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Marcinkowskiego 2–6
50-368 Wrocław, Poland
Tel.: +48 71 784 11 33
E-mail: dental@umed.wroc.pl

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# Effects of a hydroxyapatite coating on the stability of endosseous implants in rabbit tibiae

# Wpływ powłoki hydroksyapatytowej na stabilizację wszczepów śródkostnych w piszczelach królików

Magdalena Łukaszewska-Kuska[1,A–F], Piotr Krawczyk[2,A–F], Agnieszka Martyla[3,B,C,E,F], Wiesław Hędzelek[4,A,E,F], Barbara Dorocka-Bobkowska[1,A,E,F]

[1] Department of Gerodontology and Oral Pathology, Poznan University of Medical Sciences, Poland
[2] Institute of Chemistry and Technical Electrochemistry, Poznan University of Technology, Poland
[3] Institute of Non-Ferrous Metals, Central Laboratory of Batteries and Cells, Poznań, Poland
[4] Department of Prosthodontics, Poznan University of Medical Sciences, Poland

A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation;
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**Address for correspondence**
Magdalena Łukaszewska-Kuska
E-mail: m.lukaszewska.kuska@gmail.com

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## Abstract

**Background.** A dental implant surface which would promote rapid and strong osseointegration is a key factor of success in modern implantology. To achieve this goal, different implant surface modifications are developed. A hydroxyapatite (HA) coating changing a bioinert titanium surface into bioactive is one of them.

**Objectives.** The objective of the study was to investigate the effects on bone osseointegration in rabbits resulting from the application of a HA coating deposited on titanium endosseous implants using a modified electrochemical method.

**Material and methods.** Titanium implants with HA coatings and controls with $Al_2O_3$ grit-blasted surfaces were embedded into rabbit tibiae. The chemical composition, roughness and morphology of the implants were determined. Implant stability tests were performed, and the Periotest® value (PTV) and the implant stability quotient (ISQ) value for Osstell Mentor were recorded in order to evaluate the osseointegration.

**Results.** The surface characterization of the implants revealed a microstructure with an arithmetical mean height (Sa) in the range of 0.71–1.04 µm. The HA coating was free of contamination, whereas the controls were enriched with corundum. After healing, a statistically significant increase in the mean ISQ and a decrease in the mean PTV for the HA-coated implants was observed. In the case of the control implants, only PTV decreased significantly with time.

**Conclusions.** The present study found that implant surface properties affected implant stability as determined by Osstell and Periotest measurements. The HA coating reported herein was found to have chemical and physical properties which appear to improve osseointegration compared to grit-blasted implants.

**Key words:** dental implants, surface properties, electrochemical techniques, durapatite

**Słowa kluczowe:** wszczepy dentystyczne, właściwości powierzchni, techniki elektrochemiczne, durapatyt

# Introduction

The development of a firm implant/bone interface is believed to be a major prerequisite for the short- and long-term clinical function of dental implants.[1,2] Different implant geometries and surfaces as well as host site conditions may affect the interface development and its characteristics.[3,4] Many strategies have been used to improve osseointegration and to shorten the healing period of titanium implants. One of those strategies is changing bioinert titanium surface into bioactive one. Hydroxyapatite (HA) has been used for many years as a bioactive implant coating to improve osseointegration.[5–7] It has a large capacity for adsorbing proteins, improves osteoblast proliferation, enhances bone formation, and reduces bone loss.[8–10] These properties induce a more rapid fixation and stronger bonding between the host bone and the implant and are conducive to uniform bone ingrowth at the bone-implant interface.[6] The most common technique of applying HA coating is the plasma-spraying technique but several problems such as delamination and disintegration with the formation of debris particles have been reported.[11] Therefore, alternative HA coating processes have been extensively researched in order to avoid these undesirable effects of plasma application. One of the most promising methods is electrochemical deposition. Recently, we presented a modified electrochemical method of coating titanium implants with HA.[5] The obtained coating was found to be highly pure, homogenous HA, which was uniform, crack-free and thin. Moreover, its moderate surface roughness and coatings crystallinity was potentially conducive to tissue reaction.

The objective of the study was to investigate the effects on osseointegration resulting from the application of a HA coating deposited on titanium implants using a modified electrochemical method based on the changes of implants stability representing osseointegration status.

# Material and methods

## Implant design and surface preparation

Twenty-eight commercially pure titanium class IV screw implants 4 mm in diameter and 7 mm in length were used (Osteoplant®, Dębica, Poland). Implants were manufactured exclusively for the purpose of this study from commercially pure titanium class IV wire. Titanium wire, apart from titanium, consisted of 54 ppm of H, 0.285% of O, 0.01% of C, 0.14% of Fe and 0.007% of N according to the manufacturer. All the implants were sandblasted with corundum grit ($Al_2O_3$) with a diameter of 53–75 μm. The $Al_2O_3$ powder was composed of 98.5% $Al_2O_3$ with 0.18% $SiO_2$, 0.01% $TiO_2$, 0.007% $Fe_2O_3$, and 0.001% CaO. Fourteen implants were left with an $Al_2O_3$ grit-blasted surface. Fourteen other implants were coated with HA using electrochemical deposition. Prior to electrodeposition, the implants were etched with 0.5M $H_2SO_4$. The process of HA electrodeposition was carried out using an AUTOLAB PGSTAT 302N potentiostat-galvanostat (Ecochemie, Utrecht, the Netherlands) with a 2-electrode system in a galvanostatic mode, with a current of 5 mA. The implant was used as the working electrode, and a platinum mesh served as a counter-electrode. The electrolyte consisted of $2.08 \times 10^{-4}$M $CaCl_2$, $1.25 \times 10^{-4}$M $NaH_2PO_4$ and 0.1 M NaCl in distilled water. The pH was adjusted to 6.3 with NaOH solution. The process was carried out for 105 min at a temperature of 100°C. A 100 mL 3-neck flask was used as an electrochemical reactor and was immersed in a thermostated oil bath.

After surface preparation, all the implants were subjected to ultrasonic washing in a surfactant for 15 min at 55°C, followed by 2-propranol washing for 15 min at 22°C, disinfectant washing for 15 min at 22°C, and finally to washing twice in distilled water for 15 min at 55°C. The implants were then double-packed and sterilized with radiation from an electron accelerator with a radiation dose of 25 kGy.

## Physicochemical characteristics of implant surface

The chemical composition of the surface was evaluated using X-ray photoelectron spectroscopy (XPS). The measurements were made using a photoelectron spectrometer ESCALAB-210 VG Scientific Ltd., East Grinstead, UK) with Al Ka radiation (1486.6 eV) from an X-ray source, operating at 15 kV and 20 mA. Survey spectra were recorded in the energy range from 0 eV to 1350 eV, with a 0.4 eV step. High-resolution spectra were recorded with a 0.1 eV step, 100 ms dwell time and 20 eV pass energy. A 90° take-off angle was used in all measurements. Curve fitting was performed using the AVANTAGE software (Thermo Electron; Thermo Fisher Scientific, Waltham, USA), which describes each component of the complex envelope as a Gaussian–Lorentzian sum function. A constant 0.3 ±0.05 G/L ratio was used and the background was fitted using a nonlinear Shirley model. Scofield sensitivity factors and a measured transmission function were used for quantification. Aromatic carbon C1s peak at 285 eV was used as a reference of binding energy.[12]

The implant surface morphology was examined with a scanning electron microscope (SEM) Tescan Vega (Tescan, Brno, Czech Republic) and Zeiss EVO 25 (Carl Zeiss, Oberkochen, Germany).

Surfaces roughness was measured with an optical WYKO® NT1100 profilometer (Veeco Instruments, Plainview, USA) in VSI Mode, and the measured area was 0.9 × 1.2 mm under ×20 magnification. The WYKO Vision software v. 3.0 for NT-1100 was used. To remove errors of form and waviness, the removal shape function Plane Fit was used to remove linear tilt from surface

measurements. After that, the S-Parameters Analysis was used to evaluate value of parameters. The surface roughness of the examined implants was measured at 5 random locations in the integration to be part of the implant.

## Animal study

The in vivo animal study was carried out in accordance with the guidelines for the care and ethical use of laboratory animals and was approved by the regional animal Ethics Committee of the Poznan University of Life Sciences, Poland (approval No. 60/2007). Adequate measures were taken to minimize the pain or discomfort of the animals. Fourteen 6-month-old white New Zealand female rabbits weighing about 4 kg were used. Each animal received 2 implants of the same size. One implant had an HA coating prepared using the above electrochemical deposition method. The other implant had an $Al_2O_3$ grit-blasted surface. The left limb was operated in all subjects. Mesially HA-coated implant was placed and $Al_2O_3$ blasted implant was placed distally. The surgical procedure was performed under sterile conditions by 1 dental surgeon. General anesthesia was induced with an intramuscular injection of ketamine (50 mg/kg body weight) and xylozyn (10 mg/kg body weight). At the surgical site, infiltrate anesthesia was induced with an injection of 1 mL lignocain + noradrenaline 1:10,000. After gentle skin preparation, the fascia and periosteum on the medial anterior surface of the medial tibial epiphysis were exposed. For implant bed preparation, Surgical XT (NSK, Kanuma, Japan) dental unit was used. The 2 implant beds were prepared using 2 mm and 2.7 mm burs with external saline irrigation applied for drilling with a temperature of about 20°C. Osteoplant burs were used for this study. The burs used were new and every new bur after the first use was reused 6 times. The maximum 800 rpm was used. The beds were separated with a 10 mm distance. Implants were inserted into the beds with a torque spanner until the level of implants was level with the bone surface. After implant placement, the periosteum, fascia and skin were sutured with Dexon® 4.0 sutures. To prevent infection, intramuscular injections of 20 mg cefuroximum per 1 kg body weight and neomycine spray were applied at the surgical site and administered twice daily for a week. To prevent post-operative pain, ketoprofen (1 mg/kg body weight) was given for a week. After a 2-week healing period, the animals were sacrificed with pentobarbital (1 mL/kg body weight) following general anesthesia with xylozyn (10 mg/kg body weight). The implants were exposed and implant stability was evaluated (vide infra).

The rabbits' bone metabolism and healing is about twice as fast as humans, thus a 2-week healing period in rabbits may correspond with 4 weeks in humans. This healing time in humans is the most critical moment for the stability of the implants, when primary stability decreases and secondary stability is still low.

## Implant stability testing

Implant stability tests were carried out using Periotest S® (Siemens AG, Bensheim, Germany) and Ostell Mentor® (Integration Diagnostics AB, Göteborg, Sweden) devices.

The Periotest has been thoroughly studied and advocated as a reliable method for determining implant stability.[13–16] The Periotest measures implant mobility by percussing an abutment attached to the implant with an electromagnetically driven and electronically controlled rod fitted to the instrument. The contact time between the test object and tapping rod was measured with an accelerometer. The signals were then converted to a unique value called the Periotest value (PTV), which is related to the damping characteristics of tissues surrounding the teeth or implants.[17] Periotest values ranging from –8 to +50. The PTV is a measure of clinical stiffness. As the PTV values increase, implant stability is deemed to decrease. The measurements were taken at the same point of the abutment screwed to the implants. During the measurements, the Periotest handpiece was held perpendicularly to the abutment axes. All measurements were conducted by the same person. Care was taken to control the precise point and angle of the percussion unit (Fig. 1).

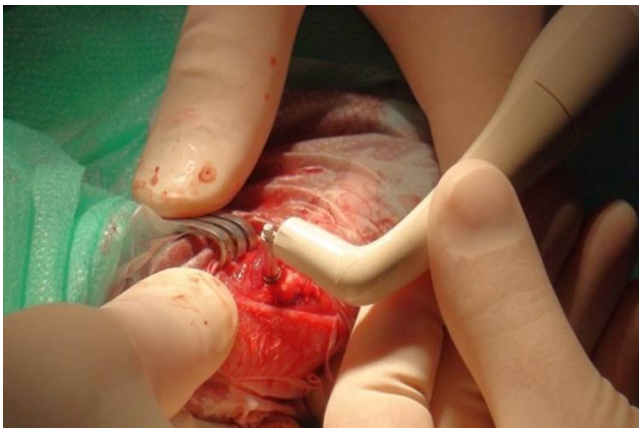

Fig. 1. Implant stability test with an Ostell device. Measurements were taken in 2 directions, with the instrument parallel and perpendicular to the longitudinal axis of the tibia. The mean ISQ values were recorded. All measurements were conducted by the same person

The Ostell Mentor is a device which uses resonance frequency analysis (RFA) to evaluate implant stability. The RFA utilizes a small Smart Peg transducer that is attached to the implant with a screw utilizing the internal threads of the implant. The transducer comprises of 2 piezoceramic elements, one of which is vibrated by a sinusoidal signal and the other serves as a receptor for the signal. Resonance peaks from the received signal indicate the first flexural (bending) resonance frequency of the measured object. The RFA values are expressed as implant stability quotients (ISQ), ranging from 1 to 100

and relate to clinical stiffness. Implant stability increases with increasing ISQ value. The measurements were taken in 2 directions, with the instrument parallel and perpendicular to the longitudinal axis of the tibia. The mean ISQ values were recorded and all measurements were conducted by the same person (Fig. 2).

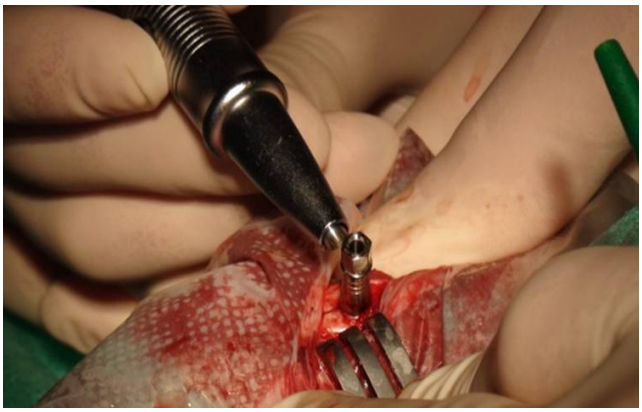

Fig. 2. Implant stability test with a Periotest device. Measurements were taken at the same point of the abutment screwed to the implants. During measurements the Periotest handpiece was always held perpendicular to the abutment axes. All measurements were conducted by the same person

## Statistical analysis

The data was reported as the mean value ± standard deviation (*SD*). The statistics software STATISTICA v. 10.0 (Statsoft, Tulsa, USA) was used for all statistical analyses. The Shapiro-Wilk test was used to determine whether ISQ and PTV results were in accordance with normal distribution. For those values which were within the normal distribution, the t-Student's test was used; for those which were not, Wilcoxon's test was used to determine the existence of statistically significant differences between the 2 groups of implants. To determine whether implants stability increased with time, Spearman's correlation was used. The level of significance was determined as $p < 0.05$.

# Results

## Surface analysis

The chemical composition analysis of coated implants revealed HA to be the principal component of the electrodeposited coating with only small amounts (up to 1%) of F, Si, N, and Na detected as surface impurities. The grit-blasted implant surface was composed of Ti and O with Al incorporated into the oxide layer during the grit-blasting procedure; in addition, carbon impurities were also detected.

Optical profilometry showed a surface roughness with Sa = 1.04 ±0.06 µm for HA-coated implants and Sa = 0.74 ±0.03 µm for the grit-blasted implants.

Scanning electron microscopy analysis revealed a uniform, integrated layer of rod-like HA crystals on the titanium surface with the longitudinal axes parallel to the implant surface for HA-coated implants (Fig. 3). In the case of the grit-blasted implants, SEM analysis of the surface revealed irregular, heterogeneous, intensively expanded, highly diverse notches and sharp edges with hollows (Fig. 4).

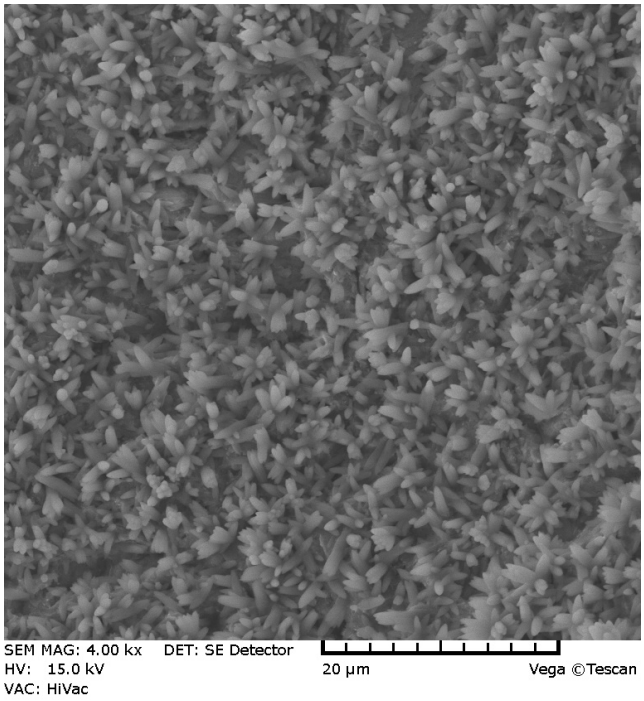


Fig. 3. SEM micrograph of hydroxyapatite coating electrochemically deposited on titanium implant (×4,000 magnification). Radiation source – Tungsten heated cathode, kV level – 15.00

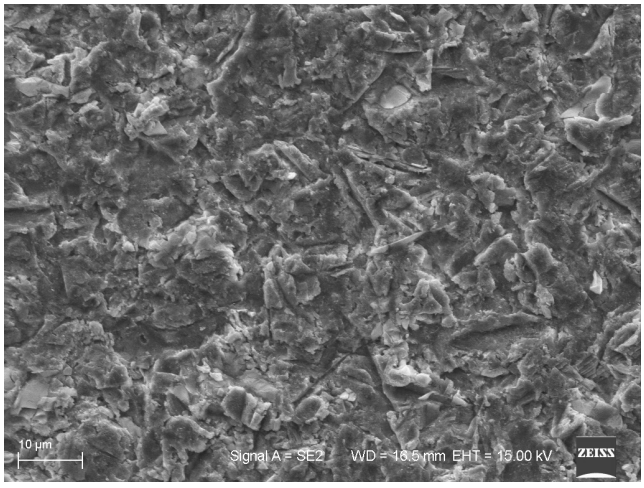


Fig. 4. SEM micrograph of Al2O3 grit-blasted surface of titanium implant (×3,000 magnification). Radiation source – Tungsten heated cathode, kV level – 15.00

A detailed analysis of hydroxyapatite coating deposited on titanium implants using a modified electrochemical method was presented in a previous article.[5]

## Implant stability tests

Implant stability tests at the time of implantation produced mean PTV values of 12.58 (*SD* = 9.04) for the implants coated with HA and 7.83 (*SD* = 5.01) for the grit-blasted implants. The mean ISQ values for the coated implants were 63.89 (*SD* = 2.17) and for grit-blasted implants 70 (*SD* = 3.74). No statistical significance was noted between the 2 groups of implants, either in the PTV values ($p$ = 0.06) or in the ISQ values ($p$ = 0.11). After 2 weeks of healing, the implant stability test revealed a decrease in the PTV values of both implant groups. Mean PTV values for the coated implants and for grit-blasted implants were 6.88 (*SD* = 8.11) and 3.13 (*SD* = 3.64), respectively. The mean ISQ values of both groups of implants increased to 69.85 (*SD* = 2.05) for the implants coated with HA and 72.25 (*SD* = 4.03) for sandblasted implants. No statistical significance was noted between the 2 groups of implants either in the PTV values ($p$ = 0.11) or ISQ values ($p$ = 0.22).

For the PTV values, statistically significant differences were noted between the measurements made between the time of implantation and the time of the animal sacrifice for the $Al_2O_3$ grit-blasted implants ($p$ = 0.01) and for the HA-coated implants ($p$ = 0.04) (Fig. 5).

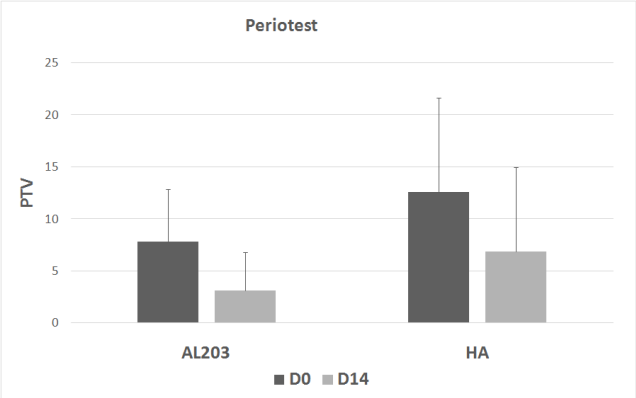


Fig. 5. Periotest values of HA-coated and sandblasted implants at the time of implantation and after 2 weeks of healing. Each value is a mean ± standard deviation (*SD*). Statistically significant differences were noted between the measurements made between the time of implantation and the time of the animal sacrifice for the $Al_2O_3$ grit-blasted implants ($p$ = 0.01) and for the HA-coated implants ($p$ = 0.04)

For the ISQ values, statistically significant differences were noted for the measurements made between the time of implantation and the time of the animal sacrifice for the HA coated implants ($p$ = 0.006), while for the grit-blasted implants the increase in the ISQ values was not significant ($p$ = 0.15) (Fig. 6).

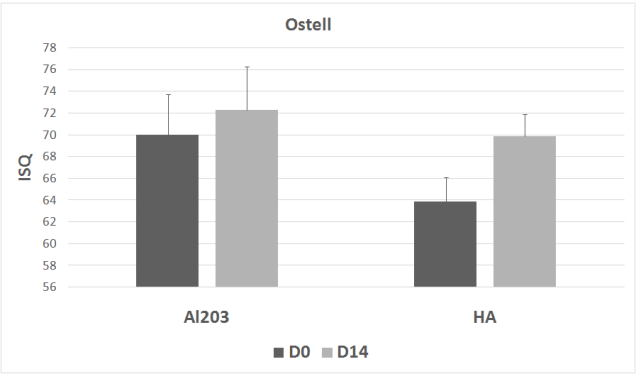


Fig. 6. Implant stability quotients values of HA-coated and sandblasted implants at the time of implantation and after 2 weeks of healing. Each value is a mean ± standard deviation (*SD*). Statistically significant differences were noted for the measurements made between the time of implantation and the time of the animal sacrifice for the HA-coated implants ($p$ = 0.006), while for the $Al_2O_3$ grit-blasted implants the increase in the ISQ values was not significant ($p$ = 0.15)

# Discussion

Development of dental implantology is focused, among other things, on designing active surfaces for the implant and conditioning the acceleration of the integration of the implant with the bone. To this end changes have been made to both the implant surface roughness and its chemical composition. Surface characterization of the implants used in this study revealed microstructured surfaces characterized as smooth for $Al_2O_3$ blasted implants and moderately rough for HA-coated implants according to Wennerberg's classification; there were also considerable differences in surface chemistry between the HA coated and $Al_2O_3$ blasted implants.[17]

With regard to implant placement, neither ISQ nor PTV values indicated significant differences in primary stability between the 2 implant groups. After a healing time of 2 weeks, the implant stability as measured by Ostell and Periotest systems significantly increased for the HA-coated implants. In the case of the $Al_2O_3$ grit-blasted implants after 2 weeks healing time, the implant stability increased significantly only in terms of the Periotest system.

A statistically significant increase of both parameters for the HA-coated implants after healing time suggested a more favorable effect on osseointegration of the implants provided by the electrodeposited HA coating. That effect is probably due to the chemical composition of the coatings. Calcium phosphates, especially HA, have the potential for adsorbing large amounts of fibronectin and vitronectin on the surface, which increases the osteoblast adhesion and bone formation.[18] Calcium phosphate also increases osteoblast proliferation, which increases the bioactivity of the coatings.[19] Calcium ions enable the formation of a biochemical bond between the implant and the bone, which results in faster and more intense osseoinegration.[18–20] Phosphate groups, on the other hand, provide potential chemical bonding sites for calcium ions and for the hydroxyapatite of the bone matrix during biological mineralization and are responsible for biochemical interaction between the implant and the bone (not just mechanical interlocking as in case of non-chemically modified surfaces).[21]

A positive HA coating effect on osseointegration was also reported by Geurs and Roynestal.[22,23] In the Geurs study, threaded and cylindrical HA coated implants were compared to threaded titanium plasma sprayed implants. HA-coated implants underwent a more rapid initial decrease in micromobility compared to their uncoated counterparts.[22] Roynesdal et al. found that HA-coated cylindrical implants had higher stability parameters than TPS or titanium threaded implants.[24] This effect in the early stages of healing may be due to a firmer bone-to-implant contact. Positive effects of Ca and P ions on implant osseointegration manifested as increased implant stabilization was also reported by Sul and derived from the removal torque values in rabbit tibiae.[24] Meraw et al., in a histological comparison, reported greater bone-to-implant contact early in the healing process for HA-coated implants in comparison with as-machined implants retrieved from dogs.[25] This difference has been associated with the chemical composition of coated implants rather than coating surface roughness.[20,26]

Implant surface roughness affects osseointegration and surfaces with Sa = 1–2 μm are most effective.[17] Although rougher HA-coated implants presented statistically significant increases of both ISQ and PTV values, an improvement in osseointegration was attributed to the surface chemistry, since the difference in the implant roughness was small. Sul et al. found no relationship between implant stability and surface roughness from 6 groups of implants with Sa ranging from 0.69 μm to 1.34 μm.[27] Instead, higher mean ISQ values were observed for surface chemistry-modified implants than for the topographically modified implants.[27] Also, no relationship between implant stability and surface roughness were noted for 6 groups of implants with Sa ranging from 0.7 μm to 1.4 μm in a different study by the same author.[28] These findings are in agreement with a review by Sennerby and Meredith, who reported that most researchers failed to establish that rough or smooth implant surfaces affected implant stability.[29]

## Conclusions

The present study found that implant surface properties affected implant stability as determined by Ostell and Periotest measurements. After 2 weeks of healing, a statistically significant increase in the mean ISQ and decrease in mean PTV values for the HA-coated implants was observed. In the case of the $Al_2O_3$ grit-blasted control implants, only the PTV values increased significantly with time. The implant surface chemistry rather than the surface roughness seams to improve implant stability. Further studies are required to evaluate the long-term bone reaction.


### ORCID iDs

Magdalena Łukaszewska-Kuska https://orcid.org/0000-0002-4163-0995
Piotr Krawczyk https://orcid.org/0000-0001-6083-9316
Agnieszka Martyla https://orcid.org/0000-0001-5205-5052
Wieslaw Hędzelek https://orcid.org/0000-0002-1644-6678
Barbara Dorocka-Bobkowska https://orcid.org/0000-0003-3659-7761


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# In vitro evaluation of the effects of frequently prescribed pediatric drugs on the microhardness of permanent tooth enamel

# Ocena in vitro oddziaływań często stosowanych leków pediatrycznych na mikrotwardość szkliwa zębów stałych

Nagehan Yılmaz[1,D], Ozgul Baygin[1,A], Tugba Nigar Cakıroglu[2,B], Tamer Tüzüner[1,C], Orhan Deger[3,E]

[1] Department of Pediatric Dentistry, Faculty of Dentistry, Karadeniz Technical University, Trabzon, Turkey
[2] Department of Biochemistry, Faculty of Medicine, Avrasya University, Trabzon, Turkey
[3] Department of Biochemistry, Faculty of Medicine, Karadeniz Technical University, Trabzon, Turkey

A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation;
D – writing the article; E – critical revision of the article; F – final approval of the article

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**Address for correspondence**
Ozgul Baygin
E-mail: dtozgul@gmail.com

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## Abstract

**Background.** Dental erosion is considered one of the oral cavity diseases. Frequent intake of liquid oral medications can be an effective factor in tooth erosion.

**Objectives.** This study aimed to evaluate the effects of frequently prescribed pediatric drugs on the permanent dental enamel microhardness over a period of 14 days in vitro.

**Material and methods.** In this study, 11 pediatric drugs with different active ingredients were used; the control group consisted of teeth immersed in distilled water. The immersion cycles were applied 3 times a day for 1 min. The measurements of the samples prepared were taken at 0 (baseline), 7 and 14 days after the immersion cycles using a Vickers hardness testing machine. The pH, titratable acidity (TA) and buffering capacity of the syrups were assessed.

**Results.** The measurements of the tooth samples that were immersed in drug solutions except Deltacortril® showed that there was a significant difference between days 0, 7 and 14. The microhardness values for the tooth samples that were immersed in the Deltacortril drug solution decreased, but no significant difference was found. There were no statistically significant differences between the day 0, 7 and 14 measurements in the control group.

**Conclusions.** Commonly used and prescribed pediatric drugs pose a risk for tooth erosion. Pediatricians should be aware of the effects of prescription drugs on erosion, and stress the need for compliancy with oral hygiene procedures.

**Key words:** microhardness, dental erosion, pediatric syrup, permanent dental enamel

**Słowa kluczowe:** mikrotwardość, erozja zębów, syrop dla dzieci, szkliwo zębów stałych

# Introduction

Oral health is known to have a direct impact on the growth, development and general health of children. Dental erosion is considered one of the oral cavity diseases[1,2] and is defined as irreversible progressive loss of hard dental tissues by chemical dissolution without bacterial involvement.[3] It has been reported that when the pH of the oral environment reaches a critical cut-off value of 5.5, the demineralization of the enamel will occur.[4] The erosion begins with the softening of the enamel surface, mainly characterized by a reduction in microhardness.[5] This erosion is a multifactorial, irreversible process resulting from internal, external or idiopathic factors.[6] Intrinsic etiological factors, which are caused by the contact of dental tissue with stomach acids, include eating disorders, regurgitation and reflux[7]; acidic drugs, diet, environment, and behavioral factors are external etiological factors.[8–12]

Changing habits in modern society, especially in children and adolescents, have increased the incidence of tooth erosion[6,12,13] and have been associated with the regular use of products with high acidity, low concentration of calcium, fluoride and phosphate ions, and low endogenous pH.[8,14] Although erosion can be seen frequently in children, it can be observed in conjunction with other forms: attrition (tooth–tooth wear), abrasion (hard surface–tooth) and abfraction. Epidemiological data collected during in vitro and in situ studies has shown that erosion, one of the 3 tooth-wear processes, is the most common cause of tooth surface loss.[15,16] Erosion was first included in the dental health surveys of children living in England in 1993 and since that time has been periodically surveyed. The prevalence of erosion has been shown to have increased in the studies between 1993 and 1997 among children aged 4–18 years.[17]

The general health of the child plays an important role in the development of tooth erosion. Liquid pediatric medicines are part of the daily routine of children with chronic illnesses. Frequent intake of drugs with erosive potential can be an effective factor in tooth erosion.[12,18] Liquid oral medications are usually prescribed to ensure compliancy with drug intake in children.[10] Acidic preparations are considered necessary for drug distribution, chemical stability, physiological adaptation, and flavor enhancement.[9,10] In addition to the acidic components, consumption frequency and duration, use between sleeping or consuming food, high viscosity, and factors that reduce the salivary flow promote tooth erosion.[12,13,19]

Therefore, due to the increased use of oral medications in recent years in children with acute or chronic diseases, this study aimed to evaluate the effects of frequently prescribed pediatric drugs on the permanent dental enamel microhardness in vitro.

# Material and methods

For the study, ethics committee approval was obtained from the Scientific Research Ethics Committee of the Faculty of Medicine at Karadeniz Technical University, Trabzon, Turkey (approval No. 2016/68).

## Experimental design

In the study, 11 pediatric drugs with different active ingredients were used; the control group consisted of teeth immersed in distilled water. The drugs used were selected among long-term and commonly used medicines that are frequently prescribed by pediatricians for acute or chronic diseases (Table 1). The immersion cycles were applied 3 times a day for 1 min. The measurements of the samples prepared were repeated at 0 (baseline), 7 and 14 days after the immersion cycles. Microhardness was measured using a Vickers hardness tester (INNOVATEST Europe BV, Maastricht, the Netherlands).

## Tooth selection

In this study, 48 non-cariogenic, healthy, permanent molar teeth that were freshly extracted were used. Once the teeth were collected, they were kept in a 0.5% chloramine T solution. Prior to use, the teeth were cleaned with a pumice/water slurry using a polishing brush with a low-speed handpiece. Teeth with molar incisor hypoplasia, or decayed and with white spot lesions were not included in the study.

## Preparation of samples

While the samples were being prepared, the teeth were separated from their roots by a transverse section through the cementoenamel junction with the help of a water-cooled diamond saw with a rapidly rotating handpiece. Subsequently, each crown was split into 2 pieces with axial cuts to obtain buccal and lingual surface measurements. Each crown was fixed with plastic wax on an acrylic plate.

Seven tooth crowns with buccal surfaces faced upward using a parallelometer (ElQuip, São Carlos, São Paulo, Brazil) to secure the flattest region of the buccal surface parallel to the plate, were fixed in each acrylic block (1 extra to substitute).

After the resin acrylic polymerization of each surface, the buccal enamel surfaces of the samples were flattened with 600, 900 and 1200 grit aluminum oxide ($Al_2O_3$) abrasive papers for non-slip measurements and to obtain a flat, stable surface without the dentin coming out. The samples were ultrasonically cleaned in deionized water for 10 min and sterilized in deionized water. The 0.5 × 0.5 mm quadratic specimens were centered at a diameter of 0.5 mm from the center (the tooth/plate sets were rendered acid-proof by coating them with 2 layers of cosmetic nail polish isolation). Prior to the immersion

Table 1. Characteristics of the studied drugs

| Therapeutic class | General name | Brand name | Pharmaceutical form | mg/mL | Manufacturer |
|---|---|---|---|---|---|
| Antibiotics | amoxicillin + clavulanic acid<br>clarithromycin<br>cefuroxime axetil | Augmentin®<br>Macrol®<br>Cefaks® | dry powder to prepare oral suspension | 600/42.9/5<br>250/5<br>250/5 | GlaxoSmithKline, London, UK<br>Sanovel, Istanbul, Turkey<br>Deva, Istanbul, Turkey |
| Antiepileptics | levetiracetam | Keppra® | syrup | 100 | GlaxoSmithKline |
| Multivitamins | multivitamins | Ferro Sanol® B | syrup | 150/5 | ADEKA, Tokyo, Japan |
| Analgesics | paracetamol<br>ibuprofen | Calpol® 6 Plus<br>Dolven® | syrup | 250/5<br>100/5 | GlaxoSmithKline<br>Sanofi, Paris, France |
| Anxiolytic | hydroxyzine HCl | Atarax® | syrup | 2 | UCB, Brussels, Belgium |
| Bronchodilator | salbutamol | Ventolin® | syrup | 2/5 | GlaxoSmithKline |
| Sympathomimetic | pseudoephedrine HCl | Sudafed® | syrup | 30/5 | GlaxoSmithKline |
| Corticosteroids | prednisolone | Deltacortril® | tablet | 5 | Pfizer, New York, USA |

cycles, the samples were kept in distilled water for 24 h at 37°C. The microhardness values of the initial enamel surfaces were evaluated using a Vickers hardness tester (INNOVATEST Europe BV). The settings for load and penetration were determined as 50 g and 10 s, respectively. The measurements were carried out at 2 separate points, each measurement at an interval of 100 µm from the center of the enamel surface, with a diamond indenter, and the average of the measurements was recorded as the Vickers hardness number.[2,12]

## Composition of artificial saliva

Artificial saliva was prepared from sodium chloride (0.4 g/L), potassium chloride (0.4 g/L), calcium chloride-$H_2O$ (0.795 g/L), sodium dihydrogen phosphate-$H_2O$ (0.69 g/L), and sodium sulfide-$9H_2O$ (0.005 g/L) in 1000 mL of distilled water.[20]

## pH measurement and buffering capacity

The pH value of the syrups used for the immersion cycles and the amount of base (acid–base titration) required to raise pH to 7.0 were measured with a pH meter (HI 2211 pH/ORP Meter; Hanna Instruments, Woonsocket, USA). To measure the titratable acidity (TA), 20 g of each solution was titrated with 0.5 M NaOH in 0.02-milliliter increments at 25°C. The buffering capacity (BC) was calculated with the following equation:

$$\beta = -\Delta C/\Delta pH,$$

where:
$\beta$ – buffering capacity;
$\Delta C$ – the amount of base used;
$\Delta pH$ – the change in pH resulting from base addition.[12]

## Immersion cycles

After the first microhardness measurements, 12 acrylic blocks were assigned randomly to 11 separate drugs for the samples to be immersed in the drugs; 1 of them was assigned to distilled water (the control group). During the immersion cycles, the samples were submerged for 1 min every 8 h, 3 times a day, in 10 mL of an undiluted drug solution. During each immersion cycle, the solutions were mixed prior to the immersion to ensure the homogeneity of the drug solutions. After each immersion cycle, the samples were washed with distilled water, and until the next immersion cycle, they were kept in 10 mL of artificial saliva at a temperature of 37°C using the method determined by McKnight-Hanes and Whitford[21] and modified by Amaechi et al.[22]

This procedure was repeated for 2 weeks and a total of 42 immersion cycles were performed. The solutions and artificial saliva were changed daily for each sample. In the control group, the distilled water was changed daily.

The surface microhardness was tested at 7 and 14 days after continuous and systematic repetition of the daily immersion cycles. The samples were kept in distilled water during the days they were tested.

## Statistical analysis

The IBM SPSS Statistics for Windows v. 23 (IBM Corp., Armonk, USA) was used in the analysis of the data. For the comparison of the data measured for 3 or more dependent groups, the analysis of variance was used. The paired comparisons of the results were evaluated using the Bonferroni test for significance. The significance level was set as $p < 0.05$.

# Results

## pH, titratable acidity and buffering capacity

The pH values ranged from 2.76 (pseudoephedrine HCl) to 6.62 (paracetamol) (Table 2). Ibuprofen had the highest TA (3750 mmol), whereas prednisolone had the lowest TA (40 mmol). Additionally, pseudoephedrine HCl had the highest BC (3246 mmol) and prednisolone had the lowest BC (71 mmol) (Table 2).

**Table 2.** The pH, titratable acidity (TA) and buffering capacity (BC) values of the drug solutions with the volume of NaOH ($V_{NaOH}$)

| Pharmaceutical name | pH | $V_{NaOH}$ | Titratable acidity | Buffering capacity |
|---|---|---|---|---|
| Keppra | 5.83 | 0.8 mL | 400 mmol | 342 mmol |
| Cefaks | 5.86 | 1.6 mL | 800 mmol | 702 mmol |
| Macrol | 5.15 | 1.5 mL | 750 mmol | 405 mmol |
| Ferro Sanol B | 2.86 | 7 mL | 3500 mmol | 845 mmol |
| Calpol 6 Plus | 6.62 | 0.4 mL | 200 mmol | 526 mmol |
| Atarax | 2.84 | 2 mL | 1000 mmol | 240 mmol |
| Ventolin | 3.58 | 6 mL | 3000 mmol | 877 mmol |
| Augmentin | 5.70 | 0.8 mL | 400 mmol | 308 mmol |
| Dolven | 4.38 | 7.5 mL | 3750 mmol | 1431 mmol |
| Sudafed | 2.76 | 6.5 mL | 3250 mmol | 3246 mmol |
| Deltacortril | 6.36 | 0.02 mL | 40 mmol | 71 mmol |
| Distilled water | 6.90 | – | – | – |

## Surface microhardness

The measurements of the tooth samples, which were immersed in the Keppra, Cefaks, Macrol, Ferro Sanol B, Calpol 6 Plus, Atarax, Ventolin, Augmentin, Dolven, and Sudafed drug solutions demonstrated a significant difference between days 0, 7 and 14 of the study ($p < 0.001$, $p = 0.037$, $p = 0.017$, $p = 0.002$, $p = 0.037$, $p < 0.001$, $p < 0.001$, $p = 0.017$, $p < 0.001$, and $p < 0.001$, respectively). The microhardness values of the tooth samples that were immersed in the Deltacortril drug solution on days 0, 7, and 14 were decreased, but no significant difference was found ($p = 0.191$). There was no statistically significant difference between the day 0, 7, and 14 measurements in the control group ($p = 0.54$) (Table 3).

**Table 3.** Measurement of the microhardness of the tooth samples immersed in the drug solutions on days 0, 7 and 14

| Pharmaceutical name | Day 0 (baseline) | Day 7 | Day 14 | *p*-value |
|---|---|---|---|---|
| Keppra | 276.7 ±28.8[a] | 181.1 ±32.4 | 197.6 ±17.0 | <0.001* |
| Cefaks | 265.7 ±62.8 | 206 ±62.9 | 229.5 ±68.7 | 0.037*[b] |
| Macrol | 313.3 ±44.0[c] | 278.0 ±52.0 | 224.0 ±42.0 | 0.017* |
| Ferro Sanol B | 243.3 ±50.3[d] | 207.1 ±43.3 | 190.0 ±51.0 | 0.002* |
| Calpol 6 Plus | 335.1 ±36.0[e] | 268.0 ±58.0 | 261.0 ±46.2 | 0.037* |
| Atarax | 282.2 ±36.0[f] | 244.0 ±55.4[g] | 167.4 ±27.3 | <0.001* |
| Ventolin | 201.1 ±36.0[h] | 155.0 ±46.5[i] | 101.0 ±39.2 | <0.001* |
| Augmentin | 293.4 ±35.5[j] | 234.0 ±53.1 | 227.1 ±83.0 | 0.017* |
| Dolven | 229.1 ±38.0[k] | 146.4 ±20.1 | 139.0 ±36.1 | <0.001* |
| Sudafed | 207.0 ±42.0[l] | 195.0 ±25.4[m] | 130.0 ±15.1 | <0.001* |
| Deltacortril | 312.1 ±21.0 | 285.0 ±49.0 | 268.3 ±61.0 | 0.191 |
| Distilled water | 146.7 ±33.6 | 162.8 ±20.6 | 163.1 ±30.3 | 0.54 |

Data presented as mean ± standard deviation (*SD*); [a] $p < 0.001$, 0 vs 7 and 14; [b] $p = 0.037$; [c] $p = 0.017$, 0 vs 7 and 14; [d] $p = 0.002$, 0 vs 7 and 14; [e] $p = 0.037$, 0 vs 7 and 14; [f] $p < 0.001$, 0 vs 7; [g] $p < 0.001$, 7 vs 14; [h] $p < 0.001$, 0 vs 7 and 14; [i] $p < 0.001$, 7 vs 14; [j] $p = 0.017$, 0 vs 7 and 14; [k] $p < 0.001$, 0 vs 7 and 14; [l] $p < 0.001$, 0 vs 14; [m] $p < 0.001$, 7 vs 14; * statistically significant.

# Discussion

In this study, it was observed that all of the drugs used, except for prednisolone ($p = 0.191$), caused a significant decrease in the tooth enamel microhardness in vitro. In the case of prednisolone, a decrease was also observed, but it was not significant ($p > 0.05$). Similarly, researchers have shown that some pediatric drugs that are given to children regularly and for a long time have the erosive potential.[23–25]

The pH values of the drugs used ranged from 2.76 (pseudoephedrine HCl) to 6.62 (paracetamol). The drug whose active substance is pseudoephedrine HCl (pH 2.76; TA 3250 mmol; BC 3246 mmol) caused a significant decrease in the microhardness of the samples. This outcome can be attributed to the lower pH and high TA and BC levels of the drug and its inactive ingredients. As it is commonly known, a drug with a low pH value and high TA has the potential to produce abrasive lesions in the teeth if used frequently and/or for a long time. Acidogenicity is defined as the production of oral acid from the sugars found in beverages or drugs, or from refined carbohydrates. Thus, it has been understood that refined carbohydrate concentration and additives modify the properties of the test media used to contribute to pH and TA.[25,26]

There are 2 ways to measure the acid content of a food or beverage: pH or total or neutralizable acidity, and pH or true acidity, which is the negative logarithm of the hydrogen ion concentration. Neutralizable acidity refers to the total number of acid molecules, both protonated and unprotonated, and determines the availability of real hydrogen ions that will interact with the tooth surface.[25,27]

In this study, pseudoephedrine HCl showed the maximum BC (3246 mmol) and ibuprofen had the greatest TA (3750 mmol). The causes of these differences were thought to be related to the syrup composition, added alcohols as well as the viscosity and surface tension of the syrups and the acids in the formulations.[12]

The liquid pharmaceutical formulations of the drugs that were included in the study are as follows: the antibiotic was clarithromycin (pH 5.15; TA 750 mmol), the antiepileptic was levetiracetam (pH 5.83; TA 400 mmol), the bronchodilator was salbutamol (pH 3. 58; TA 3000 mmol), and the sympathomimetic was pseudoephedrine HCl (pH 2.76; TA 3250 mmol), which contains citric acid as an inactive ingredient.

The effect of citric acid was thought to be important in the reduction in microhardness caused by these drugs. Citric acid is the main acid used in medicines and is a powerful erosion agent, as it has the ability to chelate calcium in hydroxyapatite.[9] Citric acid, ascorbic acid, sugar, diphenhydramine hydrochloride, and ethanol are substances that contribute to acidity, which can be neutralized at low pH.

Drugs in the form of syrups include diphenhydramine hydrochloride, ammonium chloride, sodium citrate, menthol, and ethanol.[25] In this study, hydroxyzine HCl

contained ethanol and the multivitamin ascorbic acid excipients. The microhardness reduction in the samples can be attributed to the low pH and high TA and BC values, and the inactive ingredients of the drugs.

Titratable acidity represents the total content of acids and is considered to determine the strength of the erosive potential of the syrup; BC is the time needed to neutralize the acid in the syrup by the saliva.[13]

Prednisolone (6.36), levetiracetam (5.83), cefuroxime axetil (5.86), paracetamol (6.62), and amoxicillin + clavulanic acid (5.70), which have pH values above critical pH (5.5), caused a significant decrease in microhardness, except for prednisolone. This can be attributed to high TA and high BC. A decrease in sample microhardness was observed in the prednisolone drug, but this was not significant. In this case, it is thought that the pH value of the drug (6.36) is higher than the critical acidity; the pH, TA (40 mmol) and BC (71 mmol) values are low, the sugar contains lactose, and the other excipients also play a role. At the same time, this outcome may be related to the short test duration.

In this study, despite having pH higher than prednisolone, a significant decrease in microhardness occurred in paracetamol in the applied samples. This effect can be explained by high TA (200 mmol) and BC (526 mmol). Additionally, this decrease can be linked to the inactive ingredients of the drug, such as a sorbitol solution, orange flavor, sucrose, Tween 80, and white sugar. Ibuprofen (pH 4.38; TA 3750 mmol; BC 1431 mmol), amoxicillin + clavulanic acid (pH 5.70; TA 400 mmol; BC 308 mmol), cefuroxime axetil (pH 5.86; TA 800 mmol; BC 702 mmol), and clarithromycin (pH 5.15; TA 750 mmol; BC 405 mmol) caused a significant decrease in microhardness in the samples, which can be attributed to the low pH, high TA and TK values and the inactive ingredients contained.

The samples were exposed to artificial saliva between immersion cycles. The saliva protein-based pellicle emulsifies on the tooth surface and prevents direct contact of the acids and the tooth surface, acting as a diffusion barrier and preventing the demineralization process. Therefore, an artificial saliva substance was used between the immersion cycles to protect the teeth. Care was taken to renew artificial saliva every day to ensure a remineralization effect similar to that of fresh human saliva.[13,28]

Iron(II)-glycine-sulfate complex (pH 2.86), which is frequently prescribed from the multivitamin group, was used and caused a significant decrease in microhardness. This drug has high TA (3500 mmol) and BC (845 mmol) values and inactive ingredients, such as ascorbic acid, glucose monohydrate, sorbitol, refined sugar, orange, pear essence, and sulfuric acid, which is an abrasive acid. Similarly, other in vitro studies have observed the erosive effects of certain drugs, such as bronchodilator syrups, iron supplements and antiallergic/expectorant drugs, on the surface roughness and microhardness, and morphological changes have been reported.[7–9,11,12,19]

Salbutamol from the bronchodilator group, used in the present study, has lower pH (3.58), and higher TA (3000 mmol) and BC (877 mmol) than the critical pH level. Compared to the baseline microhardness values, the decrease in the values for days 7 and 14 was associated with both low pH and high TA and TK values, and with inactive ingredients, such as salbutamol sulfate and citric acid monohydrate as well as orange flavor.

Contradictory results regarding the relationship between asthma and tooth erosion have been published in the literature.[29,30] Asthma medications used as inhalers are thought to be more protective against erosion with the help of both higher pH and the protective effect of the lips.[29]

Drugs that are acidic, however, can directly affect the teeth. When in vivo, it is thought that the long-term use of β2-adrenergic stimulants, such as salbutamol, salmeterol or terbutaline, leads to a decrease in the saliva flow and thus reduces the modifying and protective effects of saliva. At the same time, drugs used as bronchodilators are thought to enhance gastroesophageal reflux. Whether or not there is a causal relationship between both conditions is still unknown, but dentists should consider linking the possibility of erosive lesions to possible reflux disease in asthmatic patients.[26]

Sugar or other sweeteners are available in all of the medications used in the study and are thought to increase both the erosive and cariogenic potential of the medicines. Similarly, Xavier et al. found lower values for the antiemetic class drugs without sugar in their composition, and higher erosive and cariogenic potential values for antibiotics and analgesics.[31] Sugars are multifunctional ingredients added to drug formulations due to the unpleasant taste of many active ingredients. Additionally, sucrose is chemically and physically stable, functions as an oxidant and solvent, is easily processed, is found in different dry particle sizes providing viscosity for the drug, is not hygroscopic, and is less costly, which affects the final product price. Liquid medicines usually have unpleasant taste, so it is necessary to add a variety of sweeteners to the product to overcome this. Commonly used sweeteners are sodium saccharin, sorbitol and sodium cyclamate.

Most of the drugs contain sucrose and citric acid. The replacement of the acidic group, for example using malic acid instead of citric acid, and the use of sweeteners that have been proven to be less cariogenic, such as xylitol or sorbitol, may reduce the erosive and cariogenic effects of drugs. Additionally, drug companies should show the type and amount of sweetener added and label all medicines to indicate the possible adverse effects on teeth. Moreover, medicines with non-cariogenic sweeteners may be presented, and 'tooth-friendly' symbols may be placed on the packages.[14] However, studies have shown that there is a need for further research.[2,12,32]

Finally, this study imitated an in vivo environment, so it may be recommended to evaluate the effects of different active agents on the enamel of primary teeth for a longer time in further studies.

## Conclusions

Commonly used and prescribed pediatric drugs pose a risk for tooth erosion, and various protective measures should be recommended to parents, dentists and pediatricians. Parents should be informed about the importance of milk and permanent teeth. It may be advisable to rinse the mouth with water or Ca, F and ($PO_4$), 3 added formulations after drug use; to take medications during mealtimes; to pay attention to oral hygiene procedures; not to skip dental appointments in 3–6 months intervals; and to complete fluoride applications. The type and amount of sweeteners added to medications by pharmaceutical companies can be important for creating awareness among pediatricians who prescribe medicines and for parents reading labels that indicate possible adverse effects on teeth. It may also be useful to increase the number of medicines with sweeteners that do not cause erosion and to mark them with 'tooth-friendly' symbols on pharmaceutical markets. Pediatricians should be aware of the effects of prescription drugs on tooth erosion and and emphasize the need for compliancy with oral hygiene procedures. At the same time, we are of the opinion that it would be beneficial to work cooperatively with dentists.

### ORCID iDs

Nagehan Yılmaz https://orcid.org/0000-0001-9523-2899
Ozgul Baygin https://orcid.org/0000-0003-0836-7619
Tugba Nigar Cakıroglu https://orcid.org/0000-0001-9613-6911
Tamer Tüzüner https://orcid.org/0000-0001-5817-5928
Orhan Deger https://orcid.org/0000-0003-3584-6324

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# The frequency and severity of dental caries, and counts of cariogenic bacteria in rheumatoid arthritis patients

# Częstość występowania i intensywność próchnicy oraz liczba bakterii próchnicotwórczych u pacjentów z reumatoidalnym zapaleniem stawów

Rita Elizabeth Martinez-Martinez[1,2,A,E,F], Rubén Abraham Domínguez-Pérez[1,3,B–D,F], Javier Sancho-Mata[2,B,C,F], Carlos Abud-Mendoza[4,C,E,F], José Luis Ayala-Herrera[1,5,B,F], Elena Aurora Popoca-Hernandez[1,B,F]

[1] Department of Biomedical Sciences, Faculty of Medicine, Autonomous University of San Luis Potosí, Mexico
[2] Department of Advanced General Dentistry, Faculty of Stomatology, Autonomous University of San Luis Potosí, Mexico
[3] Laboratory of Multidisciplinary Dentistry Research, Faculty of Medicine, Autonomous University of Querétaro, Santiago de Querétaro, Mexico
[4] Regional Unit of Rheumatology and Osteoporosis at Dr. Ignacio Morones Prieto Central Hospital, Autonomous University of San Luis Potosí, Mexico
[5] Faculty of Dentistry, University of De La Salle Bajío, Leon, Mexico

A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation;
D – writing the article; E – critical revision of the article; F – final approval of the article

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**Address for correspondence**
Rita Martinez-Martinez
E-mail: rita.martinez35@yahoo.com

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## Abstract

**Background.** It has been reported that patients with rheumatoid arthritis (RA) are more likely to exhibit periodontitis than patients without RA. However, the frequency and severity of dental caries in patients with RA is still unknown.

**Objectives.** The aim of the study was to investigate whether higher counts of cariogenic bacteria are present in RA patients in contrast to healthy subjects, and to ascertain whether the frequency and severity of dental caries are increased in RA patients.

**Material and methods.** The study involved 160 adults: an RA group (n = 80) and a control group matched by age and gender (n = 80). The participants' dental status scores were determined based on the following indices: the Decayed, Missing and Filled Teeth (DMFT) index, the Filled and Sound Teeth (FS-T) index, Treatment Needs Index (TNI), Care Index (CI), and Integrative Dental Caries Index (IDCI). DNA copies of *Streptococcus mutans (S. mutans)* and *Streptococcus sobrinus (S. sobrinus)* were quantified using real-time polymerase chain reaction (PCR).

**Results.** The IDCI showed that the RA group was more affected, mainly presenting moderate to severe dental caries. The RA group also had higher global DMFT scores than the control group and scored higher on the decayed component of the DMFT index. The TNI and CI indicated that RA patients required more dental attention and appropriate treatment. The *Streptococcus mutans* count was significantly higher in the RA group.

**Conclusions.** A complete basic oral examination, along with oral health instruction including adequate oral and dental hygiene, is crucial to prevent dental caries and associated complications in RA patients, since they appear to be more vulnerable than the non-RA population.

**Key words:** rheumatoid arthritis, dental caries, *Streptococcus mutans*, *Streptococcus sobrinus*

**Słowa kluczowe:** reumatoidalne zapalenie stawów, próchnica, *Streptococcus mutans*, *Streptococcus sobrinus*

# Introduction

Rheumatoid arthritis (RA) is a chronic destructive inflammatory disease consisting in joint damage and bone erosion, and leading to functional disability.[1] It has a prevalence of approx. 1% and is characterized by substantial morbidity and accelerated mortality.[2,3] It is accompanied in many cases by oral manifestations, such as temporomandibular joint disorders, secondary Sjögren's syndrome and xerostomia.[4–6] Xerostomia is related to hyposalivation, which is considered a cariogenic condition, since saliva plays an important role in preventing the development of dental caries. Saliva dilutes carbohydrates and other substances, has excellent buffering capacity and antimicrobial activity, and balances the enamel demineralization and remineralization.[7]

Dental caries is regarded as a preventable non-communicable disease that affects a majority of the population across their lifespan and, along with periodontal disease, is considered the most important oral disease.[8,9] Approximately 300 bacterial species have been found to be associated with dental plaque, but only *Streptococcus mutans* (*S. mutans*) and *Streptococcus sobrinus* (*S. sobrinus*) have been consistently linked to the etiology of human dental caries.[10] Additionally, *S. mutans* and *S. sobrinus* have been associated with non-oral infections, such as subacute bacterial endocarditis, atherosclerosis, coronary artery disease, and other systemic conditions.[11,12]

It has been reported that patients with RA are more likely to exhibit advanced periodontitis than patients without RA.[13,14] However, it is still unknown whether the frequency and/or severity of dental caries is also higher in RA patients. Therefore, the aim of this study was to investigate the frequency and severity of dental caries by the use of several indices, and to detect, quantify and compare the number of DNA copies of *S. mutans* and *S. sobrinus* per milligram of supragingival dental plaque using real-time polymerase chain reaction (PCR) in RA patients and healthy subjects.

# Material and methods

## Subject population and clinical evaluation

This cross-sectional prospective study included 80 RA patients who were undergoing treatment and routine follow-up at the Regional Unit of Rheumatology and Osteoporosis at Dr. Ignacio Morones Prieto Central Hospital in San Luis Potosí, Mexico. All the RA patients had been diagnosed at least 5 years earlier by a rheumatologist, in accordance with the American College of Rheumatology (ACR) criteria[15]; they were selected for the study by non-probabilistic consecutive sampling. A control group of 80 healthy subjects (without RA), matched by age and gender with the RA group, were recruited from the waiting room of the Clinic of Oral Medicine within the Master's Degree in Advanced General Dentistry Program at the Autonomous University of San Luis Potosí. Informed and voluntary written consent was obtained prior to the clinical examinations, in accordance with the ethical principles of the Declaration of Helsinki (2013 version). The study was approved by the clinical research committee for the Master's Degree in Advanced General Dentistry Program at the Autonomous University of San Luis Potosí. In both groups, only individuals that had brushed their teeth 3 h before sample collection were included. Those who had undergone prophylaxis or antibiotic therapy in the previous 3 months, smokers, those suffering from infectious diseases or other chronic diseases, immunocompromised individuals, and those presenting <8 teeth were excluded from the study. Additionally, patients diagnosed with secondary Sjögren's syndrome and those whose RA had lasted >15 years were excluded from the RA group.

## Dental caries assessment

Each subject completed an oral health questionnaire, which included questions about the use of oral hygiene aids, the regularity of dental check-ups and oral dryness, among others. The participants were diagnosed by a single examiner using standardized dental caries detection measures, with a standard dental light, a plain mouth mirror and a probe. For dental caries diagnosis, the total surfaces of all erupted teeth were assessed by applying the Decayed, Missing and Filled Teeth (DMFT) index and the Filled and Sound Teeth (FS-T) index, determined using the previously reported criteria for healthy and functional teeth.[16,17] Treatment Needs Index (TNI), Care Index (CI) and Integrative Dental Caries Index (IDCI) were also assessed.[18,19] The IDCI included clinical findings by tooth surface and tooth area (anterior and posterior teeth) per patient.

Dental caries was evaluated on the mesial, buccal, distal, and palatine surfaces of the anterior teeth (the maxillary and mandibular central and lateral incisors as well as canines). For the posterior teeth, the occlusal, mesial, buccal, distal, and palatine surfaces were evaluated in the maxillary and mandibular premolars and molars. The third molars of the 4 quadrants were excluded. In order to provide more details, the severity of dental caries was determined using a gradient that evaluated the affected anterior and posterior surfaces: healthy (a patient without dental caries); mild (1–7 surfaces affected); moderate (8–21 surfaces affected); and severe (>22 surfaces affected by dental caries).[19]

## Sample collection, DNA isolation and quantitative polimerase chain reaction

From each participant, supragingival dental plaque was collected from 4 locations around each tooth (vestibular, mesial, palatine, and distal of anterior, posterior, upper,

and lower teeth) using a Gracey curret (Hu-Friedy Mfg. Co. LLC, Chicago, USA). Dental plaque was stored in a microcentrifuge tube containing the phosphate buffered saline (PBS) solution, then weighed in milligrams and washed; it subsequently underwent the DNA extraction in accordance with the previously reported protocol for Gram-positive bacteria.[20] All samples were processed aseptically to prevent contamination from the environment during the DNA extraction and quantitative polymerase chain reaction (q-PCR) assays.

For q-PCR, 20 μL of a mixture containing 50 ng of genomic DNA, 1× TaqMan® Universal PCR Master Mix (Applied Biosystems, Foster City, USA) and 0.5 mL of Custom TaqMan Gene Expression Assay for *S. mutans* and *S. sobrinus* (Applied Biosystems) (Table 1) was placed in each well of a 48-well plate. The amplification and detection were performed using the StepOne™ System (Applied Biosystems) with the cycling profiles indicated in the manufacturer's instructions. Standard curves were prepared using plasmidic DNA cloned into a vector in *Escherichia coli* competent cells by a 10-fold dilution series. The q-PCR results were expressed as the number of copies of bacteria DNA per milligram of dental plaque.[21]

## Statistical analysis

All quantitative data were expressed as mean, standard deviation (*SD*) and range. Qualitative data were expressed as frequency and proportion. Statistical differences between groups were determined with the Mann–Whitney *U* test for quantitative variables and the $\chi^2$ test for qualitative variables. The JMP® software v. 9.0 (SAS Institute, Cary, USA) and Stata v. 11.0 (StataCorp, College Station, USA) were used. Statistical significance was set at $p < 0.05$.

# Results

The inter-observer reliability regarding the dental caries diagnosis was analyzed with the $\kappa$ test; the obtained $\kappa$-value was 0.90. For both groups of participants, the mean age was 46 ±8 years (range: 32–60 years); the distribution was 92% females and 8% males. There were no statistically significant differences between the groups with respect to the oral behavior variables (Table 2). In the RA group, 60% of the patients reported a dry mouth sensation, whereas only 8.7% of the healthy subjects reported it ($p < 0.05$).

Regarding the DMFT index, the control group had a higher mean; however, there was no statistical difference between the 2 groups. It is important to note that the RA group had a higher rate of decayed teeth, but a lower count of filled teeth compared to the control group. These values can be translated into the high TNI and low CI found in the RA group, both with statistical significance (Table 3).

Table 4 shows IDCI, which provides more detailed information about the presence of caries by patient, tooth and surface. The dental caries gradient by patient was similar in the 2 groups, with a high frequency of mild caries (1–7 surfaces affected): 41.2% in the control group and 31.2% in the RA group. The analysis by tooth showed posterior and upper teeth as the most frequently affected, especially in RA

**Table 2.** Oral behavior overview in both groups

| Oral behavior | Control group n = 80 | RA group n = 80 | *p*-value |
|---|---|---|---|
| Tooth brushing | | | |
| ≤1 daily | 48 (60) | 51 (63.7) | 0.7449 |
| ≥2 daily | 32 (40) | 29 (36.2) | |
| Use of hygiene aids | | | |
| manual toothbrush | 77 (96.2) | 75 (93.7) | 0.7195 |
| electric toothbrush | 3 (3.7) | 5 (6.2) | |
| dental floss | 41 (51.2) | 29 (36.2) | 0.0793 |
| mouth rinse | 33 (41.2) | 44 (55) | 0.1133 |
| Last dental examination | | | |
| <12 months ago | 22 (27.5) | 31 (38.7) | 0.1788 |
| >12 months ago | 58 (72.5) | 49 (61.2) | |

Data expressed as number (percentage); RA – rheumatoid arthritis ; Fisher's exact test.

**Table 3.** Distribution of all the assessed indices and the Decayed, Missing and Filled Teeth (DMFT) index components

| Indices regarding teeth | Control group n = 80 | RA group n = 80 | *p*-value |
|---|---|---|---|
| | mean ±*SD* (range) | | |
| Healthy | 13.34 ±5.51 (2–26) | 15.25 ±5.08 (4–25) | 0.0691 |
| Decayed | 3.88 ±4.05 (0–14) | 5.79 ±3.98 (0–17) | 0.0149 * |
| Missing | 4.00 ±3.88 (0–16) | 3.90 ±3.35 (0–15) | 0.9999 |
| Filled | 6.74 ±4.98 (0–18) | 3.33 ±3.99 (0–16) | 0.0004 * |
| DMFT | 14.84 ±5.52 (2–26) | 13.02 ±4.99 (3–24) | 0.0745 |
| FS-T | 71.59 ±18.80 (21–100) | 66.02 ±19.23 (14–96) | 0.1364 |
| TNI | 35.85 ±35.22 (0–100) | 67.77 ±34.00 (0–100) | 0.0002 * |
| CI | 45.11 ±30.21 (0–100) | 23.91 ±27.00 (0–94) | 0.0009 * |

CI – Care Index; FS-T – Filled and Sound Teeth index; *SD* – standard deviation; TNI – Treatment Needs Index; * statistically significant (Mann–Whitney *U* test).

**Table 1.** Primers and probes for qualitative polymerase chain reaction (q-PCR)

| Accessed bacteria | Forward primer sequence | Reverse primer sequence | TaqMan probe |
|---|---|---|---|
| *Streptococcus mutans* | GCCTACAGCTCAGA GATGCTATTCT | GCCATACACCACTC ATGAATTGA | TGGAAATGACGGTG CCGTTATGAA |
| *Streptococcus sobrinus* | TTCAAAGCAAGA CCAAGCTAGT | CCAGCCTGAGATTC AGCTTGT | CCTGCTCCAGCGA CAAAGGCAGC |

patients: on average 5.3 and 4.0 teeth, respectively, as opposed to 2.4 teeth in the control group ($p < 0.05$). The global score of dental caries by surface showed that RA patients presented a significantly higher level of affected surfaces: about 7.9 compared to 5.6 in the control group ($p < 0.05$).

With respect to cariogenic bacteria, the results showed their presence in an order of magnitude of $10^8$ copies/mg of subgingival dental plaque (Table 5). *Streptococcus mutans* was significantly higher in the RA patients ($p < 0.05$), while *S. sobrinus* counts were higher than *S. mutans* in both study groups ($p < 0.05$).

**Table 4.** Comparisons of the Integrative Dental Caries Index (IDCI)

| IDCI components | | Control group n = 80 | RA group n = 80 | *p*-value |
|---|---|---|---|---|
| Dental caries by patient[a] | | | | |
| Gradient | healthy | 16 (20) | 11 (13.7) | 0.2314 |
| | mild | 33 (41.2) | 25 (31.2) | |
| | moderate | 18 (22.5) | 25 (31.2) | |
| | severe | 13 (16.2) | 19 (23.7) | |
| Dental caries by tooth[b] | | | | |
| Area | anterior | 0.8 ±1.0 | 1.0 ±1.1 | 0.4227 |
| | posterior | 2.4 ±2.2 | 5.3 ±3.4 | 0.0002* |
| Jaw | upper | 2.4 ±1.7 | 4.0 ±2.0 | 0.0024* |
| | lower | 1.1 ±1.0 | 2.7 ±1.6 | <0.0001* |
| Dental caries by surface[b] | | | | |
| Area | anterior | 1.2 ±1.1 | 1.4 ±1.0 | 0.7002 |
| | posterior | 3.0 ±3.2 | 6.2 ±4.2 | 0.0001* |
| Jaw | upper | 3.4 ±2.5 | 4.7 ±3.0 | 0.0264* |
| | lower | 2.2 ±1.6 | 2.8 ±1.6 | 0.2013 |
| Global score of caries by surface[b] | | 5.6 ±3.3 | 7.9 ±4.8 | 0.0228* |

Data presented as number (percentage) or mean ±*SD*
[a] $\chi^2$ test; [b] Mann–Whitney *U* test; * statistically significant.

**Table 5.** Distribution of *Streptococcus mutans (S. mutans)* and *Streptococcus sobrinus (S. sobrinus)*

| Bacteria | Control group n = 80 | RA group n = 80 | *p*-value |
|---|---|---|---|
| | mean ±*SD* (range) | | |
| Total of cariogenic bacteria | $4.6 \times 10^8 \pm 3.1 \times 10^9$ ($677.3 - 2 \times 10^{10}$) | $3.3 \times 10^8 \pm 8.2 \times 10^8$ ($6483.4 - 4 \times 10^9$) | 0.7307 |
| *S. mutans* | $1.5 \times 10^5 \pm 4.9 \times 10^5$ ($66.36 - 2.8 \times 10^6$) | $5.9 \times 10^7 \pm 1.7 \times 10^8$ ($41.72 - 8.9 \times 10^8$) | 0.0001* |
| *S. sobrinus* | $9.5 \times 10^8 \pm 5.6 \times 10^8$ ($0 - 3.8 \times 10^8$) | $5.9 \times 10^8 \pm 1.8 \times 10^9$ ($0 - 7.8 \times 10^9$) | 0.6712 |
| *p*-value | <0.0001* | 0.0421* | – |

* statistically significant (Mann–Whitney *U* test).

## Discussion

Rheumatoid arthritis is a chronic inflammatory disorder that affects joints, which results in physical disabilities, such as arm and hand dysfunction. When hyposalivation (associated or not with Sjögren's syndrome) is involved, oral hygiene becomes a particular concern.[22] In this study, we excluded patients diagnosed with secondary Sjögren's syndrome and only included patients with at least 5 years and at the longest 15 years of RA in order to limit possible injury to the salivary glands and the timespan in which the subjects could have experienced hyposialia. This was because hyposialia could affect the salivary flow and decrease the ability to neutralize acids produced as a result of bacterial metabolism, leading to changes in the oral microbiota associated with caries frequency and severity.

It is well-known that dental caries represents a major public health problem around the world, whereas RA occurs worldwide with a prevalence estimated at 0.2–1.0%, affecting mainly females at a ratio of 3:1.[23,24] The distribution of female patients in the present study (around 13:1) was higher than in the previously reported data. This can be explained by the fact that patients were selected from a rheumatology unit by consecutive sampling. Besides, it can be inferred that women generally report for more of their rheumatologic follow-ups than men do. To the best of our knowledge, there have only been 2 studies using the DMFT index to assess caries in RA patients and controls, and both reported no significant differences between the groups (11.84 vs 10.56, respectively, in one report,[25] and 17.61 vs 16.03, respectively, in the other[26]). In the present study, we obtained a slightly higher DMFT score in the control group compared to the RA group (14.8 vs 13.0, respectively). These results are consistent with other reports of DMFT scores of 13.1 in adults older than 40 years in our population.[27] Such results may be due to demographic differences. Although we found no statistically significant differences between the groups when comparing the overall DMFT index scores, analyzing each DMFT component proved that RA patients presented a higher mean of decayed teeth than the control group (5.79 vs 3.88; $p = 0.0149$), showing that RA patients had more active caries. At the same time, the mean of filled teeth was significantly lower in the RA group compared to the control group (3.33 vs 6.74; $p = 0.0004$). The TNI and CI confirmed these findings, showing that the RA patients had higher levels of active caries than the control group, which had more filled than decayed teeth.

It is important to note that the DMFT index represents the history of dental caries in the subject, which was similar in the 2 groups. Although the DMFT index is generally used in dental caries epidemiological studies, it is only focused on determining the caries experience during the subject's life. This is an important limitation, because it does not distinguish caries severity and assigns the same value to small lesions and highly damaged surfaces. For that reason, IDCI is useful to provide detailed information about dental caries. After analyzing all the indices, including IDCI, it is unquestionable that RA patients exhibited more dental caries (and often more severe) than the subjects without RA.

The present study included both bacterial species associated with dental caries, and used the golden standard – q-PCR – to quantify bacterial DNA copies per milligram of dental plaque. This method is highly sensitive and specific for the detection and quantification of bacteria, and is superior to classical methods, such as counting colony-forming units (CFUs), which is limited by a possible bias or the overestimation of the bacteria involved in dental caries.[28]

It has been widely reported that *S. sobrinus* strains are more acidogenic and aciduric, and that they exhibit a greater degree of cariogenicity in gnotobiotic animals than *S. mutans* strains. Despite these proprieties, *S. sobrinus* is much less frequently isolated from humans than *S. mutans*; and when isolated, it is almost invariably present in lower quantities.[29] However, both of our study groups presented a high amount of *S. sobrinus* compared to *S. mutans*. This can be explained by the fact that the plaque samples were obtained from supragingival smooth surfaces, where *S. sobrinus* is more frequent than *S. mutans*, which has been linked to pits and fissures on occlusal surfaces. A previous work by our research group described similar findings in systemic lupus erythematosus (SLE) patients, where moderate to severe caries and a higher number of *S. sobrinus* copies per milligram of dental plaque were observed.[19] Since both SLE and RA are autoimmune diseases, it is crucial to promote measures to prevent dental caries on all surfaces, including smooth surfaces. Oral opportunistic infections can have a considerable impact on other systemic diseases as well.

It is important to note that all the participants in our study presented both of the bacterial species that have previously been associated with a high frequency and severity of dental caries.[30,31] However, the RA group presented *S. mutans* in counts that were higher compared to the controls by at least 2 orders of magnitude, which could be clinical evidence that there is high bacterial activity in these patients. The unique difference between our groups – oral dryness in the RA group – could be related to the higher counts of *S. mutans* in that group. A limitation of this study is that we did not perform sialometry to detect the differences in saliva secretion; we only questioned patients about a dry mouth sensation. Silvestre-Rangil et al. investigated whether a dry mouth sensation was correlated to genuine hyposialia, and observed significant differences between their control group and RA group, in which saliva rates were clearly decreased.[25]

## Conclusions

Our results suggest that dental caries is more frequent and severe in RA patients, just as periodontal diseases were shown to be in previous studies. These results indicate that RA patients are not receiving adequate dental care, or they may be focusing their attention more on their systemic disease, considering dental care less important. Rheumatologists should therefore refer patients for complete basic oral examinations and promote oral health, including adequate oral and dental hygiene, in order to prevent dental caries and its complications. Dental caries must be considered an oral manifestation that accompanies RA in many cases.


### ORCID iDs

Rita Elizabeth Martinez-Martinez https://orcid.org/0000-0001-9631-2751
Rubén Abraham Domínguez-Pérez https://orcid.org/0000-0001-8979-8394
Javier Sancho-Mata https://orcid.org/0000-0001-6240-3161
Carlos Abud-Mendoza https://orcid.org/0000-0002-3749-5831
José Luis Ayala-Herrera https://orcid.org/0000-0001-7732-7974
Elena Aurora Popoca-Hernandez https://orcid.org/0000-0001-9076-0713


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# The effect of dentin pre-treatment with activated riboflavin on the bond strength of a two-step self-etch adhesive system

# Wpływ wstępnego pokrycia zębiny aktywowaną ryboflawiną na siłę wiązania dwustopniowego samotrawiącego systemu adhezyjnego

Shahin Kasraei[1,A,E,F], Maryam Mojtahedi[2,B–D], Mohammad-Taghi Goodarzi[3,A,C,E], Mohadese Azarsina[4,A,D,F], Zahra Khamverdi[1,B,E]

[1] Department of Restorative Dentistry, School of Dentistry, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[2] School of Dentistry, Hamadan University of Medical Sciences, Iran
[3] Research Center for Molecular Medicine, Hamadan University of Medical Sciences, Iran
[4] Department of Operative Dentistry, School of Dentistry, Shahid Beheshti University of Medical Sciences, Tehran, Iran

A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation;
D – writing the article; E – critical revision of the article; F – final approval of the article

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**Address for correspondence**
Zahra Khamverdi
E-mail: zkhamverdi@yahoo.ca

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## Abstract

**Background.** The cross-linking of collagen fibers in the hybrid layer has been suggested as a way to create more durable bonds.

**Objectives.** This study evaluated the effect of visible light-activated riboflavin (RF) as a cross-linking agent on the durability of the dentin microtensile bond strength (μTBS) in a 2-step self-etch (SE) adhesive system.

**Material and methods.** The occlusal surfaces of 21 human premolar teeth were ground down to expose the dentin, and were then randomly divided into 3 groups. The Clearfil® SE Bond was used in the control group. In the RF/BL group, a 0.1 wt% aqueous solution of RF was applied to the dentin surface before applying the adhesive and irradiating with blue light (BL) for 2 min. In the 3rd group, the RF-P/BL group, the RF powder was added to the adhesive primer (P) at a concentration of 0.1 wt%. The teeth were built up using composite resin. After thermocycling, 14 resin–dentin beams from each group were prepared and stored in water for 3 months. The μTBS was determined and the data was analyzed using a linear model with a generalized estimating equation (GEE) ($p = 0.05$).

**Results.** The highest and the lowest μTBS belonged to the control group (41.15 ±3.50 MPa) and the RF-P/BL group (19.84 ±3.80 MPa), respectively. The mean μTBS in the control group was significantly higher than in the RF/BL and RF-P/BL groups ($p < 0.001$), but no significant difference was found between the RF/BL and RF-P/BL groups ($p = 0.598$).

**Conclusions.** Pre-treatment of dentin surfaces with RF activated with BL had a negative impact on the μTBS of the Clearfil SE Bond as a 2-step SE adhesive.

**Key words:** collagen, riboflavin, cross-linking agents, dentin bonding agents, photoactivation

**Słowa kluczowe:** kolagen, ryboflawina, środki sieciujące, zębinowe środki wiążące, fotoaktywacja

# Introduction

The disintegration of an adhesive interface over time is an important factor influencing the longevity of adhesive restorations.[1] Unstable polymers within the hybrid layer may cause collagen fibers to be denuded. These fibers are highly susceptible to hydrolytic and mechanical fatigue as well as degradation via the collagenolytic activity of host-derived proteases.[2]

Etch and rinse (E&R)[3] and self-etch (SE)[4] adhesives activate dentin matrix metalloproteinases (MMPs) and may be responsible – at least in part – for the gelatinolytic activity observed in the hybrid layer.[5,6] Chlorhexidine has been suggested as a strong MMP inhibitor which has antibacterial properties and, when incorporated into SE adhesives, increases the long-term durability of the adhesive without jeopardizing its mechanical properties and bond strength.[7,8] Matrix metalloproteinases do not undergo turnover in dentin. Thus, inactivating these organic enzymes through the use of cross-linking agents may increase durability and may be even more effective than using MMP inhibitors like chlorhexidine.[9,10]

Increasing the cross-linking of collagen can be done by chemical or physical (photo-oxidative) means. The chemical approach includes the use of different types of cross-linking solutions, while the physical (photo-oxidative) approach is comprised of light exposure – particularly ultraviolet radiation.[9,11] The covalent cross-links created by the external cross-linkers are very stable and deactivate the active sites of dentin proteases by decreasing the molecular mobility of the active sites or by converting the negatively-charged ionized carboxyl groups into positively-charged amides.[12] Moreover, the cross-linking technique has been suggested as a way to improve collagen's stability and resistance to degradation within the demineralized dentin matrix.[13–15] Increasing the cross-linking of collagen fibrils before applying an adhesive agent may increase bonding durability.[2,16] Despite the promising results of the application of cross-linkers, there are still some problems which need to be solved before they can be extensively used in clinical situations. For instance, glutaraldehyde is effective[13] as a collagen cross-linker, but is also toxic.[12] Grape seed extract is also effective[10] and may increase the immediate bond strength to dentin.[17] However, it causes a brown discoloration in dentin and the long-term durability of the resultant bond has yet to be determined.[12]

Riboflavin (vitamin B2; RF) is a strong free-radical producer under light activation with peak absorbance at 270 nm, 366 nm and 445 nm.[18] It significantly increases the cross-linking of type I collagen.[19] When RF is photoactivated with ultraviolet A light (UVA), it produces free radicals and reactive oxygen species (ROS) such as superoxide anion ($O_2^-$) and hydroxyl radical ($^{\cdot}OH$).[20]

Some studies have shown that in E&R adhesive systems, cross-linking dentin collagen with the use of RF/UVA increases the immediate bond strength, decreases nanoleakage,[9,16,21] stabilizes the adhesive interface, and inhibits the activity of dentin MMPs.[9,22] Furthermore, using RF to change the dentin collagen matrix stabilizes the fibrillar collagen network, promotes resin infiltration and hybrid layer formation[23] and improves the mechanical properties and mechanical stability of a demineralized dentin substrate against hydrolytic or collagenolytic degradation.[21,23–25] It has been reported that visible blue light-activated riboflavin (RF/BL) enhances the resistance to biodegradation and improves the mechanical properties of dentin collagen, bond strength, durability, and interface integrity after short-term water storage.[26] Based on the literature, some protocols of RF application have succeeded in increasing microtensile bond strength (μTBS), while others demonstrated bond strength similar to or lower than that of the control group.[21,23,25,26]

Some previous studies have demonstrated that the acidic resin components present in E&R[3] and SE[4] adhesives increase the collagenolytic and gelatinolytic activity of a completely or partially demineralized collagen matrix. Moreover, it has been reported that SE adhesives upregulate MMP-2 synthesis by human odontoblasts[27] and mild acidic resin monomers can activate MMPs by inhibiting the tissue inhibitor of metalloproteinase-1 in TIMP–MMP complexes.[28] Alternatively, these acidic resin monomers may activate latent forms of MMPs (proMMPs) via a cysteine-switch mechanism.[29]

When choosing a cross-linker or a photoactivation method, some clinical and safety aspects should be considered. The blue light (BL) produced by the highly popular conventional halogen–tungsten curing units (QTH) can be a good alternative to UVA light.[26] Also, the need for a separate curing device (UVA) or for a separate curing process of the cross-linker using BL for optimal results[9,26] demands further investigation in order to achieve a clinically acceptable and applicable technique.

This study sought to assess the in vitro effect of visible light-activated RF on the μTBS of a two-step SE dentin adhesive after thermocycling and 3 months of water storage. The null hypothesis was that visible light-activated RF – whether separately or incorporated into the adhesive – would have no effect on the μTBS of a two-step SE adhesive system after thermocycling and 3 months of water storage.

# Material and methods

## Sample preparation

This experimental study was conducted on 21 sound human premolar teeth extracted in orthodontic treatment. We obtained verbal consent from the patients to use their extracted teeth in this in vitro study. The research protocol was approved by the institutional ethics committee of the Vice-Chancellor of Research, Hamadan

University of Medical Sciences, Iran (protocol No. 2015-16p 372). This research was conducted in full accordance with the Declaration of Helsinki.

The teeth were stored in a 0.2% thymol solution until 1 week prior to the start of testing, when the teeth were stored in distilled water. The occlusal enamel of the teeth was removed using a trimmer apparatus with water cooling to expose an area of dentin approx. 5 mm in diameter. The surfaces of the specimens were polished with moist 600-grit silicon carbide papers (Matador® 991A Soflex; Starcke GmbH & Co., Melle, Germany) and the specimens were randomly divided into 3 groups of 7 in each group.

In the control group, Clearfil® SE Bond (Kuraray Medical Inc., Okayama, Japan) was applied as a two-step SE adhesive system to the prepared dentin surfaces, according to the manufacturer's instructions.

In the RF/BL group, based on previous studies, a 0.1 wt% RF solution was prepared by dissolving 100 mg of riboflavin-5-phosphate powder (Sigma-Aldrich, St. Louis, USA) into 100 mL of distilled water and then adjusting the pH of the RF solution to pH = 3.[19,28] The prepared 0.1 wt% RF solutions were stored in lightproof test tubes at room temperature (24°C) in order to prevent light activation. Using a sampler, 15 µL of the RF solution was applied to the dentin surfaces. The treated dentin surfaces were gently air-dried and photoactivated using the BL generated by QTH light curing unit (Optilux® 501; Demetron Kerr, Danbury, USA) with an output light spectrum of 375–520 nm, a minimum light intensity of 600 mW/cm$^2$ and a light guide diameter of 8 mm for 2 min. The tip of the light guide was placed as close to the surface as possible. Clearfil SE primer and bonding agent were then applied to the prepared dentin surface and cured according to the manufacturer's instructions.

In the RF-P/BL group, a riboflavin–primer mixture (RF-P) was created by dissolving RF powder in the primer until reaching a concentration of 0.1 wt%. The dentin surface was treated with the RF-P for 20 s, air-dried for 5 s and photoactivated with BL for 2 min. Then, Clearfil SE Bond adhesive was applied and cured for 10 s.

All of the teeth were built up by using 4 mm of composite resin (Filtek® Z250; 3M ESPE, St. Paul, USA) in 1-millimeter increments. After being stored for 24 h in distilled water at 37°C, the prepared teeth were subjected to 5,000 thermal cycles (5–55°C, 15 s dwell time). To assess the µTBS, the teeth were then sectioned using a low-speed diamond saw with water cooling in order to obtain resin–dentin beams measuring 1 × 1 mm. Two resin–dentin beams were selected from the middle part of each specimen. Thus, each group had 14 specimens. The beams were then subjected to 3 months of water storage at 37°C.

## Bond strength evaluation

Using cyanoacrylate glue, the beams were mounted on a custom-made metal jig in a universal testing machine (STM-20; Santam, Tehran, Iran). The µTBS was measured with a 50 N load cell at a crosshead speed of 1 mm/min.

## Failure mode evaluation

All the fractured samples were evaluated twice under a stereomicroscope (SZ40; Olympus, Tokyo, Japan) at ×40 magnification to determine the mode of failure (adhesive, cohesive in dentin, cohesive in composite resin, and mixed) by 1 operator.

## Sodium dodecyl sulfate – polyacrylamide gel electrophoresis analysis

The cross-linking effect of the prepared RF solutions on type I collagen was evaluated with sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Collagen mixed with distilled water served as the negative control group and 0.1% RF/UVA (pH = 7) was used as the positive control. The samples were diluted with the sample buffer and subjected to SDS-PAGE (8%) at 150 V; the gel was stained with Coomassie blue and then photographed.

## Micromorphological assessment of the resin–dentin interface using scanning electron microscopy

Two extra resin–dentin beams from each group were selected and prepared for micromorphological assessment of the resin–dentin interface using scanning electron microscope (SEM). These beams were mounted into acrylic resin. The mounted beams were polished with 600-, 800-, 1200-, 1500- and 3500-grit silicon carbide papers (Matador 991A Soflex; Starcke GmbH & Co KG). The specimens were then etched with 35% phosphoric acid for 20 s, rinsed for 20 s and dried with oil-free compressed air. The beams were immersed in a 5.25% sodium hypochlorite solution for 20 min and were thoroughly rinsed under running water for 5 min. The specimens were then dehydrated using increasing concentrations of ethanol – i.e., 33%, 50%, 70%, and 85% – for 15 min each, followed by 90%, 95% and 100% ethanol for 10 min each. After drying, the beams were mounted and sputter-coated with a gold–palladium alloy for 180 s (Sputter Coater SC 7620; Quorum Tech, Lewes, UK). The specimens were then evaluated under SEM (LEO 1450VP; Carl Zeiss, Oberkochen, Germany) at ×5,000 magnification.

## Statistical analysis

The results of µTBS testing were analyzed with one-way analysis of variance (ANOVA) for the "dentin pretreatment" factor (without RF, with RF/BL or with RF-P/BL), followed by Tukey's post-hoc pairwise comparison tests. Statistical significance was set at $p < 0.05$.

## Results

In all columns, bands were seen at about 130 KDa, belonging to α1 and α2 monomers; in all groups, apart from the negative control group, well residuals were also seen on top of the stacking gel (Fig. 1).

The highest and the lowest bond strength belonged to the control group (41.15 ±3.50) and the RF-P/BL group (19.84 ±3.80), respectively. The generalized estimating equation (GEE) model revealed that the mean μTBS in the control group was significantly higher than in the RF/BL and RF-P/BL groups ($p < 0.001$); however, the difference in the mean μTBS between the RF/BL and the RF-P/BL groups was not statistically significant ($p = 0.598$). The mean bond strength values and the frequency distribution of the mode of failure in the study groups are shown in Table 1.

Figure 2 shows the SEM images of the resin–dentin interface in selected samples from different groups after 5,000 thermal cycles and 3 months of water storage. A uniform hybrid layer and long resin tags with relatively regular distribution and straight orientation were clearly seen in the control group (without RF). However, in the RF/BL group, a hybrid layer was formed in only a few sites and there were only a few very fine resin tags with irregular distribution. In the RF-P/BL group, the hybrid layer was less uniform. The resin tags varied in height and showed a more irregular orientation compared to the control group (Fig. 2).

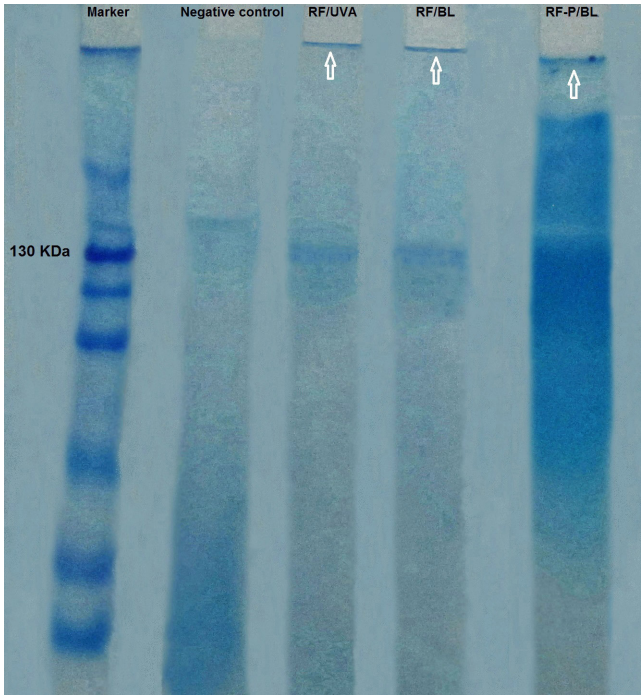


Fig. 2. Selected SEM images indicating the resin–dentin interface (at ×5,000 magnification)
A – control group; B – RF/BL group; C – RF-P/BL group.

## Discussion

In SDS-PAGE analysis, both the RF/BL and RF-P/BL groups showed stronger α-bonds and fewer fragments than the control group, which indicates the formation of intermolecular cross-links (Fig. 1). There were insoluble residuals on the wells in both groups that were identified as giant cross-linked collagen molecules. This finding was in line with the results of Chiang et al.[21]

The μTBS measured in the group without RF in our study (41.15 MPa) was similar to the value reported in previous studies using a similar test[30,31] and the failure mode was mostly cohesive in the composite resin. The μTBS value in the presence of RF alone ($p < 0.001$) or in combination with the primer ($p < 0.001$) was lower than that in the control

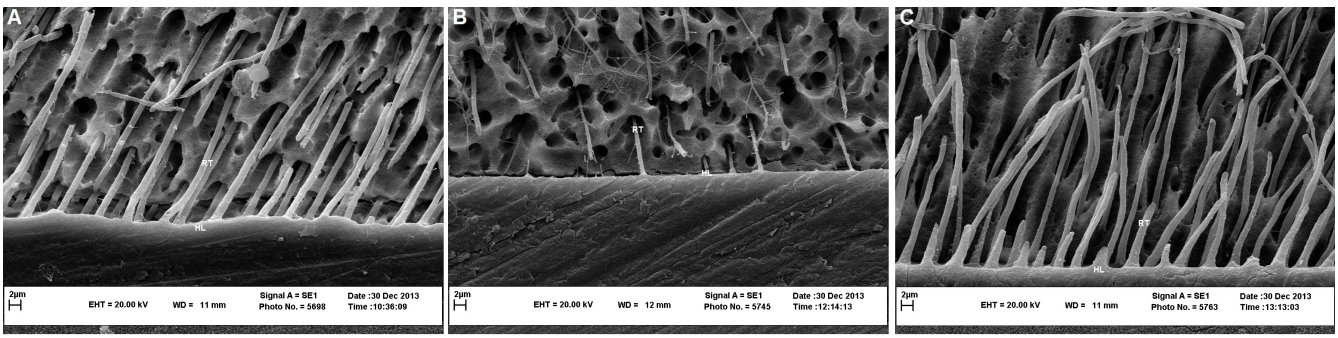


Fig. 1. Results of SDS-PAGE analysis. The arrows indicate high-molecular-weight residuals on top of the stacking gel

Table 1. Mean value (*SD*) of bond strength (in MPa) and failure modes of μTBS test

| Group | Surface pretreatment | Mean of microtensile bond strength | Failure mode (A/M/CR/CD) |
|---|---|---|---|
| Control | no pretreatment | 41.15 (3.50)a | 0/5/7/2 |
| RF/BL | application and photoactivation of RF% 0.1 (pH = 3) | 20.79 (4.41)b | 2/12/0/0 |
| RF-P/BL | application and photoactivation of riboflavin–primer mixture | 19.84 (3.80)b | 2/12/0/0 |

*SD* – standard deviation; RF – riboflavin; RF-P – riboflavin–primer mixture; BL – blue light; A – adhesive; M – mixed; CR – cohesive in resin composite; CD – cohesive in dentin. Values with different uppercase letters indicate significant differences according to Tukey's test ($p < 0.05$).

group; therefore, the null hypothesis of the study was upheld. No significant difference was found in the μTBS reduction in the understudy specimens between the 2 modes of application for the cross-linkers (p = 0.598). The majority of failures observed in these 2 groups were mixed.

One advantage of inactivating proteolytic enzymes in a dentin matrix by a cross-linking agent is its non-specific mechanism, since it cross-links all the MMPs present in dentin and collagen fibers. These cross-links are composed of covalent bonds stabilized over time.[9] Riboflavin is a non-toxic vitamin used as a food coloring agent.[9] Moreover, its light yellow color makes it more suitable for dental purposes than other photosensitizing chromophores, such as porphyrins, rose bengal and methylene blue.[21]

In contrast to our findings, some studies have reported that dentin surface pretreatment with UVA-activated RF increased μTBS immediately and after storage in comparison with a control group.[9,16,21,32] The difference between our results and those of previous studies may be attributed to the duration of exposure and the source of light used for activation, as well as the storage conditions.

As with our study, some previous investigations have reported the inefficacy of RF or even its adverse effects on bond strength.[21,26] Fawzy et al. reported that using 1% RF and single-step photoactivation with BL simultaneously with the bonding produced lower μTBS after 24 h and after 4 months of water storage compared to the control group.[26] Cova et al. reported that when comparing immediate and delayed bond strength, all specimens cross-linked with RF still demonstrated some level of reduction in the mean μTBS after 12 months.[9] It should be noted that all of these studies were conducted on E&R adhesives and did not evaluate SE adhesives.

A reduction in bond strength when RF is used may be due to the strengthening effect of hydration by RF/UVA.[33] The increased hydration of activated RF may be unfavorable to the formation of a stable hybrid layer.[21] Riboflavin can compromise the function of the adhesive system by increasing water sorption from dentin, diluting the primer and maintaining the water content of the primer.

A residual weak collagen matrix within the hybrid layer may be another possible reason for the reduced μTBS in specimens treated with RF after thermocycling and water storage found in our study. As with non-specific inhibitors, the main drawback of cross-linkers used to inactivate MMPs is that they leave a water-rich, resin-deficient collagen matrix with poor mechanical properties within the hybrid layer.[2,34] These very fragile collagen fibrils are susceptible to creep and subsequent fatigue rupture after long-term functioning.[17]

It should be noted that in this study, in contrast to the E&R systems used in previous studies, a SE adhesive system was used. In the process of SE adhesive system application, the smear layer containing proteins, collagen and debris is not eliminated from the dentin surface by the application of acidic monomers. Thus, applying activated RF can cross-link the residual proteins and collagens in the smear layer and cover the dentin surface during the application of acidic monomers of the SE adhesive, obstructing the path of the resin, hindering its penetration into the spaces between collagen fibers. The observation of a partially or deficiently formed hybrid layer and sparse fine resin tags on the SEM imagery from the RF/BL group can confirm this theory. Collagen fibrils which are not fully covered by the resin monomers during the bonding process are susceptible to mechanical fatigue as well as hydrolytic and enzymatic degradation by collagenolytic enzymes.[35]

In the RF-P/BL group, a hybrid layer was formed and the length and distribution of resin tags was less uniform than in the control group. However, due to the simultaneous use of RF and primer, the inhibition of resin penetration was not observed in the respective SEM imagery. In this group, RF was incorporated into the Clearfil SE Bond primer until a 0.1% concentration was reached, and after RF-P was applied to the dentin surface, light activation was carried out for 2 min. It has been reported that treating dentin powder with Clearfil SE Bond primer for 2 min instead of 20 s not only increases collagenolytic activity, but also disables enzymatic inhibition to the point where only 2% chlorhexidine (CHX) can still demonstrate a significant inhibitory effect.[36] Therefore, the reduction in mean μTBS observed in RF-P/BL group may be due to the long duration of dentin treatment with Clearfil SE Bond primer before the application of the bonding agent or due to the low concentration of RF used. Further studies are required to assess the surface micromorphology of treated dentin and to find the best concentration of RF in the SE primer.

The likely negative effect of directly incorporating RF into an SE adhesive primer on the degree of conversion and the mechanical characteristics of a polymerized resin may be another reason for decreased bond strength. This process negatively affects the mechanical properties of polymerized resins. For instance, it has been reported that the addition of 1% CHX to various resin mixtures with different hydrophilicities leads to a reduction in the modulus of elasticity (MOE) of polymerized resins by 27–48%.[37] When directly incorporating RF into the primer composition, the possibility that it may react with the primer components and interfere with its optimal functioning cannot be ignored. However, further tests and chemical analyses are required in order to suggest this method with confidence.

In the present study, the bond strength of the RF/BL and RF-P/BL groups was similar. Despite the vastly different microscopic images, different potential mechanisms involved in reducing the bond strength in the 2 groups may explain this similarity. As discussed earlier, the obstruction of the path of resin penetration into the spaces between collagen fibers as a result of collagen cross-linking in the smear layer may be responsible, at least in part, for the reduction in bond strength in the RF/BL group; in the RF-P/BL group, however, the reduction in bond strength may be due to the long dura-

tion of dentin treatment with Clearfil SE Bond primer before applying the bonding agent, the low concentration of RF used or the likely negative impact of directly incorporating RF into the SE adhesive primer formulation.

## Conclusions

Within the limitations of this study, cross-linking of collagen type I fibers did occur as the result of the 0.1 wt% aqueous solution of RF and the QTH light activation for 2 min. Pretreatment of the dentin surface with QTH light-activated RF decreased the μTBS of the Clearfil SE Bond as a SE adhesive system. No difference was detected between the application of 0.1 wt% RF alone to the dentin surface and the incorporation of 0.1% wt RF into the primer of the two-step SE adhesive system.

### ORCID iDs

Shahin Kasraei https://orcid.org/0000-0003-0167-4704
Maryam Mojtahedi https://orcid.org/0000-0002-7885-1847
Mohammad-Taghi Goodarzi https://orcid.org/0000-0002-5546-5812
Mohadese Azarsina https://orcid.org/0000-0001-6190-7472
Zahra Khamverdi https://orcid.org/0000-0001-9623-5573

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# Evaluation of the apical extrusion of sodium hypochlorite gel in immature permanent teeth: An in vitro study

# Ocena przepchnięcia wierzchołkowego żelu z podchlorynem sodu w niedojrzałych zębach stałych – badanie in vitro

Salma Fuad Al Nesser[B–D], Nada George Bshara[A,E,F]

Department of Pediatric Dentistry, Faculty of Dentistry, Damascus University, Syria

A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation;
D – writing the article; E – critical revision of the article; F – final approval of the article

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**Address for correspondence**
Salma Fuad Al Nesser
E-mail: salma.alnesser93@gmail.com

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## Abstract

**Background.** Sodium hypochlorite (NaOCl) gel has been suggested as a safer substitute in open apices as compared to solution, with the same antimicrobial effect.

**Objectives.** This study aimed to compare the amount of the apical extrusion of NaOCl gel and solution in immature permanent teeth.

**Material and methods.** A crossover in vitro study was conducted at the Department of Pediatric Dentistry, Faculty of Dentistry of Damascus University, Syria. Thirty freshly extracted immature single-rooted human premolars were decoronated and the cavity was then accessed. The teeth were radiographed to determine the mesiodistal dimension of the apex. In addition, the surface area of the apical foramen was calculated with Adobe Photoshop® to evaluate the amount of extrusion from the whole surface of the apex. The teeth were divided into 2 groups according to the size of the apex: ≤2.5 mm (group A) and >2.5 mm (group B); each group was irrigated with 5 mL of NaOCl solution and 2 different commercial types of NaOCl gel for 60 s, and then the extruded irrigant was measured in a plastic vial.

**Results.** The data was analyzed using the Kruskal–Wallis analysis. Based on the observed results, a statistically significant difference was noted ($p = 0$) between NaOCl solution and gel when the apical diameter was ≤2.5 mm, while there was no significant difference between the 2 types of NaOCl gel. No statistically significant difference was observed ($p = 0.2$) between NaOCl solution and gel when the apical diameter was >2.5 mm.

**Conclusions.** Sodium hypochlorite gel is safer than solution when irrigating immature teeth with the apical diameter ≤2.5 mm.

**Key words:** sodium hypochlorite, apical extrusion, immature teeth

**Słowa kluczowe:** podchloryn sodu, przepchnięcie wierzchołkowe, niedojrzałe zęby

# Introduction

Sodium hypochlorite (NaOCl) is the most commonly used irrigant in endodontic treatment[1]; it has been considered the main endodontic irrigant due to its antimicrobial effect and its ability to dissolve the soft tissue and pulpal residuals in the root canal.[2,3] However, the cytotoxic activity of NaOCl may cause acute injuries to the periapical area when the compound is extruded beyond the apex, causing hemolysis, ulcerations and the destruction of endothelial and fibroblast cells, resulting in emphysema, trismus and sensory-motor defects.[4] About 42% of endodontic practitioners described at least 1 occurrence of NaOCl extrusion beyond the apex during their career.[5]

The amount of the extruded irrigant might be related to applying high pressure on the syringes, wedging the needle and the large size of the apex, which is observed more frequently in immature teeth.[6]

Endodontic treatment of immature permanent teeth with necrotic pulp or apical pathosis has always been a challenge, since mechanical instrumentation might result in further weakening of the dentinal walls of the canal, making it more difficult to obtain an apical seal.[7]

Currently, there is a shift in treating these teeth toward revascularization, which would allow root development to continue, and according to many case reports, this procedure has proved its success.[8,9] The success is dependent on 3 factors, which are stem cells, growth factors and scaffolds.[10]

Disinfecting the canal is considered an important factor in this type of treatment.[11]

It has been found that the apical extrusion of NaOCl can harm the stem cells, affecting the success of this procedure.[12] Hence, the aim of this study was to compare the extrusion of NaOCl solution and gel.

To the best of our knowledge, this is the first study to evaluate and compare the amount of extrusion between NaOCl solution and gel in immature permanent teeth.

# Material and methods

A crossover in vitro study was conducted at the Department of Pediatric Dentistry, Faculty of Dentistry of Damascus University, Syria. The approval of the Scientific Research Committee was obtained before the initiation of the study. The sample size was calculated according to a prior pilot study using the G*Power software (v. 3.1) (Heinrich-Heine-Universität Düsseldorf, Germany; http://www.gpower.hhu.de/), and 30 single-rooted immature human premolars, freshly extracted due to orthodontic treatment, were used as the total specimen.

The criteria for tooth selection were as follows: lack of internal or external resorption as well as of visible caries, fractures or cracks under a stereoscopic microscope (Meiji Techno Co., Ltd., Saitama, Japan) at ×2 magnification.

The teeth were cleaned with the CK-6 hand scaler instrument (Zeffiro–Lascod, Florence, Italy) to remove the soft tissue residual and were stored in plastic containers with 0.5% chloramine T for 1 week, and then moved to other containers filled with 0.9% saline and stored in a refrigerator at the temperature of 4°C until used.

## Preparation of specimens

Conventional access cavity preparation was done using a 2-millimeter round bur and the roof of the pulp was removed with the Endo-Z® bur (Dentsply, Ballaigues, Switzerland). Then, the pulp was removed using barbed broaches (VDW GmbH, Munich, Germany).

The teeth were decoronated to standardize the tooth length to 15 mm and the measurements were done using a digital caliper.

The teeth were covered with 2 layers of nail polish to seal the roots.

## Determining the dimensions of the apical foramen

The surface area of the apices was measured using Adobe Photoshop® CC 2013 (Adobe Systems, Inc., San Jose, USA; www.adobe.com) by capturing the apex with a digital camera (Samsung NX500; Samsung, San Jose, USA) under a stereomicroscope (Meiji Techno Co., Ltd.) at ×2 magnification. An endodontic ruler was set beside the foramen to calibrate the pixels by measuring the logical length of each 1 mm in pixels (Fig. 1). The borders of the apical foramen were selected with a pen tool and the surface area in $mm^2$ was calculated from the measurement log after setting the measurement scale of the length in pixels according to the logical length of 1 mm (Fig. 2).

The teeth were inserted in a putty condensation silicone (ZetaPlus®; Zhermack GmbH, Marl, Germany) (Fig. 3) and radiographed in mesiodistal and buccolingual directions (imaging systems by Vatech Co., Ltd., Hwaseong,

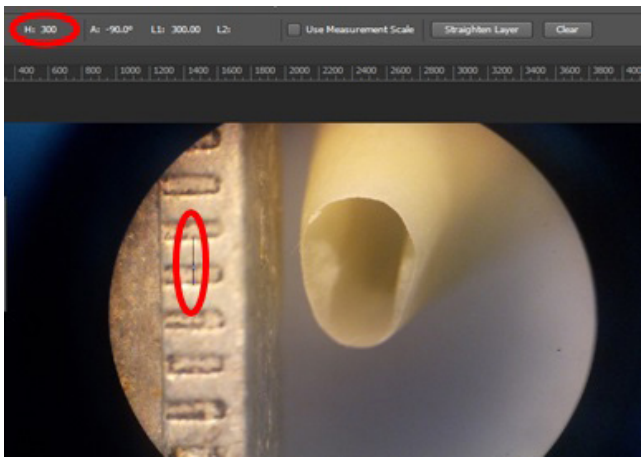


Fig. 1. Logical length of 1 mm = 300 pixels

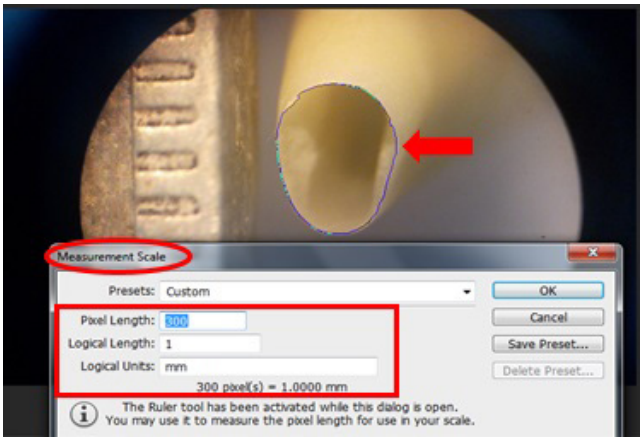


Fig. 2. Selecting the borders of the apical foramen and setting the logical length of each 1 mm to 300 pixels

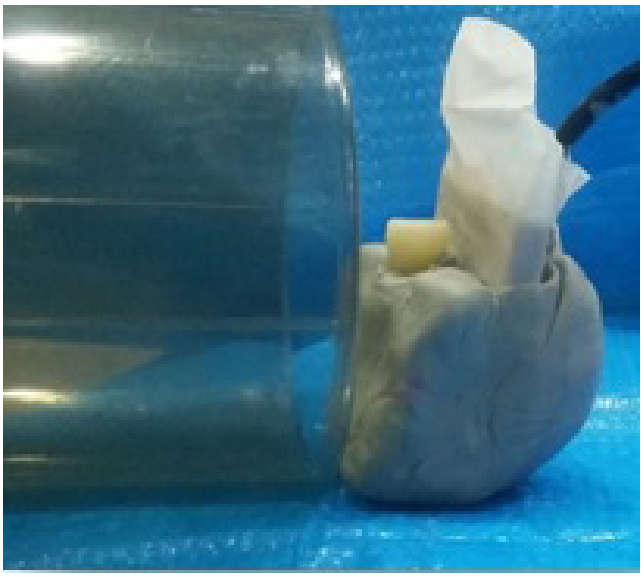

Fig. 3. Securing teeth in putty silicone before radiography

South Korea, and de Götzen® S.r.l., Olgiate Olona, Italy) to ensure that the canals are straight (<5°) according to Schneider[13] and to determine the mesiodistal dimension of the apices in order to make clinical simulation.

## Study groups and irrigation protocol

The teeth were then divided into 2 groups:

– group A: teeth with radiographically confirmed apical diameter ≤2.5 mm (surface area ≤6.9 mm$^2$);
– group B: teeth with radiographically confirmed apical diameter >2.5 mm (surface area >6.9 mm$^2$).

The teeth in each group were irrigated with 5 mL of 3 different irrigants:

– NaOCl solution 5.25% (Carmel®; Akka Brothers Co. Carmel Detergent, Damascus, Syria);
– NaOCl gel 2.25% (Harpic®; Reckitt Benckiser, PLC, Slough, UK);
– NaOCl gel 2.25% (WC Net Bleach®; Bolton Manitoba, Milan, Italy).

After each irrigation, the teeth were washed with 5 mL of saline and dried using paper points.

The irrigation was done with a 27-gauge side vent needle (Endo-Top; CERKAMED, Stalowa Wola, Poland), placed at 3 mm of the working length by adding a rubber stopper to the needle.

The irrigation protocol was employed according to the Institutional Review Board of the Oregon Health & Science University (Portland, USA) as the final irrigation; the flow rate was 5 mL/60 s with 30 s of irrigation and 60 s of waiting,[14] a vertical movement with the needle was done 1–2 mm away from the apex every 6 s.

## Collecting the extruded irrigants

The Myers and Montgomery model was used in this study (Fig. 4).[15] A hole was made in the center of a plastic lid, and the teeth were inserted up to the level of the cementoenamel junction and fixed to the vial with a composite (Tetric N-Ceram®; Ivoclar Vivadent, Zurich, Switzerland). A 22-gauge needle was bent and inserted into the lid to equalize the air pressure inside and outside the plastic vial.

An empty plastic container was weighed using a 0.01-gram balance weight. Then, 5 mL of each irrigant was placed in the weighed container and the weight of the irrigant was calculated by subtracting the weight of the empty plastic container. The irrigant extruded after the irrigation was measured in the same way as previously described. The container was replaced with a new one after each irrigation.

The weights [g] of the extruded irrigants were transformed to volumes [mL] using the following equation:

$$\text{volume of extruded irrigant} = \frac{\text{weight of extruded irrigant} \times 5}{\text{weight of 5 mL of irrigant}}$$

Fig. 4. The Myers and Montgomery model

## Test of viscosity

Kinematic viscosity was measured by determining the time it took each irrigant to flow in a glass capillary U-tube (CANNON-Fenske® and CANNON-Ubbelohde®; CANNON Instrument Company, State College, USA) inserted into a room temperature viscometer bath (JP SELECTA, Abrera, Barcelona, Spain), and multiplying this time by the calibration constant of the specific tube.

## Statistical analysis

The normality of the data was checked using the Kolmogorov–Smirnov test, and the Kruskal–Wallis test was used to determine if there was a statistically significant difference in the volume of the apically extruded irrigants between group A and group B.

In this study, the level of significance ($p$-value) was set at 0.05 and the statistical analysis was performed using the IBM SPSS software v. 23 (IBM Corp., Armonk, USA). Descriptive statistics, including minimum and maximum, means, and standard deviations (*SD*) were also calculated.

# Results

The minimum and maximum, mean, and *SD* results of NaOCl viscosity are shown in Table 1. Sodium hypochlorite gel Harpic has shown the highest viscosity, followed by NaOCl gel WC Net Bleach and NaOCl solution (Carmel).

The extrusion volumes were statistically significantly different between the 3 types of irrigants in group A (apex ≤2.5 mm) ($p$ = 0.000) (Table 2). Sodium hypochlorite solution (Carmel) had the highest amount of extrusion, followed by NaOCl gel WC Net Bleach, and the least amount was noted for NaOCl gel Harpic.

**Table 1.** Descriptive results of the viscosity test [cSt]

| Type of irrigant | Min | Max | Mean ±*SD* |
|---|---|---|---|
| NaOCl solution Carmel | 0.56 | 0.57 | 0.563 ±0.004 |
| NaOCl gel Harpic | 225 | 227.5 | 226.666 ±1.29 |
| NaOCl gel WC Net Bleach | 177.5 | 197.5 | 190 ±9.746 |

*SD* – standard deviation.

The pairwise comparison test showed that NaOCl solution (Carmel) had a significantly higher amount of extrusion than NaOCl gel WC Net Bleach ($p$ = 0.000) and Harpic ($p$ = 0.000), and it also revealed that there was no statistically significant difference between NaOCl gel WC Net Bleach and Harpic ($p$ = 0.813) (Table 3).

The extrusion volumes were not statistically significantly different between the 3 types of irrigants in group B (apex >2.5 mm) ($p$ = 0.214) (Table 2).

**Table 3.** Pairwise comparison between the 3 types of irrigants in group A [mL]

| Comparison | Difference of means | *SE* | $p$-value |
|---|---|---|---|
| NaOCl gel Harpic vs NaOCl gel WC Net Bleach | −1.133 | 4.794 | 0.813 |
| NaOCl solution Carmel vs NaOCl gel Harpic | 19.667 | 4.794 | 0.000 |
| NaOCl solution Carmel vs NaOCl gel WC Net Bleach | 18.533 | 4.794 | 0.000 |

*SE* – standard error.

# Discussion

The purpose of this study was to compare the amount of the extrusion of NaOCl solution and gel. Many studies have compared the amount of the extrusion of NaOCl solution with different irrigating systems. However, the studies have been limited to mature permanent teeth and few studies have modified mature teeth to resemble immature teeth.[16,17]

In this study, real freshly extracted immature premolars with different apical diameters were used to simulate the actual clinical situation.

Sodium hypochlorite gel was chosen, because no studies have evaluated the amount of its extrusion beyond the apex. Also, research showed its effectiveness as an intracanal irrigant; Al-Sudani and Al Omar proved its efficacy in removing the smear layer at 2.5% concentration compared to NaOCl solution at the same concentration.[18] This result was consistent with the study done by Zand et al., who observed no significant difference between 2.5% NaOCl gel and solution in smear layer removal.[19] Furthermore, Nejad Shamsi et al. reported the same effect of NaOCl gel on the growth of *E. faecalis* as in the case of NaOCl solution at the same concentration, thus considering it safe as an intracanal irrigant.[20]

**Table 2.** Descriptive results of the Kruskal–Wallis test regarding the amount of extrusion [mL]

| Group | Type of irrigant | Min | Max | Mean ±*SD* | $p$-value |
|---|---|---|---|---|---|
| A | NaOCl solution Carmel | 4.73 | 4.99 | 4.82 ±0.05 | 0.000* |
| | NaOCl gel Harpic | 0.86 | 4.99 | 4.02 ±1.07 | |
| | NaOCl gel WC Net Bleach | 1.31 | 4.86 | 4.15 ±0.89 | |
| B | NaOCl solution Carmel | 4.72 | 4.99 | 4.83 ±0.07 | 0.214 |
| | NaOCl gel Harpic | 3.06 | 4.99 | 4.57 ±0.48 | |
| | NaOCl gel WC Net Bleach | 3.81 | 4.93 | 4.71 ±0.31 | |

group A: apical diameter ≤2.5 mm; group B: apical diameter >2.5 mm; * statistically significant.

In this study, 2 different types of commercial NaOCl gel were selected, as the manufacturers always keep the additional materials added secret.

Nail varnish was used to seal the root and prevent the leakage from the lateral root canals.

Arora and Tewari found that the apical foramen would have an oval, triangular, kidney, or irregular forms.[21] For this reason, the surface area was calculated using Adobe Photoshop to evaluate the amount of apical extrusion from the whole surface of the apical foramen, regardless of its pattern and shape, and then the mesiodistal diameter was measured radiographically to obtain the results similar to the clinical situation.

A side vent needle was chosen, as it can move the irrigant sideways and reduce the extrusion.[22]

Despite the difference in viscosity between NaOCl gel and solution, the pressure applied on the plunger of the syringe would also be different; therefore, the flow rate was standardized to be 5 mL/60 s in all the specimens. Up-and-down movements were done every 6 s to evoke agitation.[22]

This study showed significantly less extrusion of NaOCl gel compared to solution in group A, where the apical diameter was less than 2.5 mm; this reduction took place despite the higher pressure applied on the plunger of the syringe when irrigating with the gel form as compared to solution at the same flow rate.

Although the results of this study showed that the mean value of apical extrusion of NaOCl gel in group A was high (4 mL), and this could be referred to the extremely immature teeth used in this study, it is thought that NaOCl gel could dissolve soft tissues to a lesser extent than solution.

The results also showed no statistically significant difference between the 2 types of NaOCl gel. These results were consistent with the results of the viscosity test, as the viscosity of NaOCl gel was 226.666 cSt for Harpic and 190 cSTt for WC Net Bleach, while the least viscosity was observed in the case of NaOCl solution (0.563 cSt).

Moreover, group B showed no statistically significant difference between NaOCl gel and solution, which indicates that all the irrigants would extrude apically when the dimensions are large enough.

# Conclusions

Sodium hypochlorite solution cannot be used as an intracanal irrigant in immature teeth because of the high risk of apical extrusion.

## ORCID iDs

Salma Fuad Al Nesser https://orcid.org/0000-0002-9492-3599
Nada George Bshara https://orcid.org/0000-0003-0055-6204

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# Comparative evaluation of the efficacy of three methods of delivering calcium hydroxide into the root canal

# Ocena porównawcza skuteczności trzech metod wprowadzania wodorotlenku wapnia do kanału korzeniowego

Roohollah Sharifi[1,A,C–F], Ehsan Bahrampour[2,A–C], Pourya Janfroozzade[3,A,B], Mohsen Safaei[4,B–F], Hamid Reza Mozaffari[5,A,D–F], Elham Soltanimehr[6,C–F], Hedaiat Moradpoor[7,A,E,F], Mohammad Moslem Imani[8,C–F]

[1] Department of Endodontics, School of Dentistry, Kermanshah University of Medical Sciences, Iran
[2] Department of Oral and Maxillofacial Radiology, School of Dentistry, Kermanshah University of Medical Sciences, Iran
[3] Student Research Committee, School of Dentistry, Kermanshah University of Medical Sciences, Iran
[4] Oral and Dental Sciences Research Laboratory, School of Dentistry, Kermanshah University of Medical Sciences, Iran
[5] Department of Oral and Maxillofacial Medicine, School of Dentistry, Kermanshah University of Medical Sciences, Iran
[6] Department of Pediatric Dentistry, School of Dentistry, Kermanshah University of Medical Sciences, Iran
[7] Department of Prosthodontics, School of Dentistry, Kermanshah University of Medical Sciences, Iran
[8] Department of Orthodontics, School of Dentistry, Kermanshah University of Medical Sciences, Iran

A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation;
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**Address for correspondence**
Ehsan Bahrampour
E-mail: e.bahrampour@gmail.com

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## Abstract

**Background.** Calcium hydroxide, due to its favorable properties, such as an antimicrobial effect as well as its ability to dissolve necrotic tissues, inhibit tooth resorption and stimulate the formation of a hard tissue barrier, is widely used in root canal treatment.

**Objectives.** The objective of this study was to compare the efficacy of 3 methods of delivery of calcium hydroxide into the canals.

**Material and methods.** This in vitro experimental study was performed on acrylic resin blocks with simulated curved canals (curvature of 24° and 44°). Calcium hydroxide was delivered into the canals with 3 different instruments : a hand file, a Lentulo and a rotary file. The data was analyzed using the three-way analysis of variance (ANOVA) and Tukey's test.

**Results.** The density of calcium hydroxide was significantly different among the 3 groups ($p < 0.001$). The manual delivery technique resulted in the lowest and using a rotary file in the highest density of calcium hydroxide ($p < 0.001$). The mean density of calcium hydroxide was significantly higher in the canals with curvature of 24°, irrespective of the delivery method ($p < 0.001$). The lowest mean density of calcium hydroxide was noted at 1 mm and 3 mm from the apex, whereas the highest mean density was noted at 11 mm from the apex, irrespective of delivery method ($p < 0.001$).

**Conclusions.** A rotary file seems to be the most efficient instrument for delivering calcium hydroxide into the canals, especially into the apical region of canals with greater curvature.

**Key words:** calcium hydroxide, hand file, rotary file

**Słowa kluczowe:** wodotlenek wapnia, pilnik ręczny, pilnik obrotowy

## Introduction

During endodontic treatment, the infected tissue is often removed using canal irrigation with disinfecting solutions and biomechanical cleaning. After the infected and necrotic tissue has been eliminated, the root canal should be disinfected, instrumented and shaped.[1] Despite efforts to completely clean and disinfect the root canal system, some microorganisms may still remain lodged in the dentinal tubules. If intracanal medicaments are not used between treatment sessions, the residual microorganisms may proliferate and soon reach their baseline count.[2]

Calcium hydroxide is extensively used as an intracanal medicament. It has many of the properties of an ideal medicament, such as the ability to physically seal the canal and prevent canal reinfection by inhibiting the proliferation of microorganisms.[3]

It also has optimal biological properties such as the ability to dissolve necrotic tissues, inhibit tooth resorption and stimulate the formation of a hard tissue barrier.[3] It has an analgesic effect and can control inflammation. It shows antimicrobial activity against the majority of endodontic pathogens, as it changes the bacterial cell wall and destructs the molecular structure of endotoxins.[4] The antimicrobial activity of calcium hydroxide is attributed to its high pH (12.5). Thus, the efficacy of calcium hydroxide depends on its dissolution and release of hydroxyl ions ($OH^-$), which can penetrate into the surrounding tissues.[5]

Calcium hydroxide should be applied in sufficient amounts into the root canal in order to be able to provide adequate concentration of calcium and hydroxyl ions to exert their biological effects.[6] Calcium hydroxide kills the microorganisms by direct contact. Thus, in order to achieve maximum biological effects, calcium hydroxide should be applied and spread uniformly and with the highest density along the canal working length.[7,8]

Uniform and high-density (condensed) filling of the root canals with calcium hydroxide to the working length depends on the technique of delivery of calcium hydroxide into the canal. Calcium hydroxide can be delivered into the canal with different instruments (e.g., a syringe, a Lentulo, hand files, rotary files, spreaders, and pluggers). To date, few studies have assessed the effect the technique of delivering calcium hydroxide into the root canal system has on its density.[9–11] Thus, this study aimed to compare the density of calcium hydroxide in simulated root canals in resin blocks following its delivery with a hand file, a Lentulo and a rotary file.

## Material and methods

This in vitro experimental study was performed on simulated root canals with curvature of 24° and 44° in resin blocks. The sample size was calculated to be 10 samples in each group according to a study by Simcock and Hicks.[7] To further increase the accuracy of results, 15 samples were evaluated in each group. A total of 45 canals with curvature of 24° and 45 canals with curvature of 44° were evaluated.

Acrylic resin blocks with simulated root canals were used. The size of root canals was 30 and they had curvature of 24° and 44°. The acrylic blocks were masked by a cover in order for the operating clinician to be blinded to the canal content.

A K-file size 10 (Mani Inc., Tochigi, Japan) exceeded the working length by 1 mm to ensure absence of canal obstruction. The canal lengths were equal (13 mm). The acrylic blocks underwent digital radiography perpendicular to the surface of the canal curvature, whereas the X-ray tube was within a distance of 10 cm from the block and at an angle of 90° relative to the sensor.

Calcium hydroxide paste (Golchai Co., Tehran, Iran) was prepared with distilled water at 44 wt% One single clinician applied calcium hydroxide into the canals in all 3 groups. He was blinded to the objectives of the study. The 3 groups were as follows:

– the hand file group – the canals were filled with calcium hydroxide paste using a hand K-file size 25 (Mani Inc.) to the working length counterclockwise;
– the Lentulo group – the canals were filled with calcium hydroxide using a Lentulo size 25 (Dentsply Maillefer US, Tulsa, USA) up to 1–2 mm to the working length with an air motor handpiece (NSK Ltd., Tokyo, Japan) at a speed of 500 rpm;
– the rotary file group – the canals were filled with calcium hydroxide using an Mtwo® rotary file size 25 (VDW, Munich, Germany) to the working length with a limited-torque electric motor (Endo E Class; Saeyang Microtech Co. Ltd., Daegu, Korea) counterclockwise at a constant 150 rpm.

The extrusion of calcium hydroxide from the canal orifice in the acrylic block indicated that the canal was filled.[7] After calcium hydroxide had been applied into the canals in each group, excess paste was removed from the canal orifice and the orifice was sealed using the intermediate restorative material (IRM) (Dentsply Caulk, York, USA).

Digital radiographs were obtained for each canal perpendicular to the canal curvature before and after root canal filling with calcium hydroxide under the same exposure settings (70 kV, 6 mA, 0.20 s). The X-ray tube was within a distance of 10 cm from the sample and at an angle of 90° relative to the sensor. The sensor was held with a cardboard positioning device and each tooth was taped to the sensor to ensure that all radiographs were taken from the same position. Fifteen radiographs were obtained for the canals with curvature of 44° in each group (a total of 45 radiographs) and 15 radiographs were obtained for the canals with curvature of 24° in each group (a total of 45 radiographs).

An expert oral and maxillofacial radiologist analyzed the radiographs using the DIGORA™ software (Soredex, Helsinki, Finland) twice with a 1-week interval. To assess the intra-examiner reliability, the intraclass correlation coefficient (ICC) was calculated (0.93).

The radiologist was not aware of the group allocation of the samples. To analyze the radiographs, first the calibration was performed using a 6-step aluminum step wedge, and then horizontal lines at a distance of 1 mm, 3 mm, 7 mm, and 11 mm from the apex were drawn on each radiograph using the ruler of the software. Using the densitometry feature, a perpendicular line was drawn at the center of the canal and density was recorded at the intersection points of this line and the previously drawn horizontal lines. The density values are between 0 and 256 shades of grey.

The data was analyzed with the PASW Statistics for Windows, v. 18.0 (SPSS Inc., Chicago, USA) using descriptive and inferential statistics. The normal distribution of data was evaluated using the Kolmogorov–Smirnov test. Since the density data was normally distributed ($p > 0.05$), the comparisons were made using the three-way analysis of variance (ANOVA). Tukey's post-hoc test was applied for pairwise comparisons. A value of $p < 0.05$ was considered statistically significant.

## Results

Table 1 shows the mean density of calcium hydroxide at different levels from the apex according to the canal curvature in the 3 groups. According to the three-way ANOVA, there was a significant difference in the mean density of calcium hydroxide among the 3 groups ($p < 0.001$). The manual technique group showed the lowest and the rotary file group the highest mean density of calcium hydroxide in the canals (Table 2).

The three-way ANOVA also showed a significant difference in density of calcium hydroxide between the canals with curvature of 24° and 44° ($p < 0.001$). The mean density of calcium hydroxide was higher in the canals with curvature of 24° than in the case of the canals with curvature of 44° (Table 3). A significant difference was revealed in the density of calcium hydroxide at different distances from the apex ($p < 0.001$) – the lowest mean density was noted at 1 mm and 3 mm from the apex, whereas the highest mean was noted at 11 mm from the apex (Table 4).

**Table 1.** Mean density of calcium hydroxide at different distances from the apex according to the canal curvature in the 3 groups

| Distance | Canal curvature | Hand file | Lentulo | Rotary file |
|---|---|---|---|---|
| 1 mm | 24° | 63.27 ±10.54 | 74.53 ±32.64 | 101.80 ±21.57 |
| | 44° | 43.67 ±20.60 | 48.87 ±21.99 | 92.80 ±23.83 |
| 3 mm | 24° | 65.13 ±7.45 | 76.33 ±32.57 | 100.27 ±19.62 |
| | 44° | 41.60 ±12.00 | 50.53 ±23.16 | 95.73 ±22.86 |
| 7 mm | 24° | 70.73 ±10.50 | 81.93 ±27.40 | 101.93 ±17.54 |
| | 44° | 56.40 ±14.24 | 57.73 ±22.04 | 100.80 ±23.79 |
| 11 mm | 24° | 71.13 ±14.98 | 93.33 ±28.59 | 103.40 ±18.23 |
| | 44° | 73.40 ±15.69 | 67.47 ±23.07 | 101.40 ±28.10 |

Data presented as mean ± standard deviation (*SD*).

**Table 2.** Mean density of calcium hydroxide in the canals in the 3 methods

| Method | Mean | *SD* | Minimum | Maximum |
|---|---|---|---|---|
| Hand file | 60.67[a] | 17.71 | 23.00 | 111.00 |
| Lentulo | 68.84[b] | 29.85 | 22.00 | 136.00 |
| Rotary file | 99.77[c] | 21.78 | 53.00 | 170.00 |

Means sharing the same superscript letter are not significantly different ($p > 0.05$).

**Table 3.** Mean density of calcium hydroxide in the canals with curvature of 24° and 44°

| Canal curvature | Mean | *SD* | Minimum | Maximum |
|---|---|---|---|---|
| 24° | 83.65[b] | 25.81 | 26.00 | 143.00 |
| 44° | 69.20[a] | 30.25 | 22.00 | 170.00 |

Means sharing the same superscript letter are not significantly different ($p > 0.05$).

**Table 4.** Mean density of calcium hydroxide at different distances from the apex

| Distance | Mean | *SD* | Minimum | Maximum |
|---|---|---|---|---|
| 1 mm | 70.82[a] | 30.84 | 22.00 | 143.00 |
| 3 mm | 71.60[a] | 29.99 | 22.00 | 148.00 |
| 7 mm | 78.26[ab] | 26.92 | 22.00 | 157.00 |
| 11 mm | 85.02[b] | 26.15 | 31.00 | 170.00 |

Means sharing the same superscript letter are not significantly different ($p > 0.05$).

## Discussion

This study compared the efficacy of 3 methods of delivering calcium hydroxide into the canal, namely the manual technique, a Lentulo and a rotary file. The results showed that the density of calcium hydroxide delivered into the canals was affected by the technique of delivery and the canal curvature.

In this study, simulated root canals in transparent resin blocks were used to assess the effect of the canal curvature on the density of calcium hydroxide. Some studies have also considered the role of the canal curvature and standardized the samples in this respect.[7,9–11] However, to the best of the authors' knowledge, no previous study has assessed the effect of the canal curvature on calcium hydroxide delivery into different parts of the canal. Thus, we evaluated canals with curvature of 24° and 44° and the density of calcium hydroxide was evaluated at different distances from the apex to assess the effect of the canal curvature on this variable. Some previous studies simulated canals with curvature of 44° (sizes 40 and 50).[9,10] It has been suggested to decrease the size of a master apical file to 20 or 25 in the preparation of severely curved or S-shaped canals to prevent procedural errors.[12,13] Simcock and Hicks showed that in a canal prepared with a master apical file size 25 (as the minimum amount of canal preparation), only 45% of the optimal amount of calcium hydroxide is delivered, but in canals prepared with a master apical file size 40, calcium hydroxide is more efficiently delivered.[7]

Staehle et al. recommended the canal preparation to size 50 in order to enhance the density of calcium hydroxide.[14] Deveaux et al. suggested canal preparation to size 50 in straight canals or those with a slight curvature to enhance the density of calcium hydroxide.[10] We considered size 30 canals in this study, since one of our objectives was to assess the quality of delivery of calcium hydroxide in severely curved canals with curvature of 44°.

It should be noted that a Lentulo may break in the canal[9]; 1 such case of a Lentulo fracture occurred in our study. A Lentulo fracture has a higher frequency in severely curved canals. In the case of a Lentulo fracture, removing the broken piece from the canal is difficult. Thus, the use of plastic syringes is often preferred to a Lentulo for the delivery of a medicament into the canal.[8–10,12]

Several studies have shown that the technique of delivering calcium hydroxide into the canal affects its density in the root canal system. The current results showed a significant difference in the density of calcium hydroxide delivered into the canal with 3 different methods of a hand file, a Lentulo and a rotary file; this finding was in agreement with those of previous studies.[6,8,9,11,12]

The current findings regarding the lowest density of calcium hydroxide delivered into the canal using the manual technique (a hand file) were in accordance with the findings of previous studies.[7,9,10] The density of calcium hydroxide delivered with a Lentulo was higher compared to a hand file; this finding also confirmed the results of previous studies.[9,11] The current results also revealed that a rotary file was superior to a Lentulo in delivering calcium hydroxide into the canal. Simock and Hicks concluded that a Lentulo is not significantly different from a rotary file for the delivery of calcium hydroxide into minimally or optimally prepared canals.[7] However, they evaluated extracted teeth in their study and excluded curved canals, since the curvature is considered an influential factor in this respect. They estimated the density of calcium hydroxide by weighing its amount delivered into the canal. Our study showed that a Lentulo and a rotary file showed a similar performance in the coronal areas of the canals with curvature of 24° with regard to the density of calcium hydroxide.

The current findings showed that the density of calcium hydroxide delivered with a rotary file was the highest compared to the other 2 methods of delivery. Rotary instruments show a fast and uniform performance in the canal preparation. Moreover, rotary files can access the entire root canal length (especially in the apical region) due to the flexibility of NiTi files in curved canals. The NiTi rotary systems are more and more frequently used for the root canal preparation. They provide clean and smooth root canal walls and create a relatively round cross-sectional shape.[12] They can be used safely in curved canals as well.[15] Rotary files can extrude dentinal debris easily from the root canal when operating clockwise.[16] They can also deliver calcium hydroxide and sealers into the canal when operating counterclockwise.[11] Thus, rotary systems and a Lentulo can efficiently deliver calcium hydroxide with optimal density into the canals.

The current results regarding the density of calcium hydroxide delivered with 3 methods into the root canals with curvature of 24° and 44° revealed that the degree of curvature can affect the density of calcium hydroxide delivered into the canal using a hand file and a Lentulo. In the canals with curvature of 24°, the density of calcium hydroxide at 1 mm, 3 mm, 7 mm, and 11 mm from the apex was higher. However, in the case of a rotary file, the curvature had no effect on the density of calcium hydroxide. The findings also indicated that in delivering calcium hydroxide by means of a hand file, a Lentulo and a rotary file into the canal, the highest density was noted at 11 mm from the apex.

The current results confirmed that the canal curvature affects the density of calcium hydroxide delivered into the canal in such a way that the calcium hydroxide density in more severely curved canals (44°) was significantly decreased compared to canals with less curvature (24°). This finding should be taken into account by clinicians. Future studies are warranted to assess the time required for delivering calcium hydroxide into the canal by rotary files and its substantivity. Also, the efficacy of different rotary files with variable cross-sectional designs for the calcium hydroxide delivery into the canals of extracted human teeth should be compared in further studies.

## Conclusions

The current results revealed that the canal curvature affects the delivery of calcium hydroxide into the canal. A rotary file operating counterclockwise yielded the highest density of calcium hydroxide in more apical regions and in the canals with greater curvature compared to Lentulo and a hand file.

### ORCID iDs

Roohollah Sharifi https://orcid.org/0000-0001-7917-5409
Ehsan Bahrampour https://orcid.org/0000-0001-7467-5770
Pourya Janfroozzade https://orcid.org/0000-0000-0000-0001
Mohsen Safaei https://orcid.org/0000-0003-3885-6640
Hamid Reza Mozaffari https://orcid.org/0000-0001-9351-1499
Elham Soltanimehr https://orcid.org/0000-0001-6207-5057
Hedaiat Moradpoor https://orcid.org/0000-0002-9161-8038
Mohammad Moslem Imani https://orcid.org/0000-0002-3982-5216

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# Bond strength and solubility of a novel polydimethylsiloxane-gutta-percha calcium silicate-containing root canal sealer

# Wytrzymałość wiązania oraz rozpuszczalność nowego uszczelniacza kanałowego zawierającego polidimetylosiloksan-gutaperkę i krzemian wapnia

Mennatullah Mohammed Khalil[1,A,C–F], Mai Hisham Abdelrahman[2,A,E,F], Sara El-Mallah[3,A,B,F]

[1] Department of Dental Biomaterials, Faculty of Dentistry, Fayoum University, Egypt
[2] Department of Dental Biomaterials, Faculty of Dentistry, Modern Science and Arts University, Cairo, Egypt
[3] Department of Endodontics, Faculty of Dentistry, Fayoum University, Egypt

A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation;
D – writing the article; E – critical revision of the article; F – final approval of the article

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**Address for correspondence**
Mennatullah Mohammed Khalil
E-mail: mml11@fayoum.edu.eg

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## Abstract

**Background.** Endodontic sealers are essential for sealing gutta-percha to the dentin walls. They help to ensure that the canal remains free of microorganisms which might lead to infection. In order to perform their intended function, the sealers should properly adhere to the dentin walls and remain insoluble when set in the canal.

**Objectives.** The purpose of this study was to evaluate the bond strength and solubility of a novel polydimethylsiloxane-gutta-percha calcium silicate-containing root canal sealer (GuttaFlow® bioseal) and compare it with the zinc oxide and eugenol sealer (Zical®).

**Material and methods.** The endodontic sealers used in this study were GuttaFlow bioseal and Zical. The bond strength was assessed using push-out bond strength test in 3 root segments: coronal, middle and apical. The solubility was tested according to the American National Standards Institute / American Dental Association (ANSI/ADA) specification No. 57 at 3 different time intervals: 1, 7 and 14 days.

**Results.** The push-out bond strength in all root segments was significantly higher in Zical compared to GuttaFlow bioseal. The solubility was significantly higher on day 1 and 7 in Zical compared to GuttaFlow bioseal, and on day 14, the difference between them was not significant.

**Conclusions.** Within the limitations of this study, the endodontic sealer GuttaFlow bioseal showed low bond strength values compared to Zical. The solubility of the set GuttaFlow bioseal and Zical were both within the recommended ANSI/ADA levels.

**Key words:** solubility, GuttaFlow bioseal, polydimethylsiloxane-guttapercha sealer, calcium silicate-containing sealer, push-out bond strength

**Słowa kluczowe:** rozpuszczalność, GuttaFlow bioseal, gutaperka na bazie polidimetylosiloksanu, uszczelniacz na bazie krzemianu wapnia, wytrzymałość wiązania w teście wypychania

# Introduction

Endodontic treatment is a 3-step process consisting of proper cleaning, shaping and obturating the root canal.[1] The most common method of obturation consists in using gutta-percha combined with a sealer.[2] The main function of the sealer is to fill the spaces between the core material and the walls of the root canal in an attempt to form a coherent mass of the obturating material. Numerous sealers with different properties are used; one of the oldest sealers is the zinc oxide and eugenol sealer, which is popular among clinicians, despite its limitations.[3] GuttaFlow® bioseal (Coltène/Whaledent AG, Altstätten, Switzerland) is a novel polydimethylsiloxane-gutta-percha calcium silicate-containing root canal sealer that obturates the root canal and has tissue-repairing properties due to the presence of calcium silicate.[4,5]

The bond strength of the obturating material to the intraradicular dentin is an important property to be assessed when evaluating an endodontic sealer. The ability of a root canal sealer to adhere to the dentin is essential in maintaining the integrity of the sealer–dentin interface while undergoing mechanical stresses.[6–8] It may also be indicative of the ability to prevent bacterial microleakage, creating a favorable environment for periapical repair and avoiding reinfection.[9–11]

Solubility is another crucial property of sealers, because it may compromise the quality and success of the endodontic treatment. Low solubility is one of the requirements for endodontic sealers according to the American National Standards Institute / American Dental Association (ANSI/ADA) specification No. 57.[12]

The aim of this study was to assess the bond strength and solubility of a novel polydimethylsiloxane-gutta-percha calcium silicate-containing root canal sealer and compare it with the zinc oxide and eugenol sealer.

# Material and methods

Two root canal sealers, GuttaFlow bioseal and Zical® (Prevest DenPro Ltd., Jammu, India), were tested in this study. Table 1 shows the chemical composition of the tested sealers.

## Push-out bond strength test

A total of 14 single-rooted human teeth were used in this study. The inclusion criteria for the selected teeth were as follows: free from root decay, root fracture or any other root defect. The teeth were stored as anonymous specimens in 0.1% thymol solution and were used within 3 months of storage. The teeth were collected on October 6th from patients undergoing routine dental treatment at the Oral and Maxillofacial Department at Modern Science and Arts University, Egypt, and kept for research purposes with the patient's prior knowledge before treatment.

The crowns were removed at the cementoenamel junction using a water-cooled precision microsaw (IsoMet® 4000; Buehler, Lake Bluff, USA). The working length was determined using an initial K-file size 15 (Mani Inc., Tochigi, Japan) to reach the apical foramen, and then subtracting 1 mm.

The preparation was carried out using the crown-down technique. All root canals were instrumented using ProTaper® rotary Ni-Ti instruments (Dentsply Maillefer, Ballaigues, Switzerland), starting with SX (0.19/0.04), followed by S1 (0.18/0.02), S2 (0.20/0.04), F1 (0.20/0.07), F2 (0.25/0.08), and F3 (0.30/0.09). The apical patency was checked using a patency file after each file.

The canals were irrigated after using each instrument with 5 mL of a freshly prepared solution of 2.5% sodium hypochlorite (NaOCl). Irrigation was performed using 5-milliliter disposable plastic syringes with 27-gauge needle tips placed passively into the canal, up to 3 mm from the apical foramen without binding. The canals were then dried with sterile paper points and the teeth were randomly divided into 2 groups (n = 7).

A trial fit of the ProTaper universal gutta-percha points size F3 was performed in all samples. The sealers were freshly prepared according to the manufacturer's instructions. Zical was applied to the canal walls with a Lentulo filler, which was then used to coat gutta-percha. The sealer-coated gutta-percha was placed in the canal space up to the working length. GuttaFlow bioseal was injected into the canal space using the tip provided by the manufacturer. The master cone was then coated with the sealer and seated inside the canal. Excess gutta-percha was removed using a heat carrier and gutta-percha was vertically compacted at the canal orifice.

The obturated teeth were embedded in chemically-cured acrylic resin and left to set. On the acrylic resin, the coronal, middle and apical thirds were defined and a section of 2 mm in thickness was cut from the center of each third using a water-cooled precision microsaw (IsoMet 4000). This resulted in 3 slices in each sample and 21 slices in each group.

Table 1. Characteristics of the tested endodontic sealers

| Commercial name | Manufacturer | Composition | Lot number |
|---|---|---|---|
| GuttaFlow bioseal | Coltène/Whaledent AG, Altstätten, Switzerland | gutta-percha powder particles, polydimethylsiloxane, platinum catalyst, zirconium dioxide, calcium salicylate, nano-silver particles, coloring, bioactive glass-ceramic | H84160 |
| Zical | Prevest DenPro Ltd., Jammu, India | powder: zinc oxide, bismuth subcarbonate, barium sulfate, sodium borate, iodoform and hydrogenated resin; liquid: eugenol | 1521802 |

The filling material was then loaded with a stainless steel plunger of a 0.9-millimeter diameter. The plunger was mounted on the upper part of a universal testing machine (model 3345; Instron®, High Wycombe, UK) and the data was recorded using computer software (Bluehill® 3, v. 3.3; Instron). The tests were conducted at a crosshead speed of 0.5 mm/min using a load cell of 500 N.[10,13,14]

The area under load was calculated as follows:

area = circumference of restoration × thickness.

The push-out value in MPa was calculated from the force in N divided by area in $mm^2$.

## Solubility test

The solubility test was conducted according to ANSI/ADA specification No. 57.[12] Split ring molds, 1.5-millimeter-thick with an inner diameter of 20 mm, were used. The rings were filled with the sealers and supported by a glass plate covered with a cellophane sheet. They were then placed in an incubator (37°C, 95% relative humidity) for a period corresponding to 3 times the setting time. The sealers were removed from the mold and weighed 3 times with an accuracy of 0.001 g. Each specimen was suspended from a string of dental floss and the floss was fixed to the stopper of a glass bottle (~40 mm in diameter). The bottle was then filled with 50 mL of distilled water pH ~5.8.

The bottles were stored for 1 week in an incubator (37°C, 95% relative humidity). The samples were rinsed with distilled water, and then blotted dry with absorbent paper. They were placed in desiccators for 24 h, and then reweighed. Seven samples for each sealer were tested at 3 time intervals: 1, 7 and 14 days. The weight loss of each sample (initial mass minus final mass), expressed as the percentage of the original mass, was recorded as the solubility of the sealer.[15]

## Statistical analysis

Statistical analysis was performed using the commercially available software program PASW Statistics for Windows, v. 18.0 (SPSS Inc., Chicago, USA). Data was expressed as mean and standard deviation, and the significant differences between the groups were compared using the independent *t*-test. Within the same sealer group, different root segments and different time observations were evaluated in terms of push-out bond strength and solubility, respectively, using the one-way analysis of variance (ANOVA), followed by Tukey's post hoc test when ANOVA revealed a significant difference. The level of significance was set at $p < 0.05$.

# Results

The results are presented in Tables 2 and 3. GuttaFlow bioseal showed significantly lower push-out bond strength in all 3 segments compared to Zical. The apical segments in both groups showed higher bond strength, followed by the middle and coronal segments, and there was no significant difference between the middle and coronal segments in Zical (Table 2). GuttaFlow bioseal showed lower solubility compared to Zical on days 1, 7 and 14, with no significant difference in both groups on day 14. In GuttaFlow bioseal there was no significant difference in solubility with time, whereas in Zical there was a significant increase in solubility after 14 days (Table 3).

# Discussion

Sealers are of crucial importance in the obturation process and can affect the quality of the endodontic treatment. Zinc oxide and eugenol sealers have represented the golden standard in endodontic treatment for many years due to their long history of successful use.[2,16] Recently, traditional sealers are being replaced with new sealers with more favorable properties; one of these new endodontic sealers is GuttaFlow bioseal. It is an intelligent obturating material that seals and fills the root canal. Upon contact with fluids, the bioactive material provides calcium silicate that forms hydroxyapatite crystals on the surface to improve adhesion and provide repair.[4,5]

In the obturation of the cleaned and shaped canal, the most common technique is cold lateral compaction.

**Table 2.** Mean values of push-out bond strength in each root segment [MPa]

| Root segment | Endodontic sealer | Mean ±*SD* | *p*-value |
|---|---|---|---|
| Coronal | GuttaFlow bioseal | $1.690^{c1}$ ±0.022 | 0.000* |
| | Zical | $4.254^{b2}$ ±0.017 | |
| Middle | GuttaFlow bioseal | $2.077^{b1}$ ±0.129 | 0.000* |
| | Zical | $4.360^{b2}$ ±0.022 | |
| Apical | GuttaFlow bioseal | $2.374^{a1}$ ±0.017 | 0.000* |
| | Zical | $7.300^{a2}$ ±0.022 | |

*SD* – standard deviation; * statistically significant; means sharing the same superscript letter and number are not significantly different.

**Table 3.** Mean values of solubility at each observation time [%]

| Time | Endodontic sealer | Mean ±*SD* | *p*-value |
|---|---|---|---|
| 1 day | GuttaFlow bioseal | $0.724^{a1}$ ±0.081 | 0.002* |
| | Zical | $0.874^{a2}$ ±0.036 | |
| 7 days | GuttaFlow bioseal | $0.697^{a1}$ ±0.098 | 0.014* |
| | Zical | $0.823^{a2}$ ±0.046 | |
| 14 days | GuttaFlow bioseal | $0.661^{a1}$ ±0.101 | 0.068 |
| | Zical | $0.749^{b2}$ ±0.044 | |

* statistically significant; means sharing the same superscript letter and number are not significantly different.

This technique has limitations, which include the following: poor adaptation of gutta-percha to the canal walls, spaces between gutta-percha and the sealer, and the presence of a large amount of the sealer in the apical region due to the inhomogeneous gutta-percha-sealer mass.[17] To help to overcome these limitations, the technique used in this study was the single-cone technique. A single gutta-percha master cone placed with a sealer provides a final compact mass without spaces.[18]

Ideal adhesion of the root canal filling material to the root dentin is one of the main criteria in evaluating the clinical efficacy of the obturation technique.[17] It helps to maintain the integrity of the dentin–sealer interface by resisting dislodgment and ensuring the proper function of the restored tooth.[4] The adhesion of the obturating material to the dentin walls is evaluated using bond strength testing. One of the most reproducible and reliable bond strength testing techniques is the push-out method. It simulates shear stresses present during functioning and the fracture of the samples occurs parallel to the dentin-bonded surface, making it a true shear test. This testing method also has an added advantage – it allows the evaluation of the bond strength in different root segments.[14,19,20]

In this study, the bond strength of Zical was significantly higher compared to GuttaFlow bioseal in all root segments. In both materials, the bond strength was the highest in the apical segment, followed by medium and the lowest bond strength, which was found coronally. The difference between all segments was statistically significant except between the middle and coronal segments in Zical. There is no agreement among authors on how the location of the root dentin segment affects the bond strength. Similarly to this study, Uppalapati and Mandava and Sly et al. found that the bond strength increases from the coronal to the apical direction.[21,22]

The low bond strength values of GuttaFlow bioseal could be due to the presence of calcium silicate in the sealer in the absence of moisture.[23] According to Nagas et al., mineral trioxide aggregate (MTA), a calcium silicate-based material, requires moisture during setting to reach high strength values and resist dislodgment forces.[24] Sarkar et al. and Prüllage et al. attributed the low bond strength values of the MTA-based sealers in their studies to the absence of phosphate-containing fluid.[25,26] Such fluids cause the release of the calcium and hydroxyl ions from the sealer and upon release of these ions, tag-like structures extend into the dentin and increase the adhesion of these sealers. Another reason for the low bond strength of GuttaFlow bioseal could be the low adhesion capacity of the tag-like structures produced by calcium silicate present in the sealer.[27]

Another essential property of endodontic sealers is solubility, because if dissolved, they may release chemicals into the periapical tissues, which could trigger an inflammatory response. The dissolution of the sealer may also result in a gap between the root canal dentine and the filling material, increasing leakage over time.[28,29] According to ANSI/ADA specification No. 57, the solubility of set sealers should not exceed 3% mass fraction after immersion in water for 24 h.[12] In both sealers, the solubility did not exceed the recommended amount over all tested time periods. The solubility of both sealers was the highest on day 1 and decreased on day 7 and 14. Comparing the 2 sealers, the solubility of Zical was higher than that of GuttaFlow bioseal at all 3 time intervals, but on day 14, the difference between both groups was not significant. The higher solubility of Zical could be related to the hydrolysis reaction of the zinc eugenolate, which is essential for setting. It could also be due to the unreacted eugenol escaping from the set cement.[30] The solubility of GuttaFlow bioseal could result from the presence of calcium silicate and its solubility, according to Hoikkala et al.[15] Biomineralization requires the dissolution of the sealer to provide the solution with ions needed for mineralization. Therefore, in the case of GuttaFlow bioseal, the solubility could be considered positive to allow ion release and hydroxyapatite formation on the dentin surface.

## Conclusions

Within the limitations of this study, the endodontic sealer GuttaFlow bioseal showed low bond strength values compared to Zical. The solubility of the set GuttaFlow bioseal and Zical were both within the recommended ANSI/ADA levels.

### ORCID iDs

Mennatullah Mohammed Khalil https://orcid.org/0000-0002-4944-7415
Mai Hisham Abdelrahman https://orcid.org/0000-0003-4934-6064
Sara El-Mallah https://orcid.org/0000-0003-2050-5877

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# SEM and EDS study of TotalFill BC Sealer and GuttaFlow Bioseal root canal sealers

# Badania SEM i EDS uszczelniaczy kanałowych TotalFill BC Sealer i GuttaFlow Bioseal

Przemysław Reszka[1,A–D,F], Alicja Nowicka[2,E,F], Włodzimierz Dura[3,C,E,F], Ewa Marek[3,C,E,F], Mariusz Lipski[3,A,D–F]

[1] Private Dental Practice, Koszalin, Poland
[2] Department of Conservative Dentistry and Endodontics, Faculty of Medicine and Dentistry, Pomeranian Medical University of Szczecin, Poland
[3] Department of Preclinical Conservative Dentistry and Preclinical Endodontics, Faculty of Medicine and Dentistry, Pomeranian Medical University of Szczecin, Poland

A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation;
D – writing the article; E – critical revision of the article; F – final approval of the article

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**Address for correspondence**
Mariusz Lipski
E-mail: lipam@pum.edu.pl

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## Abstract

**Background.** Recently, a new generation of calcium silicate-based root canal sealers has been developed. These new types of sealers have the ability to set in wet environment, have high alkalinity and present potential antimicrobial activity.

**Objectives.** The aim of this study was to determine the chemical composition and microstructure of 2 novel calcium silicate-containing root canal sealers – TotalFill® BC Sealer and GuttaFlow® Bioseal.

**Material and methods.** The tested sealers were prepared according to the manufacturers' instructions. Sixteen cylindrical dishes (inner diameter: 4 mm; height: 3 mm) were placed on a glass Petri dish and packed with the materials. The Petri dish was transferred to an incubator. After the sealers set, excess material was removed with diamond discs and polishing paste. The materials were assessed using scanning electron microscopy (SEM) and energy dispersive spectroscopy (EDS) X-ray microanalysis.

**Results.** Both TotalFill BC Sealer and GuttaFlow Bioseal contained calcium, zirconium, oxygen, carbon, silicon, and a trace of sodium. In the case of TotalFill BC Sealer, trace amounts of copper and technetium were also present, and for GuttaFlow Bioseal, iron, zinc, and a trace of magnesium and hafnium were noted. No bismuth was found. Both of the assessed sealers contained fine particles embedded in the matrix; however, GuttaFlow Bioseal also had larger particles with a diameter of 2–10 µm.

**Conclusions.** TotalFill BC Sealer represents a higher degree of purity in comparison with GuttaFlow Bioseal. The clinical implications of heavy metals present in GuttaFlow Bioseal need to be investigated. Both materials have a fine particle structure, which is desirable for root canal sealers.

**Key words:** heavy metals, scanning electron microscopy, energy dispersive spectroscopy, calcium silicate-based root canal sealers

**Słowa kluczowe:** metale ciężkie, skaningowa mikroskopia elektronowa, rentgenowska spektroskopia energodyspersyjna, uszczelniacze kanałowe na bazie krzemianów wapnia

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## Introduction

Root canal obturation is traditionally performed using gutta-percha in conjunction with a root canal sealer. The use of a sealer is necessary to fill the space between the core material and the dentin wall as well as the voids within the core material, and it also serves as a lubricating agent, thus helping to obtain a fluid-tight seal.[1,2] Without a sealer, canal fillings exhibit greater leakage.[3,4]

Nowadays, various types of root canal sealers are available, including materials based on resin, zinc oxide with eugenol, siloxane, and calcium hydroxide.[5] Recently, di- and tricalcium silicate-based root canal sealers have received significant attention due to their favorable physicochemical properties.[6–9] These sealers are biocompatible and nontoxic.[10–12] Another advantage of these materials is their ability to form hydroxyapatite during the setting process as well as their interaction with the dentin (infiltration of the mineral content of bioceramic-based sealer into the intertubular dentin) and forming the so-called mineral infiltration zone.[12,13]

One of the newer calcium silicate-based root canal sealers that has appeared on the European market is TotalFill® BC Sealer (FKG Dentaire, La Chaux-de-Fonds, Switzerland). In Canada, it has been available for several years as iRoot® BC Sealer (Innovative Bioceramix, Inc., Vancouver, Canada), and in the USA as EndoSequence® BC Sealer (Brasseler USA, Savannah, USA).[13] According to the manufacturer, it is composed of dicalcium silicate, tricalcium silicate, calcium hydroxide, monobasic calcium phosphate, zirconium oxide, tantalum oxide, filler, and thickening agents. TotalFill BC Sealer is premixed and is a ready-to-use calcium-silicate based material. This type of root canal sealer absorbs humidity during setting (dentinal fluid) and sets by itself in the root canal without previous mixing. TotalFill BC Sealer has demonstrated cytocompatibility,[14,15] bond strength and dentin penetrability.[14–16]

Another new root canal sealer containing calcium silicate is GuttaFlow® Bioseal (Coltène/Whaledent, Altstätten, Switzerland), also known as GuttaFlow 3. This material is composed of gutta-percha, polydimethylsiloxane, zirconium oxide, platinum, and bioactive ceramic glass. GuttaFlow Bioseal was developed to improve GuttaFlow (Coltène/Whaledent) bioactivity, promoting the regeneration of the periapical tissues. It shows good physicochemical properties.[16–18]

In the researchers' previous study, the chemical composition of MTA-FILLAPEX available on the market for many years and 2 novel calcium silicate-based root canal sealers (BioRoot™ RSC and Well-Root™ ST) were evaluated.[19] The aim of the present study was to determine the chemical elements in 2 other new calcium silicate-containing root canal sealers: TotalFill BC Sealer and GuttaFlow Bioseal. As in the previous study, the assessment took account of the content of heavy metals.

## Material and methods

Two root canal sealers, TotalFill BC Sealer and GuttaFlow Bioseal, were used as the experimental materials. GuttaFlow Bioseal was prepared in accordance with the manufacturer's instructions; TotalFill BC Sealer was not mixed, as it is a premixed root canal sealer, designed to set in a humid environment. Sixteen cylindrical dishes with an inner diameter of 4 mm and a height of 3 mm were placed on a glass Petri dish and filled with the materials. The Petri dish was covered with damp gauze and transported to a laboratory thermostat (37°C; 95% relative humidity). After the sealers set, excess material was trimmed to the surface level of the dishes using diamond discs and polishing paste, and characterized with scanning electron microscopy (SEM) (FE-SEM SU-70; Hitachi, Ltd., Tokyo, Japan) and energy dispersive spectroscopy (EDS) X-ray microanalysis using NORAN™ System 7 UltraDry X-ray Detector (Thermo Fisher Scientific, Grand Island, USA). The samples were coated with the gold-palladium alloy for electrical conductivity. The metals used to sputter coat the specimens were excluded from the percentage found.

### Statistical analysis

The data was evaluated for normality using the Shapiro–Wilk test. As the data did not follow normal distribution, the Mann–Whitney *U* test was used. The level of significance was set at $p = 0.05$.

## Results

The backscatter scanning electron micrographs at ×1000 and EDS profiles for the selected areas of identical sizes of the tested materials (~0.01 mm$^2$) are shown in Fig. 1 and 2. The collected EDS data is given in Table 1. The EDS microanalysis of the root canal sealers revealed high content of calcium, silicon, oxygen, zirconium, carbon, and a trace of sodium. Statistical analysis showed more calcium and oxygen in TotalFill BC Sealer than in GuttaFlow Bioseal ($p < 0.0002$), whereas GuttaFlow Bioseal contained more silicon, carbon and zirconium than TotalFill BC Sealer ($p < 0.0002$). In the case of TotalFill BC Sealer, trace amounts of copper and technetium were also present, and for GuttaFlow Bioseal, iron, zinc, and a trace of magnesium and hafnium were noted.

TotalFill BC Sealer showed relatively homogenous aggregates of very small, round particles embedded in the matrix. The EDS analysis showed that these particles were mainly composed of calcium, silicon, carbon, oxygen, and zirconium; the cementation phase was composed of calcium, silicon, oxygen, and carbon (Fig. 3).

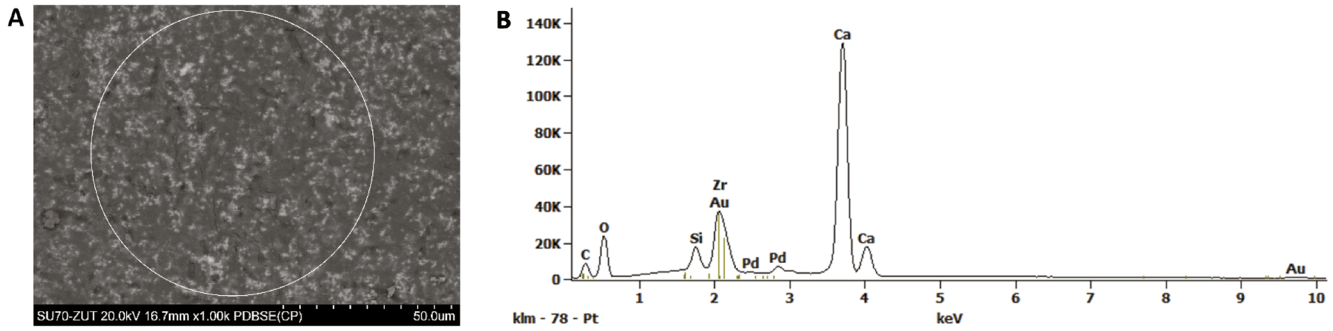


**Fig. 1.** TotalFill BC Sealer: backscatter scanning electron micrographs at ×1000 magnification (A); EDS X-ray microanalysis (B)
EDS – energy dispersive spectroscopy.

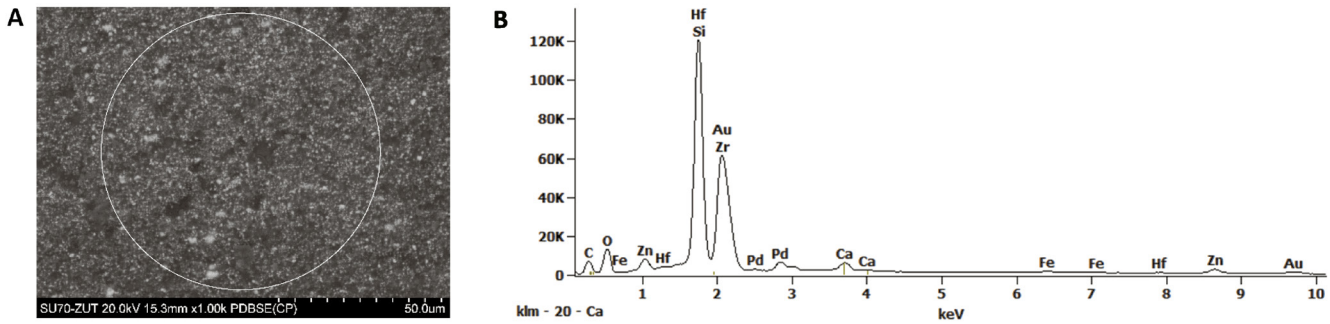


**Fig. 2.** GuttaFlow Bioseal: backscatter scanning electron micrographs at ×1000 magnification (A); EDS X-ray microanalysis (B)

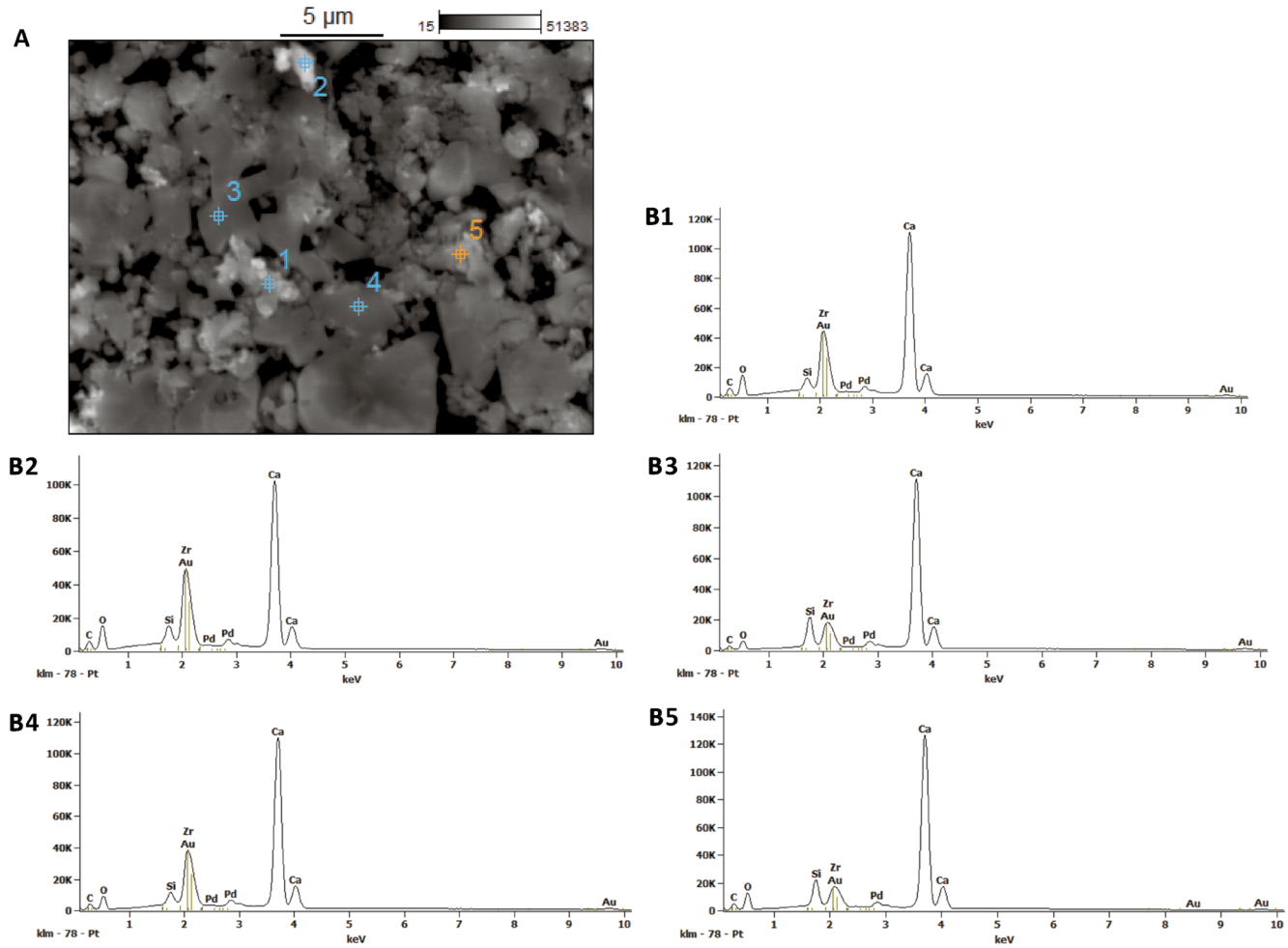


**Fig. 3.** TotalFill BC Sealer: backscatter scanning electron micrographs at ×2500 magnification (A); EDS X-ray microanalysis of particles and cementation phase (B1–B5)

GuttaFlow Bioseal showed multiple aggregates of nanoparticles. The EDS analysis showed that these very small, round particles were mainly composed of silicon, calcium, zirconium, and oxygen. Among these nanoparticles, larger roundish particles were present. The particles with a diameter of about 5–10 μm contained calcium, silicon and phosphorus; the smaller particles (approx. 2–3 μm) were rich in calcium, silicon, zirconium, and oxygen. The point analysis also showed aluminum (Fig. 4).

Neither TotalFill BC Sealer nor GuttaFlow Bioseal contained bismuth.

## Discussion

The present study tested 2 calcium silicate-based root canal sealers recently introduced on the market. As predicted, EDS showed the presence of calcium, silicon and

Table 1. Percentage (wt%) of elements in the tested root canal sealers

| Element | Root canal sealer | |
|---|---|---|
| | TotalFill BC Sealer | GuttaFlow Bioseal |
| C | 4.21 ±0.51 | 9.16 ±0.74 |
| O | 40.7 ±3.31 | 21.41 ±1.811 |
| Na | trace | trace |
| Mg | – | trace |
| Si | 1.67 ±0.88 | 26.89 ±3.52 |
| Ca | 45.1 ±3.72 | 2.09 ±0.74 |
| Fe | – | 1.02 ±0.31 |
| Zn | – | 4.31 ±1.0 |
| Zr | 7.3 ±0.72 | 32.26 ±4.73 |
| Hf | – | trace |
| Cu | trace | – |
| Tc | trace | – |

Data is presented as mean ± standard deviation (*SD*).

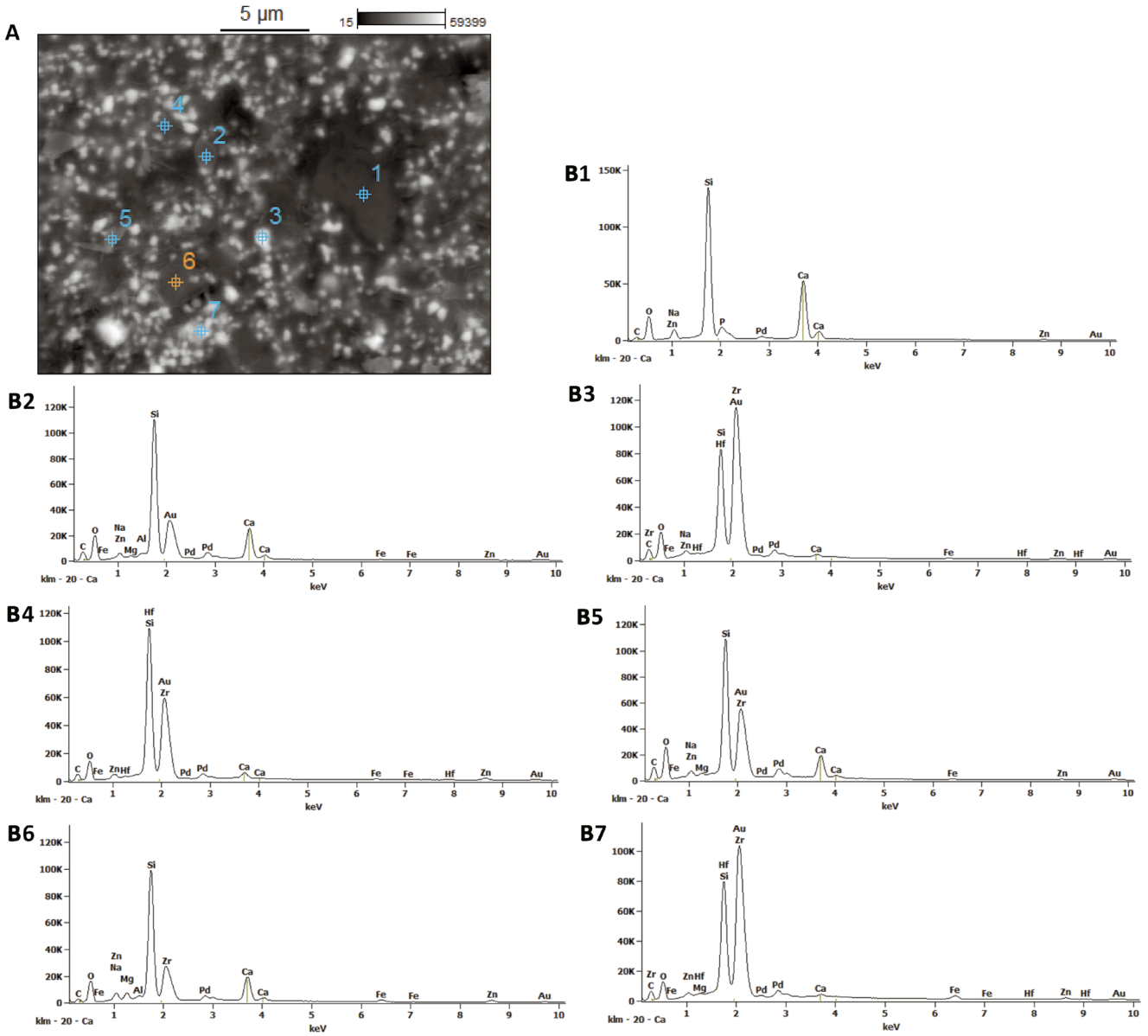


Fig. 4. GuttaFlow Bioseal: backscatter scanning electron micrographs at ×2500 magnification (A); EDS X-ray microanalysis of particles and cementation phase (B1–B7)

oxygen in the composition of both TotalFill BC Sealer and GuttaFlow Bioseal. This observation suggests that these sealers would probably favor bioactivity and would be expected to interact with the dentin. Such bioactivity has been demonstrated by other root canal sealers containing calcium silicates in a similar amount.[6,20,21]

The analysis of the chemical composition of TotalFill BC Sealer also showed the presence of traces of copper and technetium. Although they can adversely affect human health, the very small amount of them in the tested sealer is clinically insignificant.[21,22] In GuttaFlow Bioseal, zinc, magnesium, iron, and aluminum were also found. While the relatively small amount of magnesium and aluminum does not raise concerns, the relatively high content of iron (1.02 wt%) and zinc (4.31 wt%) requires testing the toxicity of these heavy metals.

Several reports have shown that calcium silicate-based root canal sealers have an unfavorable effect on tooth color, which is of clinical relevance in anterior teeth.[13,23] Causal are heavy metal compounds such as bismuth oxide, used as a radiopacifier.[23] However, increasing the concentration of bismuth oxide to increase radiopacity has no significant effect on the level of discoloration.[24] The present study showed that neither TotalFill BC Sealer nor GuttaFlow Bioseal contained bismuth. However, GuttaFlow Bioseal contains iron (1.02 wt%), which has the potential of staining the tooth. For this very reason, the chemical composition of conventional mineral trioxide aggregate (gray MTA) was changed many years ago (iron was eliminated) and an improved formulation was later introduced as white MTA.[13,25]

The EDS analysis showed that both TotalFill BC Sealer and GuttaFlow Bioseal are rich in zirconium and oxygen. Zirconium oxide is an alternative radiopacifier, which has been recently manufactured and used to limit the content of heavy metals and substitute bismuth oxide in calcium silicate-based materials. Zirconium oxide has become popular due to adequate radiopacity and lack of interference with the hydration of calcium silicate-based materials. This oxide, in comparison with bismuth oxide, is more biocompatible and does not cause tooth discoloration.[22,25]

The SEM analysis confirmed that both TotalFill BC and GuttaFlow Bioseal consisted of very small particles, although in the case of GuttaFlow Bioseal, particles of a diameter of approx. 10 μm were also observed. In an earlier study on other calcium silicate-based root canal sealers, the size of the particles was also evaluated.[19] BioRoot RCS was composed of particles of a diameter of 5–30 μm and MTA-FILLAPEX was rich in elongated particles – approx. 10–15 μm in length – and roundish particles – approx. 2–3 μm in diameter.[19] In the research by Hoikkala et al., the SEM examination of GuttaFlow Bioseal revealed bioactive glass-ceramic particles with pointed edges, embedded in a polydimethylsiloxane matrix. The particle size of this ceramic varied in the range of 20–40 μm.[26] Some authors suggest that the particle size is important, because it determines many properties of the material.[23,25] Smaller particles may better penetrate dentinal tubules, which is confirmed by the research by Akcay et al.[27] The authors tested dentinal tubule penetration of 4 different sealers: iRoot™ SP (nowadays TotalFill BC Sealer), GuttaFlow Bioseal, AH Plus™, and MTA-FILLAPEX. The iRoot SP sealer exhibited a significantly higher dentinal tubule penetration than other sealers, which can be attributed to its very small particle diameter (<2 μm). Small particles also hydrate faster than larger particles due to their higher surface-to-volume ratio and provide a low film thickness of the root canal sealer, which is suitable for this dental material and may improve the clinical performance of root canal filling.[24,28]

## Conclusions

TotalFill BC Sealer represents a higher degree of purity in comparison with GuttaFlow Bioseal. The clinical implications of metals contained in GuttaFlow Bioseal need to be investigated. Both materials have a fine particle structure that is desirable for root canal sealers.

### ORCID iDs

Przemysław Reszka https://orcid.org/0000-0001-8545-405X
Alicja Nowicka https://orcid.org/0000-0002-0455-4209
Włodzimierz Dura https://orcid.org/0000-0003-0878-528X
Ewa Marek https://orcid.org/0000-0002-5299-3185
Mariusz Lipski https://orcid.org/0000-0002-2567-3362

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# The effect of simulated erosive conditions on the frictional behavior of different orthodontic bracket-wire combinations

# Wpływ symulowanych warunków erozyjnych na charakterystykę cierną różnych kombinacji zamka i drutu ortodontycznego

Tomasz Stefański[1,A–F], Anna Kloc-Ptaszna[2,B,C,E,F], Lidia Postek-Stefańska[3,B–F]

[1] Department of Orthodontics, Medical University of Silesia, Zabrze, Poland
[2] Institute of Engineering Materials and Biomaterials, Silesian University of Technology, Gliwice, Poland
[3] Chair and Department of Pediatric Dentistry, Medical University of Silesia, Zabrze, Poland

A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation;
D – writing the article; E – critical revision of the article; F – final approval of the article

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**Address for correspondence**
Tomasz Stefański
E-mail: tomasz.stefanski@sum.edu.pl

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## Abstract

**Background.** Frictional resistance is an important parameter in orthodontics that influences the effectiveness of archwire-guided tooth movement. Since the consumption of dietary acids has increased considerably over the last 2 decades, there is a rationale for investigating the process of degradation of orthodontic materials in an acidic environment and its effect on clinical efficiency.

**Objectives.** The aim of this study was to evaluate the effect of simulated erosive conditions on the frictional behavior between the brackets of 3 different materials and 3 different wire alloys.

**Material and methods.** Three types of twin orthodontic brackets (stainless steel (SS), monocrystalline ceramic and titanium) and 3 types of archwires of the same dimension (SS, nickel-titanium (Ni-Ti) and beta-titanium (titanium-molybdenum alloy – TMA) were tested in 9 different combinations under simulated erosive and non-erosive conditions (18 groups, n = 10). Bracket-wire specimens in the erosive-condition groups were subjected to a pH cycling regimen with 1% citric acid and artificial saliva for 5 consecutive days. Bracket-wire specimens from the non-erosive-condition groups were incubated only in artificial saliva for 5 days. Static and kinetic friction were determined by measuring the force needed to move the wire through the bracket. A three-way analysis of variance and pairwise comparisons with the Student–Newman–Keuls test were performed.

**Results.** Irrespective of the conditions, SS brackets with SS wire demonstrated significantly lower mean static and kinetic frictional resistance than other bracket-wire combinations ($p < 0.01$). Ceramic and titanium brackets generated high frictional forces with all 3 types of wire tested. Erosive conditions did not significantly influence static and kinetic frictional resistance in all bracket-wire groups.

**Conclusions.** Erosive conditions do not affect the frictional behavior of SS, Ni-Ti and TMA orthodontic archwires at a clinically significant level.

**Key words:** friction, dental erosion, corrosion, orthodontic brackets, orthodontic archwires

**Słowa kluczowe:** tarcie, erozja zębów, korozja, zamki ortodontyczne, łuki ortodontyczne

# Introduction

The consumption of dietary acids has increased considerably over the last 2 decades, and is thought to be the main reason for dental erosion.[1] Erosive tooth wear is becoming a growing problem particularly among adolescents and young adults, who make up a major portion of orthodontic patients.[2] This emphasizes the need for investigating the process of degradation of orthodontic materials in an acidic environment both in terms of biocompatibility and clinical efficiency. In the literature, several studies have aimed to assess the effect of an acidic diet and soft drinks on the properties of orthodontic fixed appliances, including shear bond strength,[3–6] corrosion resistance,[7–10] metal ion release,[11–14] surface microtopography, roughness,[15] micro- and nanohardness,[16] tensile strain, modulus of elasticity, yield strength,[17] and color stability of orthodontic adhesives and elastic ligatures.[18,19] Surprisingly, very few studies have focused on frictional resistance, which is a crucial clinical parameter that influences the effectiveness of arch guided tooth movement (sliding).

Friction can be defined as the resistance to movement when one object moves tangentially against another.[20,21] In order to move a tooth using a conventional orthodontic fixed appliance, friction at the bracket-archwire interface has to be overcome. Numerous factors have been identified as influencing frictional resistance, such as archwire properties (material, cross-sectional shape or size, surface texture, stiffness), bracket properties (material, surface treatment, manufacturing process, slot dimension, bracket design, prescription), method of ligation, interbracket distance, and biological factors such as saliva (acquired pellicle), plaque, corrosion, mastication (oral forces), and temperature.[20–25]

Considering the wide variety of orthodontic wire and bracket materials, it has been observed that their various combinations exhibit different frictional behavior. Ceramic brackets are known to produce considerably higher friction than stainless steel (SS) brackets.[22] Archwires from nickel-titanium (Ni-Ti) and titanium-molybdenum (TMA) alloy demonstrate higher resistance to sliding than SS wires.[24,25] As a major reason for the abovementioned difference, a surface chemical reactivity (titanium content) is proposed rather than surface topography (roughness).[22]

Although friction could be advantageous in some situations, e.g., for maintaining the anchorage or tooth movement in a closed-loop technique, high frictional forces decrease tooth sliding, thereby prolonging treatment time.[21] Kusy and Whitley demonstrated that 12–60% of the applied orthodontic force is reduced by friction.[22,23]

The purpose of the present study was to assess the effect of the simulated erosive conditions on the resistance to sliding between brackets of 3 different materials (SS, ceramic and titanium) and 3 different wire alloys (SS, Ni-Ti and TMA).

# Material and methods

## Study design

The present study followed a 3 (bracket material) × 3 (wire material) × 2 (erosive/non-erosive condition) factorial design, hence 18 experimental groups were included, each of 10 specimens. The null hypothesis was that the tested bracket-wire combinations would not differ from each other under erosive and non-erosive conditions.

## Bracket-wire specimen preparation

Three types of preadjusted edgewise maxillary premolar twin brackets with 0.022 slot were tested:

1) stainless steel (Omniarch®; Dentsply GAC, York, USA, torque –7°, angulation 0° and distal offset 0°);
2) monocrystalline ceramic (Inspire ICE®; Ormco, Glendora, California, USA, torque –7°, angulation 0°, distal offset 0°);
3) titanium (Titanium Orthos®; Ormco, torque –6°, angulation 0°, rotation 0°).

Each bracket was positioned and bonded on the SS hex nut with composite adhesive (Transbond XT Light Cure Adhesive; 3M, Maplewood, USA). Three types of orthodontic wire alloys of the same dimensions (0.019 × 0.025 inch) and from the same manufacturer (Ormco) were tested:

a) stainless steel (SS; chromium, 17–19%, 8–10% nickel);
b) nickel-titanium (Ni-Ti; 54.9% titanium, 45% nickel, <1% other);
c) beta-titanium (TMA; 79% titanium, 11% molybdenum, 6% zinc, 4% tin).

The wires were cut into 25-mm-long segments from the straight distal ends of the archwire and tied to the brackets using a 0.120-inch-diameter elastic ligature (Ormco). A total of 180 bracket-wire specimens were prepared.

## Erosive conditions

A 1% citric acid (Sigma Aldrich, St. Louis, USA) aqueous solution with a pH of 3.2 was used as the standard erosive solution. Bracket-wire specimens in erosive-condition groups were subjected to pH-cycling. Specimens were exposed to the citric acid solution for 10 min (pH 3.2, 10 mL/specimen, at 21°C with slow stirring) and then immersed in artificial saliva for 60 min (pH 6.8, 10 mL/specimen, at 36°C without stirring). This procedure was repeated 6 times a day over 5 consecutive days. During the remaining time, specimens were stored in artificial saliva. One liter of artificial saliva contained: 2.7 g porcine gastric mucin, 1.27 g KCl, 0.580 g NaCl, 0.330 g, 0.34 $Na_2HPO_4$, 0.20 urea $KH_2PO_4$, 0.17 g $CaCl_2x2H_2O$, 0.16 g $NH_4Cl$, 0.16 g NaSCN, 0.03 g glucose, and 0.002 g ascorbic acid. The pH was adjusted to 6.8 by titrating a phosphate buffer to the solution.[26] The total time of erosion in the experiment was 5 h (6 × 10 min × 5 days). Bracket-wire specimens

from non-erosive-condition groups were only in artificial saliva for 5 days. All the solutions were renewed daily. Specimens were incubated collectively in a square-shaped Lock&Lock plastic container.

## Friction testing

Before the friction test, each specimen was washed with distilled water. The hex nuts with bracket-wire assemblies were fixed on the bolt rod perpendicularly relative to the ground in a vertical motorized testing machine (MX2; Imada, Toyohashi, Japan) equipped with a 50N load cell (ZP, Imada). A schematic diagram of the testing apparatus is shown in Fig. 1. The wire end was attached to the upper grip of the testing unit. The force needed to move the wire through the bracket at a crosshead speed of 10 mm/min over a 5-mm length of the wire was recorded continuously. Data was obtained using a computer program (ZP Recorder; Data Acquisition Software, Imada). Static friction was set as the maximum value at the beginning of movement on the force-displacement graphs. The kinetic friction was calculated as the mean of the frictional force measured after static peak at 0.5 mm, 1.0 mm, 2.0 mm, 3.0 mm, 4.0 mm, and 5.0 mm of displacement.

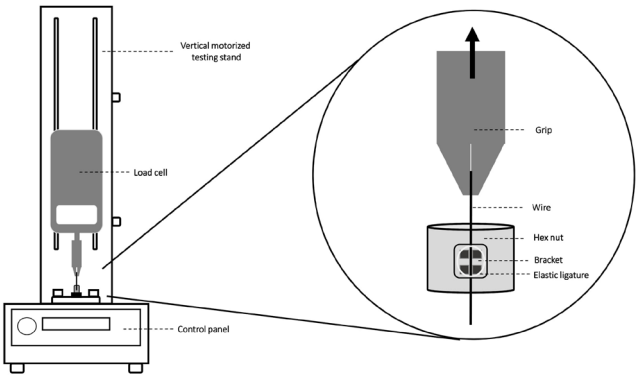


Fig. 1. Schematic diagram of testing apparatus and detailed view of bracket–archwire assembly

Each bracket/wire/ligature combination was tested once in a passive angulation (less than the critical contact angle) at a room temperature of 23°C in a dry state. New elastic ligature was placed immediately prior each test run to avoid variability in the ligature relaxation rate. All test assemblies and measurements were made by 1 operator.

## Statistical analysis

Descriptive statistics including the mean and standard deviation (*SD*) values were calculated for each bracket–archwire combination. A three-way analysis of variance (ANOVA) was used to evaluate the effects of bracket type (metallic, monocrystalline or titanium), wire type (SS, Ni-Ti or TMA) and condition (erosive or non-erosive) on frictional resistance. Pairwise comparisons were performed using the Student-Newman-Keuls post-hoc test. The level of significance was set at $p = 0.05$. Statistical analysis was performed with STATISTICA v. 8.0 software (StatSoft, Tulsa, USA)

# Results

Mean static and kinetic frictional forces between the tested brackets and wires after erosive and non-erosive conditions are shown in Table 1. Post hoc pairwise comparisons revealed that, irrespective of the conditions, SS brackets with SS wire demonstrated significantly lower static and kinetic frictional resistance than other bracket–wire combinations ($p < 0.01$). With metallic brackets the highest friction demonstrated was the TMA wire, followed by Ni-Ti (Fig. 2). Ceramic and titanium brackets generated higher frictional forces with all 3 types of wires tested. Erosive conditions did not significantly influence static and kinetic frictional forces in all bracket–wires groups. Therefore, the null hypothesis should be retained.

Table 1. Means ±*SD* of static and kinetic friction [N] between the tested brackets and wires under erosive and non-erosive conditions. Means sharing the same superscript letter within columns do not differ significantly ($p > 0.05$)

| Bracket | Wire | Condition | | | | *p*-value (static friction) | *p*-value (kinetic friction) |
|---|---|---|---|---|---|---|---|
| | | Non-erosive | | Erosive | | | |
| | | static friction | kinetic friction | static friction | kinetic friction | | |
| Metallic | SS | 1.21 ±0.79$^{A}$ | 1.02 ±0.63$^{A}$ | 1.68 ±1.13$^{A}$ | 1.51 ±0.80$^{A}$ | 0.11 | 0.09 |
| Metallic | Ni-Ti | 2.89 ±0.83$^{B}$ | 2.31 ±0.82$^{B}$ | 2.72 ±0.71$^{B}$ | 2.27 ±0.77$^{B}$ | 0.89 | 0.71 |
| Metallic | TMA | 4.15 ±0.92$^{C}$ | 3.47 ±0.74$^{C}$ | 4.13 ±1.25$^{C}$ | 3.57 ±.1.01$^{C}$ | 0.90 | 0.87 |
| Ceramic | SS | 4.47 ±1.09$^{C}$ | 4.02 ±1.21$^{C}$ | 4.89 ±1.34$^{C}$ | 4.27 ±1.34$^{C}$ | 0.84 | 0.46 |
| Ceramic | Ni-Ti | 4.93 ±0.99$^{C}$ | 4.58 ±0.67$^{C}$ | 4.55 ±1.14$^{C}$ | 4.21 ±0.89$^{C}$ | 0.58 | 0.50 |
| Ceramic | TMA | 4.84 ±1.15$^{C}$ | 4.04 ±1.10$^{C}$ | 3.99 ±1.26$^{C}$ | 3.67 ±1.14$^{C}$ | 0.11 | 0.27 |
| Titanium | SS | 3.82 ±1.02$^{C}$ | 3.62 ±0.96$^{B}$ | 3.57 ±0.99$^{C}$ | 2.99 ±1.26$^{C}$ | 0.79 | 0.22 |
| Titanium | Ni-Ti | 4.69 ±0.84$^{C}$ | 3.91 ±0.60$^{C}$ | 4.42 ±1.19$^{C}$ | 3.66 ±0.93$^{C}$ | 0.41 | 0.44 |
| Titanium | TMA | 4.52 ±1.11$^{C}$ | 4.04 ±0.97$^{C}$ | 4.69 ±0.99$^{C}$ | 3.42 ±0.73$^{C}$ | 0.88 | 0.42 |

*SD* – standard deviation; TMA – beta-titanium; Ni-Ti – nickel-titanium; SS – stainless steel.

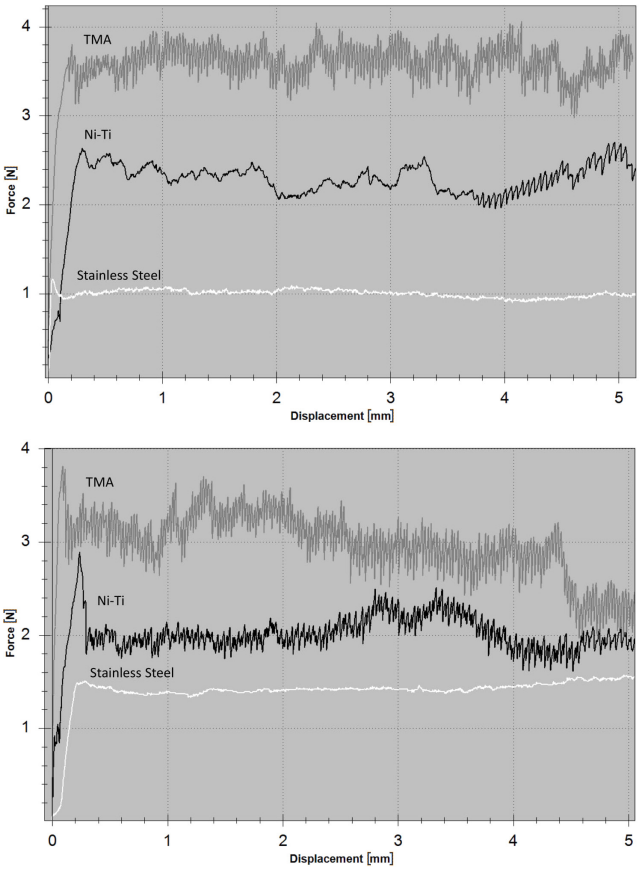


Fig. 2. A. Diagram of the frictional forces produced by randomly chosen specimens of stainless steel, Ni-Ti and TMA orthodontic wires with metallic bracket in the non-erosion group. B. Diagram of the frictional forces produced by randomly chosen specimens of stainless steel, Ni-Ti and TMA orthodontic wires with metallic bracket in the erosion group

## Discussion

The present study was designed to differentiate static and kinetic friction between different brackets and wires under erosive and non-erosive conditions. While the static friction is the smallest force needed to start the motion, the kinetic frictional force resists the sliding during a movement. Static friction seems to be more clinically relevant since orthodontic tooth movement is not continuous,[21] but involves repeated tipping and uprighting. In the present study, single bracket–wire assemblies were compared to minimize the possible variability of normal forces that can be present in multiple bracket–wire interfaces.

Under normal conditions, SS archwire had the lowest frictional forces, while Ni-Ti and TMA exhibited significantly higher friction. This observation confirms previous results.[23–25] To date, only 2 studies evaluating the frictional behavior of orthodontic archwires under erosive conditions have been published; however, none of them have investigated this effect under a simulated pH-cycling environment using conventional metallic brackets and 3 different archwire materials. Jaber et al. assessed the effects of cariogenic and erosive challenges (citric acid, pH 2.3, 60 min for 21 days) on copper-nickel-titanium (CuNiTi) wires by determining the surface roughness of the wires and friction between the wires and passive self-ligating brackets. It was observed that although surface roughness was greater after erosive challenges, the friction did not increase.[15] Nanjundan and Vimala[27] studied surface morphology and frictional forces between SS wire and metallic and polycrystalline alumina brackets after exposure to various erosive solutions (Pepsi, vinegar) and an acidulated fluoride prophylactic agent. They demonstrated higher static and kinetic frictional forces in the Pepsi group and the greatest surface roughness.[27] However, the limitation of the latter study is that it was conducted after continuous 24-hour exposure to the acidic solution, whereas intraoral pH levels rather oscillate than remain constant.

It should also be noted that archwires may exhibit various corrosion susceptibility and metal ion release due to different manufacturing processes.[7] An acidic oral environment can increase susceptibility to corrosion and breakdown failure of Ni-Ti alloys due to hydrogen absorption.[28] This process can be accelerated in the presence of fluoride, as the hydrofluoric acid (HF) disrupts the protective $TiO_2$ passive oxide surface film.[29] Moreover, Harris et al. showed decreased tensile strain, modulus of elasticity and yield strength in an acidified simulated oral environment.[17] The surface corrosion of archwire may influence the friction at the bracket-archwire-ligature interface unit, impeding the free sliding action.

Classical frictional force (F) is directly proportional to the force applied by ligation (N) and the coefficient of friction (μ), which depends on the archwire and bracket surface roughness: $F = \mu N$.[20] However, the relation between friction and surface roughness is still the subject of controversy. Some studies did not support such a relationship.[7,15,22,30–33] It should be noted that clinically, when a tooth (bracket) slides on the arch, the edges of the slot may contact the archwire, leading to its binding. Kusy and Whitley[23] have estimated the critical contact angle (θ) between archwire and bracket to be 3.7°, below which the configuration is passive and above which binding occurs (active configuration). At greater angle values, the slot edges may permanently deform the archwire, causing notching. With a 7° angulation, the binding produces 80% of the resistance to sliding.[33] It is important to highlight that in the present study only classical friction was measured, not taking into account the 2 other components of the resistance to sliding (binding and notching).

## Conclusions

In conclusion, the findings from the present study indicate that erosive conditions do not affect the frictional behavior of SS, Ni-Ti and TMA orthodontic archwire at clinically significant levels.

## ORCID iDs

Tomasz Stefański https://orcid.org/0000-0002-8133-2845
Anna Kloc-Ptaszna https://orcid.org/0000-0001-7647-9773
Lidia Postek-Stefańska https://orcid.org/0000-0002-6573-019X


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# Porcelain sectional veneers, an ultra-conservative technique for diastema closure (three-dimensional finite element stress analysis)

# Ultrazachowawcza technika zamykania diastem za pomocą częściowych licówek porcelanowych (analiza naprężenia trójwymiarową metodą elementów skończonych)

Rami Shurbaji Mozayek[1,A–F], Mirza Allaf[1,A,C,E,F], Suleiman Dayoub[2,A,E,F]

[1] Department of Fixed Prosthodontics, Faculty of Dentistry, Damascus University, Syria
[2] Department of Periodontology, Faculty of Dentistry, Damascus University, Syria

A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation;
D – writing the article; E – critical revision of the article; F – final approval of the article

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**Address for correspondence**
Rami Shurbaji Mozayek
E-mail: ramishm88@gmail.com

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## Abstract

**Background.** Diastema can be closed using conservative and non-conservative techniques. Composite resin wings and ceramic veneers are the most common treatment options if there is no indication for orthodontic treatment. A novel ultra-conservative technique has been introduced to the practice, i.e., porcelain sectional veneers can be fabricated with no or minimum preparation. However, porcelain is known for its poor mechanical properties and the long-term survival of such restorations is questionable.

**Objectives.** This paper aimed to investigate the mechanical aspects of porcelain sectional veneers by means of the finite element method (FEM).

**Material and methods.** A three-dimensional (3D) model of porcelain sectional veneers on the upper central incisors with diastema was obtained by the reversed engineering method starting from a cone-beam computed tomography (CBCT) image. A 100 N occlusal force was applied parallel and 135° to the longitudinal axis, respectively. For each direction the force was applied once with direct contact and again with no contact with the porcelain sectional veneers. For each of the resulting 4 scenarios, a 3D finite element analysis was simulated and the maximum equivalent von Mises stress was compared to porcelain flexural strength.

**Results.** Higher stresses were detected when the force was applied on the porcelain sectional veneers and they were increased dramatically with the inclined force.

**Conclusions.** Direct occlusal contact has to be avoided when using porcelain sectional veneers and the margin positions must be chosen carefully. The occlusal scheme must be noted carefully before choosing this type of restoration.

**Key words:** finite element, diastema, porcelain sectional veneers, no-prep

**Słowa kluczowe:** element skończony, diastema, częściowe licówki porcelanowe, licówki no-prep

## Introduction

The awareness of the importance of dental tissue is spreading among patients nowadays; however, increased esthetics is continuously required.[1–3]

One of the most frequent dilemmas that may be encountered in the dental office is which of the 2 common treatment plans for sound teeth to choose in the case of diastema. The first one is closing the diastema by composite resin; this technique may be more conservative for the tooth structure, but it still does not offer the greatest esthetics and the teeth are susceptible to color change overtime.[4–11] The second one – ceramic laminate veneers – offers much better esthetics than composite resin, more durability and resistance to color changes, and is considered a minimally invasive technique, which is regarded as an advantage.[12–17]

Now we are looking for techniques which might comprise the best of the above-mentioned approaches. The porcelain sectional veneer has been suggested as an innovative ultra-conservative technique that covers only part of the tooth surface without any preparation to be made and has the esthetic appearance of the ceramic laminate veneer.[18–24]

This paper aimed to study the mechanical aspects of porcelain sectional veneers by means of a three-dimensional (3D) finite element analysis and by comparing the maximum von Mises stress with porcelain flexural strength in order to predict the prognosis of such restorations.

## Material and methods

A 3D model of porcelain sectional veneers on the upper central incisors with diastema was prepared by the reversed engineering method.

A cone-beam computed tomography (CBCT) scan for a 20-year-old patient's maxilla with a diastema between the upper central incisors was obtained. The data set format known as "dicom" was imported into Mimics® v. 17.0 (Materialise Interactive Medical Image Control System; Materialise, Inc., Leuven, Belgium) in order to create separate two-dimensional (2D) masks for each of the following: the bone, periodontal ligaments, pulp, dentine, and enamel (Fig. 1). Then, the masks were converted into 3D geometric models and porcelain sectional veneers were designed digitally with a gradual thickness from 0.1 mm at the buccal surface of the tooth to 1 mm at the contact point mesially. To simplify the model, it was cropped to a block containing the upper incisors and canines (Fig. 2).

The resulting masks were exported in STL format to 3-matic® software v. 9.0 (Materialise, Inc.). The model was smoothed and converted into Non-Uniform Rational Basis Spline (NURBS), then exported as an IGS file to PowerShape 2015 software v. 15.1.4 (Delcam, Birmingham, UK), which was used to convert the NURBS model to a volumetric model, and a parasolid file (X_T format) was obtained.

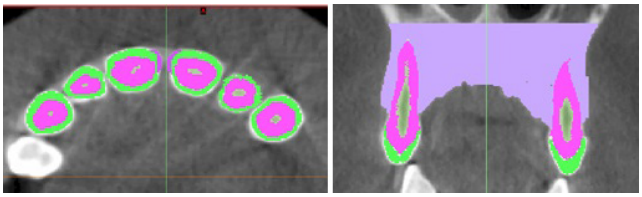

Fig. 1. Masks representing the model tissues

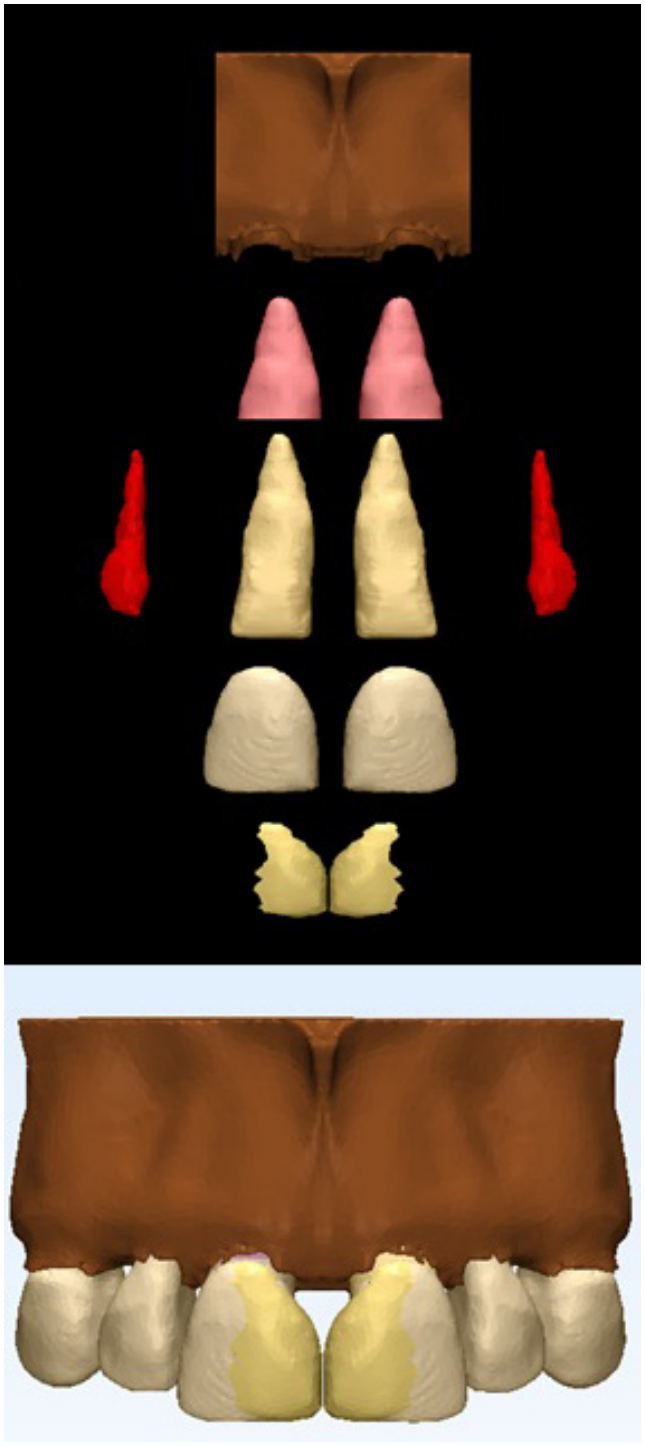

Fig. 2. 3D geometrical model

After that, the final model was imported into ANSYS® Workbench v. 15 (ANSYS, Inc., Canonsburg, USA) , and the following steps were performed:

1. Verification of contact surfaces between different bodies and bonding them together to ensure continuous displacement during loading. The cement layer was simulated by defining bonded contact between the enamel and the porcelain sectional veneers.
2. Defining material properties: bone is an inhomogeneous, anisotropic material, but since it is not possible to accurately represent the non-linear behavior and inhomogeneity of bone, it was considered to be linear, elastic, homogeneous, and isotropic in this study; thus, it can be defined with both Young's modulus of elasticity and Poisson's ratio. Table 1 shows the values for the materials used in this study.[25,26]

Table 1. Material properties of the model components

| Materials properties | Bone | Periodontal ligament | Pulp | Dentine | Enamel | Porcelain |
|---|---|---|---|---|---|---|
| Young's modulus [MPa] | 13,700 | 50 | 2.1 | 18,620 | 84,000 | 82,800 |
| Poisson's ratio | 0.30 | 0.49 | 0.45 | 0.31 | 0.31 | 0.35 |

3. Defining boundary conditions: the model was supported from the bottom to allow the bone to bend under load; the occlusal force was represented in 4 cases according to the area of effect and force direction[27–29]:
   - (a-1) the force is applied on the tooth structure only (the palatal surface) and not on the porcelain sectional veneer, and the force direction is parallel to the tooth longitudinal axis (0°) (Fig. 3);
   - (a-2) the force is applied on the tooth structure only (the palatal surface) and not on the porcelain sectional veneer, and the force direction is 135° to the tooth longitudinal axis palatally (Fig. 4);
   - (b-1) the force is applied on the tooth structure (the palatal surface) and extended to the porcelain sectional veneer, and the force direction is parallel to the tooth longitudinal axis (0°) (Fig. 5);
   - (b-2) the force is applied on the tooth structure (the palatal surface) and extended to the porcelain sectional veneer, and the force direction is 135° to the tooth longitudinal axis palatally (Fig. 6).

Force magnitude was 100 N in each case. After that, the 4 case models were meshed into 62,595 tetrahedral elements, 12,0485 nodes and processed, and the maximum equivalent von Mises stress in the porcelain sectional veneers was obtained and compared to porcelain flexural strength.

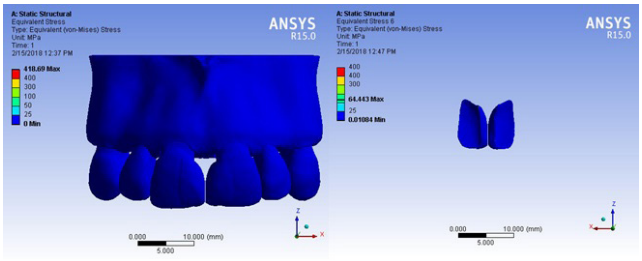


Fig. 3. Scenario a-1: The force is applied on the tooth structure only and not on the porcelain sectional veneer, and the force direction is parallel to the tooth longitudinal axis (0°)

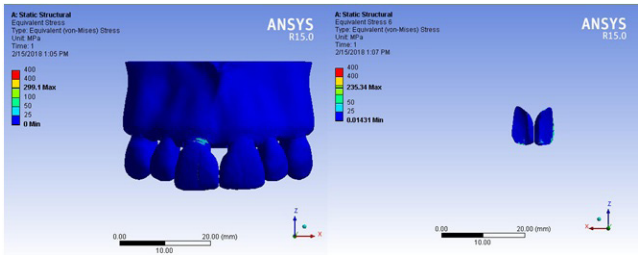


Fig. 4. Scenario a-2: The force is applied on the tooth structure only and not on the porcelain sectional veneer, and the force direction is 135° to the tooth longitudinal axis palatally

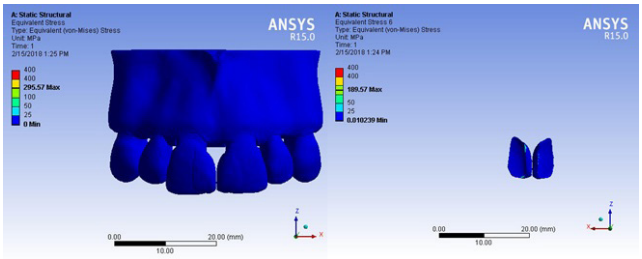


Fig. 5. Scenario b-1: The force is applied on the tooth structure and on the porcelain sectional veneer, and the force direction is parallel to the tooth longitudinal axis (0°)

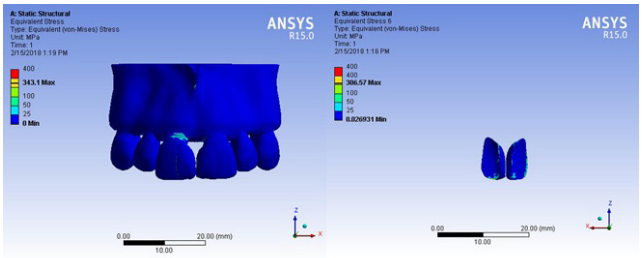


Fig. 6. Scenario b-2: The force is applied on the tooth structure and on the porcelain sectional veneer, and the force direction is 135° to the tooth longitudinal axis palatally

## Statistical analysis

Since no repetitive tests were executed in this study (n = 1), comparison of the results of each tested model was made by descriptive statistics.

## Results

The maximum equivalent von Mises stress of the porcelain sectional veneers for each case is shown in Table 2.

Higher stresses were detected when the force was applied on the porcelain sectional veneers.

Also, the inclined force caused more stress regardless of the force point of effect.

Stress distribution was similar in all cases; the thin margins of porcelain sectional veneers, especially at the buccal aspect, suffered the maximum stress. There was another concentration point at the interior angle opposite to the incisal angle of the tooth.

**Table 2.** Von Mises stress for each case

| Area of effect | Force direction compared to tooth longitudinal axis [MPa] | |
|---|---|---|
| | 0° | 135° |
| Tooth structure only | 64.44 | 235.34 |
| Tooth and porcelain | 189.57 | 306.57 |

## Discussion

Many treatment plans have been suggested for diastema closure, e.g., orthodontic treatment, laminate veneers, crowning, and composite restorations.[30] Still, there may be a gap between achieving high esthetics and conserving dental tissues.

One of the novel approaches suggested for diastema closure is using porcelain sectional veneers (also called partial veneers),[18–24] which provide ceramic esthetics and do not require dental tissue preparation.

Porcelain is known to have excellent esthetics but low strength,[31] and sectional veneers are characterized by very thin margins, so it is legitimate to question their strength and durability. This paper is concerned with studying the mechanical aspects of porcelain sectional veneers in 4 different scenarios related to force direction and force point of effect using the finite element method (FEM).

The finite element method is a virtual numerical analysis that can bring acceptable and reliable results if the conditions of simulations are as accurate as possible. On the other hand, FEM is a subjective method, which may provide different outcomes if different researchers-programers introduce their own vision of the loading conditions, material properties and boundary conditions. Therefore, FEM cannot be a complete substitute for clinical studies, but it is more like a guide, especially in cases where the studies are difficult to conduct or ethically unacceptable.

Durability of the restorations under loading conditions can be foreseen by comparing the maximum value of equivalent von Mises stress of the material to its flexural strength.

Porcelain flexural strength may vary depending on the brand and manufacturer, but generally it is accepted to be 80–110 MPa.[32,33] Based on the results obtained in the present study, it can be noted that if the porcelain sectional veneer is out of occlusion and the occlusal force is parallel to the longitudinal axis of the tooth, the equivalent von Mises stress is lower than the flexural strength and restoration survival can be expected. On the other hand, the occlusal forces acting directly on the restoration may lead to stresses which cannot be tolerated and a fracture will probably occur.

Angulated force increased the stresses dramatically, even if no direct contact to the restoration was found; the occlusal scheme of the patient could have a great influence on restoration survival, especially anterior excursions, which induce oblique occlusal forces. Parafunctional habits may also lead to such forces and have the same or even greater effect on the restoration.

By observing the stress distribution, it can be noted that the thin margins of the sectional veneer exhibit the maximum stress values, and the margins must be checked regularly to avoid any complications and to ensure good marginal integrity; it can be expected that having a strong bite might cause the chipping of the buccal porcelain margins, which may affect the esthetics dramatically. Moreover, the interior angle opposite to the incisal angle must be taken care of by providing adequate thickness and rounding the angle, which can reduce stress concentration in this area.

Porcelain sectional veneers have mechanical limitations and thus good clinical judgment must be made according to the patient's needs, occlusion and bite force. However, further clinical studies have to be conducted, and clinical evidence and long-term success must be verified.

## Conclusions

Within the limitations of this numerical analysis study, it can be concluded that porcelain sectional veneers are an esthetic option with low mechanical properties and as such must not have a direct occlusal contact. The oblique forces, which may be induced by anterior excursions or parafunctional habits for instance, threaten the survival of the restorations, even if they are not applied directly on the veneers, and may lead to failure. This feature distinguishes porcelain sectional veneers from ordinary porcelain full laminate veneers. The thin margins of porcelain sectional veneers are considered to be a weak point, and they must be positioned carefully and checked regularly. Attention must be paid due to strong occlusal forces, which may cause buccal margin chipping rather than fracture or debonding.

### ORCID iDs

Rami Shurbaji Mozayek https://orcid.org/0000-0002-8399-4025
Mirza Allaf https://orcid.org/0000-0003-1974-1995
Suleiman Dayoub https://orcid.org/0000-0003-0671-9473

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# Extracted human teeth and their utility in dental research. Recommendations on proper preservation: A literature review

# Zastosowanie usuniętych zębów ludzkich w badaniach naukowych. Wytyczne dotyczące przechowywania próbek – przegląd piśmiennictwa

Agnieszka Nawrocka[A–D], Monika Łukomska-Szymańska[A,E,F]

Department of General Dentistry, Medical University of Lodz, Poland

A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation;
D – writing the article; E – critical revision of the article; F – final approval of the article

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**Address for correspondence**
Agnieszka Nawrocka
E-mail: agnieszka_naw@wp.pl

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## Abstract

Laboratory research in dentistry and dental education use extracted human teeth as a model for simulation for ex vivo procedures. Human-borne tissues are the first choice of material for bond strength assessment. To obtain comparable results in dental material tests and to ensure microbiological safety, specimens must be stored under specific, uniform conditions. The aim of this paper was to present the contemporary view and recommendations on preserving extracted human teeth. The antimicrobial properties of the storage medium are a crucial aspect, as extracted teeth pose a risk of cross-infection. A classification of different methods (using solutions and otherwise) is presented and their sterilizing efficiency is compared based on the literature. The emphasis is put on the interaction between the storage conditions and the substrate. Tooth specimens should be biologically safe and have normal mechanical properties. The sterilizing process must be neutral for the enamel and dentin microstructure, because even a minor change can affect the adhesive bonding. Autoclave sterilization and storage in 10% formalin solution are widespread and reliable methods, although they do have their disadvantages. There is a need for further investigation in order to establish uniform recommendations on preparing and preserving extracted human teeth used for research purposes.

**Key words:** sterilization, extracted teeth, formalin, autoclave, adhesion

**Słowa kluczowe:** sterylizacja, usunięte zęby, formalina, autoklaw, adhezja

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## Introduction

Extracted human teeth are a valuable source of biological material and are indispensable for research purposes. Numerous ex vivo dentistry studies are conducted to assess the physical, chemical and biological features of various materials in order to implement the results in dental practice.[1–5] In order to obtain reliable results in such research, the tested materials or tooth samples should be properly prepared and stored.

One particularly important aspect in material science is the measurement of bond strength. Restorations bonded to extracted teeth can be tested by applying a shear load. The amount of force that leads to a fracture defines the adhesion between the 2 materials (shear bond strength test). A method which is widely used in endodontic research is the push-out test. Enough force is applied to cause the extrusion of material from the root canal.[6] Researchers can also assess the marginal adaptation by means of a dye penetration test.[7] A tooth sample is stored in a staining solution. The dye can penetrate through gaps and empty spaces between the tooth and the restoration device, indicating microleakage.[7] Vertical, horizontal and cross-sections are investigated with microscopic techniques in histological studies.[8] The crystalline structure of the teeth is revealed by observing non-decalcified sections under polarized light, and then the sample is decalcified to analyze the soft tissue component. Samples used in immunohistochemistry studies must be precisely prepared; sections can be obtained through cryomicrotomy (a freezing microtome).[8] These microscopic observations are a source of knowledge about tissue composition, developmental changes and the demineralization process, among other things.

Extracted human teeth are the material of choice for conducting laboratory tests. The proper preservation of teeth is mandatory because even slight differences in microstructure or composition can adversely affect the results. Specimens should be biologically safe with unaffected mechanical properties. The chemical composition should remain unchanged and should reflect the condition of the tooth as observed in the natural environment of the oral cavity.

## Sterilization of extracted human teeth

The usage of human teeth is not without disadvantages. The biological material poses a risk of cross-infection (the transfer of pathogenic microorganisms). Hepatitis viruses (HBV and HCV) and the human immunodeficiency virus (HIV) are particularly dangerous. Students, dental practitioners and researchers who come into contact with specimens should follow safety precautions and use personal protective equipment. The Occupational Safety and Health Administration (OSHA) of the USA considers extracted human teeth to be a potential source of blood-borne pathogens.[9] In order to ensure epidemiological safety, materials must be sterilized or disinfected before use.[10,11] The aim of disinfection is to kill pathogens by means of chemical solutions or miscellaneous inactivating agents.[12] Sterilization is a process that eradicates not only all living forms of microorganisms but their heat-resistant vegetable forms (bacterial spores) as well.[13] Upon sterilization, a specimen is free of potentially pathogenic bacteria, viruses and fungi. However, the sterilizing agent cannot interfere with dental materials or rearrange the composition of dental tissues. Centers for Disease Control and Prevention (CDC) put forward recommendations on the proper methods for storing and preparing extracted human teeth for ex vivo purposes.[12–14] Firstly, the Centers proposed the use of a standard 1:10 household bleach.[11,15] This is usually a solution of sodium hypochlorite or hydrogen peroxide. These chemical agents exert a bactericidal and virucidal action based on oxidation. However, microbiological tests revealed that not all pathogens are eliminated by them.[11] Researchers have tested various sterilizing agents to find the best measure for preserving extracted teeth. The most common methods are presented in Table 1.[8,11–23]

Dominici et al. compared the effectiveness of 5 different methods of sterilization/disinfection of extracted human teeth.[11] Antimicrobial eradication was determined by the absence of *Bacillus stearothermophilus* (heat-resistant bacteria used to validate sterilization). The results showed that preserving extracted teeth in a 10% formalin solution for 1 week or autoclave sterilization are the best antimicrobial methods (Table 2).[10,11]

Similar conclusions were reached by Sandhu et al.[10] when preserving samples for 5 days. In the first stage of their experiment, different sterilizing agents were used; the samples were then incubated on agar medium. The specimens treated with formalin, sodium hypochlorite and an autoclave did not show any signs of bacterial growth. Attam et al. described formalin as the most effective disinfectant.[24] Autoclave and formalin are the methods recommended by the CDC.[14]

An interesting alternative for formalin seems to be a household vinegar solution.[21] The liquid mainly contains 5% acetic acid in water. The greatest advantage is its accessibility and the ease of use. Teeth immersed in the solution for 1 week were as free of microorganisms as the group stored in 10% formalin and 3% hydrogen peroxide.[21,23]

**Table 1.** The most frequent methods tested for sterilizing/disinfecting extracted human teeth[10–21,23,25,27,29,30]

| Non-solution methods | Solution methods |
|---|---|
| Steam autoclave[10–14,17,25] | Ethanol[21,23] |
| YAG laser[16,29] | NaOCl[10,11,17,19,21] |
| Microwaves[20,21] | Hydrogen peroxide[10,15,18, 21,23] |
| Gamma radiation[16,27] | Thymol[10] |
| Ethylene oxide[27,30] | Formalin[10–14,19,27] |
| | Glutardehyde[10,11] |
| | Vinegar[21] |
| | Quaternary ammonium compounds[11,19] |

Table 2. The antimicrobial effectiveness of different methods[10,11]

| Substance | Concentration/conditions | Effectiveness [%] | |
|---|---|---|---|
| | | Dominici et al.[11] | Sandhu et al.[10] |
| NaOCl | 5.25% | 60 | 100 |
| Formalin | 10% | 100 | 100 |
| Quaternary ammonium compounds | 0.28% | 30 | not tested |
| Glutardehyde | 2% | 50 | 73.33 |
| Thymol | 0.1% | not tested | 13.33 |
| Hydrogen peroxide | 3% | not tested | 66.66 |
| Autoclave | 20 min | 90% (20 psi, 115.6°C) | 100% (15 psi, 120.6°C) |
| Autoclave | 40 min | 100% (20 psi, 115.6°C) | not tested |

However, this method requires further investigation in order to establish the proper concentration and its influence on tooth structure.

Another proposed substitute to formalin which maintains 100% antimicrobial effectiveness is Gigasept PA solution (Schülke & Mayr GmbH, Norderstedt, Germany). The formula, based on peracetic acid, is commonly used as a safe disinfectant for medical instruments. Freshly extracted teeth immersed in a solution for 7 days at 4°C are devoid of bacteria.[26] However, the influence of Gigasept PA on dental tissues has not been tested and is still not recommended before ex vivo tests.

The other substances presented in Table 1 did not demonstrate sufficient antimicrobial properties. In some studies, the sterilizing efficiency of NaOCl depended on the concentration.[19] Although some researchers achieved disinfection using 2.5% NaOCl,[19] Tijare et al.[21] reported that only 1 tooth out of a group of 10 samples was disinfected after immersion in a 5.25% solution for 1 week. That result corresponds with a study performed by Dominici et al.[11] (Table 2). The chemical instability of NaOCl is also important – exposure to air and high temperatures can impair its biocidal potency.[22]

Thymol, an aromatic oil derived from plants, has antiseptic properties and can palliate the inflammatory process. However, the effectiveness of 0.1% thymol as a disinfectant is poor, ranging from 0% to 13.3%.[10,18,26,27]

According to the literature, immersion in 70% ethanol disinfected only 20–30% of samples.[21,23] Quaternary ammonium compounds, although they are widely used as surface disinfectants, exhibit insufficient antimicrobial action for sterilizing extracted human teeth. Only 30–60% of samples in a solution of quaternary ammonium compounds were free from bacteria (Table 2).[11,15] A 2% solution of glutaraldehyde displays enhanced effectiveness. In the medical industry, higher concentrations of this compound are used as a "cold sterilization method" to prepare equipment that cannot be exposed to the heat of an autoclave.[14] The 2% solution is also used to eradicate bacteria from infected dental canals during endodontic treatment. There are inconsistent results regarding its ability to sterilize extracted teeth. In some studies, more than half of the samples were successfully disinfected,[10,11] while other researchers obtained only 20% of bacteria-free specimens.[18] To recommend this solution as a storage medium, it should be able to successfully disinfect all tested samples; glutaraldehyde does not meet that requirement. Additionally, the compound is highly toxic and even medical gloves may be an insufficient measure for protecting skin from irritation.[14]

Hydrogen peroxide ($H_2O_2$) is described as a high-level disinfectant.[14] The most common concentration used in research is a 3% solution. The sterilizing effect reported in different studies is compared in Table 3.

It has been claimed that 3% hydrogen peroxide led to similar levels of sterilization as 10% formalin, though its effectiveness in ex vivo studies remains controversial.[21] It is a strong oxidant that can dissolve organic matter.

Among non-solution methods, a steam autoclave is the gold standard in sterilizing extracted teeth and numerous studies have provided justification for this recommendation.[5,9,12–14] Heat is a well-known bactericidal agent causing the denaturation of bacterial enzymes and proteins. High temperatures – 121°C or 132°C – along with steam pressure kill microorganisms effectively.[13]

The antimicrobial mechanism of an Er:Yag laser has been thoroughly described, especially in reference to endodontics and periodontology. However, the application of an Er:Yag laser for disinfecting extracted teeth was unsuccessful because of damage to the superficial tissues.[16] The denaturation of dentine collagen can decrease the ability of the dentin to form a firm connection with the adhesive resin. Thus, lower shear bond strength can result not only from the properties of the tested material, but also from the imparied quality of the tooth sample. The obtained result can lead to erroneous conclusions.[25,28,29]

Table 3. The effectiveness of 3% hydrogen peroxide in sterilizing extracted teeth[10,15,18,21,23]

| Study | Period | Effectiveness |
|---|---|---|
| Chandki et al. 2013[15] | 7 days | 60% |
| Tijare et al. 2014[21] | 7 days | 100% |
| Sandhu et al. 2012[10] | 5 days | 66.6% |
| Gogineni et al. 2016[23] | 7 days | 100% |
| Lolayekar et al. 2007[18] | 7 days | 70% |

The effectiveness of ethylene oxide to sterilize extracted human teeth has also been tested.[30,31] Its ability to penetrate into internal layers and to eliminate resistant bacterial strains is insufficient, so this method is not recommended.

Microwave irradiation has been investigated to assess its sterilizing efficiency and its influence on enamel microhardness.[20] Although the mechanism is not fully understood, the method eliminates a wide spectrum of pathogens. It probably combines thermal and electromagnetic effects and leads to molecular changes in the bacterial cell.[20] Bovine enamel sections were exposed to 650 W microwaves for 3 min, which resulted in the eradication of pathogens without impairing the micromechanical properties of tissues.[20] The application of 350 W and 600 W microwaves eradicated 100% of bacteria.[19] However, these results were not supported by Tijare et al.[21] Therefore, the influence of microwaves on the mechanochemical properties of dental tissues remains unresolved.

The introduction of gamma radiation has provided promising results, but requires carefully adjusted parameters to obtain full eradication without disarranging the sterilized material. The method has been applied in the food industry because the results are satisfactory and features of the product remain unchanged.[16] However, high costs of the equipment mean that it can only be used for large-scale sterilization, not in small laboratories.

## Specific issues in laboratory practice

In laboratory practice, the chemical, mechanical and tribological characterization of human teeth and dental materials is crucial. Even a minor change in composition can be statistically significant. The storage methods which ensure 100% microbiological safety (autoclave, gamma radiation and formalin solution) require further consideration. There are some problems and limitations according to their usefulness in research.

## Autoclave

The antimicrobial effect of high temperature and steam pressure does not considerably change the mechanical properties of teeth, so they can still be used for dental training. However, the conditions of ex vivo research are rigorously applied. Changes at the microscopic level can decrease the adhesion strength between dental tissues and the material being tested. The ability to create a sound connection with the bonding material is widely described and studied in terms of permeability of the dentin.[31–33] Although Pashley et al.[32] did not report any adverse effects of autoclave sterilization, another study[33] using a modified method led to the opposite conclusion. Before sterilization, the teeth were etched with 35% orthophosphoric acid.[33] That procedure removed the smear layer and partially demineralized the intertubular dentin. The organic components responsible for creating a hybrid layer (collagen network) were exposed. Due to the temperature, collagen fibers denatured and disintegrated and the ability to form a chemical connection with the bonding resin was limited.[33]

Sound teeth can be effectively sterilized at 115.6°C at 20 psi for 40 min. Conversely, teeth with amalgam restorations may pose problems. The high temperature and pressure lead to the release of toxic mercury vapor from amalgam alloy. The tooth and amalgam exposed to heat simultaneously exibit dimensional changes to different extents. Changes in the size of tissues and restoration apparatus may lead to fractures in enamel and dentine. For restored teeth, an alternative method must be implemented – storage in a 10% formalin solution for 1 week is recommended.[11] The behavior of other dental materials (such as composites or glass ionomers) under pressurized steam has not been tested and requires further investigation.

## Gamma radiation

The influence of radiation on adhesion strength is ambiguous.[10,34,35] White et al. did not prove any adverse effect of gamma radiation on dentin properties while assessing its optical properties and specular reflectance with Fourier-transform infrared spectroscopy (FTIR).[16] Compared to other methods, tissue changes were more extensive when ethylene oxide, dry heat or an autoclave was used. A dose of $0.173*10^6$ rads (a unit of absorbed radiation dose) was applied. However, a dose of $2.5*10^6$ rads (used for instrument sterilization) decreased the adhesion in a shear bond test[35]; therefore, further investigation is recommended in order to establish the precise conditions of sterilization.[16]

## Chemical sterilization with reference to adhesion strength tests

Adhesion is a crucial factor that determines the longevity of dental restorations. It is a physicochemical process which depends on the microstructure of the surfaces that are in contact. Due to this fact, adhesion is susceptible to changes in the composition of substrates. A Shear Bond Strength test (SBS) is a universal method for ex vivo research to evaluate the connection between a tooth and dental material. The storage environment can decrease adhesion with composite resins, which has been widely proven.[17,24,34,35] Solutions used to store extracted teeth contain active agents. The ability to deteriorate the organic compounds of bacterial cells is not specific, and these substances interfere with elements forming dental tissues.

The influence of different methods of sterilization on SBS test results has been evaluated.[17] The largest reduction in adhesive properties was observed for a 5.25% solution of NaO-

Cl. This may result from enamel deproteinization and alterations of the dentin matrix.[10] Moreover, adhesion can be also altered by residual chlorine.[17] For these reasons, compounded by its insufficient antimicrobial effect, NaOCl is not recommended as a storage solution. In contrast, formalin was the only solution found to be neutral to the adhesion process.[17] The 10% concentration provides both desirable properties: the previously mentioned 100% effective sterilization and a neutral adhesion process.[10] In the second phase of this study,[17] teeth from each group were randomly selected to introduce additional sterilization. One-half of the specimens in each subgroup were autoclaved for 40 min while the second half of the specimens were immersed in 10% formalin for 14 days. Interestingly, after autoclaving SBS decreased in the control group (stored for 60 days in distilled water). On the other hand, after the previous SBS reduction in the NaOCl group, subsequent autoclaving led to an improvement in this parameter. Shear Bond Strength test performed after 60 days of storage in sodium hypochlorite yielded significantly better results after 40 min of autoclaving (almost 200%). The storage in 10% formalin also improved the previously decreased SBS, but to a lesser extent. With regard to this observation, an important conclusion can be drawn: the 2 recommended sterilizing methods – autoclave and 10% formalin – have an "equalizing effect". This means that the reduction of SBS which occurs after storage in an inappropriate medium can be balanced by a subsequent proper sterilization method.

Although another study[35] showed unpromising results in testing two-step self-etching bonding systems on teeth preserved in 10% formalin for 3 months, the adhesion obtained with an etch-and-rinse system did not change. Moreover, the special preserving properties of formalin were assessed spectroscopically. The components of formalin (formaldehyde, methyl alcohol and sodium acetate) interact with organic compounds such as proteins, glycoproteins and carbohydrates. The ability to fasten the proteins can prevent a collagen network from collapsing after etching.[24,36] Improper adhesion can also, indirectly, be defined by microleakage. Research based on the dye penetration method revealed that a sound connection between hard tissues and a restoration device was maintained when the tested samples were preserved in 10% formalin.[24]

In comparison to gamma radiation, autoclave and formalin are methods that can be successfully implemented due to their accessibility and low cost. However, the main disadvantage of formalin is the toxicity of formaldehyde (a potential carcinogen). The harmful mechanism is based on an interaction with molecules on the cell membranes, nucleic acid destruction and protein precipitation. Exposure to high concentrations of formalin vapors may lead to immediate local irritation of the eyes, nose and throat. Absorption through the respiratory epithelium is rapid. Working with teeth preserved in formalin solution requires safety precautions. A container with extracted teeth should be opened in a safe, well-ventilated area. Moreover, working under a fume hood is recommended.[37]

One scanning electron microscopy (SEM) study did not indicate an ideal method for sterilizing extracted teeth.[22] The 3 common sterilizing agents (autoclave, sodium hypochlorite and vinegar) caused changes in enamel microstructure, increasing its roughness and porosity. The dentin morphology was also altered.

Thus, a dual approach is called for. Firstly, a universal storage solution for extracted teeth should be established. The second, parallel direction in research should be focused on introducing the best alternative for human tissues for ex vivo purposes.

## Substitutes for extracted human teeth

One important aspect is the quality of material used in dental research. According to reports and clinical practice, the main cause of dental extractions is still caries and its complications.[38] The extraction of non-carious teeth due to periodontal or orthodontic indications is infrequent. Ongoing decay deteriorates the mineral and organic composition, affecting the usefulness of samples. Moreover, researchers often cannot define the source of material, so the age of the donor remains unknown. Thus, age-dependent changes in the tissues should be taken into consideration in research which includes an analysis of mineral and organic composition.[39] The size of a sample can also be problematic. A relatively small and curved surface can be a hindrance in some laboratory tests for which a flat, thick layer is required.

Taking into consideration the epidemiological safety issues in preclinical dentistry, human teeth are being replaced by typodont artificial dentition. However, models made from plastics are expensive and do not reflect the mechanical properties of natural enamel and dentin.

Whereas acrylic substitutes can be used in dental education, natural tissues are irreplaceable in laboratory tests of dental materials. Bovine teeth are widely used as an alternative substrate, and they do have many advantages. Yassen et al.[28] presented a detailed meta-analysis which included the methods of material testing used over the past 6 decades (1953–2010). Bovine teeth are easily accessible and have a more homogeneous composition. They provide a broad, flat surface without carious lesions. However, the microstructure of animal tissue is quite similar but not identical to human enamel. The lack of thorough analyses and unambiguous results comparing human and bovine material in SBS testing do not exclude animal tissues for ex vivo tests,[28] but the minimal differences should be considered when scientific conclusions are drawn. Thus, human-derived material remains the gold standard.

The last point to be raised is agreement with ethical principles. The issue of specific consent to use extracted teeth in research (human, biological material) remains unresolved. Medical experimentation requires the approval of a bioethics committee, though informed consent for tooth donation is not necessary.[28]

## Conclusions

Autoclave sterilization is a widespread and reliable method that can be used to prepare extracted teeth for educational purposes and laboratory research, excluding adhesion testing. Pressurized steam is low-cost and innocuous; moreover, its microcidal and sporicidal activity is rapid. Human-borne tissues are the first choice of material for bond strength assessment. The material should be stored in a solution that is neutral towards enamel and dentin microstructure. Numerous studies have suggested 10% formalin, though its potential health risk must be taken into account. There is a need for further investigation in order to establish uniform recommendations for preparing and preserving extracted human teeth used for research purposes.

### ORCID iDs

Agnieszka Nawrocka https://orcid.org/0000-0003-1317-9954
Monika Łukomska-Szymańska https://orcid.org/0000-0002-6110-4298

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# The effects of the headgear therapy on the airway dimensions in patients with class II malocclusion: A systematic review

# Wpływ aparatu headgear na wymiary drogi oddechowej pacjentów z wadą zgryzu klasy II – systematyczny przegląd piśmiennictwa

Mojgan Shavakhi[1,B,C], Fatemeh Mohamadian[2,C,D], Hooman Zarif Najafi[1,E,F]

[1] School of Dentistry, Shiraz University of Medical Sciences, Iran
[2] School of Dentistry, Shahid Beheshti University of Medical Sciences, Tehran, Iran

A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation;
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**Address for correspondence**
Fatemeh Mohamadian
E-mail: furstinfateme@gmail.com

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## Abstract

Class II malocclusion may be caused by the maxillary protrusion or the mandibular retrusion. One treatment method is to use a headgear, which might affect the dimensions of the patient's airway. The aim of this study was to assess the effect of a headgear on the airway dimensions in class II malocclusion patients. A digital search and a manual search were conducted for English-language articles published from January 2000 to December 2018 about human clinical trials, including the usage of a cervical headgear in class II malocclusion patients who had measurable changes in the airway and/or jaw size. The synthesis methods of the study consisted of data concerning the study design, the type of treatment device, the patient's age at the start, the sample size, the treatment duration, the type of radiography, and the results of treatment; this data was extracted and compared. The quality of the selected articles was assessed. All of the studies had a high risk of bias, providing low-quality evidence of the effectiveness of the headgear therapy on the airway dimensions. The conclusions of the articles differed from each other and there were different mechanisms of changes in the jaw or airway dimensions. Therefore, further studies are required to find the clearest results showing the effect of a cervical headgear in class II malocclusion.

**Key words:** headgear, airway dimensions, class II malocclusion

**Słowa kluczowe:** headgear, wymiary drogi oddechowej, wada zgryzu klasy II

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# Introduction

Patients with skeletal Class II malocclusion are characterized by excessive maxillary growth and/or mandibular deficiency.[1,2] It has been shown that in Class II patients, the upper airway tends to be reduced[3–5] and a correlation has been found between mandibular length and position and the size of the oropharynx and nasopharynx in these individuals.[6,7] Furthermore, airway volume is decreased in Class II patients[8] and the diminished upper airway plays an important role in oxygen saturation[9] and in the development of obstructive sleep apnea (OSA), which is known as a sleep respiratory disorder.[10,11] Since a significant relationship exists between the posterior airway and the position of the maxilla, mandible and soft palate[12], any alteration in the positioning of the skeleton might lead to changes in airway sizes.[10,13]

Two major treatment options are available for Class II growing patients: functional appliances, which stimulate mandibular growth,[14] and headgear treatment, which inhibits maxillary growth.[15]

Cervical headgear (CHG) with an expanded inner bow is commonly used for restricting maxillary forward growth and for arch expansion,[16,17] and is typically designed for nighttime usage. The distal movement of the maxilla caused by such headgear seems to have an adverse effect on the size of the upper airway.[18] However, it has been shown in some studies that CHG facilitates mandibular growth, which may lead to improved airway size.[18–20] Some studies have shown that using CHG while sleeping decreases the size of the upper airway and may contribute to the occurrence of sleep apnea in patients with a history of OSA.[19,21]

Studies concerning the effects of headgear therapy on upper airway dimensions have reported conflicting results and the systematic reviews are focused on the effect of stimulating mandibular growth on the airway size.[22,23] Based on the PICO framework, this review of the literature was aimed at assessing the changes in airway dimensions in preadolescent Class II patients treated with functional headgear.

# Material and methods

## Search strategy

A search of 5 electronic databases – PubMed/Medline, Google Scholar, EMBASE, Web of Science and Cochrane – was carried out for relevant English-language studies published between January 2000 and December 2018 using the following keywords separately and in combination: [headgear] AND [cl II malocclusion OR maxillary protrusion OR mandibular retrusion] AND [airway dimension OR upper airway size OR upper airway width] AND [jaw growth]. In addition, a manual search was also performed for publications in dental and orthodontic-related journals from January 2000 to December 2018 that were not electronically identified (including "The International Journal of Adult Orthodontic and Orthognathic Surgery" and "The Journal of General Orthodontics"). No other restrictions were established, and the free-text strategy and Medical Subject Headings terms (MeSH) were applied in the search process. This systematic review was conducted in line with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses statement (PRISMA) as illustrated in Fig. 1.

Our PICO criteria were constructed as follows: 1. Participants: humans with Class II malocclusion. 2. Intervention/Exposure: cervical head gear therapy. 3. Comparisons: treatment with headgear vs. other Class II malocclusion treatments. 4. Outcome: the effect of headgear therapy on airway dimensions

## Study selection and eligibility criteria

In order to select relevant studies, the extracted sources were evaluated by 2 independent reviewers. In case of disagreement, the final assessment was made by a 3rd reviewer. The criteria used to evaluate eligibility were defined according to the considered subject of the present article. The current article only considers original research with the following specifications: Randomized Controlled Trials (RCTs) in addition to cohort and cross-sectional sources discussing the impact of head gear therapy on the dimensions of the airway in Class II malocclusion, and human clinical studies focused on the use of headgear in patients with Class II malocclusion who did not have any respiratory problems before

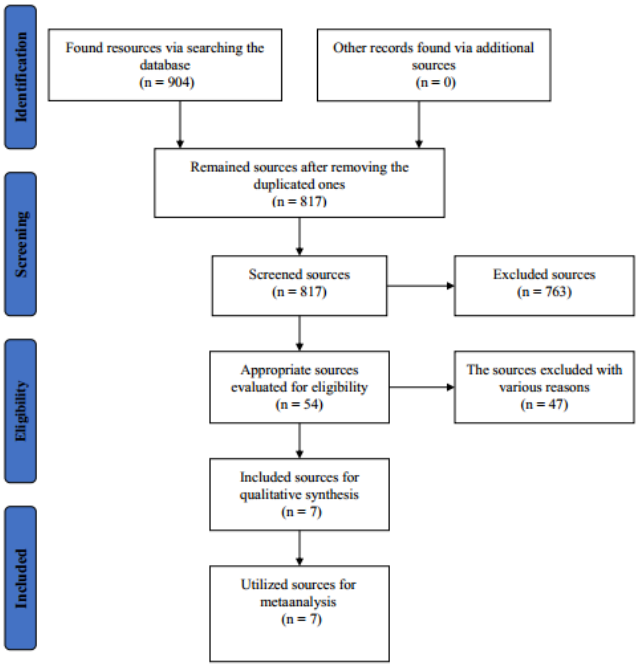


Fig. 1. PRISMA diagram of the present study

the examination. Descriptive-type observational studies (e.g., case studies) and review articles, chapters of books, reports and studies focused on nonhuman cases were excluded from the current study. Moreover, the sources which did not consider changes in the size of the airway were not included in the present review article. In addition, only sources written in English were considered. The primary screening of the subjects and abstracts was performed on the basis of the above-mentioned criteria. It should be noted that all sample sizes were considered for this review study and that any duplicate sources were identified and removed. The data was then extracted from the eligible sources and the extracted information was analyzed in the next step of the study. The features of the studies used for review and the summary of their results are presented in Table 1.

# Results

## Study selection

The search initially retrieved a total of 54 citations. Following the primary screening of titles and abstracts and the final screening of full texts, 7 studies met the inclusion criteria and were included in the final evaluation.

## Study characteristics

The data of study design, type of treatment device, patient's age at the start, sample size, treatment duration, radiographic type and the results of treatment were extracted and compared (Table 1).

## Risk of assessment bias

All selected studies were judged to have a high risk of bias (Table 2), providing low-quality evidence on the effectiveness of headgear therapy on airway dimensions.

# Data comparison

## Use of headgear and diagnostic tool

Head gear was used in all studies alone or in combination with other devices. Two studies used an activator as well[24,25] and 1 used Class II traction.[26] Each of the 7 articles selected cephalometry as a diagnostic tool.[17–19,24–27]

## Sample size

Different sample sizes were selected for the included studies, which ranged from 10 to 209 patients.[17–19,24–27]

## Patients' age

Some variation was found in the age of the patients. One study treated adults,[12] while the others treated children aged 7.2 to 11.9 years old[17,18,24,26,27]; 1 did not mention the patients' age.[25]

## Treatment duration

The duration of treatment was different, varying from 3.5 months to 6.4 years[17,18,24–27] and 1 study did not report the treatment duration time.[19]

## Treatment outcome

The outcomes of head gear treatment were reported in different ways. Two studies did not report any changes in upper airway dimensions[18,25], 1 demonstrated an increase in the upper airway size,[26] while another reported a decrease in sagittal dimensions and no change in vertical dimensions.[19] Four articles reported an increase in retropalatal[17,27] and pharyngeal airway sizes.[24,25] One experiment found a decrease in the size of the opening of the jaw after treatment.[19] On the other hand, no significant change was mentioned in another study for anteroposterior displacement of the mandible.[12] Two studies evaluated changes of the hyoid bone.[19,26] One of the studies reported a significant forward displacement of the hyoid,[19] whereas the other one just mentioned a change in the position of the hyoid.[26] One article assessed the movement of the 3rd cervical vertebra and showed that it had moved forward significantly.[19] ANB was one of the main parts that were investigated in the studies: 2 studies defined a reduction in ANB after treatment,[18,24] while the other only mentioned a change in ANB.[26] In 1 paper, a significant change was reported for SNB, A and Wits.[26] Overjet, overbite and lip measurements had decreased in 1 study.[26] In 1 paper, pharyngeal width was assessed in OSA patients and a decrease was reported.[25] A significant posterior movement of the maxilla was demonstrated among the males in one experiment.[27] A decrease in the palatomandibular angle was reported in one study.[27]

# Discussion

The development and growth of dentoskeletal features are affected by several physiological factors, such as the circadian rhythm, fluctuations in hormone levels and metabolic activity.[19] Class II malocclusion could be caused by a maxillary protrusion or a mandibular retrusion.[18] The treatment protocol focuses on restricting maxillary growth and enhancing mandibular growth, which can affect the positions of the jaw and upper airway.[18] Some studies have been performed to assess the outcomes of headgear therapy on upper airway dimensions in such patients.[18]

**Table 1.** Studied drugs' general names, brand names, pharmaceutical forms, manufacturers and mg/mL on the market

| Author/year | Study design | Device & treatment method | Radiography | Sample size | Age [years] at start | Treatment duration | Result |
|---|---|---|---|---|---|---|---|
| Kirjavainen et al.[17] 2007 | For Class II malocclusion with an overjet of more than 2 mm, a protrusive maxilla by A-point's in front of nasion-pogonion-line | CHG (Khoehne type)<br>Outer bow bent 150 upward & a large inner bow expanded 10 mm larger than intermolar distance<br>500 g per side<br>12 to 24 h a day | Lat ceph | 40 | 9.1 | 1.6 years | Retropalatal airway space: increased<br>Nasopharyngeal space: increased or no sig change<br>Oro and hypopharyngeal were narrowed |
| Hiyama et al.[19] 2001 | Subjects were instructed to stay awake all night immediately before experiment to help ensure that they could fall asleep easily. Buccal tubes were bonded to buccal surfaces of bilateral maxillary first molars. Silver/silver chloride surface electrodes to record electroencephalograms for determining sleep stage were positioned with paste. | CHG<br>Face bow & neck strap were used.<br>The force was adjusted to 700 g. | Lat ceph | 10 | adult | – | Jaw opening: decrease<br>Ant.post mandible displacement: no sig<br>Upper airway(sagittal dimension): sig reduced<br>Upper airway(vertical length): no sig<br>Hyoid: sig fwd 3rd cervical vertebra: sig fwd<br>Mandibular symphysis & hyoid relationship: no sig |
| Aksu et al.[18] 2017 | Class II patients whose upper airway sizes were not sig different at start & sagittal skeletal jaw relationships showed maxillary protrusion or mandibular retrusion. Divided into 3 groups: CHG, activator & control | CHG | Lat ceph | 57 | 10.6 | 1.1 years | ANB: sig decrease<br>Upper airway size: no sig |
| Hanggi et al.[24] 2008 | Inclusion criterion for study group was active treatment with a combined activator-high-pull headgear appliance of at least 9 months between 9 & 14 years of age. Only a moderate construction bite (typically 3–4 mm) was taken, resulting in some cases in a two-step activation. | Activator & HG | Lat ceph | 64 | 10.4 | at least 9 months | ANB: sig decrease<br>Pharyngeal airway(area,lenghth,smallest distance between tongue & post pharyngeal wall): sig increase |
| Zheng et al.[29] 2014 | Class II div 1 malocclusion were divided into 3 groups: HG & Class II traction, Class II traction & no treatment). | HG & Class II traction<br>300–350 g | Lat ceph | 90 | 11.9 | 3.5 months | A,SNB,ANB,witts: sig changed<br>L1MP,L1NP & Z angle: sig increase<br>OJ,OB,lower lip measurements: sig decrease<br>Upper airway: sig increase<br>PNS-V, PNS –U & T-V: sig increase<br>Hyoid: change |
| Godt et al.[25] 2011 | 3 groups: HG, activator & bite jumping appliance. Patients in HG group were divided into 6 subsets on basis of y-axis values | Activator & HG | Ceph | 209 | - | 5.2 to 6.4 years | V dimension of pharyngeal width: small increase<br>Upper airway: no change<br>Pharyngeal width in OSA: decrease |
| Julku et al.[27] 2018 | Class II malocclusion treated with CH & randomized into early & late treatment groups. | CHG (Khoehne type) | Lat ceph | 67 | 7.2 | 4 years | Maxilla: sig posterior change (males)<br>Palato-mandibular angle: decreased (early treatment in females & males)<br>Retroglossal airway: increased (early treatment in males).<br>Highly sig positive correlations between skeletal & upper airway dimensions (early in males) |

CHG – cervical head gear; HG – head gear; non ext – non-extraction; sig – significant; fwd – forward; V – vertical; OSA – obstructive sleep apnea; Ceph – cephalometer.

Table 2. Parameters used for quality assessment

| Study | Randomization | Control group | Blind | Follow-up | Estimated risk of bias |
|---|---|---|---|---|---|
| Kirjavainen et al.[17] | unclear | yes | yes | yes | high |
| Hiyama et al.[19] | unclear | no | unclear | yes | high |
| Aksu et al.[18] | unclear | yes | unclear | yes | high |
| Hanggi et al.[24] | yes | yes | unclear | yes | high |
| Zheng et al.[29] | unclear | yes | unclear | yes | high |
| Godt et al.[25] | unclear | no | unclear | yes | high |
| Julku et al.[27] | yes | no | unclear | yes | high |

## Effect on the upper airway

The effect of CHG on the upper airway is limited to the nose, nasopharynx and retropalatalarea.[17] Although nasal breathing can be improved by increasing the lateronasal width and retropalatal airway, this effect may not be clinically significant.[17] A few studies have investigated the features of the upper airway in a supine position — which is similar to sleep positions — because these features and the position of the tongue depend on the position of the body.[26,28] During sleep, the involvement of tonic muscles is reduced, depending on the stage of sleep.[26,28] The respiratory phase plays an important role in the position and morphology of the oropharynx.[19] Based on this rule, Hiyama et al. recorded all cephalograms at the end of expiration.[19] A decrease in the upper airway sagittal dimension after using CHG might affect the respiratory function during sleep.[19] Kirjavainen et al.[17] demonstrated that the retropalatal area was wider after CHG treatment in children; however, Godt et al.[25] reported minor or no changes in anteroposterior pharyngeal width. On the other hand, 1 study declared that there are various sizes of airways among patients and this is independent of the skeletal parameters since the mechanism is more complex; for example, different positions of the tongue or catch-up growth and its effect on oropharyngeal dimensions.[18] Therefore, there are considerable individual differences in normal growth and in the response to treatment.[24]

## Effect on the mandible

Some studies have demonstrated that CHG induces a forward displacement of the mandible in conscious patients; therefore, dilation of the upper airway can be achieved by applying pressure on the back of the neck with the neck strap.[19] Previous studies have reported that pushing the surrounding soft tissue of the neck anteriorly may cause passive superoanterior rotation of the mandible, regardless of the neuromuscular mechanism.[19] Mandibular position is affected by sleep stage, and during NREM sleep it opens progressively more.[19] Utilizing CHG during sleep does not cause significant forward mandible displacement; on the contrary, such displacement in conscious patients can facilitate mandibular growth.[19] Therefore, clinicians must pay attention to the instructions accompanying the CHG.[19] Hiyama et al. suggested that the mandible does not move anteroposteriorly and that only a counterclockwise mandible rotation was noticed, which also led to an increase in the dimensions of the upper airway.[19] On the other hand, a decrease in N-Me after wearing CHG confirms this counterclockwise rotation. In the cases of obstructive sleep apnea, the mandibles were more open than the mandibles of normal cases during sleep and such jaw opening during sleep was suggested to potentially result in the obstruction of the upper airway in patients with OSA.[19]

## Effect on the maxilla

Though there is a common assumption that CHG restricts the growth of the maxilla in the forward direction, no restriction in the growth of the palatal plane (ANS-PNS) was observed – on the contrary, palatal plane length increased.[17] The restriction of the maxillary growth after treatment is limited to the alveolar process, which can affect the growth of the nose.[17] Hanggi et al. reported that the combined activator and high-pull headgear had a significant impact on the maxilla.[24]

## Effect on the angle of soft and hard tissue

Although it has been demonstrated that the angle between the palatal plane and the tip of the soft palate decreased during treatment, this alteration does not correlate with a change in the dimensions of the retropalatal airway space.[17] Some of the decrease in this angle is due to the anterior downward rotation of the palatal plane instead of a change in the position of the uvula.[17]

## Effect on the hyoid

In children with Class II malocclusion, the hyoid bone position was higher; however, after CHG treatment it moved forward but not higher.[19] On the other hand, the third vertebra moved forward as well and resulted in the same distance between the hyoid and the third vertebra.[19] Therefore, the vertical length of the upper airway cannot be affected by wearing CHG.[19] The hyoid bone gradually moved forward and downward from mixed dentition to permanent dentition.[29]

# Conclusions

Considering the discrepancies in the literature, it can be concluded that the dimensions of different parts of the upper airway – such as sagittal dimension, vertical length and retropalatal width – demonstrated various responses to headgear treatment and that individual differences in normal growth play an important role in the response to treatment. Most of the studies agree that headgear therapy does not move the mandible anteroposteriorly and that only counterclockwise mandible rotation may be noticed. This can lead to an increase in the size of the upper airway, though the maxillary growth is limited to the alveolar process after CHG treatment.

## ORCID iDs

Mojgan Shavakhi https://orcid.org/0000-0002-7013-6048
Fatemeh Mohamadian https://orcid.org/0000-0002-8055-8762
Hooman Zarif Najafi https://orcid.org/0000-0002-1516-8880

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# Negative synergy of mental disorders and oral diseases versus general health

# Efekt negatywnej synergii zaburzeń psychicznych i chorób jamy ustnej w odniesieniu do zdrowia ogólnego

Artur Pitułaj[1,A–D], Andrzej Kiejna[2,A,C,E], Marzena Dominiak[1,A,E,F]

[1] Department of Dental Surgery, Faculty of Dentistry, Wroclaw Medical University, Poland
[2] Psychology Research Unit for Public Health, University of Lower Silesia, Wrocław, Poland

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**Address for correspondence**
Artur Pitułaj
E-mail: arturpitulaj@gmail.com

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## Abstract

In recent years, the World Health Organization (WHO) has undertaken wide-ranging epidemiological research with the purpose of discovering and confirming correlations between mental disorders and somatic diseases. Despite strong evidence for the existence of a two-way dependence between psychological disorders and general diseases, interest in studying the similar impact of oral health is still low. The purpose of this paper was to investigate the multidirectional dependencies between mental, oral and somatic health, and the importance of an interdisciplinary approach to each psychiatric patient.

The PubMed, MEDLINE, Web of Science, and Google Scholar databases were searched for articles published between 1994 and 2018 which involved studies examining the interdependencies between oral, general and mental health, using the following keywords: "comorbidities", "common mental disorders", "mental health", "oral health", "depression", "periodontitis", and "WHO". This review highlights the fact that there is still limited discussion on the importance of the impact of oral health on the general health of psychiatric patients. Data gathered in this paper suggests that an oral examination of mentally ill patients should be considered mandatory.

**Key words:** mental health, oral health, World Health Organization, interdisciplinary approach

**Słowa kluczowe:** zdrowie psychiczne, zdrowie jamy ustnej, Światowa Organizacja Zdrowia, podejście interdyscyplinarne

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## Introduction

Mental disorders are considered to be a vital component of the global burden of illnesses worldwide. They are proven to have a significant influence on constricting the physical fitness, increasing the rate of death and exacerbating other chronic diseases.[1] Neuropsychiatric diseases, including depression, bipolar disorder, schizophrenia, anxiety disorders, or developmental delays, apart from being the cause of disability on a global scale, are also responsible for reducing life expectancy.[2] According to the statistics, 1 in 6 people show the characteristics of common mental health disorders (CMD), and half of them present symptoms which justify intervention from health service employees.[3]

Every year, new dependencies are discovered between mental disorders, oral cavity health and general diseases. The aim of the following article was to analyze current activity in the literature and to present information regarding the potential influence of mental diseases on the condition of the oral cavity from the perspective of oral medicine and general medicine (Fig. 1).

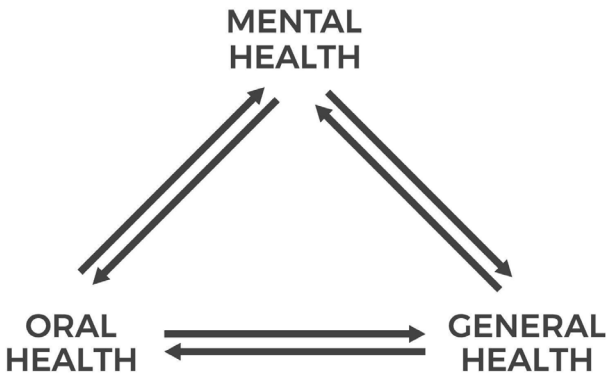


Fig. 1. Relationships between mental, oral and general health

## Mental disorders and the World Health Organization initiative

Due to the significance of mental disorders and difficulty in diagnosing them, in 2000, the World Health Organization (WHO) created the World Mental Health Survey Initiative (WMH-SI), aiming to collect detailed data from around the world on the frequency of occurrence and the correlation of mental disorders, conduct disorders and the use of psychoactive substances.[4] The WMH-SI consortium consists of a representative study on a national and regional scale in 27 countries, representing all parts of the world. The final sample exceeds 144,000 interviews, according to the data from 2015 (https://www.hcp.med.harvard.edu/wmh/).

The data collection is based on factually unified interviews performed by qualified pollsters with no clinical experience. All surveys use the same Composite International Diagnostic Interview (CIDI), designed by WHO, which assures the compatibility of the data collected worldwide.[5] The CIDI questionnaire is a multilevel screening module which evaluates diagnosis, performance, treatment, risk factors, socio-demographic correlations, and methodological factors. It also includes questions concerning somatic illnesses diagnosed by a doctor. Computer-generated diagnosis is assisted by definitions and criteria based on the 4$^{th}$ edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) and the 10$^{th}$ revision of the International Statistical Classification of Diseases and Related Health Problems (ICD-10).[6]

## Mental health and somatic health

The WMH-SI demonstrated the influence of mental disorders on the prevalence of many general diseases. For instance, anxiety disorders, depression, intermittent explosive disorders, and medication and alcohol abuse were linked with increased rates of future peptic ulcer disease, diagnosis of hypertension and heart diseases.[7–9] A general association was observed between the prevalence of mental disorders and an increase in the probability of cancer after they occurred. Depression was more strongly connected with independently reported tumors detected at the early stages of life, and with women. Post-traumatic stress disorder (PTSD) was also connected with the occurrence of tumors detected at the early stages of life.[10] Depression and impulse control disorders (especially eating disorders) correlated with an increase in the incidence of diabetes.[11] The findings of the analysis indicate a connection between these two states – mental and somatic – and support arguments in favor of the early detection and treatment of mental disorders. The data emphasizes the importance of monitoring the physical health of people with such illnesses.

## Mental and oral cavity wellness

Oral cavity diseases, including tooth decay, have a negative impact on quality of life among children and adults. Dysfunction of the masticatory apparatus translates into lingering discomfort and difficulties in one's social life.[12] Kisely indicated a two-way association between oral health and mental health.[13] It is assumed that at least half of dental patients feel anxiety before going to a dentist and some of them develop anxiety disorders – dentophobia, for instance.[13,14] In spite of the existing evidence of the poor oral cavity health of people suffering from mental disorders, that association is still marginalized.

Chronic periodontitis has been recognized as an independent factor of an increased risk of depression.[15] Among adult patients suffering from at least 1 mental disorder, there is a twofold increase in the risk of encountering dental defects that require treatment.[16]

People with diagnosed anxiety disorders or depression use dental services considerably less frequently, and are more prone to tooth loss than mentally healthy people, by about 30%.[13,17] Observed changes in behavior include a gradually increasing indifference toward their own state of wellness, changes in eating habits, leading to increased consumption of foods rich in carbohydrates, and brushing teeth less often, behavior which conduces to the development of dental caries, periodontal diseases and tooth loss.[18–20] Chronic stress, anxiety and symptoms of depression are connected with a reduction in the amount of saliva produced and a feeling of oral cavity dryness.[21] Drying of the mucous membrane renders it vulnerable to injuries and hinders self-cleaning of the tooth surfaces and of the periodontium.

Anxiety disorders and depression are notable risk factors of parafunctional activity, especially bruxism. It leads to temporomandibular joint disorders (TMDs), tooth abrasion and overextension, and subsequent loosening of teeth.[22] The inability to chew properly, arthralgia and speaking problems are the causes of both biological and interpersonal dysfunctions that affect one's mental state. Due to the multidirectional relationship between TMDs and mental health, it is often difficult to assess whether TMDs are the cause or the effect of mental disorders.[23] Moreover, the intense pain associated with arthralgia, caused by muscular disorders, in patients who also suffer from psychosomatic pathologies, is known to notably worsen oral health-related quality of life.[24] Parafunctions are also responsible for premature injuries of fixed and mobile prosthetic fillings, due to their occlusal overextension, which impedes the effective rehabilitation of patients in need of prosthetic restorations.[25] The manner of pharmacological treatment of patients with mental disorders may intensify any adverse behavior and may pose a risk of oral cavity diseases, mainly escalating the dryness of the mucous membrane.[16,26] The side effects of such medication also include inflammation of the saliva glands, gums and the mucous membrane of the oral cavity. Moreover, drugs categorized as selective serotonin reuptake inhibitors (SSRIs) may escalate bruxism, while long-term usage of heterocyclic compounds may increase tooth decay due to an increased craving for sweets.[27]

## Oral cavity health and somatic diseases

Periodontitis is a chronic bacterial disease which leads to the damage of the structures holding the teeth in the alveolus, like the gingiva, alveolar process and periodontal fibers. The pathogenesis of this particular disease includes chronic inflammation states, which change the balance between many systems, including the nervous, immune and hormonal systems.[28] There is ample evidence that existing periodontal disease affects the incidence and exacerbation of numerous systemic diseases. The most commonly described ones include diabetes, osteoporosis, respiratory system diseases, rheumatic diseases, obesity, erectile dysfunction, kidney problems, dementia, some malignant tumors, and cardiovascular diseases – primarily atherosclerosis and its complications.[29–32]

The development of periodontitis is dependent upon a specific vulnerability and the presence of bacterial dysbiotic microflora.[33] According to Socransky, *Porphyromonas gingivalis*, *Tannerella forsythia* and *Treponema denticola* belong to the red complex and are most commonly detected in the areas altered by the disease.[acc.34] However, *Porphyromonas gingivalis* is considered the primary bacterium which determines the beginning of periodontitis; this microbe is able to disturb the physiological balance between the bacteria inhabiting the oral cavity and the immune response of the host on a molecular level.[35] *Porphyromonas gingivalis* demonstrates the ability to spread throughout the body through bacteremia, causing distant infections and the exacerbation of existing diseases. The bacteria have the ability to penetrate the circulatory system through the epithelial surface altered by inflammation. Inhabiting and proliferating on the blood vessel endothelium surfaces, it initiates the process of atherosclerosis. Its DNA may be found on the atherosclerosis-affected plaques in the coronary vessels of people who died of cardiac arrest.[36] A high amount of *Porphyromonas gingivalis* in the periodontal pockets of pregnant women, or its presence in the amniotic fluid or on the surface of the placenta, increases the risk of premature birth, which in turn constitutes a major cause of death for infants.[37] Tooth loss due to tooth decay and periodontitis is a vital factor in the occurrence of many malignant tumors, including cancer of the head and neck areas, or of the esophagus and stomach.[38–40]

## Summary

Despite the major significance of mental disorders as a global health concern, their occurrence along with other comorbidities, and the possible cause–effect links between them and the most commonly occurring general diseases, they still have not been thoroughly researched. Only in recent years, due to the concerns of WHO, has wide-ranging epidemiological research aimed at discovering and confirming such correlations been undertaken. Kisely's insight shed light on the existing undisputed correlation and interaction between mental health and oral health. Although oral cavity diseases are vitally relevant to physical health, they are closely related to the occurrence and course of chronic somatic diseases which are frequently encountered in psychiatric patients.[13]

This argument and the adverse effects of mental disorders on the medical condition of the oral cavity suggest the need for increased attention and dental care in this group of patients.

The epidemiological research connected with CIDI, in relation to the somatic medical status, is based on the information about the health of the people surveyed, not on medical examinations. Broadly speaking, this may result in the collected data being imprecise, for a variety of reasons. However, most people undergo periodic health examinations, ensuring that they possess at least some general knowledge about their illnesses and overall medical condition. Using the information gleaned from a doctor, they answer the questions in epidemiological questionnaires like CIDI. Regrettably, elements of oral cavity health are often omitted from the survey.[41] In many countries, there are free preventative programs for examining children's dentition. However, adult patients are required to buy insurance or to pay for the examination, which in some parts of the world significantly decreases the availability of dental services, and consequently decreases the awareness of the oral medical condition.[42,43] There is also a tendency that, with age, the frequency of regular dental health examinations is reduced.[44]

Therefore, it is vital to collect the broadest possible scope of information concerning the magnitude and the types of dental health issues, e.g., by adding relevant questions to the main part of large epidemiological questionnaires like CIDI. This addition could facilitate the identification of further correlations between mental health and oral health. Moreover, dental problems such as halitosis, bleeding gums, pain, bruxism, tooth loosening, or tooth loss are easily recognized by patients, and provide an important and readily available source of supplementary information on the health status of the people surveyed.

The existing evidence for the multidirectional dependencies between oral, mental and somatic health disorders highlight the need for a broad view of each patient. A comprehensive examination of an individual, often exceeding the scope of knowledge of a single specialist, leads to effective preventative and curative treatment.

## Conclusions

There are multidirectional dependencies between mental disorders, oral health and general health. Psychiatric patients require special dental care, as they have a considerably higher risk of general diseases, including exacerbated secondary ones, because of the often poor condition of their oral cavity. An oral examination of people suffering from mental disorders should be considered mandatory. Adding the right questions about oral health to the general part of mental health diagnostic questionnaires would help to further study the relationship between mental health and the diseases of the stomatognathic system.

### ORCID iDs

Artur Pitułaj https://orcid.org/0000-0002-9025-2628
Andrzej Kiejna https://orcid.org/0000-0002-3708-3853
Marzena Dominiak https://orcid.org/0000-0001-8943-0549

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# Cervicofacial and mediastinal emphysema after dental extraction

# Odma szyjno-twarzowa oraz śródpiersia po usunięciu zęba

Antonino Marco Cuccia[1,A,D], Agostino Geraci[2,E,F]

[1] Department of Dental Sciences "G. Messina", Faculty of Surgery, Oncology and Stomatology, University of Palermo, Italy
[2] Department of Emergency Medicine, Civic Hospital of Palermo, Italy

A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation;
D – writing the article; E – critical revision of the article; F – final approval of the article

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**Address for correspondence**
Antonino Marco Cuccia
E-mail: antoninomarco.cuccia@gmail.com

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## Abstract

Subcutaneous emphysema (SE) is a rare but potentially life-threatening complication in dental procedures. The development of SE and pneumomediastinum (P) during tooth extraction is an uncommon complication. The roots of the second and third lower molars (and, rarely, of the premolar and first molar) communicate directly with the sublingual and submandibular spaces. Occasionally, after a dental operation, the pressurized air from the drill is forcefully injected into the surrounding subcutaneous tissues proximal to the extraction site in the facial planes. The air might pass through the sublingual and submandibular spaces to the pterygomandibular, parapharyngeal and retropharyngeal spaces, and to the mediastinum. Molar extraction is a common procedure in dental surgery.

We report a rare case of extensive cervicofacial SE as well as P, following mandibular second molar extraction with the use of a high-speed dental handpiece, which is specifically designed for restorative treatment. Careful observations of the symptoms and clinical course, and an early initiation of pharmacologic therapy are recommended.

**Key words:** pneumomediastinum, subcutaneous emphysema, dental extraction

**Słowa kluczowe:** odma śródpiersia, odma podskórna, usunięcie zęba

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## Introduction

Mediastinal emphysema or pneumomediastinum (P) is defined as the presence of air or another gas within the mediastinum. This is a rare and generally benign self-limited condition that can be categorized as spontaneous or traumatic.[1] Spontaneous P is determined by the appearance of free air in the mediastinum that is not preceded by trauma, surgery or other medical procedures, i.e., vigorous Valsalva maneuver, childbirth or injury during intubation.[2] Pneumomediastinum is often associated with subcutaneous cervical and supraclavicular emphysema.

Molar extraction is a common procedure in dental surgery. Common complications that may occur post-procedure include pain, trismus, infection, bleeding, maxillary sinus perforation, injuries to the lower alveolar nerve, and maxillary tuberosity fracture.[3] Subcutaneous facial, cervical and supraclavicular emphysema, pneumothorax and P are rare complications.[4]

A rare case of extensive cervicofacial subcutaneous emphysema (SE) and P, following second lower molar extraction with the use of a high-speed dental handpiece, which is specifically designed for restorative treatment, is reported here. Issues relating to the diagnosis, etiology and management of these complications are discussed. Occasionally, after a dental operation, the pressurized air from the drill is forcefully injected into the surrounding subcutaneous tissues proximal to the extraction site in the facial planes. The air may cause SE, airway compromise (due to the accumulation of air in the retropharyngeal, prevertebral and danger spaces), gas embolism, infections of the soft tissues, pneumothorax, P, and pneumopericardium.[5,6]

We present a case of extensive SE, which was probably caused by the use of an air-water-cooled high-speed dental handpiece when extracting a mandibular second molar.

## Case report

A 30-year-old Caucasian woman came to the Department of Emergency Medicine at the Civic Hospital of Palermo, Italy, complaining of fever, left periorbital edema, and swelling in the neck, face and chest, accompanied by audible and palpable crepitus, which is typical of SE. The symptoms appeared after dental surgery performed with a high-speed dental handpiece, normally used to dissect the tooth and to remove it from the alveolar bone tissue.

The patient had a strong sensation of pressure-aspiration in the retrosternal site during extraction, and did not suffer from lung or thoracic diseases.

When she arrived at our hospital, her vital signs were: temperature – 37.1°C; heart rate – 70 beats/min; respiratory rate – 18 breaths/min; blood pressure – 125/80 mm Hg, and oxygenation – 98%. The blood test and electrocardiogram were within normal limits. Intraoral examination revealed good oral hygiene and absence of bacterial infection. There was an enlargement of the cervicofacial soft tissues and crepitation on palpation of that region.

Computed tomography (CT) showed significant left SE in the periorbital, submandibular, sublingual, and mediastinal spaces (Fig. 1–6). Pneumothorax, parenchymal and/or pleural abnormalities were excluded.

Conservative medical treatment consisted of intravenous administration of corticosteroids (betamethasone: 4 mg / 2 mL / 8 h / 7 days) and antibiotic therapy (Tazocin®: 4 g – Cubicin®: 250 mg / 8 h / 7 days) and complete bed rest. Cough suppressants and laxatives can be used to prevent gas embolism.

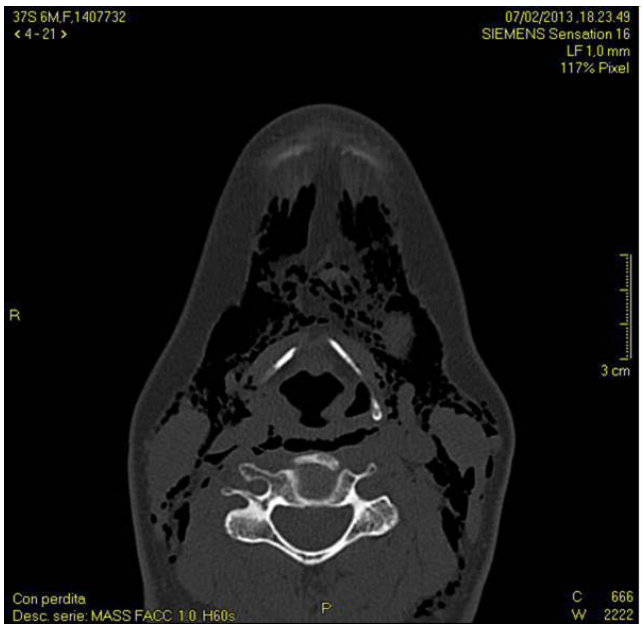


Fig. 1. Subcutaneous emphysema in the submandibular space

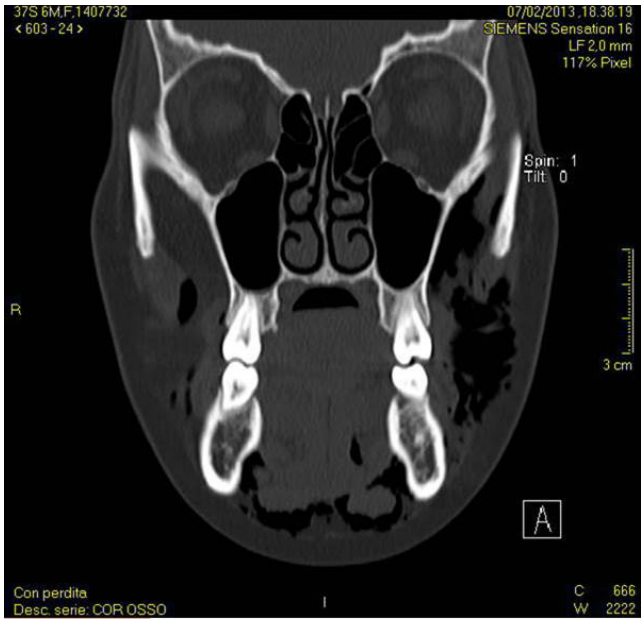


Fig. 2. Presence of air in the spaces of the left periorbital soft tissues

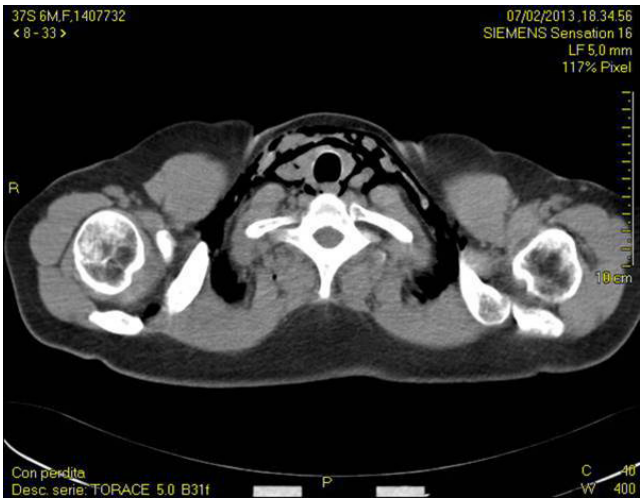


Fig. 3. Presence of air in the left subcutaneous tissues of the neck

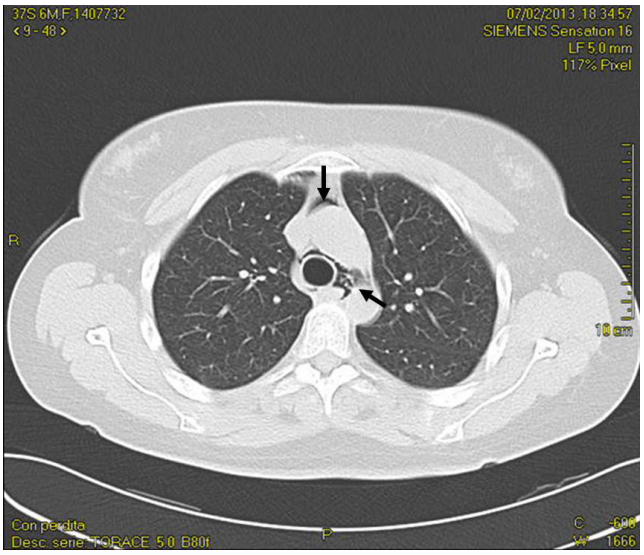


Fig. 4. Computed tomography (CT) scan of the chest revealing pneumomediastinum

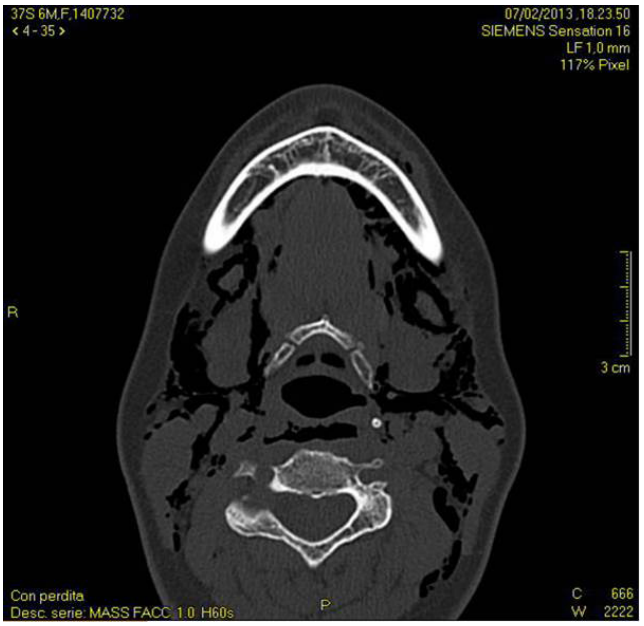


Fig. 5. Subcutaneous emphysema in the area of the pterygoid medial muscle

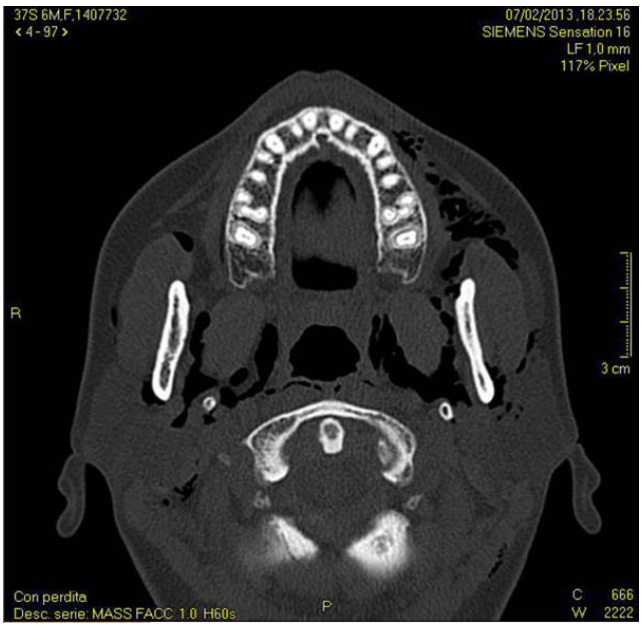


Fig. 6. Subcutaneous emphysema in the area of the masseter muscles

The CT scan taken 1 week after the medical treatment showed air reduction in the left periorbital region (Fig. 7).

The discharge prescription contained 3rd generation cephalosporins (Ceftibuten®: 400 mg / 24 h / 5 days) to prevent low respiratory tract infections in a patient who stayed in a subintensive care unit.

Another CT scan taken after 3 weeks showed air reduction in the left masseter and pterygoid muscles, and in the perimandibular space as well as the resolution of P (Fig. 8–10).

After 1 month, the patient had a complete clinical and radiological recovery without a recurrence of the disease (Fig. 11).

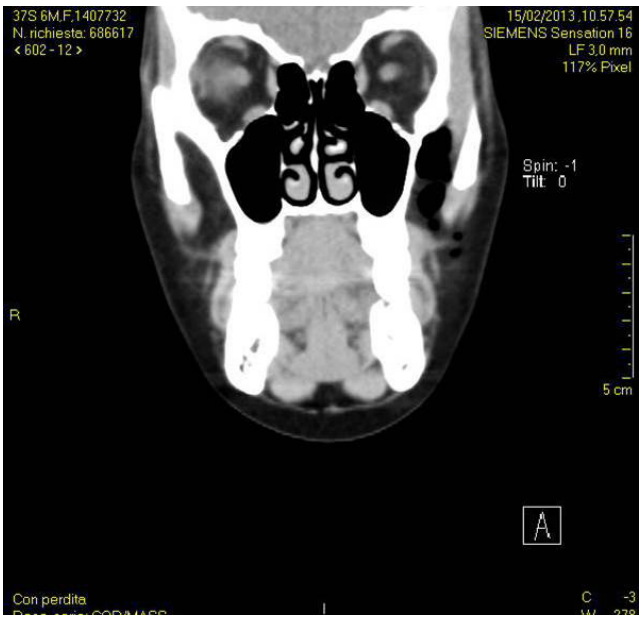


Fig. 7. Periorbital soft tissue air is partially reabsorbed after 1 week

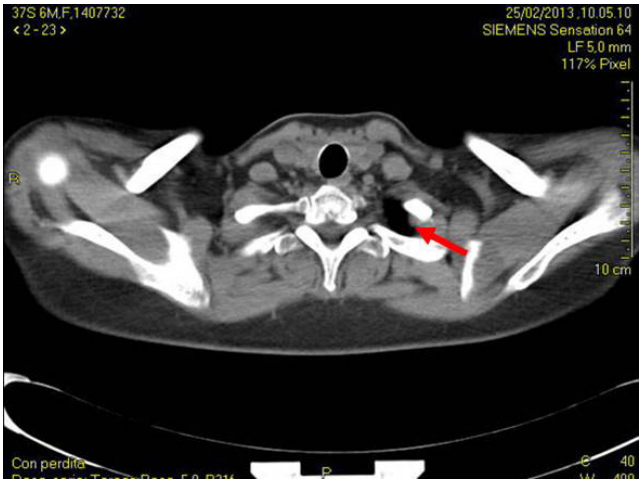


Fig. 8. CT scan showing reduced air in the subcutaneous tissues of the base of the neck after 3 weeks

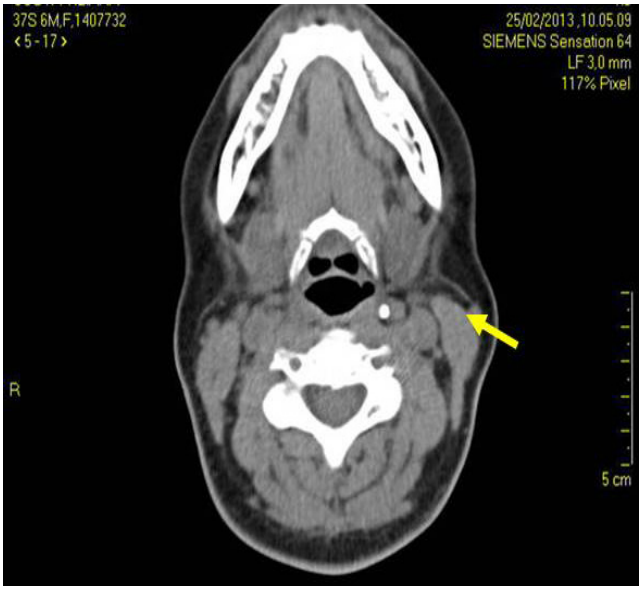


Fig. 9. Subcutaneous emphysema in the left pterygoid medial muscle regresses after 3 weeks

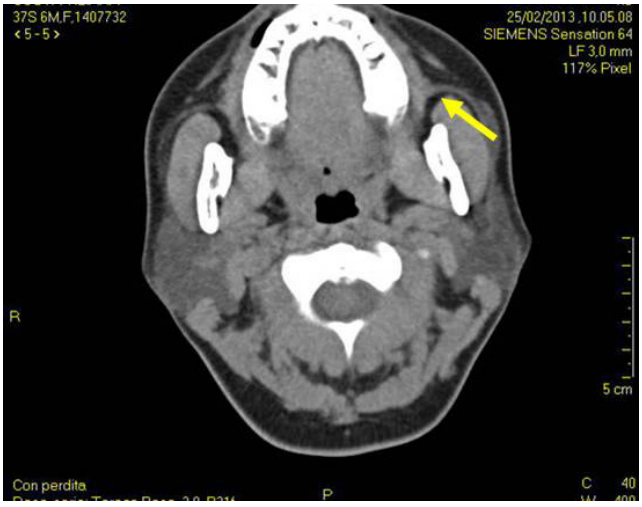


Fig. 10. Subcutaneous emphysema in the left masseter muscle regresses after 3 weeks

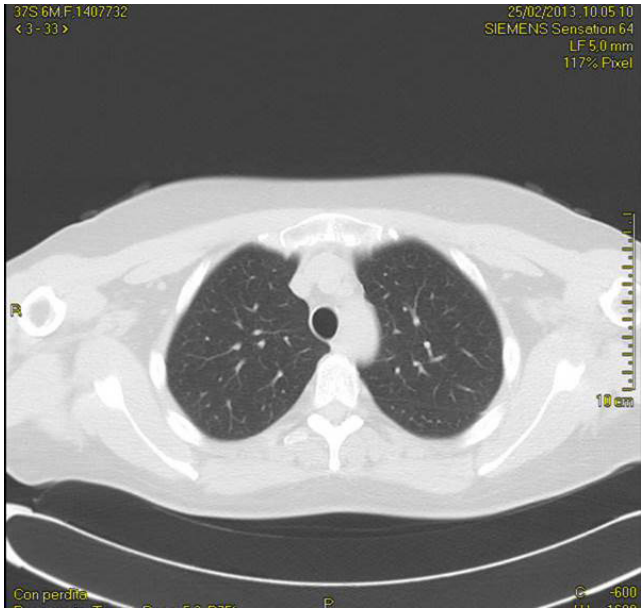


Fig. 11. CT scan showing resorption of air after 1 month

## Discussion

Subcutaneous emphysema is a complication in dental procedures (principally dental extractions and restorative procedures). The mechanism leading to SE and P after molar extraction has been identified. There may be 2 explanations for this mechanism: the compressed air procedure (high-speed dental handpiece, air-water syringe), and the communication between the oral cavity and deeper tissue.[7] Understanding the anatomy and the mechanics of injury will help to anticipate the onset of SE and P.

The roots of the second and third molars (and, rarely, of the premolar and first molar) communicate directly with the sublingual and submandibular spaces. These spaces are separated by mylohyoid muscle fibers. Only the loose connective tissue rather than true fascia actually separates one side of the floor of the mouth from the other; it is an anatomic situation that permits air to spread bilaterally with ease in the soft tissues. The sublingual space communicates anteriorly with the submental space and posteriorly with the lateral pharyngeal spaces in the neighborhood of the posterior edge of the mylohyoid muscle. This space is bounded laterally by the submandibular skin, superficial fascia, platysma muscle, the superficial layer of the deep cervical fascia, and the lower border of the mandible. These neck spaces communicate with the parapharyngeal, pterygomandibular and retropharyngeal spaces.

The retropharyngeal space (RS) extends from the skull base (clivus) to the upper mediastinum (from T1 to T6 vertebrae), lies posterior to the pharynx and esophagus, and is anterior to the prevertebral musculature. It is bounded by the buccopharyngeal fascia anteriorly, the prevertebral fascia posteriorly and the carotid space laterally. The normal contents of RS include fat, small vessels and lymph nodes.[8,9]

The alar fascia, a part of the deep cervical fascia, originates as a well-defined midline structure at the level of C1 vertebra and can be identified down to C6 vertebra, and does not reach the base of the skull.[10]

This thin fascia divides RS into 2 components: the anteriorly positioned, true or proper RS, and the posteriorly situated, danger space of Grodinsky and Holyoke. The alar fascia fuses with the visceral fascia obliterating the true RS. The danger space extends further inferiorly into the posterior mediastinum to the level of the thoracic diaphragm and is named as such, because it provides a conduit for infections to spread from the pharynx to the mediastinum.

Since the alar fascia is very thin, the danger space and the true RS cannot be distinguished by imaging in a healthy patient.[10] The symptoms depend on the amount of air, its location and the presence of infection, caused by the diffusion of microorganisms of the oral microbial flora in the submandibular space.

Patients have otalgia, Eustachian tube dysfunction with temporary hearing loss, dysphagia, dysphonia, compression of the venous trunks (congestive heart failure), compression of the trachea (asphyxia), compression of the sympathetic trunk, P, pneumopericardium, Ludwig's angina as well as mediastinal and pleural space infections.[11]

Air in the mediastinum can cause venous vasodilation and hypotension, hypercapnia, acidosis, and gaseous embolism. Death can occur due to a large gas bubble that prevents the ventricle from pumping blood into the pulmonary artery and to the lung.

The differential diagnosis of P includes Quincke's edema, allergic reactions caused by the local anesthetic, hematomas (in patients taking both anticoagulant and platelet aggregation inhibitors), and soft tissue infections.[12]

Palpable crepitus of the face and neck, crackling sounds in the lungs, the presence of Hamman's sign (crunching, rasping sound, synchronous with the heartbeat as a result of the heart beating against air-filled tissues), radiography, and cervical and thoracic CT facilitate making an accurate diagnosis.

## Conclusions

Sometimes patient can have breathing problems during or after dental and oral surgical procedures. This dyspnea can be caused by an allergic reaction to local anesthetic, but also by barotrauma and pneumothorax. Subcutaneous emphysema is a rare complication of dental procedures. The medical community should be aware of this complication. The appearance of sudden swelling and soft tissue palpable crepitus should give rise to a strong suspicion of SE. Careful observation of the symptoms and clinical course, and an early initiation of pharmacologic therapy (broad-spectrum antibiotics and 100% oxygen inhalation) are recommended. The high-speed dental handpiece and air/water syringe that exhaust air into the surgical field should not be used, even in the absence of a mucoperiosteal flap.

### ORCID iDs

Antonino Marco Cuccia https://orcid.org/0000-0003-1841-0764
Agostino Geraci https://orcid.org/0000-0002-5797-6288

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# Radiological imaging and orthodontic treatment in the case of growing patients after oncological treatment: Case reports

# Obraz radiologiczny i postępowanie ortodontyczne w przypadku rosnących pacjentów po leczeniu onkologicznym – opis przypadków

Izabela Michalak[1,A–D], Dorota Kuśmierczyk[2,C,E,F], Katarzyna Bluj-Komarnitka[3,A,D], Sadri Rayad[4,A], Małgorzata Zadurska[2,E,F]

[1] Maestria Digital Dental and Face Clinic, Warszawa, Poland
[2] Department of Orthodontics, Faculty of Medicine and Dentistry, Medical University of Warsaw, Poland
[3] Pediatric Dentistry Clinic, Medical Center in Marki, Poland
[4] Be Active Dentist Organization, Kozienice, Poland

A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation;
D – writing the article; E – critical revision of the article; F – final approval of the article

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**Address for correspondence**
Izabela Michalak
E-mail: izabelawieclaw@gmail.com

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## Abstract

According to the classification of the World Health Organization (WHO), the most common childhood malignant neoplasms include leukemia, lymphomas, and neoplasms of the central nervous system (CNS) and the sympathetic nervous system. Cancer diseases themselves as well as their treatment carry a high risk of both early and distant effects.

The most common dentition disorders resulting from the radiotherapy of the head area and chemotherapy in patients up to the age of 6 years include root agenesis, V-shaped roots, microdontia, hypoplasia of the maxilla and the jawbone, hypodontia, and enamel hypoplasia and hypomineralization.

Patients undergoing oncological treatment at the age of <6 years should receive adequate and long-term monitoring due to the possible distant effects of the underlying disease and its treatment. The radiotherapy of the head and neck region performed on a growing patient significantly worsens the patient's prognosis and results in a number of irreversible complications.

Planning appropriate orthodontic treatment in such patients contributes to increased comfort later in the patients' lives. Oncology patients do not require special orthodontic procedures; however, the high risk of complications does encourage the planning of orthodontic treatment with the least burden. Dental care for a young patient with a history of oncological disease requires the interdisciplinary cooperation of a pedodontist, orthodontist, prosthetist, and dental surgeon.

**Key words:** chemotherapy, radiotherapy, dental abnormalities, pediatric oncology

**Słowa kluczowe:** chemioterapia, radioterapia, nieprawidłowości zębowe, onkologia dziecięca

## Introduction

The World Health Organization (WHO) and the International Classification of Childhood Cancer (ICCC) state that the most common childhood neoplastic diseases include leukemia, Hodgkin's lymphomas, non-Hodgkin's lymphomas, central nervous system (CNS) tumors, and tumors of the sympathetic nervous system. Childhood tumors are rare, affecting 1 in 600 children during the first 15 years of life.[1] Early diagnosis and effective therapy of an oncological disease provides the patients with a chance for long-term survival.[2]

The main methods of treatment used in pediatric oncology include surgery, irradiation and chemotherapy. The use of combined treatment, conducted according to the latest standards of cancer treatment, increases the chances for treatment success and improves the survival rates during the developmental period after the oncological disease.[3] Both radiotherapy and chemotherapy demonstrate a number of side effects, causing damage to soft tissues and to the respiratory, cardiovascular, skeletal, and hormonal systems.[4] The destructive effect on the teeth at the developmental stage is also well-known. The complications of oncological therapy include tooth agenesis, root underdevelopment, V-shaped roots, microdontia, hypodontia, and tooth eruption disorders. These complications have a significant adverse effect on the patient's quality of life after treatment, and their severity often correlates with the intensity of therapy and the developmental stage of the patient.[5–8]

Tooth development is a long-term process which can be subject to internal and external factors. Amelogenesis and dentinogenesis may be disturbed by radiation when a beam is directed at the oral cavity or its surroundings. The teeth that are positioned along the radiation beam receive 45% of the administered dose.[9] However, radiation directed at distant areas of the body does not have a significant effect on the development of the teeth. According to Dahllöf et al., the radiation dosage which induces histological changes in the developing permanent tooth bud is 10 Gy.[10] High doses of radiation result in the death of ameloblasts and odontoblasts, regardless of their stage in the cell cycle. The development of dental tissues is suppressed, therefore the partially formed teeth, due to root agenesis, remain in the bone.[11]

The effects of direct irradiation of bones, soft tissues and blood vessels are dose-dependent and are most prominent in fast-growing patients (children under 6 years of age and children during puberty). Impaired blood supply to these areas may lead to osteoradionecrosis, but this disorder is rare in juvenile patients.[12]

Irradiation of CNS may inhibit the hypothalamic–pituitary function, resulting in reduced production of growth hormone and thyroid-stimulating hormone. Consequently, this may adversely affect odontogenesis and craniofacial development, causing asymmetrical facial bone growth, for example. Growth disorders and symptoms of premature puberty have also been observed.[13,14]

The short-term side effects of chemotherapy include cytotoxicity, bone marrow suppression, increased susceptibility to infection, xerostomia symptoms, more cariogenic bacteria in the oral cavity, and increased susceptibility to periodontal diseases and fungal infections.[15] The long-term complications of chemotherapy, reported in the literature, include missing tooth buds, microdontia, tooth crown hypoplasia, deficient enamel mineralization, and disturbed formation of the tooth root.[15–17] Chemotherapy-induced dental anomalies occur when the patient is treated before the age of 6 years. The degree of damage to the teeth and bones depends on the patient's age, and the type and duration of chemotherapy.

Despite these documented threats of temporary or permanent side effects, cancer treatment must be applied as a life-saving or necessary procedure for the patient. Modern methods of cancer treatment significantly increase the survival rates, which means that an increasing number of children are referred for orthodontic treatment after their cancer treatment.[18,19] The appropriate diagnosis of abnormalities, both systemic and local, helps in planning the proper treatment of any complications, including orthodontic ones, in oncological patients.[20] The management of patients affected by the complications in the masticatory system resulting from chemotherapy and radiotherapy is not significantly different from routine orthodontic procedures, yet it must take into account any existing disorders.

## Case presentation

### Case 1

At the age of 2 years, Patient B.D. was diagnosed with embryonal rhabdomyosarcoma, which was present in the nasal part of the throat, the paranasal sinuses, and partially in the orbits and the middle cranial fossa.

Due to the extent of the tumor, the patient was at first treated with chemotherapy, followed by surgical treatment, involving a partial removal of the neoplastic lesion. Subsequently, the chemotherapy continued along with the irradiation of the tumor-affected area, the sphenoidal sinus and the nasopharynx at a dose of up to 5.4 cGy/week. The treatment was completed in 2015.

The patient reported to the Department of Orthodontics at the Medical University of Warsaw (Poland) at the age of 8, 3 years after the end of cancer treatment (Table 1, Fig. 1–4).

Treatment with an orthodontic block appliance was planned at the first stage based on a clinical examination, and the analysis of diagnostic models and radiological scans. The construction bite was done at a 3-millimeter gap between the front teeth. The patient used the block appliance for 3 h during the day and overnight in order to relieve the front teeth and to increase the range of mandible abduction (Fig. 5).

**Table 1.** Examination of the patient, Case 1

| Case 1 | |
|---|---|
| Examination of the patient | |
| Extraoral | Intraoral |
| • asymmetry of the middle and lower face (eye socket, mouth corners and palpebral fissures)<br>• hypoplasia of the maxilla and the mandible<br>• shortened lower face | • reduction of mandibular abduction to 25 mm<br>• pink, moist mucous membrane<br>• marginal gingivitis<br>• present teeth: 21, 22, 26, 31, 32, 36, 41, 42, 46, 63, 73, and 83<br>• tooth 21 with pathological mobility (3rd degree)<br>• significant demineralization of the enamel (white and dark spots) |
| Radiological status at the age of 7 (2 years after the completion of cancer therapy) | |
| • a lack of buds for teeth 15, 25, 45, 35, 37, and 47<br>• a complete lack of roots for teeth 11, 12, 21, 22, 23, 13, 14, 24, 16, 26, and 46 (the apical foramen closed – inhibition of development)<br>• delayed tooth root development diagnosed in relation to teeth 41, 42, 31, 32, 33, 43, 34, 44, and 36 with the apical foramen open | |

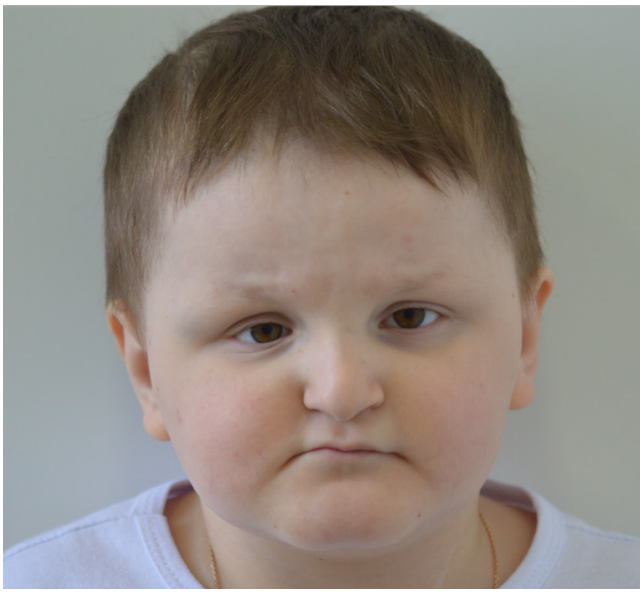

**Fig. 1.** Extraoral photo of patient B.D.

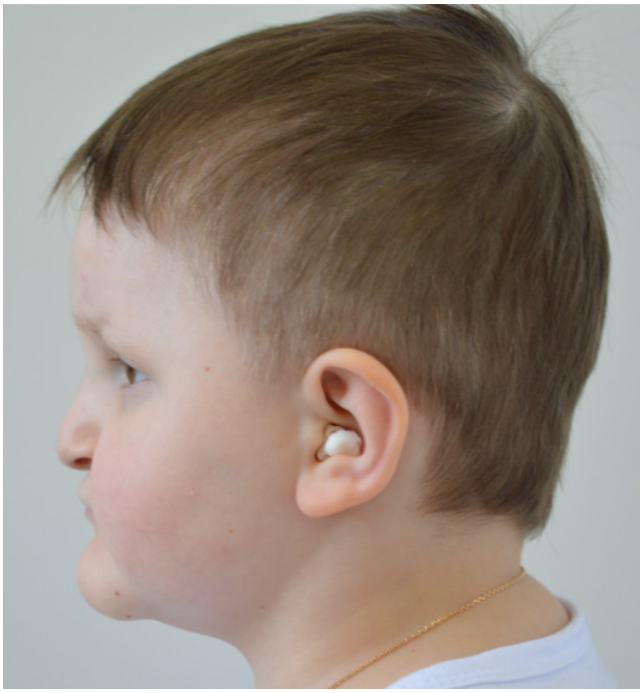

**Fig. 2.** Profile photo of patient B.D.

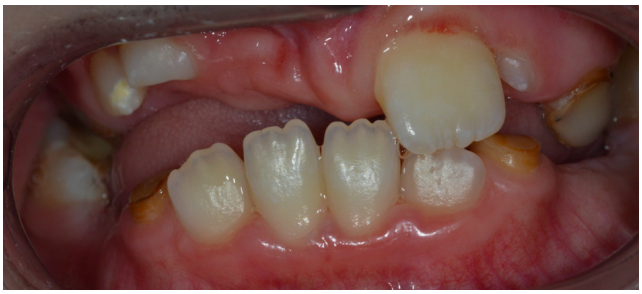

**Fig. 3.** Intraoral photo of patient B.D.

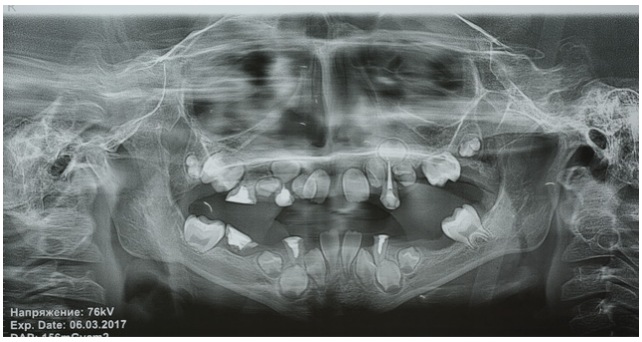


**Fig. 4.** Panoramic X-ray of patient B.D. at the age of 7

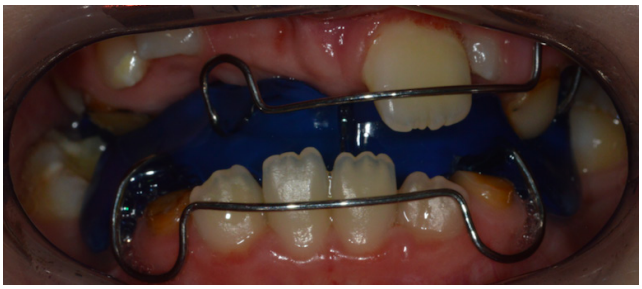

**Fig. 5.** Block appliance of patient B.D.

The patient's guardian was informed about the high risk of losing the permanent teeth with aplastic roots and the possibility of future prosthetic treatment. At follow-up, after 6 months of treatment with an orthodontic appliance, the range of mandible abduction had improved to 33 mm.

## Case 2

Patient J.G. was diagnosed with stage 4 Burkitt's lymphoma at the age of 4 years. Burkitt's lymphoma is a non-Hodgkin's lymphoma originating from B lymphocytes. The patient had neoplastic lesions in the abdominal cavity, which had additionally infiltrated the craniofacial bones and the thyroid gland. The cancer treatment included 10 months of chemotherapy (Table 2, Fig. 6–9).

Due to the low risk of complications, good overall health and the lack of other contraindications, it was possible to start orthodontic treatment earlier than after the normally required 2-year period since the completion of the oncological therapy. The first stage of treatment consisted in the preparation of child prostheses in order to restore the biting and chewing functions, to improve esthetics and to enable better pronunciation. The alveolar process of the maxilla and the alveolar part of the mandible showed signs of significant atrophy, resulting in unfavorable conditions for prosthetics. The patient's cooperation was limited, which made it difficult to perform clinical procedures (Fig. 10–12).

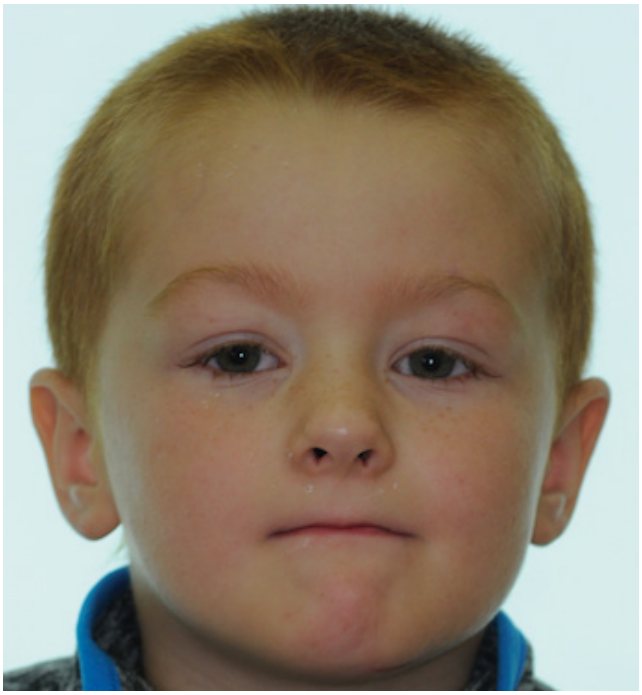

**Fig. 6.** Extraoral photo of patient J.G.

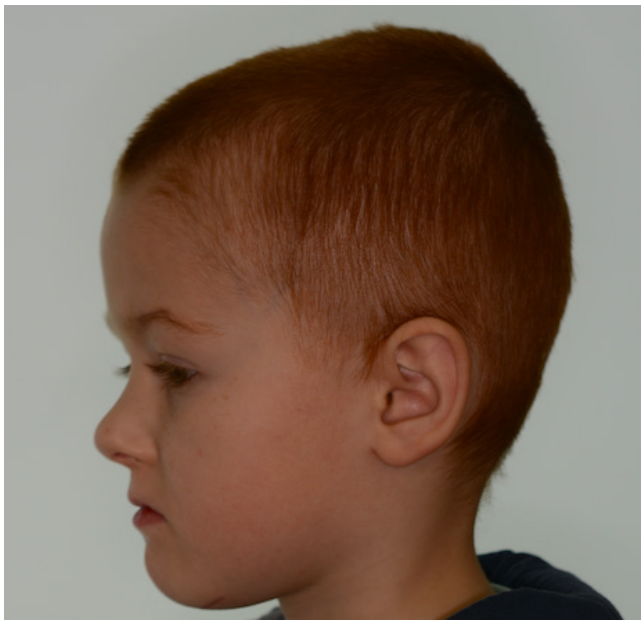

**Fig. 7.** Profile photo of patient J.G.

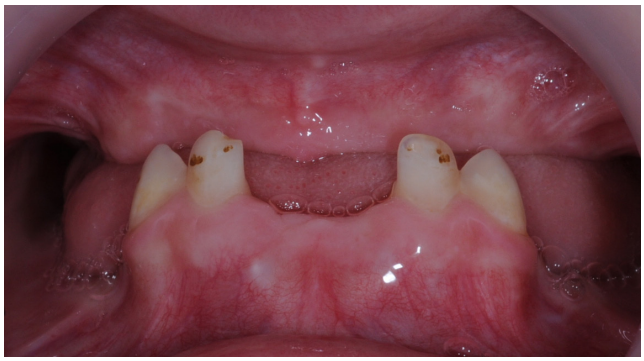

**Fig. 8.** Intraoral photo of patient J.G.

**Table 2.** Examination of the patient, Case 2

| Case 2 | |
|---|---|
| Examination of the patient | |
| Extraoral | Intraoral |
| • preserved facial symmetry<br>• sunken subnasal region<br>• shortened lower face | • extensive dental deficiencies (present teeth: 72, 73, 82, and 83)<br>• small spots of enamel demineralization<br>• significant atrophy of the alveolar process of the maxilla and the alveolar part of the mandible<br>• normal mucous membrane |
| Radiological status at the age of 6 (2 years after the completion of cancer therapy) | |
| • a lack of buds in the case of permanent teeth 45 and 15<br>• delayed development of the roots of the permanent teeth (narrow, V-shaped roots)<br>• teeth 46 and 36 in Demirjian's developmental stage F<br>• buds of teeth 17, 27, 37, and 47 in Demirjian's stage C show signs of enamel formation on the chewing surface, small amounts of dentin are visible<br>• teeth 41, 42, 31, and 32 demonstrate Demirjian's stage F – roots of an inverted funnel shape, with the apical foramen open<br>• teeth 43, 33, 34, 34, 25, 24, 45, and 14 show the beginning of root formation, corresponding to Demirjian's stage D | |

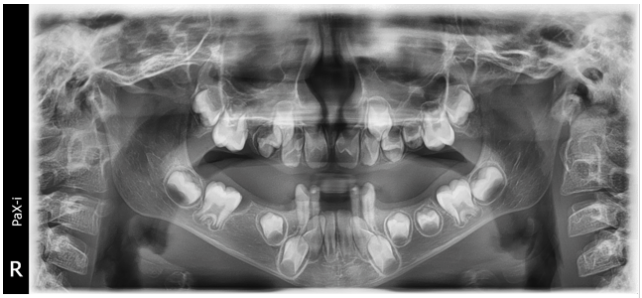


**Fig. 9.** Panoramic X-ray of patient J.G. at the age of 6

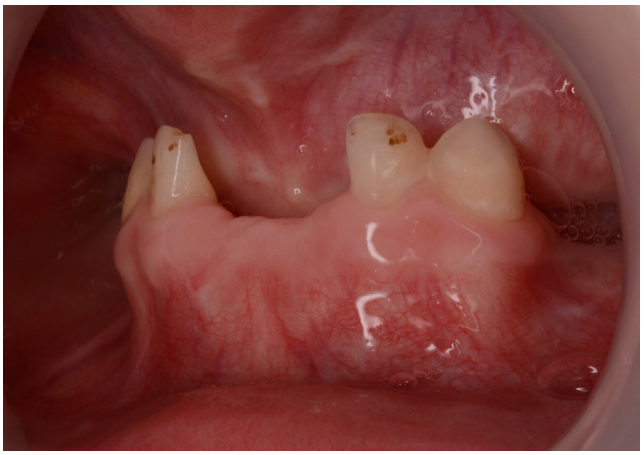

**Fig. 10.** Intraoral photo of the condition of the maxilla and the madible of patient J.G.

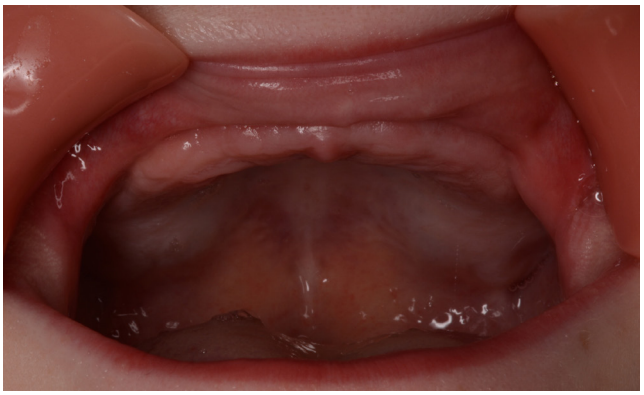

**Fig. 11.** Intraoral photo of the maxilla of patient J.G.

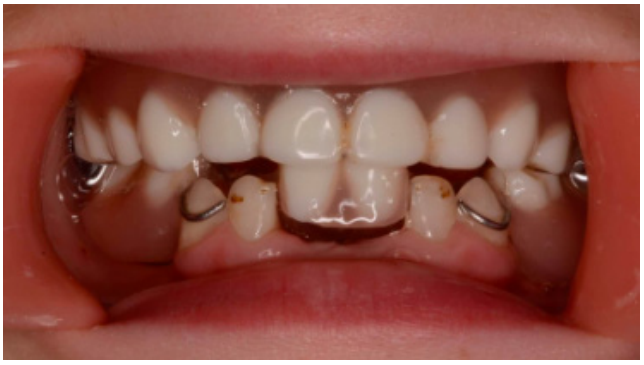

**Fig. 12.** Patient J.G. with the appliance after 6 months of use. Lack of proper stability of the appliance

After 6 months of treatment, the lower appliance showed poor stability due to the growth of the patient and the eruption of the molars. At follow-up at the age of 7, the eruption of teeth 46, 36 and 26 had begun, with minor signs of disturbed enamel mineralization in the form of small white spots.

## Discussion

When planning orthodontic treatment of a patient with a history of a neoplastic disease, it is important to work with their oncologist. The patient's health status and prognosis should be considered in their therapy.

The following factors of an increased risk of complications are mentioned in the literature: cancer diagnosis at the age of <8 years, radiotherapy of the entire body or of the head and neck (with a dose of over 2.4 cGy), a diagnosis of a solid tumor (located in the craniofacial region, in particular), hypothyroidism, hypopituitarism, <2 years passed since the end of chemotherapy or radiotherapy, stem cell transplantation, abnormal development of the tooth root, and gingival hypertrophy after treatment with drugs such as cyclosporin A.[21,22] In the literature, there can be found recommendations to start orthodontic treatment at least 2 years after cancer therapy has finished, when the patient's oncological disease is in a state of permanent remission. The recommended withdrawal period was maintained when treating the patients presented herein. When performing extractions due to orthodontic indications, the 24-month waiting period should be observed because of the increased risk of osteonecrosis after completing radiotherapy. Oncological patients whose treatment consisted only of surgical procedures, without irradiation or chemotherapy, have no indications to postpone the commencement of orthodontic treatment.[23]

Comparing the first patient, who underwent chemotherapy and radiotherapy, with the other one, who was subjected only to chemotherapy treatment, it can be concluded that radiotherapy directed at the craniofacial region at a developmental age significantly worsens the prognosis. It inhibits or significantly delays the development of the permanent teeth buds, disrupts the development of the craniofacial bone – which may lead to an unsightly asymmetry of the face – and it additionally contributes to disrupted enamel mineralization.

Due to the possible consequences of cancer therapy – increased susceptibility to infections, reduced immunity and a tendency for mucous membrane atrophy, not to mention xerostomia – appliances with the lowest possible irritation of the mucous membrane should be selected in case of such complications. When salivary secretion is disturbed, the risk of caries development is additionally increased; thus, more frequent fluoridation and more frequent oral hygiene check-ups are recommended.[24] In the cases of the patients presented herein, there was no

disturbance of salivary secretion or damage to the oral mucosa. The tolerance of both the block appliance used to treat the first patient and of the prosthetic plates in the other patient was very good.

It is important to assess the rate of growth in the patient and to consider administering growth hormone to normalize the pattern of craniofacial bone growth.[25] Craniofacial bone development disorders can be observed in the first patient, in whom the location of the tumor itself influenced the deformations. The tumor infiltrating the basal area of the skull, the effects of surgical tumor resection and radiation therapy directed at the craniofacial region all contributed to the asymmetry of the eye sockets, reduction in the length of the face and inhibited development of the mandible. On the other hand, the collapse of the subnasal area and the shortening of the lower face in the second patient were mainly due to the premature loss of the deciduous teeth, atrophy of the alveolar process of the maxilla and the alveolar part of the mandible, and reduced height of occlusion. However, skeletal changes occurring as the patient grew cannot be ruled out.

Symptoms of temporomandibular joint dysfunction are observed in patients undergoing the combination therapy of chemotherapy and radiotherapy of the entire body. Patient B.D. in this study suffered from lock-jaw, which subsided with the use of a relieving appliance. The reduced extent of the mandibular abduction caused difficulties in obtaining impressions and adjusting the orthodontic appliance.

Oncological patients do not require special orthodontic procedures, yet the high risk of complications encourage the planning of orthodontic treatment with the least burden. It is recommended to use low force in order to minimize the risk of root resorption (since the roots have already been shortened as a result of radiotherapy), to accept the compromised results of simple mechanical treatment, and to complete orthodontic treatment earlier than usual or to treat only the upper dentition.[26,27]

Orthodontic treatment must include a risk assessment regarding the loss of the teeth with significantly shortened roots. There are no contraindications to implantation in patients with a medical history of a neoplastic disease, but due to the continued growth of a minor patient, it must be postponed. Patients should have any missing teeth replaced with prostheses and plate prostheses until fixed, implant-based prosthetic restorations can be provided.[28–30] The example of patient J.G. illustrates the problems which may appear in oncological patients with extensive tooth deficiencies. The loss of the alveolar processes in the maxilla and the alveolar region of the mandible resulted in unfavorable cconditions for the prosthetic base. Initially, the stabilization of the complete superior prosthesis and partial inferior prosthesis was obtained, allowing the patient to use prostheses, but not at mealtimes – the patient was unable to adapt to eating with prostheses. Unfortunately, there was a rapid loss of the initial prosthesis stabilization due to the patient's growth and to the progressive eruption of the permanent teeth.

As cooperation with the patient is limited and he is reluctant to undergo clinical procedures, it is difficult to prepare new prosthetic restorations at the intervals of several months, though the patient does regularly report to follow-up appointments for the tooth eruption to be observed. The eruption of the first permanent molars should improve the stabilization of subsequent prostheses.

## Conclusions

Based on the analysis of the available publications, it can be concluded that among patients who were subjected to oncological treatment before they reached the age of 6 years, the following complications of the masticatory system are most commonly observed: hypodontia, abnormal structure of the tooth roots, enamel abnormalities, premature loss of the permanent teeth, xerostomia, and gingivitis. Radiotherapy and chemotherapy are independent risk factors for adverse dental and facial bone effects. Patients undergoing oncological treatment at the age of <6 years should receive adequate and long-term monitoring, owing to the possibility of distant effects of the underlying disease and its treatment. Radiotherapy of the head and neck region administered to a growing patient can significantly worsen their prognosis and may result in a number of irreversible complications.

### ORCID iDs

Izabela Michalak https://orcid.org/0000-0002-0223-4279
Dorota Kuśmierczyk https://orcid.org/0000-0003-3880-6045
Katarzyna Bluj-Komarnitka https://orcid.org/0000-0001-8664-6299
Sadri Rayad https://orcid.org/0000-0003-3415-369X
Małgorzata Zadurska https://orcid.org/0000-0002-2303-4102

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