Official Publication of
Istanbul University
Faculty of Dentistry

European Oral Research

Volume 57 ▪ Issue 2 ▪ May 2023

ISSN print 2630-6158 ▪ ISSN online 2651-2823

EOR

eor.istanbul.edu.tr

Istanbul University Press

European
Oral Research

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PUBLISHER

İstanbul University Press

İstanbul University Central Campus,

34452 Beyazit, Fatih / İstanbul, Turkiye,

Phone: +90 (212) 440 00 00

The publication languages of the journal is English.

This is a scholarly, international, peer-reviewed and open-access journal published triannually in January, May and September.

Publication Type: Periodical

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Journal Article in Print

1. Burrow MF, Tagami J, Negishi T. Early tensile bone strengths of several enamel and dentin bonding systems. J Dent Res 1994; 74: 522-8.

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2. McKeage K. Tobramycin inhalation powder: a review of its use in the treatment of chronic pseudomonas aeruginosa infection in patients with cystic fibrosis. Drugs 2013; [Epub ahead of print] Available from: http://link.springer.com/article/10.1007%2 Fs40265-013-0141-0

Book

3. Mueller HJ, Freeman D. FT-IR spectrometry in materiolography. 2nd Ed., Ohio: American Society for Metal 1994, p.51-56.

Chapter in a book

4. Alexander RG. Considerations in creating a beautiful smile. In: Romano R, editor. The art of the smile. London: Quintessence Publishing, 2005, p.187-210.
5. Hudson FB, Hawcroft J. Duration of treatment in phenylketonuria. In: Seakins J, Saunders R, editors. Treatment of inborn errors of metabolism. London: Churchill Livingstone, 1973, p.51-56.

Thesis

6. Maden I. Effect Of Nd:YAG Laser Treatment In Addition To Scaling And Root Planning. Doctoral Dissertation, Istanbul University Institute of Health Sciences Periodontology Department, 2009.

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Figure 1. Panoramic radiograph of the patient taken 6 months after surgery, note irregular borders of the lesion.

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Table 1. *Concise explanation of the table contents (SD: standard deviation, CTA: cartilage tissue area, NBA: new bone area).*

	Control group (Mean % ± SD %)	First group (Mean % ± SD %)	Second group (Mean % ± SD %)
CTA	21.41 ± 4.2	2.5 ± 2.4	11.42 ± 4.2
NBA	11.48 ± 0.2	21.41 ± 14.22	11.41 ± 4.2

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European Oral Research

Official Publication of Istanbul University Faculty of Dentistry

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Original Research Articles

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Official Publication of Istanbul University Faculty of Dentistry

Eur Oral Res 2023; 57(2): 60-67

Review

Pathway to mercury-free dentistry: an insight into past, present, and future

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Purpose
The popularity of dental amalgam arises from its excellent long-term performance, ease of use, and low cost. However, there is a concern about the potential adverse health effects arising from exposure to mercury in amalgam. This review article critically discusses the safety of dental amalgam as a restorative material and our preparedness for a mercury-free road ahead.

Materials and Methods
A database search was performed on PubMed and Google scholar using the keywords: "mercury-free dentistry", "mercury toxicity", "amalgam substitutes", "amalgam mercury toxicity". Inclusion and exclusion criteria were specified clearly. Relevant literature was also searched in the dental textbooks.

Results
Around 40 articles, highlighting mercury exposure among dental professionals and patients were included. Despite the overwhelming body of scientific evidence demonstrating amalgam to be a safe restorative material, concerns about the toxic effects of mercury persist.

Conclusion
The real challenge is to find a suitable amalgam substitute and to follow the mercury hygiene measures closely.

Keywords: *Amalgam, Mercury toxicity, Minamata Convention, Amalgam substitutes, Amalgam alternatives*

Introduction

Technically, an amalgam is a mixture of mercury (Hg) and another metal. Dental amalgam is a combination of mercury and a silver-tin (Ag-Sn) alloy (1). Amalgam was first used in Chinese literature in 659, and it has remained the most widespread and successful restorative material in dentistry for the past 160 years (2). Amalgam's popularity stems out of its superior long-term results, convenience of use, and affordability (1, 3). Prior to the 1970s, more than three-fourths of total restorations were made with amalgam. However, over the last 30 years, the use of amalgam has decreased globally. This is due to a decrease in caries incidence, an increase in the use of cast restorations such as crowns, ceramic inlays, and onlays, and the availability of direct filling tooth-coloured alternate restoratives for certain applications (2).

Despite its widespread use and prominence as a restorative material, there have been apprehensions about the potential negative health effects of mercury exposure in amalgam (2). Mercury, like all other materials, can be dangerous if not handled correctly. To ascertain that mercury does not dissipate into the oral cavity, the alloying reaction of mercury with the Ag-Sn alloy must be completed. Once the reaction is finished, in-

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Received: 2 January 2022
Revised: 20 July 2022
Accepted: 9 August 2022

DOI: 10.26650/eor.20231050091

How to cite: *Dudeja P, Kumar Dudeja K, Grover S, Singh H, Jabin Z. Pathway to mercury-free dentistry: an insight into past, present, and future. Eur Oral Res 2023; 57(2): 60-67.* DOI: 10.26650/eor.20231050091

credibly low levels of mercury, well below the existing health standard, can be released (4).

The impact of mercury obtained from amalgam to the total body load has been much debated, but it seems to be minimal. The key point to remember is that mercury gets into the body on a daily basis, irrespective of the restorative materials used in the oral cavity. Under normal physiological conditions, mercury undergoes biochemical processing and is excreted from the body. As long as the levels are low, there is no danger of mercury toxicity (4). Shortly after amalgam came into use in the United States, there were reports of mercury problems.

Material and Methods

A search of the PubMed indexed database (www.ncbi.nlm.nih.gov/pubmed) over the last 37 years (custom range: 1983 – 2020) was conducted using keywords/phrases such as "mercury-free dentistry" (yielded 23 results); "mercury-free" (195 results); "mercury toxicity" (10,862 results); "amalgam substitutes" (132 results); "amalgam mercury toxicity" (514 results); "amalgam alternatives" (455 results) and Google scholar database was conducted using phrases "mercury-free dentistry" (yielded 60,300 results); "amalgam substitutes" (31,100 results); "mercury-free dentistry era" (17,900 results); "amalgam alternatives" (71,900 results). Relevant literature on dental amalgam and its substitutes and alternatives and mercury toxicity was also searched in dental textbooks. Around 40 of these articles were deemed appropriate for inclusion. All review articles, original articles, in vivo/in vitro studies, and controlled clinical trials were considered for inclusion in this review. More emphasis was laid on studies focusing on mercury exposures among dental professionals and dental assistants and exposure to patients from mercury present in amalgam restorations. Appropriate data was collected, pooled, and finally synthesized.

Brief history of amalgam

- In 1603, Tobias Dorn Kreilius created Amalgam fillings by dissolving copper sulfide with strong acids and mercury.
- In 1818, Louis Regnart (who is called Father of Amalgam) lowered the temperature of D' Arcet's Mineral Cement by adding more mercury to it. *D' Arcet cement* was similar to the mixture produced by Kreilius and was earlier introduced in France. It had to be boiled and then poured into the tooth.
- In 1826, M. Auguste Taevaeu (5) developed the silver paste-mercury mixture.
- In 1833, the Crawcour duo (4), British businessmen, recognized the potential of Taevaeu's silver-mercury mixture, brought the concept to New York, and endorsed the material as a cost-effective and easy-to-use restoration material.

However, no consideration was given to the appropriate mercury-to-alloy proportions or the alloy form used. The alloy-mercury mix was created majority of the time by rubbing fragments of varying composition silver coins with a file. Slow-setting amalgams were created due to inconsistencies in materials and techniques, transferring mercury from the unset mass into exposed dentine. Although no casualties have been published, multiple incidents of pulp death occurred (4).

First amalgam war

The American Society of Dental Surgeons issued a warning about the dangers of amalgam in 1845 (6). This society declared all filling materials other than gold to be toxic, igniting a complex battle between dental professionals using gold foil restorations and those employing amalgam. Due to known toxicity of mercury, the members of this group swore to refrain from its use. The numerous discussions and assertions that erupted over dental mercury marked the beginning of an era known as the "Amalgam War."

The American Society of Dental Surgeons had dissolved by 1856 as a result of the Amalgam War, and the American Dental Association (ADA), an organization that endorsed the usage of amalgam, became the nation's new face. Nevertheless, concerns about dental amalgam remained. An article published in Chicago Medical Journal article in 1873 warned of *"thousands of individuals all over the globe being poisoned by pernicious sublimate produced in the oral cavity from dental amalgam inserts in the teeth"* (7).

According to Dr. E. Talbot's (8) research published in the Ohio State Journal of Dental Science (1882), amalgam will generate mercury fumes. F. Flagg's work, on the contrary, promoted the use of amalgam. In 1896, Dr. G.V Black (9) also published a comprehensive research article recommending the use of amalgam. Nonetheless, it took years for the dental profession to acknowledge Dr. Black's insights.

Second amalgam war

Due to potentially harmful mercury release, there were periodic calls to ban the use of amalgam. Dr. J. Tuthill's (7) study on "Mercurial necrosis caused by amalgam fillings," which was released in The Brooklyn Medical Journal in 1898, was a watershed moment. The dental amalgam debate raged on into the early 20th century, when technological progress allowed for numerous studies to confirm that mercury poses a hazardous risk when triturated with silver alloy and filled in teeth. In 1926, Alfred Stock, a German doctorate and chemist by profession, launched the so-called Second Amalgam War. Dr. Stock was exposed to significant amounts of mercury while operating in his facility because the amalgam tablet used at that time had to be warmed up in a ladle until the crystals of mercury popped up before being moved to a mortar and pestle for trituration. The above process resulted in the emission of a large amount of mercury vapour (9). Dr. Stock's concerns prompted the formation of an inquiry commission to look into his accusations. In its report in 1930, the commission validated the safety of newer amalgam formulations that did not require heating and were rapidly replacing older compositions (9).

Third amalgam war

Another major dispute arose in 1980, when Dr. Hal Huggins, a Colorado dentist, began spreading the notion that amalgam restorations were responsible for a variety of ail-

ments. He authored a work in 1985 stating unequivocally that amalgam restorations emit sufficient mercury to induce neural, cardiac, autoimmune, connective tissue, psychological, and inflammatory disorders (9). According to a 1995 study, 8.7% of dental professionals sought to abolish amalgam from their practices while 14.3% were unsure about its safety (9). For nearly 10 years, a dedicated group of consumers and allied health professionals worked towards getting amalgam banned.

The media played a significant role in fuelling opposition to amalgam, particularly in its "1 Hour" section that aired on television in 1990 (9). Clinicians having a large social following, such as Dr. Robert Atkins, and Dr. Andrew Weil, wrote best-selling health books warning the masses concerning possible dangers of amalgam restorations. In response to public uproar, dental professionals, the National Institute of Health-National Institute for Dental Research (NIH-NIDR), the Food and Drug Administration (FDA), and many other prominent organizations convened a meeting with world-renowned scientists and clinicians. It was unanimously agreed that, while more research into amalgam was needed, it could not be assumed that amalgam posed a serious health risk (4). Those people strongly advised against removing amalgam restorations due to this fear.

Allegations about the dangers of amalgam keep appearing in daily papers, non-scientific magazines, and, on occasion, journals. However, all documented research indicates that no causal connection exists between dental amalgam and other medical issues.

Mercury generation potential

Mercury is ubiquitous in nature. Each year, between 2,700 $x10^3$ Kg and 6,000 $x10^3$ Kg of mercury are discharged into the atmosphere from the ocean waters and the Earth's crust. Human activities such as the combustion of household and industrial wastes, as well as the combustion of fossils fuels, emit nearly 2,000 - 3,000 tonnes. In 2000, Asian countries contributed approximately 54% of global mercury emissions from anthropogenic sources, accounting for more than half of total production. Mercury waste is also produced during various dental procedures. With so many dental colleges and institutions in India, estimating the amount of mercury waste generated and its proper disposal is difficult. According to one study, dental offices produced approximately 4000 kg of mercury per year, with 1000 kg of mercury flowing into the region's waste water (10).

Mercury toxicity and exposure recommendations

Mercury can be found in three different chemical forms: elemental (valence 0), inorganic (valence +1 and +2), and organic (alkyl and aryl). The physiochemical properties, absorption and excretion levels, tissue distribution patterns, as well as toxicological profile of these forms differ (2). The most volatile of the three is elemental/metallic mercury, with mercury vapour in air being its most common form. A careful examination of mercury hygiene procedures confirms that the most dangerous times are when elemental mercury is present as a liquid or vapour. As a vapor, metallic mercury is easily absorbed through lungs at 80% efficiency (Table 1). Inorganic mercury is typically quarried as an inorganic sulphide (cinnabar ore), which is burned in air to oxidize and help push off sulphur while collecting liquid mercury. There are many other water-soluble inorganic compounds of mercury which release mercury ions into solution. They are used in pharmaceuticals and are sparsely absorbed through the respiratory tract but rapidly absorbed through the digestive system (4). The organic compounds of mercury are found in food and drinking water and are particularly associated with sea food. This type of mercury is the primary source of mercury exposure for the vast majority of people. Although methyl mercury is easily absorbed from food, it is excreted less efficiently than all the other forms of mercury. This type of mercury tends to accumulate in the brain, liver, and kidney (4). The Environmental Protection Agency (EPA) estimates total daily exposure to methyl mercury (organic mercury compound) at 5.8 micrograms (ug), while Clarkson and colleagues (2) estimate 2.3 ug. The amount of elemental mercury inhaled from the air is estimated to be between 40 and 120 nanograms each day (2).

Table 1. *Absorption efficiency of mercury*

	Skin	Lungs	Gastrointestinal Tract
Elemental	-----	80%	0.01%
Inorganic	-----	80%	7%
Organic	-----	-----	95-98%

It has been proposed that microbes in the oral or digestive tract can convert metallic mercury to methyl mercury. Specific microorganisms found in seawater are often also believed to be capable of this conversion. This organic mercury then builds up in the organs of fish and other marine creatures, eventually reaching humans who consume seafood. A classic prototype of this phenomenon is Minamata disease. For many years, waste material with significant levels of metallic mercury was dumped into the ocean surrounding Minamata Bay in Japan. Fish from all these bodies of water became adulterated with methyl mercury, resulting in severe toxicosis that caused death and prolonged overdoses that ultimately led to neurological disturbances, which is currently recognized as *Minamata disease*. Congenital Minamata disease was another teratogenic effect. The minimum dose of methyl mercury required to develop these Minamata disease symptoms is estimated to be 5 milligrams per day (9).

The toxic effects of different mercury forms have been well recorded and studied, primarily in communities exposed to high levels of occupational or environmental mercury (Figure 1). As a result, various regulations for limiting work - related mercury exposure have been established. The National Institute for Occupational Safety and Health (NIOSH), and the Occupational Safety and Health Administration (OSHA) have adopted a threshold limit value (TLV) of 50µg mercury vapour per cubic metre of the breathing zone air for eight hours per day, 40 hours per week. The World Health Organization (WHO) has established a TLV of $25ug/m^3$ for work - related mercury exposure (2).

Fawer *et al.* (11) reported in 1983 that industrial workers (n=26) exposed to work - related mercury at a time-weighted estimate of $26ug/m^3$ in the place of work for a mean of

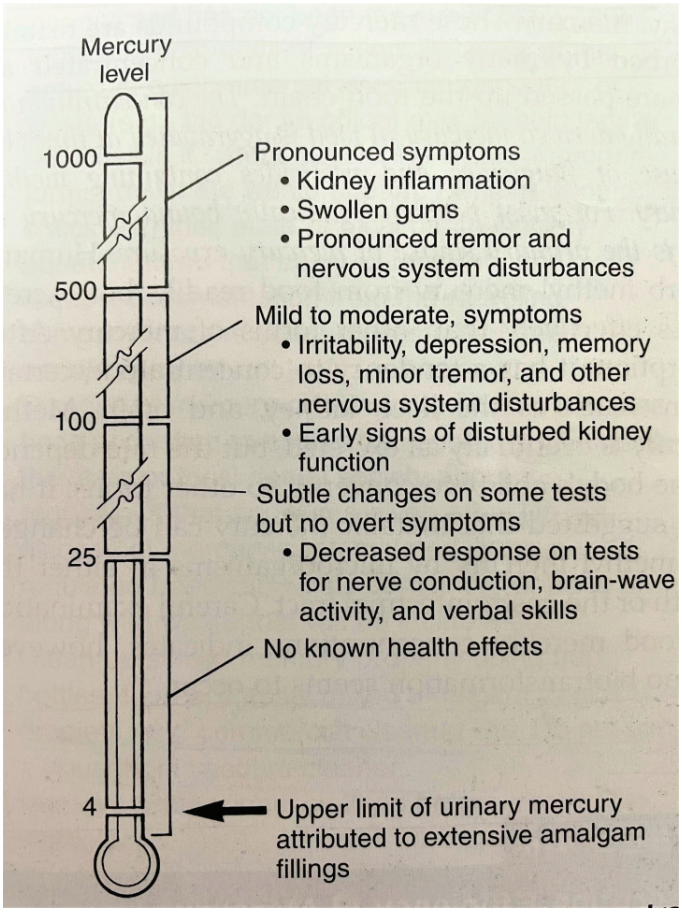

Figure 1. *Mercury thermometer portraying different levels of mercury.*

15.3 years had a substantial rise in hand tremors versus a control group. *Mackert and Berglund* (12) delved deeper and discovered that the hand-tremor trial was not blinded and the participants' medical and prior records were unknown. There was also hardly any reference of any additional sources of mercury ingestion or egestion. The specimen size was limited, and no dosimetric relationship had been identified. Using Fawer and colleagues' study as a guide, the Agency for Toxic Substances and Disease Registry established the minimum risk level (MRL) for prolonged human mercury inhalation in ambient air at 0.3ug/m^3. The MRL is defined as the level of mercury vapour to which a person can be consistently subjected without suffering any health problems. The environmental protection agency also recognizes 0.3ug/m^3 as the inhalation benchmark concentration for metallic mercury in air.

Dental professionals and mercury exposure from amalgam

The health hazards associated with amalgam use are definitely higher for dentists instead of patients. Dental personnel are exposed to mercury in several ways, including:

- Through direct physical contact with liquid mercury / newly triturated dental amalgam
- Inhaling mercury vapours (at the rate of 2-28 µg/ facet surface/ day) through
  - Inadvertent mercury splash (Figure 2)
  - Defective amalgamators
  - Faulty amalgam capsules (Figure 3)
  - Broken bulk mercury dispensers
  - Trituration and amalgam condensation
  - Polishing or removal of amalgam restorations (Figure 4)
  - Mercury vaporization from tainted instruments and
  - Open collection of amalgam scrap or used capsules with inadequate alloy to entirely consume the available mercury (Figure 5)

A case of Idiopathic Thrombocytopenic Purpura (ITP) caused by vacuuming spilled mercury has been reported in the literature. Research shows that mercury exposure among dentists has been slowly declining, most likely due to the American Dental Association's recommended mercury handling practices. Urinary mercury concentrations in dental professionals averaged 19.5ug/L in 1980, 6.7ug/L in 1986, and 4.9ug/L in 1991 (13). Decharat *et al.* (14) measured airborne and urinary mercury exposure among Thai dental

Figure 2. *Inadvertent mercury splash.*

Figure 3. *Amalgam capsules.*

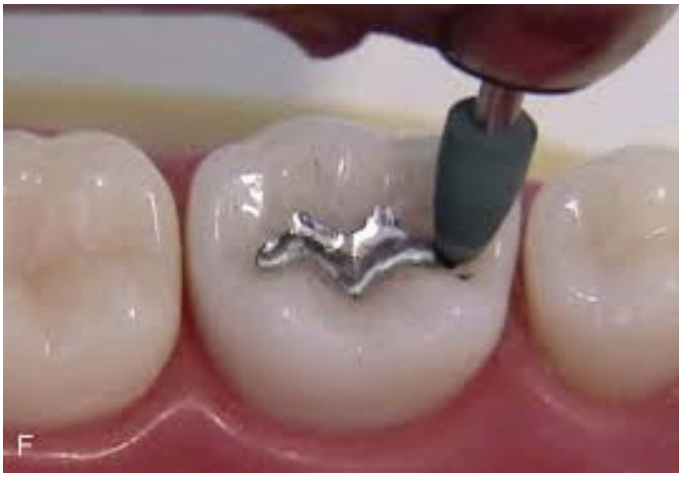

Figure 4. *Mercury vapour released during polishing of amalgam restorations.*

Figure 5. *Chair side methods of trapping amalgam.*

healthcare staff and discovered that urinary mercury levels averaged 8.24±1.89ug/g creatinine (range 2.0-22.84ug/g creatinine). The majority among them had urinary levels of mercury below 20ug/g creatinine, which is the American Conference of Governmental Industrial Hygienists (ACGIH) recommendation for mercury in urine. Urinary mercury concentrations in dental caregivers exposed to unsafe working conditions reached 22.84ug/g creatinine. According to the authors, mercury exposure was found to be directly related to hygienic practices. Ferracane *et al.* in 1994 (2) determined that exposure to metallic mercury vapour from mercury discharges in the dental clinic survived about ten to twenty minutes in a well-ventilated operatory; and in poor ventilation, mercury vapour densities reverted to levels below NIOSH's TLV in less than 30 minutes. The researchers indicated that mercury stayed in vapour state just for a short time due to its density and predilection for substrates, implying that a single unforeseen spill would presumably not constitute a key cause of mercury in a properly ventilated dental operatory. When proper infection-control practices were followed, inhalation of toxic vapours during amalgam restoration placement was found to be negligible. Even though substantial amounts of mercury may be produced during restorative treatment, high-volume evacuation can eliminate approximately 90% of them. Langworth *et al.* (1997) (15) observed that the levels of mercury generated during restorative procedures in dental clinics averaged around 2ug mercury/m^3, with no adverse health effects on the personnel. When the high-volume evacuator was used, mercury vapour thresholds with in dentist's ambient environment were minimal (1 to 2ug/m^3); without it, mercury vapour thresholds were 2 to 15 times greater than the WHO TLV. According to these researchers, the mercury level fluctuated significantly during the removal process, with peaks lasting only a few seconds (2).

Nagpal *et al.* (16) conducted a study and found that while some professional practices enhanced the level of mercury exposure, it still was well below prescribed standards. Dentists mentioned greater medical issues than the control subjects, many of which were neurological symptoms. Initial symptoms revealed by dental practitioners may be related to low-level, prolonged occupational mercury exposure, but they could even be the result of ageing, work - related abuse, and anxiety. The study encouraged dental professionals, researchers, and educators to use good work practices.

Rowland *et al.* in 1994 (2) studied female dental assistants and noted that females exposed to high levels of occupational mercury vapour were less procreant than those who were not exposed. Low mercury exposed subjects, on the other hand, were more fertile than unexposed comparison group. Mocevic *et al.* in 2017 (17) conducted a cross-sectional study in 529 males from Greenland, Poland, and Ukraine to examine semen properties and serum concentrations of androgens in connection with ambient mercury exposure. They found no evidence that environmental mercury exposure in men with median whole blood concentrations up to 10 ng ml^{-1} has an adverse effect on male reproductive health biological markers.

Mercury exposure from amalgam restorations in dental patients

It has been well understood that amalgam restoration placement and removal can lead to large intraoral mercury vapour peaks. Engle and colleagues, in an in vitro study demonstrated that dry polishing of amalgam restorations released 44ug of mercury fumes for every restoration (2). In-vivo amalgam removal on the other hand, resulted in releasing 15 to 20 ug of mercury vapour from one restoration. The brief period of these inhalation exposures, however, is deemed insufficient to induce any adverse health effects and the placement and removal of dental amalgam does not seem to pose a serious health risk to patients. Sällsten *et al.* (18) investigated the effect of protracted repeated nicotine gum chewing on plasma and urine mercury content. Mercury levels in gum chewers were significantly higher (nearly four times) than the average reading in Swedish dental personnel, but lower than the levels that could cause harmful effects. This research furthermore highlights the importance of carefully selecting a comparison group in clinical studies when establishing standard mercury exposure levels.

Chronic mercury exposure of the patients due to mercury generated from dental amalgam post insertion has also been studied. It was long believed that hardened amalgam did not generate mercury. However, numerous researches in the 1970s and 1980s found it otherwise (19). Early estimates of the average daily dose in people who have not been exposed to mercury at work ranged from 1.24 to 1.27ug/day, but latest evidence indicates a comparatively lower mercury dose from amalgam (2). According to Halbach in 1995 (20), mercury release was proportional to restoration time and surface area.

The possible implications of mercury discharge from amalgam on pregnant females' foetuses and new-borns have

been studied. The findings revealed that the mercury content in foetal and infant liver, kidney, and brain samples correlated positively with said dental amalgam restorations in mothers. However, the study design and data analysis method are highly dubious. A variety of methods can be used to estimate the average diurnal amount of mercury released from set amalgam restorations. Several variables, such as the number and age of restorations, the form of dental amalgam used, the surface area of the restoration, the quality of the restoration, methods of measuring mercury, and data analysis, all may be responsible for reported differences in results from different studies. Certain foods have been demonstrated to lower intraoral mercury vapour levels. If all masticatory period is assumed to increase vapour levels, then everyday mercury exposure from amalgam is overstated (2).

Mercury hypersensitivity

Mercury hypersensitivity is a response of the immune system to extremely low levels of mercury (4).Though poorly understood; it has been at times claimed as a potential hazard. However, the percentage of people who have been identified as plausibly hypersensitive is small, and the sensitivity reaction is mild and not serious. Mackert et al. (4) and Mandel et al. (4) studied this condition and scientifically disproved the hypothesised problems.

Amalgam illness

Amalgam illness refers to a condition that is typically self-reported by patients and is attributed to mercury vapour inhalation from established amalgam restorations (19). Symptoms differ from those seen with traditional mercury poisoning and include lethargy, poor concentration, muscle aches, and immunologic disorders. There is a lot of uncertainty about the establishment of amalgam illness because of absence of diagnostic symptoms, as well as any approved clinical test for its detection. This is complicated further by the fact that several mental illnesses demonstrate symptoms identical to ones caused by amalgam restorations. According to two surveys, 70% of sufferers asserting amalgam illness had a psychiatric condition, in contrast to 14% in a comparison group.

National and international events

The discussion over dental amalgam extends well beyond a single country's borders; in fact, countries all over the world are dealing with the same issues (21).

Denmark's regulation on mercury-containing products

According to a document released by an ad hoc working group of the European Commission (EC), the Environment Ministry released an order (No. 520) on June 9, 1994, prohibiting the trade of all mercury-containing commodities, including dental amalgam, to improve national climatic conditions. It was emphasized that the prohibition action will not go into effect unless there is a sufficient amount of clinically acceptable alternative restorative materials (non-amalgam substitutes) available.

Mercury regulations in Sweden

Barring isolated incidents of allergic reactions, there is no research evidence that mercury in dental amalgam has a negative impact on the body. According to the advisory committee, there is no medical basis to recommend amalgam removal to alleviate symptoms of general ailment.

Amalgam alerts in Germany

The German Ministry of Health issued a consensus statement on July 1, 1997, prohibiting any placement or removal of amalgam fillings from pregnant women's mouths.

Amalgam Advisory issued in Norway

The Norwegian Health Board has advised pregnant women to avoid "extensive amalgam therapy."

European Commission submit on amalgam

There is currently no evidence that mercury from dental amalgam poses an undesirable health threat to the wider public. Moreover, there is an insufficient data to suggest that clinically acceptable dental amalgam restorations should be removed unless a confirmed allergy to this material is present.

Regulation by the US Food and Drug Administration (FDA)

Encapsulated dental amalgam was classified as a Class II medical device by the US FDA in 2009. Encapsulated amalgam was previously not classified, and dental mercury (Class I) and alloy (Class II) were each given their own classifications. Amalgam is classified in the same category as the majority of other restorative materials, including composite and direct gold restorations, under this regulation. Furthermore, the FDA reaffirms that it is a reliable and practical restorative option for patients.

The Minamata convention, a historical change

The Minamata Convention on Mercury is a world treaty aimed at protecting both people and the environment from mercury's harmful consequences (22, 23). The Intergovernmental Negotiating Committee on Mercury held its fifth session in Geneva, Switzerland, on January 19, 2013. The Minamata Agreement took effect on August 16, 2017 (24).

The following are the key points of the Minamata Convention to curtail the usage of dental amalgam:

- Establishing national objectives for dental caries prevention and oral health advancement, thus reducing the requirement for dental restoration.
- Fostering research and development of high-quality non-mercury dental restoration products (25).

The World Health Organization issued a report titled *Future Use of Materials for Dental Restorations* in 2009, advising that:

- Moving away from dental amalgams was contingent on adequate quality of alternative cost- effective dental restorative materials.
- Since current restorative options are not long-lasting, it would be appropriate to reduce rather than eliminate the use of dental amalgam during this time

- Encourage professional groups and dental colleges to sensitise practitioners and students about mercury-free restoration options, as well as to promote Best Management Practices (BMP). BMPs are a set of protocols for controlling as well as disposing of amalgam waste that includes, but are not limited to:
  - Use of ISO 1114 compliant amalgam separators (Figure 5)
  - Launching large-scale mercury recovery initiatives
  - Making use of chair side traps (Figure 5)
  - Vacuum retrieval
  - Examining and cleaning traps
  - Recyclability
  - Discarding of the collected amalgam by a commercial waste disposal provider
- Opposing health coverage policies and measures that favour dental amalgam over mercury-free dental restoration.
- Promoting medical coverage initiatives that encourage the adoption of high-quality dental amalgam substitutes for dental restoration.
- Using only encapsulated dental amalgam.
- Encouraging dental services to employ best environmental measures to minimize mercury and mercury compound releases into water and soil (Figure 6) (21).

Best Environmental Practices (IISD-ELA)

The IISD Experimental Lakes Area (Figure 6) is a natural laboratory in Ontario, Canada, consisting of 58 small lakes and their watersheds reserved for scientific research. The International Institute for Sustainable Development (IISD) manages and operates the facility, which has a mandate to investigate the aquatic effects of a wide range of stresses on lakes and their catchments. According to IISD-ELA research, ecosystems can recover quickly from mercury poisoning. We simply need to reduce the amount of mercury entering that ecosystem.

Amalgam alternatives and substitutes

Amalgam alternatives are any materials that can be used to restore a tooth instead of amalgam (e.g., composite, glass-ionomer, gold, cast gold alloys, and ceramics) (4). Amalgam substitutes (e.g., cast gold alloys) are materials that are believed to have properties similar to or superior than the amalgam restoration they are replacing (4, 26). Each of these materials has relative advantages, disadvantages, and costs associated with its use. Gold is an inert metal, but it is not generally regarded as aesthetically pleasing. It is also costly. Composites and glass ionomers though aesthetic; lack the strength, durability and longevity of amalgam. Hence, they are unsuitable for large restorations. Furthermore, studies have suggested that Bisphenol A, which is present in composites, may pose a health threat. It has been identified as an oestrogen mimicker and has been linked to male infertility, as well as prostate and breast cancer (27, 28). European Commission's Scientific Committee concluded in May 2008 that dental amalgams are an efficacious and useful option for both the patients and dental professionals, and that the alternative materials have clinical limitations and toxicological risks (29). Cast/ Indirect restorations, though superior in strength and in establishing contours and contacts, are much more technique sensitive, time consuming and costlier than amalgam restorations.

Figure 6. *IISD-ELA: Promoting best environmental practices.*

A few compositions contain few amalgam constituents (e.g., Ag-Sn alloy particles) but no mercury (30). Gallium alloys made with Ag-Sn particles in Gallium-Indium (Ga- In). Gallium melts at 28°C and when combined with Indium, can create molten alloys at ambient temperature. In amalgam, Ga-In has been used in place of mercury. Other systems are being researched in which gold is combined with certain other noble metals to create the restoration structure (4). These systems seem to be extremely expensive, and little is known about their performance.The American Dental Association patented a mercury-free direct-filling alloy based on mercury-coated Ag-Sn particles that can be self-welded by compaction to create a restoration in collaboration with the National Institute of Standards and Technology. This method has been suggested as a replacement for amalgam, but is to be commercialised (4). If alloy particle sizes are carefully selected to load together well, the amount of mercury necessary for mixing can be limited to the 15% to 25% range. The clinical properties of these low-mercury amalgams are not clear (4).

Conclusion

The road towards mercury free dentistry is long and arduous and we need to tread carefully on it. Despite the fact that the enormous amount of evidence indicates amalgam to be a reliable and effective restorative material, recent publications and the Minamata Convention have put a halt to all discussions. The debate is not about whether mercury released from various sources is toxic or not or whether dental amalgam should remain the restorative material of choice or not. The consensus is now clear, but the real challenge is to find a suitable alternative restorative material that either matches amalgam in its physical properties or is superior to it. Till then, amalgam remains the undisputed king of all restorative materials. However, the point that needs consideration is that until the time amalgam is being used or even being phased-down, proper mercury hygiene measures should be employed by the entire dental office team.

Türkçe özet: *Cıva İçermeyen Diş Hekimliğine Giden Yol: Geçmiş, Günümüz ve Gelecek. Amaç:Dental amalgamın popülaritesi, mükemmel uzun süreli performansı, kullanım kolaylığı ve düşük maliyetinden kaynaklanmaktadır. Bununla birlikte, amalgamda cıvaya maruz kal-*

manın potansiyel olumsuz sağlık etkileri hakkında bir endişe vardır. Bu derleme makalesi, diş amalgamının restoratif bir materyal olarak güvenliğini ve cıvasız bir yola hazır olup olmadığımızı eleştirel bir şekilde tartışmaktadır. Gereç ve Yöntem: "cıvasız diş hekimliği", "cıva toksisitesi", "amalgam ikameleri", "amalgam cıva toksisitesi" anahtar kelimeleri kullanılarak PubMed ve Google Scholar üzerinde bir veri tabanı taraması yapılmıştır. Dahil etme ve hariç tutma kriterleri açıkça belirtilmiştir. Diş hekimliği ders kitaplarında da ilgili kaynaklar taranmıştır. Bulgular: Diş hekimleri ve hastalar arasında cıva maruziyetini vurgulayan yaklaşık 40 makale dahil edildi. Amalgamın güvenli bir restoratif materyal olduğunu gösteren çok sayıda bilimsel kanıta rağmen, cıvanın toksik etkilerine ilişkin endişeler devam etmektedir. Sonuç: Asıl zorluk, uygun bir amalgam ikamesi bulmak ve cıva hijyen önlemlerini çok yakından takip etmektir. Anahtar kelimeler: Amalgam; Cıva toksisitesi; Minamata Sözleşmesi; Amalgam ikameleri; Amalgam alternatifleri

Ethics Committee Approval: Not required.

Informed Consent: Not required.

Peer-review: Externally peer-reviewed.

Author contributions: PD, KKD, SG, HS participated in designing the study. PD, KKD, HS participated in generating the data for the study. PD, ZJ participated in gathering the data for the study. PD wrote the majority of the original draft of the paper. KKD, HS participated in writing the paper. PD has had access to all of the raw data of the study. PD has reviewed the pertinent raw data on which the results and conclusions of this study are based. PD, KKD, SG, HS, ZJ have approved the final version of this paper. PD guarantees that all individuals who meet the Journal's authorship criteria are included as authors of this paper.

Conflict of Interest: The authors declared that they have no conflict of interest.

Financial Disclosure: The authors declared that they have received no financial support.

References

1. Phillips RW, Skinner EW. Skinner's science of dental materials. 11th Ed., Philadelphia: Saunders 1991, p. 496.
2. Dental amalgam: update on safety concerns. ADA council on Scientific Affairs. J Am Dent Assoc 1998; 129: 494-503. [CrossRef]
3. U.S. Public Health Service, Committee to Coordinate Environmental Health and Related Programs. Dental amalgam: A scientific review and recommended public health service strategy for research, education and regulation (Final report of the Subcommittee on Risk Management. Washington, D.C.:U.S. Government Printing Office, 1993; PHS publication no. 342-322/60025).
4. Sturdevant. Biomaterials. In: Roberson TM, Heymann HO, Swift EJ, editors. Art and Science of Operative Dentistry. 5th Ed., India: Mosby; Reed Elsevier India Pvt. Ltd., 2006, p. 135-242.
5. Mahalaxmi S. Dental Amalgam. In: Mahalaxmi S, editor. Materials Used in Dentistry. 1st ed., India: Wolters Kluwer Health (India), 2013, p. 195-221.
6. Hyson JM Jr. Amalgam: Its history and perils. J Calif Dent Assoc 2006; 34: 215-29. PMID: 16895078. [CrossRef]
7. Tuthill, JY. "Mercurial necrosis resulting from amalgam fillings." *The Brooklyn Medical Journal* 1898; XII: 725-42.
8. Talbot ES. The chemistry and physiological action of mercury as used in amalgam fillings. The Ohio State J Dent Sci 1882; 2: 1-12.
9. Dodes JE. The amalgam controversy. An evidence-based analysis. J Am Dent Assoc 2001; 132: 348-56. [CrossRef]
10. Soni R, Bhatnagar A, Vivek R, Singh R, Chaturvedi TP, Singh A. A systematic review on mercury toxicity from dental amalgam fillings and its management strategies. J Sci Res 2012; 56: 81-92.
11. Fawer RF, de Ribaupierre Y, Guillemin MP, Berode M, Lob M. Measurement of hand tremor induced by industrial exposure to metallic mercury. Br J Ind Med 1983; 40: 204-8. [CrossRef]
12. Mackert JR Jr, Berglund A. Mercury exposure from dental amalgam fillings: absorbed dose and the potential for adverse health effects. Crit Rev Oral Biol Med 1997; 8: 410-36. [CrossRef]
13. Bernhoft RA. Mercury toxicity and treatment: a review of the literature. J Environ Public Health 2012; 2012: 460508. doi: 10.1155/2012/460508. Epub 2011 Dec 22. [CrossRef]
14. Decharat S, Phethuayluk P, Maneelok S, Thepaksorn P. Determination of Mercury Exposure among Dental Health Workers in Nakhon Si Thammarat Province, Thailand. J Toxicol 2014; 2014: 401012. [CrossRef]
15. Langworth S, Sällsten G, Barregård L, Cynkier I, Lind ML, Söderman E. Exposure to mercury vapor and impact on health in the dental profession in Sweden. J Dent Res 1997; 76: 1397-1404. [CrossRef]
16. Nagpal N, Bettiol SS, Isham A, Hoang H, Crocombe LA. A Review of Mercury Exposure and Health of Dental Personnel. Saf Health Work. 2017; 8: 1-10. [CrossRef]
17. Mocevic E, Specht IO, Marott JL, Giwercman A, Jönsson BA, Toft G, Lundh T, Bonde JP. Environmental mercury exposure, semen quality and reproductive hormones in Greenlandic Inuit and European men: a cross-sectional study. Asian J Androl 2013; 15: 97-104. [CrossRef]
18. Sällsten G, Thorén J, Barregård L, Schütz A, Skarping G. Long-term use of nicotine chewing gum and mercury exposure from dental amalgam fillings. J Dent Res 1996; 75: 594-98. [CrossRef]
19. Roberts HW, Charlton DG. The release of mercury from amalgam restorations and its health effects: a review. Oper Dent 2009; 34: 605-14. [CrossRef]
20. Halbach S. Combined estimation of mercury species released from amalgam. J Dent Res 1995; 74: 1103-9. [CrossRef]
21. World Health Organization, Petersen, Poul Erik, Baez, Ramon, Kwan, Stella & Ogawa, Hiroshi. (2010). Future use of materials for dental restoration: report of the meeting convened at WHO HQ, Geneva, Switzerland 16th to 17th November 2009. World Health Organization. https://apps.who.int/iris/handle/10665/202500
22. Wikipedia contributors. Minamata Convention on Mercury. Wikipedia, The Free Encyclopedia. December 29, 2020, 22:42 UTC. Available at: https://en.wikipedia.org/w/index.php?title=Minamata_Convention_on_Mercury&oldid=997082190. Accessed January 8, 2021.
23. Yang L, Zhang Y, Wang F, Luo Z, Guo S, Strähle U. Toxicity of mercury: Molecular evidence. Chemosphere 2020; 245: 125586. [CrossRef]
24. Balaji SM. Mercury, dentistry, Minamata convention and research opportunities. Indian J Dent Res 2019; 30: 819. [CrossRef]
25. Lutz F, Krejci I. Amalgam substitutes: a critical analysis. J Esthet Dent 2000; 12:146-59. [CrossRef]
26. "British Dental Health Foundation Policy Statement Dental Amalgam" (PDF). Dentalhealth.org. Archived from the original (PDF) on 2015-01-13. Retrieved 2015-06-13.
27. Wahl MJ. Amalgam--resurrection and redemption. Part 2: The medical mythology of anti-amalgam. Quintessence Int 2001; 32: 696-710.
28. SCENIHR (Scientific Committee on Emerging and Newly-Identified Health Risks), Scientific opinion on the Safety of Dental Amalgam and Alternative Dental Restoration Materials for Patients and Users (update), 29 April 2015.
29. Yousefi H. Replacing dental amalgam by mercury-free restorative materials; it's time to take action. Daru 2018; 26: 1-3. [CrossRef]
30. Fisher J, Varenne B, Narvaez D, Vickers C. The Minamata Convention and the phase down of dental amalgam. Bull World Health Organ 2018; 96: 436-8. [CrossRef]

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Official Publication of Istanbul University Faculty of Dentistry

Eur Oral Res 2023; 57(2): 68-74

Original research

The effects of technical factors on the fractal dimension in different dental radiographic images

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Purpose
The aim of this study was to assess the impact of exposure parameters and image formats on fractal dimension (FD) values in periapical, panoramic, and CBCT images.

Materials and Methods
Seven dry male mandibles were selected, and a Gutta-Percha was used to identify identical regions of interest. A periapical radiograph was taken with 60 kVp/7 mA and exported in DICOM, JPEG, TIFF, and PNG formats. Nine periapical radiographs (60, 65, 70 kVp; 4, 5, 6 mA) were taken from seven dry human mandibles. Additionally, 12 panoramic radiographs (60, 70, 81, 90 kVp; 5, 8, 13 mA) and 10 CBCT images (with different scanning options and FOVs) were taken from each mandible. FDs were measured from a standard area.

Results
The intra-class correlation coefficient demonstrated a high degree of agreement between observers. No significant difference was found between TIFF and PNG formats ($p > 0.05$). The highest FD mean was found in TIFF format, while the lowest FD mean was found in JPEG format ($p < 0.001$). There was no significant difference between kVp and mA settings in periapical images. In panoramic images, a significant difference was found at 90 kVp ($p = 0.001$) and 13 mA ($p < 0.001$), with lower FD values observed at these settings. There was no significant difference between FOV and resolution in CBCT images ($p > 0.05$).

Conclusion
The format of the image can influence FD. For periapical and panoramic radiographs, kVp and mA settings do not have a significant impact on FD. However, fractal analysis may not be an ideal method for evaluating three-dimensional images, such as those obtained with CBCT.

Keywords: *Fractal analysis, Exposure parameters, Image format, Dental radiography, CBCT*

Introduction

Fractal analysis (FA) is a mathematical technique utilized to evaluate complex structures and quantify their degree of irregularity through a numerical value known as the fractal dimension (FD). This method has been employed by radiologists as a tool to assess bone tissue quality and bone mineral density (1, 2), and it has been used for various purposes in dentistry (3). Previous research on FA has utilized a range of dental radiographic images, including panoramic, periapical, bitewing, cephalometric, and cone-beam computed tomography (CBCT), which are commonly employed in dentistry (1, 4).

FA can only be performed on digital radiographic images, but these images must be processed in advance. To this end, White and Rudolph developed a method using ImageJ software (https://imagej.nih.gov/ij/) with

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Received: 18 August 2021
Revised: 31 May 2022
Accepted: 24 June 2022

DOI: 10.26650/eor.2023984422

How to cite: *Amuk M., Sirin Saribal G., Ersu N., Yilmaz S. The effects of technical factors on the fractal dimension in different dental radiographic images. Eur Oral Res 2023; 57(2): 68-74.* DOI: 10.26650/eor.2023984422

the aim of making inferences about bone microarchitecture using radiographic images and FA (5). The resolution of the images is a crucial factor in ensuring accurate and realistic evaluations. However, image resolution is directly affected by scanning and exposure parameters such as peak tube voltage (kVp), tube current (mA), exposure time, scan time, the field of view (FOV), and voxel-pixel size (6). The selection of FOV has a significant impact on the quality of CBCT images and the visualization of anatomical structures (7). Moreover, several factors can influence FD, including the compression and recording format of the radiographic image, as well as the size and location of the regions of interest (ROI) within the bone being analyzed. The effect of these factors on FD has been a subject of debate in the literature, with some studies reporting minimal differences in FD associated with exposure parameters (8-13).

Digital Imaging and Communications in Medicine (DICOM) is a widely accepted image format designed for medical radiographic imaging, despite having some disadvantages. Other commonly used formats include Joint Photographic Experts Group (JPEG), Tagged Image File Format (TIFF), Portable Network Graphics (PNG), Bitmap (BMP), and Graphics Interchange Format (GIF) (14, 15). JPEG is a popular image format on all platforms, known for its compression capability. On the other hand, TIFF is an adaptable image format that uses lossless compression (LC) (14, 16). PNG was developed to replace the GIF format and is useful in medical imaging due to its LC, which results in the created image being the same as the original after decompression (16). The effects of compression on image quality or qualitative and quantitative measurements in digital radiography are important issues that need to be investigated. Although the analysis part of the FA method has some standardized steps through a computer program, there are non-standardized steps such as the methods of obtaining the radiographs, the selection of areas to be measured, and the recording formats of the images (5). The purpose of this study is to evaluate the effects of exposure parameters and image formats on FD values using periapical, panoramic, and CBCT images.

Materials and Methods

Ethical approval and study design

This study is a retrospective in-vitro study conducted in the Department of Oral and Maxillofacial Radiology at Erciyes University Faculty of Dentistry. It does not include a control group. The study was approved by the Erciyes University Clinical Research Ethics Committee. The study started with the following hypotheses: Different image formats can affect FD, exposure parameters can affect FD in periapical radiograph, exposure parameters can affect FD in panoramic radiographs and FOV and different scanning resolutions can affect FD in CBCT images.

Specimens

The present study included the use of edentulous dry mandibles from seven male cadavers that did not have any pathologies or diseases affecting bone metabolism and were aged between 50-70 years. To ensure consistency, each mandible was marked with a Gutta-Percha to identify a specific ROI. The marked area was a 6×6 mm square region located distal to the mental foramen in the periapical bone area, without any overlap with the tooth roots or periodontal space.

Radiographic imaging

The mandibles were positioned on a hard and fixed surface to ensure consistent projection geometry throughout the imaging procedures. Periapical radiographs were captured using the Kodak 2100 Intraoral X-Ray System (Kodak, New York, USA) and size 2 photostimulable phosphor plates (PSP). The dental X-ray unit was set to operate at 1.5 mm Al equivalent filtration, 0.2 s exposure time, and a focus-receptor distance of 25 cm. The parallel technique was employed to obtain all periapical images (Figure 1A-1B). Firstly, periapical radiographs were captured using 60 kVp/7 mA values, which were accepted by the device as the standard for periapical radiography in adults.

The images were exported in uncompressed DICOM, JPEG, TIFF, and PNG formats. Following that, nine periapical radiographs were taken from each mandible using 60, 65, and 70 kVp and 4, 5, and 6 mA values, respectively, resulting in 70 periapical images. The photostimulable phosphor plate (PSP) was scanned immediately after exposure using the Express Digital Imaging Plate Scanner (Instrumentarium Dental, Tuusula, Finland) in standard resolution.

Panoramic radiographs were acquired using the Instrumentarium Dental Orthopantomograph OP200 D (Instrumentarium Dental, Tuusula, Finland). The device is equipped with radial reference lines that assist in patient positioning and ensure alignment of the midsagittal plane and Frankfort plane. The position of each mandible was fixed during image acquisition with the aid of these lines (Figure 2). Initially, panoramic radiography was performed using 66 kVp/8 mA values, which were considered as the standard for panoramic radiography in adults. Subsequently, 12 panoramic radiographs were taken from each mandible, with varying exposure parameters of 60, 70, 81, and 90 kVp and 5, 8, and 13 mA values, respectively, resulting in a total of 91 panoramic images.

CBCT images were acquired using the Newtom CBCT device (Newtom5G, QR, Verona, Italy). Radial reference lines were used as a guide, similar to the panoramic radiography device. Five different scan FOVs were employed, namely 8×8, 12×8, 15×12, and 18×16, with varying scanning options. These options included standard resolution standard dose (STD), standard resolution high dose (BOOSTED), and high resolution (HIRES). However, for the HIRES resolution of the device, only 8×8 and 12×8 FOV values were available, and images were captured accordingly. A total of 70 CBCT images were produced. The exposure parameters in all radiography techniques were modified within the permissible limits of the respective devices. The images obtained through periapical, panoramic, and CBCT techniques were saved in uncompressed TIFF format.

Fractal analysis

The images were analyzed by three oral and maxillofacial radiologists using NNT software (Version 9.1), which is the

original software of the CBCT machine (Newtom5G, QR, Verona, Italy) for CBCT images, on a Dell Precision T5400 workstation with a 19-inch 1920x1080 resolution monitor (Dell E190S, China). The radiologists had three years of clinical experience and were blinded to the parameters from which the images were taken. All regions of interest (ROIs) were within the marked area borders, and the measured locations were the same regardless of technical parameter changes or imaging methods.

Firstly, FDs were calculated to assess the effects of image formats. ROIs were placed at specific X and Y coordinates using features provided by the software to ensure they were all at the same point. This ensured that ROIs in all formats (DICOM, JPEG, PNG, TIFF) were of the same size and position (see Figure 3). To evaluate the effects of exposure parameters, FD was calculated on radiographic images taken with different parameters. The size of ROIs was determined to be the maximum size allowed by the marked area in the images. The size of ROI on periapical radiographs was 70×70 pixels (see Figure 4A), on panoramic radiographs, it was 50×50 pixels (see Figure 4B), and on CBCT images, it was 40×40 pixels (see Figure 4C). FA was carried out on ImageJ version 1.53 software using the box-counting method defined by White and Rudolph (5) (see Figure 5).

Statistical analysis

The statistical analysis was performed using SPSS v26 (IBM, Armonk, New York, USA). To determine the level of agreement between observers, the intra-class correlation coefficient (ICC) with a 95% confidence interval (CI) was calculated. Statistical differences were assessed using one-way repeated measures ANOVA followed by Bonferroni's post hoc test. A p-value less than 0.05 was considered statistically significant.

Results

ICC showed a high degree of agreement between observers in all image format and imaging methods measurements (Table 1).

Image format

Based on the analysis of variance using mean FD values, it was determined that there was no significant difference between the FD values of TIFF and PNG formats ($p>0.05$) as they already had the same FD value. However, a statistically significant difference was observed between the FD values of TIFF (and PNG), DICOM, and JPEG formats ($p<0.001$). Among these formats, TIFF (and PNG) had the highest mean FD value, whereas JPEG had the lowest mean FD value, as shown in Table 2.

Exposure parameters

In the analysis of variance performed on FD measurement values obtained with the periapical radiography technique, it was found that there was no significant difference between the values of kVp and mA exposure parameters ($p > 0.05$) (Table 3). For the panoramic radiography technique, a significant difference was found in the FD values of 90

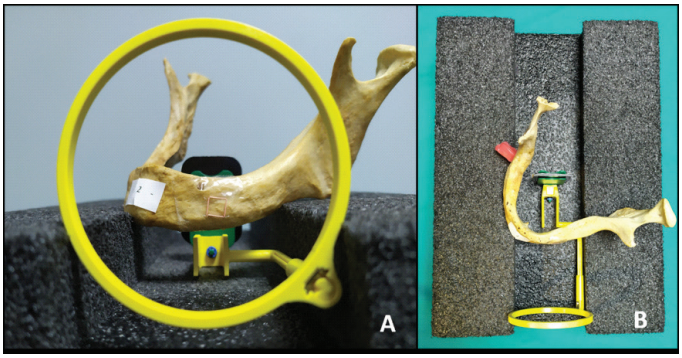

Figure 1. *A, Positioning of the mandible and apparatus for taking periapical radiographs with the parallel technique. B, mandible was placed on a hard and constant floor for stabilization.*

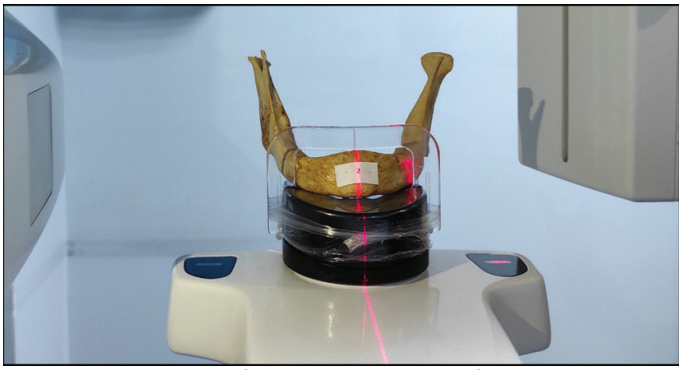

Figure 2. *Positioning of dry human mandible for panoramic radiograph.*

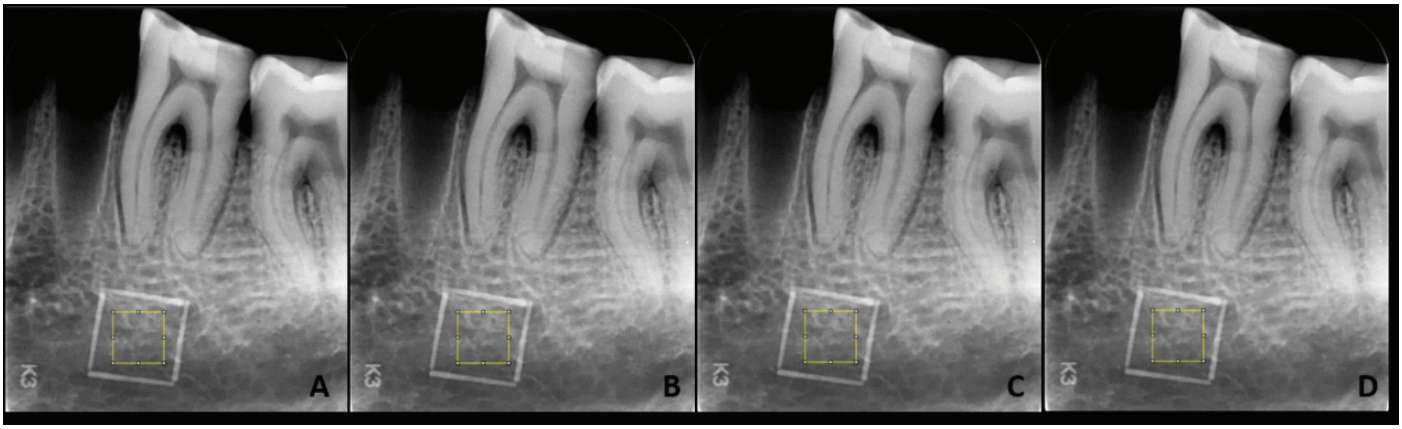

Figure3. *Periapical radiographs with different image formats placed on the marked area. A; DICOM, B; JPEG, C; PNG, D; TIFF.*

kVp/13 mA ($p < 0.05$). The FD was found to be lower in the 90 kVp/13 mA exposure parameters (Table 4). In the analysis of variance using FD measurement values in the images obtained with the CBCT technique, no significant difference was found between all groups, including FOV and resolution ($p > 0.05$) (Table 5).

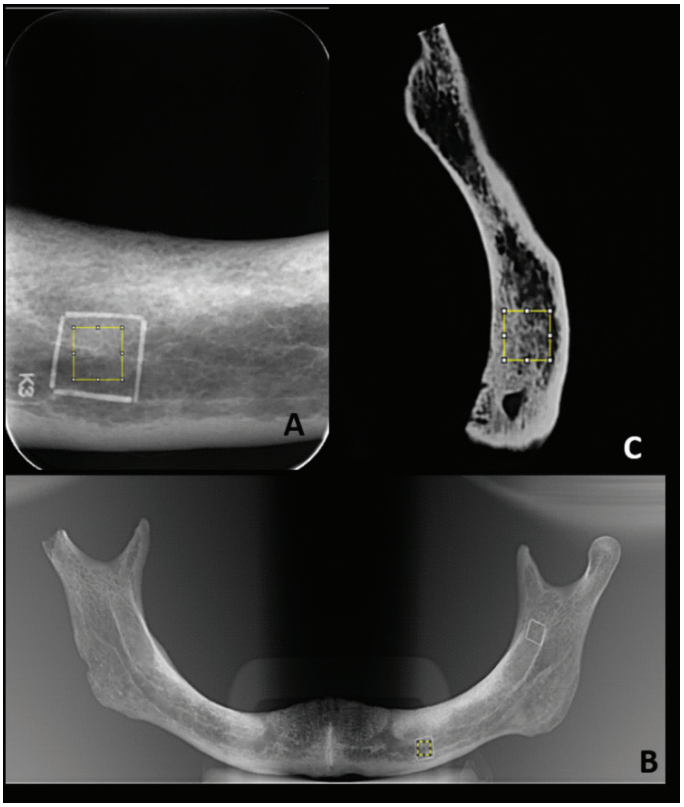

Figure 4. *A; periapical radiograph with a 70x70 ROI inserted within the marked area borders, B; panoramic radiograph with a 50x50 ROI inserted within the marked area borders, C; CBCT images with 40x40 ROI inserted within the marked area borders.*

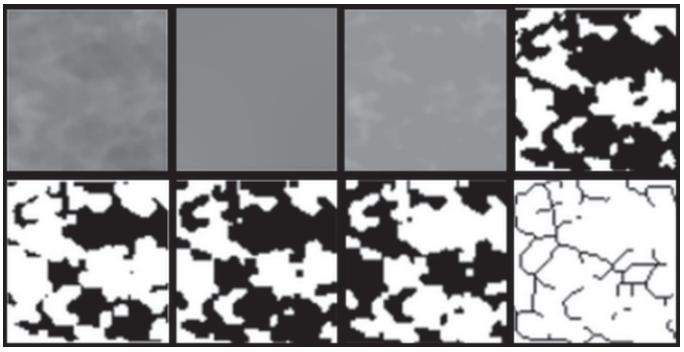

Figure 5. *Steps of fractal analysis.*

Discussion

To ensure clinical reliability and value of a bone analysis method, it should be able to operate independently of technical parameters and demonstrate stability against varying parameters, as the obtained results should remain consistent (13).

DICOM is a widely accepted image format designed for medical radiographic imaging. However, DICOM has some disadvantages. It cannot be viewed without a special viewer (such as ImageJ or DICOM Works), and it has a larger file size

Table 1. *The Intra-Class Correlation (ICC) coefficient between the raters in imaging methods*

	ICC (95%CI)	***P***
Image Formats Measurements	0.947(0.893-0.991)	0.001*
Periapical Radiography Measurements	0.932(0.807-0.988)	0.002*
Panoramic Radiography Measurements	0.947(0.893-0.991)	0.001*
CBCT Measurements	0.953(0.829-0.992)	0.001*

*ICC (%95 CI): Intra-Class Correlation with 95% confidence interval. *Significant at $p \leq 0.05$*

Table 2. *Comparison of fractal dimention results for different image format measurements on periapical radiography.*

Image Format	***n***	***mean***	***SD***	***p***	***F***
DICOM	7	1.254[b]	0.057	0.000*	F=32.70
JPEG	7	1.252[c]	0.055		
TIFF	7	1.264[a]	0.057		
PNG	7	1.264[a]	0.057		

*n: number of dry mandible, SD; standard deviation F: One-way repeated measures ANOVA, *Significant at $p \leq 0.05$, a, b, c: the difference between the averages without the common letter is significant*

because DICOM is a data set that includes different types of data, such as demographic information of the individual, imaging parameters of the device, and matrix size of the image (14, 15). JPEG is a widely used and compression-capable format. Thanks to this compression feature, JPEG offers an easy and high level of portability. However, the images produced have poorer quality than TIFF or DICOM. TIFF uses LC, making it the best choice for a master copy (14, 16). PNG is also very useful in medical imaging due to its LC. The image created after decompression is the same as the original (16).

In our study, it was found that PNG and TIFF had significantly higher FD, which was an expected result, as these formats use the LC technique. In contrast, JPEG had the lowest FD significantly, which was also an expected result because JPEG causes LC in the image.

Toghyani *et al.* (17) conducted a study comparing FD values across different image formats, resolutions, and compression levels (CL) on periapical radiographs. They found that FD increased with increasing resolution and that the most consistent and reliable FD value was obtained at high resolution. Baksi *et al.* (18) compared the effects of different compression levels on periapical radiographs using JPEG and JPEG2000 formats. They found that FA was insensitive to CL and confirmed the validity of FA, which was consistent with previous studies that reported insensitivity to changes in exposure parameters and projection geometry (8, 19). In a similar vein, Yaşar *et al.* (20) also found that the FD value was higher in the TIFF format than in the JPEG format, which is consistent with our study findings.

Fractal images exhibit a high degree of similarity between the points of the image, making them particularly susceptible to the negative effects of noise. Increased noise levels can impede correct diagnosis and early treatment, particu-

Table 3. *Comparison of fractal dimention results for periapical radiography measurements*

kVp/mA	4		5		6		7		F	p
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**		
60	1.201	0.049	1.198	0.058	1.206	0.059	1.204	0.069	0.161	0.921
65	1.187	0.034	1.217	0.039	1.189	0.070			1.548	0.260
70	1.189	0.033	1.197	0.044	1.197	0.044			0.283	0.758
F	0.489		0.788		1.001					
P	0.625		0.421		0.396					

SD; standard deviation, F: One-way repeated measures ANOVA, Significant at p ≤ 0.05.

Table 4. *Comparison of fractal dimention results for panoramic radiography measurements*

kVp/mA	5		8		13		F	p
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**		
60	1.331	0.056	1.355	0.036	1,345^{A}	0.048	0,994	0,399
66			1.352	0.044				
70	1.343	0.035	1.327	0.037	1,341^{AB}	0.045	3,043	0,085
81	1.348	0.029	1.339	0.029	1,331^{AB}	0.047	1,206	0,333
90	1.345^{a}	0.027	1.347^{a}	0.032	1,280^{Bb}	0.041	13,583	0,001*
F	0.421		2.240		13.334			
P	0.578		0.095		0.000*			

*SD; standard deviation, F: One-way repeated measures ANOVA, *Significant at p ≤ 0.05 A, B: In the same column, the difference between the mean kVp without common capital letters is significant. a,b: In the same row, the difference between the mA averages without the common lowercase letter is significant*

Table 5. *Comparison of fractal dimention results for CBCT measurements.*

FOV/Resolution	Standard		Boosted		HİRES		F *or* t	p
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**		
8x8	1.304	0.089	1.334	0.090	1.329	0.081	1.338	0.299
12x8	1.313	0.077	1.337	0.085	1.324	0.067	0.997	0.358
15x12	1.330	0.082	1.328	0.087			0.180	0.863
18x16	1.313	0.083	1.335	0.070			-1.639	0.152
F *or* t	1.326		0.160		*0.746*			
P	0.297		0.922		*0.484*			

Standard: standard dose standart resolution scannnig, Boosted: standart dose high resolution scanning, HIRES: high dose high resolution scanning, SD; standard deviation. t:Paired t-test, F: One-way repeated measures ANOVA, Significant at p ≤ 0.05

larly in the early stages of lesions (21). Previous studies have demonstrated that exposure parameters can influence the amount of noise present in images (22, 23).

In our study, there was no statistically significant difference found in the FD of periapical radiographs. This is consistent with the findings of a previous study that evaluated exposure parameters on periapical radiographs and found no significant difference in FDs (8). Given the high level of detail and clarity provided by periapical images, it is believed that FD is not affected by changes in exposure parameters. In panoramic radiographs, FD was not significantly affected up to 90 kVp and 13 mA, but there was a statistically significant difference in images taken with 90 kVp and/or 13 mA. FD changed in images taken with less than 13 mA and 90 kVp or with less than 90 kVp and 13 mA. To the best of our knowledge, there are no studies on the effects of exposure parameters on FA in panoramic images in the literature. Therefore, previous studies that have investigated the effects of exposure parameters on image quality have been reviewed in this study.

In a previous study, panoramic images that provide less detail were found to have lower FD values than periapical images that offer higher detail (24). Similarly, PSP scanned at higher resolutions were found to have higher FD values (12, 17). Although previous studies did not directly investigate the effects of different kVp-mA settings on FD in panoramic radiographs, we believe that our results are related to the varying spatial resolution, based on the information provided by these studies. However, further research is necessary to fully explore this topic.

In our study, we found no statistically significant differences in FD values between CBCT images taken with different FOV sizes. This finding is consistent with a study by Tsai *et al.* (25), which evaluated the effects of scanning protocols on bone microarchitecture in CBCT images using the ImageJ program and found that resolution mode did not affect the trabecular parameters. However, it should be noted that there are studies in the literature indicating that changes in CBCT scanning parameters can alter image quality (27-30). Furthermore, in a study by Corpas *et al.* (26) that evaluated bone density with CBCT images and histological bone sections in animal experiments, the authors found that FD values of two-dimensional CBCT image sections and histological sections were not compatible. This suggests that analyzing two-dimensional sections obtained from CBCT images using the ImageJ program may not provide accurate results. To address this issue, current publications recommend using the BoneJ extension of the ImageJ program for three-dimensional images, and using three-dimensional FD analysis (31, 32). This approach can provide more accurate and reliable results for FD analysis of CBCT images.

The goal of the researchers was to make the evaluation of bone microarchitecture with FA applicable to clinical practice. They achieved this by evaluating clinical conditions like orthognathic surgery healing and implant osseointegration using FA (33, 34). It is believed that FA can be useful in assessing bone quality before and after surgical procedures. We anticipate that this application will become more prevalent in the future. Consequently, we believe that it is necessary to standardize the use of FA in these assessments.

To improve the accuracy and reliability of the results, future studies should have larger sample sizes to assess the effects of technical parameters and the FA method on various imaging modalities. Another limitation of this study was the lack of consideration for soft tissue compensation, which may affect the results. Since our study evaluated the effects of technical features, soft tissue compensation was not taken into account. Moreover, it should be noted that each image format has its own advantages and disadvantages that should be taken into account by the radiologist according to the intended use.

Conclusion

FA is a bone analysis technique that is intended for clinical use but its standardization has yet to be clarified. The format of images used for FA can affect the FD. The study findings suggest that using higher resolution TIFF images that are suitable for black and white images can result in more accurate measurements. The parameters, such as kVp and mA, used for periapical and panoramic radiographs do not significantly affect the FD. However, for panoramic images, it is important to avoid using images that were taken with high doses, such as 90 kVp and 13 mA. It is worth noting that FA may not be appropriate for evaluating two-dimensional cross-sectional images of CBCT, and three-dimensional FA methods may be necessary.

Türkçe özet: *Farklı Dental Radyografik Görüntülerde Teknik Faktörlerin Fraktal Boyuta Etkisi. Amaç: Işınlama parametreleri ve görüntü formatının fraktal boyuta(FB) etkisini periapikal, panoramik ve KIBT görüntüleri kullanılarak değerlendirmek amaçlanmıştır. Gereç ve Yöntem: Erkek kadavralara ait, 7 adet tam dişsiz kuru mandibula seçildi ve belirlenilen ilgili alanlar Gutha-Perka kullanılarak işaretlendi. 60 kVp/7mA değerlerinde bir periapikal radyografi alındı ve DICOM,JPEG,TIFF,PNG formatlarında kaydedildi. Sonrasında her mandibuladan sırasıyla 60,65,70kVp/ 4,5,6mA değerlerinde 9 periapikal radyografi; sırasıyla 60,70,81,90kVp/ 5,8,13 ma değerlerinde 12 panoramik radyografi; farklı çözünürlük ve FOV boyutlarında 10 KIBT görüntüsü alındı. Seçilen standart bölgelerden FB ölçüldü. Bulgular: Sınıf içi korelasyon değeri gözlemciler arasında yüksek derecede uyum gösterdi. TIFF ve PNG formatları arasında istatistiksel olarak anlamlı bir fark yoktu.(p>0.05) FB değeri en yüksek TIFF formatında, en düşük JPEG formatında ölçüldü.(p<0.001) Periapikal görüntülerde farklı kVp ve mA parametreleri arasında anlamlı bir fark yoktu.(p>0.05) Panoramik görüntülerde 90 kVp/13 mA parametrelerinde daha düşük FB ölçüldü. (p<0.001) KIBT görüntülerinde farklı çözünürlük ve FOV boyutlarında anlamlı bir fark yoktu.(p> 0.05). Sonuç: Görüntü formatı FB'yi değiştirebilir. Periapikal ve panoramik radyografilerde kVp ve mA değişimleri FB'yi büyük ölçüde etkilememektedir. Fraktal analiz, KIBT gibi üç boyutlu görüntülerde değerlendirme yapmak için uygun bir yöntem olmayabilir. Anahtar kelimeler: Fractal analiz, ışınlama parametreleri, görüntü formatı, dental radyografi, KIBT.*

Ethics Committee Approval: The research protocol was approved by the Erciyes University Clinical Research Ethics Committee.

Informed Consent: Not required.

Peer-review: Externally peer-reviewed.

Author contributions: MA, GSS, NE, SY participated in designing the study. MA, GSS, NE, SY participated in generating the data for the study. GSS, SY participated in gathering the data for the study. GSS, NE, SY participated in the analysis of the data. GSS, NE wrote the majority of the original draft of the paper. MA, GSS, NE, SY participated in writing the paper. MA, GSS, NE, SY have had access to all of the raw data of the study. MA, GSS have reviewed the pertinent raw data on which the results and conclusions of this study are based. MA, GSS, NE, SY have approved the final version of this paper. GSS, NE guarantee that all individuals who meet the Journal's authorship criteria are included as authors of this paper.

Conflict of Interest: The authors had no conflict of interest to declare.

Financial Disclosure: The author declared that this study has received no financial support.

Acknowledgments: Authors would like to thank Yeliz KAŞKO ARICI for her contribution.

REFERENCES

1. Güngör E, Yildirim D, Çevik R. Evaluation of osteoporosis in jaw bones using cone beam CT and dual-energy X-ray absorptiometry. Journal of oral science 2016;58:185-94. [CrossRef]
2. Tolga Suer B, Yaman Z, Buyuksarac B. Correlation of Fractal Dimension Values with Implant Insertion Torque and Resonance Frequency Values at Implant Recipient Sites. International Journal of Oral & Maxillofacial Implants 2016;31. [CrossRef]
3. Geraets W, Van Der Stelt P. Fractal properties of bone. Dentomaxillofacial Radiology 2000;29:144-53. [CrossRef]
4. Kato CN, Barra SG, Tavares NP, Amaral TM, Brasileiro CB, Mesquita RA, et al. Use of fractal analysis in dental images: a systematic review. Dentomaxillofacial Radiology 2020;49:20180457. [CrossRef]
5. White SC, Rudolph DJ. Alterations of the trabecular pattern of the jaws in patients with osteoporosis. Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology 1999;88:628-35. [CrossRef]

6. Bechara B, McMahan CA, Moore WS, Noujeim M, Geha H, Teixeira FB. Contrast-to-noise ratio difference in small field of view cone beam computed tomography machines. Journal of oral science 2012;54:227-32. [CrossRef]
7. Ibrahim N, Parsa A, Hassan B, van der Stelt P, Aartman IH, Wismeijer D. The effect of scan parameters on cone beam CT trabecular bone microstructural measurements of the human mandible. Dentomaxillofacial Radiology 2013;42:20130206. [CrossRef]
8. Jolley L, Majumdar S, Kapila S. Technical factors in fractal analysis of periapical radiographs. Dentomaxillofacial Radiology 2006;35:393-7. [CrossRef]
9. Wilding R, Slabbert J, Kathree H, Owen C, Crombie K, Delport P. The use of fractal analysis to reveal remodelling in human alveolar bone following the placement of dental implants. Archives of Oral Biology 1995;40:61-72. [CrossRef]
10. Shrout MK, Roberson B, Potter BJ, Mailhot JM, Hildebolt CF. A comparison of 2 patient populations using fractal analysis. Journal of periodontology 1998;69:9-13. [CrossRef]
11. Shrout M, Hildebolt C, Potter B. The effect of varying the region of interest on calculations of fractal index. Dentomaxillofacial Radiology 1997;26:295-8. [CrossRef]
12. Baksi BG, Fidler A. Image resolution and exposure time of digital radiographs affects fractal dimension of periapical bone. Clinical oral investigations 2012;16:1507-10. [CrossRef]
13. Pauwels R, Faruangsaeng T, Charoenkarn T, Ngonphloy N, Panmekiate S. Effect of exposure parameters and voxel size on bone structure analysis in CBCT. Dentomaxillofacial Radiology 2015;44:20150078. [CrossRef]
14. Varma DR. Managing DICOM images: Tips and tricks for the radiologist. The Indian journal of radiology & imaging 2012;22:4. [CrossRef]
15. Tan LK. Image file formats. Biomed Imaging Interv J 2006;2:e6. [CrossRef]
16. Viswanathan GK, Lotus R. Comparison and analysis of Image File Formats.
17. Toghyani S, Nasseh I, Aoun G, Noujeim M. Effect of image resolution and compression on fractal analysis of the periapical bone. Acta Informatica Medica 2019;27:167. [CrossRef]
18. Baksi BG, Fidler A. Fractal analysis of periapical bone from lossy compressed radiographs: a comparison of two lossy compression methods. Journal of digital imaging 2011;24:993-8. [CrossRef]
19. Shrout MK, Potter BJ, Hildebolt CF. The effect of image variations on fractal dimension calculations. Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology 1997;84:96-100. [CrossRef]
20. Yasar F, Apaydın B, Yılmaz H-H. The effects of image compression on quantitative measurements of digital panoramic radiographs. Medicina oral, patologia oral y cirugia bucal 2012;17:e1074. [CrossRef]
21. Veenland J, Grashuis J, Gelsema E. Texture analysis in radiographs: the influence of modulation transfer function and noise on the discriminative ability of texture features. Medical Physics 1998;25:922-36. [CrossRef]
22. Attaelmanan AG, Borg E, Gröndahl H-G. Signal-to-noise ratios of 6 intraoral digital sensors. Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology 2001;91:611-5. [CrossRef]
23. Kocasarac HD, Kursun-Cakmak ES, Ustaoglu G, Bayrak S, Orhan K, Noujeim M. Assessment of signal-to-noise ratio and contrast-to-noise ratio in 3 T magnetic resonance imaging in the presence of zirconium, titanium, and titanium-zirconium alloy implants. Oral surgery, oral medicine, oral pathology and oral radiology 2020;129:80-6. [CrossRef]
24. Bollen A, Taguchi A, Hujoel P, Hollender L. Fractal dimension on dental radiographs. Dentomaxillofacial Radiology 2001;30:270-5. [CrossRef]
25. Tsai M-T, He R-T, Huang H-L, Tu M-G, Hsu J-T. Effect of Scanning Resolution on the Prediction of Trabecular Bone Microarchitectures Using Dental Cone Beam Computed Tomography. Diagnostics 2020;10:368. [CrossRef]
26. dos Santos Corpas L, Jacobs R, Quirynen M, Huang Y, Naert I, Duyck J. Peri-implant bone tissue assessment by comparing the outcome of intra-oral radiograph and cone beam computed tomography analyses to the histological standard. Clinical oral implants research 2011;22:492-9. [CrossRef]
27. Parsa A, Ibrahim N, Hassan B, Motroni A, Van der Stelt P, Wismeijer D. Influence of cone beam CT scanning parameters on grey value measurements at an implant site. Dentomaxillofacial Radiology 2013;42:79884780. [CrossRef]
28. Neves FS, Freitas DQ, Campos PSF, Ekestubbe A, Lofthag-Hansen S. Evaluation of cone-beam computed tomography in the diagnosis of vertical root fractures: the influence of imaging modes and root canal materials. Journal of endodontics 2014;40:1530-6. [CrossRef]
29. Schulze R, Heil U, Groβ D, Bruellmann D, Dranischnikow E, Schwanecke U, et al. Artefacts in CBCT: a review. Dentomaxillofacial Radiology 2011;40:265-73. [CrossRef]
30. Schulze R. Spatial resolution in CBCT machines for dental/maxillofacial applications—what do we know today? 2015.
31. Flaherty T, Tamaddon M, Liu C. Micro-Computed Tomography Analysis of Subchondral Bone Regeneration Using Osteochondral Scaffolds in an Ovine Condyle Model. Applied Sciences 2021;11:891. [CrossRef]
32. Min C-K, Kim K-A. Quantitative analysis of metal artefacts of dental implant in CBCT image by correlation analysis to micro-CT: A microstructural study. Dentomaxillofacial Radiology 2021;50:20200365. [CrossRef]
33. Kang H-J, Jeong S-W, Jo B-H, Kim Y-D, Kim SS. Observation of trabecular changes of the mandible after orthognathic surgery using fractal analysis. Journal of the Korean Association of Oral and Maxillofacial Surgeons 2012;38:96-100. [CrossRef]
34. Bezerra dos Santos LC, Tavares Carvalho AdA, Carneiro Leão J, Duarte Neto PJ, Stosic T, Stosic B. Fractal Measure and Microscopic Modeling of Osseointegration. International Journal of Periodontics & Restorative Dentistry 2015;35. [CrossRef]

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Official Publication of Istanbul University Faculty of Dentistry

Eur Oral Res 2023; 57(2): 75-82

Original research

The effects of sports participation on the dental age in adolescents

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Purpose
The present study aims to assess the effect of sports on the dental maturity using two different dental age assessment methods and to determine whether there is a significant correlation between dental maturity and body mass index.

Materials and Methods
One hundred and thirty-eight students from Sports High School (study group) and 126 from Fine Arts High School (control group) with standard panoramic radiographs were included in the study. Dental age was assessed using Nolla's and Haavikko's methods. Demographic information regarding the weekly training hours and sports age of the study group participants was gathered. Body mass index values of all participants were calculated. Factorial analysis of variance and Tukey's test were performed and the Pearson correlation coefficient was calculated.

Results
The mean age of the students was 15.93 ± 1.13 years for the study group and 15.99 ± 1.09 years for the control group. Mean dental age values were lower than the mean chronological age values in both high schools. The difference between the dental and chronological ages was insignificant in Sports High School ($p > 0.05$). Differences in the body mass index between high schools and genders were statistically significant ($p < 0.05$). Significant correlations were detected between the sports and dental ages and between dental age and body mass index values.

Conclusion
Sports participation could have positive effects on the dental maturity as well as on the bone development.

Keywords: *Body mass index, dental age, Haavikko's method, Nolla's method, sports*

Introduction

Numerous studies have been conducted on the influence of sports on human growth and development, with a focus on its effects on bone mineral density, body height and weight. The findings from these studies have mostly indicated the positive impact of sport participation on growth-related parameters, bone development, and overall growth (1-4). However, the relationship between sports and dental maturity is still unclear. When considering the exercise routines and diets of individuals who participate in sports, it can be inferred that sports activities may have a positive effect on dental maturity. Several studies have found that dental maturity is influenced by various factors, including gender, ethnicity, and systemic status (5-7). On the other hand, some studies have reported that environmental factors, such as nutritional status, do not have a significant impact on teeth as they are more isolated compared to other body systems (8,9).

Assessing dental age using tooth maturity and eruption stages is essential in addition to skeletal parameters when evaluating growth and

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Received: 22 June 2022
Revised: 15 August 2022
Accepted: 6 September 2022

DOI: 10.26650/eor.20231134537

How to cite: *Emek T, Ceyhan D. The effects of sports participation on the dental age in adolescents. Eur Oral Res 2023; 57(2): 75-82.* DOI: 10.26650/eor.20231134537

developmental periods. There are two primary methods for assessing dental age: visual and radiographical assessment of the physiological maturity stages of teeth, and assessment of age-related changes observed in teeth (10). In determining dental age among children, tooth calcification stages and eruption periods are typically taken into account. Calcification assessment is considered a more reliable method since tooth eruption periods are affected by local and systemic factors and cover a short period, while calcification assessment allows for an assessment without the need for tooth eruption (6,11-13).

Panoramic radiographs obtained from individuals for any reason are a convenient, easy, and reliable means of determining dental age (14). In studies on dental age determination, panoramic radiographs are frequently assessed using Demirjian's, Haavikko's, and Nolla's methods. In previous Turkish studies, mean dental age values determined by Demirjian's method were found to be more advanced than the individual's chronological age, while mean dental age values determined by Haavikko's and Nolla's methods were more consistent with the individual's chronological age. Therefore, Haavikko's and Nolla's methods are regarded as more suitable for the Turkish population (15-18). Nolla's method, which includes a total of 40 maturity stages when intermediate maturity stages are included with the primary maturity stages, is more sensitive (19). It has been found that excluding teeth with a closed apex from evaluation improves the precision of Haavikko's method (20,21).

Body mass index (BMI) is one of the fundamental measurement parameters for evaluating growth and development since it allows for assessing an individual's nutritional status (22). Physical activity is known to have a positive impact on BMI due to energy consumption (23,24).

Our study aims to assess the effect of sports on dental maturity using two different dental age assessment methods and determine if there is a significant correlation between dental maturity and BMI. The null hypotheses of the study are as follows: there is no difference in dental maturity between the sports and control groups, and there is no significant correlation between dental maturity and BMI.

Materials and Methods

Study design

A cross-sectional research design was used for this study.

Ethical approval

The approval was obtained from the Clinical Research Ethics Committee of the Faculty of Medicine, Suleyman Demirel University, Isparta, Turkey (decision no. 103) and the Provincial Directorate of National Education, Governorship of Isparta, Turkey (decision no. 11998629).

Study and control groups

During the selection process, the researchers chose students from Isparta Sports High School as the sports group. This high school is the only one in the province with regular sports participation and daily and weekly training periods within the school timetable. For the control group, students from Isparta Fine Arts High School were chosen, which is a high school where art classes such as painting and music are the main focus in the school timetable. These students were not participating in any sports activities, and they approved of this study among other high schools in Isparta, Turkey. The socioeconomic status of the students in both high schools was similar, as they shared the same campus and lived within the same region, which was classified as the low-income class according to the data from the 'Turkey Statistical Institute Income Distribution and Living Conditions Statistics'. The study group consisted of healthy students from Isparta Sports High School and the control group included healthy students from Isparta Fine Arts High School who provided informed consent to participate in the study.

Intraoral and radiographic examination

The study included intraoral and radiographic examinations of participants. Intraoral examinations were performed in a designated hall using sterile equipment. Participants who required radiographic examination and had indications for panoramic radiographs were invited to the clinic. Among the students who visited our clinic, 138 (46 female, 92 male) students from Isparta Sports High School and 126 (79 female, 47 male) students from Isparta Fine Arts High School with non-distorted, high-quality, standard panoramic radiographs (Planmeca Promax 2D, Helsinki, Finland) were included in the study. Dental age was assessed by tooth calcification levels determined from the panoramic radiographs using Nolla's and Haavikko's methods.

The study gathered information on the weekly training hours and sports age of the participants from the study group. Weekly training hours refer to the number of hours spent in sports training each week, while sports age is defined as the duration of sports involvement in years. The data was collected using prepared forms and saved in Microsoft Excel 2013 (Microsoft Corporation, California, USA).

Dental age assessment using Nolla's method

Each tooth was assessed using the ten maturity stages of Nolla's method, in which dental age is determined through the left mandibular teeth, including the third molar (19). When a radiograph image fell between two of the ten stages, intermediate maturity stage scores were utilized by adding 0.2 points if the image was closer to the lesser value's image, 0.5 points if the image fell in the middle of two values, and 0.7 points if the image was closer to the greater value's image. As this method has different scoring tables available depending on the inclusion of mandibular and maxillary third molars for females and males, the dental ages of participants were calculated by determining the sum of the points obtained from each tooth on the table. In the event of a lack of left mandibular teeth, the maturity stage of the symmetric tooth was assessed.

Dental age assessment using Haavikko's method

Haavikko's method is a dental age estimation method that involves assessing all permanent teeth, including third

molars, based on 12 maturity stages consisting of six crown and six root maturation stages (25). Any teeth with complete root maturity were excluded from dental age calculation. The maturity stage of each tooth, including left mandibular third molars, was determined, and corresponding scores for these maturity stages were obtained using tables prepared separately for male and female participants. The mean dental age was established by dividing the sum of scores by the number of examined teeth.

Assessment of BMI

Height and weight measurements for all participants were taken and recorded using a mechanical scale with a stadiometer (MESITAS AS, Mesilife JSA-180, Istanbul, Turkey) to determine BMI. Participants were advised to take off their shoes and wear lightweight clothing during the measurements. BMI for each participant was calculated by dividing weight by the square of height in meters (kg/m^2). Based on the calculated values, each participant's BMI was determined to be within or outside the normal range according to the World Health Organization (WHO) classification (26).

Statistical analysis

Statistical analyses were performed using the IBM SPSS (IBM Corp. Released 2015. IBM SPSS Statistics for Windows, Version 23.0. Armonk, NY: IBM Corp.) package. A value of $p < 0.05$ was considered statistically significant. Before the study, dental age assessments were performed by one researcher over 25 randomly chosen radiographs at two different times in order to ensure training and calibration. Intraclass correlation coefficient (ICC) was calculated to determine the researcher's internal consistency. Descriptive statistics for quantifiable parameters were obtained by calculating the arithmetic mean and standard error. This was performed in separate calculations for each high school. The Chi-square test of independence was used to determine whether the gender and high school variables were independent of each other. Differences in mean gender values were established by comparing the weekly training duration in hours and sports age data obtained from the Sports High School using the t-test. Variance analysis with the factorial design was utilized to determine the differences between high schools and mean gender values in the continuous variables that met the preconditions of parametric tests, including chronological age and BMI. The Type III sum of squares technique, utilized with imbalance correction, was used in the variance analysis with factorial design. Two levels for the high school factor (Sports High School and Fine Arts High School) and two levels for the gender factor (male and female) were present in the study. Data obtained regarding age were analyzed with repeated measures analysis of variance with factorial design. There were two levels for the gender factor (female and male), five levels for the age group factor (14, 15, 16, 17, and 18), two levels for the high school factor (Sports High School and Fine Arts High School) and three levels for the age type factor (chronological, Nolla's, and Haavikko's). Repeated measures were performed for the levels of the age type factor. Tukey's test, a multiple comparison procedure, was used to detect differences between mean values of the factor levels. A linear relationship between properties was examined by calculating the Pearson correlation coefficient for chronological age, sports age, weekly training duration in hours, BMI, and dental age determined by Nolla's and Haavikko's methods.

Results

The ICC value for the internal consistency of the single researcher who performed all measurements was determined to be 0.95, indicating substantial reliability.

The distribution of mean chronological and dental age values (determined by Nolla's and Haavikko's methods) of students by high school and gender are presented in Table 1. Although no statistically significant difference was observed in mean chronological age values between genders and high schools ($p > 0.05$), a significant difference was found in the gender distribution of high schools ($p < 0.05$). The difference between mean dental age values determined by both methods and chronological age values was statistically significant in Fine Arts High School ($p < 0.05$) and insignificant in Sports High School ($p > 0.05$). Mean dental age values assessed by both methods were significantly lower than the mean chronological age values of female students in both high school groups ($p < 0.05$). The mean dental age values assessed by Nolla's method were greater than the mean chronological age values of male students of Sports High School and lower than the mean chronological age values of male students of Fine Arts High School ($p > 0.05$). The mean dental age values assessed by Haavikko's method were also lower than the mean chronological age values of male students of both high schools. This difference was statistically significant in Fine Arts High School ($p < 0.05$) and insignificant in Sports High School ($p > 0.05$).

Table 2 shows the distribution of mean chronological age values and mean dental age values assessed by Nolla's method by age group and high school. Mean dental age values assessed by Nolla's method were lower than the mean

Table 1: *Mean chronological and dental age (by Nolla's and Haavikko's methods) values distribution of the students by high school and gender.*

High school	Chronological age			Dental age (Nolla)			Dental age (Haavikko)		
	Female	Male	Total	Female	Male	Total	Female	Male	Total
Sports High School (46 female, 92 male)	15.99±1.24$^{\#}$	15.90±1.07	15.93±1.13	14.90±1.85$^{\#}$	15.99±1.85	15.63±1.92	14.75±2.13$^{\#}$	15.47±1.82	15.23±1.95
Fine Arts High School (79 female, 47 male)	15.84±1.07$^{\#}$	16.25±1.09$^{¥}$	15.99±1.09*	14.56±1.83$^{\#}$	15.64±1.36	14.96±1.74*	14.21±2.04$^{\#}$	15.46±1.93$^{¥}$	14.68±2.08*

$^{\#,¥,*}$ $p < 0.05$

chronological age values of all age groups in both high school groups. Although this difference was not statistically significant in the 14 age group in either high school ($p > 0.05$), it was statistically significant for Sports High School in the 18 age group and for Fine Arts High School in the 15, 16, 17, and 18 age groups ($p < 0.05$).

Table 3 displays the distribution of mean chronological age values and mean dental age values assessed by Haavikko's method by age group and high school. Mean dental age values assessed by Haavikko's method were lower than the mean chronological age values of all age groups in both high school groups. This difference was statistically significant in the 14, 15, and 18 age groups in both high schools, and in the 16 and 17 age groups only in Fine Arts High School ($p < 0.05$).

Figure 1 provides the sports age distribution of Sports High School students. The mean sports age values were 4.37 ± 0.25 years for females, 4.14 ± 0.22 years for males, and 4.21 ± 0.23 years in total. Gender and sports age were found to be statistically independent of each other ($p > 0.05$). The weekly training duration distribution of Sports High School students are presented in Figure 2. The mean weekly training duration values were 7.02 ± 0.52 hours for females, 7.45 ± 0.38 hours for males, and 7.31 ± 0.43 hours in total. Weekly training duration and gender were also statistically independent of each other ($p > 0.05$).

The mean BMI value of Sports High School students was 20.66 ± 0.20 kg/m^2 (19.90 ± 0.33 kg/m^2 for females and 21.04 ± 0.25 kg/m^2 for males) and the mean BMI value of Fine Arts High School students was 21.23 ± 0.33 kg/m^2 (20.92 ± 0.43 kg/m^2 for females and 21.75 ± 0.50 kg/m^2 for males); all values were within the normal range according to the WHO classification. Differences in BMI between high

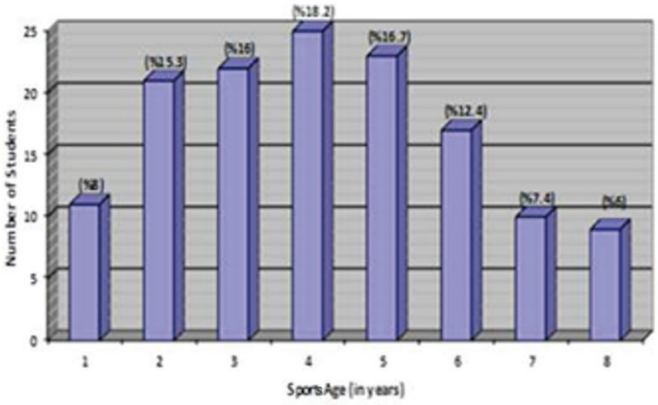

Figure 1. *The sports age distribution of Sports High School students.*

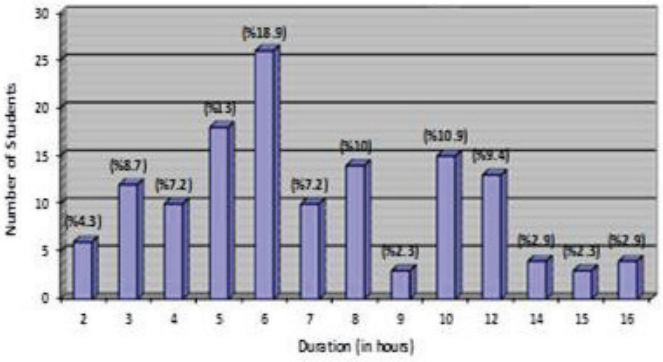

Figure 2. *The weekly training duration distribution of Sports High School students.*

Table 2: *The distribution of mean chronological age values and mean dental age values assessed by Nolla's method by age group and high school.*

Age group	Chronological age		Dental age (Nolla)	
	Sports High School	Fine Arts High School	Sports High School	Fine Arts High School
14	14.24 ± 0.12	14.24 ± 0.32	13.70 ± 0.89	13.86 ± 1.27
15	14.93 ± 0.22	15.07 ± 0.24*	14.52 ± 1.39	14.41 ± 1.66*
16	15.90 ± 0.30	15.87 ± 0.27*	15.84 ± 1.51	14.61 ± 1.59*
17	16.93 ± 0.28	16.86 ± 0.29*	16.84 ± 1.59	15.69 ± 1.26*
18	17.76 ± 0.14*	17.88 ± 0.32*	16.93 ± 2.28*	16.36 ± 2.15*

*$p < 0.05$

Table 3: *The distribution of mean chronological age values and mean dental age values assessed by Haavikko's method by age group and high school.*

Age group	Chronological age		Dental age (Haavikko)	
	Sports High School	Fine Arts High School	Sports High School	Fine Arts High School
14	14.24 ± 0.12*	14.24 ± 0.32*	12.96 ± 1.01*	13.00 ± 1.65*
15	14.93 ± 0.22*	15.07 ± 0.24*	13.85 ± 1.45*	14.07 ± 2.08*
16	15.90 ± 0.30	15.87 ± 0.27*	15.70 ± 1.55	14.16 ± 2.03*
17	16.93 ± 0.28	16.86 ± 0.29*	16.67 ± 1.03	15.62 ± 1.37*
18	17.76 ± 0.14*	17.88 ± 0.32*	16.50 ± 2.05*	16.51 ± 1.80*

*$p < 0.05$

schools and genders were statistically significant ($p < 0.05$). BMI distribution of both high school students was determined based on the WHO classification. Accordingly, 60 (43%) of the Sports High School students were found to be underweight, 71 (52%) of them were normal weight, and 7 (5%) of them were overweight, while 56 (45%) of the Fine Arts High School students were found to be underweight, 53 (42%) of them were normal weight, 14 (11%) of them were overweight, and 3 (2%) of them were obese.

Statistically significant correlations observed among the assessed parameters are shown for each high school in Table 4.

Discussion

The importance of sports activities in the growth and development of young adults is well-established (4,27). A study has shown that individuals who participate in sports exhibit greater gains in height, muscle mass, and bone mineralization rates compared to their non-sporting peers (28). Various age estimation methods are utilized to assess the level of physical growth and development in these individuals (29). Teeth contain building blocks such as calcium, phosphate, and magnesium, which are similar to bone structure and can also be utilized in these methods (30,31).

In a previous study, the importance of dental age assessment to support skeletal age assessment for fair competition in sports was emphasized, and it was suggested that third molars could be examined for this purpose (32). Several studies have reported positive correlations between dental and skeletal maturity, and it has been suggested that dental maturity could be a parameter reflecting skeletal maturity (33-36). Dental age assessment methods typically involve histological, biochemical, and radiological techniques, with the radiographic technique considered a quick, easy, and reliable method (19,37).

In a study conducted in Turkey among 425 children aged between 7 and 13 years, it was reported that mean dental age values using Nolla's method were significantly lower than mean chronological age values for both genders (17). In another study carried out in Turkey, dental ages of 719 children aged between 6 and 18 years were assessed using Nolla's method, and the mean dental age values were lower than the mean chronological age values for both genders. However, the difference was only statistically significant in females. The study reported that dental age estimation using Nolla's method is reliable for males in the Turkish population (18). In another study conducted in Turkey among 673 children aged between 5 and 16 years, the mean dental age value assessed using Demirjian's method was found to be greater than the mean chronological age value by 0.86 years. Conversely, the mean dental age value assessed using Nolla's method was lower than the mean chronological age value by 0.54 years. Based on these results, it has been concluded that Nolla's method yields more reliable results for dental age assessment in the Turkish population (16). Haavikko's method was initially used in 1970 among 885 Finnish children between the ages of 2 and 13 years, and it was found to be consistent with chronological age (38). Due to its development based on individuals from northern European countries, Haavikko's method is considered more credible in assessing dental maturity in these countries. In a study conducted in Turkey, three different methods (Nolla's, Demirjian's, and Haavikko's) were used to assess dental ages of 425 children between the ages of 7 and 13 years. According to the results, Haavikko's method was found to be more reliable than the other methods and yielded results closer to chronological age (17). Therefore, Nolla's and Haavikko's methods, which were considered the most suitable for the Turkish population compared to other methods, were used in the current study to assess dental age using tooth calcification levels (16-18). For this purpose, panoramic radiographs were used as they were found to provide more accurate results than periapical radiographs (39).

In studies conducted among children in various countries, some researchers have reported that dental age assessed by Nolla's and Haavikko's methods tend to be lower than chronological age, while other researchers have found dental age evaluated by these methods to be comparable to chronological age (40,41). In a previous study, it was reported that the mean dental age value set by Nolla's method

Table 4: *Statistically significant correlations observed among the assessed parameters for Sports High School and Fine Arts High School.*

Parameters	Pearson correlation coefficient		p value	
	Sports High School	Fine Arts High School	Sports High School	Fine Arts High School
Sports age-Chronological age	0.245		0.004*	
Sports age-Age group	0.255		0.003*	
Sports age-Haavikko's dental age	0.188		0.027*	
Sports age-Nolla's dental age	0.198		0.020*	
Chronological age-BMI	0.262		0.002*	
Age group-Haavikko's dental age	0.650	0.464	0.001*	0.001*
Age group-Nolla's dental age	0.577	0.421	0.001*	0.001*
Age group-BMI	0.287		0.001*	
Haavikko's dental age-Nolla's dental age	0.822	0.898	0.001*	0.001*
Haavikko's dental age-BMI	0.293	0.294	0.001*	0.001*
Nolla's dental age-BMI	0.345	0.284	0.001*	0.001*

* $p<0.05$

underestimated the chronological age value by 0.87 years in females and 1.18 years in males (37). Some studies utilizing Haavikko's method have reported that dental age results were consistent with chronological age, suggesting Haavikko's method to be a useful method (20,42). Other studies have found Haavikko's method to underestimate chronological age (43,44). In our study, mean dental age values found by Nolla's and Haavikko's methods were lower than mean chronological age values in both high school groups. Smaller differences between mean dental age values by Nolla's and Haavikko's methods and mean chronological age values in Sports High School students compared to Fine Arts High School students suggest that sports activities may have a positive impact on dental maturity. Mean dental age values assessed by both methods were found to be lower than mean chronological age values in female students of both high school groups. In male students of Sports High School, the difference between mean dental age values assessed by both methods and mean chronological age values was smaller than in male students of Fine Arts High School. Dental maturity was greater in males involved in sports activities compared to females, which may be associated with males taking part in more physically challenging activities.

Moreover, higher BMI values among males compared to females may indicate an acceleration in dental maturity due to increased BMI. A research has pointed out a potential relationship between skeletal and dental development and a positive effect of bone development on dental maturity (45). It may be considered that sports activities, which affect the piezoelectric effect influencing the stimulation of osteoblasts, may also have a positive effect on the stimulation of odontoblasts (46). On the other hand, while showing a tendency to underestimate mean chronological age values, mean dental age values assessed by Nolla's and Haavikko's methods were found to be consistent with each other and based on the guidance of previous studies, it was confirmed in our study that both methods are suitable for dental age estimation in children among the Turkish population (16-18).

Although the compatibility between mean dental age assessed by each method and mean chronological age differs by age group, it must be taken into consideration that consistency between dental age and chronological age may decrease as the number of teeth assessable for these methods decreases as individuals get older. In the dental age values estimated by Haavikko's method in Fine Arts High School, the tendency of dental age to be lower than chronological age among all age groups was noted. It was also observed that Nolla's method provided results closer to chronological age only in the 14 age group. Therefore, it can be inferred that Nolla's method may be suitable for use in 14-year-olds who do not participate in sports. The significantly lower dental age assessed by Haavikko's method compared to chronological age in the 14 and 15 age groups of Sports High School students may be explained by lower sports ages and BMI values seen in students among these age groups. On the other hand, because there was not a statistically significant difference between mean dental age values assessed by Nolla's method and mean chronologic age values in all age groups except the 18 age group in this high school, Nolla's method may be suitable for the dental age assessment of individuals who participate in sports. It was observed that for the 16 and 17 age groups in Sports High School, as sports age increases, dental maturity and BMI are positively influenced, and dental age values assessed by both methods tend to be closer to chronological age values. This finding in our study was compatible with a study conducted in the United States, which reported that long-term and regular sports activities had positive effects on BMI (47). In the 18 age group, the statistically significant underestimation of mean dental age values assessed by both methods compared with the mean chronological age values in both high schools may be associated with the decline in methodological sensitivity in this age group.

Approximately half of the body weight of an adult is gained during adolescence. It has been reported that having a BMI within normal ranges during this period is predictive of having healthy body measurements in adulthood (48,49). In a study conducted in Turkey among 664 adolescent participants with an average age of 14.48 years, it was reported that participants who exercise regularly had mean BMI values within normal values and these mean values were significantly lower than those who were not involved in sports and that sports activities contribute to maintaining a BMI value within normal ranges (49). In another study carried out in Turkey, BMI values of 204 adults who participated in sports and 208 adults who were not active were assessed, and it was found that BMI scores of individuals who were involved in sports were significantly lower, thus demonstrating the positive effect of sports on BMI (50). In a study performed on two Southern Californian high schools with 37 participants who were involved in sports and 37 participants who were not involved in sports between the ages of 14 and 17 years, it was found that the mean BMI value of the participants who were involved in sports was significantly lower. It was inferred that their general body health was in better condition when compared with the participants who were not involved in sports (48). In contrast, in a Canadian study in which 2278 children aged between 9 and 10 years were included, no statistically significant relationship was found between sports activities and BMI (23). In a study conducted on the Turkish population in which BMI values of 160 adults involved in sports were assessed, there was no statistically significant association between sports age and BMI (51). Despite some different findings, our study was consistent with the body of evidence indicating a positive influence of sports participation on BMI. The mean BMI value of Sports High School students in our study, who were designated as the sports group, was observed to be lower than the mean BMI value of Fine Arts High School students. The significant correlations detected between sports age and dental age and between dental age and BMI values suggest that sports participation positively affects dental maturity and bone development.

This study has several limitations that need to be considered. Firstly, there was no sports club with enough professional athletes in the province where the study was conducted, and hence, professional athletes could not be included in the study to assess the impact of sports on dental maturity. Secondly, there was only one Sports High School in the province, and the only high school that was willing to participate in the study among other high schools was Isparta Fine Arts High School. As a result, it was impossible to achieve an

equal distribution of gender among high schools. Additionally, only students who had indications for panoramic radiographs were included in the study, and their skeletal maturity was not evaluated to prevent further radiation exposure.

Conclusion

The results of this study suggest that there may be positive effects of sports on dental maturity and bone development, although these findings are subject to some limitations. Further studies are needed to confirm these results and provide more conclusive evidence on this topic.

Türkçe öz: *Adolesanlarda sporun diş yaşı üzerine etkisinin incelenmesi. Amaç: Bu çalışma, sporun diş olgunluğu üzerindeki etkisini iki farklı diş yaşı değerlendirme yöntemi kullanarak değerlendirmeyi ve diş olgunluğu ile vücut kitle indeksi arasında anlamlı bir ilişki olup olmadığını belirlemeyi amaçlamaktadır. Gereç ve Yöntem: Standart panoramik radyografileri olan Spor Lisesi'nden (çalışma grubu) 138 ve Güzel Sanatlar Lisesi'nden (kontrol grubu) 126 öğrenci çalışmaya dahil edildi. Diş yaşı, Nolla ve Haavikko yöntemleri kullanılarak değerlendirildi. Çalışma grubu katılımcılarının haftalık antrenman saatleri ve spor yaşları ile ilgili bilgiler edinildi. Tüm katılımcıların vücut kitle indeks değerleri hesaplandı. Faktöriyel varyans analizi ve Tukey testi yapıldı ve Pearson korelasyon katsayısı belirlendi. Bulgular: Öğrencilerin yaş ortalamaları; çalışma grubu için 15.93 ± 1.13 yıl, kontrol grubu için 15.99 ± 1.09 yıl idi. Her iki lisede de ortalama diş yaşı değerleri, ortalama kronolojik yaş değerlerinden daha düşüktü. Spor Lisesi'nde, diş yaşı ve kronolojik yaş arasındaki fark önemsizdi ($p > 0.05$). Liseler ve cinsiyetler arasındaki vücut kitle indeks farklılıkları, istatistiksel olarak anlamlıydı ($p < 0.05$). Spor yaşı ile diş yaşı arasında ve diş yaşı ile vücut kitle indeksi değerleri arasında anlamlı korelasyonlar tespit edildi. Sonuç: Spor, kemik gelişiminin yanı sıra diş olgunluğunu da olumlu yönde etkileyebilir. Anahtar Kelimeler: Diş yaşı, Haavikko metodu, Nolla metodu, spor, vücut kitle indeksi*

Ethics Committee Approval: The research protocol has been approved by the Clinical Research Ethics Committee of the Faculty of Medicine, Suleyman Demirel University, Isparta, Turkey (decision no. 103) and the Provincial Directorate of National Education, Governorship of Isparta, Turkey (decision no. 11998629).

Informed Consent: Informed consents were obtained from the participants and the parents or the legal guardians of the participants.

Peer-review: Externally peer-reviewed.

Author contributions: TE, DC participated in designing the study. TE, DC participated in generating the data for the study. TE participated in gathering the data for the study. TE, DC participated in the analysis of the data. TE, DC wrote the majority of the original draft of the paper. TE, DC participated in writing the paper. TE, DC have had access to all of the raw data of the study. TE, DC have reviewed the pertinent raw data on which the results and conclusions of this study are based. TE, DC have approved the final version of this paper. TE, DC guarantee that all individuals who meet the Journal's authorship criteria are included as authors of this paper.
Conflict of Interest: The authors declared that they have no conflict of interest.

Financial Disclosure: This work was supported by Suleyman Demirel University Scientific Research Projects Coordination Unit (project number: TDH-2018-6795).

Acknowledgements: The authors thank to Assoc. Prof. Dr. Ozgur Koskan from Department of Zootechnics-Biometry Genetics, Faculty of Agriculture, Isparta University of Applied Sciences, Isparta, Turkey for statistical analysis.

REFERENCES

1. Laine CM, Laine T. Diagnosis of osteoporosis in children and adolescents. Eur Endocrinol 2013;9:141-4.
2. Lin JT, Lane JM. Osteoporosis: a review. Clin Orthop Relat Res 2004;425:126-34. [CrossRef]
3. Riner WF, Sellhorst SH. Physical activity and exercise in children with chronic health conditions. J Sport Health Sci 2013;2:12-20. [CrossRef]
4. Akaike A, Suzuki D, Okuyama S, Kudo Y, Shimizu H, Takanashi S, *et al*. Associations between physical physique/fitness in children and bone development during puberty: a 4-year longitudinal study. Sci Rep 2022;12:13427. [CrossRef]
5. Atar M, Körperich EJ. Systemic disorders and their influence on the development of dental hard tissues: a literature review. J Dent 2010;38:296-306. [CrossRef]
6. Demirjian A, Levesque GY. Sexual differences in dental development and prediction of emergence. J Dent Res 1980;59:1110-22. [CrossRef]
7. Olze A, van Niekerk P, Ishikawa T, Zhu BL, Schulz R, Maeda H, *et al*. Comparative study on the effect of ethnicity on wisdom tooth eruption. Int J Legal Med 2007;121:445-8. [CrossRef]
8. Cameriere R, Flores-Mir C, Mauricio F, Ferrante L. Effects of nutrition on timing of mineralization in teeth in a Peruvian sample by the Cameriere and Demirjian methods. Ann Hum Biol 2007;34:547-56. [CrossRef]
9. Elamin F, Liversidge H. Malnutrition has no effect on the timing of human tooth formation. Plos One 2013;8:e72274. [CrossRef]
10. Uzuner FD, Kaygısız E, Darendeliler N. Defining dental age for chronological age determination. In: Dogan KH, editor. Post mortem examination and autopsy-Current issues from death to laboratory analysis. London: Intech Open, 2018, p.77-104. [CrossRef]
11. Moorrees CFA, Fanning EA, Hunt EE. Age variation of formation stages of ten permanent teeth. J Dent Res 1963;42:1490-502. [CrossRef]
12. Infante PF, Owen GM. Relation of chronology of deciduous tooth emergence to height, weight and head circumference in children. Arch Oral Biol 1973;18:1411-7. [CrossRef]
13. Eid RM, Simi R, Friggi MN, Fisberg M. Assessment of dental maturity of Brazilian children aged 6 to 14 years using Demirjian's method. Int J Paediatr Dent 2002;12:423-8. [CrossRef]
14. Chen J, Hu H, Guo J, Liu Z, Liu R, Li F, *et al*. Correlation between dental maturity and cervical vertebral maturity. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2010;110:777-83. [CrossRef]
15. Ercalikyalcinkaya S, Dumlu A, Bekiroglu N, Kizilyel G, Kargul B. Demirjian's system for estimating dental age among Northwestern Turkish children aged 4-16 years. Eur J Paediatr Dent 2013;14:225-30.
16. Nur B, Kusgoz A, Bayram M, Celikoglu M, Nur M, Kayipmaz S, *et al*. Validity of Demirjian and Nolla methods for dental age estimation for Northeastern Turkish children aged 5-16 years old. Med Oral Patol Oral Cir Bucal 2012;17:871-7. [CrossRef]
17. Kirzioglu Z, Ceyhan D. Accuracy of different dental age estimation methods on Turkish children. Forensic Sci Int 2012;216:61-7. [CrossRef]
18. Miloglu O, Celikoglu M, Dane A, Cantekin K, Yilmaz AB. Is the assessment of dental age by the Nolla method valid for eastern Turkish children? J Forensic Sci 2011;56:1025-8. [CrossRef]
19. Nolla CM. The development of permanent teeth. J Dent Child 1952;254-66.
20. Bagic IC, Sever N, Brkic H, Kern J. Dental age estimation in children using orthopantomograms. Acta Stomatol Croat 2008;42:11-8.
21. Smith BH. Standards of human tooth formation and dental age assessment. In: Kelley MA, Larsen CS, editors. Advances in dental anthropology. 1st Ed.,Washington: Wiley, 1991, p.143-68.
22. World Health Organization (WHO). Global strategy on diet, physical activity, and health. Geneva: 2004.

23. Cairney J, Veldhuizen S. Organized sport and physical activity participation and body mass index in children and youth: a longitudinal study. Prev Med Rep 2017;6:336-8. [CrossRef]
24. Ługowska K, Kolanowski W, Trafialek J. The impact of physical activity at school on children's body mass during 2 years of observation. Int J Environ Res Public Health 2022;19:3287. [CrossRef]
25. Haavikko K. Tooth formation age estimated on a few selected teeth. A simple method for clinical use. Proc Finn Dent Soc 1974;70:15-9.
26. Nuttall FQ. Body mass index: obesity, BMI, and health: a critical review. Nutr Today 2015;50:117-28. [CrossRef]
27. Felfe C, Lechner M, Steinmayr A. Sports and child development. Plos One 2016;11:e0151729. [CrossRef]
28. Brown KA, Patel DR, Darmawan D. Participation in sports in relation to adolescent growth and development. Transl Pediatr 2017;6:150-9. [CrossRef]
29. Timme M, Steinacker JM, Schmeling A. Age estimation in competitive sports. Int J Legal Med 2017;131:225-33. [CrossRef]
30. Boskey AL. Mineralization of bones and teeth. Elements 2007;3:387-93. [CrossRef]
31. Herman H, Dallemagne MJ. The main mineral constituent of bone and teeth. Arch Oral Biol 1961;5:137-44. [CrossRef]
32. Olze A, Bilang D, Schmidt S, Wernecke KD, Geserick G, Schmeling A. Validation of common classification systems for assessing the mineralization of third molars. Int J Legal Med 2005;119:22-6. [CrossRef]
33. Jeong MJ, Lee KE, Chae YK, Nam OH, Lee HS, Choi SC. Correlations between skeletal maturity and dental calcification stages in Korean children. Eur J Paediatr Dent 2022;23:101-5.
34. Jourieh A, Khan H, Mheissen S, Assali M, Alam MK. The correlation between dental stages and skeletal maturity stages. Biomed Res Int 2021;2021:9986498. [CrossRef]
35. Günen Yılmaz S, Harorlı A, Kılıç M, Bayrakdar İŞ. Evaluation of the relationship between the Demirjian and Nolla methods and the pubertal growth spurt stage predicted by skeletal maturation indicators in Turkish children aged 10-15: investigation study. Acta Odontol Scand 2019;77:107-13. [CrossRef]
36. Chaudhry K, Agarwal A, Rehani U. Interrelationship among dental, skeletal and chronological ages in urban and rural female children. Int J Clin Pediatr Dent 2010;3:79-86. [CrossRef]
37. Maber M, Liversidge HM, Hector MP. Accuracy of age estimation of radiographic methods using developing teeth. Forensic Sci Int 2006;159:68-73. [CrossRef]
38. Haavikko K. The formation and the alveolar and clinical eruption of the permanent teeth. An orthopantomographic study. Suom Hammaslaak Toim 1970;66:103-70.
39. Mani SA, Naing L, John J, Samsudin AR. Comparison of two methods of dental age estimation in 7-15 year old Malays. Int J Paediatr Dent 2008;18:380-8. [CrossRef]
40. Berkvens ME, Fairgrieve SI, Keenan S. A comparison of techniques in age estimation using the third molar. Can Soc Forensic Sci J 2017;50:74-83. [CrossRef]
41. Sachan K, Sharma VP, Tandon P. Reliability of Nolla's dental age assessment method for Lucknow population. J Pediatr Dent 2013;1:8-17. [CrossRef]
42. Galić I, Vodanović M, Cameriere R, Nakaš E, Galić E, Selimović E, *et al*. Accuracy of Cameriere, Haavikko and Willems radiographic methods on age estimation on Bosnian-Herzegovian children age groups 6-13. Int J Legal Med 2011;125:315-21. [CrossRef]
43. Butti AC, Clivio A, Ferraroni M, Spada E, Testa A, Salvato A. Haavikko's method to assess dental age in Italian children. Eur J Orthod 2009;31:150-5. [CrossRef]
44. Patnana AK, Vabbalareddy RS, Vanga NRV. Evaluating the reliability of three different dental age estimation methods in Visakhapatnam children. Int J Clin Pediatr Dent 2014;7:186-95.[CrossRef]
45. Flores-Mir C, Mauricio FR, Orellana MF, Major PW. Association between growth stunting with dental development and skeletal maturation stage. Angle Orthod 2005;75:935-40.
46. Frias C, Reis J, Capela e Silva F, Potes J, Simões J, Marques AT. Polymeric piezoelectric actuator substrate for osteoblast mechanical stimulation. J Biomech 2010;43:1061-6. [CrossRef]
47. Bailey RC, Olson J, Pepper SL, Porszasz J, Barstow TJ, Cooper DM. The level and tempo of children's physical activities: an observational study. Med Sci Sports Exerc 1995;27:1033-41. [CrossRef]
48. Ischander M, Zaldivar F Jr, Eliakim A, Nussbaum E, Dunton G, Leu SY, *et al*. Physical activity, growth and inflammatory mediators in BMI-matched female adolescents. Med Sci Sports Exerc 2007;39:1131-8. [CrossRef]
49. Tekgul N, Dirik N, Karademirci E, Dogan A. Observing the relation between body mass index (BMI) with related variables. Ege J Med 2012;51:183-6.
50. Sevimli D. Investigation of the relationship between body mass index and physical activity in adults. TAF Prev Med Bull 2008;7:523-8.
51. Sirinyildiz F, Cesur G, Alkan A, Ek RO. Determination of body mass index awareness of physical education and sports high school students. Smyrna Med 2017;3:1-6.

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Official Publication of Istanbul University Faculty of Dentistry

Eur Oral Res 2023; 57(2): 83-89

Original research

A randomized trial of the effectiveness of an ultrasonic denture hygiene intervention program among community-dwelling elders*

Purpose
This study aimed to assess the effectiveness of ultrasonic denture hygiene interventions in improving denture cleanliness among elderly individuals.

Materials and Methods
Sixty-six participants who had received upper metal framework removable partial dentures within the past 5 years were randomly allocated into three denture hygiene intervention groups: group 1 (mechanical cleaning with a toothbrush and ultrasonic cleaning with cetylpyridinium chloride), group 2 (mechanical cleaning with a toothbrush and ultrasonic cleaning with distilled water), and control (mechanical cleaning with a toothbrush only). Denture cleanliness was assessed at baseline and 1-month using: i) Denture Cleanliness Index (DCI) scores; ii) plaque coverage percentage; and (iii) microbiological samples for bacterial and yeast detection. Differences between groups were assessed with one-way analysis of variance and Chi-squared tests.

Results
Mean DCI scores and mean percentages of plaque coverage area were significantly reduced in group 1 and group 2, compared to the control group for both cobalt chromium (CoCr) and acrylic fitting surfaces ($p<0.001$). No significant differences were found between groups 1 and 2 with regard to the prevalence and viable counts of yeasts or total microbial viable counts. No significant differences in the investigated clinical and microbiological parameters were observed between CoCr and acrylic surfaces following the intervention period.

Conclusion
The ultrasonic cleaner was significantly more effective than mechanical cleaning in the reduction of biofilm coverage on metal framework removable partial dentures over a 1-month intervention period. Nevertheless, the adjunctive use of cetylpyridinium chloride with ultrasonic cleaning did not yield additional benefits.

Keywords: *Cetylpyridinium, Denture cleansers, Plaque, Randomized controlled trial, Ultrasonic*

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Introduction

Tooth loss is very common amongst elders in Hong Kong. Approximately 60% of non-institutionalized elderly (65 to 74 years old) have dental prostheses and over a third wear removable partial dentures (RPDs) (1). Denture hygiene has been increasingly recognized as a public health concern, especially with elders who, for diverse reasons, have difficulties in maintaining denture hygiene. The loss of manual dexterity, long-term diseases such as dementia, and lack of knowledge or guidance on proper cleaning methods are commonly cited factors (2, 3). The lack of regular oral hygiene practices not only leads to a build-up of dental plaque and

*This paper has previously been presented at the 65th Annual Meeting of JADR, Tokyo, Japan, November 18–19, 2017.

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Received: 17 November 2021
Revised: 12 September 2022
Accepted: 10 October 2022

DOI: 10.26650/eor.20231025114

How to cite: *Chan R, Zhang J, McGrath C, Tsang P, Lam O. A randomized trial of the effectiveness of an ultrasonic denture hygiene intervention program among community-dwelling elders. Eur Oral Res 2023; 57(2): 83-89.* DOI: 10.26650/eor.20231025114

periodontal disease, but also to the establishment of an oral reservoir of respiratory pathogens (4).

As such, the large majority of studies on oral health interventions have been targeted at improving oral care and the cleaning of the existing dentition with a wide range of mechanical oral hygiene aids such as electric toothbrushes, as well as chemotherapeutic agents delivered in the form of mouthrinses, sprays, and gels (5-8). A systematic review reported a lack of evidence, particularly in terms of randomized controlled trials, regarding the comparative effectiveness of mechanical or chemical methods to clean dentures (9). Additionally, all retrieved studies pertained to acrylic complete dentures worn by edentulous patients, and no study investigated cleaning methods for RPDs with metallic components. The latter is not suitable for cleansing with microwave irradiation or soaking in bleach, which have been suggested for the complete sterilization of complete acrylic dentures (10). Cruz et *al.* (11). published a clinical trial on ultrasonic denture cleaning and found a significant reduction in the percentage of biofilm coverage area compared to the control group; however, this study only assessed acrylic complete dentures. Currently, there is a lack of clinical studies assessing denture cleaning methods for partial dentures, especially those with a metallic framework.

The aim of this study was to investigate the effectiveness of a denture hygiene program using a combination of ultrasonic mechanical cleaning and an antiseptic agent (cetylpyridinium chloride [CPC]). Ultrasonic cleaning provides a mechanical cavitation effect which loosens and removes adherent microbial biofilms (10). CPC is a quaternary ammonium compound with established antimicrobial properties and has a long track record of *in vivo* use as a mouthrinse (12). While previous *in vitro* studies have demonstrated its compatibility with RPDs incorporating metal components such as frameworks and clasps, the utility of CPC as a denture cleanser in combination with ultrasonic cleaning has not yet been investigated (13).

The objective of this study was to compare the effectiveness of three denture hygiene interventions: (1) ultrasonic cleaning with 0.07% CPC, (2) ultrasonic cleaning with distilled water, and (3) mechanical cleaning with a soft toothbrush and liquid detergent [Control group] in improving denture cleanliness among community-dwelling elders over a 1-month period. The secondary objectives were to evaluate changes in microbial levels and to compare the effects of the interventions on acrylic and metallic denture fitting surfaces. The null hypotheses were that there would be no differences in denture cleanliness or microbial levels between the three intervention groups, as well as no differences in outcomes between acrylic and metallic denture surfaces.

Material and Methods

Ethical statement

The protocol for this randomized controlled single-blind trial was approved by the Institutional Review Board of the University of Hong Kong (approval number UW 16-266).

Sample size estimation

Denture plaque was the primary outcome variable in this study. Based on 80% power, a statistical significance level set at 0.05, and a previously documented mean plaque coverage score of 28.11 (standard deviation=19.64), we determined that 20 patients were required in each group to detect a 20% difference in denture plaque coverage scores among groups (14). Taking into account an anticipated 10% dropout rate, the proposed sample size was 66 participants (22 per group) (14).

Study population

The study population comprised elders aged 65 years old or above who were previously provided with cobalt chromium (CoCr) RPDs within the past 5 years at a dental hospital. A full list of patients meeting these inclusion criteria was generated from the computerized patient database. A total of 66 patients were selected by random sampling; these patients were contacted by letter and telephone and invited to undergo a clinical examination. Written informed consent was obtained, and ethics approval was granted by the appropriate institutional review board.

Study protocol

The baseline and 1-month review examinations included assessments of oral health and denture cleanliness. Denture cleanliness was assessed clinically and microbiologically by a single assessor, who was blinded to treatment allocation and not involved in the provision of denture hygiene instructions (conducted by a research assistant). Intra-examiner reproducibility was determined in a randomly selected subset (10%) of participants. A basic intra-oral examination was conducted and recorded with dental charts. Dental plaque levels and gingival status were assessed with the Silness and Löe Plaque Index (PI) and the Gingival Bleeding Index (GBI), respectively (15, 16). Dental caries (DMFT index) and periodontal status (Community Periodontal Index [CPI]) were assessed in accordance with World Health Organization guidelines (17). Sociodemographic characteristics and baseline PI, GBI, DMFT, and CPI scores were compared among groups to confirm the absence of bias in the randomization process.

Denture cleanliness was assessed both qualitatively and quantitatively. Qualitative assessment comprised disclosing denture plaque on the fitting surface with a plaque disclosing agent (GUM® Red-Cote® Liquid; Sunstar Americas, Inc., Chicago, IL, USA) and rating the denture according to Denture Cleanliness Index (DCI) criteria (18). The DCI score was graded by visual assessment of the stained area of the denture fitting surface. CoCr frameworks and acrylic saddles were graded separately. Quantitative analysis of denture cleanliness was determined by obtaining photographic images for planimetric assessment (4). Images of the dentures were captured by a digital camera (Nikon D80 AF-S Nikkor 60mmf/2.8G ED, Nikon Inc., Tokyo, Japan) with a fixed manual setting (aperture F29; exposure time 1/100 ISO 125). The film-object distance was standardized by a camera stand. Plaque coverage of the fitting surfaces was quantitatively determined with Adobe Photoshop® (CC2014: Adobe Sys-

tems Inc., San Jose, CA). In brief, the JPG file of each denture image was opened, and a "magnetic lasso" tool used to outline the margin of the CoCr framework, which was cut and pasted to a new file. The "magic wand" tool was used to select the plaque-stained area (threshold 30), and this was copied and pasted to a new file. The percentage of plaque coverage area was determined by total plaque pixels/total denture surface pixels. The same procedures (steps 1–4) were repeated for the acrylic saddles.

The imprint technique was used to obtain microbiological samples from denture fitting surfaces (19, 20). In brief, (2.0 x 2.0 cm) and (0.5 x 0.5 cm) sterile foam pads were pressed to the denture fitting surface of the CoCr major connector and acrylic resin saddle for 60 seconds. Imprint samples were then transferred to tubes containing 10 mL saline, vortexed, and spiral plated on Sabouraud agar (yeast) and blood agar (total microbial count). Colony-forming units (CFU) were enumerated for all samples, and pure cultures were obtained for storage (-70°C) and subsequent identification. Pure cultures were identified by colony morphology, Gram stain, and commercial identification kits (ID32C [bioMerieux Vitek; Hazelwood, MO, USA]).

Cleaning protocols

Following baseline assessments, participants were randomly allocated to one of three groups: Group 1 (Mechanical cleaning of the RPD with a soft toothbrush and liquid detergent, plus ultrasonic cleaning [42 kHz] for 7 min and 30 s with 0.07% CPC mouthrinse [Oral-B® Pro-Health™; Boston, MA, USA], once daily); Group 2 (Mechanical cleaning with a soft toothbrush and liquid detergent and ultrasonic cleaning [42 kHz] for 7 min and 30 s with distilled water, once daily); and Group 3 (Control group, mechanical cleaning with a soft toothbrush and liquid detergent, once daily).

Randomization (block randomization; random number table; sequentially numbered, sealed, opaque envelopes) was performed by a research assistant who was not involved in the clinical assessment of outcome measures. Participants were provided with verbal and written denture hygiene instructions. Customized oral hygiene instructions for participants' remaining natural dentition were provided on a one-to-one basis. The principal investigator conducting the clinical assessments was blinded to treatment group allocation. A manual toothbrush and standard sodium fluoride toothpaste were provided to participants in all groups.

Statistical analysis

Changes in continuous variables within groups from baseline to 1 month were analyzed by two-way analysis of variance (ANOVA) and paired t-tests (or nonparametric equivalent); a complete case analysis was performed. One-way ANOVA and two-sample t-tests (or nonparametric equivalent) were used to investigate differences between the three intervention groups. Comparisons of categorical variables between the three intervention groups were performed with Chi-squared tests, while changes within groups from baseline to 1 month were identified with the Cochran Q test. Multiple linear regression analyses were used to evaluate potential independent factors associated with continuous variables and categorical variables at baseline and 1 month. All analyses were conducted with the Statistical Package for Social Sciences (SPSS) for Windows software, version 16.0 (SPSS Inc., Chicago, IL,USA).

Results

During the recruitment period, a total of 84 patients were contacted by phone; 19 patients declined to participate in the study, citing reasons such as time conflicts due to employment, disabilities affecting mobility, and lack of interest. Three patients did not meet inclusion criteria. A total of 66 patients (48 female; 18 male) were recruited at baseline examination and randomly allocated into one of three groups. Two patients dropped out prior to the 1-month review. One patient reported that she could not operate the ultrasonic cleaner. Another subject did not return for review due to time conflicts. The mean age of the patients was 66.97±6.91 years, and the average denture service life was 19.05±5.63 months. There was a significantly higher percentage of females (90.9%) than males in Group 3. There were no significant differences in age, mean denture service life, Kennedy classification type, PI scores, DMFT, CPI, or loss of attachment (LOA) between groups at baseline.

DCI assessment

The mean DCI scores on CoCr major connectors were not significantly different between groups at baseline (Table 1). Within-group comparisons found significantly lower mean DCI scores at review compared to baseline, for both groups 1 and 2 ($p<0.001$). A significantly higher mean DCI score at review was found in group 3 (2.09±0.75) compared to group 2 (1.15±0.37) and group 1 (1.14±0.56) ($p<0.001$), while no significant differences were noted between groups 1 and 2. Group 1 (1.45±0.86) and group 2 (1.00±0.86) had significantly larger mean DCI change scores than group 3 (0.23±0.68; $p<0.001$) (Table 1).

The mean DCI scores on acrylic saddles did not show significant differences at baseline between groups ($p=0.189$). Significantly lower mean DCI scores were observed on review compared to baseline in both groups 1 ($p<0.001$) and 2 ($p<0.001$). At review, group 3 (1.95±0.72) demonstrated significantly higher mean scores than group 1 (1.09±0.53) and group 2 (1.15±0.59) ($p<0.001$). Mean DCI change scores in group 1 (1.68±0.95) and group 2 (1.20±1.01) were significantly higher than that in group 3 (0.36±0.79; $p<0.001$). There were no significant differences in mean DCI scores between CoCr major connectors and acrylic saddles within groups at either baseline or review. Mean change scores were not significantly different between CoCr and acrylic surfaces in any of the three groups.

Factors assessed for association with DCI scores of CoCr major connectors included age, sex, denture wearing time, PI and BI at baseline, DMFT, CPI, LOA, patient compliance, Kennedy classification of dentures, yeasts, DCI scores and plaque coverage percentage. Multiple linear regression analyses identified that denture wearing time ($p=0.014$) and CPI scores ($p=0.025$) were significantly associated with DCI scores at baseline. After the clinical trial, however, only interventions 1 and 2 ($p<0.001$), DCI scores at baseline

(p<0.001), and the presence of yeast on CoCr (p=0.024) were significantly associated with 1-month DCI scores (adjusted R^2=0.53). DCI scores (on acrylic) were not significantly associated with any of the investigated factors at baseline; interventions 1 and 2 (p<0.001) were significantly associated with 1-month DCI scores (adjusted R^2=0.38).

Plaque coverage scores

At baseline, the mean percentage of plaque coverage on CoCr major connectors was not significantly different among the three groups (p=0.596) (Table 2). All three groups demonstrated a significant reduction in mean plaque coverage scores at review compared to baseline (p<0.05). A significantly lower mean plaque coverage score was found in group 1 (17.89±12.9) and 2 (16.22±9.22) compared to group 3 on review (39.60±20.68; p<0.001). Mean changes in plaque coverage scores in group 1 (28.70±16.59) and group 2 (22.79±17.88) were significantly greater than in group 3 (8.3±11.39; p<0.001). No significant differences were observed between groups 1 and 2.

There were no significant differences in mean plaque coverage scores among acrylic saddles, between groups at baseline (Table 2). Within-group comparisons found significantly lower mean plaque coverage scores at review compared to baseline, for all three groups (p<0.05). At review, the mean percentages in group 1 (19.33±12.34) and group 2 (17.82±10.09) were significantly lower than group 3 (38.83±17.96; p<0.001). Mean plaque coverage change scores were significantly higher in group 1 (34.98±24.11) and 2 (28.40±22.58), compared to group 3 (5.21±11.23; p<0.001). Comparisons of mean plaque coverage scores between CoCr and acrylic surfaces did not yield significant differences within groups at baseline and review, or in the change scores between groups. BI (p=0.039) and DMFT (p=0.049) were significantly associated with plaque coverage percentage on CoCr major connectors at baseline; only interventions 1 and 2 (p<0.001) and plaque coverage percentage (baseline) remained significant in the final model at review (adjusted R^2=0.64). None of the investigated factors were significantly associated with plaque coverage percentage on acrylic at baseline; plaque coverage percentage at 1 month was associated with interventions 1 and 2 (p<0.001), as well as plaque percentage coverage at baseline (p=0.006; adjusted R^2=0.38).

Microbiological assessments

Median microbial viable counts (CFU/mL) on CoCr surfaces were significantly lower at review compared to baseline in group 1 (p=0.009) and group 2 (p<0.001); no significant differences were observed in group 3 (p=0.097). No significant differences were found between groups with respect to changes in microbial viable counts from baseline to 1 month (p=0.259). No significant differences were observed when comparing median microbial viable counts (CFU/mL) on CoCr surfaces between groups at baseline (p=0.940) or review (p=0.842). Group 1 demonstrated significantly lower mean yeast viable counts (CFU/mL) at 1-month review compared to baseline (p=0.004). There were no significant differences in mean yeast viable count change scores between groups. Acrylic saddles in group 1 (p=0.019) and group 2 (p<0.001) exhibited a significant reduction in microbial viable counts (CFU/mL) at 1 month compared to baseline. No significant difference was found in group 3 (p=0.153). There were no significant differences between groups in median CFU/mL at baseline (p=0.539) and after 1 month (p=0.665). Reductions in microbial viable counts were significantly greater in group 2 compared to group 3 (p=0.047). There were no significant differences in microbial viable counts on CoCr compared to acrylic surfaces, with the exception of a higher median CFU/mL on acrylic surfaces in Group 2 at baseline (p=0.035). No significant differences were observed at review.

Patient compliance

Just over half (n=38) of the subjects remembered to submit their log diary, in which they had documented their denture cleaning schedule, at the review appointment. All submitted log diaries reflected a strict compliance to daily denture cleaning, according to the proposed protocol. Of those participants who forgot to return their diary, nearly 80% (n=51) reported that they had cleaned their dentures

Table 1. *Comparison of DCI scores between CoCr and acrylic surfaces among and within groups [mean (SD)] .*

	N=64	Group 1 N=22	Group 2 N=20	Group 3 N=22	p-value†	Multiple comparisons
			Mean (SD)			
CoCr surfaces	DCI (baseline)	2.59 (0.91)	2.27 (0.94)	2.32 (0.89)	0.462	
	DCI (review)	1.14 (0.56)	1.15 (0.37)	2.09 (0.75)	P<0.001	(1)=(2)<(3)
	p-value‡	P<0.001	P<0.001	0.135		
	DCI change score	1.45 (0.86)	1.00 (0.86)	0.23 (0.68)	P<0.001	(1)=(2)>(3)
Acrylic surfaces	DCI (baseline)	2.77 (0.75)	2.45 (0.80)	2.32 (0.95)	0.189	
	DCI (review)	1.09 (0.53)	1.15 (0.59)	1.95 (0.72)	P<0.001	(1)=(2)<(3)
	p-value‡	P<0.001	P<0.001	0.062		
	DCI change score	1.68 (0.95)	1.20 (1.01)	0.36 (0.79)	P<0.001	(1)=(2)>(3)

One-way analysis of variance†; Paired-Samples T test‡; SD, standard deviation; CoCr, cobalt chromium; DCI, Denture Cleanliness Index; N, number of patients; Group 1, mechanical cleaning with a toothbrush and ultrasonic cleaning with cetylpyridinium chloride; Group 2, mechanical cleaning with a toothbrush and ultrasonic cleaning with distilled water; Group 3, mechanical cleaning with a toothbrush only.

every day. The common reasons for poor compliance for the rest of the subjects included travelling away from home during the intervention period, or being too busy or indifferent to do so. The majority of participants (95.24%) who received ultrasonic cleaners reported that they were satisfied with them. They felt that the cleaners were quiet and effective, and some participants commented that they "felt good" when they saw debris being dislodged from the dentures during the cleaning process. Negative comments were mainly related to the time-consuming operating procedure of the ultrasonic units, while a few (n=3) participants in group 1 who used the ultrasonic cleaner together with the mouthrinse (0.07% CPC) reported the noticeable accumulation of dark stains on the dentures.

Discussion

This study addressed the lack of evidence regarding the effectiveness of denture cleaning methods on partial dentures, especially those with a metallic framework. The results suggested that ultrasonic cleaning was significantly more effective than the control (cleaning with a soft toothbrush and liquid detergent) in the reduction of biofilm coverage on metal framework RPDs over a 1-month intervention period. The adjunctive use of CPC with ultrasonic cleaning, however, did not yield improved outcomes compared to water. Therefore, the null hypothesis of no differences in denture cleanliness among the three intervention groups was partially rejected.

Denture hygiene was assessed with respect to three aspects: DCI scores, plaque coverage percentage, and microbiological tests. Group 1 and group 2 showed significantly more reduction in mean DCI and plaque coverage scores than group 3, while no significant differences could be shown between groups 1 and 2. This applied to both CoCr and acrylic surfaces. These findings concur with those previously published by Cruz *et al*. (11), who reported that ultrasonic vibration could improve denture hygiene in terms of decreasing the biofilm coverage area in acrylic complete dentures. The adjunctive use of CPC (group 1) in our study, however, suggests the lack of any additive effect over water (group 2). Patient age was not a significant factor associated with denture hygiene. Denture service life, however, was significantly associated with DCI scores of CoCr surfaces, and plaque coverage scores of both CoCr and acrylic. This may be explained by a longer history of denture use and a potentially higher frequency of cumulative defects on the denture surface, which may have provided more favorable habitats for biofilm formation.

Microbiological tests indicated that group 1 had significant reductions in microbial viable counts (CoCr and acrylic) and yeasts; group 2 exhibited similar results. Significantly greater reductions in microbial viable counts were observed for acrylic saddles in group 2 compared to the control, while trends towards greater reductions were consistently observed in groups 1 and 2 compared to the control. Thus, the null hypothesis of no differences in microbial counts among the three intervention groups was partially rejected. These results could imply that the use of ultrasonic cleaners may have additional beneficial effects against microbial levels compared to manual brushing only. The lack of statistical significance might be due to the limited sample size, which was powered to detect differences in denture plaque. Previous studies conducted among patients with complete acrylic dentures have reported significantly greater reductions in total bacterial and mutans streptococci counts with combined effervescent tablet and ultrasonic cleaning, relative to ultrasonic cleaning alone, while effects on yeast counts have been inconsistent (21-24).

Previous studies have suggested a relationship between bacterial colonization and surface roughness: the rougher the surface, the more retentive and less susceptible to mechanical removal the biofilm attachment is (25). A well-polished metal surface is thought to be more resistant to biofilm attachment than acrylic. When a denture has been in prolonged use, the acrylic surface exhibits various kinds of defects such as cracks, porosities, and fractures (26). Improper denture cleaning methods, for example using abrasive dentifrice to

Table 2. *Comparison of plaque coverage scores between CoCr and acrylic surfaces among and within groups [mean (SD)].*

	N=64	Group 1 N=22	Group 2 N=20	Group 3 N=22	p-value‡	Multiple comparison
		Mean (SD)				
CoCr surfaces	Plaque coverage score (baseline)	46.59 (20.41)	41.72 (19.29)	47.96 (23.88)	0.596	
	(review)	17.89 (12.96)	16.22 (9.22)	39.60 (20.68)	P<0.001	
	p-value†	P<0.001	P<0.001	0.002		
	Plaque coverage change score	28.70 (16.59)	22.79 (17.88)	8.35 (11.39)	P<0.001	(1)=(2)>3
Acrylic surfaces	Plaque coverage score (baseline)	54.32 (20.44)	49.06 (20.71)	43.54 (20.83)	0.232	
	(review)	19.33 (12.34)	17.82 (10.09)	38.83 (17.96)	P<0.001	
	p-value†	P<0.001	P<0.001	0.041		
	Plaque coverage change score	34.98 (24.11)	28.40 (22.58)	5.21 (11.23)	P<0.001	(1)=(2)>3

SD, standard deviation; CoCr, cobalt chromium; N, number of patients; Group 1, mechanical cleaning with a toothbrush and ultrasonic cleaning with cetylpyridinium chloride; Group 2, mechanical cleaning with a toothbrush and ultrasonic cleaning with distilled water; Group 3, mechanical cleaning with a toothbrush only Paired-Samples T test†; One-way ANOVA‡

brush the fitting surface, increases the roughness of the acrylic surface. Such factors contribute to and facilitate microbial colonization and biofilm formation. In this study, however, no significant differences were shown between the CoCr and acrylic surfaces in terms of bacterial or yeast viable counts, DCI scores, or plaque coverage scores at both baseline and review; an exception was the viable bacterial count on blood agar. Thus, the null hypothesis of no differences in outcomes between CoCr and acrylic surfaces was partially rejected. This may be explained by the effectiveness of the post-operative instructions given to all patients, which included appropriate ways of handling and cleaning dentures by soft bristle toothbrushes without toothpaste. In addition, the mean denture service life was relatively short (19.05±5.63 months), and the fitting surfaces were observed to be in good condition; thus, this may have accounted for the lack of significant differences compared to the CoCr surfaces.

Some limitations in the present study are acknowledged. The first is the low rate of documented patient compliance; slightly more than half of the patients (59%) returned completed log diaries to document their level of adherence to the proposed daily denture hygiene regimen. Nevertheless, all submitted log diaries reflected strict compliance; furthermore, of those participants who forgot to return their log diary at the review assessment, the vast majority (nearly 80%) reported that they had cleaned their dentures every day using the prescribed regimen. Nevertheless, documented compliance was not found to be a significant factor associated with denture cleanliness in the regression analyses. Thus, the results suggest that the use of ultrasonic cleaning was still significantly more effective than conventional cleaning, even with the lack of strict adherence to daily use. Another limitation was that the sample size was not large enough to detect significant differences among specific pathogens. In addition, some participants (n=16) were still under professional dental care in the teaching clinics. Treatments such as scaling or OHI were prescribed during the research period, which may have provided extra positive outcomes in terms of oral hygiene condition.

Further investigation of adjunctive agents that can be used with ultrasonic cleaning, and which are also compatible with metal framework RPDs, are needed. Future studies with larger sample sizes and longer follow-up times will be required in order to determine the effectiveness of ultrasonic denture cleaning in reducing the prevalence and viable counts of oral opportunistic pathogens. This is especially pertinent in medically compromised and institutionalized elderly, who have been shown to have poorer oral health and denture hygiene. As such, the expansion of this denture hygiene intervention to other vulnerable groups is of paramount importance, and warrants further study.

Conclusion

Within the limitations of this randomized clinical trial, ultrasonic cleaning was shown to be equally effective in the reduction of biofilm coverage on both CoCr and acrylic denture surfaces during a 1-month intervention period. The adjunctive use of CPC did not provide additional benefits over distilled water, with regards to the improvement of denture hygiene.

Türkçe özet: *Toplulukta yaşayan yaşlılar arasında bir ultrasonik takma diş hijyeni müdahale programının etkinliğinin rastgellenmiş incelemesi. Amaç: Bu çalışma, yaşlı bireylerde protez temizliğini iyileştirmede ultrasonik protez hijyeni müdahalelerinin etkinliğini değerlendirmeyi amaçladı. Gereç ve Yöntem: Son 5 yıl içinde üst metal hareketli bölümlü protezleri olan 66 katılımcı rastgele üç protez hijyeni müdahale grubuna ayrıldı: grup 1 (diş fırçası ile mekanik temizlik ve setilpiridinyum klorür ile ultrasonik temizlik), grup 2 (diş fırçasıyla mekanik temizleme ve damıtılmış suyla ultrasonik temizleme) ve kontrol (yalnızca diş fırçasıyla mekanik temizleme). Protez temizliği başlangıçta ve 1. ayda aşağıdakiler kullanılarak değerlendirildi: i) Protez Temizlik İndeksi (DCI) puanları; ii) plak kaplama yüzdesi; ve (iii) bakteri ve maya tespiti için mikrobiyolojik numuneler. Gruplar arasındaki farklılıklar sırasıyla tek yönlü varyans analizi ve Ki-kare testleri ile değerlendirildi. Bulgular: Ortalama DCI skorları ve ortalama plak kaplama alanı yüzdeleri, hem kobalt krom (CoCr) hem de akrilik bağlantı yüzeyleri için grup 1 ve grup 2'de kontrol grubuna göre anlamlı derecede azaldı (p<0,001). Grup 1 ve 2 arasında mayaların prevalansı ve canlı sayıları veya toplam mikrobiyal canlı sayıları açısından önemli bir fark bulunmadı. Müdahale süresinden sonra CoCr ve akrilik yüzeyler arasında incelenen klinik ve mikrobiyolojik parametrelerde anlamlı bir fark gözlenmedi. Sonuç: Ultrasonik temizleyici, 1 aylık bir müdahale süresi boyunca metal hareketli bölümlü protezlerde biyofilm kaplamasının azaltılmasında mekanik temizlemeden önemli ölçüde daha etkiliydi. Bununla birlikte, setilpiridinyum klorürün ultrasonik temizleme ile birleşik kullanımı ek faydalar sağlamadı. Anahtar Kelimeler: Setilpiridinyum, Protez temizleyiciler, Plak, Randomize kontrollü çalışma, Ultrasonik*

Ethics Committee Approval: The protocol for this randomized controlled single-blind trial was approved by the Institutional Review Board of the University of Hong Kong (approval number UW 16-266).

Informed Consent: Participants provided informed constent.
Peer-review: Externally peer-reviewed.

Acknowledgements: The authors would like to thank the patients who participated in this study, and the staff in the Oral Bioscience Unit (Faculty of Dentistry, The University of Hong Kong) for their assistance in the culturing and identification of the microbial samples.

Author contributions: RC, CM, OL participated in designing the study. RC, JZ participated in generating the data for the study. RC, JZ, PT participated in gathering the data for the study. RC, CM, OL participated in the analysis of the data. RC, OL wrote the majority of the original draft of the paper. RC, OL participated in writing the paper. RC has had access to all of the raw data of the study. RC has reviewed the pertinent raw data on which the results and conclusions of this study are based. RC, JZ, CM, PT, OL have approved the final version of this paper. OL guarantees that all individuals who meet the Journal's authorship criteria are included as authors of this paper.

Conflict of Interest: The authors had no conflict of interest to declare.

Financial Disclosure: This study was funded by the Seed Funding Programme for Basic Research, The University of Hong Kong.

References

1. Department of Health. Oral Health Survey 2011. Hong Kong: Department of Health, Government of the Hong Kong Special Administrative Region 2011, p.1-226.
2. Murtomaa H, Meurman JH. Mechanical aids in the prevention of dental diseases in the elderly. Int Dent J 1992; 42: 365-72.
3. Jorge Junior J, de Almeida OP, Bozzo L, Scully C, Graner E. Oral mucosal health and disease in institutionalized elderly in Brazil. Community Dent Oral Epidemiol 1991; 19: 173-5. [CrossRef]
4. Coulthwaite L, Verran J. Evaluation of in vivo denture plaque assessment methods. Br Dent J 2009; 207: E12. [CrossRef]
5. Blahut P. A clinical trial of the INTERPLAK powered toothbrush in a geriatric population. Compend Suppl 1993; 16: S606-10.

6. Quagliarello V, Juthani-Mehta M, Ginter S, Towle V, Allore H, Tinetti M. Pilot testing of intervention protocols to prevent pneumonia in nursing home residents. J Am Geriatr Soc 2009; 57: 1226-31. [CrossRef]
7. Clavero J, Baca P, Junco P, Gonzalez MP. Effects of 0.2% chlorhexidine spray applied once or twice daily on plaque accumulation and gingival inflammation in a geriatric population. J Clin Periodontol 2003; 30: 773-7. [CrossRef]
8. Lopez RM, Uribe MR, Rodriguez BO, Casasempere IV. Comparison between amine fluoride and chlorhexidine with institutionalized elders: a pilot study. Gerodontology 2013; 30: 112-8. [CrossRef]
9. de Souza RF, de Freitas Oliveira Paranhos H, Lovato da Silva CH, Abu-Naba'a L, Fedorowicz Z, Gurgan CA. Interventions for cleaning dentures in adults. Cochrane Database Syst Rev 2009; 4: CD007395. [CrossRef]
10. Felton D, Cooper L, Duqum I, Minsley G, Guckes A, Haug S, Meredith P, Solie C, Avery D, Chandler ND, American College of Prosthodontists. Evidence-based guidelines for the care and maintenance of complete dentures: a publication of the American College of Prosthodontists. J Prosthodont 2011; 20: S1-12. [CrossRef]
11. Cruz PC, Andrade IM, Peracini A, de Souza-Gugelmin MCM, Silva-Lovato CH, de Souza RF, de Freitas Oliveira Paranhos H. The effectiveness of chemical denture cleansers and ultrasonic device in biofilm removal from complete dentures. J Appl Oral Sci 2011; 19: 668-73. [CrossRef]
12. Gjermo P, Baastad KL, Rolla G. The plaque-inhibiting capacity of 11 antibacterial compounds. J Periodontal Res 1970; 5: 102-9. [CrossRef]
13. Felipucci DN, Davi LR, Paranhos HF, Bezzon OL, Silva RF, Pagnano VO. Effect of different cleansers on the surface of removable partial denture. Braz Dent J 2011; 22: 392-7. [CrossRef]
14. Au KY, Cheung HC, Chung SM, Lai CF, Leung KK, Leung YF, Ng YC, Wah YS, Wong CL. Community Health Project: effectiveness of a denture hygiene intervention programme among institutionalized elders. Hong Kong: The University of Hong Kong 2015, p.1-68.
15. Silness J, Loe H. Periodontal disease in pregnancy. II. Correlation between oral hygiene and periodontal condition. Acta Odontol Scand 1964; 22: 121-35. [CrossRef]
16. Ainamo J, Bay I. Problems and proposals for recording gingivitis and plaque. Int Dent J 1975; 25: 229-35.
17. Petersen PE, Baez RJ. Oral health surveys: basic methods. 5th Ed., Geneva: World Health Organization 2013, p.1-125.
18. Mylonas P, Afzal Z, Attrill DC. A clinical audit of denture cleanliness in general dental practice undertaken in the West Midlands. Br Dent J 2014; 217: 231-4. [CrossRef]
19. Perezous LF, Stevenson GC, Flaitz CM, Goldschmidt ME, Engelmeier RL, Nichols CM. The effect of complete dentures with a metal palate on candida species growth in HIV-infected patients. J Prosthodont 2006; 15: 306-15. [CrossRef]
20. Samaranayake LP, MacFarlane TW, Lamey PJ, Ferguson MM. A comparison of oral rinse and imprint sampling techniques for the detection of yeast, coliform and Staphylococcus aureus carriage in the oral cavity. J Oral Pathol 1986; 15: 386-8. [CrossRef]
21. Nishi Y, Seto K, Kamashita Y, Kaji A, Kurono A, Nagaoka E. Survival of microorganisms on complete dentures following ultrasonic cleaning combined with immersion in peroxide-based cleanser solution. Gerodontology 2014; 31: 202-9. [CrossRef]
22. de Andrade IM, Cruz PC, da Silva CH, de Souza RF, de Freitas Oliveira Paranhos H, Candido RC, Marin JM, de Souza-Gugelmin MCM. Effervescent tablets and ultrasonic devices against Candida and mutans streptococci in denture biofilm. Gerodontology 2011; 28: 264-70. [CrossRef]
23. Duyck J, Vandamme K, Krausch-Hofmann S, Boon L, De Keersmaecker K, Jalon E, Teughels W. Impact of denture cleaning method and overnight storage condition on denture biofilm mass and composition: a cross-over randomized clinical trial. PloS one 2016; 11: e0145837. [CrossRef]
24. Papadiochou S, Polyzois G. Hygiene practices in removable prosthodontics: a systematic review. Int J Dent Hyg 2018; 16: 179-201. [CrossRef]
25. Teughels W, Van Assche N, Sliepen I, Quirynen M. Effect of material characteristics and/or surface topography on biofilm development. Clin Oral Implants Res 2006; 17: 68-81. [CrossRef]
26. Dhiman RK, Chowdhury SR. Midline fractures in single maxillary complete acrylic vs flexible dentures. Med J Armed Forces India 2009; 65: 141-5. [CrossRef]

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Official Publication of Istanbul University Faculty of Dentistry

Eur Oral Res 2023; 57(2): 90-95

Original research

Influence of home bleaching regimen on microhardness and flexural strength of two contemporary composite resins – an in vitro evaluation

Muralidasan Kalaivani[1], S Datta Prasad[2], M Indumathi[3], M Sruthipriya[4], Balachandran Janani[1], O Pavankumar[5]

Purpose
This study was to compare and evaluate the effect of home bleaching on the microhardness and flexural strength of microhybrid and nanohybrid composite resins.

Materials and Methods
The study samples were prepared using a custom-made silicon rubber mold. For microhardness evaluation, 40 disc-shaped specimens (4mm*2mm) were prepared and divided into 4 groups: GROUP A (n=10): microhybrid (GC Solaire X, GC Corporation) control group, GROUP B (n=10) nanohybrid (Tetric N Ceram, Ivoclar Vivadent) control group, GROUP C (n=10): microhybrid bleaching group, GROUP D (n=10) nanohybrid bleaching group. For flexural strength evaluation, 40 bar shaped specimens (25mm*2mm*2mm) were prepared. They were divided into 4 groups, GROUP 1 (n=10): microhybrid control group, GROUP 2 (n=10) nanohybrid control group, GROUP 3 (n=10): microhybrid bleaching group, GROUP 4(n=10) nanohybrid bleaching group. All the control groups were placed in artificial saliva and bleaching groups were exposed to home bleaching agent for 14 days according to manufacturer's instructions. The microhardness and flexural strength were evaluated for the respective specimens after 14 days and the data were statistically analyzed.

Results
Home bleaching regimen decreased microhardness of both microhybrid and nanohybrid composites whereas there was no significant effect on the flexural strength. Nanohybrid composites showed greater microhardness values before and after bleaching.

Conclusion
Bleaching agents, irrespective of their concentration can decrease the microhardness of the composite resin samples, which raises a concern about replacement of these restorations due to the effects on physical and mechanical properties.

Keywords: *Composite resin, Home bleach, Nanohybrid, Microhardness, Flexural strength*

Introduction

Esthetics is *"the science of beauty, that particular detail of an animate or inanimate object, that makes it appealing to the eye"* (1). Esthetic dentistry deals with various therapeutic techniques that can enhance or restore the shape, texture, form, color and position of the teeth (2).

Vital tooth bleaching is one of the popular treatment modalities in the management of discolored teeth that encompasses a broad array of materials ranging from over the counter (OTC) products to sophisticated in office bleaching systems. Home bleaching regimen is gaining more pop-

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Received: 7 March 2022
Revised: 30 September 2022
Accepted: 10 October 2022

DOI: 10.26650/eor.20231083203

How to cite: *Kalaivani M, Prasad SD, Indumathi M, Sruthipriya M, Balachandran J, Pavankumar O. Influence of home bleaching regimen on microhardness and flexural strength of two contemporary composite resins – an invitro evaluation. Eur Oral Res 2023; 57(2): 90-95.* DOI: 10.26650/eor.20231083203

ularity in which the patient uses custom-fitted prostheses at home for applying a bleaching solution that is generally of lower concentrations to whiten the vital teeth (3).

Inadvertent application of the bleaching agents during home bleaching procedure on teeth with existing restorations could not be excluded, especially if the procedure is not performed and monitored by a dentist, thereby affecting the mechanical properties and physical properties of the filling material. This diminutes the prognosis and longevity of restorations(4).

The aim of the present study is therefore to evaluate and compare the effects of 10% CP (Carbamide Peroxide) on microhardness and flexural strength of microhybrid and nanohybrid composite resins over a period of 2 weeks. The null hypothesis is that bleaching regimen has no effects on the properties of composite resins.

Materials and Methods

Specimen preparation for microhardness testing

Power analysis revealed 40 samples were required to achieve a power of 95% at a significance level of 0.05. Hence, 40 disc-shaped specimens from microhybrid (GC Solaire X, GC Corporation, Tokyo, Japan) and nanohybrid composite (Tetric N Ceram, Ivoclar Vivadent, Liechtenstein) resin [4×2 millimeters(mm)], were prepared using custom made silicone rubber molds. The composite material was placed as a single increment using a Teflon coated instrument (Oracraft, Punjab, India) and covered with mylar strips on both surfaces. The excess material was extruded by applying pressure on a glass plate placed over the composite material, which was light polymerized (Guilin Woodpecker Medical Instrument Co., Ltd., Guilin, China) for 40 seconds to ensure adequate polymerization (Figure 1).

Samples with visual voids or cracks were excluded. Remaining samples were painted with nail varnish on all surfaces except one flat surface that was closer to the curing light. They were placed in artificial saliva (ICPA Health Products Ltd, Mumbai, India) for 24 hours at 37°C and they were divided into 4 groups with 10 samples (n=10) in each. Group A and Group B: These control groups included disc shaped samples of microhybrid and nanohybrid composite resin respectively, which were immersed for 2 weeks in artificial saliva. Group C and Group D: These two groups included disc shaped samples of microhybrid and nanohybrid composite resin. They were bleached with a home bleaching agent (10% CP, FGM Whiteness Perfect, Brazil) for 3-4 hours per day. Specimens were then placed in artificial saliva over a period of 2 weeks.

Microhardness evaluation

A Vickers's microhardness tester (Associated Scientific Engineering Works, New Delhi, India) was used for microhardness testing. The specimens were placed underneath the indenter and a 100gm load was applied through the indenter for a dwell time of 15 seconds (Figure 2). Three consecutive indentation readings were recorded at 3 different points that are 1 mm apart from the disc margins, and the mean readings were recorded as VHN (Vickers Hardness Number).

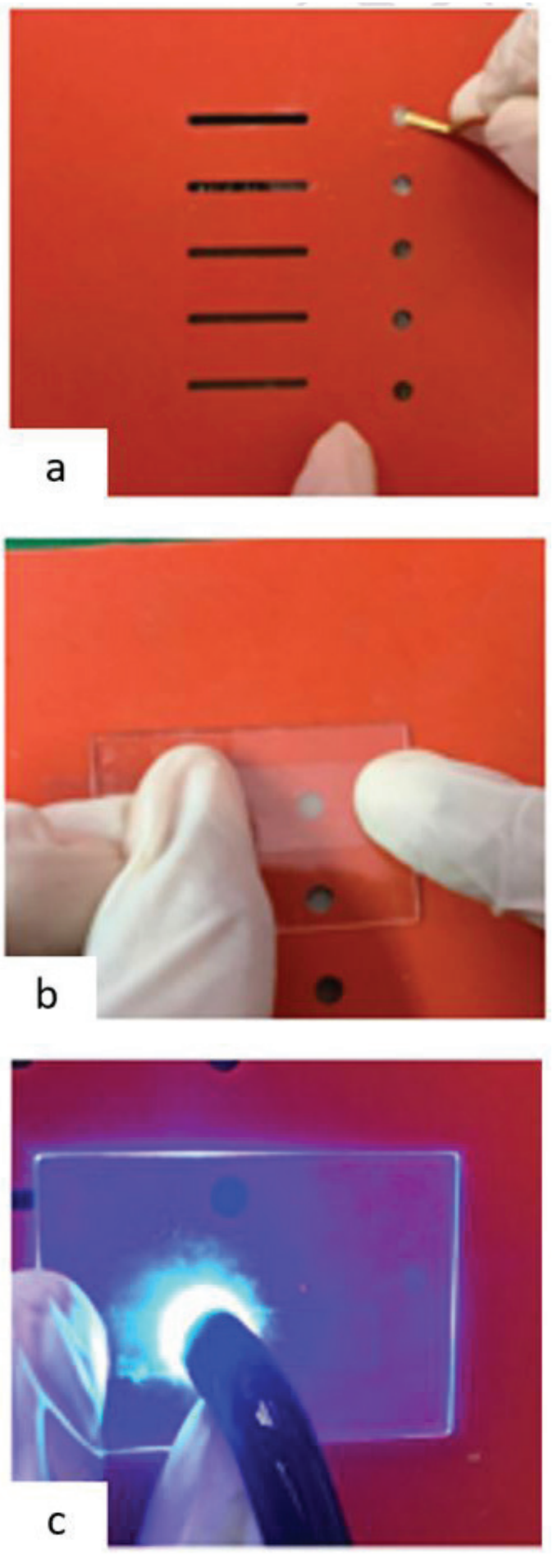

***Figure 1.* (a-c):** *Sample preparation for microhardness evaluation.*

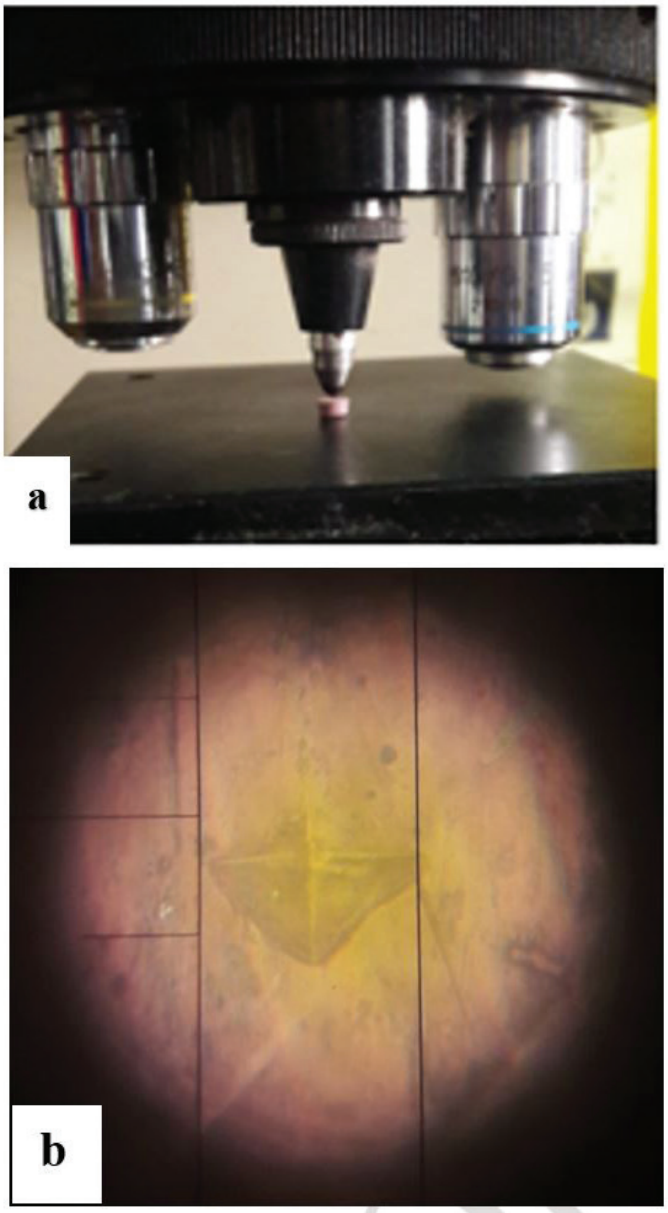

Figure 2. *(a) Vicker's Microhardness testing, (b) Rhomboid indentation on surface of sample to measure Vickers Hardness Number.*

Specimen preparation for flexural strength test

A custom made silicone rubber mold [2 × 2 × 25mm] was used to prepare 40 bar-shaped specimens from microhybrid and nanohybrid composite resins (n=20 each). The composite material was packed as a single increment using a Teflon coated hand instrument and then covered with mylar strips on both sides. Excess material was removed by application of manual pressure using a glass plate, followed by curing in 4 segments of 40 seconds each (Figure 3). Acid resistant nail varnish was applied to all the surfaces of samples except the flat surface closer to the curing light. Samples were then placed in artificial saliva for 24 hours at 37°C. The samples were then divided into 4 groups with 10 samples (n=10) in each.

Group 1 and Group 2 were designated as control groups. Bar shaped samples of microhybrid and nanohybrid composite resin were immersed for 2 weeks in artificial saliva. Group 3 and Group 4 included bar shaped samples of microhybrid and nanohybrid composite resin. Samples were

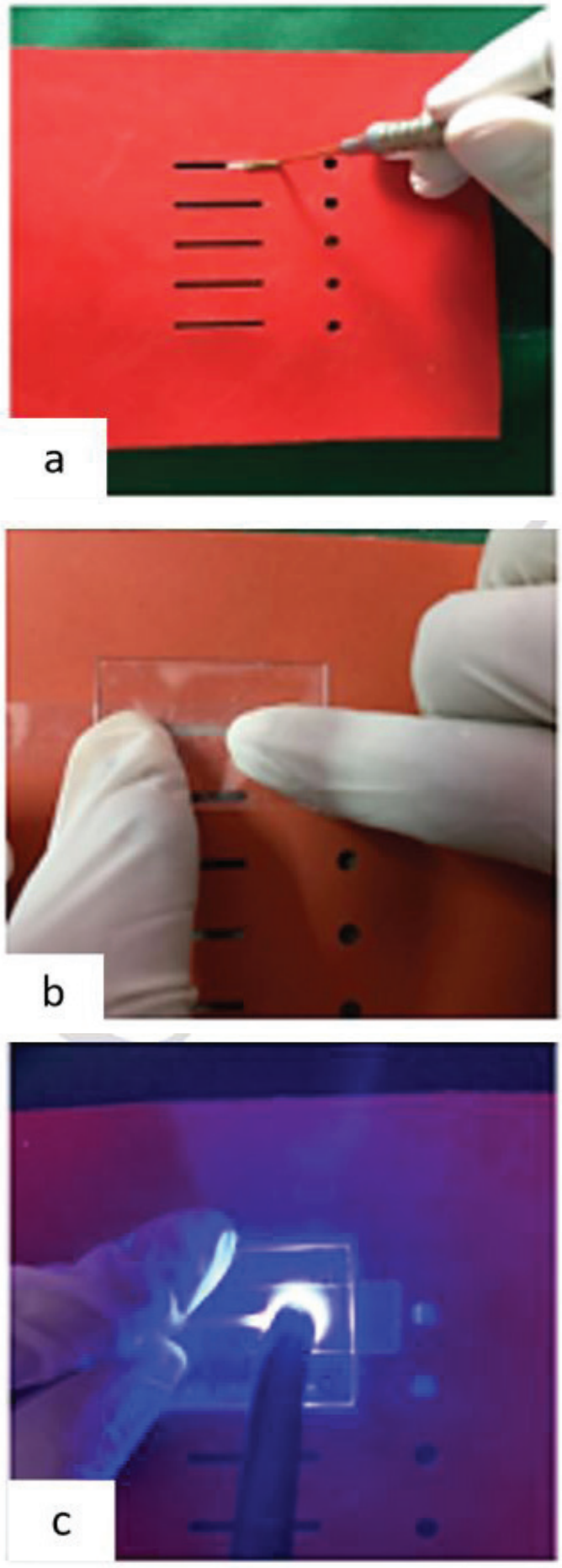

Figure 3. **(a-c):** *Sample preparation for flexural strength evaluation.*

bleached with a home bleaching agent (10% CP, FGM Whiteness Perfect, Brazil) for 3-4 hours per day and they were placed in artificial saliva over a period of 2 weeks.

Flexural strength evaluation

Universal Testing Machine (Instron 5566, Instron Inc., MA, USA) was used to perform the three-point bending test at force of 0.5mm (millimeter) of crosshead diameter per minute (Figure 4) which was applied until the observation of fracture in each sample, and it was tabulated in Newtons(N). The following equation was used to calculate FS (σ) values (MPa): $\sigma = 3\ FL / 2BH^2$, where F - failure load (N), L - distance between two jig supports, B and H - width and height of the study samples respectively (mm).

Statistical analysis

The recorded values were statistically analyzed using SPSS software 22.0 (IBM SPSS Inc., Armonk, NY, USA) by descriptive analysis, Kruskall-Wallis and Mann Whitney u-tests. The confidence interval was set to 95% and p values less than 0.05 were considered significant.

Figure 4. *Three-point bend test for flexural strength evaluation.*

Results

Microhardness test

The test statistics signify that there is a significant difference between microhardness values of microhybrid and nanohybrid composite resins with and without exposure to home bleaching regimen ($p<0.05$) which is shown in Table 1. Kruskal Wallis test showed a significant difference between control and bleached microhybrid and nanohybrid composites ($p<0.05$). The nanohybrid composite groups showed a higher sum of rank values (Group B=340, Group D=262) than microhybrid composite (Group A= 150, Group C=68), which showed that the surface microhardness value of nanohybrid composite resins is comparatively higher.

Flexural strength test

No significant difference was observed between any of the study groups. Kruskal Wallis test showed that home bleaching had no significant effects on the flexural strength of microhybrid (Group 1=165, Group 2=150) and nanohybrid (Group 3=226.5, Group 4=278.5) composite resin as seen in Table 2.

Discussion

Dental bleaching is the most conservative method of treating tooth discolouration. It usually consists of hydrogen peroxide (HP) as the active ingredient or its precursor, CP at concentrations ranging from 3% to 40% of HP equivalent. The mechanism generally proceeds via oxidation of HP into hydroxyl radicals (HO.), perhydroxyl radicals (HOO.), per-

Table 1. *Descriptive statistics of arithmetic mean values of mh and nh composite resins before and after bleaching*

Study groups	n	Mean	SD	Min	Max	Percentiles		
						25th	50th	75th
Group A Microhybrid Control group	10	42.30	4.73	35.70	50.92	38.85	41.85	45.55
Group B Nanohybrid Control group	10	55.91	3.37	50.00	60.30	54.10	55.95	57.08
Group C Microhybrid Bleached group	10	34.61	5.06	24.40	42.00	31.53	37.10	38.00
Group D Nanohybrid Bleached group	10	50.88	3.43	45.90	56.50	47.13	51.55	52.88

**n- number of samples, *sd – standard deviation*

Table 2. *Descriptive statistics of Arithmetic Mean FS values of Micro-Hybrid composite resins before and after bleaching*

Study groups	n	mean	SD	min	max	Percentiles		
						25th	50th	75th
Group 1 Microhybrid control group	10	26.00	3.16	20.00	30.00	25.00	25.00	35.00
Group 2 Microhybrid Bleached Group	10	23.75	2.04	20.00	25.00	23.62	25.00	20.00
Group 3 Nanohybrid control group	10	28.00	4.83	20.00	35.00	23.75	25.00	30.00
Group 4 Nanohybrid Bleaching Group	10	26.50	2.04	20.00	30.00	22.81	25.00	25.00

**n: Number of samples, *SD: Standard Deviation*

hydroxyl anions (HOO–), and superoxide anions (OO.) and through homolytic cleavage of either an O–H bond or the O–O bond of HP results in formation of $H^{\cdot} + {}^{\cdot}OOH$ and $2{}^{\cdot}OH$ (hydroxyl radicals), respectively (5,6). The free radicals acts on the double bonds of chromophore molecules in organic pigments, thus altering its absorption spectrum resulting in bleaching of tooth (5).

Home bleaching procedure is performed by the patients themselves under the supervision of dentists during recall visits. The peroxide agents that are applied on the tooth may also contact the pre-existing restorations causing an oxidation reaction on the restorative material surface. This reaction leads to the chemical softening, which decreases the clinical durability of these restorative materials (7,8).

Both the teeth and restorative materials are exposed to cyclic conditions of bleaching agent and saliva exposure which was formulated to simulate treatment conditions *in situ* (9). Storage of samples in artificial saliva helps by creating a surface protection layer on restorative materials (10). In contrast, the findings of the present study showed that the usage of artificial saliva as a storage medium during and after the bleaching procedure had no benefit, and there was a statistically significant ($p < 0.05$) reduction in microhardness of composite resins.

Vickers microhardness testing which was performed done in the present study is a standardized method as defined by American society for testing and materials (10). The Microhardness tester was calibrated prior to the indentation on each pellet by providing a matrix strip finished flattened surface to prevent distorted indentation (9).

The bleaching regimen (10%CP) followed in the current study was designed according to the manufacturer's recommendations to establish clinical relevance. In several studies of literature, the samples are exposed to bleaching products continuously for several days. This was done to simulate cumulative effects of bleaching over a certain period (1).

A significant reduction in hardness of samples after bleaching was noted ($p < 0.05$), when compared to the control group. Therefore, rejecting the null hypothesis is evident based on the difference in the VHN values. This was probably due to the softening effect of the bleaching agent on the resinous matrix of both MH and NH resin composites which thus decreases its surface properties (2,3,8,10–13). The extent of damage may depend on the diffusion rate, degree of conversion and water absorption of the bleaching agent (4). Further, free radicals produced by peroxides increase surface deterioration due to microscopic cracks by its action on unreacted double bonds of polymers and on the interface between resin and fillers resulting in its debonding (4,12). When the solubility parameters of bleaching products are similar to that of the resin matrix (1.82 x 104 to 2.97 x 104 $(J/m^3)^{1/2})^2$, chemical softening of the restorative materials might occur (2).

Several studies in the literature have shown consistent results (5,6,8,9,11). In contrast, some authors did not report any decrease in the microhardness of composite resin after evaluation of the effect of at home (15% CP) and in office (38% HP) bleaching agents (1,2,7,13–15). Cullen *et al.*(8) and Friend *et al.*(16) reported an increase in the tensile strength of composites. The differences in experimental methodologies, bleaching agents, frequency and restorative materials used, could have created discrepancies in results (6). In the comparison of the microhardness values of Group C (microhybrid) and Group D (nanohybrid) after bleaching, the microhybrid composites showed greater decrease in microhardness values which was probably due to the higher volume of resin matrix in microhybrid composites than in nanohybrid composites that were affected by oxidation of peroxides in bleaching agent (8). The flexural strength test was another criterion that was tested in the present study. Although several methods are widely employed, the three-point bending test was selected as recommended by the International Organization for Standardization (ISO) specification no. 4049/2008 for polymer-based restoratives and due to the coefficient of variation, lower standard deviation, and the less complex crack distribution (16).

In the present study, the dimensions of specimens [25 × 2 × 2 mm] were in accordance with the ISO 4049/2019 specification that aimed to provide the optimum rate of polymerization(17). Present results reflected no significant reduction in flexural strength values of microhybrid and nanohybrid composite resins when the home bleaching regime is used. These results might support the null hypothesis and the filler volume seems to have less correlation with fracture as the crack propagation in the specimen is intergranular (16). The filler volume-fraction of the material does not seem to be a decisive factor for the flexural strength of the evaluated materials. Also, the resistance of the silane coupling agent to the oxidative cleavage and the minimal exposure time of samples to bleaching agents could be accounted for these results (17). Similar results were presented by Firoozmand *et al.* (18), Yu *et al.* (14) and Kalaivani *et al.* (19). An increase in flexural strength values after bleaching was noticed in a study by Feiz *et al.* (20) on hybrid composites. The reason for increase in flexural strength remains unclear.

Conclusion

The home bleaching regimen (10% CP) had significant effects on the composite restorative materials. This can be clinically translated as marked weakening of composite restorations after bleaching procedures. Ideally, a complete replacement of the weakened composite restorations is warranted. However, more studies are needed to support the indication for the replacement of such restorations that are not esthetically affected or fractured.

Türkçe özet: *Ev tipi beyazlatma rejiminin sık kullanılan iki kompozit reçine esaslı dolgu malzemesinin mikrosertliği ve eğilme mukavemeti üzerindeki etkisi - bir in vitro değerlendirme. Amaç: Bu çalışma evde tipi beyazlatmanın mikrohibrit ve nanohibrit kompozit reçinelerin mikrosertliği ve eğilme mukavemeti üzerindeki etkisini karşılaştırmak ve değerlendirmekti. Gereç ve yöntem: Çalışma numuneleri özel yapım silikon kauçuk kalıp kullanılarak hazırlandı. Mikrosertlik değerlendirmesi için 40 adet disk şeklinde numune (4mm*2mm) hazırlandı ve 4 gruba ayrıldı: GRUP A (n=10): mikrohibrit (GC Solaire X, GC Corporation) kontrol grubu, GRUP B (n=10)) nanohibrit (Tetric N Ceram, Ivoclar Vivadent) kontrol grubu, GRUP C (n=10): mikrohibrit ağartma grubu, GROUP D (n=10) nanohibrit ağartma grubu. Eğilme mukavemeti değerlendirmesi için 40 adet çubuk şekilli numune (25mm*2mm*2mm) hazırlandı. GRUP–1 (n=10): mikrohibrit kontrol grubu, GRUP–2 (n=10) nanohibrit kontrol grubu, GRUP–3 (n=10): mikrohibrit ağartma grubu, GRUP–4(n) olmak üzere 4 gruba ayrıldı. =10) nanohibrit ağartma grubu. Tüm kontrol gruplarına yapay tükürük yerleştirildi ve beyazlatma gruplarına 14 gün süreyle üreti-*

ci firmanın talimatlarına göre ev tipi beyazlatma ajanı uygulandı. 14 gün sonra ilgili numuneler için mikrosertlik ve eğilme mukavemeti değerlendirildi ve veriler istatistiksel olarak analiz edildi. Bulgular: Evde ağartma rejimi, hem mikrohibrit hem de nanohibrit kompozitlerin mikrosertliğini azaltırken, eğilme mukavemeti üzerinde önemli bir etkisi olmamıştır. Nanohibrit kompozitler beyazlatma işleminin öncesinde ve sonrasında daha yüksek mikrosertlik değerleri göstermiştir. Sonuç: Beyazlatma ajanları, konsantrasyonlarına bakılmaksızın, kompozit reçine örneklerinin mikrosertliğini azaltabilir, bu da fiziksel ve mekanik özellikler üzerindeki etkilerinden dolayı bu restorasyonların sürerliği konusunda endişe uyandırmaktadır. Anahtar Kelimeler: kompozit reçine, ev tipi beyazlatma, nanohibrit, mikrosertlik, eğilme mukavemeti

Ethics Committee Approval: Not required.

Informed Consent: Participants provided informed constent.

Peer-review: Externally peer-reviewed.

Author contributions: MK, SDP, MI participated in designing the study. MK, SDP, MI participated in generating the data for the study. MK, SDP, MI, MS participated in gathering the data for the study. MK, SDP, MI, MS participated in the analysis of the data. MK, SDP wrote the majority of the original draft of the paper. MK, MI, MS, BJ, OP participated in writing the paper. MK, BJ, OP have had access to all of the raw data of the study. MK, SDP, BJ, OP have reviewed the pertinent raw data on which the results and conclusions of this study are based. MK, SDP, MI, MS, BJ, OP have approved the final version of this paper. MK, SDP, MI, MS, BJ, OP guarantee that all individuals who meet the Journal's authorship criteria are included as authors of this paper.

Conflict of Interest: The authors have no conflicts of interest to declare.

Financial Disclosure: The authors declared that this study has received no financial support.

REFERENCES

1. Yap AUJ, Wattanapayungkul P. Effects of in-office tooth whiteners on hardness of tooth-colored restoratives. Oper Dent 2002;27:137–41.
2. Polydorou O, Mönting JS, Hellwig E, Auschill TM. Effect of in-office tooth bleaching on the microhardness of six dental esthetic restorative materials. Dent Mater Off Publ Acad Dent Mater 2007;23:153–8. [CrossRef]
3. Taher NM. The effect of bleaching agents on the surface hardness of toothcolored restorative materials. J Contemp Dent Pract 2005;6:18-26. [CrossRef]
4. Hannig C, Duong S, Becker K, Brunner E, Kahler E, Attin T. Effect of bleaching on subsurface micro-hardness of composite and a polyacid modified composite. Dent Mater Off Publ Acad Dent Mater 2007;23:198–203. [CrossRef]
5. Alqahtani MQ. Tooth-bleaching procedures and their controversial effects: A literature review. Saudi Dent J 2014;26:33–46. [CrossRef]
6. Kashima-Tanaka M, Tsujimoto Y, Kawamoto K, Senda N, Ito K, Yamazaki M. Generation of free radicals and/or active oxygen by light or laser irradiation of hydrogen peroxide or sodium hypochlorite. J Endod 2003;29:141–3. [CrossRef]
7. Silva Costa SX, Becker AB, de Souza Rastelli AN, de Castro Monteiro Loffredo L, de Andrade MF, Bagnato VS. Effect of four bleaching regimens on color changes and microhardness of dental nanofilled composite. Int J Dent 2009:313845. [CrossRef]
8. Cullen DR, Nelson JA, Sandrik JL. Peroxide bleaches: Effect on tensile strength of composite resins. J Prosthet Dent 1993;69:247–9. [CrossRef]
9. Srinivasan K, Chitra S. Effects of Different Concentrations of Bleaching Agent on the Micro hardness of Restorative Materials-An In Vitro Study. J Res Med Dent Sci 2015;3:188. [CrossRef]
10. Solomon. An *in vitro* evaluation of microhardness of different direct resin-based restorative materials on using 10% carbamide peroxide gel as a bleaching agent. J Oral Res Rev 2016;8:59-65. [CrossRef]
11. Okte Z, Villalta P, García-Godoy F, Lu H, Powers JM. Surface hardness of resin composites after staining and bleaching. Oper Dent 2006;31:623–8. [CrossRef]
12. Gul P, Harorli O, Akgül N, Gundogdu M. Effect of different bleaching applications on the surface properties and staining susceptibility of dental composites. J Wuhan Univ Technol-Mater Sci Ed 2016;31:677–83. [CrossRef]
13. Effects of Two Different Bleaching Agents on the Microhardness of Composite. Pak J Med Dent 2021;10:11-16.
14. Yu H, Li Q, Lin Y, Buchalla W, Wang Y. Influence of carbamide peroxide on the flexural strength of tooth-colored restorative materials: an in vitro study at different environmental temperatures. Oper Dent 2010;35:300–7. [CrossRef]
15. Kothari. Comparison and evaluation of color stability and flexural strength of various provisional restorative materials after bleaching: An *in vitro* study. J Int Oral Health 2019;11:293-98. [CrossRef]
16. Campos I, Briso ALF, Pimenta LAF, Ambrosano G. Effects of bleaching with carbamide peroxide gels on microhardness of restoration materials. J Esthet Restor Dent Off Publ Am Acad Esthet Dent Al 2003;15:175–82. [CrossRef]
17. Yap AU. Effectiveness of polymerization in composite restoratives claiming bulk placement: impact of cavity depth and exposure time. Oper Dent 2000;25:113–20.
18. Firoozmand LM, Pagani C. Influence of bleaching treatment on flexural resistance of hybrid materials. Acta Odontol Latinoam AOL 2009;22:75–80.
19. Kalaivani Muralidasan et al. Influence of In-Office and Home Bleaching Regimen on Flexural Strength of Nano-Hybrid Composite Resins: An In-Vitro Evaluation. IOSR Journal of Dental and Medical Sciences (IOSR-JDMS) 2020;19:72-76.
20. Feiz A, Samanian N, Davoudi A, Badrian H. Effect of different bleaching regimens on the flexural strength of hybrid composite resin. J Conserv Dent 2016;19:157-160. [CrossRef]

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Official Publication of Istanbul University Faculty of Dentistry

Eur Oral Res 2023; 57(2): 96-102

Original research

Comparison of the shear bond strength of new and recycled metallic brackets using different adhesive materials. An *in vitro* study

Purpose
To evaluate and compare shear bond strength (SBS) of new and recycled metallic brackets bonded to conditioned and reconditioned enamel, using two different adhesive materials.

Material and Method
72 extracted sound human premolars were randomly divided into 6 groups. Transbond XT light cured composite (LCC) and Fuji Ortho LC resin-modified glass ionomer (RMGI), were used as adhesive materials. In groups 1 and 2 (control), new brackets were bonded to sound premolars using either LCC or RMGI, respectively. In Groups 3 and 4, new brackets were bonded to reconditioned enamel; and in groups 5 and 6, sandblasted recycled brackets were rebonded to reconditioned enamel. After 5.000 thermal cycles between 5ºC and 55ºC, SBS was evaluated and adhesive remnant on the enamel assessed using the ARI index. Statistical analyses included Shapiro-Wilk, ANOVA, Fligner-Killeen ANOVA and Tukey tests.

Results
The statistical analysis showed no significant difference in SBS comparing control and experimental groups for either new or recycled brackets ($p = 0.848$). The SBS was significantly higher in brackets bonded with LCC (15.7 MPa) than RMGI (11.6 MPa) ($p = 0.006$). Adhesive failure was the most frequent, with the adhesive remnant covering more than 50% of the bracket base.

Conclusion
No significant differences were observed in SBS using either new or recycled brackets, regardless of the dental surface treatment (conditioned or reconditioned). Significantly higher SBS values were obtained with LCC adhesive. Adhesive failure prevails in all groups.

Keywords: *Shear bond strength, Metallic brackets, Adhesive materials, Recycled brackets, Thermal cycling*

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Introduction

Bracket bond failure is a frequent complication during orthodontic treatment, which can increase its duration and represents a challenge in clinical practice (1). The most popular bracket bonding systems include light-cured composite resins (Bis-GMA) (LCC) and the resin-modified glass ionomer (RMGI) (2,3).

The adhesion strength resists shear forces naturally occurring during chewing function and can be determined by the shear bond strength (SBS), which is "the amount of force required to produce a fracture at the interface of two materials, when parallel forces are applied in opposite direction" and it is measured in Mega Pascals (MPa) (2,4,5,6,7). SBS values

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Received: 17 September 2022
Revised: 24 October 2022
Accepted: 4 November 2022

DOI: 10.26650/eor.20231163180

How to cite: *Ibarra N, Sáez M, Rojas V, Oyonarte R. Comparison of the shear bond strength of new and recycled metallic brackets using different adhesive materials. An in vitro study. Eur Oral Res 2023; 57(2): 96-102.* DOI: 10.26650/eor.20231163180

between 6 and 8 MPa are considered clinically acceptable (2,5,8,9). Some studies suggest that values higher than 13-14 MPa may increase the risk of enamel fracture (4,10,11). On the other hand, lower adhesive forces may cause bracket debonding during normal functional conditions (1,4,9). Studies have reported bracket debonding rates between 4.7% and 6% for light-curing and self-curing adhesives respectively in a 6-month treatment period (12,13).

Bracket bond failure can increase both treatment time and costs. To solve this, clinicians must replace the debonded bracket. They usually choose between rebonding with a new bracket or carry on with the same debonded bracket after recycling (4,14). In both cases, the adhesive remnant should be removed from the enamel surface and a new adhesive protocol should be performed. This procedure would not affect the SBS using LCC, but evidence is inconclusive with RMGI in this regard (15,16). Bracket recycling consists of removing the adhesive material from the bracket base, allowing rebonding in optimal conditions (17).

In *in-vitro* settings, thermocycling allows an approximation to oral conditions by simulating the permanence of orthodontic adhesive in mouth for extended periods of time (18). The results regarding SBS using new and recycled metallic brackets are still controversial, especially if different adhesive systems are used (14). The present study was conducted in order to test the null hypothesis that there were no statistically significant differences in SBS between new and recycled metallic brackets bonded with light-cured composite resin (LCC) or resin-modified glass ionomer (RMGI) before and after a thermocycling process.

Materials and Methods

Ethical statement

The present study was approved by the Scientific Ethical Committee of Universidad de los Andes, Santiago, Chile.

Sample size estimation

The sample size calculation was carried out considering a 3x2 factorial design and was calculated with the G*Power 3.1 program, aiming at a statistical power of 80%, and an alpha level of 5%. The sample size for each group was 12 teeth, with a total of 72 teeth.

Study samples and experimental design

This *in-vitro* study was performed using human premolars extracted due to orthodontic reasons. The selection criteria for the teeth included: sound enamel without cracks or fractures, hypoplastic areas, chemical pretreatments or damage during extraction. The extracted teeth were stored in tap water at room temperature (20ºC to 25ºC), which was renewed once a week. Teeth were randomly divided into 6 groups. Group 1 (control): New bracket bonded with light-cured composite resin, Transbond XT (LCC) to conditioned enamel; Group 2 (control): New bracket bonded with resin-modified glass ionomer, Fuji Ortho LC (RMGI) to conditioned enamel; Group 3: Rebonding with a new bracket using LCC to reconditioned enamel; Group 4: Rebonding with a new bracket using RMGI to reconditioned enamel; Group 5: Rebonding with a recycled bracket using LCC to reconditioned enamel; Group 6: Rebonding with a recycled bracket using RMGI to reconditioned enamel.

Bonding protocol

Orthodontic stainless-steel brackets (Abzil Kirium, slot 0.022, MBT prescription with mesh-base size 80G, 3M, Brazil) were bonded to the teeth following a standardized protocol. Each tooth was cleaned using low speed brush and pumice stone, and then dried with oil- and moisture- free air. Brackets were bonded by one calibrated operator (NI), in the center of the buccal surface of each tooth following the longitudinal axis of the crown, either with LCC or RMGI.

For groups 1, 3 and 5 brackets were bonded with Transbond XT light-curing resin (3M Unitek, Monrovia, CA, USA). The procedure included acid etching with a 37% orthophosphoric acid gel (3M Espe Scotchbond Universal Etchant, Seefeld, Germany) for 30 seconds, followed by rinsing with water spray for 30 seconds, and drying for 15 seconds with oil-free compressed air. Adhesive primer (Transbond XT, 3M Unitek, Monrovia, Ca, USA) was then applied with a microbrush and light cured, and then the brackets were bonded with composite resin. Excess resin was removed with a Hollenbeck carver (Hu-Friedy, Chicago IL, USA). Light curing was performed with a 470 nm LED light (Bluephase Style, Ivoclar Vivadent) at 1,100 mW/cm2 for 20 seconds.

For groups 2, 4 and 6 brackets were bonded with dual polymerization resin-modified glass ionomer Fuji Ortho LC (GC, Tokyo, Japan). The enamel was etched with the same 37% orthophosphoric acid (3M Espe Scotchbond Universal Etchant, Seefeld, Germany) for 30 seconds, and rinsed with water keeping the surface moist, according to the manufacturer´s recommendations. Excessive RMGI material was removed with a Hollenbeck Carver. Light curing was performed according to the manufacturer´s instructions with the same light source as previously described for 40 seconds, 10 seconds per side of the bracket.

Thermocycling

24 hours after bonding, all groups were subjected to thermocycling between 5ºC and 55ºC for 5.000 cycles, simulating a 6 to 8 months intraoral natural aging process. The time of permanence at each temperature level was 30 seconds with 10 seconds of a transfer time between baths.

Bracket rebonding

After thermocycling, all brackets from the experimental groups were debonded with bracket removal pliers (Dentamax, Santiago, Chile). The enamel in groups 3, 4, 5 and 6 was reconditioned using a tungsten carbide bur with a low-speed hand piece (Jota C21R Right Angle 012, US-No. 1158, Switzerland) and followed by the same bonding protocol as in the first bond.

For groups 3 and 4, the debonded brackets were discarded and new brackets were bonded. For groups 5 and 6, the debonded brackets were reconditioned by sandblasting with 50 µm aluminum oxide particles powder (Zeta Sand,

Zhermack, Germany) from a distance of 10 mm, under an air pressure of 5 bar, for 10 seconds or until no adhesive remained at the base of the bracket. Finally, the brackets were cleaned with acetone and dried with oil-free air. All the samples corresponding to the rebonding groups (3, 4, 5 and 6) were thermocycled again.

Shear bond strength test

The teeth were mounted in self-curing acrylic blocks (Marché®, Santiago, Chile) approximately 30 mm in diameter and 10 mm in height. Shear force was applied parallel to the bonding surface between the bracket base and enamel. A Bisco machine (Shear Bond Tester, Bisco Dental, Schaumburg, Illinois, USA) was used to determine the shear bond strength at a speed of 5 mm/min. Results were expressed in Mega Pascals (MPa). The SBS test was performed within 24 hours after the samples were removed from the thermocycling.

Bracket and enamel surface analysis

Bracket and enamel surface were examined using an optical microscope with 2.5x magnification (Leica Micosystems, Wetzlar, Germany) and photographed with a Canon DSLR 700D reflex camera attached to the microscope. The presence of enamel damage and adhesive remnant on the bracket base were recorded. The adhesive remnant was calculated as the area of the adhesive in relation to the area of the bracket base, using a morphometric Software (AmScope v3.7.13522, United Scope LLC, Irvine, CA, USA). (Figure 1). The area of adhesive remnant on the bracket base was expressed as a percentage of the total surface area, in each group, and the Adhesive Remnant Index (ARI) scale ranges from 0 to 3 was stablished (19) (Table 1).

Statistical analysis

Shapiro-Wilk test was applied to test the data for normality. The SBS data did not show a normal distribution, therefore the non-parametric Fligner-Killeen ANOVA test was used. The data on the amount of adhesive remnant was normally distributed and the ANOVA test was applied. Tukey post-hoc test was performed in case of finding statistically significant differences, and the Chi-square test was applied to the enamel damage analysis. The confidence interval was set to 95% and p values less than 0.05. The statistical analysis was performed using the RStudio statistical software (Rstudio Inc., Boston, MA, USA).

Results

There was no statistically significant difference in SBS (p=0.848) between new brackets bonded to conditioned enamel (13.1 MPa), new brackets rebonded to reconditioned enamel (14.1 MPa) and recycled brackets rebonded to reconditioned enamel (13.7 MPa). On the other hand, SBS of brackets bonded with LCC (15.7 MPa) was significantly higher than that of brackets bonded with RMGI (11.6 MPa) (p=0.006). (Table 2, Figure 2 and 3)

Regarding to the percentage of adhesive remnant on the bracket base, there was no statistically significant difference between a new bracket bonded to conditioned enamel, a new bracket bonded to reconditioned enamel and a recycled bracket rebonded to reconditioned enamel (p= 0.078). Statistically significant differences were found for the type of adhesive. The percentage of LCC remaining on the bracket base was significantly higher (p= 0.00014) than when RMGI was used (Table 3).

Table 3 shows the distribution of ARI scores and the presence of enamel fractures found during the shear bond strength test. In all groups, the most common failure was of the adhesive type located at the cement-enamel interface. Enamel fractures during the shear strength test were found

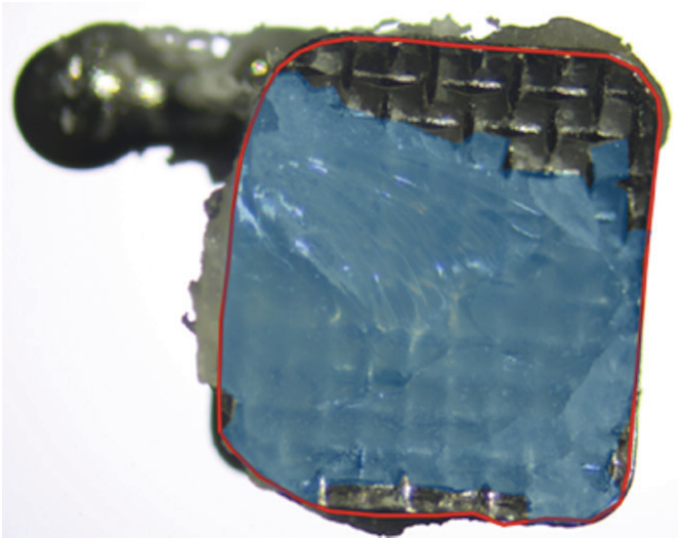

Figure 1. *Diagram of the adhesive area in relation to the bracket base .*

Table 1. *Adhesive Remnant Index score*

Score	Adhesive Remnant Index
0	0% adhesive on the enamel, 100% on the bracket. Adhesive failure
1	Less than 50% adhesive on the enamel, more than 50% on the bracket. Adhesive failure
2	More than 50% adhesive on the enamel, less than 50% on the bracket. Cohesive failure
3	100% adhesive on enamel, 0% on the bracket. Cohesive failure

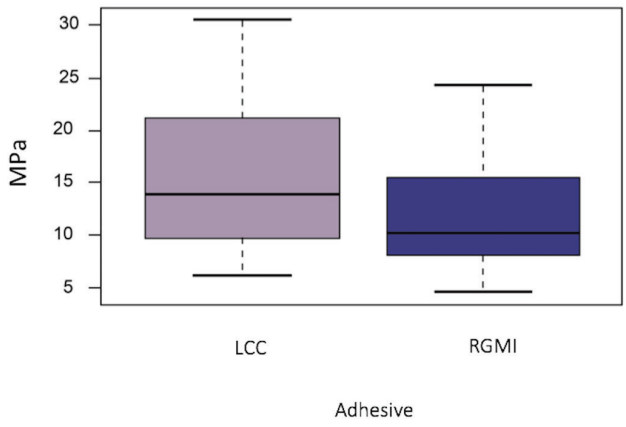

Figure 2. *Boxplot of the effect of adhesives on SBS.*

in 7 samples. Two samples belonged to group 1, one sample to group 2, three samples to group 5 and one sample to group 6 (Table 4 and Figure 4).

The Chi-square homogeneity analysis showed a homogeneous distribution among the combination of factors (type of bracket and type of adhesive), with no significant differences for the enamel fracture variable (p=0.947).

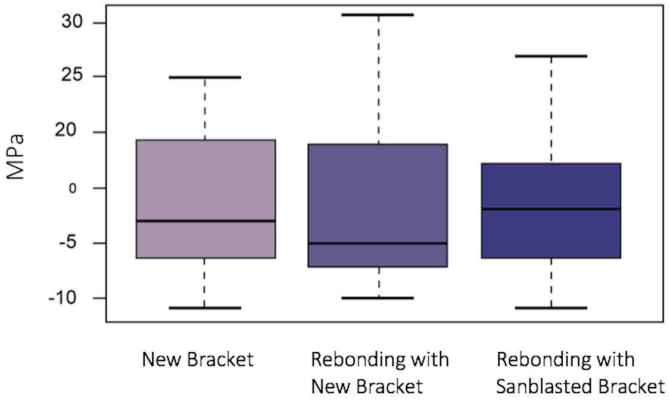

Figure 3. *Boxplot of the effect of new or recycled brackets on SBS.*

Discussion

Several authors have assessed the effects of using sandblasted recycled brackets on SBS, bonded with composite resin cement materials (17,20,21). These studies tested SBS including a thermocycling process, but the enamel was not reconditioned, as the present work did, in order to better emulate a clinical setting. Nevertheless, SBS values were similar to ours, and the mean SBS was higher than the minimum recommended for all groups.

The results of our study showed no significant difference on SBS using new or recycled brackets. Consequently, the null hypothesis of the study was accepted. Some authors reported higher SBS values with recycled brackets, which is attributed to the sandblasting process, that may increase

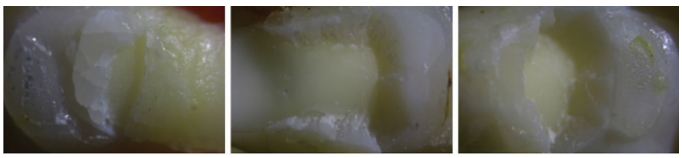

Figure 4. *Enamel fracture after SBS test.*

Table 2. *Effect of adhesives and new or recycled brackets on SBS*

Group	Adhesive	N	Mean (Mpa)	SD	Range (MPa)	*p-value*
1	LCC (Transbond XT)	36	15,7	6,7	6,1 – 30,6	0,00592
2	RMGI (Fuji Ortho LC)	36	11,6	5,3	4,5 – 24,4	
Group	**Bracket**	**N**	**Mean (Mpa)**	**SD**	**Range (Mpa)**	*p-value*
1	New Bracket	24	13,1	6,7	5,4 – 30,6	0,84803
2	Rebonding with New Bracket	24	14,1	6,5	4,5 – 26,9	
3	Rebonding with Sanblasted Bracket	24	13,7	6,2	4,5 – 24,4	

SD: Standard desviation *p-value* ≤ 0,05

Table 3. *Effect of adhesives and new or recycled brackets on the adhesive remnant in the bracket base*

Group	Bracket	N	ARI 0	1	2	3	Mean (%)	SD	Range (%)	*p-value*
1	New	24	2	22	0	0	75,7	14,4	56,1-100	0,07777
2	Rebonding with New Bracket	24	1	20	3	0	71,8	18,9	32,7 -100	
3	Rebonding with Sanblasted Bracket	24	0	19	5	0	65,2	19,6	35,8 -91,7	
Group	**Bracket**	**N**	**ARI 0**	**1**	**2**	**3**	**Mean (%)**	**SD**	**Range (%)**	*p-value*
1	LCC (Transbond XT)	36	2	32	2	0	78,6	16,3	35,2 - 100	0,00013
2	RMGI (Fuji Ortho LC)	36	1	29	6	0	63,3	16,6	32,7 - 100	

SD: Standard deviation *p-value* ≤ 0,05

Table 4. *Distribution of ARI scores and enamel fracture*

Group	Bracket	Adhesive	N	ARI				Mean Adhesive Percent (%)	SD	Range (%)	Enamel Fracture
				0	1	2	3				
1	New	LCC (Transbond XT)	12	2	10	0	0	82,7	13	63,1 - 100	2
2		RMGI (Fuji Ortho LC)	12	0	12	0	0	68,8	12,5	56,1 – 83	1
3	Rebonding with New Bracket	LCC (Transbond XT)	12	0	12	0	0	83,7	10,9	62,9 – 92,5	0
4		RMGI (Fuji Ortho LC)	12	1	8	3	0	59,9	17,9	32,7 – 100	0
5	Rebonding with Sanblasted Bracket	LCC (Transbond XT)	12	0	10	2	0	69,3	20,4	35,2 – 96	3
6		RMGI (Fuji Ortho LC)	12	0	9	3	0	61,1	18,7	35,8 – 91,7	1

SD: Standard deviation *p-value* ≤ 0,05

bracket bonding surface area (15,22,23). Conversely, several studies have reported a reduction in SBS in recycled brackets, but with values that are consistently higher than the minimum recommended SBS for bracket bonding (5,11,12,24). The variations between the studies could be explained by differences in the recycling procedure, instrumentation, storage solution and thermal cycling, among others. Despite subtle differences, the use of new or recycled brackets yields SBS values that are appropriate in a clinical setting (11,12,24,25).

It has been reported that enamel reconditioning could reduce SBS compared to when it is cemented to intact enamel conditioned for bracket bonding, which is attributed to the depth achieved by acid etching procedure, that does not allow the complete removal of the adhesive (12,24,25,26). In our study, however, no significant differences were observed in SBS neither at rebonding in reconditioned enamel nor using recycled or new brackets.

Our results did show significant differences in SBS between LCC and RMGI, which was lower in RMGI groups. Both groups, however, displayed SBS mean values higher than the acceptable limit. These results are consistent with previous studies reported in the literature (26-28). Both, LCC and RMGI appear to have appropriate adhesive properties for orthodontic bracket bonding, even on reconditioned enamel during a rebonding procedure, although RMGI could be more prone to failure if exposed to shear forces.

ARI index is frequently used for adhesive remnant assessment, but comparison between studies is difficult due to variations in their reporting methods (12,15,21,22). In the present study the adhesive remnant was first determined as a percentage of the area of the bracket base covered by the orthodontic cement after debonding. Then, the ARI index was established based on the afore mentioned percentage (19).

Some studies have reported higher adhesive remnant in the recycled brackets, because the sandblasted surface could increase the surface area for bonding, and thus the mechanical retention (9,10). It has also been suggested that bracket recycling has no apparent effect on ARI results when using LCC (12). In the present study no statistically significant difference was found between new or recycled brackets in reconditioned enamel on adhesive remnants. Most of the adhesive remaining on the base of the analyzed brackets covered more than 50% of the surface, suggesting that adhesion failure was at the adhesive-enamel interface in all groups. This can be clinically important because it would allow faster and easier reconditioning of the enamel surface. On the other hand, significant differences were found between the LCC and RMGI groups on adhesive remnant of the bracket, being higher in RMGI group.

A greater number of enamel fractures during bracket debonding has been described when SBS reaches values exceeding 13 Mpa (15). From the 7 samples with enamel fractures in the present study, 6 of them had SBS values higher than 13 Mpa. This suggests that enamel fractures are not exclusively related to shear strength and may be associated with individual variations in enamel structure or even bracket base design (29). Comparison of these results with other studies is especially difficult because most studies of bracket recycling did not report data of the presence or absence of enamel fractures (12,15,17,20-22,24,25).

During bracket bonding procedures, RMGI cements could be a better option in cases with high risk of caries and/or excessive salivation, because it releases fluoride and can be used in a humid environment. These aspects should be considered and contrasted with the more accessible pricing and the higher SBS achieved with LCC orthodontic cements (1). Both orthodontic cement systems provide the orthodontists with excellent bonding alternatives that can be selected according to clinical requirements.

Although the present article presents relevant information regarding the SBS of new and recycled metallic brackets bonded with different adhesive materials after a thermocycling process, this study was performed *in vitro*, using a specific experimental model. The limitations of this study are related to all the clinical variations that are relevant for the occurrence of bracket debonding and cannot be assessed *in vitro*. These clinical variations include masticatory force, interindividual behavioral, functional, anatomical, and intraoral factors, as well as variations in enamel thickness among

individuals. All these factors can clinically define the tendency of a bracket to debond and could not be evaluated in our *in vitro* study. Nevertheless, the results presented in this study may be relevant for the design of *in vivo* clinical studies on this topic in the future.

Conclusion

No significant differences were found for SBS between new or recycled brackets bonded in conditioned or reconditioned enamel. SBS provided by LCC (Transbond XT) was significantly higher than that achieved using RMGI (Fuji Ortho LC). Neither enamel reconditioning nor bracket recycling presented a negative impact on SBS. On the other hand, the adhesive failure prevailed at the cement-enamel interface in LCC and RMGI groups, with the remaining adhesive material covering more than 50% of the bracket surface. The presence of enamel fractures following SBS test was not associated with any adhesive system.

Türkçe özet: *Farklı adeziv malzemeler kullanılarak yapıştırılan yeni ve geri dönüştürülmüş metalik braketlerin kesme bağ dayanımının karşılaştırılması. Bir in vitro çalışma. Amaç: Bu çalışmanın amacı yeniden işlem görmüş ve görmemiş mineye iki farklı adeziv malzeme kullanılarak yapıştırılan yeni ve geri dönüştürülmüş metalik braketlerin kesme bağlanma mukavemetini (SBS) değerlendirmek ve karşılaştırmaktır. Gereç ve Yöntem: 72 adet çekilmiş sağlam insan küçük azı dişi rastgele 6 gruba ayrıldı. Adeziv materyal olarak Transbond XT ışıkla sertleşen kompozit (LCC) ve Fuji Ortho LC reçine ile modifiye edilmiş cam iyonomer (RMGI) kullanıldı. Grup 1 ve 2'de (kontrol), sırasıyla LCC veya RMGI kullanılarak sağlam küçük azı dişlerine yeni braketler yapıştırıldı. Grup 3 ve 4'te, yeniden işlem görmüş mineye yeni braketler yapıştırıldı; ve 5. ve 6. gruplarda, kumlanmış geri dönüştürülmüş braketler, işlem görmüş mineye yeniden yapıştırıldı. 5ºC ile 55ºC arasında 5.000 termal döngüden sonra, SBS değerlendirildi ve mine üzerindeki adeziv kalıntı ARI indeksi kullanılarak değerlendirildi. İstatistiksel analizler Shapiro-Wilk, ANOVA, Fligner-Killeen ANOVA ve Tukey testlerini içermektedir. Bulgular: İstatistiksel analiz, yeni veya geri dönüştürülmüş braketler için kontrol ve deney gruplarını karşılaştıran SBS›de anlamlı bir fark göstermedi (p = 0.848). SBS, LCC (15,7 MPa) ile bağlanmış braketlerde RMGI›den (11,6 MPa) önemli ölçüde daha yüksekti (p = 0,006). Yapıştırıcı ayrılması, en sık braket tabanının %50'den fazlasını kaplayan yapışkan kalıntısı ile birlikte gözlendi. Sonuç: Mine yüzeyinin gördüğü işlemden bağımsız olarak, yeni veya geri dönüştürülmüş braketler kullanılarak SBS'de önemli bir fark gözlenmedi. LCC adeziv ile önemli ölçüde daha yüksek SBS değerleri elde edildi. Adeziv kopma tipi tüm gruplarda hakimdi. Anahtar Kelimeler: Kesme bağlanma dayanımı, metalik braketler, adeziv malzemeler, geri dönüşümlü braketler, termal döngü*

Ethics Committee Approval: The present study was approved by the Scientific Ethical Committee of Universidad de los Andes, Santiago, Chile.

Informed Consent: Participants provided informed constent.

Peer-review: Externally peer-reviewed.

Author contributions: NI, MS, VR, RO participated in designing the study. NI participated in generating the data for the study.NI, MS, RO participated in gathering the data for the study. NI, VR, RO participated in the analysis of the data. NI, MS, VR, RO wrote the majority of the original draft of the paper. NI, MS, VR, RO participated in writing the paper. NI, MS, VR, RO have had access to all of the raw data of the study. NI, MS, VR, RO have reviewed the pertinent raw data on which the results and conclusions of this study are based. NI, MS, VR, RO have approved the final version of this paper. RO guarantees that all individuals who meet the Journal's authorship criteria are included as authors of this paper.

Conflict of Interest: The authors declared that they have no conflict of interest.

Financial Disclosure: The authors declared that they did not receive any financial support.

REFERENCES

1. Gange P. The evolution of bonding in orthodontics. Am J Orthod Dentofacial Orthop 2015; 47:56-63. [CrossRef]
2. Reicheneder CA, Gedrange T, Lange A, Baumert U, Proff P. Shear and tensile bond strength comparison of various contemporary orthodontic adhesive systems: an in-vitro study. Am J Orthod Dentofacial Orthop 2009;135:422-3. [CrossRef]
3. Ewoldsen N, Demke RS. A review of orthodontic cements and adhesives. Am J Orthod Dentofacial Orthop 2001;120:45-8. [CrossRef]
4. Gupta A, Kallury A, Sahu K. Influence of various factors affecting shear bond strength: A review. J Applied Dent Med Sci 2017;3:121-132.
5. Reynolds IR. A review of direct orthodontic bonding. Br J Orthod 1975;2:171-8. [CrossRef]
6. Bishara SE, Ostby AW. Bonding and debonding from metal to ceramic: research and its clinical application. Seminars in Orthodontics 2010;16:24–36. [CrossRef]
7. Finnema KJ, Ozcan M, Post WJ, Ren Y, Dijkstra PU. In-vitro orthodontic bond strength testing: a systematic review and meta-analysis. Am J Orthod Dentofacial Orthop 2010;137:615-622.e3. [CrossRef]
8. Bakhadher W, Halawany H, Talic N, Abraham N, Jacob V. Factors affecting the shear bond strength of orthodontic brackets - a Review of In Vitro Studies. Acta Medica (Hradec Kralove) 2015;58:43-8. [CrossRef]
9. Faltermeier A, Behr M. Effect of bracket base conditioning. Am J Orthod Dentofacial Orthop 2009;135:12-3. [CrossRef]
10. Eminkahyagil N, Arman A, Cetinşahin A, Karabulut E. Effect of resin-removal methods on enamel and shear bond strength of rebonded brackets. Angle Orthod 2006 Mar;76:314-21.
11. Bishara SE, Fehr DE, Jakobsen JR. A comparative study of the debonding strengths of different ceramic brackets, enamel conditioners, and adhesives. Am J Orthod Dentofacial Orthop 1993;104:170-9. [CrossRef]
12. Wendl B, Muchitsch P, Pichelmayer M, Droschl H, Kern W. Comparative bond strength of new and reconditioned brackets and assessment of residual adhesive by light and electron microscopy. Eur J Orthod 2011;33:288-92. [CrossRef]
13. Read M J F, O'Brien K D. A clinical trial of an indirect bonding technique with a visible light-cured adhesive. Am J Orthod Dentofacial Orthop 1990; 98: 259–262. [CrossRef]
14. Montero M, Vicente A, Alfonso-Hernández N, Jiménez-López M, Bravo-González LA. Comparison of shear bond strength of brackets recycled using micro sandblasting and industrial methods. Angle Orthod 2015;85:461-7. [CrossRef]
15. Rüger D, Harzer W, Krisjane Z, Tausche E. Shear bond strength after multiple bracket bonding with or without repeated etching. Eur J Orthod 2011;33:521-7. [CrossRef]
16. Kilinc D, Sayar G. Comparison of the shear bond strength of treated and untreated brackets on treated and untreated enamel surfaces in rebonding. J Orofac Sci 2018;10:69. [CrossRef]
17. Bahnasi FI, Abd-Rahman AN, Abu-Hassan MI. Effects of recycling and bonding agent application on bond strength of stainless steel orthodontic brackets. J Clin Exp Dent 2013;5:197-202. [CrossRef]
18. Sung EC, Chan SM, Mito R, Caputo AA. Effect of carbamide peroxide bleaching on the shear bond strength of composite to dental bonding agent enhanced enamel. J Prosthet Dent 1999;82:595-9. [CrossRef]
19. Bishara SE, Gordan VV, VonWald L, Jakobsen JR. Shear bond strength of composite, glass ionomer, and acidic primer

adhesive systems. Am J Orthod Dentofacial Orthop 1999;115:24-8. [CrossRef]
20. Sonis AL. Air abrasion of failed bonded metal brackets: a study of shear bond strength and surface characteristics as determined by scanning electron microscopy. Am J Orthod Dentofacial Orthop 1996;110:96-8. [CrossRef]
21. Al Maaitah EF, Alomari S, Abu Alhaija ES, Saf AA. The effect of different bracket base cleaning method on shear bond strength of rebonded brackets. J Contemp Dent Pract 2013;14:866-70. [CrossRef]
22. Salama F, Alrejaye H, Aldosari M, Almosa N. Shear bond strength of new and rebonded orthodontic brackets to the enamel surfaces. J Orthod Sci 2018;7:12. [CrossRef]
23. Montasser MA, Drummond JL, Evans CA. Rebonding of orthodontic brackets. Part I, a laboratory and clinical study. Angle Orthod 2008;78:531-6. [CrossRef]
24. Eslamian L, Borzabadi-Farahani A, Tavakol P, Tavakol A, Amini N, Lynch E. Effect of multiple debonding sequences on shear bond strength of new stainless steel brackets. J Orthod Sci 2015; 4: 37–41. [CrossRef]
25. Chacko PK, Kodoth J, John J, Kumar K. Recycling stainless steel orthodontic brackets with Er:YAG laser - An environmental scanning electron microscope and shear bond strength study. J Orthod Sci 2013;2:87-94. [CrossRef]
26. Bishara SE, Laffoon JF, Vonwald L, Warren JJ. The effect of repeated bonding on the shear bond strength of different orthodontic adhesives. Am J Orthod Dentofacial Orthop 2002;121:521-5. [CrossRef]
27. Movahhed HZ, Ogaard B, Syverud M. An in vitro comparison of the shear bond strength of a resin-reinforced glass ionomer cement and a composite adhesive for bonding orthodontic brackets. Eur J Orthod 2005;27:477-83. [CrossRef]
28. Summers A, Kao E, Gilmore J, Gunel E, Ngan P. Comparison of bond strength between a conventional resin adhesive and a resin-modified glass ionomer adhesive: an in vitro and in vivo study. Am J Orthod Dentofacial Orthop 2004;126:200-6. [CrossRef]
29. Ahangar Atashi MH, Sadr Haghighi AH, Nastarin P, Ahangar Atashi S. Variations in enamel damage after debonding of two different bracket base designs: An in vitro study. J Dent Res Dent Clin Dent Prospects 2018;12:56-62. [CrossRef]

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Official Publication of Istanbul University Faculty of Dentistry

Eur Oral Res 2023; 57(2): 103-107

Original research

Does hot coffee or cold coffee cause more discoloration on resin based composite materials?

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Purpose
This study aimed to examine the effect of beverages at different temperatures on the coloring of composites.

Materials and Methods
A total of 48 cylindrical samples, 24 of which were prepared from 2 composite materials (G'aenial; Estelite Σ Quick), were included in the study. The sample dimensions were standardized at 2 10 mm^2. After the polishing, the initial color measurements were performed using a spectrophotometer. The samples were divided into 3 subgroups as distilled water, hot coffee (60 °C) and cold coffee (0 °C) (n=8). During the 7th and 30th days, the samples were immersed in the solutions for 15 min every day. Color measurements were repeated on the 7th and 30th days. Data were analyzed using a two-way analysis of variance, followed by the Tukey post-hoc test ($p<0.05$).

Results
The highest color change was detected on the 7th and 30th days in the G-aenial anterior microhybrid composite immersed in hot coffee ($p<0.001$). The application of hot and cold coffee applications did not make a statistically significant difference in the coloration of the Estelite Σ Quick composite samples at the end of the 7th ($p=0.346$) and 30th ($p=910$) days.

Conclusion
Hot drinks had a more coloring effect on restorations. This coloration was quite evident in the microhybrid composite.

Keywords: *Coffee, cold coffee, discoloration, hot coffee, spectrophotometer*

Introduction

Dental composite materials, which are frequently used in the clinic, are highly developed in terms of mechanical, physical, and esthetic properties. In addition to physical and mechanical properties, it is desirable for composites to maintain optical color stability as a result of long-term water absorption, which is one of the critical factors for clinical success (1, 2). Composite resins can be classified according to their properties such as size, content, filler type, and physical and mechanical properties of the material (3). Most microfilled composites contain particles ranging in size from 0.4 to 0.2 µm, while nanofilled composites contain filler particles smaller than 0.1 µm (4).

The discoloration that develops over time in composite restorations is the most important factor for the renewal of these restorations. A discoloration caused by extrinsic reasons can be removed by polishing, while intrinsic discolorations that reach the depths of the restoration may cause the renewal of the restoration (5). The intrinsic factors may result from the surface properties of the material, water absorption, diet consumption,

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Received: 1 August 2022
Revised: 24 October 2022
Accepted: 10 November 2022

DOI: 10.26650/eor.20231152488

How to cite: *Ersöz B, Aybala Oktay E, Aydın N, Karaoğlanoğlu S. Does hot coffee or cold coffee cause more discoloration on resin based composite materials? Eur Oral Res 2023; 57(2): 103-107.* DOI: 10.26650/eor.20231152488

and oral hygiene. However, these factors are affected by the polymerization time, polymer composition of the matrix, and fillers (6, 7).

Beverages with high coloring pigment contents such as tea and coffee, which are frequently consumed foods, are an important factor in the coloring of composite materials (8). Coffee consumption is seen as an indispensable habit for most individuals. Around two billion cups of coffee are consumed every day around the world (9). Although coffee is traditionally a hot beverage, the popularity and place of cold coffee in the global market have increased considerably with the increasing popularity of coffee varieties and coffee culture (10). Although the color changes in the materials can be perceived visually, using various color measurement devices is recommended for an objective evaluation. During the calculation of color changes in composites, the formula in the formula CIEDE2000 (ΔE00) was used over the parameters L*, a*, and b* (11). In the coloring of materials, studies and data examining the effect of the temperature of the solution in contact with the material on the coloration are few (12, 13).

This *in vitro* study investigated the degree of the color change of two different composite resins as a result of keeping them in hot (60°C) and cold coffee (0°C) (initially, on the 7th day, and on the 30th day). The first null hypothesis of our study is that the application of hot and cold coffee will cause similar color changes in the microhybrid composite on the 7th and 30th days. The second null hypothesis is that the application of hot and cold coffee will cause a similar color change in the nanofilled composite on the 7th and 30th days.

Materials and Methods

Sample preparation

A total of 48 cylindrical composite specimens of 2 × 10 mm^2 were prepared, 24 each from two different light-cured composite resin materials (G-aenial, GC Dental Products, Tokyo, Japan; Estelite Σ Quick, Tokuyama Dental, Tokyo, Japan). The type, color, content, and manufacturer's information of the composites are given in Table 1.

While the samples were polymerized, a 1-mm glass coverslip was placed over the mylar strip after the composite resin material was placed into in the Teflon mold with a metal hand instrument. Samples were polymerized for 20 s with a 1000 mW/cm^2 light emitting diode source (DTE LUX E, Stuttgart, Germany) with the tip of the light device in contact with the glass coverslip. The light output power of the LED light device was checked at regular intervals (in each group) on the control unit (PRIMA DB685-SUPER-DUAL, COXO, China). Sample preparation and color measurements were performed by the same experienced operator (BE). After the samples were prepared, they were polished with 3M Soflex (3M ESPE, MN, USA) discs at 10,000 rpm, under water cooling, from coarse to fine grains for 20 s. Afterward, the samples were cleaned by washing them with a compressed air–water spray. The composite samples were then placed in an incubator (FN 500, Nüve, Turkey) for 24 h in distilled water at 37°C.

Colorimetric measurements

Initial color measurements of the samples were made with the help of a spectrophotometer device (VITA Easyshade V 4.0; VITA Zahnfabrik, Bad Säckingen, Germany) in a specially prepared color measurement cabinet to ensure standardization. The light power in the color-measuring cabinet was set to 6500K according to CIE standards (Master TL-D 90 Graphica 18W/965, Philips, Poland). The interior of the cabin was covered with a gray floor, and the measurements were made on a white background. The colorimeter was calibrated before each measurement. Three measurements were made for each sample, and the mean values of the L*, a*, and b* data were calculated. L*, a*, and b* color differences on the initial day, 7th day, and 30th days were calculated as three-dimensional (ΔE_{00}) in the color space, with the formula CIEDE2000 (14) where *L** is the lightness; *a** is the red (+)/green (–) color coordinate; *b** is the yellow (+)/blue (–) color coordinate.

$$\Delta E_{00} = \sqrt{\left(\frac{\Delta L'}{k_L S_L}\right)^2 + \left(\frac{\Delta C'}{k_C S_C}\right)^2 + \left(\frac{\Delta H'}{k_H S_H}\right)^2 + R_T \left(\frac{\Delta C'}{k_C S_C}\right)\left(\frac{\Delta H'}{k_H S_H}\right)}$$

For this study, K_L, K_C, and K_H were set to 1.0. The clinically accepted 50%:50% color change threshold was determined at ΔE_{00} = 1.8 (15). After the initial color measurement, 48 disk-shaped samples, 24 from each of the two composite materials, were randomly divided into three subgroups according to the type of solution (*n* = 8). Hot coffee (60°C) and cold coffee (0°C) solutions were prepared as standard in the

Table 1. *Composition of materials used in the study UDMA; urethane dimethacrylate, Bis-GMA; bisphenol A-glycidyl methacrylate, TEGDMA; tetraethylene glycol dimethyl ether.*

Composite	Brand	Material type	Filler	Matrix	Filler content (wt %)	Particle Size	Lot number
G-aenial anterior (A2)	GC Dental Product, Tokyo, Japan	Microhybrid	Pre-polymerized (silica, strontium, lanthanoid) silica, fumed silica, fluoroaluminosilicate, UDMA, dimethacrylate co-monomers	UDMA, dimethacrylate co-monomers	76	0,1-17 µm	210323C
Estelite Σ Quick (A2)	Tokuyama Dental, Tokyo, Japan)	Nanofilled	Silica-zirconia supra-nano-monodispersing spherical	Bis-GMA, TEGDMA, UDMA	78	0,1-0,3 µm	208E51

same coffee machine (Franke A800, Switzerland), with 44 mL (14 g) of coffee (Starbucks Espresso Roast, Fairtrade, USA) mixed with 311 mL of water. Before the composite samples were immersed in coffee solutions, their temperature was measured with the help of a digital thermometer (BENETECH GM1311, Shenzhen Jumaoyuan Science and Technology Co., Ltd., Shenzen, China) and the experimental procedure started when they reached the determined average consumption temperatures (0°C and 60°C). The average consumption time of one cup of coffee was 15 min (16). The samples were kept in hot coffee at 60°C, cold coffee at 0°C, and distilled water at room temperature for 15 min every day during the same time interval. After each holding, the samples were washed with distilled water and placed in fresh distilled water at room temperature for 24 h. At the end of the 7th and the 30th days, the initial color measurement procedure was repeated. ΔE values below 1.8 were considered clinically undetectable during evaluations ($\Delta E_{00} < 1.8$) (15). The color change values of the composite samples on the 7th and 30th days were calculated with the CIEDE2000 formula.

Statistical analysis

Kolmogorov–Smirnov test was used to determine whether the data had a normal distribution. A two-way analysis of variance (ANOVA) was used to examine the color change of the different materials tested. Tukey post hoc test was used for pairwise comparisons. The significance level was set to $p < 0.05$.

Results

When the results were evaluated, the most statistically significant color change on the 7th and 30th days was detected in the G-aenial anterior microhybrid composite soaked in hot coffee ($p < 0.01$) (Table 2). Hot and cold coffee applications did not make a statistically significant difference in the coloration of the nano-filled Estelite Σ Quick composite samples at the end of the 7th (p = 0.346) and 30th (p = 0.910) days (Table 2).

At the end of the 7th and 30th days, the samples kept in distilled water were significantly less colored than the samples kept in hot and cold coffee ($p < 0.01$). At the end of the 30th days, the coloration of the samples kept in distilled water, hot coffee, and cold coffee was significantly higher than the coloration at the end of the 7th day ($p < 0.01$).

At the end of the 7th day, G-aenial anterior composite specimens showed a color change below the detectable threshold in cold coffee ($\Delta E_{00} = 1.34$), while they showed a color change above the detectable threshold value in hot coffee ($\Delta E_{00} = 2.64$) (Table 2). Estelite Σ Quick composite samples showed a color change below the detectable threshold ($\Delta E_{00} \leq 1.8$) at the end of the 7th day after being kept in both hot ($\Delta E_{00} = 1.37$) and cold coffee ($\Delta E_{00} = 0.95$). G-aenial anterior and Estelite Σ Quick composite specimens showed a color change above the acceptable threshold value ($\Delta E_{00} > 1.8$) after the application of hot and cold coffee at the end of the 30th day (Table 2). At the end of the 30th day, G-aenial anterior samples ($\Delta E_{00} = 4.37$) immersed in hot coffee were significantly more colored than Estelite Σ Quick samples ($\Delta E_{00} = 2.04$) ($p < 0.01$) (Table 2).

Despite no significant difference in the coloration of the composites kept in cold coffee at the end of the 30th day, G-aenial anterior samples ($\Delta E_{00} = 2.48$) showed more coloration than Estelite Σ Quick samples ($\Delta E_{00} = 2.3$) (Table 2). Although color changes were observed in the distilled water samples at the end of the 7th and 30th days, the color changes were within the acceptable threshold ($\Delta E_{00} \leq 1.8$) (Table 2).

Discussion

The microhybrid composite was significantly more colored in hot coffee than in cold coffee at the end of the 7th and 30th days. Therefore, the first null hypothesis of our study was rejected. The nanofilled composite showed similar discoloration in hot and cold coffee at the end of the 7th and 30th days, therefore the second null hypothesis of our study was accepted.

Although dental composites are the most commonly used restorative materials in the clinic, the discoloration of these materials is still a major problem for patients and physicians. Advanced discoloration in composites is seen as a sign that the restoration needs to be renewed (17). Although intermittent polishing is recommended to remove intrinsic colorations, the main purpose is to make the composites less colored and to preserve their esthetic properties for a long time despite the consumption of coloring foods with high pigment content (18, 19).

Studies, examining the color change of composite restorative materials by keeping them in different beverages for different periods, are available in the literature (20-23). However, studies on the coloring of composite materials until today have focused on the coloration that occurs as a result of keeping colorants such as coffee, tea, wine, etc. at room temperature at usually 37°C. However, the daily consumption temperatures of these beverages are generally very different from the room temperature (37°C) and vary according to the type of beverage and the consumption. Hot beverages such as tea, hot chocolate, and coffee are often served at temperatures between 71.1°C and 85°C. However, consumers often consume their hot beverages at 60°C, which is lower than the

Table 2. *Coloration values of the composites on the 7th and 30th days. (Lower case letters a–c show statistically significant differences between rows. Upper case letters A and B show statistically significant differences between columns (p < 0.05).*

	Estelite Σ Quick 7th day	G-aenial anterior 7th day	p	Estelite Σ Quick 30th day	G-aenial anterior 30th day	p
Distilled Water	0.59a,A ± 0.1	0.49a,A ± 0.2	0.997	1.08a,A ± 0.2	0.89a,A ± 0.4	0.977
Hot Coffee	1.37b,A ± 0.6	2.64c,B ± 0.5	0.000	2.04b,A ± 0.2	4.37b,B ± 0.7	0.000
Cold Coffee	0.95ab,A ± 0.2	1.34b,A ± 0.4	0.450	2.3b,A ± 0.6	2.48c,A ± 0.6	0.984
p	0.000	0.000		0.000	0.000	

recommended serving temperature (24). Therefore, in this study, the immersion temperature of the samples in the hot coffee solution was determined as 60°C (24). Since the average consumption temperature of cold drinks was 0°C, this was determined as the holding temperature of the samples in cold coffee (25). Since the average consumption time of one glass of beverage is 15 min, the waiting time in the coloring solution was determined as 15 min per day. Thus, in the present study, the 15 min consumption time of real coffee and the temperature change at room temperature during the consumption period were also simulated, instead of keeping it at the same and constant temperature (16).

Estelite Σ Quick composite chosen for this study had nanosized fillers, while the G-aenial anterior composite consisted of microhybrid fillers. Thanks to the smaller size and better distribution of particles in the resin matrix of composite restorations, smoother surfaces were obtained (25). Studies have shown that the smaller size of nanofilled composite resin particles results in less staining (5, 26). In this study, G-aenial anterior composite samples with larger particle sizes were more colored than Estelite Σ Quick composite samples with smaller particle sizes during all time periods. However, contrary to these results, other researchers have also reported that increasing particle size causes less coloration in composites (5, 27). Another study showed that smooth surfaces were not always the most resistant to discoloring and that the staining ability was affected by the monomer and filler particles from which the composite was formed (28). In the study of Nasim *et al.* (5) which investigated the discoloration of different composite materials, the most discoloration was observed in nanofilled composite resin (Filtek Z350), followed by microfilled composite resin (Heliomolar), while the least discoloration was observed in Spectrum TPH, which is microhybrid composite resin (5). Similarly in the study of Villata *et al.* (29); nanofilled Filtek Supreme showed more discoloration than microfilled Esthet X composite.

According to the results of a study evaluating the effect of different temperatures (4^{0}C, 37^{0}C, and 60^{0}C) on the color stability of 5 different composite resins (Aelite LS Posterior, Point 4, Tetric EvoCeram, Vit-l-escence, Filtek Z350), different temperatures did not make a significant difference on the discoloration of these composites (12). In an another study on the discoloration of microhybrid composite (Filtek Z250) at different temperatures; coffee at 70°C showed significantly more discoloration than coffee at 37°C. It has been reported that high temperatures can weaken the floating properties of composite materials, reduce their microhardness and lead to more discoloration (13). In our study, G-aenial anterior composite samples kept in hot coffee on the 7th and 30th days were significantly more colored than those kept in cold coffee. Similar to the study of Tuncer *et al.* (13), in our study, coffee at 70°C may have reduced the surface hardness of the microhybrid composite and may have caused more staining. Estelite Σ Quick samples were more colored when kept in hot solutions at the end of the 7th day, similar to G-aenial samples; however, this discoloration was not statistically significant for the Estelite Σ Quick samples (7th day). The reason for the not significant difference could be the more resistance of Estelite Σ Quick to discoloration due to its nanofilled structure and small particle size.

The present study had some limitations. Oral foods are diluted with saliva under normal conditions, but this diluting effect could not be simulated in this study. In addition, as mentioned previously, further studies are needed to examine the effect of longer retention times and temperature on surface properties. Two composite types were investigated in this study. Further studies should be carried out by increasing the variety of materials. Despite all the limitations, it can be mentioned that this study was one of the limited numbers of studies examining the effect of storage conditions at different temperatures on the discoloration of composite resins.

Conclusion

Microhybrid composite resin was more colored than nanofilled composite resin. No significant differences were found in the discoloration of nanofilled composite samples kept in hot coffee and cold coffee for both the 7th and 30th days. Microhybrid composite samples kept in hot coffee were more colored than microhybrid composite samples kept in cold coffee for both the 7th and 30th days. If many composite resin restorations will be done for a person who likes to consume hot drinks, it is better to use nanofilled composite materials.

Türkçe özet: *Sıcak kahve mi yoksa soğuk kahve mi rezin esaslı kompozit materyallerde daha fazla renklenmeye neden olur? Amaç: Bu in-vitro çalışmanın amacı, farklı sıcaklık derecelerindeki içeceklerin kompozit restorasyonların renklenmesine olan etkisini incelemektir. Gereç ve yöntem: 2 kompozit materyalden (G'aenial, GC; Estelite Σ Quick, Tokuyama) 24'er adet olmak üzere toplamda 48 adet silindirik örnek hazırlandı. Örnek büyüklükleri teflon kalıp yardımıyla 2x10 mm^2 büyüklüğünde olacak şekilde standardize edildi. Örneklerin polisaj işlemi sonrası başlangıç renk ölçümleri spektrofotometre ile yapıldı. Örnekler bekletildikleri solüsyonlara göre; distile su, sıcak kahve (60 C°) ve soğuk kahve (0 C°) olarak 3 alt gruba bölündü (n=8). 7 ve 30 günlük boyunca örnekler her gün 15 dk boyunca solüsyonlara daldırıldı. 7. ve 30. günde spektrofotometre ile renk ölçümleri tekrarlandı. Veriler, iki yönlü ANOVA ve ardından Tukey post-hoc testi kullanılarak analiz edildi (p <0.05). Bulgular: Deneyin 30.günündeki materyaller üzerindeki renk değişim miktarı, 7.gündeki renk değişim miktarından anlamlı derecede fazlaydı (p>0.05). 7. ve 30. günde istatistiksel olarak en fazla renk değişimi, sıcak kahvede bekletilen G-aenial anterior micro-hibrit kompozitte tespit edildi (p<0.05). Estelite Σ Quick nano doldurucu kompozit üzerinde sıcak ve soğuk kahve uygulaması istatiksel olarak anlamlı farklılık oluşturmadı. Sonuç: Sıcak içecekler, soğuk içeceklere göre restorasyonlar üzerinde daha fazla boyayıcı etkiye sahiptir. Mikrohibrit yapıdaki kompozitte bu renklenmenin oldukça belirgin olduğu gözlenmiştir. Anahtar Kelimeler: Sıcak Kahve, Soğuk Kahve, Spektrofotometre, Kahve, Renklenme.*

Ethics Committee Approval: Not required.

Informed Consent: Not required.

Peer-review: Externally peer-reviewed.

Author contributions: BE, EAO, NA, SK participated in designing the study. BE, EAO, NA, SK participated in generating the data for the study. BE, EAO, NA, SK participated in gathering the data for the study. BE, EAO, NA, SK participated in the analysis of the data. BE, EAO, NA, SK wrote the majority of the original draft of the paper. BE, EAO, NA, SK participated in writing the paper. BE, EAO, NA, SK have had access to all of the raw data of the study. BE, EAO, NA, SK have reviewed the pertinent raw data on which the results and conclusions of this study are based. BE, EAO, NA, SK have approved the final version of this paper. BE, EAO guarantee that all individuals who meet the Journal's authorship criteria are included as authors of this paper.

Conflict of Interest: The authors declared that they have no conflict of interest.

Financial Disclosure: The authors declare that this study has not received any financial support.

REFERENCES

1. Yadav R, Kumar M. Dental restorative composite materials: A review. J Oral Biosci 2019;61:78-83. [CrossRef]
2. Demarco FF, Collares K, Coelho-de-Souza FH, *et al*. Anterior composite restorations: A systematic review on long-term survival and reasons for failure. Dental Mater 2015;31:1214-24. [CrossRef]
3. Senawongse P, Pongprueksa P. Surface roughness of nanofill and nanohybrid resin composites after polishing and brushing. J Esthet Restor Dent 2007;19(5):265-73. [CrossRef]
4. Ferracane JL. Resin composite – State of the art. Dent Mater. 2011;27:29-38. [CrossRef]
5. Nasim I, Neelakantan P, Sujeer R, *et al*. Color stability of microfilled, microhybrid and nanocomposite resins—an *in vitro* study. J Dent 2010;38(Suppl 2):e137-42. [CrossRef]
6. Stawarczyk B, Sener B, Trottmann A, *et al*. Discoloration of manually fabricated resins and industrially fabricated CAD/ CAM blocks versus glass-ceramic: Effect of storage media, duration, and subsequent polishing. Dent Mater J 2012;31(3):377-83. [CrossRef]
7. Arocha MA, Basilio J, Llopis J, *et al*. Colour stainability of indirect CAD-CAM processed composites vs. conventionally laboratory processed composites after immersion in staining solutions. J Dent 2014;42(7):831-8. [CrossRef]
8. Al Kheraif AA, Qasim SS, Ramakrishnaiah R, *et al*. Effect of different beverages on the color stability and degree of conversion of nano and microhybrid composites. Dental Mater J 2013;32:326-31. [CrossRef]
9. Poole R, Ewings S, Parkes J, *et al*. Misclassification of coffee consumption data and the development of a standardised coffee unit measure. BMJ Nutr Prev Health 2019;2(1):11-9. [CrossRef]
10. Mahmud MMC, Shellie RA, Mohebbi M, *et al*. The effect of fat and coffee concentration on the consumer acceptance of iced-coffee beverages. 2021;86(11):5004-15. [CrossRef]
11. Karaarslan ES, Bulbul M, Ertas E, *et al*. Assessment of changes in color and color parameters of light-cured composite resin after alternative polymerization methods. Eur J Dent 2013;7:110-6. [CrossRef]
12. Kim TH, García-Godoy F, Ko CC, *et al*. Effect of temperature on the mass and color stability of additional photoinitiatorcontaining composite resins. Dent Mater J 2013;32(4):628-36. [CrossRef]
13. Tuncer D, Karaman E, Firat E. Does the temperature of beverages affect the surface roughness, hardness, and color stability of a composite resin? Eur J Dent 2013 Apr;7(2):165-171. [CrossRef]
14. Luo MR, Cui G, Rigg B. The development of the CIE 2000 colour difference formula: CIEDE2000. Color Res Appl 2001;26:340-50. [CrossRef]
15. Paravına RD, Ghınea R, Herrera LJ, *et al*. Color difference thresholds in dentistry. J Esthet Restor Dent 2015;27(1):1-9. [CrossRef]
16. Noie F, O'Keefe KL, Powers JM. Color stability of resin-cements after accelerate daging. Int J Prosthodont 1995;8(1):51-5.
17. Furuse AY, Santana LOC, Rizzante FAP, *et al*. Delayed light activation improves color stability of dual-cured resin cements. J Prosthodont 2018;27(5):449-55. [CrossRef]
18. Deljoo Z, Sadeghi M, Azar M, Bagheri R. The effect of different polishing methods and storage media on discoloration of resin composites. J Dent Biomater 2016;3:226-32.
19. Spina DR, Grossi JR, Cunali RS, *et al*. Evaluation of discoloration removal by polishing resin composites submitted to staining in different drink solutions. Int Sch Res Notices 2015;20:853975. [CrossRef]
20. Ersoz B, Karaoglanoglu S, Oktay EA, *et al*. Color stability and surface roughness of resin based direct and indirect restorative materials. European Annals of Dental Science 2021;48(1):1-6. [CrossRef]
21. Aydin N, Karaoglanoglu S, Oktay EA, *et al*. Investigation of Single Shade Composite Resin Surface Roughness And Color Stability. J Dent Fac Atatürk Uni 2021;31(2):207-14. [CrossRef]
22. Iskender N, Ersoz B, Aydin N, *et al*. Investigation of Water Absorption and Color Change of Indirect Composite Resins. European Annals of Dental Science 2021;48(2):46-51. [CrossRef]
23. Aydin N, Karaoglanoglu S, Oktay EA, *et al*. Effect of Additional Polishing Application on the Surface Roughness and Color Change of Composite Resins. Turkiye Klinikleri J Dental Sci 2021;27(3):462-9. [CrossRef]
24. Lee HS, O'Mahony M. At What Temperatures Do Consumers Like to Drink Coffee?: Mixing Methods. J Food Sci 2006;67(7):2774-7. [CrossRef]
25. Reis AF, Giannini M, Lovadino JR, *et al*. The effect of six polishing systems on the surface roughness of two packable resin-based composites. Am J Dent 2002;15:193-7.
26. Barclay CW, Spence D, Laird WR. Intra-oral temperatures during function. J Oral Rehabil 2005;32(12):886-94. [CrossRef]
27. Paravina RD, Roeder L, Lu H, *et al*. Effect of finishing and polishing procedures on surface roughness, gloss and color of resin-based composites. Am J Dent 2004;17:262-6.
28. Lee YK, Powers JM. Discoloration of dental resin composites after immersion in a series of organic and chemical solutions. J Biomed Mater Res B Appl Biomater 2005;73:361-7. [CrossRef]
29. Villalta P, Lu H, Okte Z, *et al*. Effects of staining and bleaching on color change of dental composite resins. J Prosthet Dent 2006;95:137-42. [CrossRef]

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Official Publication of Istanbul University Faculty of Dentistry

Eur Oral Res 2023; 57(2): 108-114

Original research

Evaluation of the effects of thermal changes on the bond strength between zirconia framework and veneering ceramic during the firing process

Yeşim Ölçer Us[1], **Betül Tuncelli**[2]

Purpose
The aim of this in-vitro study was to evaluate the effect of thermal changes to shear bond strength during the firing process of veneering porcelain on a zirconia framework.

Materials and Methods
Single yttria-stabilized tetragonal zirconia polycrystal (Y-TZP) framework ceramic (Kavo Dental GmbH) and three different types of veneering ceramics (IPS e.max Ceram, Vita VM9, and GC Initial Zr-FS) were used. One-hundred-twenty standard disc-shaped samples were prepared from zirconia blocks by using a CAD/CAM system (Kavo Everest). Four different cooling processes (maximum, 25°C/min, 50°C/min and 75°C/min) were applied to the veneering ceramics and the shear bond strength (SBS) test was performed. Ceramic surfaces were investigated by using scanning electron microscopeic (SEM). The possible occurrence of a t–m transformation of zirconia was evaluated by X-Ray Diffraction (XRD). Two-way analysis of variance, Bonferroni correction and paired comparisons were used for statistical analysis.

Results
The main effects of veneering ceramics on shear bond strength were found to be significant ($p=0.042$). The mean shear bond strength values differ according to the cooling process ($p<0.001$). The monoclinic phase ratio increased in groups with fast cooling process.

Conclusion
The thermal changes during the firing process of veneering porcelain on a zirconia framework influenced the shear bond strength of the all-ceramic bilayered system. A slow cooling process provided higher strength for bilayer ceramic samples.

Keywords: *Zirconia, Veneering ceramic, CAD/CAM, Bond strength, Cooling process*

Introduction

The increase in the aesthetic expectations and technological developments have created an area of use for different tooth-colored, biocompatible, high-strength restorative systems as an alternative to metal-supported ceramic restorations that have been used for a long time (1, 2). However, their fragility and low resistance to stress limit the clinical applications of these materials. The use of all-ceramic systems was mostly limited to the the anterior region but thanks to the advanced dental ceramics, their use in posterior teeth has also increased. Zirconia, which exhibits high mechanical performance, durability, stress resistance, chemical and dimensional stability compared to other all-ceramic systems, has increased the reliability of all-ceramic systems (3-5).

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Received: 3 August 2021
Revised: 9 September 2022
Accepted: 10 November 2022

DOI: 10.26650/eor.2023978293

How to cite: *Ölçer Us Y, Tuncelli B. Evaluation of the effects of thermal changes on the bond strength between zirconia framework and veneering ceramic during the firing process. Eur Oral Res 2023; 57(2): 108-114.* DOI: 10.26650/eor.2023978293

In order to obtain a natural appearance, the feldspathic porcelain or various veneering ceramics specially developed for zirconia are applied over the opaque zirconia framework (6). The thermal processes applied during the production and veneering ceramic application affect the mechanical properties of the restoration. In addition, the weakness of veneering ceramics can negatively affect the clinical success of the restoration and may result in clinical failure in the form of fracture (chipping) formation in the veneering ceramics (7). This failure could be related to the difference in the thermal expansion coefficient between framework and veneering ceramics, thickness of the veneering ceramic, elasticity modulus, defective areas that may occur in the porcelain, improper firing process and the thermal changes in the cooling process after firing (8,9).

Residual stresses occur on the surface as a result of the cooling process after firing of the veneering ceramics (10). The authors argued that residual stresses could be changed using different heat treatments and these changes occur as a result of the viscoelastic behavior of the glass as a result of various cooling processes. No clear explanation has been provided so far for bonding problems in zirconia-based ceramics that result in chipping which is most likely caused by the cooling process (8,11). The aim of this in-vitro study was to evaluate the effects of thermal changes on the bond strength between the zirconia framework and the veneering ceramic during the firing process. The null hypothesis tested in the present study is that the thermal changes during the firing process does not affect the bond strength.

Materials and Methods

Sample size estimation

Using the 95% confidence (1-α), 80% test power (1-β), Partial eta squared=0.079308 and f=0.293 input, the minimum number of samples to be included was determined as 115 (12).

Table 1. *Cooling process and groups of the veneering ceramics*

IPS e.max Ceram n=40	Kavo Everest ZS n=120 / Vita VM9 n=40	GC Initial Zr-FS n=40
Max n=10 (IPS-Max)	Max n=10 (Vita-Max)	Max n=10 (GC-Max)
75°C/min n=10 (IPS-75)	75°C/min n=10 (Vita-75)	75°C/min n=10 (GC-75)
50°C/min n=10 (IPS-50)	50°C/min n=10 (Vita-50)	50°C/min n=10 (GC-50)
25°C/min n=10 (IPS-25)	25°C/min n=10 (Vita-25)	25°C/min n=10 (GC-25)

Preparation of bilayered zirconia-veneering ceramic samples

The present study was performed using single yttria-stabilized tetragonal zirconia polycrystal (Y-TZP) framework ceramic (Kavo Dental GmbH, Biberach, Germany) and three different types of veneering ceramics (IPS e.max Ceram, Ivoclar Vivadent, Schaan, Liechtenstein; Vita VM9, Vita Zahnfabrik, Germany; and GC Initial Zr-FS, GC Europe N.V., Interleuvenlaan, Leuven, Belgium) recommended for veneering the zirconia framework (Table 1, Table 2, Table 3).

One-hundred-twenty standard disc-shaped samples (height of 3mm and diameter of 10 mm) were prepared from zirconia blocks by using a CAD/CAM system (Kavo Everest). All samples were airborne particle abraded with 50 mm Al_2O_3 at 2.5 bars of pressure (15 s) at a maximum distance of 10 mm and ultrasonically cleaned in distilled water for 3 minutes followed by steam cleaning for 15 seconds before application of veneering ceramic. The samples were divided into three groups of 40 samples each and each group was layered with a different veneering ceramic (IPS e.max Ceram, Vita VM9, or GC Initial Zr-FS) The groups were further divided into four subgroups of 10 samples in order to apply four different cooling processes.

Three different veneering ceramics were applied to the groups according to manufacturers' instructions by using a layering technique. A specially designed stainless-steel mold was used to standardize the veneering ceramic size for all samples. A zirconia disc-shaped specimen was placed in this mold, with a space of 10 mm in diameter and 3 mm in height above the zirconia material to condense the veneering ceramic.

Table 3. *Technical data on the Kavo Everest ZS*

Kavo Everest ZS	
ZrO_2	99,60%
Y_2O_3	5,00%
Al_2O_3	0,25%
Other metal oxides	< % 0,15
Average grain size	0,52 ± 0,05 µm
Coefficient of Thermal Expansion (CTE)	10,0 x 10-6 K^{-1}
Thermal Conductivity	2,5 W/mK
Chemical Solubility	10 µg/cm^3
Flexural Strength	1200 MPa
Elasticity Modulus	210 GPa
Fracture Toughness	8 MN/$m^{1/2}$

Table 2. *Technical data on the veneering ceramics*

Veneering Ceramic	Coefficient of Thermal Expansion (CTE)	Glass Transition Temperature	Chemical Solubility	Flexural Strength	First Dentine Firing
IPS e.max Ceram	9,5±0,2510^{-6} K^{-1}	490±10 °C	15±5 µg/cm^3	90±10 MPa	750°C
Vita VM9	8,8-9,2 x 10^{-6} K^{-1}	600 °C	10 µg/cm^3	100 MPa	910°C
GC Initial Zr-FS	9,4 x 10^{-6} K^{-1}	550 °C	12 µg/cm^3	90 MPa	810°C

Firing process

The Austromat D4 (Dekema Dental, Freilassing, Germany) porcelain furnace was used for the slow cooling process because of the accuracy of control of this furnace in bringing the samples temperature down through the glass transformation temperature range. The firing table mechanism allows the table to exit the furnace in a vertical downwards direction. After the first dentin firing schedule, four different cooling rates; maximum, 25°C/minute, 50°C/minute and 75°C/minute through the glass transformation temperature range of veneering ceramics were used. Maximum cooling consists of immediately bringing the samples temperature down in the furnace after the end of the firing schedule. Then all samples were exposed to ambient air (~23°C).

Shear Bond Strength Test

Shear bond strengths (SBS) were determined according to ISO TR 11405, using a universal testing machine (Shimadzu AG-IS, Shimadzu, Kyoto, Japan) at a crosshead speed of 0.5 mm/min. The obtained load values were converted into the megapascals (MPa) by dividing the failure load (N) by the bonding area (mm^2).

Microstructural analysis

Microstructural characterization was conducted using scanning electron microscopy (SEM) and X-ray diffraction (XRD) analysis. Cross-sections of the samples were evaluated using SEM (JSM-7000F, Jeol, Tokyo, Japan) at ×500 magnification to observe their crack pattern and grain size.

Representative samples that showed, possible occurrence of a t–m transformation of zirconia during the firing process of the veneering ceramic were detected by using x-ray diffraction (XRD). XRD patterns were collected using a diffractometer (X'Pert Pro, PANalytical, The Netherlands) within the 2θ range of 25–65, covering the positions of the highest peaks of tetragonal and monoclinic phases of ZrO_2. CuKα radiation was generated at 40 kV and 45 mA. Mass fraction of monoclinic phase (Xm) was calculated using the methods of Garvie-Nicholson (13) and Toraya et al (14).

Statistical analysis

Data were analyzed with IBM SPSS V23 (IBM SPSS, Armonk, NY, USA). Two-way analysis of variance was used to compare mean values according to veneering ceramics and cooling process. For the comparison of the main effects, the Bonferroni correction and paired comparisons were employed. Analysis results are presented as mean and standard deviation. The significance level was set to $p < 0.05$.

Results

Shear bond strength

The main effects of veneering ceramics on shear bond strength were found to be significant ($p = 0.042$). Average shear bond strength value was 25.14 ± 7.55 MPa (Min 13.49 MPa - Max 44.01 MPa) in the Vita VM9 group, 24.14 ± 7.78 MPa (Min 10.58 MPa - Max 49,44 MPa) in the IPS e.max Ceram group and 21.12 ± 7.86 MPa (Min 10.95 MPa - Max 36.40 MPa) in the GC Initial ZR-FS group. Mean shear strength values differ according to the cooling process ($p < 0.001$). While the mean shear bond strength value was 19.68 ± 6.08 MPa (Min 10.58 MPa - Max 29.83 MPa) in Max-groups, it was obtained as 22.27 ± 5.74 MPa (Min 11,34 MPa - Max 31,96 MPa) in "75" groups, 23,88 ± 7,75 MPa (Min 11,34 MPa - Max 39,09 MPa) in "50" groups and 28,04 ± 9,22 MPa (Min 14,14 MPa - Max 49,44 MPa) in "25" groups. The average value obtained in the "25" groups is higher than "Max" and "75" groups. The value obtained in "50" groups does not differ from other groups (Table 4 and 5) (Figure 1).

Veneering ceramic and cooling process interaction did not have a significant effect on the average value ($p = 0.938$). The measurements obtained at different cooling process in Vita VM9 did not differ from the measurements in IPS e.max Ceram and GC Initial ZR-FS. The main effect of the cooling process on shear bonding strength was higher than the main effect on the veneering ceramic. While the partial eta squared value obtained for the cooling process was 0.160, the value obtained for veneering ceramics was 0.057 (Table 5).

Microstructural analysis

The monoclinic phase ratio increased in groups in which the fast-cooling process was applied (Figure 2). Therefore, an increase in monoclinic volume was accompanied with a decrease in mechanical properties. SEM photographs revealed that among the three different veneering porcelains, they

Table 4. *Descriptive statistics and multiple comparison results for shear bond strength according to vennering ceramic and cooling process*

	Vita VM9	IPS e.max Ceram	GC Initial ZR-FS	Sum
Max	20,62±5,73 MPa	20,34±6,37 MPa	18,09±6,42 MPa	19,68±6,08^{A} MPa
75	23,84±4,42 MPa	23,06±4,79 MPa	19,91±7,36 MPa	22,27±5,74^{A} MPa
50	25,32±8,45 MPa	26,10±7,09 MPa	20,21±7,02 MPa	23,88±7,75^{AB} MPa
25	30,79±7,93 MPa	27,08±10,82 MPa	26,27±9,01 MPa	28,04±9,22^{B} MPa
Sum	25,14±7,55^{a} MPa	24,14±7,78^{ab} MPa	21,12±7,86^{b} MPa	

$^{a-b}$There were no statistically difference between veneering ceramics with the same letter; $^{A-B}$There were no statistically difference between cooling process with the same letter.

Table 5. *Effects of veneering ceramic and cooling process on shear bond strength*

	F	p	Partial Eta Squared
Veneering ceramic	3,267	**0,042**	0,057
Cooling process	6,878	**<0,001**	0,160
Veneering ceramic * Cooling process	0,296	0,938	0,016

had similar microstructural appearances as a function of the cooling process. The mode of failure analysis results for the groups showed mixed failure mode with both cohesive failure within the veneering ceramic and adhesive failure with the veneering ceramic debonding from zirconia. A direct view of the fracture surface at the zirconia framework showed that a thin layer of the veneering ceramic remained on the surface with exposure of the zirconia structure in groups in which the fast-cooling process was applied (Figure 3).

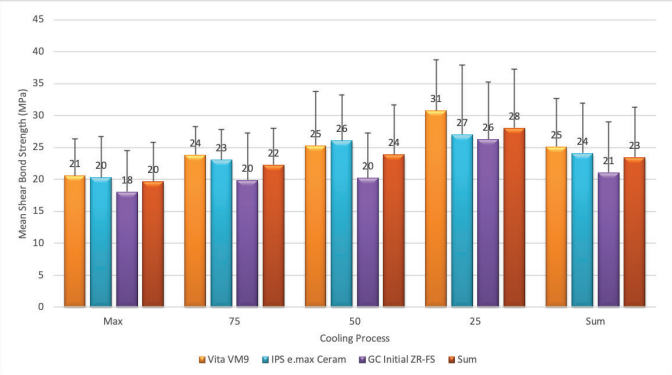
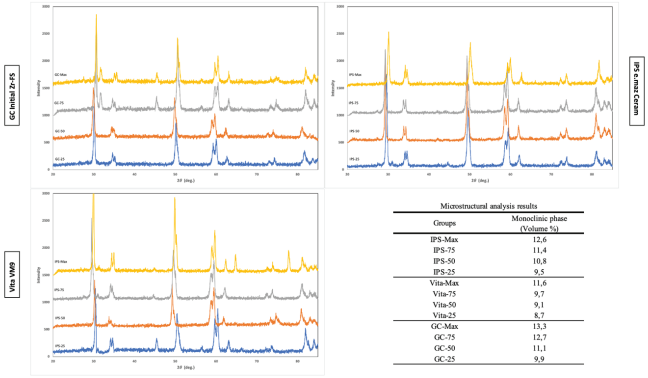


Figure 1. *Mean shear bond strength results of the groups.*



Microstructural analysis results

Groups	Monoclinic phase (Volume %)
IPS-Max	12,6
IPS-75	11,4
IPS-50	10,8
IPS-25	9,5
Vita-Max	11,6
Vita-75	9,7
Vita-50	9,1
Vita-25	8,7
GC-Max	13,3
GC-75	12,7
GC-50	11,1
GC-25	9,9

Figure 2. *XRD patterns and volume fraction (Vm) results of the monoclinic phase of the groups.*

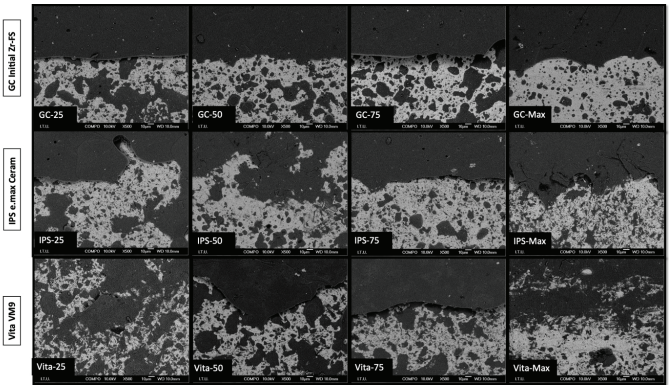


Figure 3. *SEM photographs of the groups.*

Discussion

Numerous factors have been reported to cause chipping failure in zirconia-based ceramics. Chemical bonding, mechanical bonding provided by surface roughness, the type and amount of defects at the interface, the wetting property of the veneering ceramic and the difference in the thermal expansion coefficient between zirconia and the veneering ceramic are among the factors affecting the bond strength. In addition, the thickness ratio of zirconia-veneering ceramics, the geometry of the restoration and inadequate veneering ceramic design, the magnitude of the applied force, the direction-frequency and the location of the occlusal forces, the elasticity modulus and conductivity differences of the framework and veneering ceramics, and the thermal changes observed during the firing of the veneering ceramics were indicated as other failure factors (6-9,15,16). Among these factors, the effect of the thermal changes seen during the firing of the veneering ceramics, especially the cooling process, on the bond strength between zirconia and veneering ceramics is not clear (17,18). Since the manufacturers producing veneering ceramics compatible with zirconia suggest applying a slow cooling process after firing, our research aimed to examine the effect of the thermal changes observed during the firing of ceramics applied on zirconia on the bond strength.

Previous studies investigated different cooling processes (18-20). In the present one, four different cooling processes were performed after the application of the veneering ceramics. The cooling process was applied in a similar way to the firing (similar to the heating process) applied by Tan *et al.* (21). It was stated that the samples that underwent slow heating and cooling showed higher fracture strength, but the results were not statistically significant. The failure occurred cohesively in ceramics in the region close to the zirconia framework (21). In another study, it was concluded that it was very difficult to analyze the results due to fracture patterns and factors affecting residual stress since there was no standardization regarding the cooling process and sample design (18). In our experiment, in groups that were subjected to the fastest cooling process, the samples were taken out of the furnace as soon as the firing process was completed, and the cooling process was iniated. It has been noted that this process could allow for a comparison which is similar to the steps a dental technician follows to remove restorations from the furnace immediately after firing (20).

The cooling process was performed in the furnace up to the glass transition temperature of the ceramic. The long-term cooling process is used to prevent residual stress formation in glasses and materials containing a glass matrix (22). At an annealing point above 50°C, the glass is hard enough not to deform and soft enough to relieve stresses (22-24). Therefore, the heat of glass transition had a significant effect on the magnitude of the residual stress in the ceramic and it was considerably affected by the cooling process (23,24). Above the glass transition temperature, the ceramic could make molecular movements that do not cause any stress and behave as a viscous structure that allows reshaping (8,23,25). As the temperature decreases, the viscosity of the ceramic increases and the molecular displacement becomes more difficult with the amount of thermal energy. The ceramic transforms into the elastic solid phase below the glass transition temperature (where there are no structural changes and stress accumulation may occur) (8,23).

The properties of the liquid vary, depending on the time during the transition process to the glass phase, and slow cooling should have been applied up to the glass transition temperature in order to prevent stress due to thermal transitions in ceramic and glass-containing materials. Thus, a ho-

mogeneous heat dissipation could be achieved between the inner and outer layers of the ceramic (26). For this reason, it has been concluded that the cooling process up to the glass transition temperature is more important in the formation of stress, and in our study, it was decided to perform the cooling process up to this temperature in the furnace in a controlled manner.

The stress in the veneering ceramics is an important factor in determining the longevity of the restoration. The thermal incompatibility between the framework and the veneering ceramics creates compressive or tensile stresses in the veneering ceramics, depending on whether the thermal expansion coefficient of the veneering ceramics was lower or higher than the framework. Ceramics were resistant to compressive stresses and but not to the tensile ones, and the occurrence of compressive stress in the veneering ceramic is a desirable situation, because in this way the veneering ceramic is strengthened, and the fracture resistance increases. Due to the fact that the thermal expansion coefficient of the veneering ceramic was somewhat lower than or close to that of the framework, the favorable compressive stresses may occur during the cooling process (27). When the thermal expansion coefficient of the veneering material is higher than that of the framework material, delamination and micro cracks could be observed in the veneering. In metal-ceramic systems, the excessive stresses caused by the mismatch of the thermal expansion coefficient could be partially compensated by the elastic or plastic deformation in the metal (28). However, zirconia has a lower thermal expansion coefficient than other ceramics. More destructive stresses occur in veneering ceramic in zirconia-based restorations. Therefore, it was previously reported that veneering ceramic resistance was an important parameter in long-term success (29). For this reason, in recent years, special veneering ceramics have been developed with the same or lower thermal expansion coefficient than zirconia. In our study, a zirconia framework and veneering ceramics with close and compatible thermal expansion coefficients were used.

Tempering is a method created by heating the glass to a temperature slightly below its softening degree and suddenly cooling it to room temperature and used to strengthen the ceramic (30). While this process is recommended for metal-based ceramic systems, the recommendations for veneering ceramics applied on a zirconia framework are different. Post-firing slow cooling (with the furnace closed from liquid phase to glass transition temperature) has been recommended for some ceramics (31). Manufacturers are currently concentrating their efforts on reducing the stress areas and cracks in ceramics. Previous research on this subject indicated that the fast cooling rate after firing might cause a high thermal tempering effect and residual stresses on the surface (10,32). Another factor in the residual stress formation that causes fractures in the veneering ceramic is related to the fact that zirconia is a weak conductor compared to metal alloys and even to other full ceramic frameworks. When fast cooling is applied to metal-based ceramics, veneering ceramics can cool rapidly from the surface to the inside because metal is a good conductor, but fast cooling cannot occur in zirconia-based ceramics. Veneering ceramic's surface hardens after fast cooling, but the inner part adjacent to the zirconia remains above the glass transition temperature for a long time, and wide thermal differences occur between the inner and outer layers (25,33). Benetti *et al*. (34) found that the temperature difference between the inner and outer layers was lower in the samples with slow heating and cooling compared to the samples with fast heating and cooling. In addition, the temperature difference between the layers in the metal-ceramic samples with fast cooling was found to be lower than that of the zirconia-ceramic samples. This result is thought to be due to metal being a better conductor than zirconia.

During the application of the veneering ceramic, the zirconia framework is exposed to existing humidity and increasing heat, and the zirconia is not stable due to the spontaneous t - m phase transformation. This can cause a decrease in mechanical properties. Heat, humidity, particle size, micro and macro cracks in the material can affect the t - m phase transformation of the stabilizing oxide type and amount. The most critical temperature range for this conversion is 200-300 ºC and the conversion is increased in the presence of water or steam. However, it has been stated that the effects of degradation at low temperatures on Y-TZP can only be significant after years (35,36). Tholey *et al.* detected monoclinic crystals at the zirconia-veneering ceramics interface using the SEM and XRD method (37,38). In zirconia, a volume increase of ~ 4% occurs with the monoclinic phase formed due to phase transformation. This increase in volume may develop due to the increase in humidity and temperature in the environment during the firing of the veneering ceramics (38). It was reported in another study that, the decrease in strength may depend on the amount of the structure being transformed, and there may be a significant reduction in strength when transformation occurs in a large part of the structure (39).

In the present study, the firing procedure (fast cooling after firing) decreased the shear bond strength of Vita, IPS and GC samples. XRD analysis revealed an increase in the monoclinic phase volume ratio on the surface which was observed in the groups with fast cooling after firing (in the maximum groups) compared to the groups in which slow cooling was applied (in the 25 groups). The volume increase of the monoclinic phase was also observed in the 75 and 50 group samples, and it was determined that the highest monoclinic phase volume ratio was in the GC maximum group (13.3%), and the lowest monoclinic phase volume ratio was in the Vita 25 group (8.7%). In general, it was observed that as the cooling rate increased in all groups, higher monoclinic phase volume ratios occurred. In addition, lower bond strengths were observed in the samples that underwent fast cooling (in the maximum groups). The highest monoclinic phase volume ratio was found on the surface of the GC maximum group with the lowest bond strength value, and the lowest monoclinic phase volume ratio was seen on the surface of the Vita 25 group with the highest bond strength. Taken together, it can be stated that the shear bond strength was also found to be lower in the samples with a higher monoclinic phase volume ratio. The fast-cooling process applied after the firing process may trigger the transformation of the metastable tetragonal phase into the monoclinic phase and could have weakened the structure. When the samples are evaluated in terms of failure type; combined fractures (cohesive failure in the veneering ceramic and adhesive failure at the inter-

face) were detected. In the SEM analysis, the zirconia framework in GC max. group was more exposed at the interface, although the fracture started in the veneering ceramic. This finding may support the low shear bond strength value.

There may be defects in ceramic material due to manufacturing process. Internal defects, grain size mismatches, large grain size, regions with different phases are microcracks due to the expansion and contraction of different crystal phases. External defects, on the other hand, occur after manufacturing processes and can be caused by voids, processing damage, sediments and foreign material residues. The superficial defects are always present in ceramics, and volumetric defects can re-surface with abrasion as well as during the polishing process, which could further weaken the structure (40). Despite the maximum care and attention in our study, the inherent structural defects might also have caused failure. The limitations of our study include the use of samples with different anatomical geometries to mimic the clinical situation and the ability to create fatigue by using a chewing simulator.

Conclusion

Within the limitation of this experimental study, it could be stated that the thermal changes during the firing process of veneering porcelain over a zirconia framework influenced the bond strength of the all-ceramic bilayered system. The fast cooling process reduced the strength of the bond between the zirconia framework and veneering ceramics. Slow cooling, especially after veneering firing, may reduce the bond failure, and therefore, it may prolong the longevity of the ceramic restoration.

Türkçe özet: *Zirkonya altyapı üzerine uygulanan seramiklerin ısısal değişimlerinin bağlantı dayanımlarına etkisinin incelenmesi. Amaç: Bu çalışmanın amacı; zirkonya altyapılar üzerine uygulanan seramiklerin fırınlanması sırasında görülen ısısal değişimlerin bağlantı dayanımlarına etkisinin incelenmesidir. Gereç ve Yöntem: Çalışmamızda single yttria stabilize zirkonya polikristali (Y-TZP) altyapı seramiği (Kavo Dental GmbH) ve 3 farklı üstyapı seramiği (IPS e.max Ceram, Vita VM9 ve GC Initial Zr-FS) kullanıldı. Zirkonya bloklardan CAD/CAM sistemi (Kavo Everest) kullanılarak yüz yirmi adet standart disk şeklinde örnekler hazırlandı. Üstyapı seramiklerine fırınlama sonrası dört farklı soğutma işlemi (maksimum, 25°C/dk, 50°C/dk ve 75°C/dk) uygulandı. Örneklere Makaslama Bağlantı Dayanım Testi (SBS) uygulandı. Seramiklerin yüzeyi Taramalı Elekron Mikroskobu (SEM) kullanılarak incelendi. Zirkonyadaki t-m faz dönüşümü, X-Işını Difraksiyon Analizi (XRD) ile değerlendirildi. Çalışma istatistiksel olarak, İki yönlü varyans analizi, Bonferroni düzeltmesi ve ikili karşılaştırmalar kullanılarak incelendi. Bulgular: Üstyapı seramiklerinin, makaslama bağlantı dayanımı üzerindeki ana etkilerinin anlamlı olduğu tespit edilmiştir (p=0.042). Ortalama makaslama bağlantı dayanımı değerleri soğutma işlemine göre anlamlı farklılık göstermiştir (p<0,001). Hızlı soğutma işlemi uygulanan gruplarda monoklinik faz oranında artış gözlemlenmiştir. Sonuç: Zirkonya altyapı üzerine uygulanan seramiklerin, fırınlama işlemleri sırasındaki ortaya çıkan ısısal değişiklikler, çift tabakalı seramik sistemin makaslama bağlantı dayanımını etkilemiştir. Yavaş soğutma işleminin, çift tabakalı seramik örnekler için daha yüksek bağlantı dayanımı sağladığı tespit edilmiştir. Anahtar Kelimeler: Zirkonya, Üstyapı seramiği, CAD/CAM, Bağlantı dayanımı, Soğutma işlemi*

Ethics Committee Approval: Not required.

Informed Consent: Not required.

Peer-review: Externally peer-reviewed.

Author contributions: YOU, BT participated in designing the study. YOU, BT participated in generating the data for the study. YOU participated in gathering the data for the study. YOU participated in the analysis of the data. YOU wrote the majority of the original draft of the paper. YOU participated in writing the paper. YOU, BT have had access to all of the raw data of the study. YOU, BT have reviewed the pertinent raw data on which the results and conclusions of this study are based. YOU, BT have approved the final version of this paper. YOU guarantees that all individuals who meet the Journal's authorship criteria are included as authors of this paper.

Conflict of Interest: The authors declared that they have no conflict of interest.

Financial Disclosure: This study has been supported by a research grant from Scientific Research Projects Coordination Unit of Istanbul University (Project number: 24120)

REFERENCES

1. Aboushelib MN, de Jager N, Kleverlaan CJ, Feilzer AJ. Microtensile bond strength of different components of core veneered all-ceramic restorations. Dent Mater 2005; 21: 984-91. [CrossRef]
2. Al-Dohan HM, Yaman P, Dennison JB, Razzoog ME, Lang BR. Shear strength of core-veneer interface in bi-layered ceramics. J Prosthet Dent 2004; 91: 349-355. [CrossRef]
3. Tinschert J, Zwez D, Marx R, Anusavice KJ. Structural reliability of alumina-, feldspar, leucite-, mica- and zirconia-based ceramics. J Dent 2000; 28: 529-35. [CrossRef]
4. Guazzato M, Albakry M, Ringer SP, Swain MV. Strength, fracture toughness and microstructure of a selection of all-ceramic materials. Part II. Zirconia-based dental ceramics. Dent Mater 2004; 20: 449-456. [CrossRef]
5. Kelly JR, Denry I. Stabilized zirconia as a structural ceramic: an overview. Dent Mater 2008; 24: 289-298 [CrossRef]
6. Larsson C, Wennerberg A. The clinical success ofzirconia-based crowns: a systematic review. Int JProsthodont 2014;27:33-43 [CrossRef]
7. Vult von Steyern P, Carlson P, Nilner K. All-ceramic fixed partial dentures designed according to the DC-Zirkon technique. A 2-year clinical study. J Oral Rehabil 2005; 32: 180-7. [CrossRef]
8. Swain MV. Unstable cracking (chipping) of veneering porcelain on all-ceramic dental crowns and fixed partial dentures. Acta Biomater 2009; 5: 1668-77. [CrossRef]
9. Kim J, Dhital S, Zhivago P, Kaizer MR, Zhang Y. Viscoelasticfinite element analysis of residual stresses inporcelain-veneered zirconia dental crowns. J Mech BehavBiomed Mater 2018;82:202-9. [CrossRef]
10. Taskonak B, Borges GA, Mecholsky Jr JJ, Anusavice KJ, Moore BK, Yan J. The effects of viscoelastic parameters on residual stress development in a zirconia/glass bilayer dental ceramic. Dent Mater 2008; 24: 1149-1155 [CrossRef]
11. Göstemeyer G, Jendras M, Dittmer MP, Bach F-W, Stiesch M, Kohorst P. Influence of cooling rate on zirconia/veneer interfacial adhesion. Acta Biomater 2010; 6: 4532-4538. [CrossRef]
12. Gonçalves FDCP, Bottino MA, de Melo RM, Souza RA. Effect of thickness, processing technique and cooling rate protocol on the μTBS of a bilayer ceramic system. J Adhes Dent 2015; 17: 307-312.
13. Garvie RC, Nicholson PS. Phase analysis in zirconia systems. J Am Ceram Soc 1972; 55: 303-5. [CrossRef]
14. Toraya H, Yoshimura M, Sōmiya S. Calibration curve for quantitative analysis of the monoclinictetragonal ZrO_2 system by X-ray diffraction. J Am Ceram Soc 1984; 67: 119-21. [CrossRef]
15. Guess PC, Kuliš A, Witkowski S, Wolkewitz M, Zhang Y, Strub JR. Shear bond strengths between different zirconia cores and

veneering ceramics and their susceptibility to thermocycling. Dent Mater 2008; 24: 1556-1567. [CrossRef]
16. Saito A, Komine F, Blatz MB, Matsumura H. A comparison of bond strength of layered veneering porcelains to zirconia and metal. J Prosthet Dent 2010; 104: 247-257 [CrossRef]
17. Mainjot AK, Schajer GS, Vanheusden AJ, Sadoun MJ. Influence of cooling rate on residual stress profile in veneering ceramic: Measurement by hole-drilling. Dent Mater 2011; 27: 906-914. [CrossRef]
18. Komine F, Saito A, Kobayashi K, Koizuka M, Koizumi H, Matsumura H. Effect of cooling rate on shear bond strength of veneering porcelain to a zirconia ceramic material. J Oral Sci 2010; 52: 647-652. [CrossRef]
19. Da Sılva Rodrıgues, Camila, et al. Do thermal treatments affect the mechanical behavior of porcelain-veneered zirconia? A systematic review and meta-analysis. Dent Mater 2019; 35.5; 807-817. [CrossRef]
20. Choi JE, Waddell JN, Swain MV. Pressed ceramics onto zirconia. Part 2: Indentation fracture and influence of cooling rate on residual stresses. Dent Mater 2011; 27: 1111-1118. [CrossRef]
21. Tan JP, Sederstrom D, Polansky JR, McLaren EA, White SN. The use of slow heating and slow cooling regimens to strengthen porcelain fused to zirconia. J Prosthet Dent 2012; 107: 163-169. [CrossRef]
22. Bertolotti R, Shelby J. Viscosity of dental porcelain as a function of temperature. J Dent Res 1979; 58: 2001-2004. [CrossRef]
23. Scherer GW. Relaxation in glass and composites. New York: Wiley; 1986
24. Swain, M. V., Mercurio, V., Tibballs, J. E., & Tholey, M. Thermal induced deflection of a porcelain–zirconia bilayer: Influence of cooling rate. Dent Mater 2019;35(4); 574-584. [CrossRef]
25. Zhang ZP, Zhou SW, Li Q, Li W, Swain M. Residual stresses in fabrication of core-veneered ceramic prostheses. Adv Mater Res 2010; 97: 2241-2244. [CrossRef]
26. Kingery WD, Bowen HK, Uhlmann DR. Introduction to ceramics. Vol. 17; John wiley & sons; 1976.
27. Garvie RC, Hannink RHJ, Pascoe RT. Ceramic steel? Nature 1975; 258: 703-704. [CrossRef]
28. De Jager N, de Kler M, van der Zel JM. The influence of different core material on the FEA-determined stress distribution in dental crowns. Dent Mater 2006; 22: 234-242. [CrossRef]
29. Øilo M, Gjerdet NR, Tvinnereim HM. The firing procedure influences properties of a zirconia core ceramic. Dent Mater 2008; 24: 471-475. [CrossRef]
30. Asaoka K, Kuwayama N, Tesk JA. Influence of tempering method on residual stress in dental porcelain. J Dent Res 1992; 71: 1623-1627. [CrossRef]
31. Vita VM9 working instructions. [web page on the Internet]. Erişim 04.06.2014, http://vident.com/wp-content/uploads/2011/09/VITA-VM9-Working-Instructions_1190EN_0911.pdf
32. DeHoff P, Anusavice K, Vontivillu S. Analysis of tempering stresses in metal-ceramic disks. J Dent Res 1996; 75: 743-751. [CrossRef]
33. Tholey MJ, Swain MV, Thiel N. Thermal gradients and residual stresses in veneered Y-TZP frameworks. Dent Mater 2011; 27: 1102-1110. [CrossRef]
34. Benetti P, Kelly JR, Della Bona A. Analysis of thermal distributions in veneered zirconia and metal restorations during firing. Dent Mater 2013; 29: 1166-1172. [CrossRef]
35. Piconi C, Maccauro G. Zirconia as a ceramic biomaterial. Biomaterials 1999; 20: 1-25. [CrossRef]
36. Chevalier J, Cales B, Drouin JM. Low-temperature aging of Y-TZP ceramics. J Am Ceram Soc 1999; 82: 2150-2154. [CrossRef]
37. Tholey MJ, Berthold C, Swain MV, Thiel N. Xrd2 micro-diffraction analysis of the interface between y-tzp and veneering porcelain: Role of application methods. Dent Mater 2010; 26: 545-52. [CrossRef]
38. Tholey MJ, Swain MV, Thiel N. Sem observations of porcelain Y-TZP interface. Dent Mater 2009; 25: 857-862. [CrossRef]
39. Catledge SA, Cook M, Vohra YK, Santos EM, McClenny MD, Moore KD. Surface crystalline phases and nanoindentation hardness of explanted zirconia femoral heads. J Mater Sci Mater Med 2003; 14: 863-867. [CrossRef]
40. Thompson G. Influence of relative layer height and testing method on the failure mode and origin in a bilayered dental ceramic composite. Dent Mater 2000; 16: 235-243. [CrossRef]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