws were compared, nws showing a flatter inclination as expected, which was statistically different from the steeper inclination of the ws. The same held true for NF. The assessment of the chewing tracings' areas was the most demanding and most interesting

chapter in this explorative study. Not only were

the single areas retrieved for all foods, all sides and all planes (sagittal, frontal and cranial), but ratios between the sagittal plane and the transversal planes were also calculated, so as to minimize the effect due to individual sizes, and enhance the more robust meaning of sagittal to transversal ratios. As the ratio decreases, both for the sagittal:frontal and the sagittal:cranial dimensions, then the transversal quantity of movement becomes predominant over the sagittal one. Graphical representation of areas in mm<sup>2</sup>, divided by ws and nws and molar class right and left, was difficult to interpret at a first glance and no particular difference or feature stood out. When the chewing data were assessed through the ratios one can see that the 'good' quality of the occlusion right had bigger nws ratios (more sagittal component) for SFM soft and medium (this difference was not visible for SFM hard), as compared to the average and poor right occlusions (more transversal component). A similar trend could be seen for the nws ratios for the left qualities of occlusion, but not for every single ratio and hardness of SFM.

The ws ratios were smaller in poor right occlusions as compared to the ws ratios of the the average and good occlusions, supporting the hypothesis that even in working sides there is a more transversal component in flatter occlusal qualities. The same could be seen for the left qualities of occlusion.



Similarly, when looking at the NF ratios, the poor occlusions right and left had smaller ratios compared to average occlusion, but not as much compared to the good occlusion where differences were mostly seen in the sagittal: cranial plane ratios. This sagittal:cranial ratio looks especially useful when looking at differences in NF, since the 's' displacement is already significantly shorter in NF than SFM, and perhaps more understandable on the cranial plane instead of the frontal one. For a similar reason ws ratios in NF show little or inconsequent differences among the various occlusal qualities, since the range of the areas on working sides is already quite small in mm<sup>2</sup>. Another difference between the ratios can be detected between the ws ratios of SFM and NF in subjects who have distal restorations and subjects who do not have distal restorations (NF nut is the only food which does not show this as clearly), with the Distal Restoration Group displaying smaller ratios (therefore a more transversal component). The same observation could not be made for the nws. Probands without Clear Buccal Intercuspation display smaller ratios for NF in the nws than probands with Clear Buccal Intercuspation. Ws show the same difference but only in sagittal: cranial evaluation. The above-mentioned observations have been tested (for areas and ratios, for SFM and NF) and differences have been found to be statistically significant. All differences in the sagittal and transversal areas in mm<sup>2</sup> provide food for thought in that they indicate a purely numerical size discrepancy which could hide or be related to other 'size' parameters not taken into account in this study. Anyway, it is the difference or even inversion of the sagittal to transversal ratios, in Bilateral Crossbite, Missing Molars and Asymmetric Molar Class subjects, that really supports the idea that these occlusal features and malocclusions are related to a higher transversal displacement during chewing cycles, usually with the non-working side performing very wide movements (see Figs. 9 and 10). The findings in this explorative study suggest a number of considerations. Transveral stability in occlusion (granted by one-to-two tooth contacts sagittally, and by lingual occlusion transversally) needs to be looked at and restored/provided for accordingly. The quality of occlusion is a crucial feature: restorative procedures which aim at restoring quadrants or single elements in flat occlusions need to be looked at critically. Missing and unreplaced molars correlate with dysfunctional chewing patterns. Molar Class has already shown little significance and therefore it is not a good indicator for describing malfunctions or chewing differences. Some of the unexpected chewing patterns may be due to undetected skeletal asymmetry of the cranial base which does not necessarily correlate with an asymmetry in molar class. SFM and condylographic recording can be exploited as a stress test for the detection of incapability in chewing stiff meats or other nutrient food.

### 5. CONCLUSIONS

As highlighted in previous works, the geriatric population is highly affected by cognitive impairment and more exposed to pureed, blended and mashed food. At times it is because dysphagia is present, however in many facilities prescription of pureed food is offered to many without real reasons behind it. MTF (Modified Texture Food) is often poorer in nutritional content and not very accepted and therefore eaten less despite the apparent easiness in swallowing it [49].

Like others, Dementia is a delicate Public Health issue in that it directly affects patients and families, it requires a very demanding set of measures to satisfy the need and quality of life of both, and it tackles many aspects of preventive medicine. Needless to say, it is a financial dilemma for many and it encompasses a very transversal series of medical specialties, odontology being one of the most recent. Prevention is crucial as medical therapy is at present unable to address Dementia, resulting in a frustrating series of events which lead to smaller chance of detecting other diseases, smaller response to treatments in general, reduced life expectancy and uncontrolled disease progression [6].

A better understanding of the significance and role of dental elements as singular units in neurological modulation could empower knowledge about dental risk factors and perhaps make them modifiable, and this would raise new awareness to be transferred to researchers, physicians and most importantly the population. Bearing this in mind, proper mastication should be guaranteed to avoid malnutrition in those populations where cognitive impairment has already occurred, and to at least maintain their general health status. Studies on the chewing efficiency of these very populations have shown that Dementia patients eat slowly and therefore less, have uncoordinated masticatory activities with chewing strokes accompanied by facial mimic movements, and most importantly that it is very hard to assess their chewing performance. At the same time, it is by now widely recognized that chewing positively affects cognitive functions with respect to memory and learning, and that masticatory hypofunction is independently related to cognitive impairment [50]. Mastication studies have historically been conducted by dental researchers to highlight features and risk factors related to Temporomandibular Disorders, to understand human stress-management strategies, or even to assess orthodontic treatments, full dentures, prosthodontic restorations and functions like speech and bruxism. More recent evidence on Public Health impact of mastication urges scientists to identify occlusal characteristics which interfere with a healthy masticatory function, so as to pinpoint preventive measures or minimize health impairments. The findings presented here are supported by the extensive previous works

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of authors who approached their research with different apparatuses. This study's aim was to identify a correlation between specific occlusal features and what is so far known about the characteristics of 'dysfunctional chewing patterns'. The results from chewing tests by means of a Standardized Food in combination with condylographic studies should be promoted in the future to better understand chewing patterns, but most importantly to make a statement concerning which occlusal characteristics are to be detected early, which are to be avoided in rehabilitation procedures, and which goal should occlusion therapy pursuit. In the near future, individual compensatory capabilities should not be called upon to excuse poor occlusal therapies, and other conditions within the psychosocial sphere should no longer justify a sudden or progressive onset of stomatognathic symptoms. This study's limitations are surely related to the small number of cases. The study design did not require a minimum number of occlusal patterns (such as monolateral crossbite for

instance) and even though some differences were statistically significant, a higher number of cases, a more robust study design and a less numerous choice of outcomes might allow to draw more powerful conclusions and influence clinical work.

### **CONFLICT OF INTEREST**

The authors declare no conflict of interest.

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### **AUTHOR CONTRIBUTIONS**

GT: contributed to the study design, protocol, data gathering, analysis, manuscript preparation. CT: contributed to the study design and manuscript revision. GS: contributed to the concept, study design, analysis and interpretation of data.

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### Questions

### 1. Tooth loss predicts the development of MMI among the elderly:

- □a. Absence of tooth loss and preservation of the patients' baseline status meant no increase in risk of MMI;
- □b. In case of periodontitis-induced tooth loss, cognitive function is worsened;
- □c. If teeth are lost before the age of 50;
- □d. None of the answers is correct.

### 2. Mastication influences the activity in the cerebral cortex:

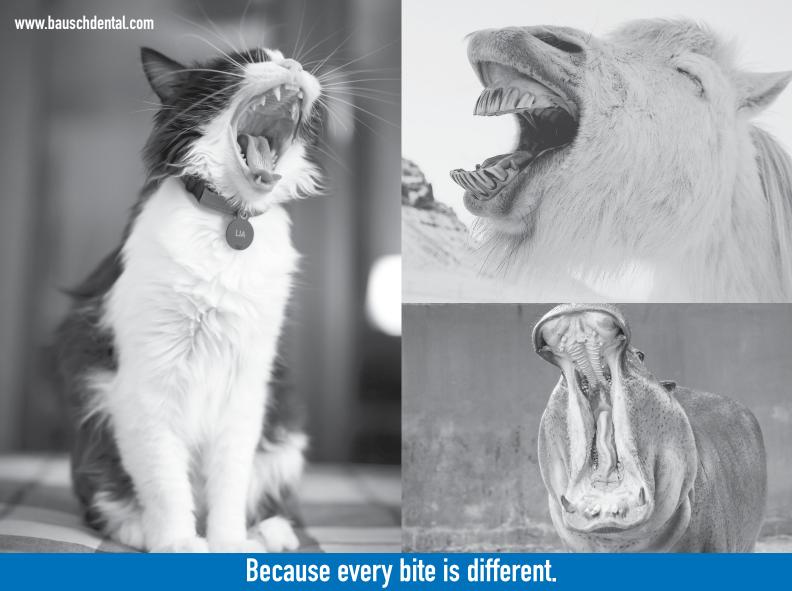
- □a. Through blood flow increase especially in the prefrontal cortex and the hippocampus, where learning and memory performance reside;
- □b. Through increase of the number of neurons and synapses;
- □c. Mastication and physical activity are rhythmic movements and have no beneficial effects on learning and spatial memory;
- □d. Regardless of the presence of teeth.

### 3. In geriatric evaluations:

- □a. Tooth count is the main predictive factor which should be assessed since patients are already being fed on mashed food;
- □b. Tooth count and chewing performance should both be considered;
- □c. Chewing performance only has relevance as a stress test for the Stomatognathic System;
- □d. Chewing performance and tooth count are both to be considered after the onset of dementia.

### 4. Recording of a healthy chewing function should possess:

- lacktrianglea. Symmetry between working and non-working sides;
- □b. A recognizable preferred chewing side;
- ☐c. No specific pattern is relevant, as long as the subject is asymptomatic;
- □d. Repetition, reproducibility and harmony in all projections, with a distinctive scheme for working and non-working sides.









### THE INFLUENCE OF THE ORAL MICROBIOME ON GENERAL HEALTH

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### **ABSTRACT**



**Background** The oral microbiome hosts a large number of microorganisms that play a critical role in oral and general health. Many factors can cause dysbiosis of the otherwise beneficial interrelationship between host and microbiome. Understanding and identifying microbial shifts and keystone pathogens that correlate with general diseases of the body holds many opportunities.

**Objective** The aim of this literature review is to present scientific evidence about disease-associated microbial shifts in the oral cavity, the effect on oral and general health and give the reader insights into alternative, new treatment approaches. This review is also intended to inspire practitioners to consider a more holistic care approach.

**Data sources** A search was performed using PubMed, Google Scholar and ScienceDirect. The general key terms used were: "Oral microbiome AND health", "Oral microbiome AND general disease". Disease-specific searches were performed. Articles found by cross-referencing were included.

**Study selection** Original studies and meta-analyses were included. Articles published in 2012 or later were preferentially considered. Reviews were included if deemed valuable and labelled in the text. Articles not written in English were excluded.

**Data extraction** The reviewer assessed each article for their relevance and methodology. Results were evaluated according to their sample size and whether or not the same result was replicated in different studies.

**Data synthesis** Individual etiological factors correlate with several, seemingly unrelated diseases. This article directs the reader towards understanding the more unusual interconnections and utilizing multidisciplinary treatment approaches, that are aimed at reestablishing an overall balance within the oral microbiome.

### **KEYWORDS**

Oral Microbiome; Systemic Diseases; Oral Health; Probiotics; Dysbiosis.

### 1. INTRODUCTION

The human body hosts many complex microbial communities that influence functions like digestion, metabolic regulation, immune response, and there is evidence that a dysfunctional microbiome promotes the development of diseases [1]. Our oral microbiome comprises at least 772 prokaryotic species, second in diversity only to that of the gut. The oral cavity exhibits a large number of surfaces and

crevices to which aerobe and anaerobe microbes can adhere, forming biofilms. These extracellular biofilms are found everywhere from the buccal mucosa to the tooth surfaces, dorsum of the tongue and gingival sulci. In addition to bacteria, diverse forms of fungi, viruses, protozoa and archaea are found as part of the normal oral microbiome [2]. Various studies have shown that an unbalanced microflora not only correlates with diseases of the oral cavity, but those of other organ systems, too [3].

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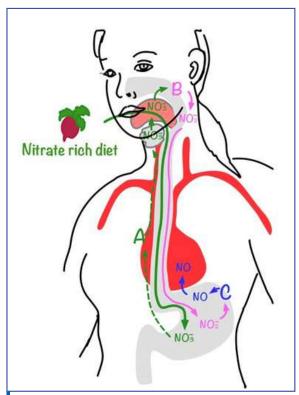


Figure 1. Enterosalivary nitrate-nitrite-nitric oxide pathway Nitrate (NO3-) is ingested and enters the stomach where it is systemically absorbed (A). It is then concentrated in the salivary glands and released into the oral cavity. Oral bacteria convert nitrate to nitrite (NO2-) (B), which is then swallowed and converted to NO when it comes into contact with the gastric juice (C). The NO in the lumen of the stomach and the nitrite enter the circulation where the nitrite also gets converted to NO. NO causes vasodilation and the reduction of blood pressure.

The "keystone pathogen" hypothesis suggests that certain pathogens that are present in low abundance can alter a benign microbiome into a dysbiotic one. Identifying and understanding keystone species is important for the development of new treatment strategies [4]. This review is intended to display these keystone species in their correlation to oral and general diseases. It also presents alternative, new treatment strategies.

Because the oral cavity acts as the port of entry to several different organs, non-oral diseases such as diabetes, cardiovascular, gastrointestinal, liver, lung and brain disease, as well as some types of cancer, Rheumatoid disease, Alzheimer's and pregnancyrelated complications are associated with oral microbiome dysbiosis [2]. Because of its direct contact with the external environment, the oral cavity faces physical, chemical, and biological variables that can have damaging effects. General health related to a healthy oral microbiome is therefore strongly influenced by factors such as diet and hygiene [2]. Constant advances in metagenomics and next generation sequencing techniques help characterize the functional role of microorganisms. Especially 16S rRNA sequencing has contributed to revealing the complexity of the oral microbial communities. Utilizing this information for diagnostic and therapeutic purposes holds many opportunities [3].

The most extensive database documenting all cultivable and uncultivable bacteria of the oral cavity is the expanded Human Oral Microbiome Database (eHOMD) [5]. It plays a valuable role for researchers to understand the composition of the microbiome in detail, and its function in health and disease.

### 2. METHODOLOGY

A literature review was performed using PubMed, Google Scholar and ScienceDirect. A detailed search using the following keywords was performed: "Oral microbiome" AND "health", "systemic diseases", "caries", "periodontal disease", "cardiovascular disease", "Bacteria AND nitric oxide", "Nitric oxide AND hypertension", "antibacterial mouthwash", "diabetes mellitus", "oral cancer", "Oral squamous cell carcinoma", "Alzheimer's disease", "respiratory disease", "pregnancy", "probiotics". Further database searches were done about specific oral bacteria and their role in certain diseases and health.

Original research articles published in 2012 or later, high-quality studies and meta-analyses were preferentially considered, review articles were added if deemed valuable for the reader and were labelled in the text. Articles found by cross referencing were also included. Articles not written in English were excluded. The studies were assessed for their statistical value and methodology. The results were evaluated according to their sample size and whether or not the same result was replicated in different studies. Ultimately, 82 publications were chosen for this review.

### 3. RESULTS

### 3.1. Composition of the oral microbiome

In the oral cavity we find an array of different microbes. In addition to the more commonly considered bacteria, different types of fungi, viruses, protozoa, and archaea are present in the oral cavity and appear to be in a symbiotic relationship with the host. Understanding the specific taxa present in the core microbiome helps to identify a state that is close to being considered "healthy". Some of these taxa will be further discussed in the sections of this review investigating specific diseases. Research has been conducted mainly focusing on the bacteria of the oral cavity. Less is known about the nonbacterial microbiome, which we will therefore not discuss further. A microbiome consists of two main parts. Data from the NIH human microbiome project (HMP) revealed that there is an identifiable core ("core" - commonly shared taxa) of microorganisms that can be found among unrelated individuals in all microbiomes of the body [6]. The largest core microbiome was found in the oral cavity consisting of 7 taxa. Those being, in the order of their prevalence from high to low: Streptococcus, Pasteurellacaceae (family), Veillonella, Fusobacterium,

Lactobacillales (family), Prevotella, and Gemella [7]. This core microbiome is common to all individuals, and together with a variable microbiome, it forms a whole microbiome. The variable microbiome of the oral cavity differs strongly from person to person depending on the diet, physiological differences, ethnicity and even socioeconomic status [8].

### 3.2. Development of the oral microbiome

The process of developing any microbiome of the body starts with the acquisition of the mother's microbes at birth. Depending on the type of delivery (vaginal vs. Caesarian section), the newborn comes into contact with somewhat different sets of microorganisms, resulting in differential development of the oral microbiome. Children that have been delivered vaginally show a larger variety of oral bacteria three months after birth (79 species compared to 54 in C-section delivery) [9]. It is however unclear if those differences have an effect on the development of diseases later in life. One major difference found was the presence of Slackia exigua in over 76% of C-section delivered children compared to absence of S. exigua in vaginally delivered children [9]. S. exigua has been associated with periodontitis, root canal infections and can be found in intestinal abscesses and extra-oral surgical wounds [10,11]. Differences in the development of the oral microbiome are also being seen in breastfed compared to formula-fed infants. A larger amount of lactobacilli colonize in the oral cavity of breastfed children, which has been found to inhibit the growth of other potentially pathogenic bacteria and likely contributes positively to the general and oral health [12]. In addition to the vertical transmission of microbes at birth and variations in breastfed vs. formula-fed children, the process of ageing and the exposure of children to different environments has been found to affect the oral microbiome as well [13].

### 3.3. Oral microbiome and oral diseases 3.3.1. Caries

Many studies show that the presence of certain bacteria is highly associated with caries in adults and children. Gram-positive bacteria of the genera Streptococcus, Actinomyces, Lactobacillus and Propionibacterium were found to be the main cause for carious lesions. Specifically, the combination of Streptococcus mutans and Scardovia wiggsiae is often detected in severe childhood caries and advanced dentinal caries [14,15]. Other than dietary adjustments and fluoride application [16], there are a number of suggested possibilities to prevent caries and aid in caries treatment. Food supplements in the form of prebiotics or probiotics have shown promising results. The prebiotic arginine has been shown to prevent dental caries. Arginine is broken down to ammonia, ornithine, CO, and ATP by commensal bacteria. Ammonia is an alkaline compound that keeps the pH value close to neutral and prevents

demineralization while promoting remineralization. An environment is created in which commensal bacteria have an ecological advantage and can outcompete acid-tolerant species like Streptococcus mutans and Lactobacilli [17]. The measurement of the reaction that causes the arginine breakdown in dental plaque, the so-called arginine deiminase system (ADS), could potentially help in determining the caries risk [18]. Similarly, adding species that are associated with a healthy microbiome may increase the buffer capacity of the microbiome and decrease the chance of dysbiosis. Two species, in particular, have shown promising results. Streptococcus dentisani and Streptococcus A12 are capable of increasing the pH of the dental plague and can inhibit the growth of caries associated to Streptococcus mutans. Streptococcus A12 produces a protease that plays an important role in the inhibition of bacteriocin production by Streptococcus mutans [19]. Bacteriocins are known to poison competitive bacteria. Streptococcus dentisani actually produces bacteriocins itself, which help limit the growth of pathogenic species [20]. Streptococcus salivarius strain M18 has also been found to be effective in reducing plaque and inhibiting Streptococcus mutans [21]. Another approach to probiotic caries treatment is supplementation with engineered microbes, especially altered Streptococcus mutans strains, that displace native Streptococcus mutans and show lower pathogenicity [22]. It is safe to say that probiotic supplementation can be effective in preventing caries development, although usage should be managed by a professional, and supplements need to be taken regularly to function.

### 3.3.2. Periodontal disease

Another disease that is highly associated with microbiome dysbiosis and presence of certain microorganisms is periodontal disease. The main pathogens involved in periodontal disease have long been known. Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis, Tannerella forsythia, and species of Treponema and Prevotella, but especially a combination of species, the so-called "red complex" consisting of Porphyromonas gingivalis, Tannerella forsythia and Treponema denticola, are associated with periodontitis [23]. According to more recent microbial analyses, Porphyromonas spp., Filifactor alocis and Tannerella forsythia and the absence of Neisseria and Prevotella denticola are considered risk factors of periodontitis, while the presence of Aggregatibacter actinomycetemcomitans, Cardiobacterium hominis, Peptostreptococcaceae sp., P. alactolyticus and absence of Fretibacterium spp., Fusobacterium naviforme/Fusobacterium nucleatum Vincentii, Granulicatella adiacens/Granulicatella elegans were associated with aggressive periodontitis [24,25]. Fusobacterium necrophorum, Lactobacillus acidophilus, Staphylococcus aureus and Streptococcus pneumoniae are associated with



periodontal health [26]. Diagnosing periodontal disease is mainly done by clinical examination and predicting its course remains a challenge. Often times, periodontitis can reoccur without symptoms, and regular examination of all tooth sites is necessary. Diagnostic tools that could give an insight into the state of disease-progression are not yet fully developed, but current findings reveal promising parameters. A recent study reveals that a significant shift in the taxonomic composition occurs between the diseased and resolved/healthy state of the periodontal pockets. While the microbiome is very similar among different tooth sites in the diseased state, a highly variable taxonomic composition has been found in the resolved state. This discovery would suggest that disrupting the synergistic state of a diseased periodontium could help to treat and prevent periodontal disease, however, this needs to be investigated further [27]. Another study investigated the diagnostic potential of analyzing the salivary proteome. Again, differences in healthy and diseased individuals were recognizable. In patients with chronic periodontitis, the amount of salivary proteins was downregulated. These proteins are associated with oral homeostasis and protective functions. The suggested mechanism is that many integral salivary proteins are suppressed by the increased bacterial load and high levels of bacterial proteases [28]. Alternative approaches to established treatment methods are based on changing the bacterial composition in the periodontium. Studies investigating the additional administration of probiotics to mechanical treatment are limited, and so far have failed in showing lasting clinical improvement compared to standard therapy. Patients in the group receiving Lactobacillus rhamnosus SP1 probiotic supplements in addition to mechanical debridement showed a greater reduction in cultivable microbiota compared to the group that only received mechanical debridement in one study, but clinical improvement showed no significant lasting differences [29]. This is in line with the outcomes of 13 other publications investigating the effect of probiotic supplementation on periodontal disease, which are discussed in a systematic review by Jayaram et al. [30]. Large-scale, long-term studies are necessary to understand to what extent probiotic treatment should be administered. Another approach to specify the state of periodontal disease is to analyze the bacteriophage community. The bacteriophage expression was found to be altered in the periodontal disease. For example, Santiago-Rodriguez et al. found that in subjects with periodontal disease, specific lytic bacteriophage genes are more highly expressed compared to healthy controls. The authors assumed that the lytic cycle of some bacteriophages might be supported by the inflammatory state of periodontal disease. Lytic bacteriophages increase the stress load on bacteria, which can increase bacterial resistance to

certain environmental conditions, like increased or decreased pH [31]. This might be one of several reasons why the periodontal disease can be hard to eradicate, and regular maintenance care is highly important.

### 3.3.3. Oral cancer

Well-known risk factors for oral cancer are tobacco, alcohol, betel-nut, and human papillomavirus (HPV) infection [32,33,34]. There is, however, increasing evidence that the oral microbiome harbors bacterial species that are highly associated with a variety of cancer types. Many recent studies investigate the connection to orodigestive cancers, predominantly cancers such as oral squamous cell carcinoma, the most common malignant transformation of the head and neck. Chronic inflammation, as present in the periodontal disease, may be considered as a risk factor in cancer development. A metaanalysis comprising 38 studies with a total of 7184 participants has found that six periodontal bacteria are associated with the occurrence and prognosis of orodigestive cancers, namely Porphyromonas gingivalis, Tannerella forsythia, Aggregatibacter actinomycetemcomitans, Treponema denticola, Fusobacterium nucleatum and Prevotella intermedia. Of those six, two, namely P. gingivalis and P. intermedia, were associated with a higher incidence of cancer. P. gingivalis infection increased the risk of cancer by a factor of 2.16 [35]. No obvious relationship between the four other species and an increase in cancer risk was found, although other studies have stated that T. forsythia, A. actinomycetemcomitans and T. denticola had positive effects on cancer progression [36,37,38]. P. gingivalis and F. nucleatum infection were additionally associated with poor overall survival 14 and might therefore be used as a marker to evaluate the overall prognosis. The authors concluded that improving oral hygiene and treating periodontal disease are important factors in the prevention and treatment of orodigestive cancers [35]. Another recent study underlines the findings of the meta-analysis and points out that P. gingivalis and F. nucleatum are the dominant bacteria correlated with oral squamous cell carcinoma (OSCC). P. gingivalis was found in significantly higher abundance in OSCC lesions compared to the tissue directly surrounding the cancer and healthy tissues. Furthermore, the bacterium was found in several layers of the tumor, from the epithelial layer to the deeper tissues. In healthy tissues, it was only found in the epithelial layer. The suggested mechanism was that "P. gingivalis-infected cells escaped the immune surveillance function of the host and appeared to proliferate rapidly, did not differentiate into maturity and then resulted in cancer progression" [39]. P. gingivalis has also been found in esophageal cancer lesions and was associated with disease progression [40]. P. gingivalis may therefore be an important marker for the severity and progression of cancers in the oral cavity and esophagus. As mentioned earlier,

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P. gingivalis is also involved in the development and progression of periodontal disease and detection of the bacterium may be useful in evaluating the disease-severity and treatment-success in both periodontitis and oral cancer. In the future, rapid chair-side testing for oral bacteria might become more widely available. One study has been conducted using a not commercially available immunochromatographic detection device, which was specifically made for the study and provided immediate chair-side results. It showed that the detection device accurately recognized all P. gingivalis-type strains. No difference to expensive laboratory real-time PCR testing was detected [41].

### 3.4. Oral microbiome and systemic diseases

Many studies suggest links between oral bacteria and diseases of the pulmonary, cardiovascular and gastrointestinal system, cancer, diabetes, Alzheimer's and even adverse pregnancy outcomes. After tooth brushing, flossing, and dental procedures, bacteremia frequently occurs. Bacteria, toxins and inflammatory products enter the systemic bloodstream and translocate to other sites in the body where they can cause disease, especially in people that are immunocompromised or have a predisposing condition [42,43].

3.4.1. Cardiovascular disease

### 3.4.1.1. Atherosclerosis

One predisposing factor for most cardiovascular diseases is atherosclerosis, a condition caused by the accumulation of atheromatous plaques in vessel walls, which have been shown to contain bacteria typically found in periodontal disease. A. actinomycetemcomitans, P. gingivalis, T. forsythia and P. intermedia were previously detected in human atheromatous plaque, which suggests that these oral pathogens translocated there from the oral cavity [44,45]. These bacteria are able to release outer membrane vesicles containing the endotoxin lipopolysaccharide (LPS). LPS's are immunomodulators that can cause an inflammatory response when binding to receptors on macrophages or dendritic cells. Atherosclerosis is in turn initiated by inflammation of arterial endothelial cells [46]. Having atherosclerosis increases the risk of stroke. Stroke has also been found to be causally linked to periodontal disease, and it has been shown that regular dental care lowers the risk of stroke [47]. 3.4.1.2. Hypertension and the link to nitric oxide Multiple recent studies have investigated a link between the composition of the oral microbiome and increased blood pressure. Two of these present a possible connection of childhood caries with arterial hypertension [48,49]. The increased inflammatory state caused by caries and periodontal disease could be causative for the increase in blood pressure. Oppositely, the presence of certain bacteria that are involved in the so called enterosalivary nitrate-nitrite-

nitric oxide pathway can affect the nitric-oxide (NO)

homeostasis and reduce high blood pressure (Fig. 1). NO is an endogenously produced molecule that mainly acts on smooth muscle cells and causes them to relax, which results in vasodilation and a reduction in systemic blood pressure. NO is normally produced in endothelial cells in an oxidation process that requires the enzyme nitric oxide synthetase (NOS). Cases, where endogenous NO generation from NOS is compromised, can occur. Endothelial dysfunction, for example, is associated with a decrease in NO generation [50]. It is recognized that oral bacteria play an important role in the physiology of NO generation that is independent of the enzyme NOS pathway. Several studies show that supplementation with nitrate (NO3-) and adhering to a diet rich in nitrate and nitrite (NO2-) is linked to blood pressure reduction [51,52]. When dietary nitrate is ingested, it enters the circulation and gets absorbed and concentrated in the salivary glands. Up to 25% of the ingested nitrate is concentrated in the salivary glands, which is approximately ten times as much as the nitrate concentration in plasma [53]. When the saliva comes into contact with commensal bacteria (mainly on the surface of the tongue), it is converted to nitrite [54]. In the stomach, nitrite is converted to nitrous acids and NO, that enters the circulation. There is a clear relationship between nitrate-reducing oral bacteria and the generation of salivary nitrite [54]. The bacteria that have been identified to contribute to optimal nitrate reduction are Actinomyces odontolyticus, Actinomyces viscosus, Actinomyces oris, Granulicatella adiacens, Haemophilus parainfluenzae, Neisseria flavescens, Neisseria mucosa, Neisseria sicca, Neisseria subflava, Prevotella melaninogenica, Prevotella salivae, Veillonella dispar, Veillonella parvula, and Veillonella atypica [55].

A recent study has shown that tongue cleaning and the use of chlorhexidine over a time period of seven days has been shown to increase systolic blood pressure. Subjects that cleaned their tongue several times a day showed a higher increase in blood pressure than subjects that cleaned their tongue irregularly. Disruption of the papillary surface by excessive cleaning of the tongue might increase penetration and efficacy of chlorhexidine and therefore reduce the amount of nitrate-reducing bacteria more than in the group that only irregularly cleaned their tongue. And in fact, differences were identified in the tongue microbiome composition between the two groups. Recovery from using chlorhexidine showed an increase in nitrate-reducing bacteria on the tongue and stabilization of the blood pressure, which underlines the importance of the oral microbiome in blood pressure regulation [56]. Furthermore, adjusting the diet in patients with increased blood pressure in combination with nitrate supplementation might play an important role in providing exogenous NO and has been found to be beneficial for cardiovascular health [57,58]. Dietary nitrate is mainly obtained from green leafy vegetables,



such as rocket, spinach, kale and lettuce and from beetroot. Nitrate is also commonly found as a food additive in processed meats [59]. Additionally, oral nitrate-reducing bacteria have an effect on the shortterm regulation of blood pressure. It was discovered that they play a role in post-exercise hypotension (PEH) and skeletal muscle oxygenation. In subjects that used antibacterial mouthwash, PEH was decreased, and so was muscle oxygenation. It seems that regular use of antibacterial mouthwash can change the composition of the oral microbiome and make subjects more prone to hypertension [60]. Not only chlorhexidine mouthwash but also the commonly used mouthwash "Listerine" (Johnson & Johnson, New Brunswick, NJ, USA), used daily, is considered an effective antibacterial agent [61]. In summary, excessive tongue cleaning and overuse of antibacterial mouthwash may be unfavorable, as it can lead to undesirable shifts in the oral microbiome. These hygiene regimens are usually considered beneficial when done in moderation. As NO is an omnipresent signaling molecule, effects caused by the oral bacteria involved in NO production may have an even more significant effect on human health than currently assumed.

### 3.4.2. Diabetes mellitus

Recently, indiscriminate use of antibacterial mouthwash has also been linked to diabetes mellitus. According to a study including 945 individuals, the diabetes risk is increased by 55% in individuals using mouthwash twice a day compared to individuals using mouthwash less frequently and is increased by 49% compared to individuals not using mouthwash at all [62]. A possible underlying mechanism was reviewed by Sansbury et al. The authors discussed that a reduction of oral bacteria that are part of the enterosalivary nitrate-nitrite-nitric oxide pathway reduces NO bioavailability which in turn might increase the risk of developing insulin resistance and obesity. The authors however concluded that additional research is necessary to substantiate this hypothesis [63]. Preshaw et al. further discussed the association of mouthwash and diabetes as suggested by Joshipura et al. and pointed out that due to methodological limitations, recommending against the use of mouthwash on the basis of this study needs to be considered individually [62,64]. If mouthwash use is recommended or not is a question that dental professionals are regularly faced with and having more background information on the topic might help in evaluating the potential downsides and benefits of mouthwash in each individual case. Correlations between diabetes mellitus and alterations of the oral microbiome have been established. In patients with diabetes mellitus, a shift in the oral microbiome has been observed. In an animal study, increased levels of oral interleukin-17, a cytokine promoting inflammation, was detected in diabetic mice [65]. Furthermore, the phylum Actinobacteria and especially the genera Actinomyces

and *Atopobium* were found to be significantly less abundant among patients with diabetes and also less abundant among obese patients [66]. And in patients with periodontitis and diabetes, *Prevotella copri, Alloprevotella rava* and *Ralstonia pickettii* numbers were increased compared to controls, but normalized after effective glycemic control [67], thus reconstructing the health of the oral microbiome. Despite well documented correlations of abnormalities of the oral microbiome and diabetes mellitus, the underlying causal relationships and potential clinical implications remain to be elucidated.

### 3.4.3. Alzheimer's disease

Several studies suggest that the presence of pathogenic bacteria in the oral cavity (mainly species also found in periodontal disease) and the development of Alzheimer's disease (AD) are related. A study with 158 participants showed that those who later developed AD (n=35) had significantly increased antibody titers against F. nucleatum and P. intermedia compared to healthy individuals, with a milder increase noted in participants that went on to develop mild cognitive impairment (n = 46). Antibody titers were also elevated for T. denticola and P. gingivalis [68]. Lipopolysaccharides of P. gingivalis have also been found postmortem in the brain tissue of AD patients, but not in the control group [69]. While abnormal growth of P. gingivalis and other periodontal pathogens appear to increase the risk to develop AD, a positive correlation between the level of pathogens and the severity of AD is not established. Periodontitis was found to increase the risk of AD in other studies as well, and it was also detected that the salivary microbiome was changed in AD patients [70,71]. One of the theories trying to explain why periodontitis increases the risk to develop AD suggests that inflammatory mediators produced by oral bacteria are able to migrate into the blood stream and cause an increased systemic proinflammatory state in the brain [70]. Patients with AD often show a decline in personal oral hygiene, a factor that might further worsen periodontal health and the systemic inflammation [72]. To break this vicious circle, AD patients should be supported with maintaining good oral hygiene and treated for periodontitis if present. Analysis of saliva contents revealed the presence of brain-proteins that are considered markers of AD. Amyloid-beta levels were higher in mild and moderate AD patients and unchanged in severe AD patients [73]. Reduced lactoferrin levels were detected in patients with mild cognitive impairment and AD. Interestingly, the level of accuracy those samples provided was higher than that obtained from cerebrospinal fluid biomarkers [74] C. Tau-proteins were also found in higher levels in AD patients compared to the healthy control group [75]. Sampling these from saliva rather than from CSF and/or blood samples is less invasive and therefore an interesting and promising method to diagnose

and monitor the disease, however, as of now, it is less well established. The bottom-line criterion so far is that oral hygiene is highly important in AD patients and needs to be included in AD therapy along with the periodontal disease treatment.

### 3.4.4. Pneumonia

Unhealthy oral cavities host bacteria that are involved in respiratory infections like pneumonia. Periodontal disease has been associated with an increased risk of acquiring nosocomial pneumonia [76]; in fact, one study concludes that individuals with periodontitis were almost three times as likely to present with nosocomial pneumonia compared to those without periodontitis [77]. Several oral pathogens, including *P. gingivalis* and *T. denticola* have been associated with pneumonia [78] and it has been shown that increased oral hygiene reduces the incidence of ventilator associated pneumonia [79].

### 3.4.5. Pregnancy-related conditions

Maternal infections can lead to adverse pregnancy related outcomes, including preterm birth and decreased birth weight. The levels of P. gingivalis, Tannerella forsythia, Prevotella intermedia and Prevotella nigrescens were found to be significantly higher in preterm births compared to those with term deliveries. Additionally, a low maternal IgG antibody response to the above-mentioned pathogens increases the risk of a preterm birth [80]. A recent meta-analysis of 20 articles including 10215 women concluded that the risk of preterm birth is doubled in mothers with periodontitis [81]. The results of one study indicate that preventive dental treatment has a beneficial effect on prolongation of the pregnancy and birth weight [82]. These results clearly justify the need for preventive dental care and periodontitis treatment in pregnant women.

### 4. DISCUSSION

Advances in the understanding of oral microbial communities and their dynamics have revealed a large level of biodiversity among oral bacteria and are continuously enriching the study of microbiomeassociated diseases. Next-generation sequencing techniques and especially 16S rRNA gene sequencing have helped to identify and characterize complex bacterial communities [3]. For both dental professionals and patients alike, it is of high importance to realize that maintaining a balanced oral microbiome is part of maintaining health. Understanding the consequences of individual habits is in direct connection to that. It has been established that a large number of oral bacteria are directly

involved in disease development and progression. The traditional treatment of periodontitis possibly combined with alternative treatments is important in restoring oral health, and in preventing and treating oral and extra-oral diseases. Ongoing research of probiotic supplementation is revealing more and more beneficial effects and probiotics specifically designed to restore a healthy oral microbiome have recently been developed. In this article issues like diet, stress and smoking have not been discussed extensively. It is very well known that stress and smoking are etiological factors for oral microbial dysbiosis. Diet can affect the microbial communities of the body in different ways and it would be worth devoting a separate article to these topics.

### 5. CONCLUSION

Advanced screening of the oral microbiome has made it possible to tie seemingly unrelated diseases to the same etiological factors. This holds considerable potential for early diagnosis and disease prediction. Increasing numbers of keystone pathogens and their specific role are being identified. Their presence in the oral cavity often correlates with microbial dysbiosis and the development of caries, periodontal disease, oral cancer and a number of extra-oral diseases as described in detail above. Maintaining good oral hygiene appears to be key to reduce the risk of developing microbial dysbiosis in the oral cavity, which includes not overly reducing oral bacteria. Many new treatment approaches that directly influence the bacterial composition of the oral microbiome have been discovered in recent years. The overall understanding of the oral microbiome and its role in general health is still limited. Further research is therefore indispensable.

### **CONFLICT OF INTEREST**

The authors declare no conflict of interest.

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### **AUTHOR CONTRIBUTIONS**

JR:Data gathering, data analysis, data interpretation, manuscript drafting. AR:contributing to the concept and conduction of the manuscript. GS:critical revision of the manuscript. IC: scientific and technical reviewer of the manuscript.



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### Johannes Friedrich Carl ROHR

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### Questions

### 1. The oral microbiome consists of

- □a. Random bacteria that are different in every individual;
- □b. A core microbiome that is very similar among different individuals, along with a variable microbiome;
- □c. Only bacteria and no viruses;
- □d. B and C are correct.

### 2. Increased oral hygiene is highly recommended for:

- □a. Alzheimer's patients;
- □b. Patients with severe dental abrasion;
- □c. A generally healthy patient;
- □d. All of the above are correct.

### 3. Oral probiotics can...

- □a. Kill specific keystone pathogens directly;
- □b. Replace the need of brushing your teeth;
- □c. Be effective as an adjunctive treatment;
- □d. Have severe side effects.

### 4. Indiscriminate use of antibacterial mouthwash has been linked to:

- □a. An increase in blood pressure and a decrease in post-exercise hypotension;
- □b. A decrease in blood pressure and an increase in post-exercise hypotension;
- □c. Nothing, using mouthwash as much as possible is beneficial for the oral flora;
- ☐d. Periodontal disease.







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### DRAL AND MAXILLOFACIAL SURGERY

# case Repor

### A RARE CASE REPORT OF SYPHILIS MIMICKING AN OROPHARYNGEAL NEOPLASM

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### **ABSTRACT**



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Aim Syphilis is a widely spread, sexually transmitted disease that is often considered archaic, but it has been on the rise in recent years. The oropharyngeal region is an uncommon location for treponema pallidum to present itself. It is even more uncommon when, on a radiological evaluation, it is diagnosed as a neoplasm or squamous cell carcinoma. This case report discusses a rare case of syphilis that mimics an oropharyngeal carcinoma.

Summary The patient presented himself initially with cervical lymphadenopathies and an oropharyngeal lesion. On the MRI scan, the lesion was suspicious for an oropharyngeal squamous cell carcinoma. Multiple negative biopsies (2X) urged the search for an alternative diagnosis. After serologic testing, it became clear the patient was suffering from syphilis. Syphilis is known as the "great pretender" and can present in a nonspecific manner.

### **Key learning points**

- i) Syphilis is known as the "great pretender".
- ii) Treponema pallidum infections need to be taken into account as a differential diagnosis in patients with an oropharyngeal lesion.
- iii) Syphilis is on the rise.
- iv) Syphilis has the ability to mimic a malignancy upon clinical presentation.
- v) Syphilis has the ability to mimic a malignancy on CT and MRI-imaging.

### **KEYWORDS**

Syphilis; Cervical Lymph Node; Oropharyngeal Ulceration; Carcinoma; Mimicking.

### 1. INTRODUCTION

An increasing incidence of syphilis is inherently related to an increasing number of atypical presentations of the disease. Syphilis is on the rise and, with the increase in cases, there has been an increase in different and unusual presentations of the disease.

Since 2010, reports of syphilis incidence rates in the European Union (EU) have been ever increasing. This trend seems to be accelerating, predominantly amongst male homosexuals [1]. Syphilis is a sexually transmitted infection caused by Treponema pallidum, a well-known pathogen that can cause pathology in the maxillofacial area. Although there is little

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literature describing oropharyngeal treponema pallidum infections as mimicking an oropharyngeal squamous cell carcinoma (OPSCC), it has been described in rare cases [2]. This case report presents such a case.

Ulcers of the oral cavity and the oropharynx are frequently encountered in maxillofacial practices around the world. Consequently, the differential diagnosis of oral and oropharyngeal ulcers is an important one. A wide range of causes for oral or oropharyngeal ulcers are reported, such as aphthous, traumatic, malignant, tuberculosis, HIV and AIDS (Table 1).

Ulcers can also present associated with skin lesions [3]. Most of these ulcers are promptly diagnosed and treated because the oral or oropharyngeal subsite and the anamnesis are often positive indicators for diagnosis. An important differential diagnostic criterium for traumatic ulcerations is if healing occurs after removal of the probable cause. It is important however to distinguish innocent solitary ulcerations from malignant lesions, such as squamous cell carcinomas. Lesions that persist longer than 3 weeks should be considered as malignant until proven otherwise.

A tuberculous ulcer is rare, almost always secondary to lung tuberculosis and preferably located on the tongue (or lips). It is often not painful and regional lymphadenopathy is usually present. A rare cause of chronic solitary ulcers is syphilitic ulceration. Ulcer usually develops on the lips and, rarely, on the tongue, the pharynx, or the tonsils [4]. In even more rare cases, the ulcer will present itself mimicking a neoplasm in the oropharynx on a clinical and a radiological basis. Hence, it has been named "the great pretender" [5].

### 2. CASE REPORT

A 35-year-old male with no significant medical history was referred to the Cranio-Maxillo-Facial department of the University Hospital of Antwerp, Belgium with swallowing difficulty and minor pain in the throat for the last two months. He suffered from significant fatigue and unexplained weight loss of 10 kg in the previous months. He noticed a swelling of the left oropharynx and multiple bilateral lumps in his neck. There was no history of smoking, oncological disorders, or a familial predisposition to cancer. The patient claimed to have no risk factors for sexually transmitted diseases and no sexual contact for a longer period of time.

Clinical examination revealed an ulcerative mass of 30 mm by 50 mm in size in the left oropharynx (Fig. 1). The ulceration was associated with moderate pain complaints.

The patient had multiple swollen cervical lymph nodes, which were painless. No facial deformities or any neurological abnormalities were noted. The thyroid gland presented normal upon swallowing and was not enlarged. Further intra-oral examination

of the tonsils, mucosa, hard and soft palate, floor of the mouth, dentition, and tongue showed no abnormalities.

### 3. RADIOGRAPHIC EXAMINATION

A multi-slice computerized tomography scan (CT) with iodine contrast revealed an irregular lining on the left of the posterior wall of the oropharynx (Fig. 2). Furthermore, the scan showed a slight retropharyngeal gray area without any collection or fluid build-up.

There were multiple lymphadenopathies in the neck, at levels II to III on the left side and on level II on the right side. In addition, a magnetic resonance imaging (MRI) scan was performed to further investigate this soft tissue lesion. The MRI scan showed an oropharyngeal lesion compatible with an oropharyngeal carcinoma (Fig. 3). There was no extranodal extension in the neck. As an oropharyngeal squamous cell carcinoma was suspected, a diagnostic biopsy was performed.

### 4. PATHOLOGY

Two negative biopsies were taken by an ENT surgeon before referral. Because the clinical presentation was very suggestive for malignancy, a fiberoptic inspection under general anesthesia with biopsies was performed at our center.

The biopsy showed mucosal ulceration and a dense chronic inflammation with mixed lymphoplasmacytic infiltrate. No monoclonal cell population was detectable, and there was no evidence of dysplasia or epithelial malignancy.

### 5. DIAGNOSIS

Although the clinical and radiologic presentation was very suspect for an oropharyngeal carcinoma, multiple negative biopsies ruled out a malignancy. Alternative diagnoses were investigated and a differential diagnosis was made. After performing extensive laboratory tests, the diagnosis of an active syphilis was confirmed.

Blood tests showed a positive Treponema pallidum RPR (Rapid Plasma Regain) in combination with highly elevated titers in the Treponema Pallidum particle Hemagglutination Assay (TPHA). The TPHA test is a very sensitive treponemal test (sensitivity >95% and specificity >99%, qualitative mean accuracy, 91.4%; range, 56.1 to 98.2%; quantitative mean accuracy, 75.4; range, 55.5 to 95.5%) [6].

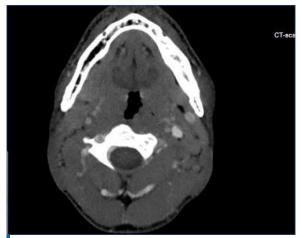
In this case, the TPHA was >20280 which indicated the presence of treponema pallidum. In addition to the TPPA result, a RPR (Rapid Plasma Regain, nontreponemal test) was performed, with a positive result (titer 2).

The results of this nontreponemal test is semiquantitative, reflecting the activity of the

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Figure 1. Ulcerative lesion seen in the left oropharynx.



**Figure 2.** Axial section of a CT scan of the head and neck showing an irregular posterior wall of the oropharynx.

 Table 1. Differential diagnosis for oral/oropharyngeal ulcers..

Traumatic	Syphilis
Malignant	Tuberculosis
latrogenic (radiotherapy, chemotherapy, antiresorptive medication, etc.)	Recurrent, painful ulcerations (Behçet's disease, chemotherapy, radiotherapy, herpetic, aphtous, etc.)

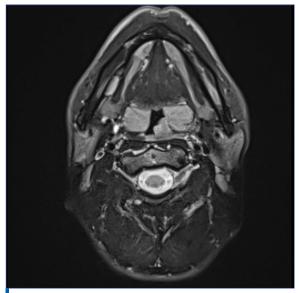


Figure 3. Axial section of an MRI scan of the head and neck showing an oropharyngeal lesion compatible with an oropharyngeal carcinoma.

infection [7]. Given the clinical presentation and serologic testing, the diagnosis of a primary/early secondary active syphilis was made. HIV testing was negative, as was the screening for Chlamydia, Hepatitis B and C, and gonorrhea.

### **6. TREATMENT**

The patient was referred to the infectious disease specialist and his general practitioner (GP) for treatment with benzathine penicillin intramuscular (2.4 million IE, one day treatment) [8].

After 3 weeks the patient was seen on consultation, and the lesion and the discomfort had disappeared.

### 7. DISCUSSION

Due to the rising number of cases of syphilis, syphilitic lymphadenitis is an important differential diagnosis for patients presenting with cervical lymphadenopathy and oropharyngeal lesions [9]. Clinicians should be aware that syphilis can also present as a squamous cell carcinoma-like lesion in most oral subsites [10]. In this case, the patient presented with weight loss, fatigue, cervical lymphadenopathies, an oropharyngeal lesion, and an MRI scan that suggested a possible oropharyngeal squamous cell carcinoma. Because multiple biopsies were negative for malignancy, an alternative diagnosis was eventually made.

It has to be mentioned, that there are different techniques to obtain an oropharyngeal biopsy, and a false-negative result may be possible if the technique employed is not optimal. In case of doubt, a new biopsy should be taken and retaken by the same surgeon, taking into account that an incision biopsy at the exact location of the lesion is the most sensitive. We advise against fineneedle aspiration cytology of ulcerative lesions because of its lower sensitivity and specificity, which are respectively 71.4% and 97.8% [11]. Syphilis can evolve into four stages, each with certain characteristics (Table 2). Given the absence of mucous patches or maculopapular lesions, and the local presentation, a working diagnosis of primary syphilis was made [12].

Tamura et al. (2008) described a case similar to the one presented in this report [13]. His patient presented with an oropharyngeal tumor and a cervical lymphadenopathy with the primary lesion being



Table 2. Most important characteristics of the four syphilitic stages.

Stages	Timing	Characteristics
Primary	3-4 weeks incubation	Painless chancre at the site of infection
Secondary	4-10 weeks after infection	Rash, systemic symptoms (fever, weight loss, fatigue), headache, mucous membrane lesions throughout body
Latent	Early latent (< 1 year after infection) Late latent (> 1 year after infection)	Asymptomatic, diagnosis through serologic testing
<u>Tertiary</u>	1-10 years after infection	Gummatous lesions, Cardiovascular issues, CNS disorders

strongly suggestive for oropharyngeal cancer. Eventually this case was diagnosed with oropharyngeal syphilis. As a FDG-PET scan was performed as part of a staging procedure, their work-up slightly differed from ours. Since FDG is not specific for malignancy, it will also accumulate in many benign processes such as inflammatory, post-traumatic or benign tumours. Preferably incisional qualitative biopsy should be taken before imaging FDG-PET scan is considered.

### 8. CONCLUSION

Treponema pallidum infections need to be taken into account as a differential diagnosis in patients with an oropharyngeal lesion when histopathological results exclude malignancy. Syphilis is known as

the 'great pretender', in their ability to mimic a malignancy upon clinical presentation and on CT and MRI-imaging.

### **CONFLICT OF INTEREST**

The authors declare that they have no competing interest.

### **ACKNOWLEDGMENTS**

There are no conflicts of interest and no financial interests to be disclosed.

### **AUTHOR CONTRIBUTIONS**

SK, CP, HV wrote the manuscript in consultation with WD and GV. All authors provided critical feedback and helped shape the research and the manuscript. SS and MV aided in interpreting the results.

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### **Sofia KALANTARY**

MD, DDS, Maxillofacial Surgery Resident ZMACK / Associatie MKA AZ Monica Antwerp, Belgium



### CV

Dr. Sofia Kalantary is a resident in Oral and Maxillofacial Surgery at the University of Antwerp, Antwerp, Belgium. She has a clinical focus in orthognathic and dento-alveolar surgery. During her training she spent two years at the St. Elisabeth Hospital, Tilburg in the Netherlands where she was trained by JPO Scheerlinck. For the last two years of her residency she specialized in the field of orthognathic surgery and 3D surgical planning at the ZMACK / MKA association of Antwerp, Belgium.

### Questions

### 1. Syphilis is a sexually transmitted infection caused by

- □a. Treponema pallidum;
- □b. Actinomyces;
- □c. Streptococcus salivarius;
- □d. Neisseria gonorrhoeae.

### 2. Primary syphilis has an incubation time of

- □a. 6 months;
- □b. 3-4 weeks;
- □c. 4-10 weeks;
- □d. 1 day.

### 3. Syphilis has the ability to mimick

- □a. Dental caries;
- □b. Periodontal disease;
- □c. Neuropathic pain;
- ☐d. A malignancy on CT- and MRI-imaging.

### 4. An oral/oropharyngeal ulcer cannot be one of the following

- □a. Syphilis;
- □b. Traumatic ulcer;
- □c. Malignant;
- □d. Hemangioma.



### A new disinfection device - Deactivate™ by Xenex

Since the onset of the COVID-19 pandemic, researchers and manufacturers of products and equipment have been frantic to develop new methods, products and equipment to effectively limit the causes.

PubMed has over 111,190 articles published on COVID-19, of which over 1,820 articles on dentistry, to which one can add the over 5 articles published by the Stomatology Edu Journal (*Stoma Edu J*).

WHO, FDI, ERO, ADA and other professional associations have developed a series of rules on protection against COVID-19, itineraries to be followed by patients, protection of patients and medical staff, disinfection of surfaces and equipment. Xenex Disinfection Services Inc., a global provider of UV-based disinfection strategies and solutions, is known for LightStrike™ Germ-Zapping rob robots, which are used by many medical institutions around the world to the room no-touch disinfection. Following the COVID-19 pandemic, LightStrike robots are now used in airports, schools, hotels, sports arenas, police stations and correctional facilities, convention centers and more to quickly disinfect rooms and large areas.

Recently, Xenex Disinfection Services Inc. has provided an efficient technology for disinfecting small spaces that are difficult to clean, a high-power LED device, Deactivate™, designed to quickly disinfect enclosed spaces, such as dental examination rooms, ambulances and pilot cabins.

Deactivate™ is a portable device that uses highpower LEDs to create ultraviolet (UV) light that deactivates pathogens, including severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), the virus that causes COVID-19. Deactivation does not require heating or cooling time and leaves no chemical residue. After disinfection, the area is immediately available for use.

Evidence-based tests performed at the Texas Biomedical Research Institute have shown that the DeactivateTM device has the following performance:

- a 99% disinfection level against SARS-CoV-2 in 30 seconds at 1 meter
- a 99% disinfection level against vegetative bacteria (methicillin-resistant Staphylococcus aureus) such as MRSA, Escherichia coli in 1 minute at 1 meter
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The manufacturer of the portable LED disinfection device, Deactivate™, recommends it for a variety of applications:

- Rapid disinfection of frequently affected areas between uses



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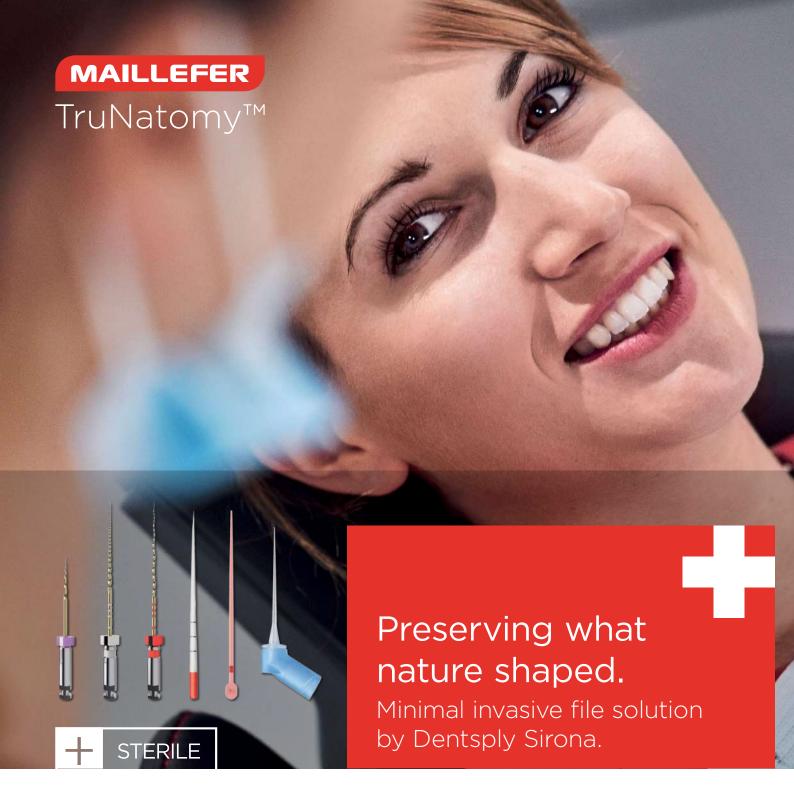
Three concerns need to be taken into account when working with UVC rays to disinfect dental rooms:

- The sensitivity of microorganisms to UVC rays is highly variable and also dependent on environmental factors such as temperature, relative humidity, environment (air, water, ...) and on the condition of the organism. Coronaviruses do not seem be among the most sensitive organisms. In other words: for most systems with usual irradiance values, the air will have to pass more than once through to achieve a useful effect.
- Regarding the health risks of UVC radiation: studies report adverse effects on the human eyes and skin with direct exposure; in addition, UVC rays have genotoxic and carcinogenic properties.
- Another practical point is the maintenance of the UVC systems, which requires qualified personnel with specific protective equipment to carry out the regular cleaning of the lamps and check their efficiency, as well as replace the lamps.

For the practitioner to be fully convinced, I would like the manufacturer to provide me with a device to be tested so as to compare its efficiency with the other devices we are currently using in our clinic.

Florin - Eugen Constantinescu DMD, PhD Student Editorial Director, Product News

https://doi.org/10.25241/stomaeduj.2021.8(1).prodnews.1



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