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**Dear Colleagues,**

Dicle Dental Journal, formerly known as Dicle University Faculty of Dentistry Journal, which started its publication life in 1984, it is a scientific publication of Dicle University Faculty of Dentistry. It will continue its publication life electronically under the name Dicle Dental Journal.

It is with great pleasure that I welcome you to the first issue of the 2024. In this first issue, reviews of new developments in the field of dentistry and original researches are included. One of our most important goals is to mediate appropriately the sharing of knowledge and experience among dental professionals, researchers and academicians.

We are pleased to present you with five valuable articles on the 40th anniversary of our journal. The first article of our journal "Investigation of stress occurring on fixation systems and mandibular bone in fixation of mandible symphysis fractures with different plate systems" is an original article. The authors aimed to investigate the effectiveness of CFR-PEEK plates by comparing titanium plates with CFR-PEEK plates in the fixation of mandibular symphysis fractures. The second article "Investigation of the knowledge level of allied health professionals in a faculty of dentistry about dental avulsion" is an original article. The authors have evaluated the level of knowledge about dental avulsion among dental school allied health professionals who may encounter dental avulsion trauma in the first moment.

It is claimed in the literature that ellagic acid has effective anti-oxidant activity, radical scavenging capacity, chemopreventive and anti-apoptotic, anti-mutagenetic, anti-viral, anti-fibrosis and anti-inflammatory properties. The aim of the third article, "Experimental investigation of the effect of ellagic acid on bone healing", is to investigate the effect of ellagic acid on hard tissue healing after tooth extraction in rats. The fourth article "The use of gingival crevicular fluid as a potential biomarker for periodontal disease" is a review article. Finally, the fifth article "Immediate loading of All-on-4 concept : 12 months follow-up" is a case report. This study aims to show 12 months follow-up of the treatment of an edentulous patient with concerns about using removable dentures with immediately loaded fixed prosthesis with All on four treatment concept.

I would like to thank the authors, reviewers, editorial team and publisher for their hard work and dedication in bringing this issue to fruition. We look forward to providing you with the latest insights and developments in dentistry, and we welcome your feedback and suggestions.

**Sincerely**

**Assoc. Prof. Elif Pınar BAKIR, PhD**

**Editor-in-Chief**

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# Investigation of stress occurring on fixation systems and mandibular bone in fixation of mandible symphysis fractures with different plate systems

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## ABSTRACT

**Aims:** The aim of this study is to investigate the effectiveness of carbon fiber-reinforced polyetheretherketone (CFR-PEEK) plates by comparing titanium plates with CFR-PEEK plates in the fixation of mandibular symphysis fractures.

**Methods:** In the study, a model that imitates the mandible was obtained with the finite element analysis method. A fracture line was created in the symphysis on the model, and a double mini-plate was applied to this line with the Champy method. Comparisons were made by assigning CFR-PEEK and titanium material properties to the plates.

**Results:** Von Mises stresses on screws and plates were found to be lower than those on titanium plates when CFR-PEEK plates were applied. When the stresses in the screws were examined, it was observed that the highest stresses occurred in the screws adjacent to the fracture line.

**Conclusion:** CFR-PEEK materials provide a more stable fixation by reducing the stresses in the fixation systems and may be a good alternative to titanium materials with their advantageous properties.

**Keywords:** Mandible fractures, CFR-PEEK plate, titanium plate, finite element analysis

## INTRODUCTION

Mandibular fractures constitute 19-40% of fractures in the maxillofacial region.<sup>1</sup> The mandibular corpus, angle, and symphysis are the most frequently fractured parts of the mandible due to maxillofacial trauma, while the ramus and coronoid process are the least commonly affected regions.<sup>2</sup> Closed reduction and open reduction methods are employed in the treatment of mandibular fractures.<sup>3</sup> The use of plate and screw systems in open reduction with internal fixation has become a significant milestone in maxillofacial surgery, evolving into a routinely preferred and successful technique.<sup>4,5</sup> However, there is no consensus in the literature regarding the ideal treatment method for mandibular fractures. Fixation systems made from titanium alloys, due to their advantages such as high biocompatibility, ease of handling and use, and sufficient hardness and durability, are widely used in internal fixation.<sup>6,7</sup> But the hardness of titanium alloys is 6-7 times higher than that of the cortical bone.<sup>8</sup> Because it is so hard in relation to bone

tissue, the titanium plates absorb all incoming stresses and prevent the formation of physiological forces in the bone, causing resorption to the bone over time.<sup>8</sup> In recent years, researchers have focused on investigating polymer composites with an elasticity modulus much closer to bone, aiming to overcome the disadvantages of titanium systems. For this purpose, carbon fiber-reinforced polyetheretherketone (CFR-PEEK) materials, known as “isoelastic”, are becoming increasingly widely used in neurological and orthopedic surgeries, with a cortical-like elasticity module and high biocompatibility.<sup>9</sup> However, CFR-PEEK materials have not yet been routinely used in maxillofacial traumatology and oral surgery. Nevertheless, a review of recent literature reveals a limited number of studies evaluating the effectiveness of CFR-PEEK plates in the fixation of mandibular fractures. Therefore, the aim of this study is to investigate whether CFR-PEEK plates can serve as an alternative to titanium systems in symphysis fractures through finite element analysis.

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## METHODS

Since this is a three-dimensional analysis study carried out on digital models, there is no need to obtain ethics committee permission. All procedures were carried out in accordance with the ethical rules and the principles.

In this study, the tomography of a young, healthy adult was obtained to create a geometric model of the mandible. Cone-beam computed tomography (CBCT) was utilized for scanning the jawbone. The 3M Iluma CBCT device was employed for the tomography, using a 40-second exposure mode with 120 kVp and 3.8 mA values. The captured images were transferred to the 3D-Doctor software (Able Software Corp., MA, USA). Sections obtained from the reconstruction were transferred to the 3D-Doctor software in DICOM 3.0 format, where bone tissues were separated from the sections. The segmented sections were transformed into a 3D model. The resulting 3D model underwent further processing in the 3D-Doctor software, transforming it into a smooth surface composed of elements with proportional dimensions, completing the mandibular modeling process.

For the creation of the 3D solid model and finite element stress analysis, a computer equipped with an Intel Xeon® R CPU 3.30 GHz processor, 500GB hard disk, 14GB RAM, and Windows 7 Ultimate Version Service Pack 1 operating system was used. The 3D scanning was conducted using the Activity 880 optical scanner (smart optics Sensortechnik GmbH, Sinterstrasse 8, D-44795 Bochum, Germany). Software tools such as Rhinoceros 4.0 (3670 Woodland Park Ave N, Seattle, WA 98103 USA), VRMesh Studio (VirtualGrid Inc, Bellevue City, WA, USA), and Algor Fempro (ALGOR, Inc. 150 Beta Drive Pittsburgh, PA 15238-2932 USA) were utilized for 3D modeling and analysis.

All models were considered to have linear, homogeneous, and isotropic materials. Material values defining the physical properties of structures forming models, such as cortical bone<sup>10</sup>, cancellous bone<sup>11</sup>, callus<sup>12</sup>, and titanium<sup>12</sup> fixation systems, along with CFR-PEEK<sup>13</sup> plates, were applied as shown in Table 1.

**Table 1. Mechanical properties of simulated materials in models**

Material	Young's Modulus (MPa)	Poisson's Ratio
Cortical bone	14 000	0.30
Cancellous bone	1 500	0.30
Callus	3	0.40
Titanium (plate, screw)	110 000	0.35
CFR-PEEK (plate)	15 000	0.356

The model was fixed at the anterior, posterior, superior, and lateral sides of the temporomandibular joint with zero degrees of freedom (DOF). Muscle forces were applied according to the literature.<sup>9,11</sup> The right side was considered as the balancing side, and the left side was

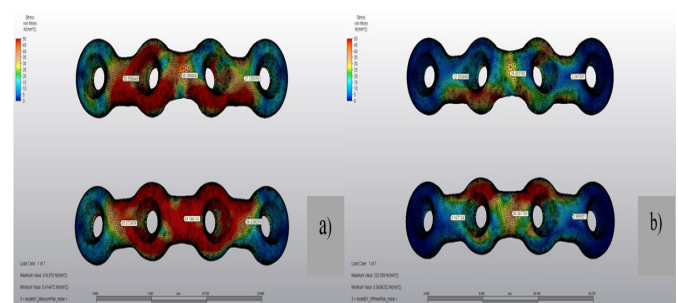
considered as the working side. Von Mises stresses in plates and screws, maximum and minimum principal stresses in cortical bone, were measured in megapascals (MPa) (N/mm<sup>2</sup>), and the displacement between fracture fragments was measured in millimeters (mm) as a result of the forces applied in the study.

After creating a realistic representation of the entire anatomy of the mandible and performing 3D solid modeling, a linear fracture line with no gap between fragments in the mandibular symphysis was generated. Following the Champy method for fixation, two four-hole miniplates were placed on the superior and inferior borders of the fracture line. Two different material properties (Titanium, CFR-PEEK) were assigned to the plate material, and two different loading conditions, simulating molar and incisal loads, were applied to ensure fixation.

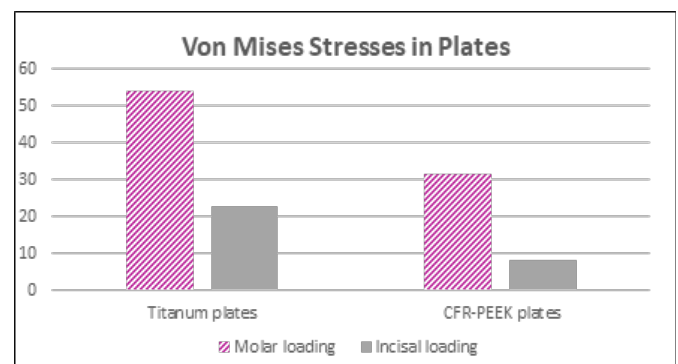
## RESULTS

### Von Mises Stresses in Plates

Under both molar and incisal loading conditions, stresses in both plate materials were concentrated at the midpoint of the plate, with stresses accumulating in the inferior plates observed to be higher than those in the superior plates (Figure 1). Von Mises stresses in the plates were found to be higher under molar loading conditions compared to stresses under incisal loading conditions. It is notable that stresses accumulated in CFR-PEEK plates under both loading conditions were significantly lower than those in titanium plates (Figure 2).



**Figure 1. Von Mises stresses in plates a) Titanium b) CFR-PEEK**



**Figure 2. Von Mises stresses in plates**

### Von Mises Stresses in Screws

Under both loading conditions and in both plate materials, the highest screw stresses were observed in screws close to the fracture line, with the amount of stress decreasing as the screws moved away from the fracture line. When CFR-PEEK plates were applied under both loading conditions, Von Mises stresses in the screws were found to be lower (Figure 3).

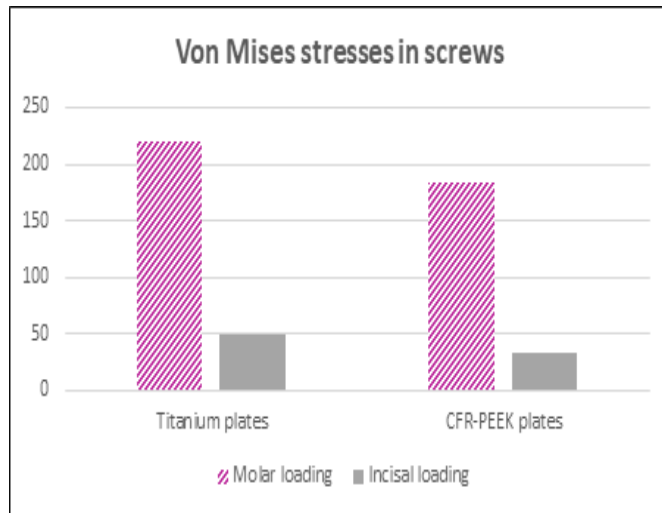


Figure 3. Von Mises stresses in screws

### Maximum and Minimum Principal Stresses in Bone

The highest maximum principal stresses (Pmax, strain) in cortical bone were observed in the superior band of the titanium plate under molar loading conditions (39.095 MPa) and in the inferior band of the titanium plate under incisal loading conditions (9.855 MPa) (Figure 4). The highest minimum principal stresses (Pmin, compression) under molar loading conditions were observed in the superior band of the CFR-PEEK plate (-32.887 MPa), and under incisal loading conditions, they were observed in the inferior band of the CFR-PEEK plate (-7.039 MPa) (Figure 5). According to the study results, compressive stresses in all groups under both loading conditions were found to be below the physiological limit of 11-56 MPa.

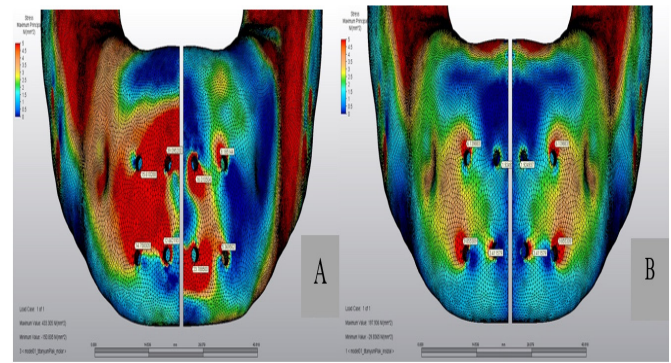


Figure 4. Highest maximum principal stresses in cortical bone  
A: Titanium plate under molar loading conditions  
B: Titanium plate under incisal loading conditions

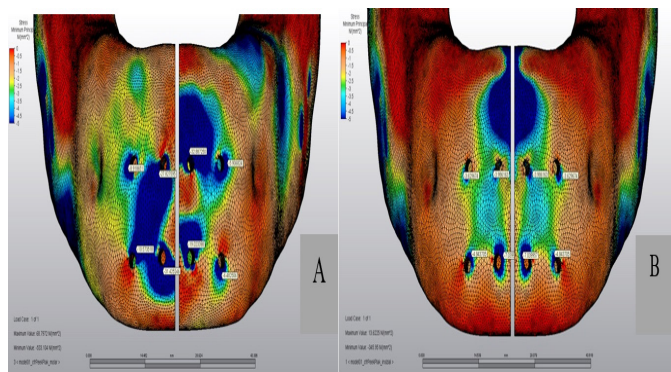


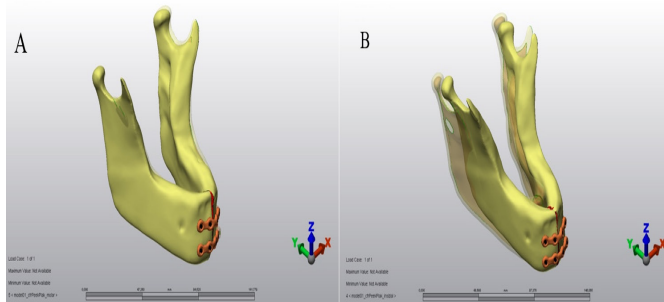
Figure 5. Highest minimum principal stresses in cortical bone  
A: CFR-PEEK plate under molar loading conditions  
B: Titanium plate under incisal loading conditions

### Displacement Amounts Between Fractured Segments

The three-dimensional displacement amounts between the fragments were determined for the fixed ends at the inferior and superior ends of the fracture line for all three axes (Table 2). Generally, when titanium plates were applied, less intersegmental mobility was observed compared to CFR-PEEK plates. The highest displacement amounts were observed in the inferior end of the symphysis fracture with CFR-PEEK plate application under molar chewing conditions, and in the superior end with CFR-PEEK plate application under incisal biting conditions (Figure 6).

Table 2. Displacement amounts between fractured segments

			X	Y	Z	Total
Molar chewing	Titanium plate	Superior end	0.1073	0.3710	0.2399	0.4547
		Inferior end	0.4955	0.4234	0.2878	0.7125
	CFR-PEEK plate	Superior end	0.1911	0.4215	0.2645	0.5331
		Inferior end	0.5331	0.5183	0.3639	0.8279
Incisal biting	Titanium plate	Superior end	0.0293	0.4881	0.0060	0.4890
		Inferior end	0.0063	0.4407	0.0126	0.4409
	CFR-PEEK plate	Superior end	0.0384	0.4902	0.0065	0.4917
		Inferior end	0.0148	0.4505	0.0130	0.4509



**Figure 6.** Displacement amounts between fractured segments with CFR-PEEK Plate application A: Molar loading B: Incisal loading

## DISCUSSION

The differences in bending properties between bone and plate-screw systems cause the majority of stresses, even when excellent bone healing is achieved through internal fixation, to be borne by the plates. According to Wolff's law, the density of bone is dependent on the amount of stress accumulated on the bone, and cortical bone under very rigid plates will adapt to these conditions, leading to a more porous structure compared to healthy bone, which may result in delayed or unsuccessful bone healing.<sup>14</sup> Therefore, bone fragments fixed with open reduction after trauma should be exposed to physiological forces after a healthy bone healing. This is why the idea of treating fractures with isoelastic fixation systems, which have biomechanical properties close to the mechanical properties of healthy bone, has gained prominence nowadays.<sup>9</sup>

CFR-PEEK is an isoelastic material that is resistant to sterilization conditions, has high biocompatibility, does not create artifacts in radiographs, and most importantly, has a Young's modulus very close to cortical bone.<sup>8</sup> Studies have shown that CFR-PEEK is an increasingly promising fixation material, especially in orthopedic and brain surgeries, particularly in spinal cage applications.<sup>15,16</sup> However, there are very few studies on the use of CFR-PEEK in plate-screw systems in maxillofacial surgery.

The load-bearing properties of plates in fixation systems should be at a level that balances mechanical stability allowing bone healing with the disadvantages of excessive rigid fixation. According to our study results, the stresses accumulated in titanium plates are higher than those in CFR-PEEK plates. Similarly, under the conditions where titanium plates are applied, the average stress in screws was found to be higher than in CFR-PEEK plates. These results parallel the studies of Diker and Bayram,<sup>9</sup> comparing CFR-PEEK and titanium in atrophic mandible fractures, and Avcı et al.'s<sup>17</sup> investigation of

the biomechanical stability of CFR-PEEK in angulus fractures.

One of the most important complications leading to failure in open reduction with internal fixation is screw loss. If compressive stresses in screw sockets in bone exceed physiological values, there is bone resorption and loosening of the screw. Schilemann et al.<sup>18</sup> reported that complications of screw loss could be reduced with the use of CFR-PEEK materials. When evaluating the possibility of bone resorption in finite element analysis, Minimum Principle stresses in cortical bone are examined. The accepted Minimum Principle threshold value for bone to survive without resorption in cortical bone is -56 MPa.<sup>11</sup> In our study, it was found that the highest stresses generally occurred around the screw sockets close to the fracture line, and compressive stresses in the bone in both plate materials were below the physiological threshold.

As the rigidity in fixation systems increases, the displacement amounts between fractured segments decrease.<sup>9</sup> In our study results, the displacement amount in CFR-PEEK plates was found to be higher than in titanium plates. However, the semi-rigid fixation feature promised by CFR-PEEK materials allows for more micro-movement between fragments, making this outcome expected.

## Limitations

Finally, regarding the limitations of the current study, although we attempted to create a mandible model very close to reality with finite element analysis, to achieve more realistic results about the long-term stability and success of plate systems, we believe that this study needs to be supported by in vivo studies.

## CONCLUSION

Within the limitations of the study, it has been determined that CFR-PEEK materials can provide appropriate bone healing at the fracture line and yield better results in symphysis fractures compared to titanium materials. CFR-PEEK materials are promising in the field of biomechanics and offer more advantages over traditional fixation materials due to their biomechanical properties. However, we believe that these advantageous results need further support in the field of maxillofacial surgery with more in vitro and in vivo studies.

## ETHICAL DECLARATIONS

### Ethics Committee Approval

The study does not require an ethics committee approval.

### Informed Consent

The study does not require an informed consent.

## Referee Evaluation Process

Externally peer-reviewed.

## Conflict of Interest Statement

The authors have no conflicts of interest to declare.

## Financial Disclosure

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

## Author Contributions

All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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# Investigation of the knowledge level of allied health professionals in a faculty of dentistry about dental avulsion\*

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## ABSTRACT

**Aims:** The aim of this study is to investigate the effectiveness of CFR-PEEK plates by comparing titanium plates with CFR-PEEK plates in the fixation of mandibular symphysis fractures.

**Methods:** In the study, a model that imitates the mandible was obtained with the finite element analysis method. A fracture line was created in the symphysis on the model, and a double mini-plate was applied to this line with the Champy method. Comparisons were made by assigning CFR-PEEK and titanium material properties to the plates.

**Results:** Von Mises stresses on screws and plates were found to be lower than those on titanium plates when CFR-PEEK plates were applied. When the stresses in the screws were examined, it was observed that the highest stresses occurred in the screws adjacent to the fracture line.

**Conclusion:** CFR-PEEK materials provide a more stable fixation by reducing the stresses in the fixation systems and may be a good alternative to titanium materials with their advantageous properties.

**Keywords:** Mandible fractures, CFR-PEEK plate, titanium plate, finite element analysis

\* This study was presented as an oral presentation at the "11th International Hippocrates Congress on Medical and Health Sciences".

## INTRODUCTION

Traumatic dental injury (TDI) have been reported to affect more than one billion people worldwide and are the fifth most common injury in the world.<sup>1</sup>

Avulsion of permanent teeth occurs in 0.5-16% of all dental injuries. This injury is one of the most serious dental injuries, and its prognosis largely depends on the interventions performed at the scene of the accident. Replantation of the tooth is the preferred treatment method in most cases, but it cannot always be performed immediately.<sup>2</sup>

Proper management, rapid repositioning and splinting, and prevention of infection during replantation and handling procedures for avulsed teeth are extremely important to improve the long-term prognosis and reduce future problems for all trauma victims.<sup>3</sup>

Patients with TDI are likely to present at healthcare facilities, including general dental practices, emergency medical services, and pharmacies. Consequently, every health professional should have the appropriate knowledge, skills, and training to manage TDIs, but

studies have reported a lack of knowledge among ordinary people and health professionals about managing TDIs.<sup>3,4</sup>

In this study, basic information about dental avulsion was investigated for all auxiliary healthcare personnel, especially those working at the faculty of dentistry and who first encountered the parents or were consulted by their relatives.

## METHODS

"Avulsion Questionnaire" was administered face-to-face individually to 54 allied health personnel working at Dicle University Faculty of Dentistry. The necessary ethics committee decision was obtained for the study (Date: 22.02.2023, Decision No: 2023-10). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki. In this study, Dicle University Faculty of Dentistry; oral and dental health personnels (OHP), medical secretaries, nurses, X-ray technicians, and emergency medical

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technicians were surveyed as allied health professionals. A questionnaire consisting of 14 questions about demographic information, personal experience in the treatment of dental injuries, ways of accessing theoretical knowledge about dental injuries, and the interventions to be performed in the first emergency intervention was applied to the volunteers after obtaining the necessary consent form. The necessary statistical analyses were performed for the answers obtained.

### Statistical Analysis

In this study, frequency (n) and percentage (%) values were given as descriptive statistics. Chi-square analysis was used to examine the relationships between groups of nominal variables. The confidence interval was set at  $p < 0.05$ . SPSS 20 (IBM Corp., Armonk, NY, USA) package program was used for statistical analyses.

## RESULTS

Of the participants, 48.1% were OHP, 22.2% were nurses, 11.1% were X-ray technicians, and 18.5% were medical secretaries. 51.85% of the participants were male, and 48.15% were female. Those who thought that oral, dental, and surrounding tissue injuries constituted an emergency were significantly higher in all occupational groups ( $p < 0.05$ ) (Table 1). Medical secretaries were less knowledgeable about oral, dental, and surrounding tissue injuries ( $p < 0.05$ ) (Table 2). Those who had information about injuries were 48% of the participants. And they mostly obtained this information from dentists (Table 3). The level of knowledge about immediate tooth replacement at the scene was significantly lower ( $p > 0.05$ ) (Table 4). The majority of the participants knew that a health institution should be consulted without waiting for the result of an avulsion of a permanent tooth (Table 5). The employees were aware of the need to apply to the OHP and the Faculty of Dentistry (Table 6). X-ray technicians and medical secretaries were more likely to be aware of transporting teeth in a dry environment and had a low level of awareness (Table 7). Nurses and OHP had a good awareness of transporting avulsed teeth in milk and saline (Table 8). OHP were more aware of not replacing avulsed deciduous teeth (Table 9).

**Table 1. Distribution of the answers to the question “Do you think injuries to the mouth, teeth, and surrounding tissues constitute an emergency?”**

Question 4	Yes	No
OHP	22 (40.8)	0 (0)
Nurse	10 (18.5)	1 (1.9)
X-ray technician	13 (24.1)	1 (1.9)
Medical secretary	5 (9.3)	2 (3.7)
OHP: Oral health personnels		

**Table 2. Distribution of the answers to the question “Have you ever been informed about injuries to the mouth, teeth, and surrounding tissues?”**

Question 5	Yes	No
OHP	14 (26)	12 (22.2)
Nurse	6 (11.1)	6 (11.1)
X-ray technician	3 (5.6)	3 (5.6)
Medical secretary	3 (5.6)	7 (13)
OHP: Oral health personnels		

**Table 3. Distribution of the answers to the question “If yes, where did you learn?”**

Question 6	Internet/ TV	Training program/ brochure	Dentist	First aid course	Previously encountered
OHP	2 (3.7)	2 (3.7)	5 (9.3)	2 (3.7)	1 (1.9)
Nurse	0 (0)	1 (1.9)	4 (7.4)	0 (0)	0 (0)
X-ray technician	2 (3.7)	0 (0)	1 (1.9)	1 (1.9)	0 (0)
Medical secretary	1 (1.9)	1 (1.9)	3 (5.6)	0 (0)	0 (0)
OHP: Oral health personnels					



**Table 4. Distribution of the answers to the question “Would you consider replacing the permanent tooth if it is completely dislocated as in the image above?”**

Question 10	Yes	No	No opinion
OHP	9 (16.7)	12 (22.2)	5 (9.3)
Nurse	7 (13)	4 (7.4)	1 (1.9)
X-ray technician	3 (5.6)	3 (5.6)	0 (0)
Medical secretary	3 (5.6)	4 (7.4)	3 (5.6)
OHP: Oral health personnels			

**Table 5. Distribution of the answers to the question “Which health institution do you first apply to when your tooth is completely dislodged as a result of trauma as in the image above?”**

Question 8	Emergency Service	ODHC	Family Physician	Faculty of Dentistry	Private Dental Office
OHP	3 (5.6)	14 (25.9)	0 (0)	7 (13)	2 (3.7)
Nurse	2 (3.7)	6 (11.1)	0 (0)	4 (7.4)	0 (0)
X-ray technician	1 (1.9)	2 (3.7)	0 (0)	1 (1.9)	2 (3.7)
Medical secretary	1 (1.9)	3 (5.6)	0 (0)	6 (11.1)	0 (0)
OHP: Oral health personnels					

**Table 6. Distribution of answers to the question “If a permanent tooth is dislodged (avulsed), when do you go to a dentist or health care provider for help?”**

Question 9	Right now	In a few hours	The next day	No opinion
OHP	23 (42.6)	1 (1.9)	0 (0)	2 (3.7)
Nurse	10 (18.5)	1 (1.9)	0 (0)	1 (1.9)
X-ray technician	6 (11.1)	0 (0)	0 (0)	0 (0)
Medical secretary	8 (14.8)	1 (1.9)	1 (1.9)	0 (0)
OHP: Oral health personnels				

**Table 7. Distribution of answers to the question “You could not place the dislodged tooth. How do you take it to the dentist?”**

Question 13	In any liquid	Wrapped in cotton or napkin	In the ice	In the mouth	In plastic container
OHP	15 (27.8)	3 (5.6)	1 (1.9)	3 (5.6)	4 (7.4)
Nurse	7 (13)	1 (1.9)	1 (1.9)	2 (3.7)	1 (1.9)
X-ray technician	1 (1.9)	2 (3.7)	0 (0)	2 (3.7)	1 (1.9)
Medical secretary	4 (7.4)	3 (5.6)	0 (0)	0 (0)	3 (5.6)
OHP: Oral health personnels					

**Table 8. Distribution of answers to the question “Which liquid do you prefer to wash or carry the tooth?”**

Question 14	Alcohol	Tap water	Physiological serum	Milk	Antiseptic solution
OHP	3 (5.6)	1 (1.9)	7 (13)	12 (22.2)	3 (5.6)
Nurse	0 (0)	2 (3.7)	4 (7.4)	6 (11.1)	0 (0)
X-ray technician	0 (0)	3 (5.6)	0 (0)	3 (5.6)	0 (0)
Medical secretary	0 (0)	1 (1.9)	4 (7.4)	4 (7.4)	1 (1.9)
OHP: Oral health personnels					

**Table 9. Distribution of answers to the question “Do you think deciduous teeth should be replaced if they are dislodged?”**

Question 11	Yes	No
OHP	3 (5.6)	23 (42.6)
Nurse	4 (7.4)	8 (14.8)
X-ray technician	0 (0)	6 (11.1)
Medical secretary	1 (1.9)	9 (16.7)
OHP: Oral health personnels		

## DISCUSSION

This cross-sectional study was conducted to evaluate the level of knowledge of all ancillary personnel (OHP, X-ray technician, nurse, medical secretary) working in a dental school about dental avulsion, its management, and its relationship with their educational background. An avulsion of permanent teeth is considered the most serious dental injury. The success of treatment depends on the correct intervention after trauma and emergency management and treatment plans have been established with international guidelines.<sup>2</sup>

In the literature, attempts have been made to evaluate the knowledge of various populations (including dentists, physicians, school teachers, parents, students, dental students and athletic trainers) regarding emergency management of avulsed teeth. Most of these studies have emphasized the need for better communication between dental professionals and the community and the effective implementation of educational campaigns.<sup>5-10</sup> Dental assistants, who are an integral part of the auxiliary dental team, can be consulted directly or by telephone during the first emergency intervention after trauma. Studies evaluating dental assistants' knowledge of dental avulsion and its treatment have been found to be rare.<sup>11</sup> In this study, all dental assistants who have not been consulted much before but who can be consulted directly or by telephone during the treatment of avulsed teeth because they work in the faculty of dentistry were selected as the target population.

In a study conducted in Chile among healthcare personnel, including doctors, nurses, and other support ambulance staff, 90.2% of participants had no formal training on how to act in the event of dental injuries.<sup>12</sup> Halawany et al.<sup>11</sup> reported that in their study of dental assistants, the likelihood of answering most knowledge-based questions correctly was significantly higher among respondents who had received advice or training on tooth avulsion. In our survey, 26% of OHP with 2 years of dental education reported having received information about injuries to the mouth, teeth, and surrounding tissues. This rate was even lower among other allied health professionals. Most of the allied health personnel stated that they obtained information from dentists, not from official sources.

Loh et al.<sup>13</sup> reported that all dental therapists (school dental nurses) agreed that avulsion requires 100% immediate action, and 94.6% agreed that intervention should be done within 30 minutes. In Halawany's<sup>11</sup> survey, dental assistants reported that 65.3% of avulsed teeth should be implanted immediately, and 81.3% reported that a professional referral should be made as soon as an avulsion occurs (within 30 minutes). In our study, 92.7% of the participants agreed that oral and dental injuries constitute an emergency. 40% of the participants emphasized that they could immediately reimplant the avulsed tooth. 87% of the participants agreed that they should immediately contact an aid organization, especially oral and dental health care providers.

Current IADT guidelines mention HBSS, milk, saline, and saliva as examples of osmolality-balanced media suitable for storage of avulsed teeth.<sup>2</sup> In a study by Halawany et al.<sup>11</sup>, 70.9% of the participants recommended storage of avulsed teeth in fresh milk, 58% in saline, and

43.8% in saliva. In the survey by Loh et al.<sup>13</sup>, 54.8% of the participants were not sure about the most appropriate storage medium for avulsed teeth. In our study, 46.3% of the participants recommended milk, 27.8% serum, and 13.1% tap water as the transport solution for avulsed teeth.

Regarding avulsed deciduous tooth replantation in accordance with the current IADT guidelines,<sup>14</sup> the majority (85.2%) of the participants in this study reported that they would not perform avulsed deciduous tooth replantation. In the Halawany et al.<sup>11</sup> survey, the majority of respondents (72.5%) were of the opinion that they would not perform deciduous tooth reimplantation.

### Limitations

As the study was cross-sectional, participation depended on the availability and willingness of volunteers. The fact that there was only one dental school in the city prevented us from reaching more employees. We consider these limitations of our study.

### CONCLUSION

The educational qualifications of allied health workers were associated with their level of knowledge about dental avulsions and their management. It was observed that the basic knowledge of dental allied health professionals about dental avulsion was not sufficient. It was thought that regular in-service training for dental assistants may be useful for the first emergency intervention approach to dental trauma.

### ETHICAL DECLARATIONS

#### Ethics Committee Approval

The study was carried out with the permission of Dicle University Faculty of Dentistry Local Ethics Committee (Date: 22.02.2023, Decision No: 2023-10)

#### Informed Consent

All patients signed and free and informed consent form.

#### Referee Evaluation Process

Externally peer-reviewed.

#### Conflict of Interest Statement

The authors have no conflicts of interest to declare.

#### Financial Disclosure

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

#### Author Contributions

All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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# Experimental investigation of the effect of elagic acid on bone healing

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## ABSTRACT

**Aims:** Some of the most effective medicines discovered to date have been derived from natural products derived from microorganisms, plants and animals. Phenolic compounds have been recognized as the most important bioactive compounds responsible for bone health effects. Ellagic acid (EA), a member of the flavonoids, is a natural phenol antioxidant found in many fruits and vegetables. The anti-proliferative and antioxidant properties of ellagic acid have prompted research into its potential health benefits.<sup>1</sup> The aim of our study is to investigate the effect of EA on hard tissue healing after tooth extraction in rats.

**Methods:** For this purpose, the lower left mandibular teeth of 16 Wistar Albino male rats were extracted under general anesthesia. Among the rats randomly divided into four groups, the 1st study group was given EA by gavage once a day for 14 days (14EA), and the 2nd study group was given EA by gavage once a day for 21 days (21EA). The 1st control group was given 0.9% Isotonic NaCl by gavage once a day for 14 days (14K), the 2nd control group was given 0.9% Isotonic NaCl by gavage once a day for 21 days (21K). Afterwards, the rats were sacrificed and prepared for histopathological examination.

**Results:** As a result of histopathological examination, the inflammation level of the 21EA group was found to be significantly lower than the 14K (p:0.042) and 21K (p:0.004) groups (p<0.05). The epithelial proliferation level of the 21EA group was found to be significantly higher than the 21K, 14K (p:0.001) and 14EA (p:0.009) groups (p<0.05). The new bone formation level of the 21EA group was higher than the 14K (p:0.001), 21K (p:0.008) and 14EA (p:0.018) groups; The new bone formation level of the 14EA group was statistically significantly higher than the 14K group (p:0.014; p<0.05). The healing score of the 21EA group was found to be significantly higher than the 14EA (p:0.013) (14K (p:0.017) and 21K (p:0.009) groups (p<0.05).

**Conclusion:** As a result of the study, it was found that EA had a statistically significant positive effect on bone healing, especially on the 21st day.

**Keywords:** Mandible fractures, CFR-PEEK plate, titanium plate, finite element analysis

## INTRODUCTION

In dentistry, it is important to preserve sufficient alveolar bone volume in order to make a prosthesis that can provide the increasing demand for aesthetics and function. For this reason, research to accelerate the healing process of the alveolar bone has become an important topic of discussion. Therefore, it is increasingly important that the healing process promotes the formation of alveolar bone with sufficient amounts of hard and soft tissue to allow for an ideal implant-supported restoration.

Tooth extraction is a surgical procedure that is frequently performed in clinics and involves hard tissue and soft tissue. However, tooth extraction can cause

complications such as bleeding, infection, fracture and alveolitis. The healing process of the alveolar bone is affected by osteoblasts, osteoclasts and osteocytes, as well as hormones, nutrients, growth factors and inflammatory cytokines.<sup>2</sup> Impaired wound healing after tooth extraction may hinder subsequent treatments and the patient's ability to function. For this reason, it is important that the wound healing process after tooth extraction occurs smoothly.

Some of the most effective drugs discovered to date have been obtained from microorganisms, plants and animals.<sup>3</sup> Polyphenols constitute one of the most common and widely distributed groups of substances

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found in plants and are considered the most important bioactive compounds responsible for bone health effects.<sup>4</sup> They are secondary metabolites involved in the chemical defense of plants against predators and in plant-plant interventions. Several thousand plant-derived polyphenols are known, covering a wide variety of molecules containing at least one aromatic ring with one or more hydroxyl groups. The biological properties of polyphenols include anti-oxidant, anti-cancer and anti-inflammatory effects.<sup>5,6</sup>

EA was first discovered by French chemist and pharmacist Henri Braconnot in 1831.<sup>7</sup> EA is a dimeric derivative of gallic acid and is rarely found free in dietary products and usually forms part of ellagitannins.<sup>8,9</sup> Considering the level of conversion of ellagitannins to EA, the highest concentrations of EA are found in berries, pomegranates, muscadines, and tropical fruits in plants of the raspberry rubus genus.<sup>7</sup> In addition, dates, goji berries and green tea also contain high amounts of EA.<sup>10,11</sup>

EA is a highly thermostable molecule with a melting point of 350°C, a molecular weight of 302.197 g/mol, slightly soluble in water, alcohol and ether, and soluble in potassium hydroxide. EA is a weak acid that ionizes at physiological pH. Structurally, it has four rings representing the lipophilic domain, four phenolic groups, and two lactones representing the hydrophilic domain, which function as electron acceptors, forming the hydrogen bond sides, respectively.<sup>12</sup> The hydrophilic part of the EA molecule plays an important role in biological activity due to the presence of both hydrogen-bonding acceptor (lactone) and donor (–OH) sites (phenolic hydroxyl groups that can dissociate into negatively charged phenolate ions under physiological conditions).<sup>7</sup> EA has effective anti-oxidant activity, radical scavenging capacity, chemopreventive and anti-apoptotic, anti-mutagenetic, anti-viral, anti-fibrosis and anti-inflammatory properties.<sup>4</sup>

EA scavenges free electrons present in the last orbital shell and therefore acts as a potent antioxidant and therefore reduces oxidative stress of the cellular component.<sup>13</sup>

Ionic metals such as copper, iron, nickel and cadmium are a potential source of oxidative stress, and EA provides an additional protection mechanism against this situation by chelating these metals.<sup>14</sup>

EA can not only clear pro-oxidant agents, but also increases the expression/activity of enzymes such as antioxidant superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase.<sup>15</sup>

Bone damage triggers the bone healing process, which begins with inflammation. Inflammation is a normal

part of the bone healing process. Long-term inflammation causes bone resorption due to osteoclast activity modulated by pro-inflammatory cytokines such as TNF- $\alpha$ .<sup>15</sup> This inflammation increases the activity of osteoclasts, which play a role in bone resorption. Suppression of inflammation is expected to reduce osteoclast activity and therefore increase the rate of new bone formation.<sup>16</sup>

## METHODS

The study was carried out with the permission of İstanbul University Animal Experiments Local Ethics Committee (Date: 29.01.2021, Decision No: 2021/01). All procedures followed are based on animal rights (Guide for the Care and Use of Laboratory Animals).

Our study was carried out in the Laboratory of Experimental Medicine Research Institute and the experimental animals were housed in the Experimental Animal Care and Housing Unit of Experimental Medicine Research Institute, Department of Laboratory Animal Science. In our study, Ketanest vial (Ketamine hydrochloride, Parke Davis, Berlin, Germany) (50 mg/kg) and Rhompun vial (Xylazine hydrochloride, Bayer, İstanbul, Turkey) (5 mg/kg) were administered intramuscularly (i.m.) to rats under the supervision of a veterinarian for general anesthesia. 2% lidocaine (Lidocaine 2% E80, Vem, Ankara, Turkey) containing 1:100,000 epinephrine was applied to the lingual fold to create local anesthesia and hemostasis. After the left mandibular first molar tooth of each rat was extracted with the help of a hemostat, a defect was created in the extraction socket with a 2 mm steel round bur under cooling. The extraction wound was sutured with 4.0 silk suture (Doğsan, İstanbul, Türkiye). Compression was applied with a sterile tampon for 2 minutes. Xylazine HCl at a dosage of 5 mg/kg was administered intramuscularly for pain control in the pre-operative period. In the post-operative period, Cefazolin at a dosage of 50 mg/kg and Tramadol HCl at a dosage of 5 mg/kg were administered intraperitoneally twice a day for pain and infection control for 2 days.

The ellagic acid (EA) solution was prepared by dissolving 12.5 mg of EA powder (E2250, Sigma Aldrich, USA) in 1 ml of distilled water, following the methodology described in the studies of Al-Obaidi et al.<sup>4</sup> The solution was shaken before each application to ensure homogeneous distribution and was administered to rats at a dosage of 10 ml/kg.

1. The control group received 0.9% Isotonic NaCl by oral gavage once a day for 14 days.
2. The study group was administered ellagic acid powder (E2250, Sigma Aldrich, USA) dissolved in distilled water (10 ml/kg) via oral gavage once a day for 14 days.

3. The control group was given 0.9% Isotonic NaCl by oral gavage once a day for 21 days.
4. Ellagic acid was administered to the study group via oral gavage once a day for 21 days.

Sacrifice was performed by administering 150 mg/kg of sodium pentothal (Pental Sodium, Ulagay, Istanbul, Turkey) to all groups on the 14th or 21st day. The alveolar bone in the extraction area was then removed and sent to the I.U. Oncology Institute Pathology Laboratory in formaldehyde solution for histopathological examination.

### Histopathological Evaluation

The mandibles of the animals were extracted, and the soft tissues and skin were meticulously cleaned before being transported to the pathology laboratory in 10% formaldehyde solution. The bone tissue surrounding the extraction sites of the mandibular left first molar teeth was fixed in formalin and subsequently immersed in solutions containing 32 sodium citrate (20%) and formic acid (50%) for the decalcification process. Following decalcification, the rat mandibles underwent standard tissue processing procedures and were then embedded in paraffin blocks. Sections measuring 5 µm in thickness were obtained from the paraffin blocks, followed by deparaffinization and transparency procedures prior to mounting on slides. These sections were then stained with hematoxylin eosin dye and examined under a light microscope. Digital photographs were taken and analyzed using the Olympus ANALYSIS 5 (Tokyo, Japan) program. Inflammation, new bone formation, fibrosis, epithelial proliferation, necrosis, and healing scores were assessed by a single researcher across predetermined areas within the sections using the software.

**Inflammation:** Scored from 0 to 4, with increasing scores indicating higher levels of inflammation.

**Epithelial proliferation:** Scored from 0 to 3, with increasing scores indicating greater epithelial proliferation.

**Necrosis:** Scored as 0 for absent and 1 for present.

**Fibrosis:** Scored from 0 to 3, with increasing scores reflecting greater amounts of fibrosis.

**Bone healing score:** Graded from 0 to 6, with the following interpretations:

- 0: No defect healing
- 1: Incomplete cartilage healing
- 2: Complete cartilage healing
- 3: Incomplete bone healing in its initial stage
- 4: Moderate incomplete bone healing
- 5: Late incomplete bone healing
- 6: Complete bone healing

### Statistical Analysis

The findings obtained in this study were evaluated using IBM SPSS Statistics 22 (IBM SPSS, Tukey) program for statistical analysis. The suitability of the data parameters in the study to normal distribution was evaluated with the Shapiro Wilk test. While evaluating the study data, descriptive statistical methods (Mean, Standard deviation, frequency) were used, as well as when comparing quantitative data, one-way ANOVA test was used to compare normally distributed parameters between groups, and TukeyHSD test was used to determine the group causing the difference. Kruskal Wallis test was used for intergroup comparisons of parameters that did not show normal distribution, and posthoc Dunn's test was used to determine the group causing the difference. While evaluating the study data, Mann Whitney U test was used to compare the parameters between two groups. Kruskal Wallis test (post hoc Dunn's test) was used to compare the parameters between the four groups. Fisher's exact test, chi-square test and Fisher Freeman Halton exact test and chi-square test were used to compare qualitative data. Significance was evaluated at  $p < 0.05$  level.

### RESULTS

This study was conducted on a total of 16 male Wistar Albino rats weighing between 250-300 g. The control group, named "14K," received 0.9% isotonic NaCl for 14 days after tooth extraction and was sacrificed on the 14th day post-extraction. The study group, named "14EA," received EA solution for 14 days and was also sacrificed on the 14th day. Similarly, the control group named "21K" received 0.9% Isotonic NaCl for 21 days and was sacrificed on the 21st day, while the study group named "21EA" received EA solution for 21 days and was also sacrificed on the 21st day.

#### Inflammation

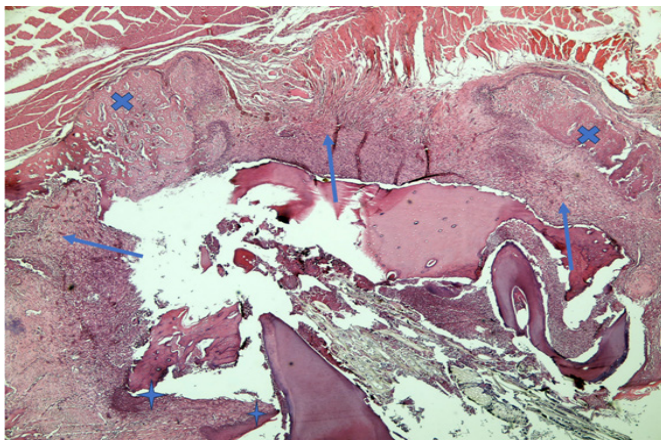
Inflammation plays a crucial role in biological processes aimed at eliminating pathogens and maintaining tissue homeostasis. In the context of bone defect regeneration through osteogenesis, the process begins with inflammation. During the inflammatory phase, phagocytic cells such as monocytes, macrophages, and neutrophils situated on the bone surface generate oxygen-based free radicals. These free radicals contribute to the formation of osteoclasts and subsequent bone resorption. This process hinders the remineralization of the bone defect or tooth extraction socket.

In our study, significant differences in inflammation levels between groups were observed ( $p:0.026$ ;  $p<0.05$ ) (Table). Post hoc analyses conducted to determine significance revealed that the inflammation level of the 21EA group was notably lower compared to both the 14K ( $p:0.042$ ) and 21K ( $p:0.004$ ) groups ( $p<0.05$ ) (Figure 1, Figure 2).

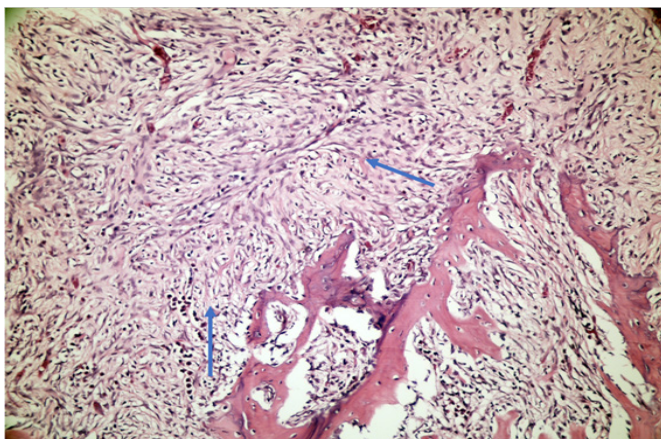
Table. Comparison of four groups in terms of study parameters

	Study 14 (n=4)		Control 14 (n=4)		Study 21 (n=4)		Control 21 (n=4)		
	Min-Max	Mean±SD (median)	Min-Max	Mean±SD (median)	Min-Max	Mean±SD (median)	Min-Max	Mean±SD (median)	p
Inflammation	2-4	2.8±0.84 (3)	3-4	3.25±0.5 (3)	1-3	2±0.71 (2)	3-4	3.75±0.5 (4)	0.026*
Fibrosis	2-3	2.2±0.45 (2)	1-2	1.75±0.5 (2)	2-2	2±0 (2)	2-3	2.5±0.58 (2.5)	0.138
Epitel proliferation	0-2	1.4±0.89 (2)	1-1	1±0 (1)	3-3	3±0 (3)	1-3	2±0.82 (2)	0.007*
New bone formation (%)	0.5-0.75	0.66±0.09 (0.68)	0.02-0.08	0.05±0.03 (0.05)	0.8-1.7	1.14±0.35 (1.1)	0.11-0.3	0.21±0.08 (0.21)	0.001*
Healing score	3-4	3.4±0.55 (3)	1-3	2.25±0.96 (2.5)	4-6	5±0.71 (5)	2-3	2.25±0.5 (2)	0.004*
Necrosis n (%)	4	%80	4	%100	2	%40	4	%100	+0.137

Kruskal Wallis Test, +Fisher Freeman Halton Exact Ki-kare test, \*p&lt;0.05



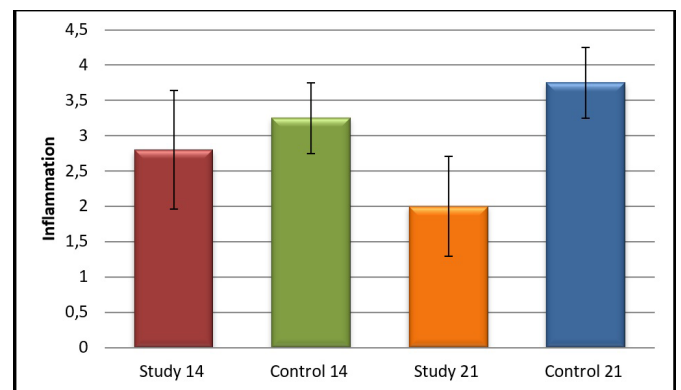
**Figure 1.** Study group sacrificed on the 14th day (14EA): food residues in the cavity, new bone formation (x's) in the fibrous connective tissue (arrows) at the cavity floor and in the surrounding area. Surface epithelium (stars) are observed at the edges of the cavity surface (H&E X40).



**Figure 2.** Control group sacrificed on the 21st day (21K): New bone formation within the active fibrous tissue (arrows) within the extraction socket (H&EX200).

Additionally, the inflammation level of the 21EA group was statistically significantly lower than that of the 21K group ( $p:0.016$ ;  $p<0.05$ ). However, there were no statistically significant differences in inflammation levels among the other groups ( $p>0.05$ ) (Figure 3).

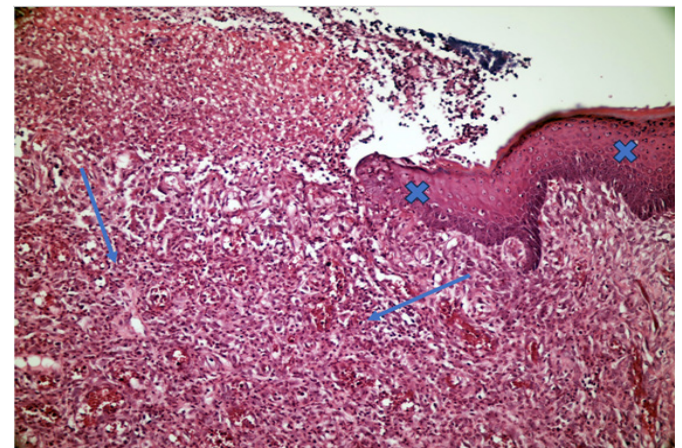
21EA group was statistically significantly lower than that of the 21K group ( $p:0.016$ ;  $p<0.05$ ). However, there were no statistically significant differences in inflammation levels among the other groups ( $p>0.05$ ) (Figure 3).



**Figure 3.** Graph showing inflammation levels across all groups

### Epitel Proliferation

A statistically significant difference was observed in epithelial proliferation levels between groups ( $p:0.007$ ;  $p<0.05$ ). Post hoc analyses were conducted to determine significance, revealing that the epithelial proliferation level of the 21EA group was significantly higher than that of the 21K group ( $p:0.007$ ) (Figure 4), as well as the 14K group ( $p: 0.001$ ) and the 14EA group ( $p:0.009$ ) ( $p<0.05$ ). However, there were no statistically significant differences observed between the other groups in terms of epithelial proliferation levels ( $p>0.05$ ) (Figure 5).



**Figure 4.** Control group sacrificed on the 21st day (21K): Connective tissue (arrows) containing dense lymphocyte, plasma cell, neutrophil polymorphous infiltration in the cavity and stratified squamous epithelium (x's) proliferating in the direction of closing the extraction socket on the surface (H&EX200)

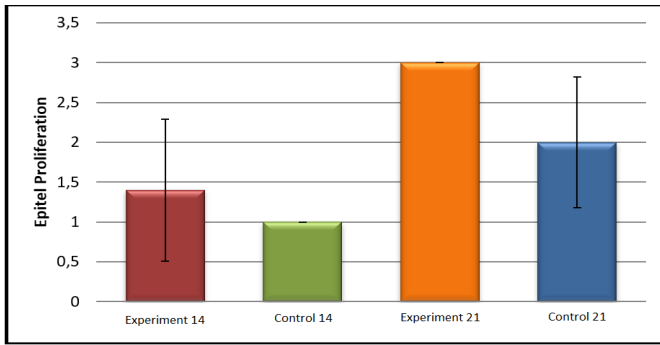


Figure 5. Graph showing epithelial proliferation levels between all groups

There is no statistically significant difference between the 14EA and 14K groups in terms of epithelial proliferation levels ( $p>0.05$ ). The epithelial proliferation level of the 21EA group is statistically significantly higher than the 21K group ( $p:0.028$ ;  $p<0.05$ ).

### New Bone Formation

A statistically significant difference was observed between the groups in terms of new bone formation levels ( $p:0.001$ ;  $p<0.05$ ). Post hoc analyses were conducted to determine significance, revealing that the new bone formation level of the 21EA group (Figure 6, Figure 7, Figure 8) was significantly higher ( $p<0.05$ ) than that of the 14K group ( $p:0.001$ ), the 21K group ( $p:0.008$ ), and the 14EA group ( $p:0.018$ ) (Figure 1, Figure 9, Figure 10). However, there was no statistically significant difference in new bone formation levels between the other groups ( $p>0.05$ ).

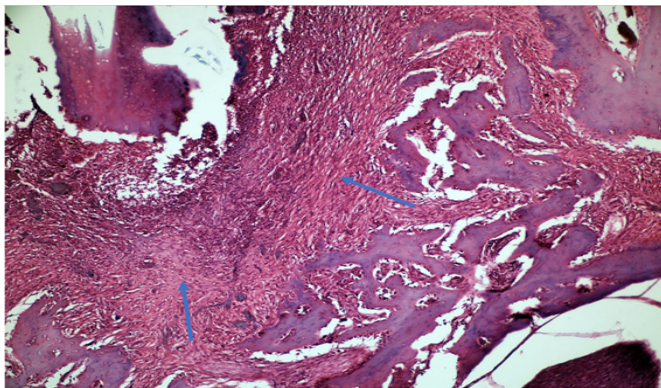


Figure 6. 21. Control group sacrificed on the first day (21EA): Fibrous connective tissue (arrows) and new bone trabeculae (H&EX100) in the extraction cavity

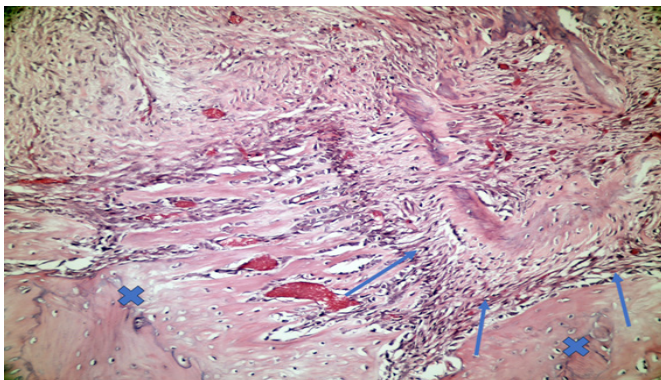


Figure 7. EA group sacrificed on the 21st day (21EA): Fibrous connective tissue (arrows) within the extraction cavity and new bone trabeculae (x's) filling most of the cavity (H&EX200)

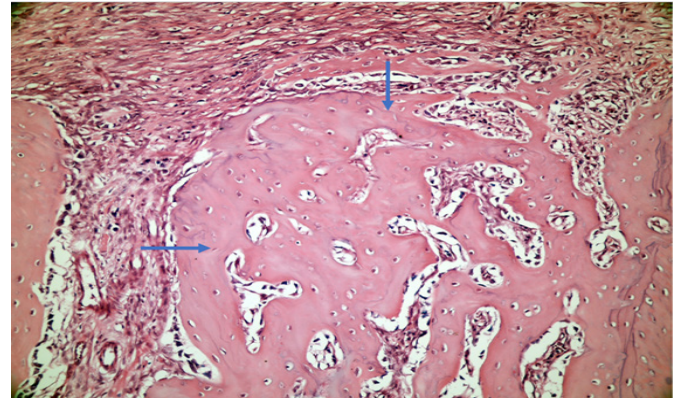


Figure 8. Study group sacrificed on the 21st day (21EA): New bone formation filling the cavity in the extraction socket (arrow marks) (H&EX200)

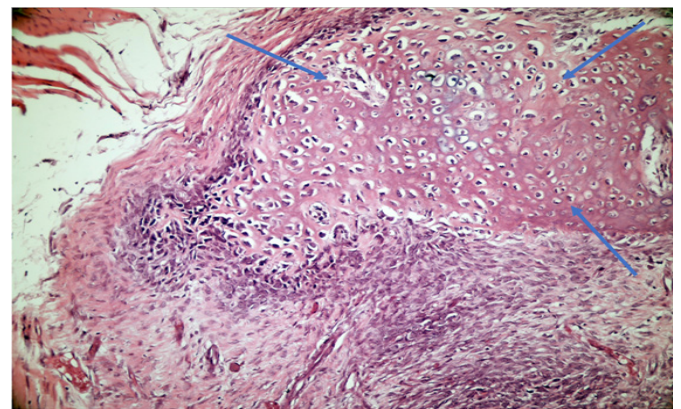


Figure 9. Study group sacrificed on day 14 (14EA), new bone formation (arrow marks) (H&EX200)

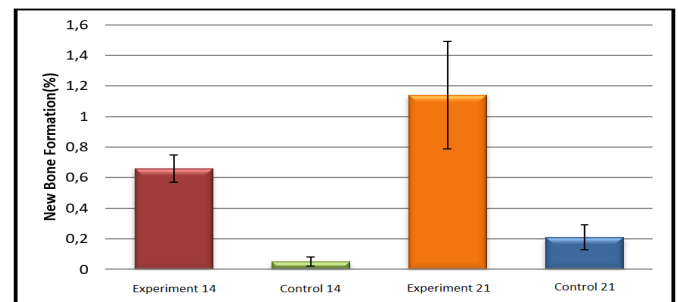


Figure 10. Graph showing new bone formation levels among all groups

The new bone formation level of the 14EA group was statistically significantly higher than the 14K group ( $p:0.014$ ;  $p<0.05$ ). The new bone formation level of the 21EA group is statistically significantly higher than the 21K group ( $p:0.014$ ;  $p<0.05$ ) (Figure 11).

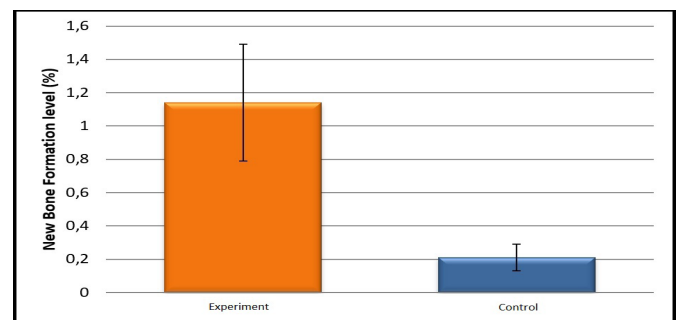


Figure 11. Graph showing new bone formation levels between 21EA and 21K groups

## Bone Healing Score

In this study, a statistically significant difference was observed between the groups in terms of healing ( $p:0.004$ ;  $p<0.05$ ). Post hoc analyses were conducted to determine significance, revealing that the healing score of the 21EA group was significantly higher than that of the 14K group ( $p:0.017$ ) and the 21K group ( $p:0.009$ ) ( $p<0.05$ ) (Figure 12).

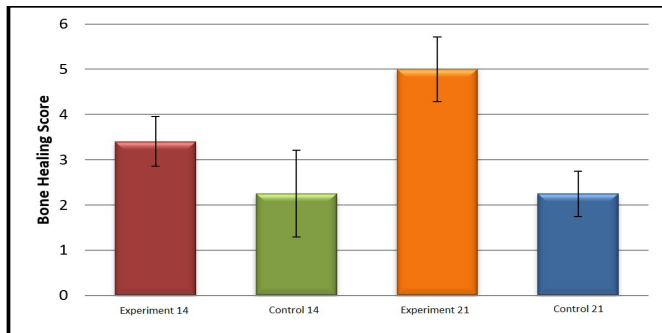


Figure 12. Graph showing healing score levels among all groups

Although the healing score of the 14EA group was higher than that of the 14K group, this difference was not statistically significant, although it was close to significance ( $p>0.05$ ).

The healing score of the 21EA group is statistically significantly higher than the 21K group ( $p:0.011$ ;  $p<0.05$ ).

## Fibrosis

There are studies showing that EA has a stimulating effect on the proliferation of osteoblasts and fibroblasts.<sup>4,10</sup> However, in this study, no statistically significant difference was seen between the groups in terms of fibrosis level. ( $p>0.05$ ) (Table).

Between 14EA and 14K groups; Between 21EA and 21K groups; Between 14EA and 21EA groups; There is no statistically significant difference in fibrosis levels between the 14K and 21K groups ( $p>0.05$ ).

## Necrosis

Although the rate of necrosis did not show a statistically significant difference between the groups in this study ( $p>0.05$ ), the rate of necrosis was observed as 80% in the 14EA group, 100% in the 14K group, 40% in the 21EA group, and 100% in the 21K group.

## DISCUSSION

Since bone healing can be influenced by sex hormones, our study experiments were conducted on male rats. This choice was made because sex hormones are generally less impactful in males compared to females, and male rats do not experience monthly periods.<sup>17</sup> However, it's important to note that bone healing was evaluated in adult, mature rats.

In their study involving mice, Ito et al.<sup>8</sup> observed significantly different pathological features during the healing processes of semi-stabilized femur fractures, attributed to the absence

of cartilage appearance in tooth extraction socket healing. They highlighted the distinct cartilage plasticity between periodontium and periosteum, noting the absence of cartilage formation in socket healing post tooth extraction.<sup>18</sup> This absence of cartilage appearance and the evident pathological features observed during extraction socket healing served as the rationale for utilizing a tooth extraction model in our study.

In their study involving 24 male rats, Al-Obaidi et al.<sup>4</sup> divided the rats that underwent left upper incisor tooth extraction into two groups, each consisting of twelve rats. The first group served as the control group and received only normal saline, while the second group received ellagic acid (EA) intragastrically once a day for 28 days. Their histopathological examination concluded that EA administration on days 14, 21, and 28 accelerated the healing process of tooth extraction sockets in rats. Similarly, in our study, the healing score of the 21EA group was significantly higher compared to the 14K group ( $p:0.017$ ) and the 21K group ( $p:0.009$ ) ( $p<0.05$ ). Although the healing score of the 14EA group was higher than that of the 14K group, this difference was close to significance but not statistically significant. In our study, molar tooth extraction was performed in rats due to the difficulty in extracting anterior teeth caused by their root length, which significantly affected post-operative nutrition. However, as the molar teeth in rats have a root form that prevents complete removal, the extraction socket was milled after extraction. To eliminate the possibility of residual roots affecting the study results, the socket was meticulously cleaned from any remaining roots using a round bur. Plant-derived drugs are seen as a promising source with fewer side effects and their compounds are non-toxic.<sup>2</sup> In our study, no rats were lost during the experimental procedures, and it was observed that EA did not cause any side effects, toxicity, or allergic reactions on rats.

In their study, Dede et al.<sup>18</sup> aimed to evaluate the effect of ellagic acid (EA) on the periodontal repair process associated with experimental periodontitis in rats. They measured the levels of alveolar bone resorption, inflammatory markers, and oxidative stress markers in periodontal tissue and serum. The results of their study revealed that EA led to significant positive improvements in gingival oxidative stress, inflammatory markers, and alveolar bone resorption during the repair process associated with experimental periodontitis. Therefore, they concluded that EA may hold therapeutic potential for periodontitis. In our study, consistent with the findings of Dede et al.<sup>18</sup>, the inflammation level of the 21EA group was significantly lower compared to the 14K group ( $p:0.042$ ) and the 21K group ( $p:0.004$ ). Additionally, the epithelial proliferation level of the 21EA group was significantly higher than that of the 21K group, the 14K group ( $p:0.001$ ), and the 14EA group ( $p:0.009$ ). These results suggest that EA may have a positive effect on periodontitis and bone healing by reducing inflammation levels and increasing epithelial proliferation.

Nirwana et al.<sup>19</sup> aimed to investigate the effect of pomegranate extracts on the wound healing process in tooth extraction wounds in their experimental study on *Cavia Cobayas*. Their statistical analysis revealed significant differences between the control and treatment groups, leading to the conclusion that the application of pomegranate fruit extract increased the expression of vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF) in the wound after tooth extraction. In our study, similar to the findings of Nirwana et al.<sup>19</sup>, the recovery score of the 21EA group was significantly higher compared to the 14K group ( $p:0.017$ ) and the 21K group ( $p:0.009$ ). Additionally, the epithelial proliferation level of the 21EA group was found to be significantly higher compared to the 21K group, the 14K group ( $p:0.001$ ), and the 14EA group ( $p:0.009$ ). Therefore, it can be concluded that both the healing score and epithelial proliferation increase with the duration of ellagic acid (EA) use in our study.

In their study, Nirwana et al.<sup>20</sup> investigated the effect of the combination of hydroxyapatite (HA) grafts and ellagic acid (EA) in preventing graft-induced inflammation. They demonstrated that the HA+EA combination could effectively prevent bone damage and promote bone tissue renewal after tooth extraction. Similarly, Primasari et al.<sup>21</sup> explored the effectiveness of EA and hydroxyapatite in bone remodeling. Their findings revealed that the combination of HA+EA reduced the levels of TNF- $\alpha$ , the primary pro-inflammatory cytokine involved in bone inflammation, leading to increased bone growth factors.<sup>21</sup> In our study, consistent with the findings of Primasari et al.<sup>21</sup> and Nirwana et al.<sup>20</sup>, the inflammation level of the 21EA group was significantly lower compared to both the 14K group ( $p:0.042$ ) and the 21K group ( $p:0.004$ ). Additionally, the new bone formation level of the 21EA group was significantly higher ( $p<0.05$ ) than that of the 14K group ( $p:0.001$ ), the 21K group ( $p:0.008$ ), and the 14EA group ( $p:0.018$ ). Moreover, the new bone formation level of the 14EA group was statistically significantly higher than that of the 14K group ( $p:0.014$ ;  $p<0.05$ ). Based on these findings, it is suggested that the use of EA in combination with grafts may accelerate bone healing and mitigate graft-induced inflammatory reactions.

In the study by Gül et al.<sup>22</sup>, they conducted an experiment involving 24 male Wistar rats, wherein a 7 mm critical size calvarial bone defect was surgically created. The rats were divided into three groups: the first group had the defect left empty as a control, the second group received only a bone graft placed in the defect, and the third group had 0.325 mg/kg ellagic acid (EA) applied topically to the defect in addition to the bone graft. Following histological and biochemical examinations, the researchers observed that the topical application of 0.325 mg/kg EA graft material did not yield a positive effect on the applied area. However, significant positive differences were noted in the analyses conducted on serum samples obtained from

rats in both the graft group and the EA+graft group. As a limitation of their study, Gül et al.<sup>22</sup> highlighted the need for further investigations at various doses to elucidate the relationship between topical application of EA and bone healing.

In another study conducted by Al-Obaidi et al.<sup>23</sup>, involving 24 rats as a diabetes model, maxillary incisor teeth were extracted from the rats. Subsequently, the sockets were filled with Rosuvastatin. The animals were then divided into three groups: the first group served as the control and received normal saline, the second group, where a diabetes model was induced using streptozotocin, also received normal saline, and the third group, with a streptozotocin-induced diabetes model, received ellagic acid (EA) via gastric feeding. The rats were sacrificed on the 14th and 28th days. The study concluded that EA is an effective natural compound in preventing bone loss caused by tooth extraction in diabetic rats. The treatment with EA resulted in reduced levels of pro-inflammatory cytokines in the serum of diabetic rats after tooth extraction, and a decrease in oxidative stress levels in the gingival tissue of diabetic rats was also observed. In our study, similar to the findings of Al-Obaidi et al.<sup>23</sup>, the new bone formation level of the 21EA group was significantly higher compared to the 14K group ( $p:0.001$ ), the 21K group ( $p:0.008$ ), and the 14EA group ( $p:0.018$ ) ( $p<0.05$ ). In a study by Wardhana et al.<sup>24</sup>, a bone defect was created in the left femur of 30 Wistar rats, where the defect in the study group was filled with EA-HA in powder form mixed at a ratio of 3:97, while the control groups were filled with polyethylene glycol (PEG) or HA in gel form. It was observed that defects treated with EA-HA exhibited fewer osteoclasts and decreased RANKL staining on days 7 and 14. The findings of our study support the positive effect of EA on bone healing, consistent with the studies by Wardhana et al.<sup>24</sup> and Al-Obaidi et al.<sup>23</sup> The new bone formation level of the 21EA group was significantly higher than the 14K group ( $p:0.001$ ), the 21K group ( $p:0.008$ ), and the 14EA group ( $p:0.018$ ). Furthermore, the epithelial proliferation level of the 21EA group was significantly higher compared to the 21K group, the 14K group ( $p:0.001$ ), and the 14EA group ( $p:0.009$ ) ( $p<0.05$ ).

### Limitations

The limitations of this study include the fact that tooth extraction in experimental animals is more difficult and not standardized compared to humans and factors such as wound healing disorders and infection that may occur in experimental animals cannot be fully prevented. In addition, since the genetic and physiological structure of experimental animals is not exactly similar to humans, the clinical generalization of the results of the study to humans is limited. In addition, the complexity of the models used

in experimental animals may not fully reflect real clinical situations and therefore the clinical applicability of the findings may be limited.

## CONCLUSION

According to the histopathological findings of this study, EA was observed to decrease the level of inflammation on days 14 and 21.

In this study, larger areas of thin bone trabeculae and areas covered by connective tissue and osteoblasts were observed around the newly formed bone trabeculae in the EA groups compared to the control groups. Based on histological observations, this study suggests that EA could expedite the healing process by reducing inflammation and promoting epithelial proliferation on days 14 and 21.

Although there are currently food supplements and cosmetic products containing EA, further studies and clinical trials are necessary to fully comprehend their effects.

Future studies could include longer observation periods, exploration of conditions that hinder wound healing such as diabetes, investigating the combination of EA with other biomaterials, prolonged use of EA, and examining both topical and systemic applications.

Consequently, it has been observed that the administration of EA for 21 days post tooth extraction accelerates wound healing.

## ETHICAL DECLARATIONS

### Ethics Committee Approval

The study was carried out with the permission of İstanbul University Animal Experiments Local Ethics Committee (Date: 29.01.2021, Decision No: 2021/01).

### Informed Consent

Since this is an animal study, there is no need for an informed consent form.

### Referee Evaluation Process

Externally peer-reviewed.

### Conflict of Interest Statement

The authors have no conflicts of interest to declare.

### Financial Disclosure

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

### Author Contributions

All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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# The use of gingival crevicular fluid as a potential biomarker for periodontal disease

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## ABSTRACT

Gingival crevicular fluid (GCF) is an extremely valuable research material in the detection of periodontal diseases. The study of GCF components contributes to clinical diagnosis and helps us understand the mechanism of periodontal diseases. GCF is important for non-invasive analysis of periodontitis as it shows markers of connective tissue and bone destruction. GCF can be used in the future as a diagnostic tool for the identification of periodontitis, but it can also help in the detection of periodontitis progression. Early detection of periodontitis progression provides clinical benefit by allowing better control of disease activity and may improve patient follow-up. The aim of this review is to investigate the various enzymes and biomediators released in the GCF in periodontal disease and to provide an update on their role in inflammation.

**Keywords:** Mandible fractures, CFR-PEEK plate, titanium plate, finite element analysis

## INTRODUCTION

The diagnosis of periodontal disease is based on traditional diagnostic parameters such as pocket depth, bleeding on probing, clinical attachment level, and assessment of alveolar bone levels. These parameters do not indicate current disease status but only reflect past periodontal destruction. Therefore, several clinical studies have been conducted to identify potential biomarkers of periodontal disease activity.<sup>1</sup> The diagnostic potential of GCF has been investigated by experts since the 1950s, but to date there is no consensus on its acceptance as a clinically important diagnostic tool.<sup>2</sup>

GCF is a biological monitoring fluid that is crucial for the identification of dental conditions, particularly gingivitis and periodontitis.<sup>2</sup> Deficits in GCF cause issues for proteomic and biochemical analyses.<sup>3</sup> Tissue inflammation and sulcular epithelium ulceration are directly correlated with GCF volume.<sup>4</sup> Greater GCF volume is seen in areas that are moderately or severely inflamed than in areas that are not.<sup>5</sup> Nevertheless, no research has demonstrated a link between a higher sulcus fluid volume and the likelihood of periodontal tissue deterioration.<sup>4</sup>

The progression and severity of periodontitis are linked to a combination of genetics, host response, microbial diversity, and local environmental factors.<sup>6</sup> The release

of biological mediators from host tissue cells or the disruption of host tissues are the two main ways whereby bacterial virulence factors result in the demise of host tissue. Tissue lysis is facilitated by mediators generated as part of the host response, such as prostaglandins, cytokines, and proteinases. Furthermore, a number of enzymes generated by periodontal microbes can demolish tissue.<sup>7</sup> Thus far, a number of inflammatory factors, such as proteins, phosphatases, proteinases, cytokines, and products of local tissue breakdown, have been identified from GCF.<sup>8</sup> Prior research has examined over 65 GCF components as potential indicators of the advancement of periodontitis.<sup>9</sup> It has been suggested that these variables could serve as diagnostic indicators for periodontitis. GCF comprises possible indicators generated from subgingival microbial plaque as well as host tissues, serum, and other sources because it accumulates in the gingival sulcus. Variations in GCF components may serve as a possible indicator of the advancement of periodontitis.<sup>6</sup> Therefore, the GCF is regarded as a window for non-invasive periodontitis analysis that takes into consideration signs and indicators of bone and connective tissue degradation.<sup>10</sup> In addition to being a potential diagnostic tool for periodontitis, GCF can also be used to track the disease's advancement.<sup>2</sup> Clinically speaking, early detection of periodontitis

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advancement is advantageous since it allows for greater control over the disease's activity and may enhance patient follow-up.<sup>11</sup> Consequently, the targeted risk zone may be effectively managed, additionally disease progression can be stopped, and high vulnerability to disease activity in these sites can be identified with the aid of GCF collection from numerous sites.<sup>12</sup>

Potential biomarkers in GCF are grouped into three broad groups (Figure):<sup>4</sup>

1. Host-derived enzymes and inhibitors.
2. Inflammatory mediators and products
3. Markers of tissue lysis products

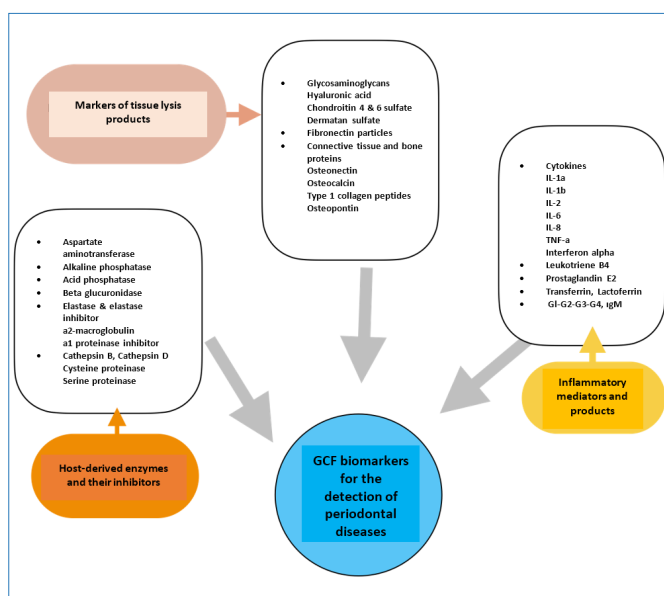


Figure. GCF biomarkers used for the detection of periodontal diseases.<sup>13</sup>

## HOST-DERIVED ENZYMES AND THEIR INHIBITORS

The regulation of periodontal tissue turnover in healthy individuals and tissue death in periodontitis is largely dependent on enzymes, particularly proteinases. The overwhelming presence of enzymes linked to tissue degradation demonstrates how crucial the pathophysiology of periodontitis is what causes the inflammatory response.<sup>6</sup> Some of these markers have received special attention for clinical applications, and commercial test kits have been developed.<sup>4</sup>

### Aspartate Aminotransferase (AST)

Aspartate aminotransferase (AST), collagenase, prostaglandin E2, beta-glucosidase, lactate dehydrogenase, arylsulfatase, and elastase are the most noticeable indicators in active periodontal lesions. In the affected areas, neutrophils, epithelial cells, dead cells, and connective tissue produce these enzymes. AST, which used to be known as serum glutamic oxaloacetate transferase (SGOT), has been used successfully to help doctors figure out what's wrong with people who have heart and liver tissue necrosis.<sup>14</sup>

AST levels are elevated at sites of active periodontitis. In cases of clinical attachment loss and inflammation, a significant increase in GCF AST levels has been observed.<sup>6</sup> In a study by Priti et al.<sup>14</sup> it was shown that AST levels increased as the severity of clinical inflammation increased. Chambers et al.<sup>15</sup> found an increase in AST levels in the GCF in their study, in which they induced experimental periodontitis using a beagle dog model.

### Alkaline Phosphatase (ALP)

Membrane-bound glycoprotein alkaline phosphatase (ALP) contributes to both the regeneration of the periodontal ligament (PDL) and the preservation of alveolar bone.<sup>4</sup> All types of cells, including leukocytes, osteoblasts, macrophages, and fibroblasts, produce ALP, but polymorphonuclear leukocytes (PMNL) are assumed to be the primary source. ALP is also produced by bacteria in the gingival groove or pocket, which raises the ALP levels in the GCF. It has been proposed that ALP levels in GCF could serve as a possible periodontitis diagnostic marker.<sup>6</sup>

ALP enzyme levels in the GCF and saliva of 23 patients with severe periodontitis and 23 healthy patients were tested by Rasaei et al.<sup>16</sup> ALP levels in salivary were measured as 24.78 per liter in the healthy group and 80.<sup>17</sup> in the periodontitis group, while ALP levels in GCF were assessed as 19.43 in the chronic periodontitis group and 12 in the healthy group. The mean of this enzyme in the gingival gingival fluid and saliva of patients with chronic periodontitis differed significantly from that of healthy subjects ( $P < 0.001$ ).

A study by Nakashima et al.<sup>17</sup> over a long period of time showed that high levels of ALP can be found before clinical attachment loss and that the total amount of ALP in the GCF is much higher in areas of active destruction.

### Beta Glucuronidase (BG)

Beta glucuronidase (BG) is an enzyme that helps break down non-collagen parts of the extracellular matrix. It is thought to show or predict the activity of periodontal disease.<sup>4</sup> Proteoglycans are broken down by BG, a lysosomal enzyme that is mostly secreted from PMNs. BG has a high sensitivity and specificity for clinical attachment loss, according to Lamster et al.<sup>18</sup> In a 6-month, multicenter investigation examining the correlation between BG and the advancement of periodontal disease, elevated BG levels in the GCF were linked to deeper probing depths or different thresholds of clinical attachment loss. The study's findings showed that the relative chance of the disease progressing increased six to fourteen times when the enzyme was present in high concentrations. The correlation between high PMNL cell counts in the periodontal pocket (a marker of the severity of the initial inflammatory response) and destructive periodontitis was also highlighted in this study.<sup>4</sup>

## Elastase

Elastase is a serine endopeptidase that breaks down a variety of collagen and non-collagen substrates. It is found in the azurophil granules of PMNs. Giannopoulou et al.<sup>19</sup> showed that elastase increased during experimental gingivitis and returned to baseline levels with the resumption of tooth brushing. High levels of the elastase enzyme in GCF have been reported in periodontitis, and high elastase levels are considered a risk factor for the development of periodontitis.<sup>20</sup> Eley and Cox<sup>21</sup> demonstrated in longitudinal research that a rise in GCF elastase is a sign of periodontal attachment loss. In a long-term follow-up study of adult patients with periodontitis who were getting supportive periodontal treatment, Bader et al.<sup>22</sup> found a link between elastase in the GCF and clinical attachment loss.

## Cathepsin B

The cysteine proteinase enzyme cathepsin B is secreted, particularly by macrophages in the GCF. Patients with periodontitis were shown to have elevated levels of GCF cathepsin B.<sup>23</sup> The degree of periodontitis has been directly correlated with the cathepsin B level.<sup>21</sup> In cases of periodontal disease, the amount of GCF cathepsin can be used as a prognostic sign as well as an indicator of attachment loss.<sup>24</sup> Kunitatsu et al.<sup>25</sup> observed that cathepsin B levels were higher in periodontitis than in gingivitis, although the amount of GCF was similar. Analysis of GCF cathepsin B levels seems to differentiate chronic gingivitis from periodontitis.<sup>26</sup> Also, GCF cathepsin B levels are strongly linked to clinical parameters both before and after periodontal treatment. This means that cathepsin B can be used to measure how well treatment worked.<sup>24</sup>

## Matrix Metalloproteinase (MMP)

PMNs are thought to be the main source of matrix metalloproteinase (MMPs), a collagenolytic enzyme that is present in many cells and is in charge of breaking down extracellular matrix constituents such collagen, proteoglycans, lamina, elastin, and fibronectin.<sup>4,6</sup> MMPs are essential for the remodeling of the periodontal ligament (PDL) in both pathological and physiological settings.<sup>6</sup> Patients with periodontal disease have tissue degradation due in part to MMP-8, MMP-9, and elastase.<sup>27</sup> Patients with periodontitis also have increased levels of MMP-2 and MMP-9, two MMPs involved in tissue degradation.<sup>28</sup> In the gingival groove, bacterial plaque causes a first infiltration of inflammatory cells, such as lymphocytes and macrophages. The inflammatory mediators produced by these activated inflammatory cells encourage fibroblasts, epithelial cells, and PMNs to manufacture MMPs.<sup>6</sup> Lee et al.<sup>29</sup> observed a substantial increase in MMP-8 activity in patients with

active periodontitis in a longitudinal study comparing patients with gingivitis, stable periodontitis, and active periodontitis. MMP-3 levels were also investigated in individuals with periodontal disease in a recent study by Beklen et al.<sup>30</sup> In particular, it was discovered that MMP-3 generated from living gingival tissue fibroblasts activated pro-MMP-8 and pro-MMP-9 that were formed from neutrophils in GCF, underscoring the significance of MMP cascades in the etiology of periodontitis.

## Leptin

The inflammatory response is influenced by leptin, a polypeptide that regulates body fat. Adipose tissues secrete a glycosylated polypeptide called leptin, which was first shown to be a hormone linked to obesity.<sup>31</sup> Obesity is caused by disturbances in the leptin signaling system in both humans and animals.<sup>6</sup> Because leptin shares structural and functional similarities with the cytokine family, it plays a role in the host response to inflammatory and infectious stimuli. Because it promotes phagocytosis and the creation of cytokines, it also boosts the immune system.<sup>32</sup> Leptin is known to affect bone metabolism both through the central nervous system and directly through bone stimulatory and inhibitory activity.<sup>30</sup> Leptin can be detected in GCF, and it has been observed that GCF concentration is higher in individuals with healthy gingival tissues compared to individuals with periodontal disease.<sup>33</sup> This is because leptin concentration decreases in inflammation as a result of vascular endothelial growth factor-induced expansion of the vascular network.<sup>34</sup> GCF leptin levels can be utilized as a marker and have been demonstrated in studies to decline as periodontal disease progresses.<sup>6</sup> Moreover, leptin receptors have been found in the gingiva and GCF in both healthy and diseased conditions.<sup>35</sup>

## INFLAMMATORY MEDIATORS AND PRODUCTS

### Cytokines

Numerous factors influence the delicate balance between periodontal health and disease. Increased synthesis of inflammatory cytokines like IL-1, IL-6, TNF- $\alpha$ , PGE<sub>2</sub>, and MMP leads to periodontal damage.<sup>36</sup> A healthy periodontium produces large amounts of anti-inflammatory cytokines, including interleukin-1 receptor antagonist (IL-1R), IL-4, IL-10, and tissue inhibitors of matrix metalloproteinases (TIMP).<sup>37</sup> Alveolar bone resorption, collagen degradation, and loss of periodontal attachment are among the processes linked to periodontal deterioration that IL-1 $\beta$  directly affects. Patients with periodontal disease have higher levels of IL-1 $\beta$  in GCF, which are considerably lowered following treatment.<sup>38</sup> IL-1 and

TNF- $\alpha$  are examples of inflammatory stimuli that stimulate the release of IL-6. In addition to promoting osteoclast development, IL-6 also causes bone loss.<sup>39</sup> Compared to people with healthy periodontal tissues, patients with chronic periodontitis have higher levels of IL-8 protein and gene expression in their gingival tissues.<sup>40</sup>

There is evidence to suggest that proinflammatory cytokines, especially, IL-1 $\beta$  play an integral role in the etiology of periodontal disease. As the gingival index and probing depth went up, Liu et al.<sup>41</sup> found that IL-1 $\beta$  levels went up in both GCF and gingival tissue. In a longitudinal study by Engebretson et al.<sup>42</sup> IL-1 $\beta$  levels in the GCF following scaling and root planning (SRP) were evaluated in patients with periodontal disease of different severities. An important observation was that IL-1 $\beta$  levels were higher in shallower areas of patients with severe periodontitis compared to shallower areas in patients with mild or moderate periodontitis. This suggests that IL-1 $\beta$  expression in the GCF is genetically influenced and is not solely a consequence of current clinical parameters.

TNF- $\alpha$  promotes cell permeability by controlling the expression of adhesion molecules in vascular endothelial cells and upregulating the expression of proteolytic enzymes, collagenases, and PGE2. This results in the loss of periodontal tissue adhesion and the development of osteoclasts. PGE2 expression is elevated in inflammatory gingival tissues, and this has led to reports that it serves as a marker for the severity and progression of periodontal disease.<sup>43</sup>

In a study by Hwang YS.<sup>28</sup> IL-6, IL-8, MMP-2, MMP-9, and TNF- $\alpha$  levels in GCF samples obtained from periodontitis patients were found to be higher than those obtained from healthy individuals. The author stated that the use of a combination of multiple biomarkers in periodontitis may increase diagnostic accuracy.

The association between IL-1 $\beta$  and C-reactive protein (CRP) in GCF in Australian patients with periodontitis was assessed by Tracy et al.<sup>44</sup> Every biomarker was found to have an independent association in cases of clinically severe periodontitis. This suggests that those who have higher levels of biomarkers showing systemic (CRP) or local (IL-1 $\beta$ ) inflammation may have a higher risk of developing periodontal disease.

## MARKERS OF TISSUE LYSIS PRODUCTS

### Laminin

Laminin is mainly produced by epithelial cells. Laminin is believed to be a critical component in the development of periodontal pockets, the apical migration of epithelial

cells, and the course of periodontitis.<sup>45</sup> Some laminin isoforms are produced by PDL fibroblasts.<sup>7</sup> It is thought that various laminin isoforms play a role in tissue remodeling in periodontal diseases.<sup>46</sup>

### Osteopontin (OPN)

Osteoblasts and macrophages, while osteoclasts can also create osteopontin (OPN), a protein found in the bone matrix. Studies have shown that OPN levels in the GCF increase in periodontitis and decrease after periodontal treatment.<sup>13,47</sup>

### Osteocalcin (OC)

The most precise indicator of osteoblast function, it is produced by osteoblasts.<sup>13,48</sup> A non-collagen matrix protein called osteocalcin (OC) is present in both calcified and non-calcified tissue. It participates in bone resorption as well as mineralization structurally by binding to the two main components of bone, collagen and apatite.<sup>6</sup> Elevated levels of OC in GCF are linked to elevated rates of bone turnover and are observed in conjunction with heightened activity of periodontal disease.<sup>13</sup>

The degree of alveolar bone damage and/or healing may be correlated with the elevated GCF OC levels observed in cases of periodontitis. OC and OC fragments are anticipated to be released into the GCF from the extracellular matrix during vigorous bone resorption.<sup>6</sup> The average concentration of OC in GCF was found to be 10 times higher than in serum by Nakashima et al.<sup>49</sup> They also believed that periodontal tissues produce OC locally. Numerous studies that have looked at OC levels in GCF from patients with periodontitis demonstrate the recent interest in OC as a potential measure of bone turnover in periodontal disease. According to their research, OC levels in GCF might be an indicator of inflammation in sick locations.<sup>50</sup>

## CONCLUSION

GCF analysis contributes to our understanding of the role of the inflammatory response in periodontitis and provides clinicians with useful information about the location and severity of existing periodontal disease. There is a wide range of periodontal diseases that require different types of treatment. The success of periodontal treatments lies in establishing accurate diagnoses, which is best achieved with the help of GCF. The aim of this study is to develop an understanding among dentists about how to increase the use of GCF for diagnosis and treatment.

GCF has promising possibilities as a diagnostic tool as it shows biomarkers of inflammation and bone resorption in periodontal tissues, is easy to collect, can distinguish

areas of active inflammation, and allows for the diagnosis of early signs of periodontitis.

## ETHICAL DECLARATIONS

### Referee Evaluation Process

Externally peer-reviewed.

### Conflict of Interest Statement

The authors have no conflicts of interest to declare.

### Financial Disclosure

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

### Author Contributions

All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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# Immediate loading of all-on-4 concept: 12 months follow-up

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## ABSTRACT

This study aims to show 12 months follow-up of the treatment of an edentulous patient with concerns about using removable dentures with immediately loaded fixed prosthesis with All on four treatment concept. Our patient, a 55-year-old female patient without any systemic disease was referred to İstanbul University, Faculty of Dentistry, Oral and Maxillofacial Surgery Clinic. The intraoral examination and radiographic evaluation had been done. All on four treatment concept had been applied. The All-on-4 concept is a highly effective treatment option for complete edentulism that yields excellent results and does not require major surgeries. Due to the low number of implants, it is a cost-effective alternative. Given these advantages, it is often a preferred treatment modality. In summary, the All-on-4 treatment requires careful planning and consideration of patient expectations, offering a reliable and cost-effective solution for complete edentulism without the need for extensive surgical procedures.

**Keywords:** All-on-four, immediate loading, implant

## INTRODUCTION

The All-on-Four concept is based on placing of four implants in a single jaw, with the posterior implants angled for optimal support. This principle was first introduced by Dr. Paulo Malo in 1993, and he and his colleagues formally described this technique in 2003.<sup>1</sup>

The “All-on-Four” treatment concept refers to the application of two vertical implants in the anterior region and two distally angled implants in the posterior region, with a fixed restoration placed on these implants.<sup>2,3</sup> After concepts that involve a higher number of implants for a single jaw, the “All-on-Four” application, which requires only four implants, is frequently implemented in clinical practice.

This system has been developed to maximise the utilisation of limited bone volume in atrophic jaws, allowing for rapid functionality post-surgery and preventing complications inherent in major surgical procedures that increase treatment costs and patient morbidity.<sup>4</sup> According to Capelli et al.<sup>5</sup>, the bone loss resulting from stresses in the cortical bone of the implant neck region is similar between straight and angled implants. Short implants could be used for the atrophic mandible and maxilla, but even for use, a minimum of 7 mm vertical bone height is needed.<sup>6,7</sup>

The crucial aspect of this treatment concept is the angled placement of posterior implants. This allows for the use of longer implants and increases the implant-bone interface, thereby positively influencing osseointegration. Additionally, it enhances anchorage obtained from the bone, eliminating the need for grafting.<sup>8-10</sup> In the All-on-Four system, the long-term survival rate of implants in the mouth ranges between 92.2% and 100%.<sup>11-13</sup>

This study aims to show 12 months follow-up of the treatment of an edentulous patient with concerns about using removable dentures with immediately loaded fixed prosthesis with All on four treatment concept.

## CASE

Our patient, a 55-year-old female patient without any systemic disease was referred to İstanbul University, Faculty of Dentistry, Oral and Maxillofacial Surgery Clinic. The patient came to the clinic to have oral rehabilitation treatment. An informed consent form was obtained.

The intraoral examination and radiographic evaluation had been done ([Figure 1](#)). It had seen that there was some bone loss. Impressions for the model cast and photographs had taken. After understanding the patient's needs, All on four treatment concept had been decided to be performed.

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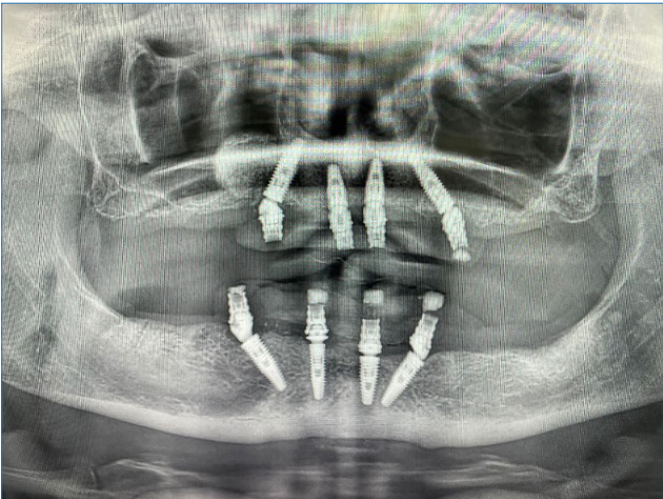


Figure 1. First orthopantomographic image of the patient

An overview of the surgical and prosthetic procedures in the described case is as follows:

**Preoperative Preparation**

Before the surgical operation, the patient used chlorhexidine oral rinse, and its continued use was recommended during the healing process.

**Initial Surgical Phase**

Flap elevation and extraction of existing teeth.  
Re-evaluation of implant positions.

Anterior implants were placed as vertically and parallel to anatomical structures as possible, while posterior implants were placed with a distal inclination (Figure 2).



Figure 2. Orthopantomographic image of the patient after implant application with All-on-4 concept

**Postoperative Care and Temporary Prosthesis**

Three months after the surgical operation, the temporary prosthesis was removed.  
Impressions were taken for the fabrication of a metal framework.

Fit and passive seating of the metal framework on abutments were verified.  
Occlusal alignment and closure were checked.  
Porcelain was applied to the metal framework for both aesthetic and functional purposes.  
The restoration was secured by screwing it onto the abutments with a torque of 30 Ncm.  
The screw access holes were filled with composite matching the colour of porcelain.

**Patient Education and Follow-up**

The patient received instruction on proper oral hygiene practices. Follow-up appointments were scheduled at intervals of 1 week, 1 month, and 1 year (Table).  
During all follow-up appointments, the patient expressed satisfaction with both aesthetics and functionality.  
Evaluation after 1 year revealed excellent outcomes in terms of function, aesthetics, and osseointegration.

Table. Follow-up evaluation			
Duration	1 week	1 month	1 year
Evaluation	Oedema on peri-implant soft tissue	No inflammation or swelling on peri-implant soft tissue	No inflammation or swelling on peri-implant soft tissue

**RESULTS**

With ageing, molecular and morphological changes in bone structure can lead to observed bone loss.<sup>14</sup> In our case, our patient is 55 years old and exhibits bone loss in both the maxilla and mandible.  
In the All-on-Four concept, four implants are placed to support a fixed prosthesis without the need for a removable prosthesis. Two of these implants are positioned in the anterior region vertically and parallel, while the posterior implants are placed at a distal angle.<sup>2,3</sup> In this technique, the distal angulation of the implants in the posterior is primarily done to ensure proper load distribution. This way, the distance between the anterior and posterior increases, balancing the load distribution from the prosthesis and allowing the placement of 12 teeth with a relatively short cantilever length.<sup>15-17</sup> We were able to provide fixed prosthodontic treatment for our patient by implementing the All-on-Four treatment, as her priority was not to use a removable prosthesis.  
The prostheses that had placed on the All-on-Four system can be made of metal substructures with porcelain, zirconium, or titanium materials, and they can be either fixed, removable, or hybrid prostheses. Long implants placed at an angle in the posterior region allow for immediate loading due to increased primary stability.<sup>18</sup>

We aimed to increase the primary stability by placing the implants in the posterior region at a long and approximately 45-degree angle for our patient. Taking advantage of this approach, we were able to perform immediate loading.

The All-on-Four concept is a highly effective treatment option for complete edentulism that yields excellent results and does not require major surgeries. Due to the use of a minimal number of implants, it is a cost-effective alternative. Given these advantages, it is often a preferred treatment modality.

## DISCUSSION

The All-on-Four concept is a highly effective treatment option for complete edentulism that yields excellent results and does not require major surgeries. Due to the use of a minimal number of implants, it is a cost-effective alternative. Given these advantages, it is often a preferred treatment modality.

One of the primary concerns among clinicians in All-on-four treatment is the potential loss of prosthetic support even with the loss of a single implant. However, implant loss is extremely rare in this treatment method, and if it occurs, it can be addressed by placing another implant in a nearby area of the same jaw. This underscores the importance of meticulous treatment planning.

Since All-on-Four treatment is typically employed in cases with limited bone support, the patient's aesthetic expectations should be clearly defined before the treatment begins. If the patient has expectations such as increasing vertical dimension or significantly enhancing lip support, alternative options like removable prostheses should be discussed with the patient.

## CONCLUSION

The All-on-Four treatment requires careful planning and consideration of patient expectations, offering a reliable and cost-effective solution for complete edentulism without the need for extensive surgical procedures.

## ETHICAL DECLARATIONS

### Informed Consent

The patient signed the free and informed consent form.

### Referee Evaluation Process

Externally peer-reviewed.

### Conflict of Interest Statement

The authors have no conflicts of interest to declare.

## Financial Disclosure

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

## Author Contributions

All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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