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CE PROGRAM FAQs



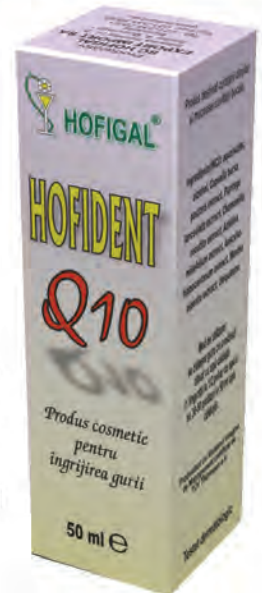
## Hofident Q<sub>10</sub>

**Product presentation:** Solution for oral hygiene.

**Composition (INCI):** aqua/water, alcohol, *Capsella Bursa Pastoris* extract, *Plantago Lanceolata* extract, *Chamomilla Recutita* extract, *Achillea Millefolium* extract, *Aesculus Hippocastanum* extract, *Mentha Piperita* extract, Ubiquinone.

**Action:** The product has antiseptic, healing, hemostatic, anti-inflammatory action, it acts as a antioxidant, detoxifier, deodorant. It is strongly recommended in gingivitis, stomatitis, thrush, compression pain caused by dental prostheses, after tooth extraction, in case of nipple lesion, bleeding gums, mouth and gum ulcers.

**Recommendations:** It delays dental plaque formation, it prevents bad odour and provides daily mouth hygiene.



## HofImun® FORTE

**Product presentation:**

Chewable tablets to stimulate the immune system

**Composition:** Each chewable tablet contains raspberry fruit extract (*Rubii idaei fructus*), Echinacea extract (*Echinacea purpurea*), concentrated extract of licorice root (*Glycyrrhiza radix*), magnesium ascorbate and excipients.

**Action:** It stimulates the immune system, it is antiinflammatory, antiviral, antiseptic, it fluidifies the bronchial and pharyngeal secretions, antioxidant, cardioprotective, vasoprotective, it has antineoplastic antileukemic action, (due to the ellagic acid), it contributes to wound healing, fortifies and remineralizes (it regulates the potassium balance), it has antiulcer effects and is an overall body tonic.

**Recommendations:** to supplement the diet with nutrients and bioactive substances in: acute and chronic infections of the upper airways (angina, pharyngitis, laryngitis, bronchitis), prophylactic during periods with increased risk of infection with influenza viruses, it has sweating effects in fever, in recurrent herpes episodes of mucocutaneous rash, frequent urinary tract infections, inflammatory urogenital processes; immunodepression after radiotherapy or chemotherapy, bacterial skin infections, psoriasis, neurodermitis, chronic cardiovascular diseases associated with hypercholesterolemia, adjuvant in the diet indicated in the treatment of gastroduodenal ulcers, tonic during periods of physical and mental strain, exhaustion.



## Bucoprotect gel

**Product presentation:** Gel for oral hygiene.

**Composition (INCI):** aqua, *capsella bursa pastoris*, *calendula officinalis*, *achillea millefolium*, *hippophae rhamnoides*, *olea europea*, *hypericum perforatum*, carbomer, triethanolamine, collagen, *foeniculum vulgare*, *mentha piperita*, *citrus amara*.

**Action:** Antiseptic, anti-inflammatory, healing, stimulates the inside lining of the mouth and gums trophicity, reduces pain caused by specific oral diseases (gingivitis, stomatitis, lesions of the prosthesis, thrush, periodontitis).

**Recommendations:** Fights against bad breath (halitosis).

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# Contents

## Editorials

- 138 Fake news, fake science – the black side of the internet  
Jean-François Roulet

- 140 The key to improving the peer-review process and rewarding the reviewer's activity: Publons registration  
Marian-Vladimir Constantinescu

## Letters

- 142 In Celebration of Professor Adrian Bejan on his 70th birthday  
Maria Greabu

## News

- 144 Impressions from London at the 96th General Session meeting of the International Association for Dental Research (IADR)  
Andrei C. Ionescu

- 145 The European Society of Cosmetic Dentistry (ESCD) celebrated its 15<sup>th</sup> anniversary in Lisbon, from the 20<sup>th</sup> to the 22<sup>nd</sup> of September  
Florin Lăzărescu

## Continuing Education Online

- 147 JADA CE Online

## Original Articles

- 148 **DENTAL MATERIALS:** Light transmission through resin composites  
Nicoleta Ilie, Eva-Maria Plenk

- 155 **DENTAL MATERIALS:** The effect of ceramic light scattering on an inhomogeneous beam profile  
Jean-François Roulet, Marwah Majid Khudhair, Chiayi Shen

- 161 **DENTAL MATERIALS:** Effect of whitening on the optical and mechanical properties of aged resin composites  
Ingrid Fernandes Mathias-Santamaria, Ayla Macyelle de Oliveira Correia, Débora Cristina Barbosa Dantas, Márcia Carneiro Valera, Renata Marques de Melo, Eduardo Bresciani

- 168 **ORAL MEDICINE:** Salivary matrix metalloproteinases activity levels in autoimmune diseases  
Iulia-Ioana Stănescu, Alina Dima, Eugenia Bălănescu, Daniela Miricescu, Alexandra Totan, Radu Rădulescu, Bogdan Calenic, Paula Perlea, Daniela Bălan, Maria Greabu

- 173 **MAXILLOFACIAL SURGERY:** Efficacy of a dental extraction policy designed to prevent osteoradionecrosis: a retrospective study in 100 oral cancer patients treated with intensity-modulated radiotherapy  
Constantinus Politis, Paul Deckers, Matthias Schol, Daan Nevens, Sandra Nuyts, Joseph Schoenaers, Reinhilde Jacobs

- 179 **ORAL IMPLANTOLOGY:** The efficacy of bone scraper for bone window osteotomy in the course of sinus augmentation  
Liat Chaushu, Hadar Better, Samuel Porphirio Xavier, Sarit Naishlos, Joseph Nissan, Gabi Chaushu

## Case report

- 184 **RESTORATIVE DENTISTRY:** Resin infiltration as a minimal invasive esthetic treatment for a mild fluorosis case  
Rasha Owda, Hande Şar Sancaklı

## Dentistry Conferences

## Product News

- 193 Gold standard in dental photography: digital camera for professional dental photography, EyeSpecial C-III  
Florin - Eugen Constantinescu

## 196 Author Guidelines

## 198 Subscription

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# Fake news, fake science – the black side of the internet

Jean-François ROULET  
DDS, PhD, Dr hc, Prof hc, Professor  
Editor-in-Chief



Dear readers,

The internet is a fantastic world. It is an unbelievably deep source of information. On top of it, with the social media it gives to you access to basically millions of people. Sounds nice, doesn't it? But as a critical mind, I must ask myself: is this beneficial only? I doubt it. Since information is neutral, it can be good or bad, or in other words, true, false or even worse: manipulated. I definitely know that social media are used to target people in order to make business with them. The mechanism is simple: you create first a group of followers (the larger the better), then you feed them with a recommendation to buy something you want them to do in order to increase the profit of your institution. With so-called hashtags (# name) you can throw out some concentrated slogans hoping to create a community of followers. The objective is to have the mass to ask for changes. All these mechanisms run independently, without anybody checking if the contents are true or false.

This summer, Germany has experienced a wave of news about fake science by German scientists. An international group of journalists had investigated the topic and the results were made public by the Süddeutsche Zeitung Magazin and the public broadcasters Norddeutscher Rundfunk (NDR) and Westdeutscher Rundfunk (WDR). The journalists evaluated 175,000 scientific articles by 5 of the most important pseudo-scientific platforms. They had successfully published numerous non-scientific papers, even computer-generated fake papers with these pseudo-scientific publishing platforms. Furthermore, they found that employees of big pharmaceutical companies had used these pseudo-scientific platforms to publish data ([www.dw.com/en/germany-sees-sharp-rise-in-fake-science-journal-publications-report/a-44742014](http://www.dw.com/en/germany-sees-sharp-rise-in-fake-science-journal-publications-report/a-44742014); [www.ndr.de/der\\_ndr/presse/More-than-5000-German-scientists-have-published-papers-in-pseudo-scientific-journals,fakescienc178.html](http://www.ndr.de/der_ndr/presse/More-than-5000-German-scientists-have-published-papers-in-pseudo-scientific-journals,fakescienc178.html)). The original report had been picked up by multiple news publications and had triggered a vast public discussion about this phenomenon, known for years, but no one had realized its gravity.

The reasons for this are the so-called predatory journals that take advantage of the pressure on scientists to publish. "Publish or perish" is still very valid. The traditional way of publishing is through well-known journals which have a well-established rigorous peer review process that functions as a quality control that guarantees the validity of the content. This process is sometimes painful and slow. The authors must react to the comments of their peers and modify their papers. Sometimes papers are rejected, which requires even more work and with a submission to another journal the process starts again, which means that publishing is hard work. These "traditional" journals are usually printed and financed by subscriptions mainly sold to university libraries. On the other side, there are the so-called open access journals. The idea of open access to knowledge is noble and at first glance correct. Readers get access via internet and are free to read online or download the content as pdf files.

However, someone must pay for the production costs, which are substantial, even if the journal is not printed. This approach has created a reverse of the fee structure. Usually, in open access journals, the authors must pay for their submission. With this vicious trade, a big problem was created. Publishers have very quickly realized that such an approach can easily make big money by keeping the effort low (minimal or no peer review process) publishing fast and requiring substantial submission fees. These so-called predatory journals are characterized by usually having names very similar to renowned journals, have the look of a serious journal, and have editorial boards, but very often the individuals are not known in the field. The predatory journals usually aggressively ask for manuscripts by contacting researchers via e-mail. The problem we are faced with now is “You simply don’t know if the studies, which are published with open access journals are good, worthless, or bad, because you cannot be sure if and what kind of editorial process or peer review takes place there. That is the problem with the predatory publishers in the end: eroding trust in science. A slowly creeping poison. Something might look like a study, but is not worth the paper it is written on.”([www.theguardian.com/technology/2018/aug/10/predatory-publishers-the-journals-who-churn-out-fake-science](http://www.theguardian.com/technology/2018/aug/10/predatory-publishers-the-journals-who-churn-out-fake-science)). Unfortunately, since the world is not black and white, but a sum of grey shades, the situation is more complicated. There are open access journals that have a well-established and functioning peer review process, and therefore are publishing valid information. On the other hand, also “traditional” journals feel the economic pressure and I have noticed that sometimes they publish manuscripts that definitely could have been improved with relevant recommendations by reviewers. So what to do as a reader? First be very critical, when you read a paper. Check, if what you read makes sense, if you compare it to your knowledge, check if the language is correct and be suspicious, if everything is too smooth and if there are no figures. If in doubt, there is no way around the effort to look up the journal in the internet, check who is the publisher, the editor and who is in the editorial board. You should recognize some names of scientists that have a record of good contribution to the research field the journal is reporting about.

The sad reality is that everybody working in an academic institution or having the obligation of continuing education in his/her profession is confronted with this. So am I as well. This summer I got a new teaching assignment. Teaching students dental materials by reading the literature. For this class, every student must read 3 scientific papers and present a critical review of each paper as a power point presentation. My predecessor has assigned the papers from the literature of the years 2017 and 2018 to the students, a total of 276 papers. In order to be able to grade the students, I must read all these papers carefully as well and I was confronted with many journals unknown to me. Reading the papers with an editor’s eyes revealed a scary amount of errors which should have been detected with a peer review process. Here are some examples: The reader cannot exactly understand what was done; for the methods used, the authors refer to other publications, however when looked up they were not found or showed different methods; poor experimental designs which do not allow to properly discriminate the different groups; wrong materials used that were not what the authors claimed; faulty statistics, or statistics reports that do not allow to see where the significant effects were, or no statistics at all; conclusions that were not based on the hard data of the experiment; poor discussions; or very poor English.

And finally, I got a sore finger from deleting all the requests by unknown journals to submit a paper or become a member of the editorial board and solicit papers. There is no day, where I do not get multiple requests. So far there was only bad news to report in this editorial. The good news is that yes Stomatology Edu J is an open access journal. It is even an exotic journal, since we print it as well. The very good news is that we have a rigorous peer review process and dedicated editors which improve the quality of manuscripts and eliminate errors. In the final phase, every manuscript is run through plagiarism software. And, I am proud to say that we rejected a paper that had been published already in Russian. This, dear readers is our commitment to you, so you can read valid content.

Sincerely yours,

J-F Roulet   
Editor-in-Chief

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# The key to improving the peer-review process and rewarding the reviewer's activity: Publons registration

Marian-Vladimir CONSTANTINESCU  
DDS, MSc, PhD, Professor  
Editor-in-Chief



Dear readers,

As editor of the Stomatology Edu Journal, I attended the webinars organized by Clarivate Analytics. This is where I found out a series of extremely useful information in my editorial task. This is how I learned about Publons, a site which is part of Clarivate Analytics, and its importance in an editor's activity. Therefore, to improve the peer-review process and to make the activity of the reviewers more visible, we have registered the Stomatology Edu Journal in Publons. <https://publons.com/journal/70355/stomatology-edu-journal>

Opening access to reviews and assigning publication credits to the best reviews is one of the latest achievements of the current digitization.

The proliferation of scientific journals poses a lot of problems to editors in recruiting reviewers. An estimated 63 million hours were devoted to peer-review in 2015, out of which 18.9 million hours were provided by the top 5% contributing reviewers [1].

From now on, the Stomatology Edu Journal belongs to the family of over 250 dental journals included in the constellation of over 2,400 Publons partner journals.

We recommend that you stop being a reluctant reviewer and join the more than 490,000 Publons experts to monitor, review and submit your review contributions to our field.

From now on, any review can be automatically added to your profile by submitting a "Thank you for reviewing" email to [reviews@publons.com](mailto:reviews@publons.com). Publons will privately check your activity and get credible evidence for your CV.

From the next issue of the Stomatology Edu Journal, we recommend that you do not allow any of your reviews unregistered. If you are not registered as a reviewer in Publons yet, after reading this Editorial, you can create a free reviewer profile as early as today by signing up for <https://publons.com/account/signup/>.

By registering with Publons you will meet the world's best reviewers in our field and you will get quantitative data on your research work in case global research is cited, reused, read and so on.

Publons is integrated with all peer-review systems to provide peer-reviewers with instant recognition of their contributions in line with the journal review policies.

Publons is integrated into the reviewer workflow so academics can monitor and verify every review and editorial contribution on the fly and in complete compliance with the journal review policies.

By 2013 Publons has archived and processed 66,224 reviews from 3,675 journals. Reviewers were granted credits for the posted items and were able to export their records to their IDs for editorial and academic promotion and research funding applications [2]. In recognition of an "outstanding contribution" on "time, quality and frequency", Elsevier awarded certificates of excellence to 25 of the best reviewers for each title in 35 journals [3].



Reviews posted on academic platforms, like Publons, can identify the best contributors, whom we invite to continue reviewing and to whom we want to offer significant editorial positions in the new editorial board.

Every year Publons rewards the best reviewers in the world honoring them with the Peer Review Awards to certify the quality, rigor and innovation in their field [4]. In its methodology to assess researchers, Publons uses the 22 Essential Science Indicators (ESI), as they stand for broad research areas that are aligned with the Web of Science - the most trusted citation index for scientific and scholarly research.

Our goal is clearly defined, namely to assist the authors who support the over 180,000 readers of the Stomatology Edu Journal by streamlining peer-review process and by reducing the processing time from four weeks to two weeks and finally seven days. By improving the peer-review process management, as of 2019, the loyal readers of the Stomatology Edu Journal can hope to receive a new issue from the new editorial team every two months.

So help us, God!

M-V Constantinescu 

Editor-in-Chief

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# In Celebration of Professor Adrian Bejan on his 70th birthday



Now, when Professor Adrian Bejan, honorary member of the Romanian Academy and Deputy Editor-in-Chief of the Stomatology Edu Journal, is celebrating his 70th anniversary, all his colleagues and friends would like to warmly congratulate him and also take this opportunity to show their deep appreciation and give you an idea of just how great this man is in various areas of engineering science and applied physics.

He was born on 24 September 1948 in Galați (Romania). At age 19 Bejan won a scholarship for the United States and enrolled in the Massachusetts Institute of Technology (MIT) in Cambridge, Massachusetts. Adrian Bejan specialized in cryogenics and heat transfer and completed all his studies at MIT. There, he managed to acquire all academic degrees at the same university (B.Sc. with Honors, 1972; M.Sc. with Honors, 1972; and Ph.D., 1975)

Between 1975-1976 he was a Lecturer and Research Associate with the Department of Mechanical Engineering at the Massachusetts Institute of Technology. Then, between 1976-1978, he was a Fellow of the Miller Institute of Basic Research in Science at the University of California-Berkeley. As of 1978 until 1984 he was an Assistant and Associate Professor with the Department of Mechanical Engineering at the University of Colorado. And from 1984 until 1989, he was a Tenured Professor with the Department of Mechanical Engineering and Materials Science at Duke University. And from 1989 until the present, he is a J.A. Jones Distinguished Professor of Mechanical Engineering at Duke University.

Professor Bejan's research covers engineering science and applied physics: thermodynamics, heat transfer, fluid mechanics, convection, porous media. His first book *Entropy generation through heat and fluid flow* (Wiley, 1982) shifted the attention of thermal engineers from the global calculation of the entropy generation rate of the entire system, considered as a 'black box', to the calculation of the local rates, clarifying the physical correlation between irreversible entropy generation and efficiency and paving the way to a new branch of Thermodynamics. It was in 1984 that he published his second book entitled *Convection Heat Transfer* (Wiley, 1984). That book focused on new research methods, which were totally different from the ones used back then to solve various problems. His innovative methods are based on the intersection of asymptotes, heatlines, and scale analysis. Later on, in 1988, the first edition of his textbook entitled *Advanced Engineering Thermodynamics* (Wiley, 1988) was also

published. That book was a combination between the theory of thermodynamics, engineering heat transfer and fluid mechanics. The author also introduced a new method of organization, namely the minimization of entropy generation.

In the years to come, more precisely between 1992-2006, professor Bejan published four more books, as follows: Convection in Porous Media (Springer, 1992, 1999, 2006), Heat Transfer (Wiley, 1993), Thermal Design and Optimization (Wiley, 1996) and Entropy Generation Minimization (CRC Press, 1996).

One of Adrian Bejan's greatest achievements is that he is the discoverer of the Constructal Law of design evolution in nature. Bejan has identified a basic Law of Physics that describes and predicts how design patterns emerge over time. The Constructal Law is his statement that there is a universal tendency (a phenomenon) toward design in nature, in the physics of everything.

Bejan has also written two popular science books targeting a general audience, as follows: first of all, Design in Nature: How the Constructal Law Governs Evolution in Biology, Technology, and Social Organization (Doubleday, 2012) and secondly, The Physics of Life: The Evolution of Everything (St. Martin's Press, 2016).

Professor Bejan has received 18 honorary doctorates from universities in 11 countries. In 2001 he was ranked among the 100 most highly cited authors worldwide in engineering (all fields, all countries) by the Institute for Scientific Information. You can read in his curriculum that Adrian Bejan is the author of 30 books and over 600 peer-referred articles. In August 2017, his h-index is 87 on Google Scholar and 60 on the Web of Science. But what you shall not find there is that several of his books and papers represent a break with the previously established knowledge and a proposal for a new 'paradigm'.

And then, in November 2017, there came the great piece of news from the Franklin Institute in Philadelphia. The Institute announced that professor Adrian Bejan would be awarded the 2018 Benjamin Franklin Medal in Mechanical Engineering. The Institute cited him for "his pioneering interdisciplinary contributions in thermodynamics and convection heat transfer that have improved the performance of engineering systems, and for the constructal theory, which predicts natural design and its evolution in engineering, scientific, and social systems."

Thus, since 2018, Adrian Bejan has joined the ranks of other geniuses such as Nikola Tesla, Marie and Pierre Curie, Thomas Edison, Albert Einstein, Stephen Hawking, Jane Goodall, and Bill Gates – all of them laureates of the Franklin Institute founded in 1824.

His accomplishments are not limited to the 'printed paper': many students are inspired every day by professor Bejan's lectures and seminars. We find his words very inspiring and worthy to follow: I have chosen to be happy. The idea is to look into the future with hope.

Maria Greabu , MD, PhD, Professor, Head  
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Faculty of Dental Medicine  
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Associate Editor of Stomatology Edu Journal

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# Impressions from London at the 96th General Session meeting of the International Association for Dental Research (IADR)

The International Association for Dental Research (IADR) General Session was held in conjunction with the Pan European Regional (PRE) Congress on July 25-28, 2018 in London, England. The congress was a major meeting, encouraging the presentation and discussion of novel findings and clinical approaches among more than 5000 scientists and researchers coming from almost 90 countries. The very high amount of data presented included the most recent research findings, developments and trends in all the sub-disciplines of dentistry. In fact, the programme included more than 2200 poster and 780 oral presentations, 92 symposia, several lunches & learning, hands-on workshops and two Distinguished Lecture Series plenary sessions. Condensing the whole meeting in just four days was a major challenge. This could be accomplished by conducting several sessions at the same time, thanks to the London Excel congress venue that provided enough room for all the events.

The meeting was a huge opportunity especially for young researchers to present their findings at the beginning of their careers, with the organization providing many possibilities to support them, especially at the European level, by means of travel grants, company and Group-sponsored competitions covering different topics, and the Robert Frank awards for both clinical and basic science. Winners of the award were selected to represent each Division at the upcoming General Session meeting, which will be held in Vancouver, BC, in June 19-22, 2019. Several dinner events, mainly organized by the different Groups, Universities and Companies, but also a dinner reception specifically dedicated to the first-time attendees, were excellent occasions for further networking and getting in touch with each other. The Continental European Division (CED) also organized a meeting of the CED-IADR

Ambassadors in order to facilitate the primary aim of the organization, namely to encourage high-quality Oral Health Research and to facilitate networking across Europe. The Ambassadors Group also takes an active part in organizing the Young CED-IADR Symposia at the upcoming CED/PER-IADR meetings. Each time the Symposia address a specific topic of frontier research and are totally organized, chaired and have speakers who are less than 35 years old, with a brilliant experience in research. Their aim is to promote young researchers and to encourage networking especially among people at the beginning of their research career. In particular, at the next CED-IADR meeting, the Young Symposium title will be: "New insights on peri-implantitis".

The next European meetings of the Association are scheduled to take place as CED-IADR, meeting of the Continental European Division in Madrid, Spain, September 19-21, 2019 and as PER-IADR, meeting of the Pan-European Region in Marseille, France, September 10-12, 2020.

For any information, please refer to the website of the organization: <https://ced-iadr.eu/> and the secretariat email: [ced.iadr@uzleuven.be](mailto:ced.iadr@uzleuven.be).

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# The European Society of Cosmetic Dentistry (ESCD) celebrated its 15<sup>th</sup> anniversary in Lisbon, from the 20<sup>th</sup> to the 22<sup>nd</sup> of September

The festive moment of the European Society of Cosmetic Dentistry (ESCD) arrived in Lisbon during its most important congress ever organized. More than 650 participants from 50 countries gathered in Lisbon to celebrate 15 years of friendship, high-end dentistry and education. This anniversary edition of the ESCD Meeting was really outstanding taking in consideration the feedback given by the attendees as well as the very complex organization and number of participants. We had two parallel main panels with speakers from all over the world talking about a broad range of topics surrounding aesthetics and implantology, two hands-on sessions and of course the impressive live studio, from where the interviews with various speakers, representatives of the industry and organizers were broadcast live on ESCD's Facebook page. Experienced speakers all over the world shared their knowledge with the public: Dr. Mauro Fradeani, Prof. Dr. Marius Steigmann, Prof. Dr. Rade Paravina, Dr. Paulo Monteiro, Dr. Eric Van Dooren, Prof. Dr. Louis Hardan, Dr. Javier Tapia Guadix, Dr. Miguel Stanley, Dr. Maciej Zarow, Prof. Dr. Nitzan Bichacho & Dr. Mirela Feraru.

ESCD is also known for their unforgettable social events, starting Thursday, with a very friendly night on a roof top bar in the middle of Lisbon, Friday with the classy and ceremonial atmosphere during the President's Dinner and the Certified Members' Ceremony and Saturday with a Portuguese night accompanied by live fado music.

Next year ESCD will move to Saint Petersburg, so we are looking forward to having a very busy, interesting and extremely productive year ahead of us.

The European Society of Cosmetic Dentistry (ESCD) was founded in 2003 by a group of



ESCD Board & Country Chairpersons with special guest Dr. Mauro Fradeani

practicing dentists, dental technicians and dental professors from different universities all over Europe. The ESCD is a logical process, responding not only to the growing interest by the public in cosmetic and aesthetic dentistry, but also to the respective needs of the European dental practitioners to enhance their skills and knowledge in the field. The purpose of the society is to promote dental education in the area of cosmetic dentistry. ESCD strongly supports and facilitates the promotion and cooperation with regards to practical, scientific and ethical aspects.

Florin Lăzărescu, DDS  
President, ESCD

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July 2018

Niodita Gupta, MD, MPH, PhD, Marko Vujcic, PhD, Andrew Blatz, MS

**MULTIPLE OPIOID PRESCRIPTIONS AMONG PRIVATELY INSURED DENTAL PATIENTS IN THE UNITED STATES  
Evidence from claims data**

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<http://jada.ada.org/ce/home>.

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[https://jada.ada.org/article/S0002-8177\(18\)30130-2/fulltext](https://jada.ada.org/article/S0002-8177(18)30130-2/fulltext)

## LIGHT TRANSMISSION THROUGH RESIN COMPOSITES

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## ABSTRACT

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**Introduction:** The study aimed to quantify the amount of light that passes through different resin-based composite (RBC) types and to assess if a clinically used polymerization procedure in curing incrementally filled deep cavities is justified.

**Methodology:** Light transmission through 2-mm thick specimens made of three regular RBCs - a nano, a flowable nano and a microhybrid - of the same shade A3, was analyzed under 24 different curing conditions, that resulted by varying the curing mode, exposure distance and exposure time when using a violet-blue LED light curing unit. Incident and transmitted irradiances were assessed in real-time on a spectrophotometer and radiant exposure, transmittance (T) and absorbance (A) were calculated. A multivariate analysis assessed the effects of various parameters on T and A.

**Results:** Incident irradiance varied among 656.4 (8.1) mW/cm<sup>2</sup> (Standard mode, exposure distance = 7 mm) and 3361.5 (33.6) mW/cm<sup>2</sup> (Plasma Emulation mode, 0 mm). The filler amount (weight and volume %) exerted a significant effect on transmitted irradiance ( $p < 0.001$ ; partial eta squared  $\eta^2 = 0.400$  and 0.362, respectively) while the effect of exposure distance was low ( $p < 0.001$ ,  $\eta^2 = 0.141$ ). Light transmittance was material-dependent and very low. The significant lowest absorbance was identified in Filtek Supreme XTE flow ( $1.11 \pm 0.09$ ), followed by Filtek Silorane ( $1.21 \pm 0.03$ ) and Filtek Supreme XTE ( $1.62 \pm 0.13$ ). Incident and transmitted radiant exposure correlated exceptionally well in each RBC (Pearson correlations coefficient  $> 0.99$ ).


**Conclusion:** When restoring a deep cavity with regular RBCs, each increment needs to be cured adequately, since final curing to compensate for deficits in polymerisation is insufficient.

**Keywords:** resin-based composites, light curing unit, irradiance, radiant exposure, transmittance, absorbance.

## 1. Introduction

The optical properties of light cured resin-based composites (RBC) are essential material characteristics that are relevant for both the esthetical appearance of a restoration [1,2] and the quality of curing in depth [3]. The latter is fundamentally related to the translucency of the material and thus to the amount of light (photons) that is allowed to pass through the RBC during polymerization. While the surface of an RBC filling is prevalently sufficiently cured, the polymerization of deeper increments is decisively influenced by the light transmitted through the material [4]. Yet, an insufficient polymerization may not be immediately noticeable. It is evidenced later in the reduced mechanical properties [5], low degree of conversion [6], elution of unreacted monomers [6], increased toxicity [7] and potential hypersensitivities. The transmitted light through a material sums the remaining light, after the incident light, striking the surface of the RBC, was reflected, absorbed and scattered. Light absorption occurs when atoms or molecules of the RBC's constituents, such as monomers [8], filler particles [9,10], photo-initiator molecules [11], dyes and pigments [8,12,13] take up the energy of a photon of light. In contrast, scattering take place on reinforcing particles or porosity voids [9]. The extent of scattering at the interfaces between

reinforcing particles and polymer matrix is minimized when the mismatch in refractive index between each material is reduced [14]. It is thus a function of the chemical composition of both constituents and differs accordingly within individual RBCs. In addition to the refractive index, also the filler dimension exerts a significant effect on light scattering, that was shown to be highest when the filler diameter approaches approximately one-half the wavelength of incident light, i.e.  $\sim 0.2\text{--}0.3 \mu\text{m}$  [3]. It must also be noted that large variation in light transmission was observed also within resin composite of similar shade [15]. Moreover, light transmittance varied during polymerization, while increasing or decreasing during curing as a function of the RBC type and composition [16]. In clinical dentistry, many of the aspects described above are not sufficiently considered when making recommendations on restoration techniques. It is, for instance, occasionally acclaimed to only "pre-cure" the first, lowest increment for 2-3 seconds, when restoring incrementally a deep cavity with an RBC. This proceeding is justified by the additional amount of light that would pass through the filling when the upper RBC increments are exposed to light or, as usual clinically, when the entire filling is exposed again to light at the end of the restoration. Therefore it was the aim of the present study to

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**Table 1.** Resin composite brand, type, chemical composition of matrix and filler as well as filler content by weight (wt.) and volume (vol.) %. All materials are manufactured by 3M ESPE.

RBCs	RBC-Type	Batch	Shade	Resin Matrix	Filler	Filler wt%/vol%
Filtek™ Supreme XTE	Nano	N229448	A3 Dentin	Bis-GMA, Bis-EMA, UDMA, TEGDMA, PEGDMA	ZrO <sub>2</sub> , SiO <sub>2</sub> , ZrO <sub>2</sub> /SiO <sub>2</sub>	78.5/63.3
Filtek™ Supreme XTE flow	flowable Nano	N236527	A3	Bis-GMA, Bis-EMA, TEGDMA, PEGDMA	ZrO <sub>2</sub> , SiO <sub>2</sub> , ZrO <sub>2</sub> /SiO <sub>2</sub>	65/55
Filtek™ Silorane	Microhybrid	N225426	A3	3,4-Epoxy cyclo-hexylethylcyclopolymethylsiloxane Bis-3,4-epoxy cyclo-hexylethylphenyl-methylsilane	SiO <sub>2</sub> , YF <sub>3</sub>	76/55

simulate and quantify the amount of light that would pass through 2 mm thick increments of different RBC types - a nano, a flowable nano and a microhybrid - as it would be received from a lower layer in a resin composite filling. To simulate clinically relevant curing conditions, 24 different radiant exposures were considered for each material, that were obtained by varying the curing mode of a modern, high-performance LED light-curing unit (LCU), the exposure distance and the exposure time.

The null hypotheses assume: a) similar light transmittance (= ratio of transmitted to incident radiant power) through all material types; b) within one material, similar transmittance for all curing modes of the LCU; c) similar absorbance in all materials.

## 2. Materials and Methods

Light transmission through three regular RBCs (Table 1) was analyzed under different curing conditions at a specimen thickness of 2 mm. Therefore the violet-blue LED LCU VALO (Ultradent, South Jordan, USA, serial number VO 7710) was applied in three different exposure modes (Standard, High Power and Plasma Emulation), at various exposure times (5 s, 10 s, 15 s, 20 s and 40 s in the Standard mode; 1 s, 2 s, 3 s, 4 s and 12 s in the High Power mode and 3s and 6s in the Plasma Emulation mode) and exposure distances (0 mm and 7 mm). This resulted in 24 different curing conditions.

### 2.1. Spectrophotometry: measurement of the Incident irradiance and Light Transmittance

Incident irradiance and light transmittance through the analyzed RBCs were assessed on a laboratory-grade National Institute of Standards and Technology (NIST)-referenced USB4000 Spectrometer (MARC (Managing Accurate Resin Curing) System, Blue light Analytics Inc., Halifax, NS, Canada). The incident irradiance (the irradiance reaching the specimen's surface) was determined on five occasions, by applying the curing unit directly to the sensor. With each program (standard, high power, plasma emulation) and material (Table 1) the maximum irradiance reaching the sensor was measured in a random order. The exposure distance was set at 0 mm and 7 mm. Specimens were prepared in cylindrical Teflon molds

(6 mm diameter, increment thickness 2 mm, n = 5), and cured by applying the aforementioned curing unit directly, perpendicularly and centered on the surface of the sample using a mechanic arm. While the specimens were cured, the spectrophotometer measured in real-time the irradiance at the bottom of the specimens. The cylindrical Teflon molds containing the material were aligned centered on the round detector of the spectrometer, which had a diameter of 3.9 mm. Consequently, the irradiance and radiant exposure reaching this area were considered. The miniature fiber optic USB4000 Spectrometer employs a 3648-element Toshiba linear Charge-coupled Device (CCD) array detector and high-speed electronics (Ocean optic, Largo, FL, USA). The spectrometer was calibrated using an Ocean Optics' NIST-traceable light source (300–1050 nm). The system uses a CC3-UV Cosine Corrector (Ocean optic, Largo, FL, USA) to collect radiation over a 180° field of view, thus mitigating the effects of optical interference associated with light collection sampling geometry. Irradiance and radiant exposure at a wavelength range of 360–540 nm were individually collected at a rate of 16 records/s. The sensor was triggered at 20 mW. The radiant exposure was calculated by integrating the irradiance versus the wavelength at the used exposure time.

### 2.2. Transmittance and absorbance

Transmittance (T) is defined as the ratio of transmitted irradiance (radiant power) to incident irradiance:  $T = I_t/I_0$ , where  $I_t$  is the irradiance after the beam of light passes through the specimen and  $I_0$  is the irradiance of the incident light.

Transmittance is related to absorbance by the expression: Absorbance (A) =  $-\log(T) = -\log(I_t/I_0)$ , where absorbance stands for the amount of photons that are absorbed.

Being defined as ratios of irradiance values, transmittance and absorbance are dimensionless.

### 2.3. Statistical Analysis

A Shapiro–Wilk test verified the normal distribution of the data. A multivariate analysis (general linear model) assessed the effects of various parameters as well as their interaction terms on the transmitted irradiance and absorbance. The partial eta-squared statistic reports the practical significance of each term, based on the ratio of the variation accounted

for by the effect. Larger values of partial eta-squared indicate a greater amount of variation accounted for by the model effect, to a maximum of 1. Correlation among incident and the transmitted radiant exposure was assessed by a Pearson correlation analysis. In all statistical tests, p-values < 0.05 were considered statistically significant when using SPSS Inc. (Version 24.0, Chicago, IL, USA).

### 3. Results

The irradiance of the analyzed LED LCU at an exposure distance of 0 mm amounted to 1174.1 (12.4) mW/cm<sup>2</sup> in the Standard mode, 1760.3 (9.8) mW/cm<sup>2</sup> in the High Power mode and 3361.5 (33.6) mW/cm<sup>2</sup> in the Plasma Emulation mode. The incident irradiance decreased at an exposure distance of 7 mm to 656.4 (8.1) mW/cm<sup>2</sup>, 986.3 (10.6) mW/cm<sup>2</sup> and 1917.8 (31.6) mW/cm<sup>2</sup>, respectively (Fig. 1).

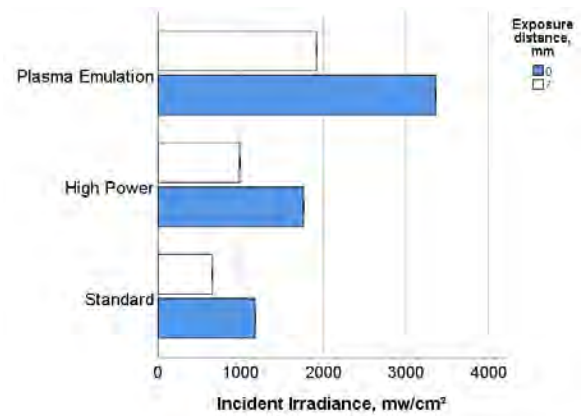
The effect of the parameter filler amount (weight and volume %) was proved to be significant on the transmitted irradiance ( $p < 0.001$ ; partial eta squared  $\eta^2 = 0.400$  for weight % and 0.362 for volume %), while the effect of exposure distance was low ( $p < 0.001$ ,  $\eta^2 = 0.141$ ).

The Plasma Emulation mode induced the highest transmitted irradiances in each analyzed RBC, while the lowest values were identified when the LCU was run in the Standard mode. Increasing the exposure distance from 0 mm to 7 mm lowered the transmitted irradiance by 43 % to 49 % (Table 2), while the incident irradiance was lowered by 42.9% to 44.1%.

Within each curing mode and exposure distance, the significant highest transmitted irradiances were identified in the Filtek Supreme XTE flow, followed by the Filtek Silorane, while the significant lowest values were identified in the Filtek Supreme XTE ( $p < 0.001$ ). Within the analyzed incident irradiances, which varied in the range 656.4 (8.1) mW/cm<sup>2</sup> (Standard mode, 7 mm exposure distance) to 3361.5 (33.6) mW/cm<sup>2</sup> (Plasma mode, 0 mm exposure distance), the transmitted irradiance was reduced within the range 16.5 (1.3) mW/cm<sup>2</sup> to 71.7 (4.9) mW/cm<sup>2</sup> in Filtek Supreme XTE, 49.5 (2.3) mW/cm<sup>2</sup> to 217.9 (13.0) mW/cm<sup>2</sup> in Filtek Silorane and 49.7 (2.4) mW/cm<sup>2</sup> to 249.1 (16.3) mW/cm<sup>2</sup> Filtek Supreme XTE flow (Fig 2). This means that within the above-mentioned intervals the percentage of transmitted irradiance relative to the incident irradiance amounted 1.4% to 2.1% in the Filtek Supreme XTE, 4.2% to 6.5% in the Filtek Silorane and 4.2% to 7.4% in the Filtek Supreme XTE flow. Within these limits, by trend, the lower the incident irradiance, the higher the % transmitted light related to the initial irradiance.

The significant lowest absorbance was identified in the Filtek Supreme XTE flow (1.11±0.09), followed by the Filtek Silorane (1.21±0.03) while the highest absorbance was measured in the Filtek Supreme XTE (1.62±0.13). A strong influence on absorbance was identified in the parameter RBC ( $p < 0.001$ ; partial eta squared  $\eta^2 = 0.744$ ), followed by the parameter curing mode ( $p < 0.001$ ;  $\eta^2 = 0.364$ ) while the exposure distance exerted only a low influence ( $p < 0.001$ ;  $\eta^2 = 0.117$ ) (Table 2).

Within the 12 analyzed curing conditions at an

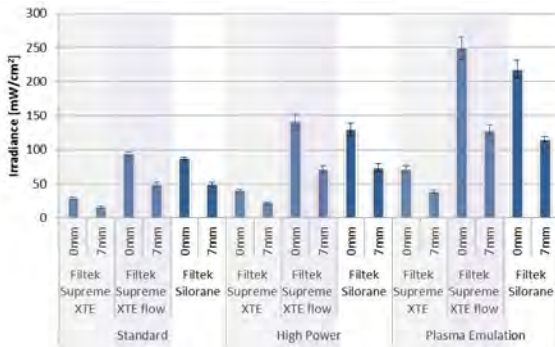


**Figure 1.** Incident irradiance as a function of the curing mode and exposure distance.

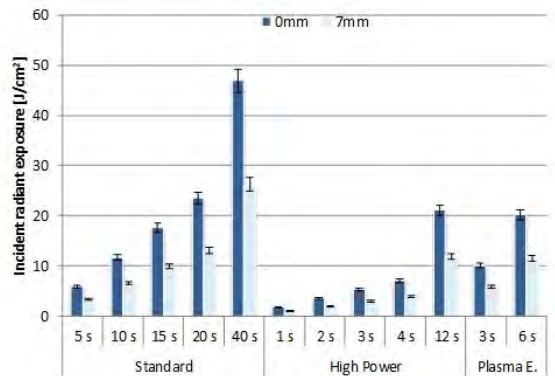
**Table 2.** Absorbance as a function of RBC, curing mode and exposure distance as well as loss in transmitted irradiance ( $\Delta I$ , %) at an exposure distance of 7 mm related to 0 mm within each RBC and curing mode.

Curing mode	RBC	Exposure distance	Absorbance	$\Delta I$
Standard	Filtek™ Supreme XTE	0mm	1.58 (0.2)	43.3 %
	Filtek™ Supreme XTE	7mm	1.57 (0.21)	
	Filtek™ Supreme XTE flow	0mm	1.08 (0.14)	47.1 %
	Filtek™ Supreme XTE flow	7mm	1.10 (0.14)	
	Filtek™ Silorane	0mm	1.1 (0.15)	43.1 %
	Filtek™ Silorane	7mm	1.10 (0.143)	
High Power	air	0mm	-	44.1 %
	air	7mm	-	
	Filtek™ Supreme XTE	0mm	1.63 (0.03)	46.6 %
	Filtek™ Supreme XTE	7mm	1.65 (0.006)	
	Filtek™ Supreme XTE flow	0mm	1.1 (0.0001)	49.3 %
	Filtek™ Supreme XTE flow	7mm	1.14 (0.003)	
Filtek™ Silorane	0mm	1.13 (0.0001)	42.9 %	
Filtek™ Silorane	7mm	1.13 (0.013)		
Plasma E.	air	0mm	-	44.0 %
	air	7mm	-	
	Filtek™ Supreme XTE	0mm	1.66 (0.01)	45.1 %
	Filtek™ Supreme XTE	7mm	1.69 (0.008)	
	Filtek™ Supreme XTE flow	0mm	1.13 (0.006)	48.5 %
	Filtek™ Supreme XTE flow	7mm	1.17 (0.006)	
Filtek™ Silorane	0mm	1.14 (0.2)	47.0 %	
Filtek™ Silorane	7mm	1.22 (0.02)		
Plasma E.	air	0mm	-	42.9 %
	air	7mm	-	

exposure distance of 0 mm, the highest incident radiant exposure was identified when the LCU was run in the Standard mode and an exposure time of 40s (46.96 J/cm<sup>2</sup>), followed by the 20s exposure in the same curing mode (23.48 J/cm<sup>2</sup>), the 12 s exposure in the High Power mode (21.12 J/cm<sup>2</sup>), and the 6s exposure time in the Plasma Emulation mode (20.17 J/cm<sup>2</sup>). The lowest radiant exposure resulted at an exposure time of 1s in the High Power mode (1.76 J/cm<sup>2</sup>) (Fig. 3). For an exposure distance of 7 mm, the



**Figure 2.** Transmitted irradiance through 2 mm thick specimens of the analysed RBCs as a function of curing mode and exposure distance.

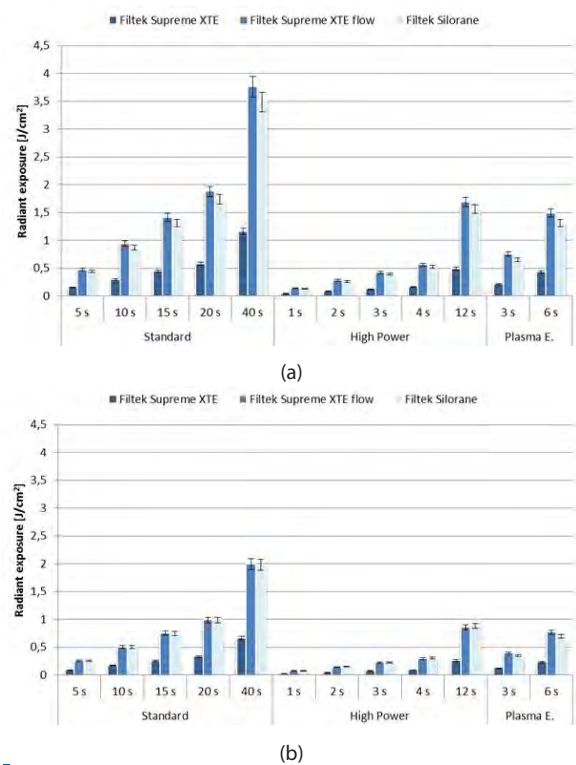


**Figure 3.** Radiant exposure received by the top-surface of the analysed specimens as a function of exposure time, distance and curing mode.

radiant exposure varies in the same sequence at lower values, which varied from  $26.26 \text{ J/cm}^2$  (40s, standard mode) to  $0.99 \text{ J/cm}^2$  (1s, High Power mode). An excellent correlation was found in each RBC between the incident and the transmitted radiant exposure (Fig. 4 a, b) (Pearson correlations coefficient = 0.997 for Filtek Supreme XTE, 0.999 for Filtek Supreme XTE flow and 0.925 for Filtek Silorane).

#### 4. Discussion

The present study quantifies the attenuation of light when traveling through different types of resin composite specimens of a predetermined thickness of 2 mm. This thickness was chosen owing to the fact that all three analyzed materials are regular RBCs that need to be placed incrementally, while the thickness of an increment should not exceed 2 mm. The selected materials belong to different RBC types - nano, flowable nano and microhybrid. Two of the analyzed RBCs - Filtek Supreme XTE flow and Filtek Supreme XTE – have a quite similar chemical composition of all their constituents and primarily differ with respect to the filler amount (Table 1). Both are methacrylate-based RBCs. The difference in filler amount resulted in significantly lower light transmittance and a ca. 50% higher absorbance in the higher filled material. Although light transmittance was higher in the Filtek Supreme XTE flow, it amounted to less than  $250 \text{ mW/cm}^2$  also at the highest analyzed incident irradiance ( $3361.5 \pm 33.6 \text{ mW/cm}^2$ , Plasma Emulation). Considering that the polymerization at such high irradiances should not exceed a few seconds, due to the increased risk to over-heat the pulp, the amount of light available to potentially complete the curing of an underneath pre-cured increment might be far insufficient. There are



**Figure 4.** Radiant exposure as recorded at the bottom of 2-mm thick specimens of the analysed RBCs, as a function of exposure time and curing mode at an exposure distance of a) 0 mm and b) 7 mm.

more reasons against exposure to high irradiance, as curing fast at high irradiances leave no room to relieve internal stresses accumulated during shrinkage [17]. To address light transmittance also from the perspective of a different chemical composition of the organic matrix, the silorane based material Filtek Silorane was additionally selected. It must be noted that this material is no longer on the market. The reasons therefore were not motivated in the physical properties or curing behavior of Filtek Silorane, since there were comparable to regular methacrylate-based RBCs [18,19]. The siloran monomer was obtained from the reaction of oxirane and siloxane molecules, thus combining the two key advantages of the individual components: low polymerization shrinkage due to the ring-opening oxirane monomer and increased hydrophobicity due to the presence of the siloxane species [20]. It is a four-branched monomer (methacrylate monomers are only two-branched) which suggest a high crosslinking density of the final polymer and, as a result, good chemical stability. A further particularity of this system is the cationic initiated polymerization which is less sensitive to oxygen compared to the radical polymerization of the methacrylate-based RBCs. The cationic polymerization initiation system consists of camphorquinone, an iodonium salt, and an electron donor. In the redox process, the electron donor decomposes the iodonium salt to an acidic cation which then starts the ring-opening polymerization process [20]. The transmittance and absorbance characteristics measured in the present study for Filtek Silorane were rather comparable to the flowable than to the nano RBC Filtek Supreme XTE. Apart from differences in the refractive index and filler size that influence light scattering and thus light transmittance [3,14] as mentioned above, the results may be explained by the similar volumetric filler amount of both materials (Table 1).

The 24 different curing conditions analyzed in the present study were simulated by using a violet-blue LED LCU that offers three different curing programs of medium and high irradiances: 1174.1 (12.4) mW/cm<sup>2</sup> in the Standard mode, 1760.3 (9.8) mW/cm<sup>2</sup> in the High Power mode and 3361.5 (33.6) mW/cm<sup>2</sup> in the Plasma Emulation mode. The LCU is equipped with four high-power LED chips emitting three different wavelength ranges (two chips with a peak at 465 nm, one chip with a peak at 445 nm and 405 nm, respectively) and placed directly into the head of the LCU.

In addition to the exposure distance, the angle at which an LCU is placed on the restoration plays a major role for the quality of polymerization. Particularly in posterior cavities that are difficult to access, the LCU may not be placed perpendicularly on the restoration. In this case, the RBC may cure inhomogeneously in depth, according to the placement of the LCU, even if the surface appears to be well cured. This situation is a good indication for using a "pin-shaped" LCU, like the one analyzed in the present study. Furthermore, in a clinical situation, if the composite surface is larger than the light exit window of the LCU, it must be polymerized in an overlapping manner to cover the entire composite surface. It must also be taken into account that the amount of light emitted by an LCU is not equal to the amount of light a restoration receives. Apart from angulation, the access to lower increments in deep restorations in a clinical situation may be impeded by the presence of cusps. Therefore, the exposure distance was set in the present study either at 0-mm, to simulate the closest contact between restoration and LCU, or at 7 mm. As shown in the present study the enlarged exposure distance lead to a loss of almost 50% of the incident irradiance related to the closest contact, also when using a modern, high-performance LCU.

For economic reasons, clinicians often demand short curing times when polymerizing RBCs. This led to the development of the concept of "exposure reciprocity". This concept considers the product of the incident irradiance (mW/cm<sup>2</sup>) and exposure time (s), which is denominated as radiant exposure (J/cm<sup>2</sup>). It assumes that, at a given radiant exposure, the effect induced in the RBC would be similar, irrespective of the radiant exposure is reached by lowering the exposure time and increasing the irradiance or vice versa. Even if this simple construct sounds plausible and useful in a clinical situation, it is not universally valid. It does not apply especially if very high or very low irradiances are used. [21]. Numerous studies in recent years have clearly shown that efficient polymerization, especially in depth, is achieved with LCU of moderate irradiance (maximum 1200 mW/cm<sup>2</sup>) and exposure times of at least 20 s [5].

The recommendation to pre-cure the lowest increment for only few seconds as justified by the additional amount of light supplied during the cavity restorations must be declined. As summarized in Fig. 2, the light that passes through 2 mm thick layers of various RBCs after light exposure by means of a high-performance LCU is too low. It must be pointed out that, within the analyzed curing conditions, the transmitted light related to the incident light amounted only 1.4% to 2.1% in the Filtek Supreme XTE, 4.2 to 6.5 in the Filtek Silorane and 4.2% to 7.4% in the Filtek Supreme XTE flow. In terms of radiant exposure measured at the bottom of 2-mm

thick increments, this amounted to less than 4 J/cm<sup>2</sup> in the most translucent RBCs at highest incident radiant exposure (40s, standard mode, exposure distance 0 mm) and less than 2 J/cm<sup>2</sup> at an exposure distance of 7 mm at similar curing conditions. A general value for the radiant exposure that is needed to adequately cure a 2 mm increment of an RBC is indicated as 16 J/cm<sup>2</sup>, thus the transmitted values measured in the present study are far away from fulfilling this requirement (Fig 4).

To transfer these data to a clinical relevant situation, we may consider the Standard mode of the LCU used in the present study (ca. 1200 mW/cm<sup>2</sup>), which would correspond to a modern and well-working LCU, as used by many clinicians. For this curing settings and under ideal laboratory conditions (exposure distance 0 mm, LCU placed perpendicularly on the RBC's surface) significantly less than 100 mW/cm<sup>2</sup> pass through a 2 mm increment of a flowable RBCs (Filtek<sup>TM</sup> Supreme XTE flow), which was the most translucent material analyzed in the present study. These values are even reduced to 87.2 (1.9) in the Filtek<sup>TM</sup> Silorane and to 29.1 (1.3) in the Filtek<sup>TM</sup> Supreme XTE. Thicker composite layers, as they may result in a clinical situation during restoring a cavity, are even completely impermeable to light [22]. Under clinical relevant conditions, light transmittance may be even lower, accounting for numerous factors related to the LCU or clinician. This comprises the use of LCUs with low irradiance, defect or contaminated waveguides, improper polymerization due to angulation and high exposure distances. Thus, the present study provides clear arguments against the above-claimed curing techniques and reinforces the recommendation to sufficiently polymerize each RBC layer. The data also suggest that a subsequent polymerization of an RBC filling to potentially alleviate tooth sensitivity due to insufficient polymerization in depth is useless. Besides, it should also be mentioned that an important requirement for a successful polymerization is an intact and clean waveguide. This needs to be checked clinically before each exposure, as residues of composite or adhesive, which often sticks to the waveguide, leading to a strong reduction in the irradiance of the LCU, as the results of the present study suggest. It should also be borne in mind, that the variation of irradiance with exposure distance is LCU-specific. What is especially fast is the reduction of the irradiance with the distance for LCUs when using a so-called "turbo" waveguide.

## 5. Conclusions

All null hypotheses must be rejected. Light transmittance was shown to be material dependent and very low in regular RBCs, irrespective of RBC composition and type. It is therefore indicated to adequately cure each increment when restoring a deep cavity, and not to rely on a final curing of a restoration to compensate for deficits in polymerisation in deeper increments.

## Author contributions

NI: designed the study, designed and established the methods and infrastructure, analysis and interpreted the data, made statistics and wrote the manuscript. EP: collected and analyzed the data.

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## CV

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## Questions

### 1. Which parameters do not affect light transmittance through resin composites?

- a. Reflection, absorption and scattering of the incident light;
- b. Refractive index of filler and matrix;
- c. Filler size;
- d. Type of resin composites.

### 2. Which type of resin composites has been analyzed in the present study?

- a. A bulk-fill resin composite;
- b. A macro-fill resin composite;
- c. A resin-modified glass-ionomer;
- d. Nano and microhybrid resin composites.

### 3. Light transmittance through the analyzed 2-mm thick resin composite increments amounted:

- a. > 50% of the incident light;
- b. > 25% of the incident light;
- c. < 10% of the incident light;
- d. 0% of the incident light.

### 4. When restoring a deep cavity with resin composites incrementally:

- a. The lower increment needs to be cured adequately;
- b. The lower increment must only be pre-cured for few seconds since it will receive sufficient light at the end of the restoration;
- c. At very high irradiance, curing each increment for 1-2 s is sufficient, since exposure reciprocity is a valid concept;
- d. Curing at very high irradiance will reduce shrinkage stress.



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## THE EFFECT OF CERAMIC LIGHT SCATTERING ON AN INHOMOGENEOUS BEAM PROFILE

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## ABSTRACT

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**Aim:** The study aimed to measure light scattering of a broad spectrum light curing unit (LCU) as influenced by ceramic type, shade and thickness as well as exposure distance and LCU's position.**Methodology:** A broad spectrum LED LCU (ASCENT OL5) was mounted above a spectrometer (MARC Resin Calibrator, Blue, Light Analytics) at exposure distances of 1.0, 1.5 or 2.5 mm. The position of the center of the head was aligned with the spectrophotometer's sensor and then moved in 1 mm increments in the X-Y plane, while concomitantly recording the irradiance. The process was repeated with lithium disilicate and leucite glass ceramic slabs of similar thicknesses. The loss in irradiance related to the value measured at center position was analyzed by means of linear regressions and multiple ANOVA analysis.**Results:** The regressions showed a good fit (90% - 99%). Moving away from the center showed decreased irradiance. Values of slope obtained were divided by their respective intercept to eliminate the influence of the irradiance measured at the center. Two three-way ANOVA's were performed. One examined the influence of ceramic slab, direction and translucency/shade. It shows only the direction of measurement exhibited significant influence ( $p < 0.0001$ ) on the mean normalized slope values. The other one examined the influence of ceramic slab, direction and slab thickness. It shows the mean normalized slope values are significantly influenced by the direction of measurement and the slab thickness ( $p < 0.0001$ ). Values of the slopes indicated the ceramic scattering effect of the light. Thicker samples showed more scattering.**Conclusion:** The ceramic types, translucency/ shade had no significant effect on the light scattering. The thicker the ceramic the less irradiance changes were found indicating that the ceramics were scattering the light and thus slightly alleviating the effect of the inhomogeneous beam profile.**Keywords:** light curing, beam profile, glass ceramics, light scattering.**OPEN ACCESS** This is an Open Access article under the CC BY-NC 4.0 license.**Peer-Reviewed Article****Citation:** Roulet J-F, Khudhair MM, Shen C. The effect of ceramic light scattering on an inhomogeneous beam profile. *Stoma Edu J.* 2018;5(3):155-160**Academic Editor:** Nicoleta Ilie, Dipl-Eng, PhD, Professor, Ludwig-Maximilians-Universität München, München, GermanyReceived: June 19, 2018  
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## 1. Introduction

Bonded ceramic restorations have been used in dentistry for many decades and the first application was the resin bonded ceramic veneer [1,2]. A few years later, resin bonded ceramic inlays were tested first in vitro [3] and later in vivo [4]. In order to have enough time for the cementation process, clinicians prefer light-cure resin-based composites (RBC), which usually have a lower viscosity (luting agents) to facilitate the process of bonding veneers to teeth [5]. For inlays, which are usually thicker, dual-cure resin-based luting agents are preferred [5], because it is not clear if the blue light may penetrate the ceramic sufficiently to cure the resin-based luting agent. A clinical study has shown that after 12 years of observation, glass ceramic inlays and onlays (Empress, Ivoclar Vivadent) luted with dual-cure resin-based luting agent showed significantly fewer bulk fractures than those luted with a light-cured RBC (Tetric, Ivoclar Vivadent) [6]. Today we know that glass ceramics (leucite and lithium disilicate ceramics) are absorbing the blue light to a considerable amount [7-9]. The degree of light attenuation by overlying ceramics depends on the characteristics of the ceramic

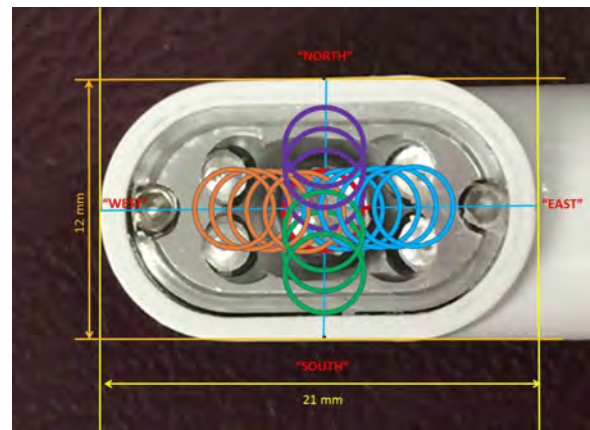
restoration such as optical behavior, crystalline structure, grain size, defects, intrinsic porosity, thickness and shade [10]. After passing 0.5 mm thick ceramic, the irradiance of the light reaching the resin-based luting agent would be reduced by approximately 75% [7]. In order to fulfill its function as luting agent, the resin-based luting agent must be adequately polymerized. This depends on its composition, e.g. the resin mix, the refractive index of the filler, the filler size and size distribution, pigment, and the photo initiators used [11,12]. Using microhardness measurements, for Variolink Estetic (Ivoclar Vivadent) the minimum energy required for adequate curing was found to be approximately 5 J/cm<sup>2</sup> [13].

Modern broad spectrum LED light curing units (LCU), use different types of LEDs, emitting light with different wavelengths (violet light (380 – 420 nm) and blue light (420 – 495 nm) [14,15]. Therefore, they are able to activate different types of photoinitiators [16]. Historically, the most used photoinitiator is champhorquinone (CQ) which has an absorption peak at 470 nm and requires a tertiary amine as co-initiator, which reacts with the activated CQ to create a free

radical used for the polymerization of the resin [17]. These amines, unfortunately, create a yellowing effect on the material over time [17]. Recently manufacturers started to use alternative photoinitiators, such as phenylbis-(2,4,6-trimethylbenzoyl)-phosphine-oxide (TPO) [18] or bis-(4-methoxybenzoyl)diethyl-germane (Ivocerin) [19,20]. These photoinitiators are much more effective than the combination of CQ and tertiary amine, but have absorption peaks below 410 nm (TPO) or 430 nm (Ivocerin), thus requiring broad band LCUs which usually have two different LEDs, (blue and violet light). However, these LED LCUs show more or less pronounced inhomogeneity of the beam profiles [21-24]. This means that not every point on an irradiated surface gets exposed to the same level of irradiation from the different wavelengths, especially in depth [25-27]. Since ceramics scatter the light because its direction is changed at the grain boundaries, one can assume that the local irradiances may vary less, once the light has passed through the ceramic, thus moving the beam profile more towards homogeneity. Furthermore, since the light is absorbed and scattered more with the thicker ceramic, this should affect the homogeneity of the beam profile as well. The objective of this study was to measure the beam homogeneity of an LCU with known inhomogeneous beam profile after the light had passed ceramic slices of different thickness. The null hypothesis tested is that (a) the ceramic has no effect on the beam profile and (b) the thickness of the ceramic has no effect on the beam profile as well.

**2. Materials and Methods**

A broad spectrum LED LCU (ASCENT OLS, CAO Group South Jordan, UT, USA) was attached to an x-y-z positioning device mounted on an optical bench in order to standardize the positioning of the light beam centered above the cosine corrector light signal collector of a spectrometer (MARC® Resin Calibrator, Blue light Analytics, Halifax, Canada) with the handle towards the right side ("EAST", Figure 1) at an exposure distance of 1.0, 1.5 or 2.5 mm. The diameter of the cosine corrector was 3.9 mm. Using the translation stage, the position of the geometrical center of the LCU was first aligned with that of the cosine corrector and then moved in 1-mm steps in the x-y plane ("EAST" – "WEST" and "NORTH" – "SOUTH") (Fig. 1). At each position, the irradiance was measured in triplicates. The process was repeated with ceramic slabs of 1.0, 1.5 or 2.5 mm thickness while applying the LCU directly on the ceramic slabs. The ceramics used and their translucency/shade are listed in Table 1. IPS Empress CAD slabs were used as cut from blocks with a diamond saw (Isomet 100, Buhler, Lake Bluff, USA), IPS e.max CAD slabs were cut from blocks and processed according to manufacturer's instructions in an oven (Programat P-500, Ivoclar Vivadent), under vacuum, using the following parameters: heating rate of 90°C/min up to 820 °C, holding time 0:10 min, then heating rate of 30°C/min up to 840°C hold for 7:00 min, followed by long-term cooling. The irradiance was assessed at each condition described above and related to the highest irradiance



**Figure 1.** Measuring points as related to the position within the light exit window. Blue cross = geometrical center of the light exiting window. Orange circles = "WEST"; Light blue circles = "EAST"; Purple circles = "North" and green circles = "SOUTH" (From Roulet et al 2018).

**Table 1.** The ceramics used and their translucency/shade.

Material	Shade and Translucency					
IPS Empress CAD	A1LT	B1LT	C2LT	D3LT	B3HT	C2HT
IPS e.max CAD	A1LT	B1LT	C2LT	D3LT	B3HT	C2HT

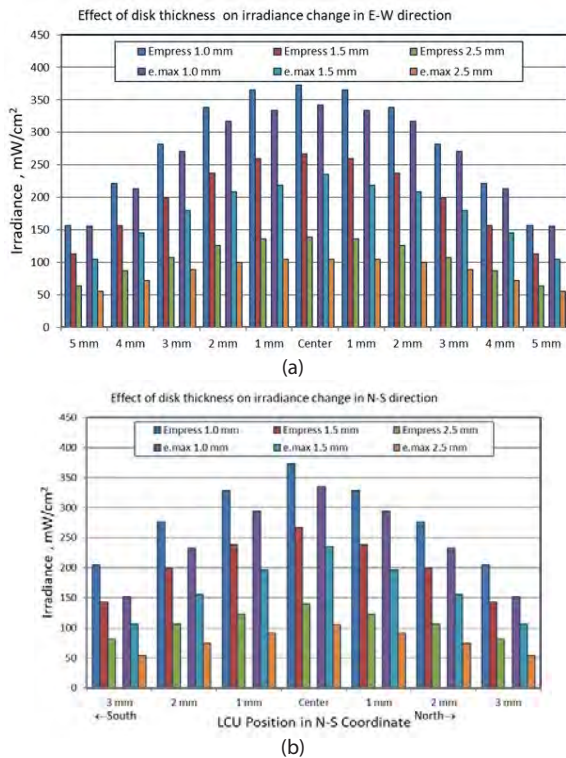
**Table 2.** The ratio of slope to the intercept of linear regression for all ceramics.

Direction	Translucency /Shade	Empress			e.max		
		1.0 mm	1.5 mm	2.5 mm	1.0 mm	1.5 mm	2.5 mm
East	HTB3	0.135	0.130	0.115	0.135	0.130	0.115
	HTC2	0.129	0.123	0.126	0.129	0.123	0.126
	LTA1	0.130	0.136	0.126	0.130	0.136	0.126
	LTB1	0.132	0.123	0.123	0.132	0.123	0.123
	LTC2	0.134	0.128	0.123	0.134	0.128	0.123
	LTD3	0.130	0.134	0.119	0.130	0.134	0.119
West	HTB3	0.169	0.165	0.141	0.169	0.165	0.141
	HTC2	0.163	0.148	0.129	0.163	0.148	0.129
	LTA1	0.129	0.193	0.149	0.129	0.193	0.149
	LTB1	0.158	0.145	0.142	0.158	0.145	0.142
	LTC2	0.172	0.169	0.146	0.172	0.169	0.146
	LTD3	0.159	0.164	0.157	0.159	0.164	0.157
North	HTB3	0.141	0.140	0.120	0.141	0.140	0.120
	HTC2	0.146	0.132	0.132	0.146	0.132	0.132
	LTA1	0.156	0.141	0.141	0.156	0.141	0.141
	LTB1	0.145	0.142	0.132	0.145	0.142	0.132
	LTC2	0.144	0.137	0.129	0.144	0.137	0.129
	LTD3	0.149	0.143	0.137	0.149	0.143	0.137
South	HTB3	0.159	0.154	0.137	0.159	0.154	0.137
	HTC2	0.168	0.148	0.143	0.168	0.148	0.143
	LTA1	0.170	0.120	0.124	0.170	0.120	0.124
	LTB1	0.168	0.143	0.140	0.168	0.143	0.140
	LTC2	0.167	0.142	0.150	0.167	0.142	0.150
	LTD3	0.162	0.162	0.147	0.162	0.162	0.147



**Table 3.** The ratio of slope to the intercept of linear regression (pooled shades and translucencies).

Direction	No ceramic slabs			Empress			e.max		
	1.0 mm	1.5 mm	2.5 mm	1.0 mm	1.5 mm	2.5 mm	1.0 mm	1.5 mm	2.5 mm
East	0.117	0.122	0.091	0.132±0.002	0.129±0.005	0.122±0.004	0.132±0.020	0.124±0.014	0.116±0.015
West	0.190	0.175	0.141	0.158±0.015	0.164±0.017	0.144±0.009	0.193±0.009	0.187±0.009	0.168±0.009
North	0.163	0.161	0.156	0.147±0.005	0.139±0.004	0.132±0.007	0.155±0.009	0.151±0.008	0.141±0.006
South	0.187	0.172	0.186	0.166±0.004	0.145±0.014	0.140±0.009	0.139±0.015	0.130±0.005	0.114±0.010

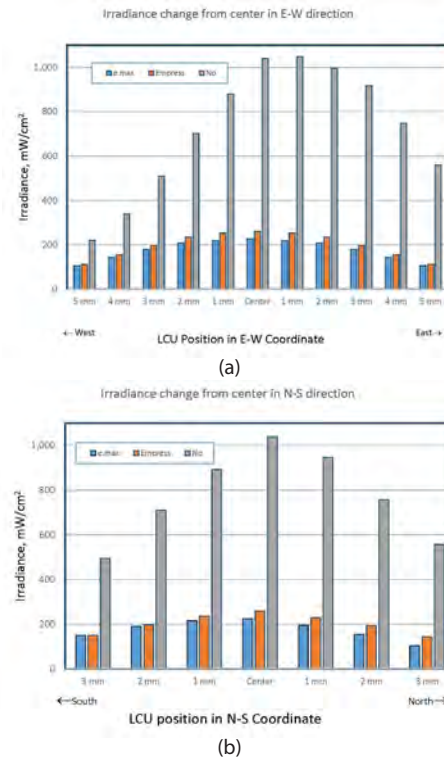


**Figure 2.** Effect of disk thickness on irradiance change in E-W direction (a) and N-S direction (b).

value which was identified in the present study when positioning the LCU directly and centered on the sensor. The percentage of irradiance loss was statistical analyzed by means of linear regression for each material, shade, thickness and direction. The effect of vertical position (distance between LCU and bottom of ceramic or sensor surface), horizontal position or off-set (X-Y plane), glass ceramic type, thickness and shade on the irradiance were analyzed using multiple ANOVA's (SAS 9.4, Cary, NC, USA).

### 3. Results

The numerical data show that there is a linear relationship between the irradiance measured by the MARC unit and the offset distance from the center, excluding the irradiance value at the center. A total of 144 linear regressions (SAS 9.4) were performed from a combination of two ceramic, four directions, three slab thicknesses and six translucency/shade of each ceramic. The degree of fit was in general greater than 90% with the majority in the 99% range. The value of intercept of linear regression is the calculated irradiance at the center and can influence the values of the slope. To normalize the irradiance at the center, each value of slope was divided by the respective value of intercept of the linear regression resulting



**Figure 3.** Irradiance change from center in E-W direction (a) and N-S direction (b).

normalized slope values with a unit of  $\text{mm}^{-1}$ . Although decreasing irradiance with offset distance yielded negative value of the slope; to avoid potential confusion of negative sign in the text absolute value of the normalized slope is used (Table 2).

Two three-way ANOVA's were performed. One examined the influence of ceramic slab, direction and translucency/shade. It shows only the direction of measurement exhibited significant influence ( $p < 0.0001$ ) on the mean normalized slope values. The other one examined the influence of ceramic slab, direction and slab thickness. It shows the mean normalized slope values are significantly influenced by the direction of measurement and the slab thickness ( $p < 0.0001$ ). The mean normalized slope value is in the decreasing order of 1.0, 1.5 and 2.5 mm. For the effect of direction of measurement, the mean normalized slope value is in the decreasing order of North, West, South and East. It means the inhomogeneity of the light curing depends on the device and the thickness of the ceramic slabs. The optical characteristic of the ceramic would have no influence on the inhomogeneity as determined by MARC. Figure 2 shows the values of irradiance with respect to the offset distance and direction of measurements for each thickness of the two ceramics.

The mean and standard deviation of normalized slope values by offset distance and slab thickness are pooled

together for each ceramic in Table 3 along with those when no ceramic slabs were present. The values of no ceramic slabs were means of taken from a previous study [28]. Figure 3 shows the mean irradiance values measured at each location. The values in the figure are means of all thickness and translucency/shade for both ceramics.

#### 4. Discussion

The LCU used in this study was selected on purpose because it is known to have a quite inhomogeneous beam profile as shown in a previous study [28]. The objective of this study was to show the effect of ceramics on the inhomogeneity of the beam profile.

Therefore, it makes sense to use an LCU, where this characteristic is to be expected. As shown in the previous study, the blue range the LCU used was so dominant (irradiance: blue 1088 mW/cm<sup>2</sup> vs violet 71 mW/cm<sup>2</sup>) [28], that the observed effects may be mainly for the blue light portion only. However, two factors need to be considered, that may influence the outcome. First, it is known that the violet light is scattered differently from the blue light by ceramic, which is seen by the different rate of attenuation [29]. Second, one should remember that the sensitivity of photo initiators sensible in the violet range is much higher than the one of camphorquinone for the blue light. Thus, despite the less amount of violet light reaching the resin-based composite, due to the better efficiency, the initiation of the polymerization is effectively enough to cure the RBC [30], which may influence the degree of conversion of an RBC at least under thin layers of the ceramic [29].

In the present study, the LCU was laterally moved in a controlled way in the X- and Y-direction of a coordinated system. This creates a decrease in the irradiance as a function of the offset from the center value, which can be determined as a slope, as seen in Figs 2 and 3, and Tab. 2, which is the expression of the inhomogeneity of the beam profile.

With the method used (controlled lateral movements), the inhomogeneity of the beam could be roughly reproduced. However, the cosine corrector light signal collector used has a diameter of 3.9 mm, which limits the precision. It is suggested for future measurements to use a smaller sensor diameter.

The ceramics used for the present study (Empress and IPS e.max) were chosen because they are sufficiently translucent for resin-bonded restorations (inlays, onlays, veneers and crowns) where light curing of the resin bonded luting material may be an option [31,32]. Therefore, the scattering behavior of the blue (and violet) light is important if the beam profile is inhomogeneous).

It is known that the irradiance at the target surface received is a function of the exposure distance [21]. Therefore, the decrease in irradiance reported in Fig. 2 is not only due to the increased thickness of the ceramic but also to the increasing distance from the sensor to the light exciting window of the LCU. Figure 3 confirms the fact that interposing ceramics in the light beam attenuates a substantive amount of light irradiance [7,33].

The decrease in irradiance as the measuring point moves farther away from the center is due to the design of the LCU and representative for its beam profile. A steeper slope (higher value) means a greater rate of irradiance

reduction relative to the offset distance. A flatter slope determined from the same experimental configuration but with the presence of ceramic slabs would mean that increased scattering of the light has occurred. As seen in Fig. 3, the absolute irradiance values without ceramic were substantially higher than those with ceramic interposition. Therefore, for direct comparisons, the slopes must be normalized as described in Materials and Methods. The ANOVA's showed that there were no significant differences with respect to the ceramic materials and translucency/shade; however, highly significant differences for the position (design of LCU) and thickness of the ceramic. It is known to clinicians that the translucency and the shade have a high impact on the aesthetical outcome of a restoration. This is the reason why ceramics are not only produced in different shades, but also in different translucencies. Therefore, it is interesting to see that the shades/translucencies have little effect of scattering the light in the blue or purple range. This confirms that yellow colors are more important to reach good esthetics with restorations of teeth.

In Table 2 where the data of all ceramics tested are displayed, the slopes with thick slabs are always lower, which means that there is a light scattering effect by the thickness of the ceramic; the thicker the ceramic the more light scattering. The same is visible in Table 3, where the ceramic shades and translucencies were pooled.

Based on the outcome of the ANOVA's, the ceramic had no effect on the beam profile, which can also be seen in Tab. 3. Therefore, the first null hypothesis must be accepted. However, one must note that the ceramic had a strong effect when one looks at the attenuation of the light, which was not the topic of the present investigation. The second null hypothesis can be rejected, since thicker ceramic slabs showed more scattering of the light.

#### 5. Conclusion

The thicker the ceramic the fewer irradiance changes were found as a function of the position indicating that the ceramics were scattering the light and thus slightly alleviating the effect of the inhomogeneous beam profile.

#### Author contributions

JFR: Idea, experimental design, wrote the manuscript. CS: Performed data analysis, substantially contributed to writing manuscript. MMK: Performed spectrometer experiment.

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## CV

Jean-François Roulet, DDS, Dr med dent, PhD, is the former chair and current professor of the Department of Restorative Dental Sciences at the University of Florida. Professor Roulet is author/coauthor of more than 180 papers, edited/contributed to 27 textbooks and mentored more than 150 theses. He is a renowned international lecturer with over 800 appearances to date. Dr. Roulet is a member of many professional organizations, has won numerous awards, and holds four patents. He is editor of the Prophylaxe Impuls and Stomatology Edu Journal. His areas of interest include minimally invasive dentistry, dental materials (ie, composites and ceramics), adhesive dentistry, esthetic dentistry, and application concepts in preventive dentistry.

## Questions

### 1. Why are amine free resin-based composites preferred to bond veneers?

- a. They allow for a longer working time;
- b. Tertiary amines used in combination with campherquinone tend to discolor over time;
- c. Tertiary amines and campherquinone require a broadband light curing unit;
- d. Amine free resin-based composites provide a stronger bond.

### 2. Which ceramics were used for the experiment?

- a. Leucite reinforced ceramic and lithium disilicate ceramic;
- b. Translucent Zirconium oxide ceramic;
- c. Feldspathic ceramic;
- d. None of the above.

### 3. The results were analyzed with:

- a. ANOVA and Wilcoxon test;
- b. ANOVA and t-test;
- c. ANOVA and Kruskal Walls Test;
- d. ANOVA and linear regression.

### 4. Which was the main outcome of the experiment?

- a. Ceramic color, shade and type had a significant effect on the scattering of light;
- b. Ceramic did not alter the irradiance;
- c. All of the above;
- d. The thicker the ceramic, the more light-scattering occurred.

## GREATER NEW YORK DENTAL MEETING



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## EFFECT OF WHITENING ON THE OPTICAL AND MECHANICAL PROPERTIES OF AGED RESIN COMPOSITES

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### ABSTRACT

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**Objectives:** To evaluate the effect of bleaching on colour, gloss, translucency, and microhardness of two types of resin composites (Charisma Classic and Filtek Z350 XT), previously aged.

**Materials and methods:** Forty specimens of each material were artificially aged for 300 hours (UV-accelerated aging). Specimens were then treated with 35% hydrogen peroxide (Whiteness HP) in four 15-minutes sessions, totaling one hour of bleaching. Colour coordinates CIE L\*a\*b\* and parameters of translucency (PT) were measured using a spectrophotometer (CM2600d – Konica Minolta), surface gloss readings were performed with a glossmeter (Novo-Curve – Rhopoint TM), and microhardness was assessed using a hardness device (FM-700, Future-Tech). Measurements have been performed after artificial aging as well as after the first and last bleaching protocol. Data were analyzed by two-way ANOVA and two-way repeated measures ANOVA, followed by Tukey's test (5%).

**Results:** The bleaching procedures of aged resin composites decreased the gloss and increased the translucency, but did not alter colour and microhardness.

**Conclusion:** Bleaching procedures should be used carefully when resin composite restorations are present.

**Keywords:** colour, hardness, gloss, bleaching agents, composite resins.

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 **Peer-Reviewed Article**

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### 1. Introduction

Dental bleaching is a conservative alternative with high success rates for treating discoloured teeth [1]. Tooth bleaching is a chemical process, which is performed with peroxide, chlorine, or chloride based-products. Among the products, hydrogen peroxide is the most commonly used agent. The bleaching process relies on the decomposition of peroxide into free radicals, while unstable molecules, such as tooth pigments, uptake electrons for stabilization [2]. Basically, the formed free radicals break up the double-bonds of the pigment's complex chains, resulting in a lighter pigment. This process represents the oxidative reaction of pigments within tooth structures.

Studies have reported that bleaching agents might affect the properties of resin composites; this influence is dependent on the bleaching agent and its concentration, as well as on the type of restorative material tested [3-6]. Microhybrid resin composites seem to undergo greater colour changes than nanohybrid ones; and the concentration of the bleaching agent seems to be less important than the time exposed to such products, although there are reports stating the higher the concentration the greater the bleaching efficacy on a determined period of time [5]. Regarding surface gloss assessment, previous

studies [7-9] concluded that bleaching agents are able to reduce significantly the gloss of resin composites, thus changing the aesthetic property of restorations. Bleaching procedure alters the colour of resin composite materials [6], but not the translucency. The literature is still controversial regarding the microhardness of resin composites after bleaching procedures. It has been reported that bleaching treatment with 10% carbamide peroxide decreased the surface microhardness of resin composites by 15% of its baseline value [10]. However, there are available studies on reduced [9,11,12] or even increased [13] microhardness, when testing nanofilled resin composites.

The influence of bleaching on resin composites is explained by a surface degradation and the presence of microcracks on the surface, which is aggravated over time [14]. Despite those studies, considering colour, gloss, translucency and surface hardness, the literature is not conclusive about the influence of bleaching treatment on the surface of different resin composites [3,5].

To simulate clinical condition several in-vitro studies uses artificial aging of resin composites. For those, artificial aging by UV light and moisture or water immersion protocols [15] have been proposed [16,17]. By employing artificial aging, the mechanical properties

of resin composites are negatively influenced over time due to filler degradation [18], or matrix plasticization [19]. The influence of isolated artificial aging or bleaching procedures over resin composites is well documented. The association of both parameters is usually tested using a bleaching procedure followed by artificially accelerated aging. Studies with that purpose have shown that colour and roughness of resin composites were significantly changed depending on resin type [16,17].

To the best of our knowledge, there are limited studies identifying potential problems that bleaching may result on the properties of previously aged resin composites, especially regarding the optical properties. Bleaching employed over aged resin composite restorations is a usual scenario faced in clinical practice. Therefore, the aim of this study is to evaluate the optical (colour, gloss and translucency) and mechanical (microhardness) properties of two types of resin composite, previously aged and whitened. The Null hypothesis is that none of the variables (colour, gloss, translucency microhardness) is affected by the bleaching procedure considering the independent variables tested (type of resin composite and time of bleaching).

## 2. Materials and methods

### 2.1. Sample preparation

The characteristics of the tested resin composites are shown in Table 1.

A total of 20 samples of each material were prepared for assessing colour, translucency, and gloss. Resin composite was placed in a single increment into a standardized circular matrix, 6 mm in diameter and 1 mm thick for specimen preparation. For the microhardness assessment, a total of 20 samples of each material were prepared, 3 mm in diameter and 1 mm thick. The molds and materials were covered with Mylar strips on the top and bottom and placed between two cover glasses. Finger pressure was then applied to extrude the excess of material and to promote an even and smooth surface. Specimens were light-cured (Radii Cal curing light, SDI, Victoria, Australia; 440-480 nm, 1200 mW/cm<sup>2</sup>) according to the manufacturer's instructions (20 seconds for both materials). The irradiance of the light-curing unit was assessed with a radiometer (Ecel, Ribeirão Preto, Brazil) prior to the fabrication of specimens. The samples were stored in ultrapure water for 24 hours at 37°C, to allow post-curing.

The specimens were positioned inside a metal holder, and the surface that would be treated with the bleaching therapy was polished with sequential (P1200, P2400, and P4000 grit) aluminum oxide abrasive papers (FEPA-P, Struers, Ballerup, Denmark) in a polishing device (DP-10, Panambra Industrial e Técnica SA, São Paulo, SP, Brazil) for 30 seconds on each paper (Fig. 1).

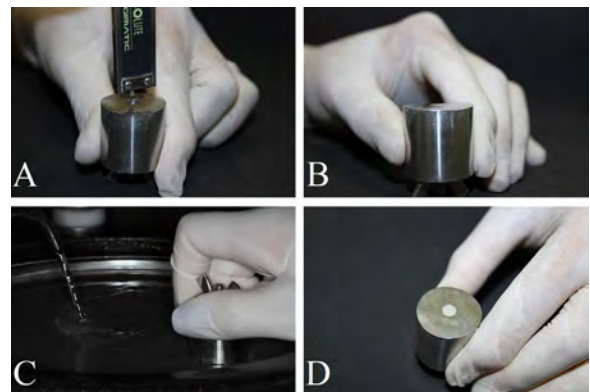
### 2.2. Accelerated artificial aging

After polishing, the samples were artificially aged in a weathering machine (SUNTEST CPS+ - Atlas Material Testing Technology, Mount Prospect, Illinois, USA), following ISO 7491 standard. One hundred aging cycles were performed (300 hours in total). Each cycle was composed of two hours at 55 ± 5°C and

**Table 1.** Characteristics of resin composites tested in the present study.

Composite	Shade	Type	Composition	Manufacturer	LOT
Charisma Classic	A2	Micro-hybrid	Bis-GMA, TEGDMA, barium aluminium fluoride glass (0.02–2 microns) and highly dispersive Siliciumdioxide (0.02–0.07 microns), with 78% filler content by weight	Heraeus Kulzer, Hanau, Germany	010027A
Filtek Z350 XT	A2	Nano-hybrid	Bis-GMA, Bis-EMA, UDMA, and TEGDMA; 20-nm nanosilica, and 5- to 20-nm zirconia nano agglomerates, with 78.5% filler content by weight	3M ESPE, St Paul, MN, USA	96374

Bis-GMA: bisphenol A-glycidyl ether dimethacrylate; UDMA: urethane dimethacrylate; TEGDMA: triethylene glycol dimethacrylate; Bis-EMA: ethoxylated bisphenol A-dimethacrylate.



**Figure 1.** Sequence of the polishing step. A. Thickness standardization; B. Specimen in position inside the metal holder; C. Polishing; D. Final aspect.

**Table 2.** Composition of the artificial saliva according to Göhring et al., 2004.

Component	mmol/l
Hydrogen carbonate	22.1
Potassium	16.1
Sodium	14.5
Hydrogen phosphate	2.6
Boric acid	0.8
Calcium	0.7
Thiocyanate	0.4
Magnesium	0.2
pH	7.4-7.8

irradiation at 765 W/m<sup>2</sup>, followed by one hour at 37 ± 5°C and no light irradiation, totaling 3 hours each cycle. That protocol simulates exposure of 160 klux, corresponding to intense natural light, equivalent to one year of clinical use [20]. During the artificial aging, samples were immersed in artificial saliva at 37 ± 1°C (Table 2) [21].

### 2.3. Colour assessment

The colour coordinates of each specimen were assessed, under standardized ambient conditions, according to the CIE  $L^*a^*b^*$  system, using a reflectance spectrophotometer (CM2600d, Konica Minolta, Osaka, Japan). The device was adjusted to D65 standard illuminant with 100% UV and specular component included (SCI). The observer angle was set at 2° and the device was adjusted to a small area view (SAV). The colour of each sample was measured three times, obtaining an average of  $L^*a^*b^*$  chromatic coordinates, as established by the Commission Internationale de l'Eclairage (CIE), which locates the colour of an object in a three-dimensional colour space. The  $L^*$  axis represents the degree of lightness within a sample and ranges from 0 (black) to 100 (white). The  $a^*$  axis represents the degree of the green/red colour within the sample, while the  $b^*$  axis represents the degree of the blue/yellow colour within the sample. The colour was measured over white ( $L^*$ : 84.95;  $a^*$ : -0.38;  $b^*$ : 2.93) and black ( $L^*$ : 2.58;  $a^*$ : -0.15;  $b^*$ : -0.24) standard backgrounds (Ceram, Staffordshire, UK) [22]. Optical contact between the specimen and the backgrounds was improved by using an interfacing layer of a polyethylene glycol 400 solution (LabSynth, Diadema, São Paulo, Brazil) [22]. From the colour measurements after the artificial aging and after the first or last week of bleaching, the values of the changes of  $L^*$  ( $\Delta L$ ),  $a^*$  ( $\Delta a$ ), and  $b^*$  ( $\Delta b$ ) were calculated. Next, the total change in colour or the variation in perception of colour of each specimen was calculated, designated by the abbreviation  $\Delta E_{ab}$ . Data obtained using white background were used for colour assessments. Colour variation was calculated using the following equation:

$$\Delta E_{ab}^* = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$$

### 2.4. Translucency assessment

Translucency was expressed using the translucency parameter (TP), which was determined by calculating the colour difference between the  $L^*$ ,  $a^*$  and  $b^*$  coordinates obtained over black and white backgrounds for each specimen, using the formula:

$$TP = [(L_B^* - L_W^*)^2 + (a_B^* - a_W^*)^2 + (b_B^* - b_W^*)^2]^{1/2}$$

The subscript B refers to the colour coordinates of the specimens over the black background and the subscript W refers to those measurements over the white background.

### 2.5. Gloss analysis

Surface gloss was measured using a glossmeter (Novo-Curve, Rhopoint TM, East Sussex, England), with a 2 mm × 2 mm area and a 60° geometry (light incidence), with values expressed in Gloss Units (GU). The measuring principle of this device is based on a light beam incident to a surface at 60°, and the glossmeter measures the intensity of the reflected light and compares it to a reference value. Three readings were carried out of each specimen and they were averaged to obtain a single value for each specimen during each assessment period.

### 2.6. Microhardness analysis

The surface microhardness (KHN) was assessed using a Knoop Microhardness Tested (FM-700, Future-Tech,

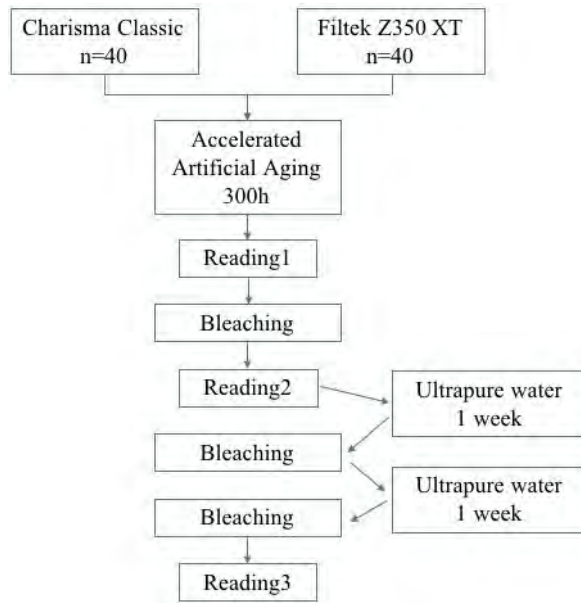


Figure 2. Schematic chart.

Tokyo, Japan), with a 25 g load for 10 seconds. Three random measurements were carried out on each specimen and the average represented each data point.

### 2.7. Bleaching

Bleaching procedures were performed in three sessions one week apart from each other. In each session, specimens received a layer of 35% hydrogen peroxide gel (Whiteness HP, FGM Ltda., Joinville, Santa Catarina, Brazil) during four sessions of 15 minutes, totaling 1 hour. In between sessions, the specimens were stored in ultrapure water under constant temperature in a bacteriological oven (37°C + 1°C).

Colour, translucency, gloss and microhardness were analyzed considering the baseline (Reading 1), the first (Reading 2) and last (Reading 3) bleaching sessions, being the last two readings performed right after the bleaching protocol. Figure 2 shows a schematic chart illustrating the experimental design of this study.

### 2.8. Statistical Analysis

Assumptions of the normal distribution (Kolmogorov-Smirnov test) were checked for all the variables tested. Data were statistically analyzed using STATISTICA software (StatSoft, version 8.0, 2010). Two-Way ANOVA was performed for colour analysis, followed by Tukey's test with 5% significance. All other parameters were analyzed by Two-way repeated measures ANOVA, followed by Tukey's test with 5% significance.

## 3. Results

### 3.1. Colour change

The mean and standard deviation values of the colour change ( $\Delta E$ ) for tested resin composites during bleaching related to the artificial aging are presented in Table 3. In relation to colour changes ( $\Delta E$ ) after bleaching (Reading 1-Reading 2 and Reading 1-Reading 3), both materials tested did not present significant differences ( $p > 0.05$ ).

### 3.2. Translucency

The two-way repeated measures ANOVA showed significant differences in TP for resin composites ( $p <$

0.01) and readings ( $p < 0.01$ ), with no interaction of factors ( $p = 0.901$ ). Charisma Classic overall presented lower TP in comparison to Filtek Z350 XT. For both resin composites, increased translucency was observed over time; this increase was detected after the first week after bleaching for Charisma Classic, while Filtek Z350 XT presented increased TP only in the third week of bleaching (Table 4).

### 3.3. Gloss

Gloss values as a function of time (baseline (before bleaching and after aging), 1 and 3 weeks after bleaching) are shown in Table 5. Reduction of gloss values was noticed after 1 week for Charisma Classic with no further reduction over time ( $p < 0.01$ ). For Filtek Z350 XT, surface gloss reduction was detected only after the third week of treatment ( $p < 0.01$ ). In terms of absolute gloss values, resin composite Charisma Classic presented lower gloss values.

### 3.4. Microhardness analysis

The values obtained for KHN are shown in Table 6. Two-way repeated measures ANOVA showed significant differences between the resin composite ( $p < 0.01$ ) while the time (baseline, 1 and 3 week) was not ( $p = 0.63$ ), nor was the interaction between factors ( $p = 0.87$ ). Charisma Classic presented KHN mean values lower than Filtek Z350 XT.

## 4. Discussion

The influence of bleaching and artificial aging procedures on the optical and mechanical properties of resin composite has been investigated [6,9,16]. It was expected that the bleaching procedure had no effects on the properties studied. However, this was not observed and, consequently, the null hypothesis was rejected. The results revealed that 35% hydrogen peroxide bleaching procedure was able to increase the translucency and reduce the gloss of aged resin composites. No differences were detected regarding colour and microhardness variations.

The optical and mechanical properties of resin composites can be influenced by several factors, including the aging process, bleaching procedures, as well by characteristics related to restorative material [5,7,23-25]. The UV-accelerated aging has a photo-oxidative potential, by means of the action of both UV radiation and water, inducing the cleavage of simple and double carbon bounds found in the resin matrix, such as Bis-GMA, UDMA and TEGDMA, being responsible for the material deterioration and changes in properties [9,23,26,27]. Regarding bleaching procedures, oxidative cleavage of polymer-chains by means of free prehydroxyl ( $\text{HO}^{\cdot}$ ) radicals, resulting from the degradation of the  $\text{H}_2\text{O}_2$ , have been suggested to explain the colour changes of restorative materials [28]. Water and molecular oxygen are also released during the reaction [29]. Such release may accelerate the hydrolytic degradation, and lead to colour changes of resin composites. In addition, factors related to composites can influence colour, including wear, matrix structure, volume and type of filler, and weakening of matrix-filler bonding [22,25,30]. Thus, a composite with higher resin matrix content is expected to be more prone to degradation and colour changes after bleaching treatment [31]. Although they presented

**Table 3.** Mean values ( $\pm$ SD) of colour change ( $\Delta E$ ) and results of Tukey's test.

	Charisma Classic	Filtek Z350 XT
Reading1-Reading 2	2.21( $\pm$ 0.95) Aa	1.92 ( $\pm$ 0.44) Aa
Reading1-Reading 3	2.08( $\pm$ 1.14) Aa	2.44 ( $\pm$ 0.66) Aa

In each row, distinct capital letters indicate significant difference between resin composite while distinct lowercase letters in each column, indicate significant difference among weeks ( $\alpha = 0.05$ ).

**Table 4.** Mean values ( $\pm$ SD) of translucency parameter (TP) and results of Tukey's test.

	Charisma Classic	Filtek Z350 XT
Reading 1	23.02 ( $\pm$ 5.62) Aa	26.4 ( $\pm$ 5.11) Ba
Reading 2	33.16 ( $\pm$ 1.95) Ab	37.31 ( $\pm$ 3.88) Bb
Reading 3	30.9 ( $\pm$ 2.39) Ab	35.42 ( $\pm$ 4.18) Bb

In each row, distinct capital letters indicate significant difference between resin composite while distinct lowercase letters in each column, indicate significant difference among weeks ( $\alpha = 0.05$ ).

**Table 5.** Mean values ( $\pm$ SD) of gloss and results of Tukey's test.

	Charisma Classic	Filtek Z350 XT
Reading 1	79.53 ( $\pm$ 8.54) Aa	84.77 ( $\pm$ 4.03) Aa
Reading 2	66.57 ( $\pm$ 12.15) Ab	84.37 ( $\pm$ 4.52) Ba
Reading 3	63.42 ( $\pm$ 9.1) Ab	67.5 ( $\pm$ 5.24) Ab

In each row, distinct capital letters indicate significant difference between resin composite while distinct lowercase letters in each column, indicate significant difference among weeks ( $\alpha = 0.05$ ).

different fillers, both resin composites present Bis-GMA in its composition, and it might have influenced the presently assessed colour stability for both nanohybrid and microhybrid aged resin composites. Previous studies on the effect of bleaching on the properties of aged composites reported conflicting results. The discrepancies between those studies may be caused by the type of aging protocol, bleaching agents used and total application time of agents or the type of resin composite tested. Some studies have shown a significant colour change for resin composite Charisma [16] or Filtek Supreme [9], while others did not [16]. Comparing the present data on aged resin composites to other studies in which aging was not performed [5,7], the bleaching process resulted in similar patterns of colour alterations, suggesting bleaching may behave similarly to aged and non-aged resin composite.

As reported by other studies [3,6], the potential effect of bleaching treatment depends on the type, concentration of bleaching agent, and the resin composite brand. For instance, it has been reported that Filtek resin composite presented different behavior to colour change after bleaching when compared to another nanohybrid resin composite [3]. Moreover, 16% carbamide peroxide is reported to be more effective for bleaching therapy than 35% hydrogen peroxide, and stained resin composites for enamel are more prone to bleaching. Besides that, the exposure time of resin composites to bleaching agent plays an even more important role to the material properties, since an extended exposure is more deleterious than higher concentration [6]. This can be attributed to the colour results of the present study. Translucency is related to light transmission, absorption, scattering and reflection [22]. This property is influenced by the difference between the refractive index of the resin matrix and filler particles, filler size, distribution,



concentration, pigments and defects like voids [30,32]. The increase of particles weight percent in resin composite (ranging from 10 to 70 %) reduced the translucency of the material. Additionally, the increase in particle size is also reported to result in increased translucency when comparing 0.77  $\mu\text{m}$  with 0.50  $\mu\text{m}$  fillers [32]. In the present study, an increased TP for Filtek Z350 XT was observed. It was reported that aging can change the translucency of the composites due to changes in scattering and absorption properties [33].

Previous studies reported that water from the bleaching agent is released during the bleaching procedures [29,34] and it is able to penetrate within the polymer chains and stay between the layers [27,35]. This process can induce hydrolytic degradation of the material [29,34,36], leading to filler–matrix interface debonding and changes in optical properties due to an altered pattern of light diffusion [32,33,36]. One might question this statement regarding the presence of water in the oral environment during the whole restoration service. The authors believe that the bleaching therapy exacerbates the potential of water influence on resin composites. In addition, the hydrophilic characteristics of the monomers present in resin composites may have contributed to translucency changes [36]. The presence of Bis-GMA and TEGDMA monomers, which present great susceptibility to hydrolysis, may lead to a water uptake and monomer elution and consequently an alteration in the light refraction index of the polymer matrix [37]. Those considerations might contribute to explain the translucency behavior detected. However, this condition needs to be further investigated.

One of the reasons for studying the surface gloss of materials relies on the fact they might influence colour matching/selection in clinical conditions [38]. It has been reported that the gloss is altered after bleaching procedures [7,9]. Once the material already presents a state of degradation, bleaching may worsen this condition. In theory, the deterioration or wear of materials can produce changes in surface gloss, resulting in esthetic changes [23,39]. Light reflection is related to filler size [40]. Small filler sizes are reported to present greater surface gloss than large fillers under the same polishing procedures, leading to greater specular reflection [41]. In the present study, the bleaching decreased gloss for all tested materials, and no differences were detected between nanohybrid and microhybrid aged composites. Possibly, the superficial layer was degraded due to the erosion of the resin matrix [7], a fact that might be the reason for the present results. Thus, the fillers were exposed, altering the surface topography, and therefore less gloss was detected.

Comparing the present data on aged resin composites to non-aged resin composite studies [7,8], the bleaching process resulted in similar patterns of surface gloss decrease, suggesting bleaching may behave similarly to aged and non-aged resin composite regarding such property.

It was observed that the application of the bleaching agent was not able to change the microhardness of the tested composites, confirming our expectations. The effect of the bleaching agent on microhardness seems to be related to the oxidation process, as previously described [14]. The oxidative cleavage of polymer chains induced by the free radicals released by hydrogen peroxide leads to chemical softening of the dental

materials [14]. In this study, different results would possibly be detected with increased bleaching time. It is important to emphasize that the microhardness of the two tested resin composites was different. The higher microhardness values of the Filtek Z350 XT are expected due to the presence of nanoclusters combined with nanoparticles, which reduce the interstitial space, improving the physical properties [42]. From these results, it was found that microhardness results were only material dependent.

Notwithstanding the low amount of studies and methodological variability, it has to be highlighted that aging and bleaching procedures produce relevant changes in the properties of the resin composite, which are material dependent. Although these changes might not be clinically observed, bleaching agents can lead to microcracks in the resin composite's surface. This might influence the clinical acceptability of resin composite restorations concerning the longevity, and further necessity of repair or replacement. Therefore, this study provides interesting information and support further investigations to complement the present obtained results.

## 5. Conclusion

Considering the limitations, the results of this in vitro study provide useful additional information on the optical and mechanical properties of aged resins after bleaching procedures. It is concluded that colour and microhardness were constant, before and after bleaching procedures. The bleaching agents were able to increase translucency and reduce the gloss of aged resin composites. Microhybrid composite showed lower values for all the parameters assessed when compared to the nanohybrid composite.

## Author Contributions

IFM: Study design, laboratory testing, discussion of results, manuscript writing, and final manuscript approval; AMC: Study design, laboratory testing, discussion of results and manuscript writing, and final manuscript approval; DCD: Study design, laboratory testing, discussion of results, and final manuscript approval; MCV: Study design, and discussion of results, and final manuscript approval; RMM: Study design, and discussion of results, and final manuscript approval; EB: Study design, statistical analysis, interpretations of data, and manuscript writing, and final manuscript approval.

## Disclosure

The authors do not have any financial interest in the companies whose materials are included in this article.

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**Questions**

**1. Which method was used to assess colour and gloss?**

- a. Spectrophotometer and Glossmeter, respectively;
- b. Glossmeter and Spectrophotometer, respectively;
- c. Spectrophotometer only;
- d. None of above.

**2. Which was the result of the study?**

- a. All the parameters were influenced by the bleaching therapy;
- b. None of the parameters were influenced by the bleaching therapy;
- c. Gloss decreased, and colour and microhardness were not influenced by the bleaching therapy;
- d. None of the optical parameters changed after bleaching therapy.

**3. Which was the bleaching therapy used?**

- a. 35% hydrogen peroxide for 20 minutes per application, three sessions;
- b. 35% carbamide peroxide for 15 minutes per application, three sessions;
- c. 35% carbamide peroxide for 15 minutes per session, three sessions;
- d. 35% hydrogen peroxide for 60 minutes per session, three sessions.

**4. Regarding the artificial accelerated aging used, choose the correct alternative:**

- a. Resin composites were not aged in this study;
- b. Aging was performed for 300 h using a UV-light chamber, simulating one year of clinical service;
- c. Aging was performed for 300 h using a UV-light chamber, simulating one year and a half of clinical service;
- d. Aging was performed for 300 aging cycles using a UV-light chamber, simulating one year of clinical service.



<https://members.perio.org/am2018/home?ssopc=1>

## SALIVARY MATRIX METALLOPROTEINASES ACTIVITY LEVELS IN AUTOIMMUNE DISEASES

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### ABSTRACT

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**Introduction:** It is widely recognized that saliva represents a solid alternative as a diagnostic fluid in a wide range of oral and general diseases. Autoimmune diseases constitute an important health threat to both men and women worldwide with matrix metalloproteinases (MMPs) playing significant roles in pathogenesis and development of these diseases. The specific aim of the present study is to assess, for the first time, salivary levels of a selected panel of MMPs in several autoimmune diseases.

**Methodology:** The study included 30 patients divided into groups such as: systemic sclerosis, vasculitis and healthy subjects. Salivary levels of MMP-1, -2, -7, -9 and -10 were analyzed using magnetic bead-based multiplex assays and Luminex technology.

**Results:** MMP-2 salivary levels were statistically elevated in systemic sclerosis, while MMP-10 were also increased in vasculitis patients. Salivary levels of MMP-9 were found significantly increased in all analyzed groups.

**Conclusion:** Taken together, our results promote saliva as a reliable diagnostic fluid for quantifying MMPs in autoimmune diseases.

**Keywords:** saliva, autoimmune diseases, matrix metalloproteinase, vasculitis, systemic sclerosis.

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### 1. Introduction

Over the past decades, saliva has become a hot topic in medical research related to discovering alternative diagnostic methods. Whole saliva is a complex fusion of the fluid produced by major and minor salivary glands with gingival crevicular fluid, oral desquamated cells, bacteria, as well as with food remains [1,2]. The advantages provided by saliva as a diagnostic fluid include non-invasive and effortless collection procedures, the need for small samples for analysis, along with easy storage and an established correlation between salivary and blood biomarkers' levels [3]. In addition to these benefits, its rich composition consisting of electrolytes, proteins and hormones deriving not only from oral sources, but also from systemic production, promote saliva as a remarkable diagnosis and monitoring fluid for both local and general diseases [4-6]. However, few studies have focused on the potential saliva has as a diagnostic fluid for autoimmune diseases [7,8].

Scleroderma is considered a rare disease that affects the connective tissue. Depending on the extent of the manifestations, scleroderma can be classified either as localized- when only the skin and in some cases the tissues underneath are involved, or systemic sclerosis, a more complex form in which cutaneous manifestations are associated with visceral impairment. Theories regarding the etiology of this affliction have incriminated genetic factors, trauma, viral or bacterial infections, neurological damage or vascular abnormalities. However, an increased level of antinuclear antibodies, as well as the frequent association with autoimmune diseases such as systemic vasculitis or systemic sclerosis, plead for an autoimmune disease characterized by an inflammatory phase followed by fibrosis, causing atypical collagen to replace normal dermis [9]. Vasculitis is a rare disease characterized by an inflammatory process located within the walls of blood vessels, affecting mainly small calibre vessels. The great diversity of the vessels affected

explains the clinical heterogeneity of this disease that involves a great variety of organs [10]. Meanwhile, matrix metalloproteinases (MMPs) represent a group of proteases produced by several types of cells, including pro-inflammatory cells with multiple roles such as leukocyte infiltration in conditions with an inflammatory component [11]. To the best of our knowledge up to the present, there are no scientific studies focusing on the salivary MMP levels in patients with autoimmune diseases. Taking all this into consideration, the main objective of the present research is to assess the potential use of saliva as a diagnosis and monitoring fluid for autoimmune diseases by evaluating salivary levels of a group of MMPs in patients with systemic sclerosis and vasculitis and compare them with those of healthy subjects.

## 2. Material and methods

### 2.1. Patient selection

Our study included a total of 30 patients: 10 patients with systemic sclerosis and 10 vasculitis patients. 10 healthy subjects, with no autoimmune disease, represented the control group. Clinical data including age, gender, current treatments, as well as a series of biochemical and immunological parameters have been collected for all the participants to the study. All subjects included in our research agreed to participate voluntarily and signed an informed consent. The present research was approved by the ethics board of Colentina Clinical Hospital, Bucharest, Romania. All patients were diagnosed by the same experienced medical professional.

### 2.2. Samples collection

For all participants in the study, saliva samples were collected at the moment of inclusion in the present study. Their written consent was taken prior to collecting the samples. The collection method followed a well-established protocol and took place as follows: 2-5 mL of unstimulated whole saliva was given by each participant in the morning around 9 AM. The participants were asked to refrain from eating, drinking or smoking prior to saliva collection. All subjects rinsed with 5 mL of distilled water before collection. The patients were also asked to sit down and be relaxed during saliva sampling as well as not to communicate with each other. Immediately after the collection, the samples were kept on ice and transported in an isotherm box and subsequently, they were centrifuged at 5000 rpm for 10 minutes. Samples were aliquoted and stored at -80°C until further determinations.

### 2.3. Matrix metalloproteinases detection

All MMPs were detected using Luminex technology and Magnetic Bead-Based Multiplex assays. This method enables the simultaneous detection of multiple salivary human biomarkers. Thus salivary levels of MMP-1, -2, -7, -9, -10 were assessed using MILLIPIX MAP Human MMP Magnetic Bead Panel 2 - Immunology Multiplex Assay. All samples and controls were processed following the manufacturer's specifications. The assay required 25 µL of saliva diluted at 1:20; each MMP had its own detection interval as follows: MMP-1: 27 –

**Table 1.** Clinical data of patients included in the study (autoimmune disease group and control group).

	Cases	Inclusion age, Years	Disease duration, Years	BMI, kg/m <sup>2</sup>
<b>Total subjects</b>	63	53.0 (37.5 – 60.5)	8.0 (4.0 – 14.0)	25.5 (21.8 – 29.9)
<b>SS</b>	10	52.0 (40.0 – 60.0)	2.0 (0.5 – 9.0)	25.9 (22.4 – 30.9)
<b>VA</b>	10	47.0 (42.0 – 70.0)	8.0 (2.0 – 14.0)	25.8 (24.8 – 35.1)
<b>Control</b>	10	45.0 (30.0 – 50.5)	-	21.9 (19.5 – 22.6)

SS – systemic sclerosis; VA – vasculitis; BMI – body mass index; kg – kilogram; m – meter;

**Table 2.** MMP levels in Systemic Sclerosis. SS n-10 cases.

	MMP1	MMP2 *	MMP7	MMP9 *	MMP10 *
<b>SS</b>	1424.43 (1009.22-1994.30)	24.3125 (19.32-29.33)	1093.68 (1003.02-1903.10)	6571.25 (5894.31-7043.44)	1796.62 (1221.33-1943.01)
<b>Control</b>	3565.167 (1237.5-4422.5)	10.3 (3.45-14.32)	1521.45 (854.3-2321.59)	1148.5 (843.4-2749.48)	943.82 (754.28-1232.37)

Data are expressed as median (inferior; superior limits); \*Mann-Whitney test; p – value < 0.05 is considered statistically significant

**Table 3.** MMP levels in Vasculitis. VA n-10 cases.

	MMP1	MMP2	MMP7	MMP9 *	MMP10 *
<b>VA</b>	1899.7 (1002.54-2540.9)	25.4 (10.22-38.43)	1071 (920.32-1643.39)	5551.7 (4988.38-5903.80)	2372.2 (2082.10-3002.48)
<b>Control</b>	3565.167 (1237.5-4422.5)	10.3 (3.45-14.32)	1521.45 (854.3-2321.59)	1148.5 (843.4-2749.48)	943.82 (754.28-1232.37)

Data are expressed as median (inferior; superior limits); \*Mann-Whitney test; p – value < 0.05 is considered statistically significant

20,000 pg/mL; MMP-2: 68 – 50,000 pg/mL; MMP-7: 548 – 400,000 pg/mL; MMP-9: 14 – 10,000 pg/mL; MMP-10: 27 – 20,000 pg/mL. The assay involved a two-hour multistep procedure involving preparation of reagents, samples and controls, preparation of the 96 well-plate and analysis using a Luminex® 200™ and the xPONENT® software for data acquisition and analysis.

### 2.4. Statistical analysis

The statistical analysis was performed using SPSS software. The characteristics were expressed as median (quartile 1; 3). Statistically significant correlations were found using the Mann-Whitney test (a two-sided p-value less than 0.05 was noted as statistically significant). A Spearman test was used to evaluate possible correlations between salivary MMPs levels, as well as for other significant bivariate (p-value < 0.05 was considered statistically significant).

## 3. Results

### 3.1. Clinical data

Several clinical parameters such as body mass index, age at the moment of the diagnostic and at the inclusion as well as disease duration were recorded for all subjects included in the study (see Table 1).

### 3.2. MMP-1, -2, -7, -9, 10 levels in Systemic Sclerosis

In the present study patients with SS showed a statistical increment in salivary levels of MMP-2, -9 and -10 when compared with respective controls. MMP-7 was also increased, but with no statistical significance. MMP-1 were statistically lower in the SS group vs the control group (Table 2).

### 3.3. MMP-1, -2, -7, -9, 10 levels in Vasculitis

Patients suffering from vasculitis had statistically increased levels of MMP-9 and -10. At the same time, the data were not statistically relevant for MMP-1, -2 and MMP-7 (Table 3).

## 4. Discussion

In the past, blood serum has routinely been used in the diagnostic process. However, most of the blood constituents are found in the saliva, as they pass through transcellular and paracellular routes. Nowadays saliva could be introduced as a diagnostic tool in many medical fields including microbiology, immunology, oncology, endocrinology, etc. Saliva is easy to collect, ship and store; it could be obtained in sufficient quantities at a low cost via non-invasive methods [12,13]. Saliva-testing kits have been presented on the market, although some of them are still waiting for approval. Intense efforts have been undertaken to detect markers that reflect tumor pathologies such as oral cancers. The composition of saliva also reflects pathological changes in salivary glands. Salivary tumors can release in the salivary flow important levels of stathmin or maspin, tumor necrosis factor, Dim1p, v-Ha-ras oncogene, type I collagen pro alpha or pirin [14]. Systemic cancers such as breast, gastric or larynx neoplasm can also be detected in saliva. *p53* is a tumor suppressor protein capable of blocking the tumor formation. Mutations in *p53* gene are found in most of the tumors and are associated with tumor development and poor prognosis. *p53* antibodies are found in the serum and saliva of patients with oral squamous cell carcinoma [15]. Another marker for oral squamous cell carcinoma identification, with significantly increased levels in saliva, is cortisol. The marker is even frequently used for tumor staging. Some growth factors concentrations in saliva also correlate with the development of neoplasms. Elevated levels of Fibroblast growth factor 2 (FGF2) correspond to salivary gland tumor growth. Sjögren syndrome, an autoimmune disease which among other effects alters the composition of saliva and tears is also studied in association with reactive species of oxygen (ROS) changes [16]. Several recent studies have shown that the biomarker composition is different in saliva from patients with Sjögren syndrome versus controls. Other studies analyzed saliva in connection with diseases such as Alzheimer, anorexia, bulimia of mental stress [17]. MMP-1 is a collagenase that normally can be found in low quantities in the cells, but increases its levels in autoimmune diseases as well as in inflammation. This protease that degrades collagen plays an important role in wound healing, immune response, inflammation, fibrotic disorders and cardiovascular diseases. Moreover, MMP-1 could be involved in cancer. Our data did not show an increased activity of MMP-1 in any included

**Table 4.** Increased salivary MMPs levels in autoimmune diseases (increments are based on own results and are statistically significant at a  $p < 0.05$ ).

	SS	VA
<b>MMP-1</b>	-	-
<b>MMP-2</b>	↑ 2 fold	-
<b>MMP-7</b>	-	-
<b>MMP-9</b>	↑ 6 fold	↑ 4 fold
<b>MMP-10</b>	↑ 2 fold	↑ 2 fold

autoimmune disease. Moreover, salivary MMP-1 was decreased SS and VA when compared to their controls, but the results were not statistically significant. These findings corroborated with existing scientific literature may suggest that MMP-1 does not play an active role in the pathogenesis of autoimmune diseases. Considering their organization, MMP-2 and MMP-9 are considered gelatinases. MMP-2 plays important roles in both physiological processes such as angiogenesis and in pathological situations like varicose veins disease, inflammation, and cardiovascular diseases. Meanwhile, MMP-9 is involved in cell apoptosis and osteoarthritis. MMP-9 was shown to present higher levels in patients with systemic sclerosis [18]. Interestingly, in our study salivary MMP-9 levels were found statistically higher in all patient groups included in the study when compared to the respective controls. Knowing that MMP-9 is generally involved in programmed cell death we can infer that this marker is involved in tissue remodeling processes occurring in these diseases. MMP-7 belongs to the matrilysins subgroup and has been linked to inflammation, lung disease, cardiovascular afflictions and to physiological cell apoptosis. In the present study, MMP-7 salivary levels were not statistically elevated in any studied disease. MMP-10 or stromelysin 2 has been linked to liver disease, viral infections, lung disease, tissue remodeling and cell apoptosis and is considered to play an important role in peripheral arterial disease. Our findings show that MMP-10 salivary levels were significantly elevated when compared to controls in both systemic sclerosis and vasculitis patients.

The present study has several limitations that will be addressed in further studies: a larger number of patients will be needed in the future to validate the present findings. Future correlations with MMP serum levels in all patients groups are needed in order to address the possible utility of salivary MMPs in clinical settings.

## 5. Conclusion

In conclusion, the present study shows for the first time, that important matrix metalloproteinase is increased and can be detected in saliva from patients with autoimmune diseases. Thus, salivary determination of MMP-1, -2, -7, -9 and -10 promotes saliva as a viable alternative when determining the activity of these enzymes in systemic sclerosis or vasculitis.

## Conflicts of Interest

The authors declare no conflict of interest.

### Author Contributions

All authors equally contributed to the present manuscript. IIS: participated in study design, data collection, data interpretation and manuscript writing; AD: participated in study design and sample collection; EB: participated in sample collection; DM: participated in sample analysis and manuscript writing; AT: participated in manuscript writing; RR: participated in data collection, data interpretation; BC: participated in study design, data collection, data interpretation and manuscript writing; PP: participated in critical review of the manuscript; DB: participated in critical review of the manuscript; MG: participated in study design, data collection, data interpretation and manuscript writing.

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### CV

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## Questions

### 1. Saliva is:

- a. A reliable diagnostic fluid containing biomarkers that statistically correlate with those found in blood;
- b. Not a reliable diagnostic fluid containing biomarkers that statistically correlate with those found in blood;
- c. A fluid produced by major and minor salivary glands with gingival crevicular fluid, oral desquamated cells, but not oral bacteria;
- d. An invasive method of collection.

### 2. Vasculitis:

- a. Is a common autoimmune disease;
- b. Usually affects large blood vessels;
- c. Can affect only a few organs;
- d. Is located in the walls of blood vessels.

### 3. MMP-2 is:

- a. Increased 2 fold in saliva from patients with vasculitis;
- b. Increased 2 fold in saliva from patients with systemic sclerosis;
- c. Not statistically increased in saliva samples taken from patients with autoimmune diseases;
- d. Not a gelatinase.

### 4. MMP-9 is:

- a. Increased 6 fold in saliva from patients with systemic sclerosis;
- b. Increased 2 fold in in saliva from patients with systemic sclerosis;
- c. Statistically decreased in saliva samples taken from patients with autoimmune diseases;
- d. Not involved in apoptosis.




The advertisement features a portrait of Ueli Grunder, DMD, on the left. The background is a gradient of dark red to orange. Text includes the Quintessence Publishing USA logo, the course title 'Esthetics in Implantology', the dates 'NOVEMBER 29-30, 2018', and the location 'QUINTESSENCE SEMINARS, BATAVIA, IL'. A circular badge on the right indicates 'UP TO 12 HRS OF CE CREDIT'.

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## EFFICACY OF A DENTAL EXTRACTION POLICY DESIGNED TO PREVENT OSTEORADIONECROSIS: A RETROSPECTIVE STUDY IN 100 ORAL CANCER PATIENTS TREATED WITH INTENSITY-MODULATED RADIOTHERAPY

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### ABSTRACT

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**Introduction:** The aim was to determine the efficacy of an extraction policy designed to prevent osteoradionecrosis (ORN) in dentate areas of the jaw after intensity-modulated radiotherapy (IMRT). A secondary aim was to establish whether our extraction policy risked unnecessary tooth extractions for areas designated to be not-at-risk of ORN.

**Methodology:** Data were retrospectively collected from 100 oral cancer patients, including the fate of 1430 individual teeth, from diagnosis to follow-up.

**Results:** Eight percent of IMRT-treated patients developed ORN; spontaneous cases (5) outnumbered those provoked by dental issues (3). All cases of ORN arose in regions irradiated with > 60 Gy, with the posterior mandible as preferred location, with non-spontaneous cases primarily due to progressive periodontitis. No correlation was found between the likelihood of extraction and cancer stage.

**Conclusions:** A more robust extraction policy of teeth affected with periodontitis and pocket depths of 4 - 5 mm might be advocated in molar areas predicted to receive > 60 Gy. Unfortunately, our study's retrospective design precluded any detailed analyses of the underlying reasons for the 88 extractions that occurred at lower radiation dose thresholds; a more conservative approach may therefore be warranted for these low-risk areas.

**Keywords:** osteoradionecrosis, intensity-modulated radiotherapy, dosimetric distribution, tooth extraction, oral cancer.

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### 1. Introduction

Osteoradionecrosis (ORN) of the jaw is a serious complication that can arise following radiotherapy for head and neck cancers [1]. ORN is defined as a slow-healing radiation-induced ischemic necrosis of the bone, associated with varying degrees of soft tissue necrosis in the absence of local primary tumor necrosis, recurrent, or metastatic disease [1]. When radiation is delivered in standard fractions, bone irradiated at doses in excess of 60 Gy appears to be the most vulnerable to ORN lesions [1]. However, occasionally, lesions arise in bone exposed to lower doses (usually above 50 Gy but below 60) [2]. Furthermore, ORN lesions may occur spontaneously after radiotherapy, or after trauma, particularly after dental extractions [1].

Pre-treatment dental screens aim to reduce the risk of developing ORN following radiation therapy by removing teeth predicted to be at an increased risk of harboring infection or becoming infected [3].

Frequently, these screens are based on the presumption that conventional external beam radiation therapy will be used, as is the case in our Department of Oral and Maxillofacial Surgery, University Hospitals of Leuven (Leuven, Belgium). However, with the introduction of intensity-modulated radiotherapy (IMRT), a significantly higher proportion of normal tissue is spared from high-dose radiation [4], with these high doses restricted to a smaller area. This leads us to suggest that a modified extraction policy might be appropriate for IMRT patients [5].

To determine which teeth should be left in situ versus those that should be extracted (as a precautionary measure in terms of ORN prevention), clinicians in our department base their decision-making on the outcome of a "single tooth risk assessment" (Table 1), which is used for areas of the mouth at a low risk of developing ORN (i.e. areas < 50 Gy).

Teeth which are designated "irrational to treat" in Table

1) are extracted (leaving those with good prognoses in-situ. The fates of teeth with doubtful prognoses are then based on levels of oral hygiene, any limitation in the ability to open the mouth, and the clinical experience of the supervising surgeon: whenever possible these teeth are treated and retained.

For those areas at a high risk of developing ORN (> 50 Gy), extractions are completed whenever the tooth demonstrates one of the signs/conditions listed in Table 2 [6]. Some teeth are also necessarily sacrificed as part of the surgical resection procedure.

The main objective of this study was to determine the efficacy of the tooth extraction policy in preventing ORN at the Department of Oral and Maxillofacial Surgery, University Hospitals of Leuven (Leuven, Belgium). A high-performance extraction policy would accurately target only those teeth that constitute a risk of promoting ORN. However, two other scenarios, should they be identified, would be of concern as these would indicate failings in the current preventative extraction protocol. These scenarios include a failure to extract teeth that subsequently provoke ORN, or the identification of extractions in the absence of risk (i.e. non irradiated teeth, teeth irradiated with < 50 Gy radiation, or teeth with none of the indications in Table 1).

## 2. Materials and Methods

### 2.1. Subjects

Our 100-patient cohort (with 1430 individual teeth under consideration) comprised patients diagnosed with cancer of the oral cavity between January 2012 and September 2016, at the Department of Oral and Maxillofacial Surgery, University Hospitals of Leuven (Leuven, Belgium). These patients were subsequently treated with IMRT at the Department of Radiotherapy and Oncology, University Hospitals of Leuven (Leuven, Belgium).

All patients underwent pre-IMRT dental screening, after which extractions of compromised teeth were performed: the "irrational to treat" as listed in Table 1, the indications as listed in Table 2, and extractions deemed necessary because of the planned resection procedure. Since clinical, radiographic, and radiotherapeutic documentation were available for all patients, we could retrospectively determine which teeth remained in-situ and were present at the time of IMRT. These teeth were subsequently denoted as "not being considered a risk factor for ORN development after IMRT".

Using the individual IMRT plans for each patient, all teeth (including those that were, and were not extracted) were subdivided according to maximum dose, with categories of < 50 Gy (1), 50 - 60 Gy (2), and > 60 Gy (3). This distinction was made because there is still a risk of ORN, albeit reduced [2], even if the total radiation dose delivered by standard fractionation is below 60 Gy [1].

Patients who developed ORN prior to February 2017 were categorized according to two types of ORN: spontaneous ORN that occurred post IMRT, and ORN caused by a dental issue.

The following data were also collected: patient identification number, date of cancer diagnosis, start date for IMRT, the cause of ORN and its date of diagnosis. To avoid bias, clinicians involved in gathering and processing data were not involved in treatment.

**Table 1.** Single tooth risk assessment.

Prognosis	Dental field	Criteria
Good	-	-
Doubtful	Periodontal	Furcation involvement (levels II and III)
		Angular bone defects
	"Horizontal" bone loss exceeding 2/3 of the root	
Endodontal	Incomplete root canal therapy	
	Periapical pathology	
Dental	Presence of voluminous posts/screws	
	Extensive root caries	
Irrational to treat	Periodontal	Recurrent periodontal abscesses
		Periodontic-endodontic lesions
	Endodontic	Attachment loss to the apex
		Root perforation in the apical half of the root
	Dental	Periapical pathology in the presence of obturating post and core
		Vertical fracture of the root
Functional	Oblique fracture in the middle third of the root	
	Caries lesions that extend into the root canal	
		Third molars without antagonist and with periodontitis/caries

**Table 2.** Tooth extraction policy for areas predicted to receive a radiation dose > 50 Gy.

Lesions induced by deep caries that could expose the pulp during treatment	
Active periodontitis with:	pockets > 6 mm
	furcation involvement > = level I
	mobility > level I
	gingival recession > = 6 mm
	any combination of these periodontal criteria
	in patients that demonstrate poor cooperation, any tooth with active periodontitis in the field of radiation is extracted
Non-restorable teeth with large and/or subgingival restorations	
Root caries	
Teeth with large restorations, combined with severe erosion and abrasion	
Teeth with a periapical granuloma	
Avital teeth	
Partially impacted or partially erupted teeth that are not fully covered by bone, or those showing a radiolucency above the crown	
Teeth with cyst formation	
Teeth showing radiographic abnormalities	
Teeth with cracked tooth syndrome	

### 2.2. Statistical Analyses

The primary objective of this study was analyzed statistically by evaluating patient subsets with percentages and confidence intervals, as well as plots of an empirical distribution function. Statistical analyses were supervised by a certified statistician.

### Ethical Approval

This study was approved by the ethical committee of UH Leuven (S54701).

### 3. Results

In 100 patients, 1770 teeth were absent at intake (Table 3). Management decisions for the 1430 remaining teeth were then made prior to surgery and/or radiation therapy. In total 1031 teeth were left in situ, with 399 extractions. The respective dose predictions for the 399 extracted teeth were as follows: 156 were destined to receive a radiation dose of < 50Gy; 59 were projected to receive > 50Gy but < 60 Gy; and 48 extractions were in an area that would receive a dose of > 60 Gy. Another 136 teeth were removed during the surgical procedure due to their position in the resection zone or immediately opposite the free flap.

In those areas predicted to receive a radiation dose of less than 50 Gy, 156 teeth were also extracted: 75 for dental reasons (indications listed in Table 1), and 81 for (other) reasons that could not be established following review of the medical files. Due to the retrospective character of this study, it was impossible to identify the relevant decision-making criteria for these 81 extractions (i.e. limited cooperation of the patient, predictions of poor oral hygiene, or limited ability to open the mouth). Irrespective of the underlying reasons, the extraction policy in these areas more closely resembled the extraction policy used to prevent ORN. Collectively, 22 patients underwent 81 extractions from areas that were either not destined for irradiation, or were destined to be irradiated with a dose of < 50 Gy. One hypothesis that was tested was whether patients with an advanced stage of cancer would undergo more extractions in areas not prone to exhibit ORN of dental origin? Figure 1 shows a scatterplot depicting the correlation between tooth extraction (in each patient) and cancer staging at the tumor level. A linear correlation could not be found. The Spearman's rank correlation coefficient was -0.0314 (p-value = 0.7557). If only those patients that underwent tooth extractions were included, the Spearman's rank correlation coefficient was 0.285 (p-value = 0.1465). Consequently, no correlation was found to exist between the stage of the oral cancer and the tooth extraction policy.

The patients in this study had an average follow-up period of 815 days, or close to 2 years and 3 months after starting IMRT. In total, 8 out of 100 patients developed an ORN lesion, 5 spontaneously, and 3 after an extraction. For the five spontaneous cases (with no link to dental problems), 1 lesion occurred in the maxilla and the other 4 cases were in the mandible. All lesions arose in the body of the mandible, in areas where radiation levels exceeded 60 Gy. Lesion incidence showed a preference for the posterior parts of the jaw in areas corresponding to (6) molars, (3) bicuspid, and (1) canine.

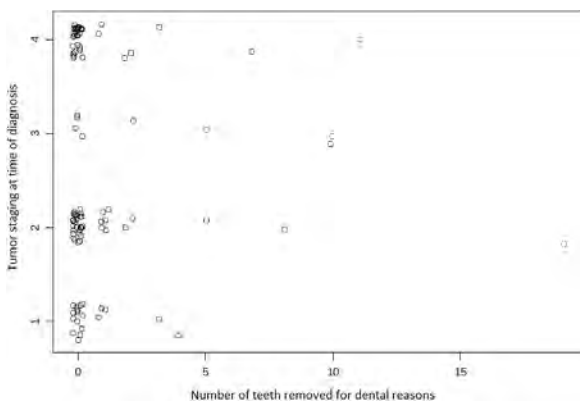
Three patients developed ORN lesions caused by dental problems from the retention of 6 teeth that were presumed to pose (at the time of the extraction decision-making) no risk of ORN.

The average period between commencing IMRT and developing ORN was 438 days, or just over 1 year and 2 months; onset data for individuals (all 8 patients that developed ORN) with follow-up periods for the entire cohort are shown in Figure 2.

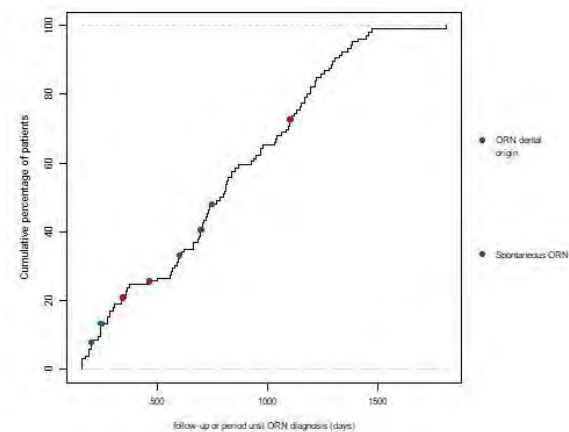
All three ORN lesions of dental origin arose in the mandible in areas exposed to a radiation dose of 60 Gy

**Table 3.** Details of all extractions.

Area	Tooth status	Number of teeth
	Absent at intake	1770
	Present at intake	1430
Resection site	Peroperative removal in the resection specimen	136
Irradiated area > 50 Gy	Left in situ	397
Irradiated area > 50 Gy	Extracted for ORN prevention	76
Irradiated area > 50 Gy	Extracted for a dental reason	31
Non-irradiated or <50 Gy	Left in situ	634
Non-irradiated or <50 Gy	Extracted for a dental reason	75
Non-irradiated or <50 Gy	Extracted, reasons not specified	81



**Figure 1.** Scatter plot showing the relationship between the number of teeth removed and tumor size.



**Figure 2.** Empirical distribution function showing the follow-up period prior to developing ORN.

or higher. The causal teeth for these lesions were canine (1), bicuspid (3), and molar (2). The medical files did not mention any specific pathological findings at the canine or bicuspid at intake. The molars showed signs of periodontitis with pocket depths of 4 and 5 mm, respectively, at intake. Eventually, these teeth had to be removed as periodontitis had progressed. Either this infection, or the subsequent extraction, triggered ORN.

### 4. Discussion

As early as 1922, the first case of ORN was described by Regaud [7]. In 1926, Ewing followed with an article

describing bone changes associated with radiation therapy that were termed "radiation osteitis" [8]. Despite the extensive research provoked by these early reports, ORN still poses a substantial threat in patients that have undergone radiation therapy in the head and neck region, especially given the absence of any standard conservative treatment [9], and the frequent requirement for extensive surgery [10].

The first category of ORN that should be addressed is the spontaneous lesion. These can, by definition, not be prevented, even with an adequate extraction policy, as their origin is unrelated to trauma [1]. In this study, all but one spontaneous lesion arose in the mandible, with these results in line with the higher susceptibility of the mandible to ORN versus (vs.) the maxilla [1, 10]. The overall ratio for ORN incidence for the mandible vs. maxilla was 24:1 [11]. All spontaneous lesions in this study were found in the alveolar ridge, with a preference for more posterior regions. The lesions occurred in areas corresponding to 6 molars, 3 bicuspid, and 1 canine. These findings are also in line with the general consensus that the posterior regions of the jaw are more susceptible to developing ORN lesions [1, 10].

The second category of ORN lesion would appear to be preventable given that this category arises following an extraction or by dental infection that occurs in areas of bone exposed to a high level of radiation [1]. The risk of developing ORN because of dental problems in highly irradiated alveolar bone persists for life [12]. Furthermore, there is an increased risk of developing dental caries [13], and periodontal defects [14] after radiation therapy. Teeth predicted to lie in the path of high doses of radiation (> 50 Gy) should be in good condition in order to satisfy the criteria for being left in situ without posing a risk of developing ORN [6, 15, 16]. In this study, ORN caused by dental problems still developed, with three cases identified. This finding indicates that more teeth should have been extracted from the area of high radiation in order to prevent ORN. These findings point to periodontal disease as the greatest hazard, with periodontal pockets of 4-5 mm present at dental screening that subsequently progressed during follow-up.

It was noted that ORN lesions caused by extractions showed a slight preference to develop in the posterior regions of the alveolar bone, although not to the same extent as spontaneous ORN lesions. The teeth that provoked ORN in the 3 patients were a canine, 3 bicuspid, and 2 molars.

Given that IMRT spares many teeth from high doses of radiation when compared to conventional external beam radiation therapy [4], less teeth are predicted to be at risk to develop ORN lesions. However, the findings of this study fail to account for the higher number of extractions in areas considered to pose no risk of developing ORN (81) vs. the 76 teeth that were at risk (Table 3). It is unclear, due to the retrospective design of this study, as to what triggered the extraction decisions in these scenarios. However, possibilities include poor oral hygiene, future prospects of a limited ability to open the mouth, poor surgical access, an uncooperative patient, or a mistaken evaluation of the area of high radiation. A prospective study should now

clarify this aspect of the decision-making process. For those areas not destined to receive > 60 Gy by IMRT, a more heuristic approach might be beneficial in terms of improving decision-making without bias.

Due to the elevated incidence of oropharyngeal cancers caused by the human papilloma virus (HPV) [17, 18], the number of patients at risk of ORN is predicted to rise. The highest doses of radiation will be in the oropharynx itself [19], with a smaller amount affecting the anterior area of the mandible. Therefore, there should be a focus on maintaining the strict extraction criteria for ORN prevention [6] for posterior teeth, particularly those in the mandible, whereas areas with a low risk of ORN should be treated with the less austere general practice guidelines as outlined in Table 1.

In total, 8 out of 100 patients developed an ORN lesion. Recent studies have reported an overall incidence of ORN in IMRT patients treated for oral cavity and oropharyngeal cancer of 25.5% (mean follow-up of 41 months) [20] and 40% (after 5 years of follow-up) [21]. The 8% incidence reported in this study is therefore much lower than average. However, the short average follow-up period of 815 days (just under 2 years and 3 months) may explain this low incidence rate that is predicted to increase with lengthier follow-up.

## 5. Conclusions

This study revealed that, in spite of an existing extraction policy, ORN lesions caused by dental problems still occurred. Since ORN in these patients only developed in areas in receipt of > 60 Gy, primarily in the molar areas, a more robust extraction policy could be warranted for these areas.

This study made a distinction between areas receiving a radiation dose of between 50 and 60 Gy, and those areas that received a higher radiation dose. The results show that all ORN lesions caused by a dental problem occurred in areas of 60 Gy or higher. As such, a cut-off value of 60 Gy of radiation may be a good guideline to establish for decision-making processes. The overall incidence of 8% (8/100) ORN after IMRT for oral and oropharyngeal cancer, after a follow-up period of 2.3 yrs., is considered to be low, although this figure is expected to increase with time. Spontaneous ORN (5 patients) was more common than ORN caused by dental problems (3 patients).

As for extractions in areas not destined for irradiation, or predicted to receive less than 50 Gy, a heuristic approach to extraction decision-making is warranted. Future prospective studies could clarify the reasoning for those extractions that failed to meet the expected extraction criteria. We anticipate that this approach will benefit patients given that the loss of multiple teeth is debilitating to the irradiated patient, both physically and emotionally.

## Author contributions

CP, PD, MS: Substantial contributions to the design of the work; JS: Drafting the work; SN, DN: Substantial contributions to the acquisition of data; RJ: reviewed the manuscript.

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## Questions

### 1. ORN occurred in areas irradiated with:

- a. < 40 Gy;
- b. < 50 Gy;
- c. < 60 Gy;
- d. > 60 Gy.

### 2. IMRT causes ORN in irradiation of oral and oropharyngeal cancer:

- a. within the first 3 months after irradiation;
- b. within the first 6 months after irradiation;
- c. within the first 12 months after irradiation;
- d. usually after the 1st year of irradiation.

### 3. ORN usually does not occur in:

- a. in the upper jaw;
- b. in the ascenic ramus of the lower jaw;
- c. in the dentate area of the lower jaw;
- d. in the area of the lower jaw where extractions have been done.

### 4. The following teeth need not to be extracted if they occur in a zone with 72 Gy of irradiation:

- a. mobility > 6 mm;
- b. periodontal pocket of 2 mm;
- c. furcation > 1 mm;
- d. root caries.



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## THE EFFICACY OF BONE SCRAPER FOR BONE WINDOW OSTEOTOMY IN THE COURSE OF SINUS AUGMENTATION

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### ABSTRACT

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**Introduction:** The aim was to determine the efficacy of the bone scraper in the course of sinus augmentation.

**Methodology:** A total of 50 sinus augmentation procedures performed in 35 patients were included in this study. The outcome parameters included membrane perforation and bone volume collected.

**Results:** Twenty-two were female, and 13 males. The age ranged from 55 to 80 years. The average osteotomy site dimensions were  $(12 \pm 2) \times (10 \pm 2)$  mm. There were no perforations of the sinus membrane during the creation of the bony window. The average bone volume from the 50 osteotomies was  $0.75 \pm 0.25$  cm<sup>3</sup>. There were no statistically significant differences between edentulous vs. partially edentulous patients, men vs. women, or time length of edentulism.

**Conclusions:** The proper use of the bone scraper allows autogenous bone harvesting and prevents sinus membrane perforation during the creation of the bony window and is cost-effective financially.

**Keywords:** bone scraper, sinus floor, augmentation, perforation, membrane, bone graft.

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### 1. Introduction

Implant dentistry in the posterior atrophic maxilla still poses a challenge, due to lack of alveolar bone height. Sinus augmentation procedures are done to generate sufficient bone to place an implant. Bone graft may be autogenous bone and/or xenografts and/or alloplasts to fill in the volume created. For the autogenous donor site, the iliac crest or the mandibular symphyseal area are often used [1-9]. Autogenous bone graft particles still serve as the gold-standard of bone grafting [8-12]. Particulate bone grafts are often used alone or in combination with xenografts or allografts during sinus augmentation procedures [13]. Estimating the bone volume to be harvested prior to surgery for maxillary sinus floor bone grafting might help in selecting the donor site, minimizing complications following bone harvesting, and reducing expenses [14].

The bone scraper (Ebner™ grafter, Maxilon Laboratories, Inc. Hollis, NH, USA) is an instrument comprised of a reusable stainless-steel handle and a disposable blade. The blade shaves bone from cortical surfaces producing

short convoluted ribbons. While being cut, shavings combine with blood and flow into the handle's head. This graft material, an osseous coagulum, is then delivered with the handle directly to the recipient site, or to a bowl.

The object of this study was to measure the amount of bone that can be harvested from common bony window osteotomy prior to sinus membrane elevation dental implant osteotomies using the bone scraper in order to give the surgeon an idea of how much bone can be collected. Thus, the surgeon will be able to predict in advance how much additional graft material will be needed to fill the sinus.

### 2. Methodology

Patients undergoing sinus augmentation at the Tel Aviv University or the private practice of the authors were included in the study. Inclusion criteria were absence of clinically significant health problems, use of bone scraper, valid documentation regarding sinus

membrane perforation and bone volume. The study was approved by the ethical committee of the Tel Aviv University.

Surgery commenced as a routine lateral approach sinus augmentation. Following local anesthesia and flap elevation, the entire head of the bone scraper was dipped into sterile saline solution before starting to cut and collect bone from the window osteotomy. Force was applied perpendicularly to the lateral sinus wall surface and the instrument was pulled backwards. The instrument cut with straight-back or side motion. Repetitive strokes were used to cause bone shavings and blood to flow through the aperture into the head. Looking through the view slots monitored progress. Over-filling was avoided to prevent aperture clog. The blade was removed with the aid of a curved 12.5 cm Halstead Mosquito hemostat. The head was placed close to a bowl and the graft material was spooned out with a curette (Figs. 1-3). In order to harvest additional graft material, the blade was reattached and the procedures were repeated as required. Membrane perforations were registered. Mesiodistal and vertical dimensions of the lateral bony window were measured using a periodontal probe. The samples were lightly packed into a 1 mL syringe for volumetric evaluation. Chi square test was used for statistical analysis.

### 3. Results

Thirty-five patients were included in the study. Twenty-two were females and 13 males. The average age was  $62 \pm 3$  years, with a range of 55 to 80 years. A total of 50 augmented sinuses were included. The average window osteotomy site dimensions were  $(12 \pm 2) \times (10 \pm 2)$  mm. There were no perforations of the sinus membrane during the creation of the bony window. The total number of major ( $> 5$  mm) sinus membrane perforations reached 5%. The average bone volume was  $0.75 \pm 0.25$  cm<sup>3</sup> per osteotomy window. There were no statistically significant differences between males and females, partially edentulism and complete edentulism. When comparing length of edentulism,  $< 5$  years to  $> 5$  years, no statistically significant differences were noted.

### 4. Discussion

When using a bone scraper during window osteotomy sites, a predicted  $0.75 \pm 0.25$  cm<sup>3</sup> of bone can be obtained from an osteotomy site of approximately  $(12 \pm 2) \times (10 \pm 2)$  mm. Autogenous grafts can often be combined with xenografts or alloplastic materials to provide extra bulk to fill the sinus or peri-implant defects when implants are placed simultaneously. Collecting graft material in this way can often spare the patient a separate surgical donor site. Surgical expense and time can also be saved when this technique is used, which is beneficial to both the patient and the surgeon. When combining xenografts or alloplastic material with autogenous bone, decreased amounts of xenografts or alloplastic material will have to be used to increase the bulk of the graft. This also reduces the expense for xenografts or alloplastic materials.



Figure 1. Bone harvested from one window.



Figure 2. Bone scraper after blade removal.



Figure 3. Bone spooned out with a curette.

In the present study, a volumetric measurement was used because it is more closely associated with xenografts or alloplastic materials, which are often purchased by volume rather than weight.

A recent study [15] described the use of a mini bone scraper for a lateral bone window approach and simultaneous bone harvesting during sinus floor elevation. There was no injury of the Schneiderian membrane, and the mean volume of particulate bone collected from the anterior wall of the maxilla using this technique was 0.74 cm<sup>3</sup>. Supplementary allogeneic materials were not required in all cases. They concluded that the use of bone scraper is a simple and safe procedure in lateral bone window approach and simultaneous bone harvesting during sinus floor elevation, because it is performed under better



visualization of the membrane without irrigation. The maxillary sinus [14] volume was measured as an aid to determine the volume of graft bone needed before grafting the autogenous bone to the maxillary sinus floor. Maxillary sinus volumes were measured from computerized tomographic images of 38 sinuses using a 3-dimensional reconstruction system. When the sinus-lift procedure was simulated, the mean volume for bone grafting was 0.70 cm<sup>3</sup> for 5 mm lifting, 1.92 cm<sup>3</sup> for 10 mm lifting, 4.02 cm<sup>3</sup> for 15 mm lifting, and 6.19 cm<sup>3</sup> for 20 mm lifting. Combining the data from this study [14] with the mean volume obtained in the present study one can deduce that for a 5 mm lifting there is no need for additional bone, for 10 mm lifting an additional 1 ml of bone substitute will be required. The cost of 0.75 cm<sup>3</sup> bone substitute outweighs the cost of one blade. Therefore, the use of bone scraper is also advantageous from a financial point of view. Using a bone scraper minimized sinus membrane perforations during the creation of the bony window. Sinus membrane perforations may reach up to 44% [16]. We have previously reported [17] an incidence of 28% significant (> 5 mm) membrane perforations, observed intraoperatively, during sinus augmentation procedures. The access to the sinus was achieved by the use of rotatory instruments. The use of the bone scraper highly reduced the incidence of significant (> 5 mm) membrane perforations, to a merely 5%. A recent study [18] compared bone scrapers versus piezoelectric surgery in the lateral antrotomy for sinus floor elevation. The scraper was compared in terms of efficacy, speed, and safety to an ultrasonic insert for osteoplasty, in a randomized controlled clinical trial with a split-mouth design. Twenty-five patients were included. The occurrence of membrane perforation, laceration of vascular branches, and surgical time were recorded. No significant differences were found in terms of surgical time, incidence of membrane perforation during antrostomy (4.3% in both groups), or other intraoperative complications between the 2 techniques. The authors concluded that both surgical approaches represent effective options for performing lateral antrostomies during sinus floor elevation procedures in a safe and predictable way.

## 5. Conclusions

1. When using a bone scraper to harvest bone from the bony window osteotomy prior to sinus membrane elevation, an average of 0.75 ± 0.25 cm<sup>3</sup> of bone can be obtained from a site approximately (12 ± 2) x (10 ± 2) mm.
2. Its proper use minimizes sinus membrane perforations.
3. The use of bone scraper is cost-effective financially.

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## Questions

### 1. Sinus membrane perforations in general may reach up to:

- a. 10%;
- b. 22%;
- c. 44%;
- d. 63%.

### 2. Sinus membrane perforations using bone scraper may reach up to:

- a. 5%;
- b. 10%;
- c. 44%;
- d. 63%.

### 3. The average bone volume collected from an osteotomy was:

- a. 0.25 cm<sup>3</sup>;
- b. 0.5 cm<sup>3</sup>;
- c. 0.75 cm<sup>3</sup>;
- d. 1 cm<sup>3</sup>.

### 4. The average bone volume collected from an osteotomy in males vs. females is:

- a. Higher;
- b. Lower;
- c. Similar;
- d. Not statistically significant different.



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## RESIN INFILTRATION AS A MINIMAL INVASIVE ESTHETIC TREATMENT FOR A MILD FLUOROSIS CASE

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### ABSTRACT

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**Aim:** The present case report aimed to describe a minimally invasive method to mask the white opaque lesions of enamel in a mild fluorosis case (Dean's Index code 3) to improve its esthetic outcome.

**Summary:** Dental fluorosis (DF) is a developmental disturbance of enamel caused by excessive ingestion of fluoride on ameloblasts during enamel formation. The clinical manifestations depend on the severity of fluorosis. In mild cases, there are white opaque striations across the enamel surface, whereas in more severe cases, the porous regions increase in size, with enamel pitting, and secondary discoloration of the enamel surface. Patients often suffer from the discoloration and the pitted surface of the teeth which are the major characteristics leading to an unaesthetic appearance. A minimally invasive treatment approach of in-office bleaching followed by a resin infiltration technique was applied to enhance the porous fluorosed enamel surface. The combination of the two techniques resulted in a perfectly satisfactory aesthetic outcome with a clinical follow-up for 12 months.

**Key learning points:** fluorosis, minimal invasive approach, bleaching, resin infiltration, esthetic.

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### 1. Introduction

Enamel pathologies like enamel hypoplasia, amelogenesis imperfecta and dental fluorosis occur as a result of disturbances during the last stage of sound enamel formation. In the maturation stage; the ameloblasts lose their protein secretory properties due to increased growth activity [1]. Dental fluorosis is caused by excessive intake of fluoride in drinking water during enamel formation [2]. When the fluoride concentration is 1 ppm, fluorapatite is formed instead of hydroxapatite which has more protein content, is hypermineralized and thereby has increased appetite crystallinity. The clinical manifestations of dental fluorosis depend on its severity [3]. In mildest fluorosis form, the enamel is characterized by white lines that represent accentuated perikymata or rod ends. The white lines maybe confluent or discrete areas with white lines in between. However, in moderate dental fluorosis cases, the entire enamel surface maybe chalky white and the sub-surface porosities may attract extrinsic stains and cause enamel discoloration. In sever dental fluorosis cases post-eruptive trauma of the extensive subsurface porosity enamel surface causes detachment and pitting enamel surfaces [4].

The treatment option of dental fluorosis depends on its severity as well. For severe fluorosis cases, invasive approaches such as resin composite veneers, ceramic veneers, or ceramic crowns, are generally chosen. But for mild to moderate fluorosis cases more conservative treatment options are preferred. In addition to the

microinvasive treatment approach (micro or macro abrasion) other ultra- minimal invasive treatment options such as bleaching and resin infiltration technique should be considered [5].

Patients with fluorosed teeth usually complain of the unaesthetic appearance of teeth and seek aesthetic treatments. The unaesthetic fluorosed teeth discoloration maybe due to enamel hypomineralization and the subsequent extrinsic stains into the porous subsurface enamel that range from yellowish, light brown to dark brown or black. This type of discoloration could be treated by in-office or at home bleaching or a combination of both using different bleaching agents. Hydrogen peroxide and carbamide peroxide are the commonly used bleaching agents [6].

A resin infiltration technique was developed for the treatment of incipient caries lesions by using a low-viscosity resin that fills the porous structure of the carious lesions and when light cured can stop caries, inhibit further demineralization and mask white spot lesions. The resin infiltration technique showed good applicability and high acceptance by dentists [7-9].

The purpose of this article is to discuss the clinical result with a 12-month follow-up of a mild fluorosis case treated by resin infiltration proceeded by dental bleaching.

### 2. Case Report

A 23-year old male patient presented to the Conservative Dentistry Department at Istanbul



Figure 1. Mild fluorosis, Dean's Index code 3.



Figure 2. The patient's teeth after bleaching with 35% Hydrogen peroxide gel.



Figure 3. Preoperative view, teeth were isolated by rubber dam.



Figure 4. 15% HCl acid gel application.

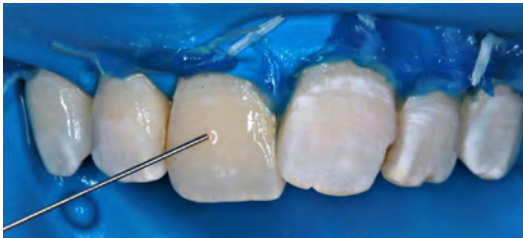


Figure 5. 100% Ethanol dehydration, Alcohol index.



Figure 6. Resin infiltration application.

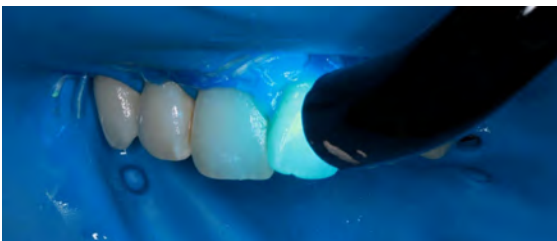


Figure 7. Resin infiltrant polymerization.



Figure 8. Resin infiltration re-application.



Figure 9. Resin infiltrant polymerization.



Figure 10. Resin infiltration re-application.

University complaining of unaesthetic appearance of his teeth due to the presence of white lesions and discoloration. After his clinical examination this patient was diagnosed as mild fluorosis Dean's index code 3. (Fig. 1).

For this patient a combination of in-office bleaching and resin infiltration was planned. First, one session in-office bleaching was applied to the patient using the 35% Hydrogen peroxide bleaching gel (Whitness HP, FGM, Joinville, SC, Brazil) (Fig. 2).

Resin infiltration was applied according to the manufacturer's instructions; first the teeth were cleaned and polished. Then rubber dam and floss were

used for isolation (Fig. 3).

The bleached fluorosed enamel surface was etched with 15% HCl gel (ICON etch<sup>®</sup>, DMG) for 2 minutes (Fig. 4) as instructed by the manufacturer.

After etching, the enamel surface was rinsed with air-water spray for 30 s, dried, and then dehydrated with 100 % ethanol (ICON dry<sup>®</sup>, DMG) for 30 s (Fig. 5).

Alcohol is an efficient visual examination way to control the effectiveness of penetration capacity of the etched enamel by its high infiltrative capability. When 100% Ethanol (ICON dry<sup>®</sup>, DMG) used the white spot lesions on the fluorosed enamel surface should have almost disappeared as a result of porous lesions, otherwise the

etching step should be repeated.

Resin infiltration (ICON®, DMG) was applied on the etched surface using the applicator with rubbing for 2 minutes, then one should wait 1 minute before the surface is slightly dried with compressed air for 10 s. (Fig. 6).

Then it should be light-cured for 40 s (Fig. 7) and reapplied for 1 minute more to compensate the polymerization shrinkage (Fig. 8).

Changes were evident and immediate improvement could be observed after resin infiltration in this case (Fig. 9). The aesthetic outcome improvement and durability could be observed 12 months after the treatment (Fig. 10).

### 3. Discussion

Discoloration stands as the main concern of patients suffering from fluorosis and the reason why they seek treatment. Regarding mild fluorosis, bleaching is indicated to enhance the natural appearance of the discolored superficial porosities (mean depth approx. 200 µm). If the lesion displays deeper porosities in conjunction with the entrapped extrinsic stains then it should be removed by micro or macro abrasion depending on the surface characteristics of the lesion that should be taken in consideration. However, some fluorosis cases reveal deep subsurface porosities (300 µm) which could not easily be removed by minimal invasive treatment methods. In these cases, resin composite or porcelain veneer restorations could be indicated as an invasive treatment modality. Considering much more severe cases existing more than 50% of fluorosed enamel surface with loss of enamel and compromised remaining substrate structure, the adhesion capacity of the dental hard tissues threatens the adhesive efficiency thus crowning of the tooth could be considered [10].

The basic philosophy of minimally invasive dentistry is the integration of prevention, remineralization and minimal intervention for placement or replacement of restorations [11]. The aim of minimal invasive intervention is tissue preservation by prevention of the disease, intercepting its progression and applications of treatment techniques with the possible least tissue loss [11]. With respect to the present fluorosis case treatment outcome, an outstanding aesthetic improvement had been achieved immediately following bleaching and resin infiltration treatment, which both are considered as minimal invasive treatment approach. These treatment approaches are especially tailored for young patients with optimum oral health care, sound non-carious teeth without any pathological signs or further periodontal abnormalities.

The discoloration of mild fluorosed enamel could be enhanced by in-office or at-home bleaching or a combination of both. Bleaching aims to match the color and opacity difference of the natural unaffected portion of enamel structure and the opaque white spot lesions part of the affected enamel surface by exerting camouflage effect [6,12,13]. The camouflaging effect tries to elevate the opacity of the bleached enamel thus lowering the contrast of the distinction of the unaffected and effected enamel [13].

The resin infiltration technique was considered a novel approach of dental fluorosis when compared to the other treatment options [14]. The resin infiltration system (ICON) was produced by DMG company (Hamburg, Germany) for non-cavitated carious lesions in proximal and smooth surfaces in which the resin seals the lesion and works as a barrier on the lesions surface [16,17]. This technique aims to fill the subsurface lesions porosities (with depth up to 450 µm) by the low-viscosity and high penetration coefficient resin [17]. Before the application of the resin infiltrant the carious lesions surface should be prepared by an acid etch agent to eliminate the hypermineralized superficial layer (average thickness 30 - 40 µm) and allow the resin to infiltrate deep into the subsurface porosities. Usually 2 minutes of 15% HCl acid is used for this purpose [18]. In addition to caries progression inhibition resin infiltration could be indicated to enhance and restore the natural enamel appearance of enamel surface in cases of fluorosis and enamel hypoplasia. The resin infiltrant has a refractive index (RI = 1.62) similar to that of sound enamel furthermore allowing the masking effect over the subsurface enamel porosities [19]. In this case a combination of two treatment options were applied for the discolored mild fluorosis teeth, first in-office bleaching using 35 % hydrogen peroxide was applied. Thus, it was possible to attenuate the contrast between the opaque white spot lesions part and the healthy luminous enamel parts. For some instances, the camouflaging effect could be adequate for satisfying the patients. But when bleaching is not sufficient as demonstrated in the present case, resin infiltration could be chosen in consequence. However, since the infiltration would be in conjunction with the bleaching, it is mandatory to wait for two weeks before application of the resin infiltrant in order not to interfere with the resin curing capability [20].

The resin infiltrant penetration capacity into the carious lesions had been investigated in many in vitro studies and had shown almost complete penetration depth [21,22]. However, the histopathological features of enamel affected by fluorosis, especially the presence of hypermineralised surface layer reduces the penetration capacity of the resin infiltrant into the subsurface lesions body of the fluorosed enamel. The resin infiltration penetration depth was limited in moderate fluorosis and a little higher in mild fluorosis teeth [23]. Many clinical studies have demonstrated the efficacy of resin infiltration technique in arresting carious lesions as well as improvement in esthetics of white spot lesions, fluorosis, MIH and other enamel hypoplasia stains [9,24-26]. The aesthetic improvement was noted over time, but this might be as a result of water absorption by resin, which was not completely removed by ethanol. This absorption reduces the optical interfaces in the light path. The aesthetic outcome of many clinical cases followed for more than 12 months showed adequate durability [9,25].

### 4. Conclusions

In this case, a 12-month follow-up of the resin infiltration technique after bleaching showed a satisfying outcome

with enhanced aesthetic and function. Thus, resembling a minimal invasive treatment approach, a combination of resin infiltration technique following bleaching should be considered for the treatment of such mild fluorosis teeth. It is considered as an economic and effective solution. Furthermore, beside the expanded recall intervals to evaluate the treatment outcome, in vitro studies are also required to discuss the effect of the bleaching agents on the penetration depth of the resin infiltrant into the lesions and its effect on the fluorosed enamel surface mechanical properties.

### Author contributions

RO: contributed to the case treatment, follow-up control, data collection, analysis and drafting the manuscript; HŞS: contributed to the design of the work, treatment plan, analysis of data, revising the draft for important intellectual content and giving the final approval for the version to be published.

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## Questions

### 1. Which are the clinical manifestations of mild fluorosis enamel:

- a. Confluent or discrete white lines on the enamel surface;
- b. Chalky white smooth enamel surface;
- c. Chalky white enamel surface with sub-surface porosities;
- d. Chalky white pitted enamel surface with stains and discoloration.

### 2. Resin infiltration technique is used for all except:

- a. Smooth surface incipient caries treatment;
- b. Pit and fissure incipient caries treatment;
- c. Dental fluorosis;
- d. Enamel hypoplasia.

### 3. The resin infiltrant (ICON) has a refractive index which is similar to:

- a. Water;
- b. Enamel;
- c. Air;
- d. Dentin.

### 4. In this case, we assessed:

- a. The efficacy of the invasive treatment approach in dental fluoroses cases;
- b. The efficacy of bleaching camouflage on mild fluorosis teeth;
- c. The efficacy and durability of a combination of bleaching and resin infiltration technique in mild fluorosis case;
- d. The treatment options based on dental fluorosis severity.



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## Gold Standard in Dental Photography: Digital Camera for Professional Dental Photography, EyeSpecial C-III

While visiting the dental exhibition in Cologne, IDS 2017, I tried to explore as many stands as possible belonging to the major equipment and material manufacturers present. We also went to the stand of Shofu Dental Corporation, the well-known producer coming from the "Land of the Rising Sun".

There, besides abrasives, diamonds & carbids, cements, dental materials for both laboratories and dentists, we noticed the new digital camera for professional dental photography, EyeSpecial C-III. Because there were many practitioners asking for information about it, I gave up and continued my journey to collect information.

This year, during the FDI World Dental Congress 2018 in Buenos Aires, 5-8 September, I managed to hold this digital camera in my hand, to gather information and now I have the pleasure to introduce it to you.

In our current practice, clinicians and team members tend to circumvent the moment of taking pictures when they think about pulling out and holding up a big and heavy camera, finding help, selecting its functions, and hoping the captured images will be of high quality.

Shofu Dental Corporation (Kyoto 605-0983, Japan, <http://www.shofu.co.jp>) designed the camera EyeSpecial C-III especially for dentistry, as it is used for dental photography, case presentations in orthodontics and also in the dental laboratory.[1]

Designed for dentistry, this digital camera[1] [2] has a number of qualities:

- **easy to use**, so that anyone can capture high quality images;
- **preset shooting modes**, allowing faster and more reproducible images for a wide range of applications;
- **image quality**, allows exceptional photos to be taken without initial preparation;
- **overall satisfaction**, provides excellent service, saves user time.

### Easy to use

The camera offers one-touch intuitive operation and a large LCD touch screen for viewing and scrolling, even when wearing gloves. Easy to handle, independent photo-taking, low shooting time, water and chemical resistant, easy disinfection, weighing only 590 g, ergonomic design, easy to hold with only one hand - these are all qualities that make the camera user-friendly and easy to use.

### Preset shooting modes

EyeSpecial C-III is ideal for treatment planning, case documentation, patient education, case presentation

and collaboration with the dental laboratory. The camera is equipped with eight preset shooting modes (Standard, Surgery, Mirror, Face, Low Glare, Whitening, Tele Macro, Isolate Shade) to make images easier, faster and more reproducible for a wide range of applications.

### Image quality

The camera offers twelve megapixels, an exceptional field depth, a 49 mm lens, fast autofocus, a proprietary FlashMatic system (4 LEDs around the lens and 4 side flashes) and an anti-shock mode, allow exceptional photos to be taken without photographic preparation previous. With Wi-Fi access, images can be instantly uploaded to a tablet, computer, or smartphone. It provides quality photos in compliance with OSHA and HIPPA protocols.

### Overall satisfaction

The EyeSpecial C-III is a durable, easy-to-use product that delivers consistent results, preprogrammed modes make life easier and saves user time.

In conclusion, I recommend that you use the EyeSpecial C-III, a digital camera designed for professional dental photography, with which any member of the dental team without any photographic training can contribute to the high-quality photographic documentation of all activities in daily practice.

### References

1. <http://www.shofu.com/en/wp-content/uploads/sites/2/2014/05/EyeSpecial-C-III-Brochure.pdf>
2. <http://www.thedentistryshow.co.uk/press-releases/eyespecial-c-iii-by-shofu>

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DMD, PhD Student  
Editorial Director, Product News

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EyeSpecial C-III

Digital Camera for Professional Dental Photography EyeSpecial C-III  
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Stomatology Edu Journal (Stoma Edu J) publishes:

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- letters to the editor.

All articles must be up to 3,000 and 5,000 words for meta-analysis (the word count is for the manuscript text only). Letters to the editor must not exceed 400 words of text and have no more than 3 authors. Letters to the editor can be related to an article already published in the journal or it can represent original scientific contributions or events news/presentations etc. of interest for the reader. If, following the peer-review process, the article requires only minor changes (language changes etc.) then the manuscript is accepted for publication in its revised form without further input from the author. In case the changes are considered more important (scientific errors or an incorrect use of the language that can affect the quality of the scientific message) the author will be contacted by a member of the editorial committee and it will only be published after he approves the changes considered necessary by the peer reviewers. In some cases, based on the written approval of the author(s), the peer-reviewers and the chief-editor or the publisher the article may be published alongside the comments of the reviewer(s).

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For original articles:

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**Results** - the results of the study will be presented in a descending order of importance. An interpretation of the results will not be done in this section.

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For all other types of articles we recommend the use of a clear structure based on sections and sub-sections.

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