T.C.

YEDITEPE UNIVERSITY
INSTITUTE OF HEALTH SCIENCES
DEPARTMENT OF ORTHODONTICS

EXAMINATION OF CORROSION EFFECTS ON ORTHODONTIC BRACKETS UNDER SIMULATED GASTROESOPHAGEAL REFLUX DISEASE (GERD) – AN *IN-VITRO* STUDY

DOCTOR OF PHILOSOPHY THESIS

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APPROVAL

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Prof. Dr. Bayram YILMAZ

Director of Institute of Health Sciences

BEYAN

Bu tezin kendi çalışmam olduğunu, planlanmasından yazımına kadar hiçbir aşamasında etik dışı davranışımın olmadığını, tezdeki bütün bilgileri akademik ve etik kurallar içinde elde ettiğimi, tez çalışmasıyla elde edilmeyen bütün bilgi ve yorumlara kaynak gösterdiğimi ve bu kaynakları kaynaklar listesine aldığımı, tez çalışması ve yazımı sırasında patent ve telif haklarını ihlal edici bir davranışımın olmadığını beyan ederim.

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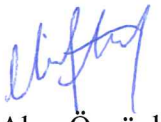

Elif Akın Özgünler

DECLARATION

I hereby declare that this thesis is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person nor material which has been accepted for the award of any other degree except where due acknowledgment has been made in the text.

26/12/2019

Elif Akın Özgünler

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LIST OF SYMBOLS AND ABBREVIATIONS

AAS	Atomic Absorption Spectroscopy
AFM	Atomic Force Microscopy
AISI	American Iron and Steel Institute
Cd	Cadmium
Co	Cobalt
CO_2	Carbon Dioxide
Cr	Chromium
Cu	Copper
DNA	Deoxyribonucleic Acid
EDS	Energy Distribution Spectrometer
EGJ	Esophagogastric Junction
FDA	U.S. Food and Drug Administration
Fe	Iron
FSR	Final Surface Roughness
FSR-ISR	Final Surface Roughness - Initial Surface Roughness
g/l	Gram/Liter
GER	Gastroesophageal Reflux
GERD	Gastroesophageal Reflux Disease
GIS	Gastro-intestinal System
H^+	Hydrogen
H2RA	Histamine-2 Receptor Antagonist
H_2S	Hydrogen Sulfide
HCl	Hydrochloric Acid
Hg	Mercury
HNO_3	Nitric Acid
IARC	International Agency for Research of Cancer
ICP	Inductively Coupled Plasma
ICP-MS	Inductively Coupled Plasma – Mass Spectrometer
ICP-OES	Inductively Coupled Plasma Optical Emission Spectrometry
IR	Ion Release
ISR	Initial Surface Roughness
KCl	Potassium Chloride

L	Liter
LES	Lower Esophageal Sphincter
mg/L	Miligram/Liter
MIM	Metal Injection Molding
Mn	Manganese
Mo	Molybdenum
MS	Mass Spectrometry
NaCl	Sodium Chloride
NERD	Non-Erosive Reflux Disease
ng/ml	Nanogram/Mililiter
NH_3	Ammonia
Ni	Nickel
Ni-Ti	Nickel Titanium
nm	Nanometer
NSAID	Non-steroidal Anti-inflammatory Drugs
O_2	Oxygen
pg/l	Picogram/Liter
pH	Power of Hydrogen
ppb	Parts Per Billion
PPI	Proton Pump Inhibitors
ppm	Parts Per Million
ppt	Parts Per Trillion
Ra	Roughness Average
RNA	Ribonucleic Acid
SEM	Scanning Electron Microscopy
Si	Silicium
SR	Surface Roughness
SS	Stainless Steel
TLESR	Transient Lower Esophageal Sphincter Relaxation
Zn	Zinc
µg	Microgram
µg/kg	Microgram/Kilogram
µg/l	Microgram/Liter
µg/ml	Microgram/Mililiter

µm	Micrometer
μm^2	Square Micrometer

ABSTRACT

Akın Özgünler, E. (2019). Examination of Corrosion Effects on Orthodontic Brackets under Simulated Gastroesophageal Reflux Disease (GERD) – An *In-Vitro* Study. Yeditepe University Institute of Health Sciences, Department of Orthodontics, PhD thesis, Istanbul.

The aim of this study was to investigate the surface roughness and ion release of stainless steel brackets (N=180, upper central incisor) after corrosion under simulated Gastroesophageal Reflux Disease. The effects of different pHs and durations were evaluated. Brackets were randomly divided into 3 main groups and 15 subgroups (n=12). The pH solutions forming the main groups were: simulating severe (pH 1.5) and moderate (pH 3.0) forms of gastric regurgitation and artificial saliva (pH 7.0) as the control group. Each pH group was divided into subgroups according to the brackets' elapsed times in the solutions (30 minutes, 12 hours, 24 hours, a week, a month). All of the specimens were kept in an incubator, at specified times. The average surface roughness of bracket slots was examined with optical profilometer. Inductively coupled plasma mass spectrometry (ICP-MS) was used to determine the metal ions (Fe, Ni, Cr) released to the solutions from brackets. Before immersions, initial surface roughness (ISR) of each braket slot was measured. After immersions, final surface roughness (FSR) and the difference of FSR-ISR of each bracket slot were examined, and the results were compared statistically in all main pH groups and time subgroups. Also, ion release at 30 minutes, 24 hours and 1 month were examined and the results were compared statistically in all main pH groups and specified time subgroups. When the pH groups were evaluated in terms of FSR at different time intervals, the statistically significant difference in all pH groups firstly occured after 24 hours ($p=0.0001$). The FSR-ISR was statistically significant in all main pH groups and time subgroups ($p=0.0001$). As the acidity of the environment and the immersion time increased, increased corrosion of brackets was observed. The highest FSR, FSR-ISR and ion release were found in pH 1.5 group at 1 month. pH 7.0 group's ion release at all time subgroups (except 30 minutes for Ni) was found to be lower than gastric solutions. Ion release from the brackets occurred in all pH groups at all time intervals, but the amounts of ion release were below the toxic limits.

Key words: Corrosion, GERD, Surface Roughness, Ion Release

ÖZET

Akın Özgünler, E. (2019). Gastroözofageal Reflü Hastalığı (GÖRH) Taklit Edilmiş Ortamda Ortodontik Braketlerdeki Korozyon Etkilerinin İncelenmesi – *İn-Vitro* Çalışma. Yeditepe Üniversitesi Sağlık Bilimleri Enstitüsü, Ortodonti ABD., Doktora Tezi, İstanbul.

Bu çalışmanın amacı taklit edilmiş Gastroözofageal Reflü Hastalığı ortamında paslanmaz çelik braketlerin (N=180, üst santral kesici diş) korozyonu sonrası yüzey pürüzlülüğü ve iyon salınımını incelemektir. Farklı pH'lar ve sürelerin etkileri değerlendirilmiştir. Braketler rastgele 3 ana gruba ve 15 alt gruba ayrılmıştır (n = 12). Ana grupları oluşturan pH çözeltileri: gastrik regürjitasyonun şiddetli (pH 1,5) ve orta (pH 3,0) derecesini simüle edenler ve kontrol grubu olarak yapay tükürüktür (pH 7,0). Her pH grubu, braketlerin çözeltilerde geçen sürelerine (30 dakika, 12 saat, 24 saat, 1 hafta ve 1 ay) göre alt gruplara bölünmüştür. Örneklerin tümü belirtilen zamanlarda inkübatörde tutulmuştur. Braket slotlarının ortalama yüzey pürüzlülüğü optik profilometre ile incelenmiştir. Braketlerden çözeltilere salınan metal iyonlarını (Fe, Ni, Cr) belirlemek için indüktif olarak eşleşmiş plazma kütle spektrometresi (ICP-MS) kullanılmıştır. Deneyler öncesi her braket slotunun başlangıç yüzey pürüzlülüğü (ISR) ölçülmüştür. Deneylerden sonra, her braket slotunun final yüzey pürüzlülüğü (FSR) ve FSR-ISR farkı incelenmiştir ve sonuçlar, tüm ana pH gruplarında ve zaman alt gruplarında istatistiksel olarak karşılaştırılmıştır. Ayrıca, 30 dakika, 24 saat ve 1 aydaki iyon salımı incelenmiş ve sonuçlar tüm ana pH gruplarında ve belirtilen zaman alt gruplarında istatistiksel olarak karşılaştırılmıştır. pH grupları farklı zaman aralıklarında FSR açısından değerlendirildiğinde, tüm pH gruplarındaki istatistiksel olarak anlamlı fark ilk olarak 24 saat sonra ortaya çıkmıştır ($p = 0,0001$). FSR-ISR, tüm ana pH gruplarında ve zaman alt gruplarında istatistiksel olarak anlamlı bulunmuştur ($p = 0,0001$). Ortamın asiditesi arttıkça ve çözeltilerde geçen süre uzadıkça, braketlerin korozyonunun arttığı gözlenmiştir. En yüksek FSR, FSR-ISR ve iyon salımı, 1 ayda pH 1,5 grubunda bulunmuştur. pH 7,0 grubunun tüm zaman alt gruplarındaki iyon salımının (Ni için 30 dakika hariç) gastrik çözeltilerdeki iyon salınımından daha düşük olduğu bulunmuştur. Braketlerden iyon salınımı tüm zaman aralıklarında ve tüm pH gruplarında meydana gelmiştir, ancak iyon salınım miktarları toksik sınırların altında bulunmuştur.

Anahtar kelimeler: Korozyon, GÖRH, Yüzey Pürüzlülüğü, İyon Salımı

1.INTRODUCTION and PURPOSE

Gastroesophageal reflux disease (GERD) is a widespread disease and affects approximately 1 in each 4-5 adults. The incidence of the disease in Turkey is between 19-25% (1,2). Many patients suffer from the presence of typical symptoms such as heartburn, regurgitation and aggravation of symptoms with exacerbating factors, whereas swallowing, peristalsis and saliva are important factors in preventing the formation of GERD (1,3).

Transient relaxation of the lower esophageal sphincter leads to GERD, which is identified by esophageal escape of the stomach contents, where the refluxate lastly gets through to the oral cavity (3).

Since the pH of the gastric content is below 1, the repeated acid attack can damage the soft and hard tissues of the oral cavity by generating a permanent acidic environment (4,5). Although saliva has a protective effect on teeth due to its diluent and buffering properties, long-term gastric reflux can lead to dental erosion and caries, especially the erosion of posterior teeth. Reflux frequency and duration, pH, acid type, and quality / quantity of saliva determine the severity of the condition (6).

Recent studies have shown that, an increase in pepsin concentration was found in saliva in patients with GERD compared to healthy individuals. Therefore, detection of pepsin in the esophagus and proximal is thought to be taken as a biomarker for the diagnosis of GERD (7).

Taking into account the effects of GERD to the oral cavity, the studies clearly show that not only the soft and hard tissues, but also the dental materials used can be effected. Biocompatibility should be one of the major characteristics of dental materials. Various types of corrosion, material degradation and surface properties are the most important factors that determine the biocompatibility of the material (8). Saliva, which is a hypotonic solution due to its content, creates an electrochemical environment to the dental materials. Materials in the oral cavity get into reaction with these physiological fluids over and over again (9). In paralel with this, corrosion occurs by the gradual deterioration of materials in the electrochemical environment (10,11). Thus, the phenomenon of corrosion is considered as an electrochemical reaction (9).

During orthodontic treatment, by means of fixed appliances using bands or brackets placed on the teeth and the wires passing through them, tooth movement is obtained (12). Even though the ortho brackets are manufactured as corrosion resistant

(10), the oral environment affected by factors such as quantity and quality of saliva, pH of food and beverages, affect the corrosion resistance of orthodontic materials (11). Depending on the duration of orthodontic treatment, metal brackets may remain in the mouth for 1-2 years and due to this reason they may be subject to corrosion (10). At the same time, corrosive nature of the oral environment can cause orthodontic materials to release metal ions into the saliva, and this may arose concern for biological and cytotoxic side effects. As a result of the corrosion that increases the friction between the bracket slot and the wire, tooth movement will be affected and enamel discoloring will begin (8).

The most widely used orthodontic material in orthodontic practice is stainless steel (SS) which has a high corrosion resistance and is a biocompatible alloy (8). A highly reactive base metal chromium provides corrosion resistance. The spontaneously formed passive film layer in air and wet conditions provides corrosion resistance (9). The presence of oxygen is inevitable for the formation and preservation of this layer. On the contrary, acidity and chloride ions may disrupt this film (8,13). Depending on the nature of the oral environment, corrosion can affect oral hard and soft tissues, as well as corrosion-resistant orthodontic materials. Therefore, in patients with GERD with low oral pH, the acidic value may impair or affect the aesthetic, integrity and biocompability of orthodontic brackets that remain in the mouth 1-2 or more years (14). However, there is no study showing this effect in literature.

In the light of these findings, the purpose of this *in-vitro* study is to investigate the surface roughness and metal ion release of brackets after corrosion under simulated GERD episodes. The null hypothesis of this study is, there is no corrosion effect of GERD on the brackets systems.

2.LITERATURE REVIEW

2.1. Gastroesophageal Reflux Disease (GERD)

2.1.1. Definition of GERD

Gastroesophageal reflux (GER) that occurs many times during the day is actually a normal physiological event. When GER causes irritating symptoms and complications, pathological table is revealed and it is called Gastroesophageal reflux disease (GERD) (15). GERD is a gastrointestinal system disorder defined as the abnormal reflux of the gastric substances into the esophagus and sometimes even to the oral cavity causing to several symptoms or complications (1). There are typical (heartburn, regurgitation, chest pain) and extraesophageal symptoms (chronic cough, laryngitis, asthma, and dental erosions) of GERD (16).

In the past GERD was described as an erosive esophagitis and considered as an acute condition that was treated for a short-term. Now, it is accepted that there are clinical findings requiring chronic treatment strategies (1). Due to the chronic nature of GERD, it requires continued management using medications and lifestyle modifications. Therefore, there are many disadvantages such as financial burden and reduction in quality of life in these patients (16).

2.1.2. Epidemiology of GERD

It has been found in recent studies that the prevalence of GERD is the most common in North America, ranged from 18.1% to 27.8%, 8.8% to 25.9% in Europe, 2.5% to 7.8% in East Asia, 8.7% to 33.1% in the Middle East, 11.6% in Australia, and 23.0% in South America. Much increase has observed in prevalence especially in North America and East Asia since 1995 (17,18). Global prevalence is influenced from geographical region (from 2.5% in China to 51.2% in Greece) and the criteria used to define gerd symptoms. Age ≥50 years, smoking, nonsteroidal anti-inflammatory drugs (NSAID) and/or aspirin use, obesity, and low socio-economic status were modestly, but significantly associated with GER symptomps and prevalence was higher in these subjects (16).

Turkey is situated between the eastern and western countries and epidemiological studies in our country have shown that Turkey's GERD epidemiology and complications are similar with both groups as of its location. GERD prevalance is lower in Eastern

Countries and higher in Western Countries. In addition to this, regurgitation is mostly seen in Eastern Countries and heartburn is mostly seen in Western Countries. Complications (erosive esophagitis, Barrett's esophagus, esophageal adenocarcinoma) are quite common in Western Countries. Turkey's GERD prevalence is similar to the Western Countries (19,20), but it is higher than Eastern Countries. In Turkey, regurgitation is more common than heartburn likewise Eastern Countries (21).

The prevalence of reflux in the East Anatolia, Central Anatolia, Mediterranean and Black Sea region was found higher than in other regions of Turkey and was higher in females than males (22).

2.1.3. Risk Factors of GERD

The body mass index, family history of GERD, and alcohol use are factors that are supported by evidence pose a risk to the GERD (23). It is stated that some drugs and foods may increase the likelihood of GERD development and might have a place in the pathophysiology of reflux. Possible drugs that might provoke GERD symptomps involved such as aspirin and other NSAIDs, nitroglycerin, calcium channel blockers, anticholinergics, antidepressants, sildenafil, albuterol, and glucagon. Coffee, chocolate, and fatty meals are considered as food types that might cause GERD symptoms. However, research is inconsistent about their contribution to GERD, as well as regarding tobacco smoking's. Carbonated soft drinks, overeating, and eating rapidly are weakly associated with GERD according to the findings of Kahrilas (24).

2.1.4. Pathogenesis of GERD

Many mechanisms are effective in the pathophysiology of GERD (25). The stomach's columnar mucosa has properties to withstand low pH, the stratified squamous mucosa of the esophagus is easily destroyed by these low pHs (26). Normally, the anatomical and physiological mechanisms in the gastroesophageal junction try to prevent reflux (25). Esophageal protective defense consists antireflux mechanisms, luminal clearance mechanisms and tissue resistance (27). Pathological reflux occurs as a result of breakdown of the balance between the protective and reflux facilitating mechanisms against reflux (25). Eventually, reflux of acid, bile, pepsin and pancreatic enzymes forms cause esophageal mucosal damage (28). Amongst these gastric acid is the most detrimental substance. The presence of bile and pancreatic enzymes with acid are factors

that increase the severity of the damage. Contrary to this, bicarbonate and growth factors in the content of saliva play a protective role against the destructive effects of reflux. The degree of damage to the esophagus and also the severity of the symptoms are different in every human after exposure to the same severity of acid because of the different mucosal sensitivity against acid (29,30). The mucosal defensive factors have an important role in preventing GERD formation by neutralizing the backdiffusion of the hydrogen ion to the esophagus tissue (28).

Factors that have been triggered reflux are sliding hiatus hernia, low LES pressure, transient lower esophageal sphincter relaxation (TLESR), the acid pocket, obesity, increased distensibility of the esophagogastric junction (EGJ), prolonged esophageal clearance, and delayed gastric emptying (31).

The EGJ is responsible for the antireflux barrier and also consists of LES (32). Physiologically, before contraction of esophagus, LES relaxes and provides food to move into the stomach. LES is a high-pressure zone in rest conditions. The low chronic resting pressure of LES is usually linked with esophagitis (28). LES pressure is under the control of neurogenic, myogenic and humoral mechanisms. Fatty foods, cigarettes, some drugs (calcium channel blockers, beta-adrenergic blockers, nitrates and anticholinergics) lower the sphincter pressure (30). TLESRs are short sequences of LES relaxation unassociated to swallowing or peristalsis. TLESRs occur usually postprandial period and in the upright position (28,31).

Studies indicated that after meals the body of the stomach is too often less acidic than esophageal refluxate. While the stomach is empty, the gastric juice's pH is close to 1, but in the postprandial period, with the buffering effect of the foods, the acidity level decreases and so pH is between 3 and 5. Acidic refluxate of esophagus derives from proximal stomach distal to the EGJ and named as an acid pocket which reduces the buffering effect of the meals and behaves like a reservoir (26,31,33).

Cigarettes, alcohol, very hot beverages, salty and spicy foods, tetracycline, doxycycline, vitamin C, bisphosphonates and potassium chloride (KCl) might decrease the resistance of the esophageal mucosa to acid (29,34).

Pathophysiology of mild reflux disease: Mild reflux occurs when an adequate amount of acid refluxes into the esophagus and esophageal pH is less than 4 between 4 and 15 percent of the time, and nearly all of it during TLESRs. This is the most frequent type of reflux. Mild reflux occurs generally post-prandial during the day and there is little or no reflux during the night (26).

Pathophysiology of severe reflux disease: There is more esophageal acid exposure exist. Severe reflux can cause more critical problems such as Barrett's esophagus or severe erosive esophagitis. Severe reflux can be seen at times when the LES pressure has fallen for a long time and during swallowing and is also relevant with nocturnal reflux (26).

2.1.5. Classification of GERD

Montreal Consensus Group has divided the clinical picture of GERD into two subgroups according to symptoms and complications: esophageal or extraesophageal syndromes, and also extraesophageal symptoms have divided into established and proposed associations. Esophageal symptomatic syndromes and esophageal syndromes with esophageal injury are the subgroups of esophageal syndromes. Chest pain syndrome is recorded apart from the group of esophageal symptomatic syndromes in order to identify a group of patients who do not have typical reflux symptoms with chest pain or who have pain that exceeds typical reflux symptoms (35).

Esophageal damage causes esophageal syndromes such as: reflux esophagitis, reflux stricture, Barrett esophagus, and esophageal adenocarcinoma. The term reflux esophagitis is favoured instead of erosive esophagitis. Non-erosive reflux disease (NERD) is a separate category of esophageal syndromes and is not used in the classification scheme as they are depended on a diagnostic test (endoscopy) that may not be applied in many patients. Although typical symptoms of GERD (regurgitation, heartburn or chestpain) are seen in NERD, there is no esophageal mucosal damage on the endoscopic observation. Upper gastrointestinal endoscopy and esophageal manometry results are normal in these patients. The answer of NERD to proton pump inhibitors (PPIs) is weak. Contrary to NERD, erosive symptoms are seen with esophageal mucosal damage (35). Depending on the severity of esophagitis, there is potential for chronicity and recurrence (36). Most of the patients recover as a result of treatment, but when patients do not continue treatment, it is known that the recurrence rate is 80% within 30 weeks (37). Esophagitis may not coincide with the severity of symptoms (38). The response of NERD patients to treatment is similar to that of patients with erosive reflux (35,37).

Extraesophageal syndromes involves dental erosions, laryngitis, cough, asthma, pharyngitis, sinusitis, idiopathic pulmonary fibrosis, and recurrent otitis media (33,35).

Performing a single classification of this disease has been difficult due to the diversity of clinical findings, disease grade and diagnostic methods in each individual. Classification should be made according to the findings observed during the first examination. Akyüz and Mutluay (39) have made symptomatic classification and endoscopic classification.

Symptomatic Classification: Considering the severity, frequency, intensity and duration of symptoms, they have evaluated GERD as mild or moderate according to their effect on daily living activities. It is called as mild disease if these features are involved: less than three in a week, minimal effect on daily activities, short-lasting. If there are features like more than three in a week, excessive effect on daily activities, long-lasting, it is called as moderate disease (39).

Endoscopic Classification: GERD can be divided into 3 categories in the presence of reflux symptoms according to the existence of endoscopic evidence: erosive, non-erosive reflux disease (NERD) and complications. Barrett's esophagus, stricture, hemorrhage and adenocarcinoma are complications of GERD (39).

2.1.6. Clinical Manifestations of GERD

2.1.6.1. Extraesophageal Symptoms of GERD

Etiology of extraesophageal symptoms of GERD is still unknown. But, two possible hypothesis are considered: vaso-vagal reflex and direct contact of aspirated gastric refluxate with the upper airway. The most common extraesophageal complications are noncardiac chest pain, chronic hoarseness, chronic cough, and asthma (40). If acid reflux reaches into the lungs, it causes pulmonary symptoms such as chronic cough, intermittent wheezing, asthma, bronchitis, aspiration or recurrent pneumonia, and interstitial fibrosis. When it reaches oral cavity, dental erosion, tooth decay, gingivitis, halitosis, aphthous ulcers, and water brash can be seen. Sore throat and globus sensation can be seen if acid reflux exists in throat. Chronic posterior laryngitis and hoarseness may occur due to inflammation of the vocal cord. Other potential extraesophageal symptoms include Otalji and hiccups (41).

2.1.6.2. Esophageal Complications of GERD

Erosive esophagitis due to erosion and ulcers of the esophageal mucosa is one of the most important complications of GERD. Although patients may be asymptomatic, complaints which are similar to the typical symptoms of GERD may also occur (42).

Esophageal strictures may occur due to chronic acid irritation and irregular healing process of esophagus. These strictures are generally seen in squamocolumnar junction. Patients suffer from dysphagia and food absorption (43).

Barrett's Esophagus which has a risk of malignancy due to tendency to esophageal adenocarcinoma, is one of the complications of GERD. Stratified squamous epithelium is transformed into metaplastic columnar epithelium. Chronic exposure to gastric acid and other refluxate materials, age more than 50 years, obesity, smoking, hiatal hernia, male sex, and Caucasian race are the risk factors for developing this metaplastic transition. Barrett's esophagus may be prevented with the help of diet regulation, H. pylori infection and use of NSAIDs. It may be seen as asymptomatic besides the typical GERD symptoms. These patients also have esophageal ulcers, strictures and hemorrhage and should be routinely monitored (44,45).

2.1.7. The Clinical Spectrum of GERD

GERD has a wide variety of clinical symptoms and complications, depending on the degree of to which refluxed acid reaches other tissues (24).

The frequency of symptoms varies from patient to patient. Some patients may report a symptom frequency daily or weekly, whereas some of them may report a few times per month. Changes in the esophageal mucosa seen on endoscopy may not always be compatible with symptom frequency and severity (46).

2.1.7.1. Heartburn

Heartburn, described as burning behind the sternum, is the most common manifestation of GERD. This symptom often occurs at post-meal (usually 30-60 minutes after eating) especially after fatty foods or upon reclining at night. The pain is increased in the excessive bending position, while relaxation is observed in an upright position and after antacid intake (24,47).

Heartburn has a high level of reliability when diagnosing GERD. If both heartburn and regurgitation are observed, GERD can be diagnosed precisely with greater than 90% (41).

2.1.7.2. Regurgitation

Regurgitation is defined as reflux of acidic gastric fluid from esophagus to the oral cavity without an effort, nausea, wretching or abdominal contractions. Also, it generally appears after meals, likewise heartburn. Complaints may be increased during recumbency, straining, or bending over. Regurgitated stomach contents generally have a bitter or acidic taste. There may be a history of stain on the pillow when regurgitation occurs at night (24,48).

2.1.7.3. Dysphagia

Dysphagia is the difficulty in swallowing and the perception of the impaired movement of the material during swallowing. It has an impact on more than 30% of patients with GERD. The possibility of esophageal cancer should not be ignored in severe cases. Dysphagia is a rarely seen symptom in reflux disease (24).

2.1.7.4. Odynophagia

Odynophagia is described as painful swallowing. A sharp substernal pain is felt. Esophageal ulceration is considered to be the cause. Ingestion of caustic substances, or corrosive injuries caused by drugs may also cause this condition (24,47).

2.1.7.5. Non-cardiac Chest Pain

Non-cardiac chest pain is a substernal chest pain that mimics myocardial infarction but has no underlying coronary artery disease. The closeness of esophagus to the heart and visceral innervation are thought to cause this condition. The response of the patient to exercise is important in distinguishing of noncardiac chest pain and myocardial infarction (24).

2.1.8. Clinical Diagnosis and Diagnostic Methos of GERD

There are many methods used in the diagnosis of reflux, but it is not essential to apply to each patient. Anamnesis and PPI test is extremely helpful in making a diagnosis when GERD is suspected. It is indicated in the Montreal classification that in the presence of typical symptoms (heartburn, regurgitation) a reasonable GERD diagnosis can be made (high recommendation level, moderate evidence level). While PPI treatment is suggested empirically in patients diagnosed, also response to PPI treatment supports confirmation of diagnosis (78% sensitivity and 54% specificity) (35,43,49). In the absence of typical symptoms, we cannot ignore the diagnosis of GERD.

Two types of algorithms are recommended when the possibility of GERD diagnosis is high: 1) Standard dose PPI is implemented as a pretreatment option (without endoscopy), 2) Endoscopy is performed before PPI treatment. If symptomatic improvement is seen with PPI, treatment should be quitted. When the symptoms remained or recurrence is occured, endoscopy should be performed. The differential diagnosis of erosive GERD and non-erosive GERD is possible only with endoscopy before PPI (50).

The use of barium radiography for diagnostic purposes is not recommended, but may be helpful for differential diagnosis in some cases. Upper gastrointestinal endoscopy should not be routinely recommended in the existence of typical reflux symptoms but should be applied to patients with alarm symptoms (weight loss, dysphagia, odynophagia, hematemesis, anemia, fecal occult blood), those with a high risk of complications and in case of unresponsive cases. In addition, routine biopsy from the distal esophagus, esophageal manometer, ambulatory esophageal reflux moniterization, h. pylori screening and eradication are not the suggested methods for routine evaluation of GERD (35,43). Ambulatory esophageal reflux monitoring enables convenience in terms of observation the duration of the acid exposure to esophagus and number of reflux episodes. It can be recommended in such cases that where optimal treatment is applied but reflux symptoms are continued, where surgical treatment can be performed and where the diagnosis of GERD is suspected. The acid reflux, defined as the drop of esophageal pH below 4, is measured by esophageal pH monitoring. This technique measures the esophageal pH, impedance and manometry (43).

2.1.9. Treatment of GERD

In the treatment of reflux, control of symptoms, healing of esophagitis, prevention of complications and maintenance of remission are aimed. Treatment is divided into three types: clinical, surgical or endoscopic. The therapeutic approach can be examined in two groups as behavioral and pharmacological measures. The treatment of each patient should be different due to differences in the course of the disease. Another point to be considered in the treatment of GERD is that the disease has a chronical nature, therefore when the treatment is stopped recurrent relapse can be seen (17,43,50,51).

Symptoms may improve with lifestyle modifications, antacids and histamine2-receptor antagonist (H2RA), when mildly symptomatic and infrequent GERD is seen. However, aggressive and persistent acid suppression is necessary when more severe and frequent symptoms are seen (1).

2.1.9.1. Lifestyle Changes

Dietary changes and social measures might be therapeutic in patients with mild GERD symptoms such as elevation of the head of the bed, giving up smoking and alcohol, nutrition with low fat diet, decreasing the amount of food in each meal, giving up eating and drinking at least 3 hours before bedtime, losing weight, keeping away from tight clothes, keeping away from precipitating food (chocolate, spicy foods, coffee, tea, coke, tomatoes, acidic fruit juices) and medicines (anticholinergics, theophylline, diazepam, narcotics, calcium channel blockers, beta adrenergic agonists, progesterone, alpha adrenergic antagonists) that lower LES pressure, chewing gum and reducing stress level (17,43,50,51).

2.1.9.2. Medication

Medical therapy is recommended if the patient's complaints do not change as a result of lifestyle changes. The drugs used in the treatment were divided into three groups: gastric acid suppressants (histamine-2 receptor antagonists (H2RA), proton pump inhibitors (PPI)), prokinetic agents, sodium alginate and antacids.

PPIs which are recommended as the first-line medication in the treatment of GERD are known to be significantly more effective than H2RA and randomized-controlled studies have demonstrated that they are recommended for maintenance therapy

because of its efficiency and cost- effectiveness (17,25,30,43,50–52). The irreversible blockade of the H^+ K^+ ATPase proton pump activated in gastric parietal cells is the mechanism of action of PPIs. PPIs convert the pH of refluxate to weakly acidic or alkaline. It is recommended to take 30-45 minutes before meals. According to a meta-analysis study examining different PPIs (omeprazole, lansoprazole, rabeprazole, pantoprazole, esomeprazole), no major differences were found in efficacy between different PPI agents (1,53).

Gastric acid is produced by means of histamine which sends signals to the parietal cells via the H2 receptor. H2RAs that bind to histamine receptors in the parietal cell of the stomach act by reducing acid secretion. The use of H2RA in addition to the use of PPI may have a reducing effect on symptoms. Ranitidine, famotidine, cimetidine and nizatidine are four H2RAs approved for use in the treatment of GERD in the United States (1,53).

The antacids which are used to control intermittent esophageal symptoms (especially heartburn), are essentially aluminium, calcium and magnesium compounds. Rapid recovery of symptoms is one of the most important advantages of antacids. It does not eliminate long-term symptoms and provide healing of erosive esophagitis, nor does it prevent GERD complications. Alginates, which can form a physical barrier against reflux by increasing the viscosity of the gastric content, are especially useful in neutralizing acid pocket. They are more effective when used with antacids. Sodium alginate and antacid which are effective in the temporary healing of symptoms are not appropriate for severe cases. Gaviscon (Reckitt Benckiser) is from this group (1,53).

Prokinetic agents (metoclopramide, domperidone, mosaprid and itopride) are used in cases with GERD symptoms from time to time. It was stated that only a modest reduction in symptoms was observed when using in combination with PPI (1,53).

There is no study in the literature regarding the effect of duration of drug utilization on treatment success and endoscopic or symptomatic recurrence. In addition, symptoms after discontinuation of long-term PPI treatment are controversial due to a sudden increase in acid release. PPI treatment is not terminated suddenly, the dose is gradually decreased. Long-term studies are required about medication (17,25,30,43,50–52).

Baclofen which has an ability to reduce transient LES relaxations and reflux episodes is another alternative medication for refractory GERD treatment. The use of

baclofen, which is not approved by the U.S. food and drug administration (FDA), is limited because of the lack of long-term studies (43).

2.1.9.3. Surgical Treatment

Desire to quit medication, non-cooperation, side effects associated with drug treatment, a major hiatal hernia, esophagitis resistant to medical treatment and persistent symptoms caused by GERD can be accepted as an surgical indication. There are many methods in antireflux surgery such as; bariatric surgery, roux-en-Y surgery, laparoscopic fundoplication, open surgical Nissen fundoplication, LinxTM magnetic ring (33).

2.1.10. Characteristics of the Refluxate in GERD

Harmful substances escaping from the stomach to esophagus include hydrochloric acid (HCL), pepsin, bile salts and pancreatic enzymes such as trypsin. Acid is the harmful constituent of the gastric fluid. On the other hand it provides a suitable chemical environment for pepsin activation while destroying the tissue. Pepsin causes damage to the esophagus mucosa by ingesting the epithelial protein (54). Gastric acid secretion capacity which determines the severity of the disease is different in each individual (55).

The most important difference was found between pH 2 and 4. Among these values, the passed time to pain sensitivity increased, ultimately reaching a balance at a pH of more than 4. Likewise, if the lumen has a pH of less than 2 or if pepsin is present in the reflux content, the degree of mucosal damage can be significantly accelerated. It has been shown that the association of acid and pepsin is the most damaging combination to the esophageal mucosa (24). A pH of 4 appears as the optimal threshold value to separate aggressive and non-agressive reflux throughout the 24-hour period (56). Gastric refluxate with a pH less than 4 includes active pepsin. Pepsin is activated in an acidic pHs. The refluxate of bile and pancreatic secretions may also play a role in some cases. However pepsin obviously is the major factor. The effect of bile has not been determined (24).

According to pH monitoring, which has importance in terms of indicating whether the patient's symptoms are related to reflux and determining pathological reflux, normally, the pH in the esophagus should be above 4 (pH 6-7), and a decrease in pH below 4 is considered a reflux episode. In a normal patient, in 24 hours, the total time

under pH 4 in the esophagus should be less than 60 minutes (less than 4% of the total 24 hours) and there should be no reflux episode lasting more than 5 minutes (30).

For dental erosion, Bartlett and Coward (57) stated that, gastric fluid, which has in it primarily HCl, was more erosive than acidic carbonated drinks, which usually consists phosphoric or citric acid. West et al. (58) also found that, in various circumstances, pure HCl was more erosive than phosphoric acid. In spite of the prevalence of this disorder, its clinical senses associated with orthodontic treatment are nearly unexplored (59).

2.1.11. Back-up Mechanism in GERD

The reflux has a role in stimulating the peristaltic wave in the esophagus, and the peristaltic wave provides clearance of reflux toward stomach. Secondary peristalsis can also be induced, especially during sleep when swallowing is suppressed. The swallowing also allows the bicarbonate content of the saliva to reach the esophagus, thereby contributing to the neutralization of reflux. In addition, bicarbonate released from the submucosal glands of the esophagus also provides protection (60–63).

2.2. Corrosion in Orthodontics

As a result of the electrochemical attack, the gradual decay of metal or metal alloys is called corrosion. Orthodontic appliances are exposed to chewing forces, appliance loading, temperature changes, foods and saliva in the oral cavity. In such a case, the release of a number of ions from the material, soft tissue coloration, allergic reactions may occur. As a result, a mechanical breakdown can be seen in the material and at the same time, the physical, chemical and electrical properties of metal or alloy may vary (64,65).

Certain variables such as pH, concentration, surface tension, buffering capacity, enzymatic activity, organic acid and bacterial flora that affect the electrochemical structure of saliva can alter the corrosion process. Studies have shown that the most substantial variable is the saliva flow rate (65).

Acidic foods and beverages, toothpastes and mouthwashes contribute to the corrosive effect in the oral environment. House et al. (64) stated that microorganisms

accumulated on orthodontic materials acidify the environment through the metabolic products they produce and cause corrosion.

In orthodontics, chemically stable and corrosion resistant high noble alloys and metals can be used. Protective surface layer formation is another factor that provides corrosion resistance during orthodontic treatment. Considering biocompatibility and orthodontic appliance durability in orthodontics, the corrosion resistance of the metals to the oral environment is of great importance (65). Therefore, it is important to have information about the formation and types of corrosion.

2.3. Corrosion

Corrosion is the deterioration of metal and / or metal alloy as a result of chemical or electrochemical reactions between it and the surrounding environment (8,66). Both the type of metal and the environmental conditions affect the form and grade of deterioration. As a result, the physical, chemical, mechanical or electrical properties of the metal or alloy get changed (67).

When necessary conditions for the formation of corrosion mechanism such as material, medium and interface are met, the material reacts with the medium. In order for the corrosion to occur electrochemically, there must be potential difference , load transfer reaction and a continuous current transmission path in the environment. When these conditions are taken into consideration, it is seen that the most corrosive medium is liquid media with different contents (67). There are 2 different procedures of corrosion occurrence by the transition of the metal ions to the liquid and by the gradual dissolution of the superficial film layer which are mostly oxide or sulfate layers (64).

Corrosive effect is increased when oxygen (O_2), carbon dioxide (CO_2), hydrogen sulfide (H_2S), oxidizing agents, ammonia (NH_3), acid, base and acid salts are present in medium (67).

There are two concurrent reactions in the corrosion process, oxidation and reduction. The degree of corrosion depends on the structure of the liquid in which it is placed (64). For example, iron in weak acid, will cause the iron to dissolve by producing ferric ions ($Fe \rightarrow Fe^{2+} + 2e^-$) by oxidation reaction. In the reduction reaction, hydrogen ions are reduced to hydrogen gas ($2H^+ + 2e^- \rightarrow H_2$). This corrosion process continues until the metal is completely consumed, until it forms a protective surface layer on the metal surface or the dissolved oxygen in the environment is exhausted (64).

2.3.1. Protective Surface Layer

Corrosion resistance of stainless steel, chrome-cobalt and titanium alloys used in orthodontic appliances is based on passive surface oxide film formation. This protection layer, which is affected by mechanical and chemical deterioration, may lose its integrity. Even if no deterioration occurs, the oxide film will slowly dissolve (passivation) and then regenerate (repassivation) when the metal surface is exposed to oxygen. Acidic environments and chlorine ions can accelerate the passivation procedure (64).

2.3.2. Corrosion Types

Corrosion types, chemical and physical processes are listed below and the most common types are stress, pitting, uniform and galvanic corrosion (65).

- Uniform Corrosion
- Pitting Corrosion
- Crevice Corrosion
- Fretting and Erosion-Corrosion
- Intergranular Corrosion
- Galvanic Corrosion
- Stress Corrosion
- Hydrogen Damage
- Microbial Corrosion in Orthodontic Appliances

2.3.2.1. Uniform Corrosion

In addition to being the most frequent type of corrosion, it can be observed where the corrosive environment has equal accessibility to the material and the metal is uniform. The metal is separated from the surface regularly and may not be detected without dissolving a large amount of metal (65).

2.3.2.2. Pitting Corrosion

Pitting corrosion which is usually occurring on base metals is observed in brackets and wires depending on the roughness of the surface of these materials, and also the

growth of pits may cause holes in the metal surface. The presence of chloride in the environment increases the rapid breakdown of metal (65).

Liu et al. (68) stated that the use of titanium aluminum nitride coating significantly reduced the pitting corrosion. Es Souni et al. (69) noted that Cr-Co alloy showed great pitting corrosion compared to Ni-Ti alloy. Kim and Johnson (70) noted that Ni-Ti and SS wires were prone to pitting and localized corrosion.

2.3.2.3. Crevice Corrosion

Crevice corrosion is a type of corrosion seen in interfaces or narrowed surfaces where oxygen substitution is not possible. It can be seen in where the elastomeric ligatures are applied to the brackets. The onset and progression of this corrosion depends on the decrease in pH and the increase of the chloride ion concentration. In other words the passive layer dissolves as the pH of the medium decreases (65).

Recycling can include heat chemical and mechanical process which could be a reason to speeding up of crevice corrosion of brazed joints (8). The crevice corrosion in removable appliances can be seen as a brown staining at the junction of acrylic and screw/metal, and it is thought to be caused by bacteria and biofilm on the junction (64,71,72).

2.3.2.4. Fretting Corrosion

Metal ion transition to the tissues is mostly caused by fretting corrosion which is formed as a result of the incorporated effect of chemical and mechanical attack. It arises at contact points of metals under long-term load. It is corresponded to bracket slot-archwire interface in orthodontics (8,64,65).

2.3.2.5. Erosion Corrosion

Corrosive fluid erodes the material surface and high flow velocity of this fluid accelerates corrosion process. In the discrimination of erosion corrosion and cavitation, the difference of the environment and the dissimilar materials are important (65,73).

2.3.2.6. Intergranular Corrosion

Intergranular corrosion can be seen especially while brazing and welding of stainless steel. Heating at temperatures as low as 350 degrees results in the reaction of chromium and carbon, and the formation of chromium carbide, which leads to increased brittleness of the alloy and reduced corrosion resistance (64).

2.3.2.7. Galvanic Corrosion

Galvanic corrosion arises in the conductive liquid or electrolyte, when 2 or more metals or dissimilar alloys (or in fact same alloy) which have different potentials get in connection with each other. This electrochemical potential difference leads to the formation of electrical current between the materials and cause to corrosion and eventually release of metal ions. Orthodontic bracket – wire combination is an example to this corrosion type. Removable appliances with soldered joint are more prone to galvanic corrosion (8,64,65).

2.3.2.8. Stress Corrosion

The stress corrosion occurring in the corrosive environment under the influence of tensile and compresive stresses can be seen at the twisting points or during the application of the wire to the crowded teeth due to loading. It may disrupt the mechanical integrity of the archwires (8,64,65).

Repeated cyclic stress (fatigue) enhances the risk of fracture in metals. Corrosion fatigue is a status which is increased in a corrosive environment. It may be seen on wires which stay in the oral cavity for an extended period under stress (64).

2.3.2.9. Hydrogen Damage

Hydrogen can be absorbed under certain circumstances by archwires and as a result ductility of metals are decreased. Embrittleness of reactive metals such as titanium, vanadium, niobium can be seen (8,65).

2.3.2.10. Microbiologically Influenced Corrosion

Microorganisms can corrode metal alloys through 2 pathways. Some types of microorganisms do this by absorbing and metabolizing metal from alloys. Others do this by making environmental conditions more favorable to corrosion, through their normal metabolic by-products (eg. by increasing acidity in the region) (64). The presence of microbes on metals might be disturb the passivity of passive metal and may reduce pH by forming organic acids during glucolysis. Low pH provides a suitable environment for corrosion process (65).

The resistance of some bacteria to antibiotics might be increased by the corrosion products. Therefore, further exposure to metals and corrosion products has a potential to cause the spread of resistant genes among bacteria, including into pathogens of medical and dental significance (64).

Using antibiotic spray and dips helps controlling the degree of microbial corrosion by cleaning the area (65). Chang et al. (74) showed that, the presence of Streptococcus mutans and its growth by products increase the corrosion behaviour of metalic materials.

2.4. Ion Release and Cytotoxicity in Orthodontics

Metal release in orthodontics is important, due to the possibility of cytotoxicity caused by ions released into the oral environment (75), and the changes in the physical properties of orthodontic appliances which affects their clinical performance (64). Since patients are exposed to these metals for a long time after prolonged use of orthodontic appliances, there is concern about patient safety due to potential toxic effects (76).

The degree of effect of the released ions on the patient, locally and systemically, is not fully known (77). Some researchers have reported that metal release from orthodontic appliances has occurred, but also have stated that these values are not sufficient to create toxic effects (78). In addition, even the non-toxic ions released by appliances has potential to be sufficient to induce biological changes (79).

Stainless steel and Ni-Ti alloys are generally used in orthodontics and potentially chromium (Cr), iron (Fe) and nickel (Ni) ions are corrosion products released from stainless steel alloys, while nickel (Ni) and titanium (Ti) ions can be released from Ni-Ti alloys (78). Despite the negative effects of each ion, the most striking ions were nickel and chromium because Ni and Cr have also mutagenic, cytotoxic and carcinogenic effects

in addition to their allergenic properties (76,80). Faccioni et al. (81) argued that the released metal ions may affect the deoxyribonucleic acid (DNA) of oral mucosa cells and may create toxic effects on living tissues. Ortiz et al. (82) concluded that the SS alloy and Ni-free allow generates DNA damage in buccal mucosa cells. On the contrary, Eliades et al. (80) argued that stainless steel and NiTi-containing orthodontic appliances do not affect the DNA synthesis of any cell. Also, Eliades et al. (83) and Huang et al. (84) reported that short-term studies do not reflect reality and that symptoms may occur in the long term because orthodontic treatments last for 2-3 years.

Some researchers, in their *in-vitro* studies, tried to understand the leading causes that could impact corrosion resistance or negatively affect the release of elements. Suárez et al. (85) and Hwang et al. (86) reported that SS archwires (8% Ni) released the highest amount of Ni compared to NiTi and NiTiCu archwires (which both have 50% Ni content). Ortiz et al. (82) stated that during the first week of immersion, greater quantities of Ni, Fe, Cr, Mn released into the environment because of materials with high Ni content are more prone to corrosion. Sfondrini et al. (87) and Huang et al. (88,89) reported that the release of Ni from three types of brackets (new conventional SS, recycled SS, and Ni-free brackets) was significantly high for recycled brackets, while the lowest was from Ni-free brackets. Huang et. al. (88) also stated that for all types of brackets, acidic environments (pH 4.2) exhibited the highest Ni release, a consequence similar to results obtained by Milheiro et al (90). Bhaskar and Subba Reddy (91) reported in their study in which orthodontic space maintainers were evaluated that the release of Cr and Ni ions from bands reaching its greatest amounts at the end of 7 days. Danaei et al. (92) investigated the release of Cr, Cu, Fe, Mn and Ni from SS orthodontic brackets after 45 days of immersion in 3 different mouthwashes and maximum ion release was observed with chlorhexidine mouthwash. Sheibaninia (93) assessed the pH values and stated that Ni release from NiTi wires is accelerated in acidic pHs. An *in-vitro* study regarding the release of elements from stainless steel orthodontic appliances performed by Mikuliewicz et al. (94) supported the opinion that Ni and Cr ions need more attention, since their concentrations indicated that these ions were released together.

Regarding *in-vivo* studies (95), the general conclusions were that metal ion release is seen just in the initial stage of the orthodontic therapy. In general, most of them have reached the conclusion that the metal ion quantities do not arrive the normal daily dietary intake.

2.5. General Features of Metal Ions

Elements whose atoms all have the same number of protons are chemically the simplest substances, and therefore cannot be broken down using chemical reactions. The elements form compounds by reaction with different elements, and at the same time they become stable (ion) by taking or giving electrons. Ion is the name given to atoms or groups charged with '+' or '-'. Heavy metals are metals with a density greater than 5 g / cm^3 in terms of physical properties. This group includes over 60 metals including iron (Fe), copper (Cu), nickel (Ni), chromium (Cr), cobalt (Co), cadmium (Cd), mercury (Hg) and zinc (Zn). These elements, by their nature, are found stable in the form of carbonates, oxides, silicates and sulphides, or are trapped in silicates, in the earth. Heavy metals' negative effects gradually increase based on the accumulation of them which is higher than other metals in living organisms in a certain period of time. In parallel with this, it is thought that these metals are toxic (96). A localized increase of these metallic ions or a systemic distrubiton by increased debris in other area might cause toxic reaction (97). According to the Council of Europe Directive (98), in which the quality of drinking water was evaluated in 1980, Ni and Cr ions were classified as toxic substances, while Fe and Mn were classified as potentially toxic substances. Ni have been regarded as carcinogen, and the International Agency for Research of Cancer (IARC) accepted Ni compounds as group 1 carcinogens (99).

2.5.1. Iron (Fe)

Iron element, which is rarely found as an element in nature, has an atomic number of 26 and is used in steelmaking (100).

Iron element, which is vitally important, has a role in oxygen transport, energy production, deoxyribonucleic acid (DNA), ribonucleic acid (RNA) and protein synthesis. The total amount of iron present in the human body is about 4 g (100). 70-90% of the body's total iron is composed of functional iron which is found in hemoglobin, myoglobin, cytochrome, and enzymes that use iron as cofactors (100,101), while the remaining 10-30% of the iron is stored as ferritin and hemosiderin in the liver, spleen, bone marrow and muscle. Approximately 3 mg which is transferrin-linked circulates in plasma (100).

It must be taken in a certain amount each day to be used in physiological events for cell metabolism, in contrast, high doses affect almost all organs. Heart, kidneys, lungs, hematological system are mainly affected and especially liver is the most affected organ. Depending on the amount absorbed, cytotoxic and lethal effects may also ocur (102,103).

When the amount of elemental iron taken is more than 20 mg / kg, the toxic effects of iron can be seen (104). These effects can be graded according to different classifications; <30 mg / kg mild, 30-60 mg / kg medium, 60-150 mg / kg heavy,> 150 mg / kg can be defined as fatal poisonings (103).

2.5.2. Nickel (Ni)

Nickel, one of the transition metals, is the 24th element found naturally in the earth's crust. It increases the hardness of alloys. Nickel which includes silver white, shiny and golden yellow color is heavy and water-resistant. Some metals that can form alloy with nickel are iron, copper, chrome and zinc. Alloys generally do not smell and taste. It is generally found in nature as a mixture of iron and nickel (105).

Nickel is the most commonly used in stainless steel and other metal materials. People might be exposed to nickel in various ways; by inhalation, drinking water, consumption of food, smoking or contact. Foodstuffs naturally contain small amounts of nickel. Foods containing most nickel; canned vegetables, sugars, jams, bread and cereal foods. Since most nickel molecules are tightly bound to other substances, the transition to foods is difficult, so it does not affect human health. Since it is easily soluble in water, it is generally found in water and waste water (106). Human body takes the 15-50% of the nickel found in drinking water. In food, this is less than 15%. 30% of inhaled nickel reaches the lungs, 20% involves in the bloodstream (107).

The daily amount of nickel consumption with drinking water and meals is 200-300 μg (108). Nickel is most commonly found in the lung, thyroid, adrenal and bone, with a small proportion of hair, breast milk and bile; may exceed the placenta. Nickel is not an accumulating toxin. Nickel is absorbed and metabolically eliminated (109). An adult weighing 70 kg has 0.5 mg of nickel in his body. The toxic dose is 1 mg / l (110). Plasma concentration of nickel does not change much in healthy individuals (107).

In addition to acute poisoning, exposure to excess nickel leads to important conditions such as lung cancer, laryngeal cancer, kidney diseases and nickel allergy caused by skin contact (111). The first signs of acute poisoning are nausea, vomiting,

headache and dizziness. In severe cases, death may occur as a result of diffuse pneumonia and brain edema due to shortness of breath, mental confusion and tremor (108).

Cytotoxic, mutagenic and allergic effects of nickel have been demonstrated by studies conducted for many years (108,112). In a study by Greig (113), an allergic reaction was observed in a 16-year-old girl due to nickel-plated headgears. Herpes lesions were observed. It has been proposed that these reactions can be prevented by polishing the metal and by applying non-allergic coatings. According to the study of Grimsdottir et al. (114), nickel released from orthodontic devices causes lymphocyte proliferation in individuals with nickel sensitivity. Contrary to these studies, there are studies indicating that nickel-based orthodontic and prosthetic applications do not show any allergic reactions in patients with nickel allergy (115,116).

2.5.3. Chromium (Cr)

The chromium element is a steel gray colored, bright and hard metal. It is also tasteless, odorless and soft. It increases the corrosion resistance and stain resistance of the alloys. Therefore, it is added to iron and nickel as ferrochrome in order to obtain alloys which are resistant to corrosion and oxidation. Today, the most commonly used metal structure is stainless steel and chrome plated metals (117,118).

Chromium exists in nature in different structures. It is most commonly found in the structures Cr^{0}, Cr^{+3} and Cr^{+6}. Cr^{0} and Cr^{+6} structures are formed as a result of industrial processes. The Cr^{+3} element, naturally found in the environment and in foods, acts as a promoter of insulin activity in humans and animals so as to help the body to use the fat, carbohydrates and protein (117,118). Therefore, it is an essential element for human metabolism and it has an important role in glucose and lipid metabolism. Cholesterol levels increase in chromium deficiency and glucose tolerance is impaired, symptoms mimic diabetes disease (119,120). The daily chrome (Cr^{+3}) requirement in adults is 50-200 micrograms (119). This amount ensures that blood sugar and cholesterol levels are kept in balance (117,120). The most common way of enterence of Cr^{+3} to human body is food consumption. Cr^{+3} is found in fresh vegetables, fruits, meats, yeast and cereals. In the human body, chromium is present intensively in the kidney and skin. The place and mechanism of absorption of chromium in humans is unknown (121). It is reported that Cr^{+3} compounds binds to plasma proteins and accumulates in the skin, lungs, muscles and fat (109).

The toxic dose of chromium (Cr^{+3}) is 0.5 milligrams. In case of exposure to Cr^{+6} more than 2 micrograms; nasal discharge, pruritus, nosebleeds, ulcers and nasal septum holes can be seen. Prolonged exposure to Cr^{+6} leads to lung cancer, especially for chrome-weighted workers (117). The relationship between lung and gastro-intestinal system tumors and chromium levels is emphasized. Chromium must be in the form of oxidation to show carcinogenic properties. Especially hexavalent oxidation form of chromium (Cr^{+6}) is very harmful because it is directly absorbed (122).

It is stated that the amount of chromium in the blood is 2.2-95.4 μg / L. 80% of the chromium entering the body is excreted with urine (123). In a study conducted by Basketter et al. (124) in 1993, the allergic effect of nickel and cobalt as well as chromium was mentioned.

2.6. Bracket System in Orthodontics

Brackets are used to transmit the biomechanical force from the wire to the teeth in fixed orthodontic treatment, so they need to have correct hardness and strength (125,126). Various types of materials and manufacturing processes have utilized in the progression of brackets (126).

Metal brackets have been preferred clinically for many years in orthodontic treatment because of their strong retention property, resistance to mastication forces and easy debonding at the end of treatment (127). At first, the metal brackets were made of various stainless steel alloys in which the base and wings were made by casting and / or machining and were assembled by soldering the different parts. By means of the progresses in technology such as laser welding and metal injection molding (MIM) and additionally in new materials, brackets made of titanium and its alloys, cobalt-chromium alloys and gold alloys have entered the orthodontic market (128). Worries about the biocompatibility of nickel-containing appliances due to metal ion release have created an attempt to get over this trouble, stainless steel alloys with reduced nickel content and nickel-free ferrous alloys have been employed. Also titanium brackets, which have biocompatible, corrosion resistant, good mechanical properties, have manufactured as an alternative from commercially pure titanium and its alloys (127). Because of the esthetic concerns in metallic orthodontic materials, ceramic and plastic brackets have preferred cosmetically. Following the introduction of polycrystalline alumina brackets in the late 1980s, both polycrystalline and single-crystal alumina brackets are commercially

available. Although it offers excellent aesthetics, there are disadvantages such as bracket fracture during ligation, fracture due to the strength of the archwires and enamel fracture at debonding phase (129). Also plastic brackets have shortcomings such as reduced hardness and wear resistance in the long term, intraoral softening, coloration, and difficulty in providing the clinically desired amounts of torque (130).

2.6.1. Stainless Steel Brackets

The majority of metal brackets are made from stainless steel (131). The predominant alloying element of stainless steels, which are iron-based alloys, is chromium, a concentration of at least 11 wt % Cr being required. Corrosion resistance can be increased by the addition of nickel and molybdenum.

Stainless steels are divided into 3 classes on the basis of their microstructure: martensitic, ferritic, or austenitic at normal temperature. The stainless steel used for orthodontic brackets is an austenitic alloy, which is nonmagnetic and is highly corrosion resistant to all media, except hydrochloric acid and other halide acids. Most commercially available stainless steel brackets are made from American Iron And Steel Institute (AISI) classification type 303, 304, 304L and 316L steels in which the amount of carbon decreases as the numerical nomenclature increases. The suffix "L" denotes low carbon. The decrease in carbon supports to increased passivating properties, rendering the austenitic alloy less susceptible to corrosion (132).

AISI type 304L SS contains 18–20 % chromium and 8–10 % nickel with a low quantity of manganese and silicon, and has a low carbon content, typically less than 0.03 % and many of orthodontic brackets are made from this type of SS. Manufacturers use different types of them according to their properties such as 316 L SS is used for its better welding and intergranular corrosion resistance, 17-4 PH SS is used for its higher mechanical property and corrosion resistance. Nevertheless, localized corrosion of these materials usually can develop in the oral environment due to their low localized corrosion resistance in a solution containing aggressive chloride ions (133).

Maijer and Smith (66) found in their *in-vitro* study evaluating the biodegradation of the orthodontic bracket system that bond strength and corrosion resistance were noticeably reduced in recycled brackets.

There are main elements added to the alloy in the production of stainless steel. Carbon increases the hardness and durability of the alloy. The risk of chromium-carbide

formation due to localized corrosion in oral fluids increases with increasing carbon content. Chromium increases oxidation resistance, the thin passive chromium oxide layer prevents oxygen diffusion and surface corrosion. Nickel forms austenitic phase and improves corrosion and oxidation resistance. Due to the weak bonds between the atoms of nickel, the possibility of the release of nickel ions into the oral environment is high. Manganese is used as an alternative element to nickel. Nitrogen has the effect of increasing the stability of the austenitic phase. Molybdenum increases the resistance to corrosion caused by chloride ions. Titanium increases carbide stabilization and corrosion resistance. Phosphorus increases corrosion resistance and resistance. Niobium and Tantalum are added to stabilize carbon and increase corrosion resistance. Copper is added to produce the precipitation hardening properties. Selenium and Sulfur are added to make the steel more workable and usable; it also reduces hardness and strength (134).

For stainless steel brackets, Ni, Cr and Fe; for Ni-Ti wire alloys, Ni and Ti are the major corrosion products (135).

2.6.2. Elemental Content of Brackets

Elemental content of some types of brackets used in orthodontics is shown in Table 2.1. (109,136).

Table 2. 1. The percentages of elemental content of some types of brackets used in Orthodontics (109).

	Ormco	Dentaurum	Forestadent	Forestadent (Ti-coated)	Unitek
Nickel (Ni)	8	10	8	9	8
Chromium (Cr)	18	19	19	18	19
Iron (Fe)	72	69	69	70	71
Manganese (Mn)	2	2	2	2	2

2.7. Examining the Surface Roughness of Orthodontic Brackets

Due to the adsorption and calcification of the biofilm layer, the increase in porosity and roughness can occur in orthodontic appliances, resulting in increased friction between bracket-arch wire and false torque expression (11,137,138). The brackets are the longest remaining orthodontic appliance in the mouth (139). In addition to the deterioration of the aesthetics due to coloration on the brackets, the changes in the surface roughness also affect the performance of the sliding mechanics by changing the friction coefficient. Friction force can reduce the effectiveness of the applied orthodontic force by 50% or more (140).

In previous studies, surface roughness of brackets and wires were examined with these devices: scanning electron microscopy (SEM) (141,142), a contact surface profilometer (138,143), atomic force microscopy (AFM) (138,144–147) and optical profilometer (139,148).

When SEM is used, surface morphology is obtained in two dimensions and quantitative information is not obtained about the selected region. For AFM, where quantitative measurements are obtained in addition to the 3D configuration, it is disadvantageous that the sample preparation is required and the measurement range is in the micrometer scale. Depending on these, grinding of the bracket wings can damage the slot surface and the macroscopic properties may not be well defined (139,148).

The most commonly used technique for determining the surface roughness is the surface profilometer(138). Surface profilometers can be examined under three headings: contact tip profilometer, laser profilometers and non-contact optical profilometers (149). The surface roughness parameter values are visualized and defined, by contact profilometer (139). The contact profilometer scans with a sharp-tipped pencil on the surface to be examined (138). The calculated region is in the form of a line and the sample may be damaged due to the diamond tip (139). The laser profilometer may form a surface topography without contacting the surface, either by measuring the deviation of the laser beam or by using the confocal principle with white light. It is affected by the color of the surface (150,151).

In non-contact optical profilometers, the surface roughness profile can be determined in three dimensions as a result of the reflection of the light beams sent from the optical end of the device to the surface (152,153). The surface roughness results obtained while the scanner is moving on the sample surface are digitally calculated and

recorded (138). These devices have high stability and are able to easily view scratch ranges which are 0.5 microns. For stylus devices, stability falls below 2.5 microns. The main advantages of non-contact optical profilometers are: it can easily obtain detailed images even if the surface is complicated in the investigated area, does not damage the samples to be examined and the device does not require repeated calibrations (152,153).

Numerical measurements with optical profilometer are expressed in various parameters: Ra (arithmetical mean value of the movement of the profile above and below the center line of the surface); Rt (depth of the maximum roughness); Rp (roughness with maximum depth within the Rz); and Rmax (mean depth of roughness among five adjacent spaces) (154). Liu et al. (139) reported that the optical profilometer is faster and non-destructive compared with the stylus profilometer, provides a larger area that does not require sample preparation compared to AFM, and their data may better show the entire characteristics of the bracket slot.

2.8. Investigation of Ion Release from Orthodontic Brackets

2.8.1. Inductively Coupled Plasma – Mass Spectrometer (ICP-MS)

ICP-MS consists of a combination of Inductive Coupled Plasma (ICP) and Mass Spectrometry (MS) and has the ability to analyze a large number of elements simultaneously, quickly and precisely compared with atomic absorption techniques. The elements in the sample to be analyzed are sent to mass spectroscopy after ionization in ICP and measured by mass / charge (m / z) ratios (155). ICP-MS is suitable for direct determination of trace element concentrations in solution. The working range is quite wide compared to other methods and calibration graphs between pg-mg / l can be drawn for many elements. It allows simultaneous analysis of several elements with different concentrations. In addition to liquid samples, it is frequently used in the analysis of solid samples. ICP-MS analysis is widely used in medical and forensic fields, as well as environmental studies. In summary, ICP-MS is used for counting and conducting elemental analysis of metals, proteins and biomolecules (136,156).

One of the most important steps of the ICP-MS analysis technique is the sample preparation step. It is preferred that certain substances in the liquid sample to be analyzed are below certain limit values in order not to damage the device. The samples should also not contain suspended solid particles. Some of these samples to be analyzed must be passed through a 0.45 micron filter. To eliminate the matrix effect, it is important that

samples and standards are prepared in the same acid solution (typically 2% HNO_3). Since the ICP-MS measuring range is in the parts per billion (ppb) – parts per trillion (ppt) range, dilution is required to bring the more concentrated analytes into this measuring range (136,156).

3.MATERIALS and METHODS

3.1. Materials

3.1.1. Simulated Gastric Solution

Simulated gastric solutions (experimental groups) (Figure 3.1.) was prepared at Yeditepe University Faculty of Pharmacy by dissolving 2.0 g of sodium chloride (NaCl) and 3.2 g of pepsin (Pepsin from gastric mucosa, P6887, Sigma, Steinheim, Germany) (Figure 3.2.) in 7.0 mL of hydrochloric acid (HCl)(d=1.15 37%) and water to make up 1.000 mL (157,158). The pH of the solutions were measured and adjusted to pH 1.5 (severe form of gastric regurgitation) and pH 3.0 (moderate form of gastric regurgitation) by using pH meter (Mettler Toledo MP220, Switzerland) (159).

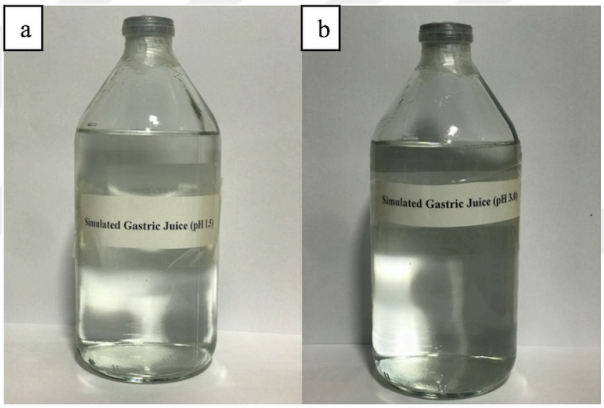

Fig. 3. 1. Simulated gastric solutions (a) pH 1.5, (b) pH 3.0

Fig. 3. 2. Pepsin from gastric mucosa

3.1.2. Artificial Saliva

Artificial saliva (control group) (Figure 3.3.) was prepared at Yeditepe University Faculty of Pharmacy. The formulation of the saliva-substitute solution used in the study was modified according to related manuscript which contains 0.65 g/L potassium chloride, 0.058 g/L magnesium chloride, 0.165 g/L calcium chloride, 0.804 g/L dipotassium hydrogen phosphate, 0.365 g/L potassium dihydrogen phosphate, 2 g/L sodium benzoate, 1 g/L hydroxypropyl cellulose, 0.0003 g/L sodium fluoride, deionized water to make 1L and 1% methyl cellulose was also added to obtain similar viscosity (160). The pH of the artificial saliva was measured and adjusted to pH 7.0 by using pH meter (Mettler Toledo MP220, Switzerland).

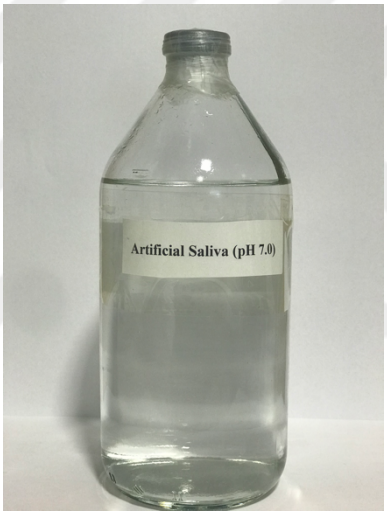

Fig. 3. 3. Artificial Saliva pH 7.0

3.1.3. Brackets

This study was conducted on 180 stainless steel (SS) upper central incisor brackets (Dyna-lock Standard Edge-wise 0.018) (3M, Unitek, Puchheim, Germany) (Figure 3.4. a, b). Elemental composition analysis of the brackets were measured by Energy Distribution Spectrometer (EDS) (JEOL/JSM-5410). The percentages of silicium (Si), chromium (chrome), manganese (Mn), iron (Fe), nickel (Ni), copper (Cu), molybdenum (Mo) elements are given in Table 3.1. and Figure 3.5..

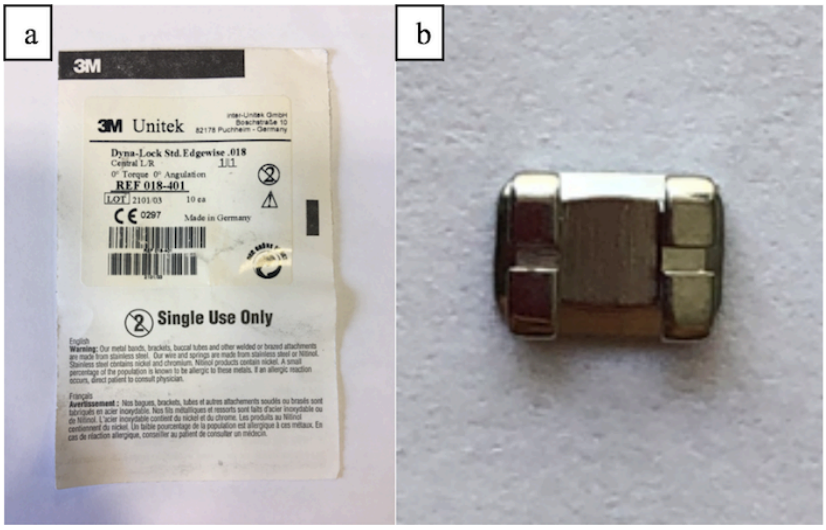

Fig. 3. 4. Stainless steel upper central incisor brackets (a) Dyna-lock Standard Edge-wise 0.018 (3M, Unitek, Puchheim, Germany), (b) close view of the bracket

Table 3. 1. The percentages of the elements measured by EDS.

Element	Si	Cr	Mn	Fe	Ni	Cu	Mo	Total
Atomic %	2.179	18.8520	0.923	69.4935	7.639	0.532	0.3815	100

kV 15.0, Takeoff Angle 25.0°, Elapsed Livetime 30.0

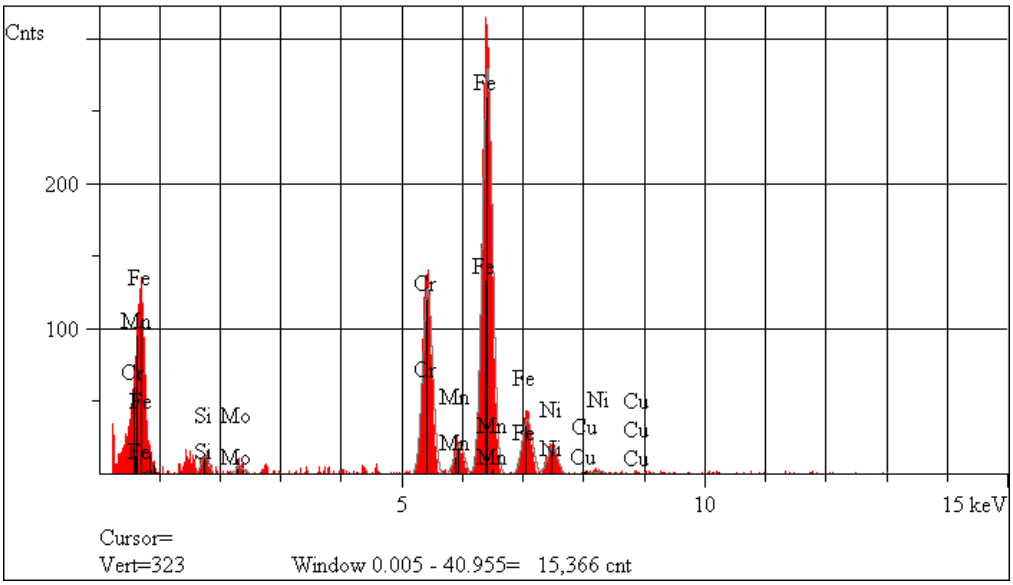

Fig. 3. 5. The elemental spectrum measured by EDS.

3.1.4. Airtight Glass Tubes

1.5 ml airtight glass tubes (La Pha Pack, Germany) (Figure 3.6.) (161) were used to store the study samples. Each glass tube contained one bracket with in 1 ml solution.

Fig. 3. 6. 1.5 ml airtight glass tubes (161)

3.1.5. Incubator

Each bracket was immersed in the prepared test solutions and all of the specimens were kept in an incubator (Memmert, Germany) (Figure 3.7.) all throughout the experiment at 37±0.1 °C at Yeditepe University Soft Tissue Laboratory.

Fig. 3. 7. Incubator

3.1.6. Optic Profilometer

The average surface roughness (Ra) of the bracket slot base was examined with a 3D non-contact optical surface profilometer machine by one examiner (S.S.Ö.) at the Department of Metallurgical and Materials Engineering at Istanbul Technical University (Veeco NT1100, Wyco, New York, USA) (Figure 3.8.). The device is placed in a silent room on a vibration isolation table.

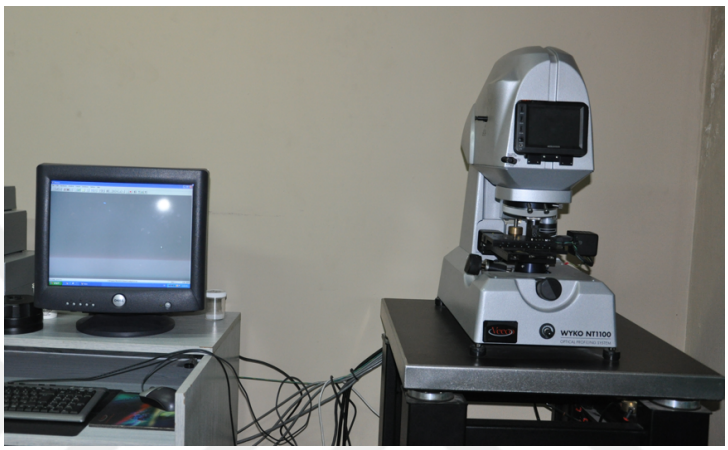

Fig. 3. 8. Optic Profilometer

3.1.7. Inductively Coupled Plasma – Mass Spectrometer (ICP-MS)

In order to determine the metal ions released from the brackets to the solutions, the ICP-MS (Agilent 7700x, USA) (Figure 3.9.) (162) device was used which is found in the AND Analysis and Laboratory Services.

Fig. 3. 9. Inductively Coupled Plasma – Mass Spectrometer (ICP-MS) (162)

3.2. Methods

3.2.1. *In-vitro* Study

3.2.1.1. Experimental Groups

Brackets (N=180) were randomly divided equally into 3 main groups (according to the pH) and 15 subgroups (according to the time elapsed in solutions) (n=12). The pH values forming the main three groups contains hydrochloric acid: simulating severe (pH 1.5) and moderate (pH 3.0) forms of gastric regurgitation and artificial saliva (pH 7.0) as the control group. Each pH group were then divided into 5 subgroups according to the immersion time in the solutions; at the end of 30 minutes, 12 hours, a day (24 hours), a week (7 days), a month (30 days). Schematic description of experimental groups are shown in Table 3.2. in terms of surface roughness (SR) parameter and ion release (IR) parameter.

Table 3. 2. Schematic description of experimental groups in terms of surface roughness (SR) and ion release (IR) parameter.

		Solutions		
Duration	**Parameter**	pH 1.5	pH 3.0	pH 7.0
30 minutes	SR	n=12		
	IR	n=9		
12 hours	SR	n=12		
24 hours	SR	n=12		
	IR	n=9		
7 days	SR	n=12		
30 days	SR	n=12		
	IR	n=9		

3.2.1.2. Preparation of the Experimental Environment

The brackets were washed with distilled water (Figure 3.10. (a)) in order to form a better relationship with the environment before they were exposed to solutions, and then they were dried with a cold dryer (Figure 3.10. (b)).

After the brackets were placed in glass tubes, 1 ml of solution was added to each 1.5 ml glass tube. A separate syringe was used for each pH solution. Each group was kept in the incubator at 37°C for the time indicated for itself.

After the specified times were completed, the brackets were removed from the glass tubes by a thin tweezer. The same procedure, washing the brackets with distilled water and air-drying, was repeated after exposure to solutions, the brackets were kept in paper envelopes to avoid scratching their surfaces (Figure 3.10. (c)). These procedures were conducted by one investigator (E.A.Ö.). The average surface roughness of the bracket slots was examined using an optical profilometer by one investigator (S.S.Ö.). The solutions with the corrosive product extracts were stored in glass tubes until the metal ions in the solution were examined. The solutions were evaluated for the amount of ions released from samples by using ICP-MS.

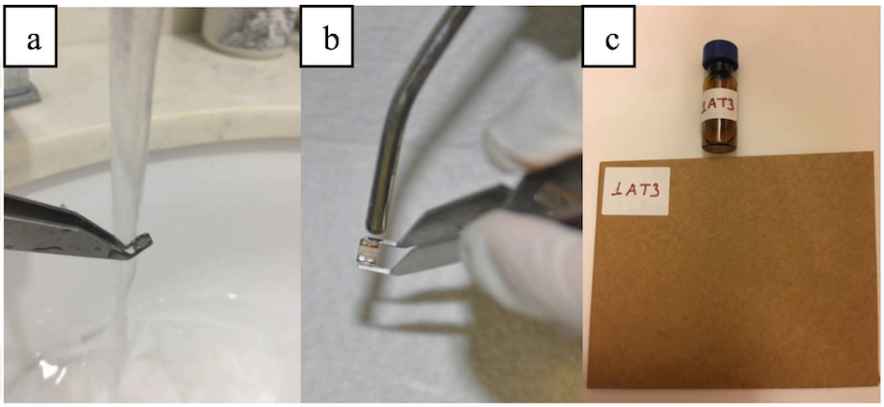

Fig. 3. 10. Procedures after exposure to solutions. (a) washing the bracket with distilled water, (b) air-drying, (c) keeping the brackets in paper envelopes

3.2.2. Surface Roughness Examination with Optical Profilometer

The initial surface roughness (ISR) of all brackets were measured before the experiment. After immersion periods of 30 minutes, 12 hours, a day (24 hours), a week (7 days), a month (30 days) the brackets were removed from the solutions and the final surface roughness (FSR) of each bracket slot were examined by optical profilometer (Figure 3.11. (a)). Measurements were performed on all samples which kept at pH 1.5, pH 3.0 and pH 7.0, respectively. Each of the samples was placed on a flat surface of the profilometer machine with a tweezer.

Non-contact, white-light vertical interferometer was used for measurements (Figure 3.11. (b)), using these parameters: magnification 5.1x; sampling 1.64 um and array size 736 x 480 (Figure 3.11. (c)). The device is operated in VSI mode. Three

measurements were made on the floor of the bracket slots, and the mean value was calculated. All measurements were saved in nanometers (nm). 3D images were captured using Wyko Vision 32® (New York, USA). Thermal was selected in the color settings when the image was saved.

Fig. 3. 11. Surface roughness measurements were obtained by optical profilometer. (a) Optic Profilometer Machine, (b) Non-contact, white-light interferometer, (c) Device setting parameters

3.2.3. Inductively Coupled Plasma – Mass Spectrometer (ICP-MS) Measurements

For ICP-MS examination, 1 ml solutions were taken from 9 samples in all pH groups after incubation for 30 minutes, 24 hours and 1 month. Immersion solutions were analyzed for iron (Fe), nickel (Ni) and chromium (Cr) ions (Figure 3.12.) (163).

Autotune was performed using commercially available tune solution and P / A solution to perform device optimization. After autotuning, the tune report was evaluated which was obtained by reporting tune data. The performance of the device was found to be suitable for operation. A 6-point calibration curve was drawn in accordance with the elements (Fe, Ni, Cr) to be analyzed in the sample. Then, the results were obtained in miligram/liter (mg/l) by analyzing the sample. Once the ICP device was calibrated and a standard calibration curve obtained, the samples were analyzed and the readings were recorded. Before each reading, 2 ml of 2% nitric acid was added to the 1 ml samples in order to make the metals soluble.

The results obtained were used to compare the ion release at different times and to compare the effect of different pHs on metal release.

Fig. 3. 12. Released metal ions in the solutions were analyzed with ICP-MS. (163)

3.2.4. Statistical Analysis of Results

In this study, statistical analysis was performed with NCSS (Number Cruncher Statistical System) 2007 Statistical Software (Utah, USA). Descriptive statistical methods (mean, standard deviation, median, interquartil range) as well as Shapiro - Wilk normality

test were used to evaluate the distribution of variables. One-way analysis of variance in intergroup comparisons for variables showing normal distribution, Tukey multiple comparison test was used for subgroup comparisons, Kruskal Wallis test for intergroup comparisons, and Dunn's multiple comparison test for subgroup comparisons. The results were evaluated at $p < 0.05$ level of significance.

4.RESULTS

4.1. Surface Roughness Findings

The mean initial surface roughness (ISR) of all untreated brackets (N = 180), measured by optical profilometer was shown in Table 4.1. and no statistically significant difference was observed between the mean values of ISR of all untreated brackets (p> 0.05).

Table 4. 1. Evaluation of mean initial surface roughnesses (ISRs) (nm) of all untreated brackets.

Initial Surface Roughness (ISR) (nm)	pH 1.5	pH 3.0	pH 7.0	p*
30 minutes	610.13±52.55	601.74±55.16	596.5±38.92	0.794
12 hours	603.77±54.41	605.32±55.8	593.99±62.75	0.874
24 hours	608.57±63.63	614.51±35.86	584.31±69.56	0.414
1 week	608.62±46.22	586.64±76.66	627.97±56.13	0.266
1 month	627.22±62.8	586.8±55.5	618.28±52.6	0.255
p*	0.549	0.508	0.311	

*One-way ANOVA test, *p<0.05*

After immersions, final surface roughness (FSR) of each bracket slot were examined and the results were compared statistically in all main pH groups and time subgroups. Mean FSRs were shown in Table 4.2..

When the pH groups were evaluated at different time intervals, statistical differences were observed between them, when the mean values of pH 1.5, pH 3.0 and pH 7.0 groups' FSR at 30 minutes and 12 hours were evaluated, no statistically significant difference was observed (p>0.05) (Table 4.2.).

On the other hand a statistically significant difference was observed between the mean values of pH 1.5, pH 3.0 and pH 7.0 groups' FSR at 24 hours (p = 0.0001) (Table 4.2.). The mean values of pH 7.0 group's FSR was found to be significantly lower than the mean values of pH 1.5 and pH 3.0 groups' FSR at 24 hours (p = 0.0001, p =0.001) while no statistically significant difference was observed between the mean values of pH 1.5 and pH 3.0 Groups' FSR at 24 hours (p = 0.417) (Table 4.3.).

A statistically significant difference was observed between the mean values of pH 1.5 , pH 3.0 ve pH 7.0 groups' FSR at 1 week (p=0.0001) (Table 4.2.). The mean values

of pH 1.5 group's FSR was found to be significantly higher than the mean values of pH 3.0 and pH 7.0 groups' FSR (p=0.0001, p=0.0001), while no statistically significant difference was observed between the mean values of pH 3.0 and pH 7.0 groups' FSR (p=0.990) (Table 4.3.).

A statistically significant difference was observed between the mean values of pH 1.5, pH 3.0 and pH 7.0 groups' FSR at 1 month (p=0.0001) (Table 4.2.). The mean values of pH 1.5 group's FSR was found to be significantly higher than the mean values of pH 3.0 and pH 7.0 groups' FSR (p=0.0001, p=0.0001), while no statistically significant difference was observed between the mean values of pH 3.0 and pH 7.0 groups' FSR (p=0.963) (Table 4.3.).

At different time intervals, each pH group also showed statistical differences within its group (Table 4.2.).

A statistically significant difference was observed between the mean values of pH 1.5 group's FSR at 30 minutes, 12 hours, 24 hours, 1week and 1 month (p=0.0001) (Table 4.2.). The mean values of 30 minutes group's FSR was found to be significantly lower than the mean values of 24 hours, 1 week and 1 month groups' FSR (p=0.009, p=0.0001, p=0.0001), likewise the mean values of 12 hours group's FSR was found to be significantly lower than the mean values of 24 hours, 1 week and 1 month groups' FSR (p=0.007, p=0.0001, p=0.0001). Also the mean values of 1 month group's FSR was found to be significantly higher than the mean values of 24 hours and 1 week groups' FSR (p=0.0001, p=0.0001), whereas no statistically significant difference was observed between the other times (p> 0.05) (Table 4.4.).

Significant differences were observed between the mean values of pH 3.0 group's FSR at different time intervals (p=0.01) (Table 4.2.), but only the mean values of 30 minutes group's FSR was found to be statistically significantly lower than the mean values of 24 hours and 1 month groups' FSR (p=0.021, p=0.049) (Table 4.4).

A statistically significant difference was observed between the mean values of pH 7.0 group's FSR at all time subgroups (p=0.0001) (Table 4.2.). The mean values of 30 minutes group's FSR was found to be significantly lower than the mean values of 1 week and 1 month groups' FSR (p=0.011, p=0.001), the mean values of 12 hours group's FSR was found to be significantly lower than the mean values of 1 week and 1 month groups' FSR (p=0.021, p=0.002), the mean values of 24 hours group's FSR was found to be significantly lower than the mean values of 1 week and 1 month groups' FSR (p=0.006,

p=0.0001), whereas no statistically significant difference was observed between the other times (p> 0.05) (Table 4.4.).

Table 4. 2. Evaluation of mean final surface roughnesses (FSRs) (nm) of each bracket slot after immersions.

Final Surface Roughness (FSR) (nm)	pH 1.5	pH 3.0	pH 7.0	p*
30 minutes	627.59±52.63	619.82±58.37	600.65±38.86	0.416
12 hours	624.39±54.71	638.55±59	605.95±61.74	0.403
24 hours	729.81±63.23	698.54±44.36	596.07±69.2	**0.0001**
1 week	811.54±75.36	674.42±78.55	678.25±54.14	**0.0001**
1 month	991.6±105.1	690.28±59.99	698.37±51.44	**0.0001**
p*	**0.0001**	**0.01**	**0.0001**	

*One-way ANOVA test, *p<0.05*

Table 4. 3. Comparison of pH groups at different time intervals.

Tukey multiple comparison test	30 minutes	12 hours	24 hours	1 week	1 month
pH 1.5 / pH 3.0			0.417	**0.0001**	**0.0001**
pH 1.5 / pH 7.0			**0.0001**	**0.0001**	**0.0001**
pH 3.0 / pH 7.0			**0.001**	0.990	0.963

p<0.05

Table 4. 4. Comparison of time subgroups at different pH values.

Tukey Multiple Comparison Test	pH 1.5	pH 3.0	pH 7.0
30 minutes/12 hours	0.999	0.943	0.999
30 minutes/24 hours	**0.009**	**0.021**	0.999
30 minutes/1 week	**0.0001**	0.198	**0.011**
30 minutes/1 month	**0.0001**	**0.049**	**0.001**
12 hours/24 hours	**0.007**	0.129	0.993
12 hours/1 week	**0.0001**	0.605	**0.021**
12 hours/1 month	**0.0001**	0.245	**0.002**
24 hours/1week	0.059	0.868	**0.006**
24 hours/1 month	**0.0001**	0.997	**0.0001**
1 week/1 month	**0.0001**	0.968	0.903

p<0.05

After immersions, the difference between final and initial surface roughness (FSR-ISR) of each bracket slot were also examined and the results were compared

statistically in all main pH groups and time subgroups. Mean FSR-ISR were shown in Table 4.5..

When the pH groups were evaluated at different time intervals, statistical differences were observed between them (Table 4.5.).

A statistically significant difference was observed between the mean values of pH 1.5, pH 3.0, pH 7.0 groups' FSR-ISR at 30 minutes (p=0.0001) (Table 4.5.). The mean values of pH 7.0 group's FSR-ISR at 30 minutes was found to be significantly lower than the mean values of pH 1.5 and pH 3.0 groups' FSR-ISR (p=0.0001, p=0.0001), no statistically significant difference was observed between pH 1.5 and pH 3.0 groups' FSR-ISR at 30 minutes (p=0.978) (Table 4.6.).

A statistically significant difference was observed between the mean values of pH 1.5, pH 3.0, pH 7.0 groups' FSR-ISR at 12 hours (p=0.0001) (Table 4.5.). The mean values of pH 3.0 group's FSR-ISR at 12 hours was found to be significantly higher than the mean values of pH 1.5 and pH 7.0 groups' FSR-ISR (p=0.035, p=0.0001), whereas no statistically significant difference was observed between the mean values of pH 1.5 and pH 7.0 groups' FSR-ISR (p=0.186) (Table 4.6.).

A statistically significant difference was observed between the mean values of pH 1.5, pH 3.0, pH 7.0 groups' FSR-ISR at 24 hours (p=0.0001) (Table 4.5.). The mean values of pH 1.5 group's FSR-ISR at 24 hours was found to be significantly higher than the mean values of pH 3.0 and pH 7.0 groups' FRS-ISR (p=0.0001, p=0.0001), and likewise the mean values of pH 3.0 group's FSR-ISR at 24 hours was found to be significantly higher than the mean values of pH 7.0 group's FSR-ISR (p=0.0001) (Table 4.6.).

A statistically significant difference was observed between the mean values of pH 1.5, pH 3.0, pH 7.0 groups' FSR-ISR at 1 week (p=0.0001) (Table 4.5.). The mean values of pH 1.5 group's FSR-ISR at 1 week was found to be significantly higher than the mean values of pH 3.0 and pH 7.0 groups' FRS-ISR (p=0.0001, p=0.0001), the mean values of pH 3.0 group's FSR-ISR at 1 week was significantly higher than the mean values of pH 7.0 group's FSR-ISR (p=0.002) (Table 4.6.).

A statistically significant difference was observed between the mean values of pH 1.5, pH 3.0, pH 7.0 groups' FSR-ISR at 1 month (p=0.0001) (Table 4.5.). The mean values of pH 1.5 group's FSR-ISR at 1 month was found to be significantly higher than the mean values of pH 3.0 and pH 7.0 groups' FSR-ISR (p=0.0001, p=0.0001), whereas

no statistically significant difference was observed between the mean values of pH 3.0 and pH 7.0 groups' FSR-ISR at 1 month (p=0.110) (Table 4.6.).

At different time intervals, each pH group also showed statistical differences within its group (Table 4.5.).

A statistically significant difference was observed between the mean values of pH 1.5 group's FSR-ISR at all time subgroups (p=0.0001) (Table 4.5.). The mean values of 30 minutes group's FSR-ISR was found to be significantly lower than the mean values of 24 hours, 1 week and 1 month group's FSR-ISR (p=0.0001, p=0.0001, p=0.0001), the mean values of 12 hours group's FSR-ISR was found to be significantly lower than the mean values of 24 hours, 1 week, 1 month groups' FSR-ISR (p=0.0001, p=0.0001, p=0.0001), the mean values of 24 hours group's FSR-ISR was found to be significantly lower than the mean values of 1 week and 1 month groups' FSR-ISR (p=0.0001, p=0.0001), the mean values of 1 week group's FSR-ISR was found to be significantly lower than the mean values of 1 month group's FSR-ISR (p=0.0001), whereas no statistically significant difference was observed between the other times (p>0.05) (Table 4.7.).

A statistically significant difference was observed between the mean values of pH 3.0 group's FSR-ISR at all time subgroups (p=0.0001) (Table 4.5.). The mean values of 30 minutes group's FSR-ISR was found to be significantly lower than the mean values of 24 hours, 1 week and 1 month group's FSR-ISR (p=0.0001, p=0.0001, p=0.0001), the mean values of 12 hours group's FSR-ISR was found to be significantly lower than the mean values of 24 hours, 1 week and 1 month groups' FSR-ISR (p=0.0001, p=0.0001, p=0.0001), the mean values of 24 hours group's FSR-ISR was found to be significantly lower than the mean values of 1 month groups' FSR-ISR (p=0.0001), the mean values of 1 week group's FSR-ISR was found to be significantly lower than the mean values of 1 month group's FSR-ISR (p=0.001), whereas no statistically significant difference was observed between the other times (p>0.05) (Table 4.7.).

A statistically significant difference was observed between the mean values of pH 7.0 group's FSR-ISR at all time subgroups (p=0.0001) (Table 4.5.). The mean values of 30 minutes group's FSR-ISR was found to be significantly lower than the mean values of 1 week and 1 month group's FSR-ISR (p=0.0001, p=0.0001), the mean values of 12 hours group's FSR-ISR was found to be significantly lower than the mean values of 1 week and 1 month groups' FSR-ISR (p=0.0001, p=0.0001), the mean values of 24 hours group's FSR-ISR was found to be significantly lower than the mean values of 1 week and 1 month

groups' FSR-ISR (p=0.0001, p=0.0001), the mean values of 1 week group's FSR-ISR was found to be significantly lower than the mean values of 1 month group's FSR-ISR (p=0.0001), whereas no statistically significant difference was observed between the other times (p>0.05) (Table 4.7.).

Table 4. 5. Mean change of surface roughness (FSR-ISR) (nm).

Change of SR (FSR-ISR)(nm)	pH 1.5	pH 3.0	pH 7.0	p*
30 minutes	17.46±9.25	18.07±7.96	4.14±4.32	**0.0001**
12 hours	20.62±14.03	33.23±13.77	11.96±5.62	**0.0001**
24 hours	121.24±14.58	84.03±15.52	11.76±7.75	**0.0001**
1 week	202.92±38.85	87.78±10.92	50.28±14.1	**0.0001**
1 month	352.38±59.71	113.48±22.64	80.09±23.65	**0.0001**
p*	**0.0001**	**0.0001**	**0.0001**	

*One-way ANOVA test, *p<0.05*

Table 4. 6. Comparison of pH groups' FSR-ISR at different time intervals.

Tukey multiple comparison test	30 minutes	12 hours	24 hours	1 week	1 month
pH 1.5 / pH 3.0	0.978	**0.035**	**0.0001**	**0.0001**	**0.0001**
pH 1.5 / pH 7.0	**0.0001**	0.186	**0.0001**	**0.0001**	**0.0001**
pH 3.0 / pH 7.0	**0.0001**	**0.0001**	**0.0001**	**0.002**	0.110

p<0.05

Table 4. 7. Comparison of time subgroups' FSR-ISR at different pH values.

Tukey multiple comparison test	pH 1.5	pH 3.0	pH 7.0
30 minutes/12 hours	0.999	0.111	0.597
30 minutes/24 hours	**0.0001**	**0.0001**	0.621
30 minutes/1 week	**0.0001**	**0.0001**	**0.0001**
30 minutes/1 month	**0.0001**	**0.0001**	**0.0001**
12 hours/24 hours	**0.0001**	**0.0001**	0.999
12 hours/1 week	**0.0001**	**0.0001**	**0.0001**
12 hours/1 month	**0.0001**	**0.0001**	**0.0001**
24 hours/1 week	**0.0001**	0.973	**0.0001**
24 hours/1 month	**0.0001**	**0.0001**	**0.0001**
1 week/1 month	**0.0001**	**0.001**	**0.0001**

p<0.05

4.2. Ion Release Findings

4.2.1. Iron (Fe)

When Fe release of pH groups were compared at different time intervals, a statistically significant difference was observed between the mean values of all the pH groups at 30 minutes (p=0.0001) (Table 4.8.). The mean values of pH 1.5 group's Fe release at 30 minutes was found to be significantly higher than pH 3.0 and pH 7.0 groups' mean values (p=0.005, p=0.0001). Likewise the mean values of pH 3.0 group's Fe release at 30 minutes was found to be significantly higher than pH 7.0 group's mean value (p=0.0001) (Table 4.9.).

A statistically significant difference was observed between the mean values of all pH groups' Fe release at 24 hours (p=0.0001) (Table 4.8.). The mean values of pH 1.5 group's Fe release at 24 hours was found to be significantly higher than pH 3.0 and pH 7.0 groups' mean values (p=0.0001, p=0.0001), the mean values of pH 3.0 group's Fe release at 24 hours was found to be significantly higher than pH 7.0 group's mean value (p=0.0001) (Table 4.9.).

A statistically significant difference was observed between the mean values of all pH groups' Fe release at 1 month (p=0.0001) (Table 4.8.). The mean values of pH 1.5 group's Fe release at 1 month was found to be significantly higher than the mean values of pH 3.0 and pH 7.0 groups' Fe release (p=0.0001, p=0.001). Also, the mean values of pH 3.0 group's Fe release at 1 month was found to be significantly higher than pH 7.0 group's mean value (p=0.0001) (Table 4.9.).

When the Fe release at different time subgroups were compared, statistically significant differences were observed at each pH value. A statistically significant difference was observed between the mean values of pH 1.5 group's Fe release at 30 minutes, 24 hours and 1 month (p=0.0001) (Table 4.8.). The mean values of 30 minutes group's Fe release was found to be significantly lower than the mean values of 24 hours and 1 month groups' (p=0.0001, p=0.0001), whereas no statistically significant difference was observed between the mean values of 24 hours and 1 month groups' Fe release (p=0.354) (Table 4.10.).

A statistically significant difference was observed between the mean values of pH 3.0 group's Fe release at 30 minutes, 24 hours and 1 month (p=0.0001) (Table 4.8.). The mean values of 30 minutes group's Fe release was found to be significantly lower than the mean values of 24 hours and 1 month groups' (p=0.001, p=0.0001). Also, the mean

values of 24 hours group's Fe release was found to be significantly lower than 1 month group's mean value (p=0.0001) (Table 4.10.).

A statistically significant difference was observed between the mean values of pH 7.0 group's Fe release at 30 minutes, 24 hours and 1 month (p=0.0001) (Table 4.8.). The mean values of 1 month group's Fe release was found to be significantly higher than the mean values of 30 minutes and 24 hours groups' Fe release (p=0.0001, p=0.001), whereas no statistically significant difference was observed between 30 minutes and 24 hours groups' mean values (p=0.092) (Table 4.10.).

Table 4. 8. Evaluation of Fe ion release (mg/l).

Fe (mg/L)		pH 1.5	pH 3.0	pH 7.0	p‡
30 minutes	**Mean±SD**	0.091±0.014	0.055±0.026	0.015±0.003	**0.0001**
24 hours	**Mean±SD**	6.62±3.159	0.108±0.022	0.041±0.03	**0.0001**
1 month	**Mean±SD**	8.478±4.987	0.481±0.083	0.089±0.01	**0.0001**
	p‡	**0.0001**	**0.0001**	**0.0001**	

‡Kruskal Wallis test, *p<0.05*

Table 4. 9. Comparison of pH groups' Fe ion release at different time intervals.

Dunn's Multiple Comparison Test	30 minutes	24 hours	1 month
pH 1.5 / pH 3.0	**0.005**	**0.0001**	**0.0001**
pH 1.5 / pH 7.0	**0.0001**	**0.0001**	**0.001**
pH 3.0 / pH 7.0	**0.0001**	**0.0001**	**0.0001**

p<0.05

Table 4. 10. Comparison of time subgroups' Fe ion release at different pH values.

Dunn's Multiple Comparison Test	pH 1.5	pH 3.0	pH 7.0
30 minutes / 24 hours	**0.0001**	**0.001**	0.092
30 minutes / 1 month	**0.0001**	**0.0001**	**0.0001**
24 hours / 1 month	0.354	**0.0001**	**0.001**

p<0.05

4.2.2. Nickel (Ni)

When Ni release of pH groups were compared at different time intervals, no statistically significant difference was observed between the mean values of all the pH groups at 30 minutes (p=0.203) (Table 4.11.).

A statistically significant difference was observed between the mean values of pH 1.5, pH 3.0 and pH 7.0 groups' Ni release at 24 hours (p=0.001) (Table 4.11.). The mean values of pH 7.0 group's Ni release at 24 hours was found to be significantly lower than pH 1.5 and pH 3.0 groups' mean values (p=0.0001, p=0.005), whereas no statistically significant difference was observed between pH 1.5 and pH 3.0 groups' mean values (p=0.352) (Table 4.12.).

A statistically significant difference was observed between the mean values of all pH groups' Ni release at 1 month (p=0.002) (Table 4.11.). The mean values of pH 7.0 group's Ni release at 1 month was found to be significantly lower than the mean values of pH 1.5 and pH 3.0 groups' Ni release (p=0.043, p=0.005), whereas no statistically significant difference was observed between pH 1.5 and pH 3.0 groups' mean values (p=0.133) (Table 4.12.).

When the Ni release at different time subgroups were compared, statistically significant differences were observed at each pH value. A statistically significant difference was observed between the mean values of pH 1.5 group's Ni release at 30 minutes, 24 hours and 1 month (p=0.0001) (Table 4.11.). The mean values of 30 minutes group's Ni release was found to be significantly lower than the mean values of 24 hours and 1 month groups' (p=0.0001, p=0.001). Also, the mean values of 24 hours group's Ni release was found to be significantly lower than 1 month group's mean value (p=0.031) (Table 4.13.).

A statistically significant difference was observed between the mean values of pH 3.0 group's Ni release at 30 minutes, 24 hours and 1 month (p=0.0001) (Table 4.11.). The mean values of 1 month group's Ni release was found to be significantly higher than the mean values of 30 minutes and 24 hours groups' Ni release (p=0.004, p=0.004), whereas no statistically significant difference was observed between 30 minutes and 24 hours groups' mean values (p=0.757) (Table 4.13.).

A statistically significant difference was observed between the mean values of pH 7.0 group's Ni release at 30 minutes, 24 hours and 1 month (p=0.001) (Table 4.11.). The mean values of 1 month group's Ni release was found to be significantly higher than the

mean values of 30 minutes and 24 hours groups' (p=0.004, p=0.005), whereas no statistically significant difference was observed between 30 minutes and 24 hours groups' mean values (p=0.171) (Table 4.13.).

Table 4. 11. Evaluation of Ni ion release (mg/l).

Ni (mg/L)		pH 1.5	pH 3.0	pH 7.0	p‡
30 minutes	**Mean±SD**	0.178±0.089	0.296±0.257	0.135±0.096	0.203
24 hours	**Mean±SD**	2.247±2.881	0.293±0.318	0.179±0.07	**0.001**
1 month	**Mean±SD**	8.796±6.066	2.469±1.315	0.292±0.061	**0.002**
	p‡	**0.0001**	**0.0001**	**0.001**	

‡Kruskal Wallis test, *p<0.05*

Table 4. 12. Comparison of pH groups' Ni ion release at different time intervals.

Dunn's Multiple Comparison Test	30 minutes	24 hours	1 month
pH 1.5 / pH 3.0		0.352	0.133
pH 1.5 / pH 7.0		**0.0001**	**0.043**
pH 3.0 / pH 7.0		**0.005**	**0.005**

p<0.05

Table 4. 13. Comparison of time subgroups' Ni ion release at different pH values.

Dunn's Multiple Comparison Test	pH 1.5	pH 3.0	pH 7.0
30 minutes / 24 hours	**0.0001**	0.757	0.171
30 minutes / 1 month	**0.001**	**0.004**	**0.004**
24 hours / 1 month	**0.031**	**0.004**	**0.005**

p<0.05

4.2.3. Chromium (Cr)

When Cr release of pH groups were compared at different time intervals, a statistically significant difference was observed between the mean values of all the pH groups at 30 minutes (p=0.001) (Table 4.14.). The mean values of pH 1.5 group's Cr release at 30 minutes was found to be significantly higher than pH 3.0 and pH 7.0 groups' mean values (p=0.003, p=0.0001), likewise the mean values of pH 3.0 group's Cr release

at 30 minutes was found to be significantly higher than pH 7.0 group's mean value (p=0.004) (Table 4.15.).

A statistically significant difference was observed between the mean values of all pH groups' Cr release at 24 hours (p=0.0001) (Table 4.14.). The mean values of pH 1.5 group's Cr release at 24 hours was found to be significantly higher than pH 3.0 and pH 7.0 groups' mean values (p=0.001, p=0.0001). Also, the mean values of pH 3.0 group's Cr release at 24 hours was found to be significantly higher than pH 7.0 group's mean value (p=0.0001) (Table 4.15.).

A statistically significant difference was observed between the mean values of all pH groups' Cr release at 1 month (p=0.0001) (Table 4.14.). The mean values of pH 7.0 group's Cr release at 1 month was found to be significantly lower than the mean values of pH 1.5 and pH 3.0 groups' Cr release (p=0.0001, p=0.0001), whereas no statistically significant difference was observed between pH 1.5 and pH 3.0 groups' mean values (p=0.329) (Table 4.15.).

When the Cr release at different time subgroups were compared, statistically significant differences were observed at each pH value. A statistically significant difference was observed between the mean values of pH 1.5 group's Cr release at 30 minutes, 24 hours and 1 month (p=0.0001) (Table 4.14.). The mean values of 30 minutes group's Cr release was found to be significantly lower than the mean values of 24 hours and 1 month groups' (p=0.0001, p=0.0001). Likewise, the mean values of 24 hours group's Cr release was found to be significantly lower than 1 month group's mean value (p=0.005) (Table 4.16.).

A statistically significant difference was observed between the mean values of pH 3.0 group's Cr release at 30 minutes, 24 hours and 1 month (p=0.0001) (Table 4.14.). The mean values of 30 minutes group's Cr release was found to be significantly lower than the mean values of 24 hours and 1 month groups' (p=0.0001, p=0.0001). Also, the mean values of 24 hours group's Cr release was found to be significantly lower than 1 month group's mean value (p=0.004) (Table 4.16.).

A statistically significant difference was observed between the mean values of pH 7.0 group's Cr release at 30 minutes, 24 hours and 1 month (p=0.017) (Table 4.14.). The mean values of 1 month group's Cr release was found to be significantly higher than the mean values of 30 minutes group's Cr release (p=0.009), whereas no statistically significant difference was observed between the other groups' mean values (p>0.05) (Table 4.16.).

Table 4. 14. Evaluation of Cr ion release (mg/l).

Cr (mg/L)		pH 1.5	pH 3.0	pH 7.0	p‡
30 minutes	**Mean±SD**	0.09±0.08	0.027±0.011	0.022±0.009	**0.001**
24 hours	**Mean±SD**	2.205±0.662	0.159±0.058	0.028±0.01	**0.0001**
1 month	**Mean±SD**	4.441±2.899	1.041±0.452	0.037±0.012	**0.0001**
	p‡	**0.0001**	**0.0001**	**0.017**	

‡Kruskal Wallis test, *p<0.05*

Table 4. 15. Comparison of pH groups' Cr ion release at different time intervals.

Dunn's Multiple Comparison Test	30 minutes	24 hours	1 month
pH 1.5 / pH 3.0	**0.003**	**0.001**	0.329
pH 1.5 / pH 7.0	**0.0001**	**0.0001**	**0.0001**
pH 3.0 / pH 7.0	**0.004**	**0.0001**	**0.0001**

p<0.05

Table 4. 16. Comparison of time subgroups' Cr ion release at different pH values.

Dunn's Multiple Comparison Test	pH 1.5	pH 3.0	pH 7.0
30 minutes / 24 hours	**0.0001**	**0.0001**	0.184
30 minutes / 1 month	**0.0001**	**0.0001**	**0.009**
24 hours / 1 month	**0.005**	**0.004**	0.132

p<0.05

5.DISCUSSION

5.1. Discussion of Aim and Method of the Study

In our study, surface roughness and ion release of SS orthodontic brackets that are used for a long time in the mouth during treatment were compared at simulated GERD medium with different acid concentrations (pH values) and different durations of acid exposure (times). Various investigators have reported that the alloys used in dentistry undergo corrosion in oral environment (10,164). However, in the literature, there is no *in-vitro* study evaluating the corrosion in brackets due to low oral pH in patients with GERD.

Although there are many studies investigating corrosion in SS brackets in the literature (9,75,78,84,86,139,146,165–173); these studies mostly examined surface roughness in bracket slot due to sliding mechanics or ion release from brackets in environments such as artificial saliva, toothpastes and mouthwashes, spicy foods, acidic beverages and various pH buffers. In studies where the quantities of metal ions released from orthodontic appliances were examined, various types and numbers of orthodontic appliances were used such as molar bands, brackets, archwires, and maxillary expansion devices, or pieces of these items (94,174). Also, there are studies in the literature about corrosion resistance of SS orthodontic brackets (75,131,175–177). Reports about the *in-vitro* corrosion resistance of SS orthodontic brackets have concentrated primarily on the metal ions released using different durations of exposure in various solutions. (75,84,178,179).

Corrosion is affected by many variables such as different oral environment, saliva concentration, saliva pH, saliva flow rate, saliva buffering capacity, different temperatures, acidic / basic food and beverages, toothpastes and mouthwashes (10,64,65,109,180,181). In our study, *in-vitro* method was preferred as it was aimed to provide standardization and to make comparison without being affected by such variables. Due to the fact that, some *in-vivo* conditions cannot be fully reflected in this study.

In our study, pH values were selected as 1.5 and 3.0 for simulated GERD medium and pH 7.0 for artificial saliva. Similarly, Mann et al. (159) used the same pH values; pH 1.5 for severe reflux, pH 3.0 for moderate reflux. Likewise, in the study of Cengiz et al. (157), the same formulation as in our study was used for artificial gastric fluid, but the

pH was adjusted to 1.14. Roque et al. (182) exposed their specimens to acid challenge with HCl and pH was adjusted to 1.2. Derceli et al. (183) simulated the reflux medium at pH 2.0. These acidic pHs were chosen in our study to symbolize different levels of acidity of the gastric refluxate in the oral environment as in Mann et al.'s (159) study. Also similar to Mann et al.'s (159) study, artificial saliva was not used in simulated gastric solutions, to keep the number of variables as low as possible. Since the demineralization-remineralization cycle of the dental tissues did not occur in the metal alloys used in orthodontic treatment, the buffering property of the saliva was neglected in our study when forming the *in-vitro* environment. In our study, artificial saliva was used as a control group to mimic GERD-free oral conditions and the formulation was modified according to Preetha and Banerjee's (160) manuscript. However there are a lot of formulations of artificial saliva in the literature. In addition to this, there is a lack of instruction about the standardization of type of saliva for use in *in-vitro* protocols for erosive studies (184).

In our study, pepsin was also added to simulated gastric solutions because of its promising diagnostic role in saliva and the presence of active pepsin in the gastric refluxate (with a pH less than 4). Likewise, Aytaç et al. (185) in their *in-vitro* investigation of the effect of artificial gastric fluid on enamel surface roughness and Cengiz et al. (157) in their study about the impacts of simulated gastric solution on laboratory-processed composites have used the same formulation with this study. In contrast, Backer et al. (186) have used pepsin-free simulated GERD medium in their study about impacts of simulated gastric solution on CAD/CAM resin composites. There are conflicting results in the literature regarding the diagnostic role of pepsin.

In our study, the material variable was kept constant while the different duration and pH variables were examined in order to better simulate the GERD. Because in these patients, contact between the hydrochloric acid and the oral cavity occurs for a few seconds, several times a day (183). For this reason, we evaluated the corrosion of brackets in different time intervals; at the end of 30 minutes, 12 hours, 24 hours, 1 week and 1 month. In the literature, different durations were examined in terms of metal ion release in different solutions with different types of orthodontic materials (92,94,174,179). Erdoğan (187) kept the silver soldered and laser welded samples in different types of mouthwashes for 24 hours. While Mikulewicz et al. (94) kept the samples in artificial saliva for 30 days, Gürsoy et al. (179) and Danaei et al. (92) kept for 45 days. Ağaoğlu (109) reported the release of nickel and chromium in an *in-vivo* study in which salivary samples were examined by patients who had undergone orthodontic treatment for 1 week,

1 month, 1 year and 2 years. Huang et al. (84) kept the new and recycled brackets for 12 weeks at different pHs. Jang et al. (172) kept different SS brackets and Kang et al. (173) kept different Ti-based brackets in acetic NaF solutions with different pHs for 3 days. Shahabi et al. (9) exposed the brackets to different acidic environments for 6 weeks. Costa et al. (167) maintained different brackets in artifical saliva for 21, 42, and 63 days. Acidic gastric contents may remain on the orthodontic appliances, but standardization of the time is very difficult and almost impossible since the frequency and degree of symptoms of each patient is different. Also, in addition to these difficulties in *in-vitro* studies, there is no standard practice regarding the immersion time in solutions.

In our study, we selected bracket material because it remains on oral cavity longer than other materials and our aim was to calculate the precise corrosion of the brackets, so the whole appliance was not tested in our study. Similarly, many researchers (9,14,84,166,171–173) have used bracket material kept in different solutions and different durations in their studies about corrosion. Borg et al. (188) investigated removable prosthesis in patients suffering from GERD. Behroozi et al. (168) investigated the corrosion of different bracket-archwire complexes that were kept in artificial saliva. Staffolani et al. (75) investigated the release of metal ions from one simulated fixed appliance (steel molar bands, steel brackets, Ni-Ti archwire and a brazing alloy). Mann et al. (159) and Aytaç et al. (185) used extracted human teeth in order to evaluate the surface roughness of enamel in artificial gastric fluid, while Derceli et al. (183) used bovine teeth. Cengiz et al. (157) have used laboratory-processed composites about the impacts of simulated gastric solution. Roque et al. (182) used resin composites to determine the influence of hydrochloric acid on surface roughness. Matasa (13) stated that corrosion is the primary reason of the progressive breakdown of brazing filler metal, causing to seperation between wing and base during treatment or at debonding. Therefore, it was more reasonable to evaluate the bracket material in patients with GERD. Furthermore, the reason for selecting the upper central incisor bracket is that, it provides convenience during measurement with optical profilometer because of the larger area examined. Choi et al. (165) also used maxillary central incisor brackets in their *in-vitro* study which was used optical profilometer due to same reasons.

In our study, the brackets were immersed in acidic solutions that mimic GERD. Because of the acid content of the solutions, the environment in which the solutions were preserved had to be durable and should not be degraded due to environmental factors. For this reason, airtight glass tubes were used in our study to store the study samples.

Likewise study groups, the brackets without any treatment were examined after exposure to solutions by 3D optical profilometer, which is a surface roughness examination device. Many techniques have been used in literature to investigate the surface roughness in brackets like SEM, contact profilometer, AFM and optical profilometry (75,78,84,86,139,146,165–169,171–173). SEM and contact profilometer project the surface morphology 2D, while AFM and optic profilometer provide 3D configuration. Except SEM, the other techniques offer quantitative information. But despite the advantages of AFM, there are handicaps, for instance bracket wings need to be grind in order to evaluate the slot surface and this may be destructive for bracket and its measurement range is on a micrometer scale and therefore macroscopic features of brackets might not be well defined (139). Among all these, optical profilometry gave a quantitative aspect through the calculation of Ra value. Sample preparation is not required and a larger area scanned compared to AFM, and working fastly according to contact profilometer (148). Therefore, 3D optical profilometer machine was used in our study. Until today, there have been few reports on examination of the surface roughness of bracket slots using this type of device. Lin et al. (14) used AFM for evaluation of bracket wing surfaces. Lee et al. (144) and Choi et al. (147) examined the surface roughness of bracket slots by AFM. However, the images scanned were approximately 30 × 30 and 32 × 32 μm^2 respectively, and before scanning grinding was performed. As in our study, Liu et al. (139), Agarwal et al. (148) and Choi et al. (165) evaluated the surface roughness of bracket slots by using noncontact optical profilometer. Compared to 736 x 480 μm^2 area in our study, Liu et al.'s (139) observed range was 376 × 260 μm^2. The area was larger, the bracket wings needed no grinding, and therefore our measurements might better show the total features of the bracket slot according to AFM.

Roughness Average (Ra) is a parameter that usually used to measure the surface texture and also it measures the absolute magnitude of the surface height and represents the average surface roughness (139). In our study, for the surface roughness, Ra parameter which is considered to be the most representative was taken into account.

In our study, nickel (Ni), chromium (Cr) and iron (Fe) levels were evaluated due to their higher presence in SS brackets with ICP-MS device. Metal ion release are measured by different devices such as atomic absorption spectroscopy (AAS), inductively coupled plasma optical emission spectrometry (ICP-OES), or inductively coupled plasma mass spectrometry (ICP-MS). Similar to our study, Luft et al. (166) used ICP-MS in their *in-vitro* evaluation of the corrosion behavior of orthodontic brackets and the artificial

saliva was analyzed in terms of nickel ion concentration. Behroozi et al. (168) also used ICP device and the levels of Fe, Cr, Ni, and Mn ions were measured in their bracket-archwire complex immersed in artificial saliva. Jang et al. (172) and Kang et al. (173) used ICP-OES, while Huang et al. (84), Costa et al. (167) and Staffolani et al. (75) used AAS for determination of released ions from brackets in their *in-vitro* studies. For many years, researchers who have analyzed the metal release of orthodontic appliances have preferred AAS (10,107,109,164,189). In recent years, AAS has been replaced by ICP devices used for the same purpose. ICP-MS has the ability to analyze a large number of elements simultaneously, quickly and precisely compared with atomic absorption techniques (155), based on all these advantages, ICP-MS was prefered in this study.

There have been *in-vitro* studies investigating corrosion in SS brackets in solutions with different contents, however no study examines and compares the effect of gastric regurgitation on brackets (9,75,78,84,86,139,146,166–169,171,172). It is very difficult to fully mimic this oral environment. *In-vitro* tests have numerious disadvantages in simulating the intraoral conditions, in which a bacterial biofilm or a thermocycling could not be examined. In addition, frictional force between bracket and archwire could not be tested. A change of the pH level because of the mechanical loading, orthodontist activities, saliva, oral hygiene or some foods and drinks, drugs, toothpastes etc. was not simulated in our study. Factors like these may lead to the slots getting rougher after oral exposition, therefore it is logical to complete the results of this *in-vitro* study with an *in-vivo* investigation. Nevertheless, since *in-vitro* studies cannot be performed under oral conditions, the results obtained from this study, which is performed under controlled conditions, should be evaluated with these aspects; to aid clinical performance and to shed light on events occurring *in-vivo*.

5.2. Discussion of the Results

In our study, we concluded that pH and duration are effective parameters on surface roughness and ion release of SS brackets. There are no studies in the literature examining the effects of reflux on metals used in orthodontic treatment.

It was observed in our study that in the images obtained by optical profilometer, there were pits on the surface of the untreated brackets. Also, it was noted that these pits deepened after the experiments depending on the pitting corrosion. In parallel with this, Gwinnett (176) found that pitting corrosion could be seen on SS brackets, and in some

cases great quantities of metal are missing, causing to the persistence of substantial quantities of Fe, Cr, and Ni elements in the bonding, discolored resin. Furthermore, Maijer and Smith (131) reported that the presence of voids, together with poor oral hygiene, causes to the appearance of crevice corrosion of SS brackets and the accumulation of colored products, which makes enamel stains. Also, Loreille (177) stated that one of the most important reason for the unpredictable control of orthodontic forces could be the surface corrosion of wires and brackets. We cannot evaluate crevice corrosion, discoloration or tooth movement, because only the bracket is used in our experimental setup, the oral environment is not fully imitated and our study is *in-vitro*. Similarly, Chaturvedi (171) examined the *in-vitro* corrosion of orthodontic SS metallic brackets submerged in solutions of salt and spices in artificial saliva using electrochemical techniques and surface analysis test. They indicated that certain spices such as turmeric and coriander are effective in reducing corrosion, whereas salt, red chilies and black pepper have been found to enhance it for metallic brackets. As a result, surface analysis of small pits present on the surface of the as-received brackets will initiate corrosion which leads to more pitting. Since the contents of the solution in which the samples were stored, bracket brands used or examination methods were different, the comparison of the obtained data with our study would not be correct. However, we conclude that the pits may deepen due to the environment the surface is exposed to.

In our study, at different time intervals, pH 7.0 group also showed statistical differences within its group for FSR ($p=0.0001$) (Table 4.2.) and the mean values of both 1 week and 1 month groups were found to be statistically higher than all other groups when compared with other time subgroups ($p<0.05$), whereas no statistically significant difference was observed between 1 week and 1 month ($p=0.903$) (Table 4.4.). Costa et al. (167) evaluated the *in-vitro* corrosion of SS and low-nickel orthodontic brackets with SEM immersed in 6.75 ± 0.15 pH artificial saliva for 21, 42, and 63 days. SS brackets showed increased surface roughness as saliva exposure increased, while low-nickel brackets remained unchanged, indicating a higher corrosion resistance. Despite the methodological differences such as duration, bracket material or examination method, presence of the connection between duration and corrosion degree is evident.

In our study, when the pH groups were evaluated in terms of FSR at different time intervals, statistical differences were observed between time subgroups ($p<0.05$), except for 30 minutes and 12 hours ($p=0.416$, $p=0.403$) (Table 4.2.). The mean values of pH 7.0 group's FSR was found to be significantly lower than the mean values of pH 3.0 groups'

FSR at 24 hours (p=0.001), but no statistically significant difference was observed between the mean values of pH 3.0 and pH 7.0 groups' FSR for both 1 week (p=0.990) and 1 month (p=0.963) time subgroups (Table 4.3.). We interpret that it is obvious that at first (for 30 minutes and 12 hours) there was a relative resistance to corrosion for almost all the specimens and that was clearly because of the chrome oxide layer on the brackets. In addition to this, there was a change in 24 hours but there was no difference between pH 3 and pH 7 groups when duration time increased. On the contrary, Jang et al. (172) assessed SS orthodontic brackets' surface morphology immersed in acetic NaF solutions (pH 3.0, pH 6.0) for 3 days, by using SEM. The brackets did not show any visible surface modification compared with their control surface regardless of product and pH value. In another study, Kang et al. (173) examine the effects of acetic NaF solutions on titanium and Ti alloy brackets' surface morphology for 3 days by using SEM. In the acetic NaF solution of pH 3.5, severe corrosion were seen on the brackets, based on the results of this study, the use of Ti-based brackets did not appear to be safe if they are used with products that contain a high concentration of fluoride for a long time under acidic circumstances. Although it is not possible to make a one to one comparison of these studies with our study, many factors, such as duration, pH, acid type and bracket material affect the surface, providing the basis for achieving different results.

When the pH groups were evaluated in terms of FSR at different time intervals, the statistically significant difference in all pH groups firstly occurs after 24 hours (Table 4.2.). In previous studies, corrosion was tested on different time intervals. Park and Shearer (164), Barrett et al. (10) and Hwang et al. (86) indicated that, the amount of metal ions released from the fixed orthodontic appliances reaches its maximum in 7 days and will be completed in about 4 weeks. In our study, corrosion was evident in terms of surface roughness after 24 hours. Although there are methodological differences, our study is consistent with the results of these studies.

According to our results, the lowest and highest ISR values were found to be 509.98 nm and 690.02 nm respectively. After the experiments, the lowest and highest FSR values were found to be 526.87 nm and 1096.7 nm, respectively. To date, there have been few reports on the measurement of surface roughness of bracket slots using that type of profilometer device and no study similar to ours was found in the literature. Agarwal et al. (148) conducted a study to examine the surface roughness of the bracket slot floor of the conventional SS bracket with optical profilometer. Differences were seen in the surface roughness among the 4 groups, group D has the smoothest surface, with values

0.74 and 0.75 μm for the mesial and distal slots, respectively. Another *in-vitro* study conducted by Choi et al. (165) examine the surface roughness of the slot floors of modern plastic brackets using an optical profilometer. SS brackets (control group) demonsrated significant increases in surface roughness parameters after the archwire sliding test, measured as 0.28 μm before sliding and 0.49 μm after sliding. When we evaluate these studies with our study, we concluded that the surface roughness values differ according to the bracket brand. When we evaluate our ISR values, we observed that similar values are observed on the same bracket types unless there are manufacturing defects or deep scratches.

In our study, we found that the change of surface roughness (FSR-ISR) was statistically significant in all main pH groups and time subgroups (p=0.0001) (Table 4.5.), which means that orthodontic tooth movements may be affected by increased slot surface roughness and lead to increased frictional force. Moreover, the other surfaces of the tested brackets were also irregular and these changes might affect the prescription characteristics of bracket slot and therefore affect the rotation, tip and torque movements. Liu et al. (139), in their *in-vivo* study, examined the surface roughness of bracket slots, using optical profilometer as in this study, before and after orthodontic treatment. 4 groups were: 2 different brands, both of two contains new and retrieved brackets. Brackets were exposed to the oral cavity through 21.5 ± 3.3 months, and as a result experienced the leveling and space closure stages. Orthodontic treatment caused to significant increases in surface roughness for both brands of brackets. But, no significant difference was observed between brands for new or retrieved brackets (139). Although the study types and materials and methods used were different, and even if one to one comparison cannot be made with this *in-vivo* study, we can conclude that slots may be rougher after intraoral exposure due to biofilm accumulation, saliva, and carbonic acid drink erosion, tooth-brushing, orthodontist activities, friction between brackets and archwires. Consequently, surface alterations may affect bracket performance and so tooth movement.

The values for released ions in our study do not exceed recommended daily intake even for the longest duration (1 month) or lowest pH (pH 1.5). Similarly, the study by Staffolani et al. (75) demonstrated that the amounts of metal ions released from SS bracket in acidic environment should not be the reason for concern about orthodontic treatment. Huang et al. (175) claimed that the recycled stainless steel brackets release more metal ions than the new stainless steel brackets, but the values at the end of 12 week

submersion in artificial saliva does not pass the recommended daily intake. Our study is consistent with these studies that the total amount of ion release was less than the cumulative daily intake and the toxic concentration, however, it may have some adverse effects on the oral mucosa cells, including changes in DNA synthesis and in enzyme activity and suppression of the chemotaxis of leukocytes (190).

In our study, when ion (Fe, Ni, Cr) release of pH groups were compared at different time intervals, a statistically significant difference was observed between the mean values of all the pH groups at all time subgroups ($p<0.05$) (Fe: Table 4.8., Ni: Table 4.11., Cr: Table 4.14.) except for Ni at 30 minutes ($p=0.203$). For Fe ion, at all time subgroups; mean values from the highest to the lowest are pH 1.5, pH 3.0, pH 7.0, respectively ($p<0.05$) (Table 4.9.). For Ni ion, both at 24 hours and 1 month; pH 7.0 group's mean values are the lowest ($p<0.05$), but there is no statistically significant difference between pH 1.5 and pH 3.0 groups ($p=0.352$, $p=0.133$) (Table 4.12.). For Cr ion, both at 30 minutes and 24 hours; mean values from the highest to the lowest are pH 1.5, pH 3.0, pH 7.0, respectively ($p<0.05$), but at 1 month; pH 7.0 group's mean values are the lowest ($p<0.05$), but there is no statistically significant difference between pH 1.5 and pH 3.0 groups ($p=0.329$) (Table 4.15.). In addition, when the ion (Fe, Ni, Cr) release at different time subgroups were compared, statistically significant differences were observed at each pH value ($p<0.05$) (Fe: Table 4.8., Ni: Table 4.11., Cr: Table 4.14.). For Fe ion; no statistically significant difference was observed between the mean values of 24 hours and 1 month at pH 1.5 ($p=0.354$) and between 30 minutes and 24 hours at pH 7.0 ($p=0.092$) (Table 4.10.). For Ni ion; no statistically significant difference was observed between the mean values of 30 minutes and 24 hours at pH 3.0 ($p=0.757$) and at pH 7.0 ($p=0.171$) (Table 4.13.). For Cr ion; no statistically significant difference was observed between the mean values of 30 minutes and 24 hours ($p=0.184$) and between 24 hours and 1 month ($p=0.132$) at pH 7.0 (Table 4.16.). There is a statistical difference between other comparisons and mean values increase with durations increase ($p<0.05$) (Fe: Table 4.10., Ni: Table 4.13., Cr: Table 4.16.). Although, there are different results between comparisons of our study, in general, the ion release increases as the pH value decreases and the duration increases. Huang et al. (84) compared the release of metal ions from new and recycled metal brackets after immersion in artificial saliva and buffers with various pH values (pH 4, pH 7, and pH 10) incubated at 37°C for 12 weeks. Ni, Cr, Fe, and Mn ions were measured with atomic absorption. Contrary to our results, it was observed that excess amounts of Ni, Fe, and Mn ions were released in artificial saliva

compared with the other buffers tested. This is thought to be due to the fact that the artificial saliva formulas used are different and duration time is longer than our study. They also concluded that, as immersion time increased and pH decreased, ion release increased. As it is in line with the results of our study that metal ion release was found to be higher in gastric solutions compared to artificial saliva and this is thought to be due to the increased acidity level of gastric solutions. Acidic conditions create a less stable and reducing environment for the SS oxide film which is required for corrosion resistance. In addition, although 12 weeks is much longer than the durations in our study, the same conclusion was reached. Whereas, Borg et al. (188) investigated the surface microstructural changes and the release of ions from metal alloys used in removable dental prostheses and the potential effects of acidic reflux found on patients who have GERD. Comparison of saliva metal ion levels of patients using a metal denture was made among patients with GERD and without GERD. No significant difference was found in salivary metal ion levels between these two groups. They interpreted that GERD appeared to have slight affect on salivary ion release. Although the method used is different and our study is *in-vitro*, we interpreted all these as GERD is not a stable disease, and further investigation is needed about the affect of gastric acid on metal ion release. In parallel with our study, Staffolani et al. (75) also stated that corrosion increased due to the decrease in pH and the daily release of Ni and Cr was well below that ingested with a normal daily diet. They investigated the release of metal ions from one orthodontic appliance which was immersed in both inorganic (HCl)(pH 3.5–6.5) and organic acid solutions (stored for 1 day, then transferred to new solution for the following 27 days). The release of Ni and Cr was noticeably less at pH 6.5 than at pH 3.5 at all time periods in acid solution. Similar to our study, Sfondrini et al. (87) studied Ni release at three different pH values (pH 4.2, 6.5 and 7.6) and stated that the maximum release was at pH 4.2. In another study conducted by Shahabi et al. (9) compared the corrosion degree of lemon juice (pH 2.7), vinegar (pH 2.5) and cola (pH 2.5) on orthodontic brackets in 6 weeks *in-vitro* and demonstrated the amount of corrosion was the most for cola followed by vinegar and then lemon juice. They interpreted that, the excessive level of corrosion seen in cola could not entirely be imputed to its low pH, in addition to acidity, other parameters concerned in the process of corrosion should be search. Although there is no orthodontic study performed in the reflux environment in the literature, when we compare our findings with these studies performed in acidic environment, while parallel results were found with Sfondrini et al. (87) and Staffolani et al.'s (75) findings, it is seen that

different results were found in Borg et al.'s (188) and Shahabi et al.'s (9) findings and some of Huang et al.'s (84) findings. In our study, at the end of 24 hours, 1 week and 1 month period, rust colored precipitates was observed in all the sample tubes, with the darkest in pH 1.5 group and lightest in pH 7.0 group. This was due to a high Fe concentration, as noted by studies (191,192). Also, differences in mean values of Fe ion release in our study support this finding. In our study, when comparing the changes in time subgroups (30 minutes, 24 hours, 1 month) in itself, it was found that there were statistical differences in the mean values of all pH groups' Fe ($p<0.05$) (Table 4.8.). In all time subgroups in itself, mean values of Fe were statistically the highest in pH 1.5 group and the least in pH 7.0 group (Table 4.9.). In case of comparing the changes in pH groups in itself, statistically significant difference was observed at 30 minutes, 24 hours and 1 month in the mean values of Fe (Table 4.8.). In pH 1.5 group, the mean values of 30 minutes group's Fe was found to be significantly lower than the other times. In pH 3.0 group, the mean values of Fe was found to be significantly the lowest in 30 minutes group and the highest in 1 month group. In pH 7.0 group, the mean values of Fe was found to be significantly higher in 1 month group than other time groups (Table 4.10.).

In our study, Fe, Ni and Cr mean values at 24 hours are 0.041±0.03, 0.179±0.07 and 0.028±0.01 mg/L for pH 7.0, 0.108±0.022, 0.293±0.318 and 0.159±0.058 mg/L for pH 3.0, 6.62±3.159, 2.247±2.881 mg/L and 2.205±0.662 mg/L for pH 1.5, respectively. Staffolani et al. (75) found that the variations in Ni and Cr at the end of the first day ranged from 0.41 and 0.09 mg/appliance respectively for pH 6.5, to 2.75 and 1.43 mg/appliance for pH 3.5. The reason why the results are higher than ours may be one orthodontic appliance used in this study, rather than a bracket. Jang et al. (172) examined the effect of acetic NaF solutions of different pH values (pH 3.5 and pH 6) on two different SS orthodontic brackets (similar elemental composition) in terms of element release and surface modification. The brackets were submerged in solutions for 3 days and the concentration of Fe, Cr, Ni, and Mn ions evaluated. Both brackets released an excessive amount of ions just in pH 3.5 solution, but both brands of brackets released less than 1 ppm in most test solutions after 3 days. Ion release values from 2 different brackets at pH 3.5 are respectively; for Fe; 2.36 ± 0.23 ppm and 151.46 ± 12.41 ppm, for Cr; 0.11 ± 0.02 ppm and 33.63 ± 2.60 ppm, for Ni; 0.14 ± 0.01 ppm and 19.37 ± 1.34 ppm, while at pH 6 are respectively; for Fe; 0.56±0.01 ppm and 0.56±0.03 ppm, for Cr; 0±0 ppm and 0±0 ppm, for Ni; 0.08±0 ppm and 0.06±0 ppm. It is thought that, the difference between the values of this study with our values may be due to differences in bracket types, pH,

acid type, duration or using 5 brackets for each test. Although the materials and methods used is different, from these studies, we conclude that pH value and duration affects corrosion and ion release. But still, daily release of Ni and Cr does not reach the daily consumed dose.

For all three ions the highest mean values were found at pH 1.5 in 1 month group. The mean values for Fe, Ni, Cr were 8.478 ± 4.987 mg/l, 8.796 ± 6.066 mg/l, 4.441 ± 2.899 mg/l, respectively (Fe: Table 4.8., Ni: Table 4.11., Cr: Table 4.14.). It was observed that among the comparison of all pH groups and time groups in itself, Ni was released more than the other ions on average. This is consistent with the results of Huang et al. (84) and in contrast with the findings of Behroozi et al. (168) in which iron release was more than others. This difference is thought to be due to the difference in bracket materials. Especially, Ni is more allergic than other ions found in orthodontic appliances. The amount of Ni in the oral mucosa of the treated patients was higher than the control group, as supported by previous studies (193). Daily consumption of Ni with nutrients and beverages is nearly 300–500 μg (86,92,164). Additionally, it has been approved that if the absorption of nickel exceeds 2.5 μg/kg, allergic symptoms could be seen (194). The amount of Ni ions released in our study was smaller than the mentioned threshold. However, even this low Ni ion concentration may result in allergic reactions or can induce biological effects and caused to DNA modifications mostly by DNA strand scission and DNA base damage in the oral buccal mucosa cells, in view of the fact that patients usually treat about 2-3 years and have nearly 20 brackets during treatment period (92,193,195).

In our study, the mean values for Ni at pH 7.0 were 0.135±0.096 mg/l (30 minutes), 0.179±0.07 mg/l (24 hours), 0.292±0.061 mg/l (1 month). In other study, Costa et al. (167) evaluated the *in-vitro* corrosion of SS and low-nickel orthodontic brackets immersed in pH 6.75 ± 0.15 artificial saliva and keeped at 37°C under stable conditions for 21, 42, and 63 days. It was stated that Ni ion concentrations, detected from 63-day, suggested that SS brackets showed less corrosion resistance compared to low-nickel brackets. The highest concentration of nickel was detected from the SS-bracket extracts after 42-day (4.46 ± 0.68 μg/mL), and from the low-nickel SS-bracket extracts after 63 days (0.07 ± 0.01 μg/mL). In our study, the highest concentration of Ni at pH 7.0 was detected after a 1 month immersion period. Luft et al. (166) evaluated the Ni ion release of 9 different bracket systems in artificial saliva during the immersion period of 1 week. The nickel ion release ranged from a minimum of 0.01 μg/day to a maximum of 5.24 μg /day. The Ni ions released in our study was less than these studies. It is thought to be that

bracket material, artificial saliva, durations and examination methods may be caused this difference. Also, these values are less than the daily dietary intake level and the critical concentration necessary to generate Ni allergy. Luft et al. (166) also stated that in some cases the surface changes could be correlated with the measured Ni ion release. Likewise in our study, when Ni ion release and surface roughness values were examined, it was found to be consistent with each other.

Although brackets with different brands are not examined in our study, it was observed that, there are also differences between the brackets with same brands. Matasa (13) stated that, corrosion of orthodontic appliances and metal ion release is directed by two primary factors. These are manufacturing process (type of alloy, metal features) and environmental factors (mechanical stress, diet, time of the day, salivary flow rate, health and mental condition). It has been reported by Hensten-Pettersen et al. (189) that internal stress or irregular microstructure features of alloy or both of them could increase the corrosion occurence in metal brackets. Hence, the variations in corrosion resistance between the tested SS brackets with the same surface passive film may be associated to the different surface pictures, for instance surface residual stress and metallurgical factors, developed during manufacturing processes, rather than the surface roughness or preexisted defect. In the light of all these, it is thought that in addition to environmental factors (pH, acid type, duration) affecting corrosion there are also metallurgical factors.

Comparison of results is difficult because all the experiments were performed in various immersion media, different materials and analytical techniques were used. Also each method has different sensitivity and detection limits. The comparison of the given *in-vitro* studies shows us which material is more biocompatible. In the systematic literature review of Mikulewicz and Chojnacka (78), which supports our view, it was mentioned about the difficulty of interpretation of the results because of the same reasons. According to this review's general conclusions from the papers discussed were that; the reported concentrations of metal ions ranged excessively, for instance, the concentration of Ni remained within the range 1.62–11,000 ng/ml., the less biocompatible material was SS which released the highest amount of Ni and Cr, acidic environment significantly increased the degree of metal ions release (30– 50 times as compared with acidic vs. alkaline media). In another systematic review of Mikulewicz and Chojnacka (95), it was stated that *in-vivo* works evaluated only short-term effects associated with orthodontic treatment, the studies were accomplished about 1–2 months and the greater part of them emphasized the trouble with Ni ion release, this supports the findings of *in-vitro*

experiments, and both of them concluded that the amounts of metal ions were less than the toxic limits. They also stated that, there must be a standardization about methods and procedures to have results which can be comparable (78).

In our experiment, we evaluated only the amount of bracket corrosion in different pHs, while in the oral environment other conditions were the leading factors in terms of corrosion resistance such as galvanic and crevice corrosion. In addition to this, our *in-vitro* study was carried out in static conditions, however, orthodontic appliances are mechanically activated in the oral cavity and the movement of archwires might lead to further corrosion. Furthermore, the safety and risks or doses and long-term effects associated with orthodontic treatment were not possible to assess due to *in-vitro* conditions. Another drawback to this study is that we did not evaluate the effects of different brackets or wire-bracket combinations. This was because of the reason that our main purpose is to see the corrosion changes of bracket in different times and pHs. While in many senses the *in-vitro* design is a drawback, in measuring ion release such a setup is rather useful. Not all of the ions released from orthodontic brackets are found in the oral fluids, some of the ions are absorbed into the oral and gingival tissues, while others are ingested and could disperse into distant organs (168). The advantage of the *in-vitro* design is that it presents the total ions released from the bracket, which could better show the true effect of this phenomenon.

As a result, changes in surface roughness and ion release are observed in the environment where GERD is simulated with different durations and pH's, but the amount of metal ion release still does not reach toxic doses. However, since the appliances used in our *in-vitro* study do not fully simulate the ones that are used in fixed orthodontic treatment, as well as the effects of the oral environment on them, further research is required to clarify the corrosion effects of GERD on orthodontic brackets. In the future, this study can be improved in retainers which stay in the mouth for a long time after orthodontic treatment or in different types of brackets. Furthermore, crevice and galvanic corrosion can be evaluated by simulating oral environment and fixed orthodontic treatment. In GERD, firstly the palatinal surfaces of the upper incisors and mostly the palatinal regions are affected, so the assessment of the corrosion in the brackets in patients with GERD receiving lingual treatment may contribute to the literature. Aligners or ceramic brackets may also be recommended for the treatment of these patients. Although standardization is difficult to achieve in *in-vivo* studies, this would contribute to literature and clinical practice.

6.CONCLUSION

Within the limitations of this *in-vitro* study;

1. When the pH groups were evaluated in terms of FSR at different time intervals, the difference in all pH groups firstly occurs after 24 hours.
2. As the pH of the solutions decreased (acidic environment increased), corrosion of brackets increased.
3. The longer the immersion time of the brackets in the solutions, the higher the corrosion of brackets were examined.
4. The highest final surface roughness (FSR), change of surface roughness (FSR-ISR) and ion release (Fe, Ni, Cr) were found in pH 1.5 group at 1 month.
5. pH 7.0 group's Fe, Ni, Cr ion release at all time subgroups (except 30 minutes for Ni) was found to be less than gastric solutions.
6. Ion release from the brackets occurred in all pH groups at all time intervals, but the amounts of Fe, Ni, Cr release were below the toxic limits and cumulative daily intake.

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8. CURRICULUM VITAE

EK 13. Özgeçmiş

Kişisel Bilgiler

Adı	Elif	**Soyadı**	Akın Özgünler
Doğum Yeri	Alanya	**Doğum Tarihi**	10/10/1991
Uyruğu	T.C.	**TC Kimlik No**	17347459088
E-mail	dtelifakin@gmail.com	**Tel**	05339581142

Öğrenim Durumu

Derece	Alan	Mezun Olduğu Kurumun Adı	Mezuniyet Yılı
Doktora	Ortodonti	Yeditepe Üniversitesi	2019
Yüksek Lisans	Diş Hekimliği	Hacettepe Üniversitesi	2014
Lisans	Diş Hekimliği	Hacettepe Üniversitesi	2014
Lise	Sayısal	Manavgat Anadolu Lisesi	2009

Başarılmış birden fazla sınav varsa(KPDS, ÜDS, TOEFL; EELTS vs), tüm sonuçlar yazılmalıdır

Bildiği Yabancı Dilleri	**Yabancı Dil Sınav Notu (#)**
İngilizce	

Bilgisayar Bilgisi

Program	**Kullanma becerisi**
Microsoft Office	İyi
Dolphin	İyi

*Çok iyi, iyi, orta, zayıf olarak değerlendirin

Bilimsel Çalışmaları

SCI, SSCI, AHCI indekslerine giren dergilerde yayınlanan makaleler

Diğer dergilerde yayınlanan makaleler

Nalbantgil D(1), Burcu Nur Yılmaz R(1), Akın E(1), Erden MA(1), Yılmaz S(1), Özdemir F(2). Evaluation of the impact of interdisciplinary case-based courses in dental education on smile evaluation skills of undergraduate students. Turkish Journal of Orthodontics. 32(1):11-15. doi:10.5152/TurkJOrthod.2019.18012.

Uluslararası bilimsel toplantılarda sunulan ve bildiri kitabında (*Proceedings*) basılan bildiriler

1-5 Ekim 2016 XV. Uluslararası Türk Ortodonti Derneği Kongresi, Antalya - Sözlü Bildiri - Doğal ve Dijital Olarak Tasarlanmış Gülüşlerin Diş Hekimleri, Diş Hekimliği Öğrencileri ve Meslekten Olmayan Kişiler Tarafından Değerlendirilmesi – Nalbantgil D, Nur Yılmaz RB, Yılmaz S, Akın E, Erden MA

5-7 Kasım 2017 15. Uluslararası Türk Ortodonti Derneği Sempozyumu, Ankara - Poster - Yüz maskesi tedavisinin etkilerinin ebeveynlerin bakış açısından değerlendirilmesi - Nalbantgil D, Hepdarcan SS, Özcan M, Akın E.

June 17-21, 2018 94th European Orthodontic Society Congress, United Kingdom, Edinburgh - Poster - Assessment of the Effects of Facemask Therapy from Parents' Perspective - Nalbantgil D, Hepdarcan SS, Özcan M, Akın E.

Hakemli konferans/sempozyumların bildiri kitaplarında yer alan yayınlar

Diğer (Görev Aldığı Projeler/Sertifikaları/Ödülleri)

5-7 Kasım 2017 Türk Ortodonti Derneği Sempozyumu, Ankara – Poster bildiri 2. lik ödülü – Yüz maskesi tedavisinin etkilerinin ebeveynlerin bakış açısından değerlendirilmesi – Nalbantgil D, Hepdarcan SS, Özcan M, Akın E.