

**T.C.  
BAHCESEHIR UNIVERSITY  
GRADUATE SCHOOL OF EDUCATION  
BIOMEDICAL ENGINEERING HEAD OF THE DEPARTMENT**

**THE EFFECT OF SURFACE MODIFICATIONS FOR THE AIM OF  
DECREASING BACTERIAL ADHESION ON TITANIUM IMPLANTS**



**MASTER'S THESIS  
ARMIN RAHMANI**

**ISTANBUL 2023**

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**THESIS ADVISOR**  
**ASSIST. PROF. AYŞE SENA SARP**

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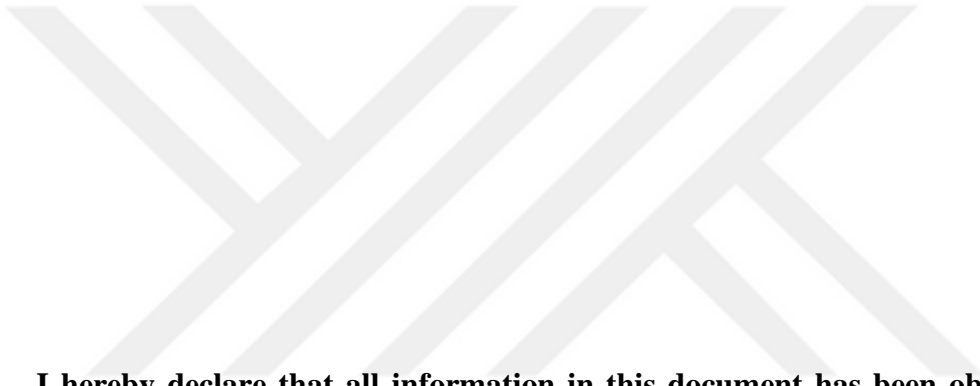
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	<b>Title/Name</b>	<b>Institution</b>	<b>Signature</b>
<b>Thesis Advisor's</b>	Assist. Prof. AYŞE SENA SARP	Bahçeşehir University	
<b>Member's</b>	Assist. Prof. ELİF ÖZCAN	Bahçeşehir University	
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## **ABSTRACT**

The Effect of Surface Modifications for the Aim of Decreasing Bacterial Adhesion  
on Titanium Implants

ARMIN RAHMANI

Master's Program in Bioengineering

Supervisor: Assist. Prof. AYŞE SENA SARP

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Surface modification of titanium surfaces offers a potential solution to reduce the risk of bacterial adhesion and extend the lifespan of implants. This study aimed to explore the impact of different surface patterns on bacterial adhesion and develop an appropriate pattern to minimize such adhesion on titanium plate surfaces. The honeycomb pattern was specially selected for its various applications in the field of biomedical engineering and the four distinct pattern groups were generated, including Plain, Line Type 1, Line Type 2, and Honeycomb Patterns. Preliminary experiments were conducted to compare Line Type 1 and Line Type 2 groups. Statistical analysis revealed no significant difference in bacterial colony-forming units (CFU) values, and based on the results, Plain, Line Type 1, and Honeycomb pattern groups were further evaluated. Although a minor difference in mean CFU values was observed in the honeycomb group compared to other groups, the difference was not significant enough to support the adoption of honeycomb patterns as a definitive solution for effectively minimizing bacterial adhesion on titanium and its alloys surfaces. These findings provide useful insights into the selection and effectiveness of surface patterns in reducing bacterial adhesion on titanium plates.

Key Words: Pattern, Titanium Plates, Bacterial Adhesion

## ÖZ

### Titanyum İmplantlarda Bakteriyel Adezyonu Azaltmak Amacıyla Yüzey Modifikasyonlarının Etkisi

ARMIN RAHMANI

Biyomühendislik Yüksek Lisans Programı

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Titanyum yüzeylerin yüzey modifikasyonu, bakteriyel adezyon riskini azaltmak ve implantların ömrünü uzatmak için potansiyel bir çözüm sunmaktadır. Bu çalışma, farklı yüzey desenlerinin bakteriyel yapışma üzerindeki etkisini araştırmayı ve titanyum plaka yüzeylerinde bu tür yapışmayı en aza indirmek için uygun bir desen geliştirmeyi amaçlamıştır. Bal peteği deseni, biyomedikal mühendisliği alanındaki çeşitli uygulamaları nedeniyle özel olarak seçildi ve Düz, Çizgi Tipi 1, Çizgi Tipi 2 ve Bal Peteği Desenleri dahil olmak üzere dört farklı desen grubu oluşturuldu. Çizgi Tipi 1 ve Çizgi Tipi 2 gruplarını karşılaştırmak için ön deneyler yapılmıştır. İstatistiksel analiz, bakteriyel koloni oluşturma birimi (CFU) değerlerinde önemli bir fark olmadığını ortaya koymuş ve sonuçlara dayanarak Düz, Çizgi Tipi 1 ve Petek desen grupları ayrıca değerlendirilmiştir. Bal peteği grubunda diğer gruplara kıyasla ortalama CFU değerlerinde küçük bir fark gözlemlense de, fark, titanyum ve alaşımlarının yüzeylerinde bakteriyel yapışmayı etkili bir şekilde en aza indirmek için bal peteği desenlerinin kesin bir çözüm olarak benimsenmesini destekleyecek kadar önemli değildi. Bu bulgular, titanyum plakalar üzerinde bakteriyel yapışmayı azaltmada yüzey desenlerinin seçimi ve etkinliği konusunda faydalı bilgiler sağlamaktadır.

Anahtar Kelimeler: Desen, Titanyum Plakalar, Bakteriyel Yapışma



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## LIST OF ABBREVIATIONS

(BIC)	Bone-to-Implant Contact
(RTV)	Removal Torque Values
(SLA)	Sandblasting
(TiO <sub>2</sub> )	Titanium Dioxide
(Cp-Ti)	Commercially Pure Titanium
(HA)	Hydroxyapatite
(SiO <sub>2</sub> )	Silicon Dioxide
(Al <sub>2</sub> O <sub>3</sub> )	Aluminum Dioxide
(CFU)	Colony-forming Units
(EPS)	Extracellular Polymeric Substances
(EF)	Enterococcus Faecalis
(BHI)	Brain Heart Infusion Broth
(BHI)	Brain-Heart Infusion Agar
(PBS)	Phosphate Buffered Saline

# Chapter 1

## Introduction

### 1.1 Dentistry

Dentistry is a branch of medicine that deals with the detection and treatment of oral disorders, such as diseases of the teeth and supporting structures. Dentistry mainly includes diagnosing and treating jaw deformities, malocclusions, and congenital anomalies of the oral cavity, including cleft palate. In other words, dentistry examines, prevents, and cures diseases, abnormalities, and problems of the oral cavity, most commonly of the teeth, and studies the oral mucosa and adjacent structures and tissues, especially in the maxillofacial region. In dentistry, the responsibility of a dentist is to be well-versed in the functionality and principles of dental morphology and dental care. Dental practitioners must develop physical skills to replicate any part of an individual tooth while maintaining an accurate connection to adjacent structures; therefore, a scientific understanding of most dental areas' functions and anatomical relationships is vital (C Bhaskor Rao, 2013).

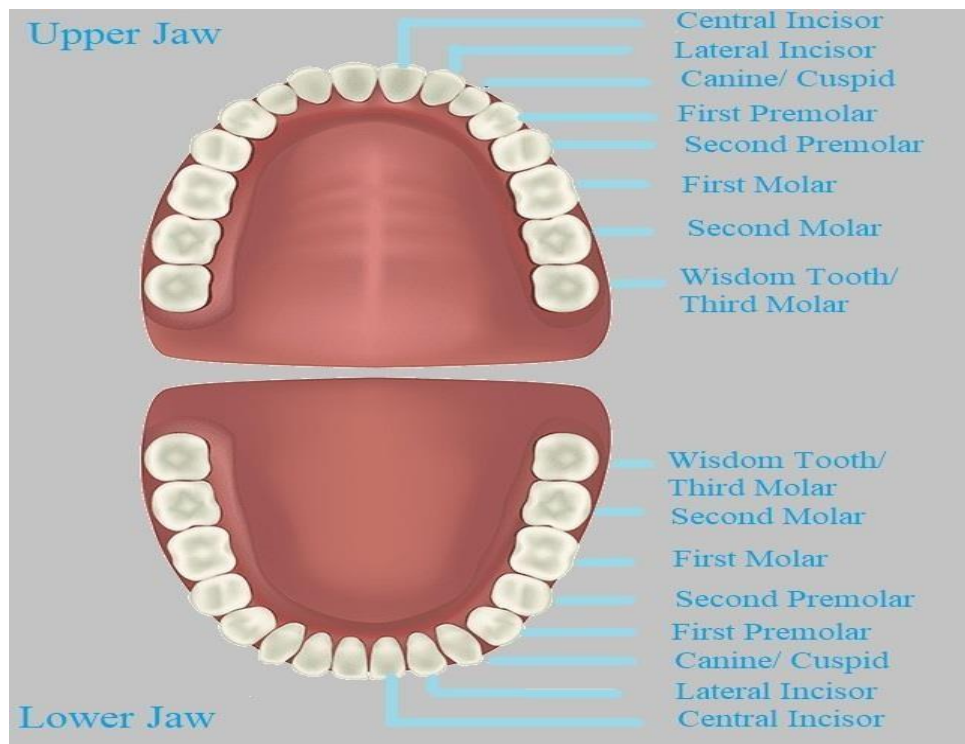


Figure 1: Human Dentitions (Group, 2019).

## 1.2 Dental Anatomy and Oral Physiology

Dental anatomy focuses on the external structure and shape of the tooth. In addition, dental anatomy is a vital topic in dentistry that examines teeth' morphology and associated systems, such as the chewing process. In other words, the science of dental anatomy is dedicated to the detailed study of the structural and potential interactions of the jaws and related parts that are required for the proper dental balance of the patient (C Bhaskor Rao, 2013).

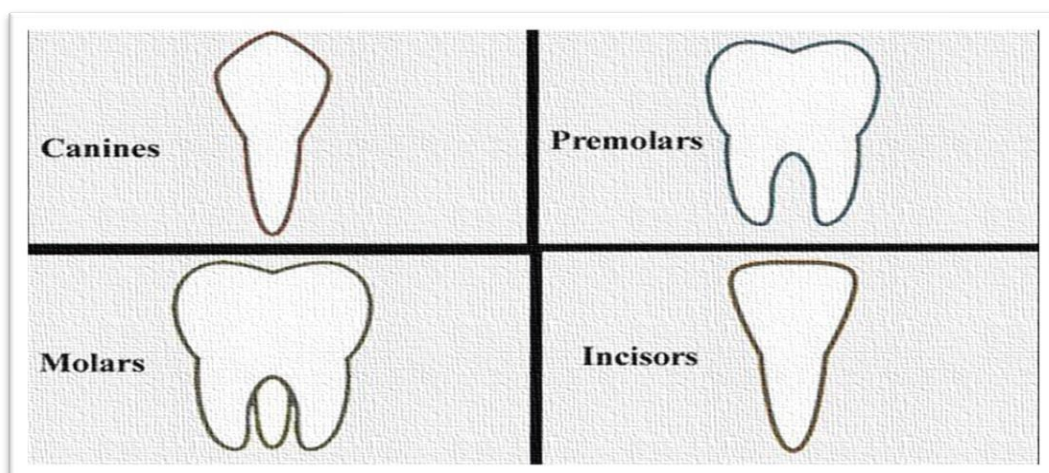
### 1.2.1 Tooth Classifications

**Incisors** are in the middle front of the upper and lower jaws and have sharp thin cutting edges. Besides, incisors get their name because they are designed to cut or incise food (C Bhaskor Rao, 2013).

**Canines** are positioned at the corners of the alveolar crest beside the incisor. Instead of an incisal edge, every tooth has a single cusp perfectly designed for tearing and cutting (C Bhaskor Rao, 2013).

**Bicuspid**s are like cuspids that are commonly known as premolars. These teeth have two cusps for cutting and tearing and a broader occlusal surface for crushing food (C Bhaskor Rao, 2013).

**Molars** are positioned in the rear of the mouth. From the first to the third molar, their size decreases gradually. Each molar contains four or five cusps, which are shorter and blunter in form than the rest of the teeth and support a broad area when crushing and eating massive amounts of food (C Bhaskor Rao, 2013).



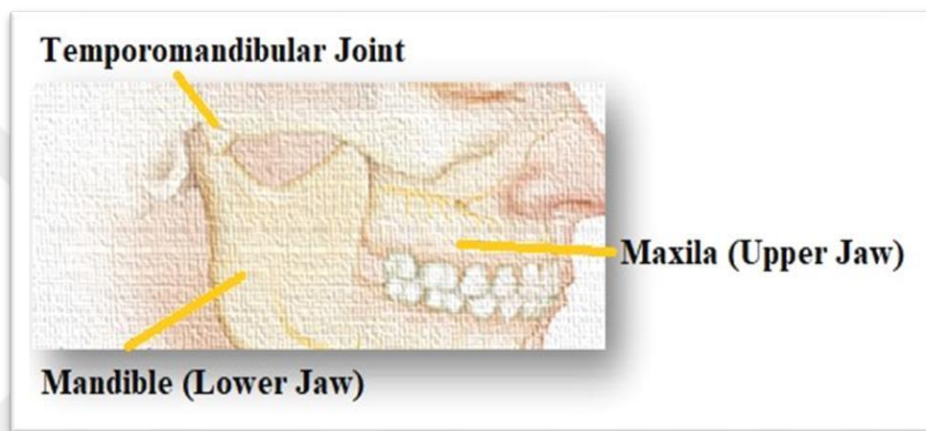
*Figure 2: Human Teeth Diagram (Humagain, 2021).*

### 1.2.2 The Parts of the Human Jaw

**Temporomandibular Joints (TMJ):** The connections of the jawbone to the skull that act as a sliding hinge are known as temporomandibular joints (DANIEL TALMACEANU, 2018).

**Mandible (Lower Jaw):** This part comprises a single pair of bones. In addition, this part is a complete lower jaw (C Bhaskor Rao, 2013).

**Maxilla (Upper Jaw):** This is a bone or portion of the upper jaw containing premolar, molar teeth, and canines (C Bhaskor Rao, 2013).



*Figure 3: Human Jaw Illustration (Library, 2021).*

### 1.2.3 Tooth Anatomy

Through a slightly thinner part called the neck, each tooth includes a root and a crown, which have been perfectly connected.

The gum or gingiva is the tissue surrounding a tooth's neck and covering the jawbone (C Bhaskor Rao, 2013).

**Root:** this portion of the tooth is embedded in the jaw to keep the tooth in place within the bone (C Bhaskor Rao, 2013).

**Crown:** the visible portion of a tooth above the gum line (C Bhaskor Rao, 2013).

### 1.2.4 The Structures of a Tooth

Teeth are structurally made up of four different tissues, which are as follows:

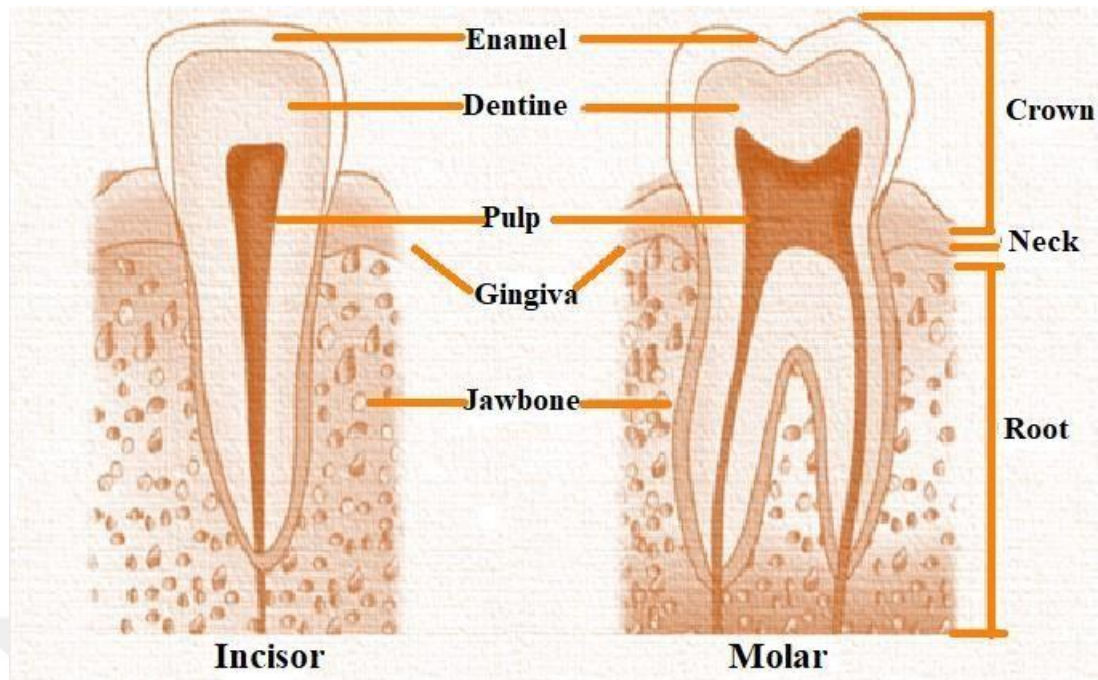
**Enamel** is one of the toughest tissues in the body and the outermost part of the tooth's crown. It is not a living tissue since it is avascular and acellular. Enamel acts as a dental safeguard that protects the teeth from damage. For example, when the teeth are exposed to the acid of food and bacteria, enamel can cause them to dissolve (C Bhaskor Rao, 2013).

**Dentin:** this tissue, found beneath the enamel and cementum, is the second most rigid tissue that makes up most of the tooth. Dentin, on the other hand, is a living tissue that is less rigid than enamel. Also, dentin completely covers the pulp of a tooth's root and crown (C Bhaskor Rao, 2013).

**Cementum:** This tissue surrounds the bone-embedded root of a tooth (C Bhaskor Rao, 2013).

**Pulp:** this tissue is formed of loose connective tissue and is in the middle of the tooth as well as beneath the layer of dentin, which is called dentin-pulp, also known as the periodontium, which means that the survival and developmental rates of dentin-pulp are dependent and interrelated on each other. Pulp also enters the tooth via the apical foramen at the tip of the root, which contains nerves and blood vessels (C Bhaskor Rao, 2013).

The only soft tissue of the tooth is the pulp. The rest of the tissues are all rigid. The cement-enamel junction, also known as the cervical line, joins the crown and the root and is visible as a dividing line on a tooth specimen with the naked eye (C Bhaskor Rao, 2013).



*Figure 4: Microscopic Anatomy of a Tooth (C Bhaskor Rao, 2013).*

### 1.3 Branches of Dentistry

The specialty of dentistry typically involves dental orthodontics, endodontics, prosthodontics, periodontics, restorative dentistry, pediatric dentistry (pedodontics), oral and maxillofacial surgery, and oral radiology, which are defined below:

- **Orthodontics** is a branch of dentistry that focuses on diagnosing, preventing, and treating teeth and jaws positioned improperly (Simon J. Littlewood, 2019).
- **Endodontics** is a treatment responsible for maintaining the health of the entire dental pulp and designing and developing the process of maintaining the well-being of a portion of the dental pulp (Walker, 2014).
- **Prosthodontics** focuses on treatment to improve oral function and patient satisfaction with beautiful and healthy teeth. This technique can restore and replace natural and artificial missing teeth (Sangappa, 2011).
- **Periodontics** is also known as periodontology. Periodontology is the study of support tissues and/or structures of teeth and is responsible for curing injuries.

and diseases that impact them. In addition, the periodontium is a group of supporting tissues/structures that contain the gingiva (gums), cementum, alveolar bone, and periodontal ligament (Reddy, 2018).

- **Restorative dentistry** mainly concentrates on enhancing oral health, which means it can provide dental health by replacing or repairing damaged teeth. The most common dental restorations are implants, bridges, and crowns (Rafael Sarkis-Onofre PhD, 2019).
- **Pedodontics** is a dentistry specialty that concentrates on the teeth treatment of children (Rao, 2012).
- **Oral & Maxillofacial Radiology** is the dental specialty that prescribes, creates, and analyzes medical testing for assessing and monitoring craniofacial illnesses (MacDonald, 2020).
- **Oral and maxillofacial surgery** is a specialty of surgery that is well-known across the world. This branch of dentistry concentrates on the prognosis and diagnosis of a wide range of ailments, traumas, and abnormalities affecting both soft and hard tissues of the mouth, jaw, and face region as well as the head and neck (Manuel, 2021).

All branches of dentistry mentioned above work for the long-term oral health prognosis. As missing teeth is one of the major problems in dentistry, dental implants are popular as one of the most significant treatments for the replacement of both teeth and implant loss.

#### **1.4 Dental Implants**

The dental implant is one of the materials used in dentistry that allows substituting of missing or damaged teeth with artificial teeth that function and appear like natural ones. In addition, dental implants provide a satisfying alternative to unshapely or oversized bridgework or prostheses and an opportunity whenever an absence of natural tooth roots prevents the construction of bridgework or prostheses that demand tooth substitution (Staff M. C., 2019).

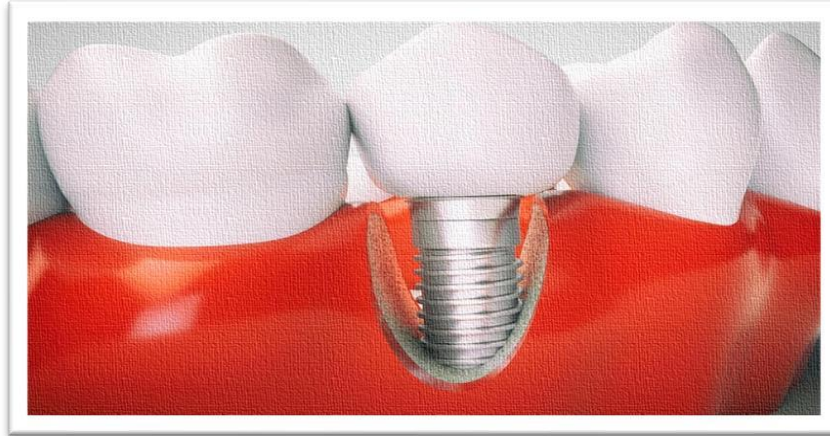
Taking advantage of dental implants has outstanding benefits, which are as follows (Staff F. V., 2021):

- Long-term study of titanium and its alloys for good osseointegration
- Provide an opportunity for permanent tooth substitution.
- Prevent Bone Loss
- Look and feel natural: It cannot be recognized by others that the teeth are artificial.
- Straightforward to care for
- There is no difference in eating and drinking with dental implants compared to natural teeth.

Besides its advantages, severe issues of implant failure have emerged. The issues that cause implant failure are as follows (Jordi Gargallo-Albiol, 2019):

- Damage to the structures around the teeth and blood vessels.
- Numbness and discomfort in natural teeth, gums, lips, and chin are caused by nerve damage.
- When dental implants inserted in the upper jaw intrude into one of the sinus cavities, it may lead to sinus difficulties.

In addition to the issues mentioned above, increased bacterial adhesion in the oral cavity can develop in common oral diseases known as periodontitis and periimplantitis leading to implant failure. Periodontitis is a chronic gum disease that has a detrimental impact on the bone responsible for supporting the tooth and consequently provides an environment leading to tooth loss. At the same time, peri-implantitis is the most well-known oral disease, which emerges as a result of a specific infectious disease in a particular part of the implant leading to bone loss around the bone implant and creating an inflammatory process in the soft tissues, which has a destructive effect on the overall function of the Osseo-integrated implant (Dinesh Rokaya, 2020). Consequently, periodontitis and peri-implantitis, marginal bone resorption, and unfavorable osseointegration can negatively impact the overall effectiveness of dental implants (Pejeva E1, 2018).



*Figure 5: A Titanium implant is Screwed into the Jaw (Staff F. V., 2021).*

The figure above shows an example of a titanium implant procedure (Fig.5). If the osseointegration capacity of the implant surface is high and bacterial adhesion capacity is low, better performance can be expected from this prosthesis with good biocompatibility.

A sessile bacterial community characterized by cells permanently attached or fixed to a substrate or interacting with one another and immersed in a substrate of extracellular polymeric compounds created by microorganisms is well known as a biofilm (Karthikeyan Subramani, 2009). Biofilms may occur on a broad range of surfaces, including tooth surfaces and implants. Dental plaque has been defined as biofilm produced on tooth surfaces. Plaque, on the other hand, has come to refer to biofilms on all oral surfaces. Biofilms in the oral cavity are comprised of diverse microbial populations embedded in a matrix of polymers derived primarily from saliva and bacteria (Pratten, 2003).

Periodontitis, peri-implantitis, stomatitis, caries, and gingivitis are all caused by bacteria in the dental plaque (Alexandros Besinis, 2014). Peri-implant disease is identified as one of the leading reasons for implant failure.

Two primary sources of microbes' cause peri-implant ailments (Belibasakis, 2013):

1. The presence of biofilms on dental implant surfaces
2. Prosthetics restorations

The emergence of biofilm on a titanium dental implant is extremely significant in recognizing whether the implantation was successfully embedded. Due to its outstanding surface characteristics and biocompatibility, titanium is commonly used in dental implants. The adsorption of the salivary pellicle, followed by bacterial accumulation and biofilm formation, induces an inflammatory reaction throughout the transmucosal curative phase of titanium dental implants (Birte Groessner-Schreiber, 2004).

Biofilm formation can be directly influenced by several factors (Karthikeyan Subramani, 2009):

1. The biofilm composition and speed of formation
2. Chemical characteristics, roughness, and surface energy of the implant
3. Abutment materials, as well as prosthetic components

New techniques such as surface modifications have emerged to tackle these problems to reduce or eliminate bacterial adhesion, enhance osseointegration, and decrease marginal bone resorption around dental implants to achieve favorable biocompatibility (Francesca Accioni, 2022). In addition, surface roughness and surface-free energy characteristics are thought to impact bacterial adhesion to implant surfaces. Accordingly, Pier-Francesco et al. (Amoroso Pier-Francesco, 2006) observed that implant surface roughness levels below those commonly found in collars/abutments in  $R_a$  350 nanometers decreased the degree of *P. gingivalis* on titanium implants.

The study by Subramani et al. (Karthikeyan Subramani, 2009) indicated that smoother abutment surfaces and implants could inhibit biofilm formation, whereas rougher implant surfaces allow for more bacterial colonization because the area of adhesion is increased by 2 to 3-fold. Therefore, the adhered bacteria are protected against shear forces in the rapidly altering oral environment.

The implant surface design can be advantageous in establishing a secure part to prevent many oral bacteria, which may even have a decontaminating impact (Stefanie Kligman, 2021).

In a preclinical investigation conducted in the 1990s, Buser et al. (D. Buser, 1991) examined the effects of surface features on bone osseointegration across five distinct titanium surfaces by increasing the number of physiological procedures in

the peri-implant alveolar bone, such as calcifying osteoblasts, matrix synthesis, attachment, proliferation, and differentiation.

Another research hotspot in dental implants is the micro-nano structural implant surface alteration, which may enhance the implant's hydrophilicity and bone conductivity while minimizing stress conduction. Furthermore, the new and different technologies of surface coatings are constantly expanding. These technologies for surface coatings involve interdisciplinary research, especially in biomaterials. Such technologies can influence bacteria interactions and have the potential to improve implant surface properties such as surface roughness, charge, wettability, and chemical composition (Heng Dong, 2020).

As illustrated below (Fig. 6), to enhance the overall effectiveness of titanium and its alloys as well as zirconia implants, the technology of surface modifications has emerged to modify and/or alter the characteristics of the implant surfaces in a manner that can simultaneously develop to better osseointegration, less bacterial adhesion capacity, and good biocompatibility.

On the one hand, to improve the osseointegration rate between the implant and the jawbone, popular surface coatings of implants known as hydroxyapatite, magnesium, graphene, growth factors, and extracellular matrix proteins have recently become the most reliable and effective materials to be used in the biomedical engineering field. On the other hand, antibacterial coatings materials have now attracted much attention to lessen or minimize the bacterial adhesion capacity on the implant surfaces to achieve a desirable performance of titanium and zirconium implants. These popular antibacterial coatings are well-known as antibiotics, antimicrobial peptides, metal elements, and polysaccharides.

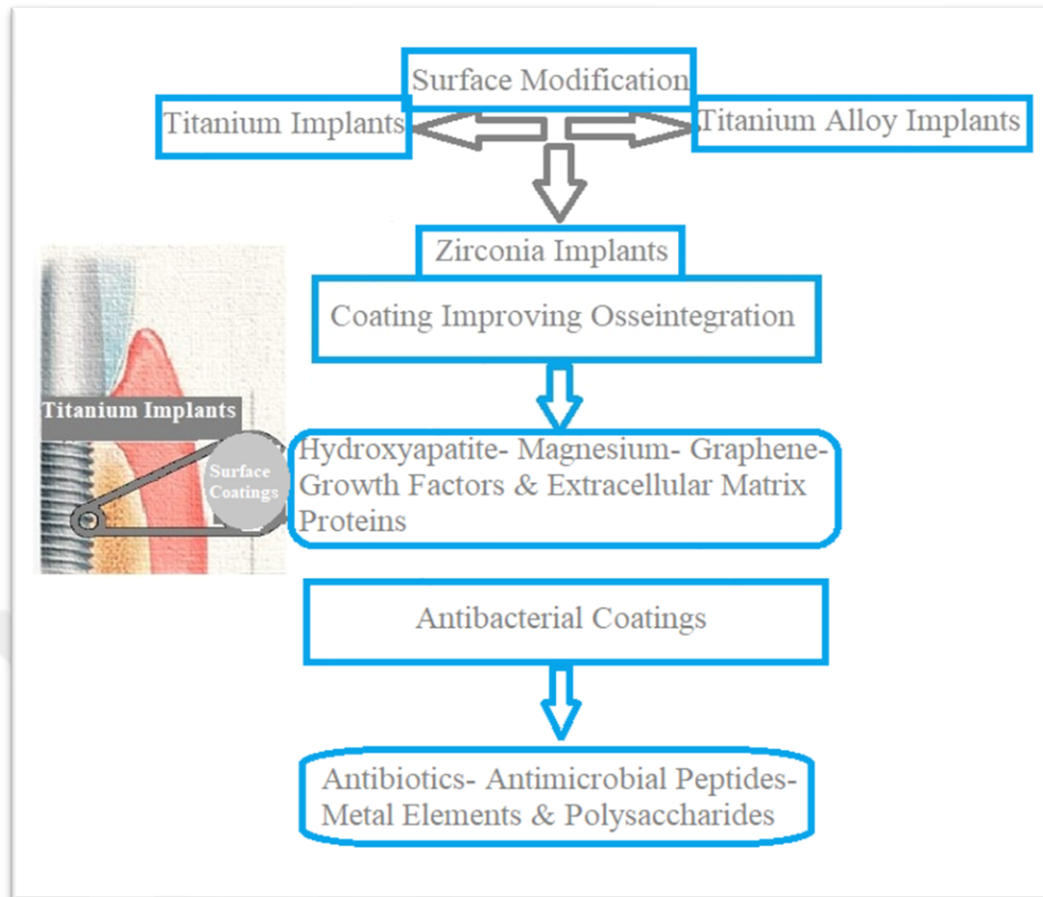


Figure 6: Surface Modifications and Effective Coatings of Dental Implants (Heng Dong, 2020).

#### 1.4.1 Titanium Dental Implants

Titanium's characteristics have the potential to respond satisfactorily to a wide range of dental demands, such as excellent biocompatibility, corrosion resistance, relatively high strength, desirable formability, good machinability, and low modulus of elasticity. Besides, both bone-to-implant contact (BIC) and removal torque values (RTV) are the targeted benefits that can be attained through modifying, adjusting, and changing the texture of surface titanium implants (Qingge Wang, 2020). Furthermore, many dental implant manufacturers take advantage of titanium and its alloys because of their osseointegration capacity, which histologically means the interaction between the treated surface and the alveolar bone to optimize the contact (Meirelles, 2014). Fundamentally, biocompatibility as well as both chemical and mechanical properties are essential when selecting the material for dental implantology.

For instance, one of the most used titanium in the industry is commercially pure titanium (Cp-Ti) which plays an essential role in engineering materials, particularly because of its anti-toxic characteristics, favorable corrosion resistance, and good biocompatibility (Parashar, 2022).

#### **1.4.2 Zirconia Dental Implants**

Because of its good biocompatibility, thermal conductivity, and desirable characteristics, zirconia has recently been brought to dental implantology. The reason that zirconia implants have attracted the attention of researchers compared to titanium implants is that they have a higher organization of collagen fibers and a lower sulcus depth in the gingival tissue leading to likely developed soft tissue integration as well as extra enhancing the aesthetic results of the implantation (Antonio Linares, 2016). However, some controversial debates are being continued because of a scarcity of long-term studies on the osseointegration of zirconia implants. Nowadays, various physicochemical methods are designed to enhance zirconia implants' biological and osseointegration properties, such as laser treatment, grit blasting, ultraviolet treatment, acid-etching, and machining (Fernanda H. Schünemann, 2019).

#### **1.5 Common Technologies of Surface Modifications**

The implant surface has been modified to increase biological surface properties that promote osseointegration (Ralf Smeets B. S.-B., 2016). When comparing a rough surface with a smooth surface, improved osteoblast activity has been revealed at 1 to 100 meters of surface roughness, which verifies the importance of surface roughness in implant osseointegration (Cornelius von Wilmowsky, 2013). Rough surfaces are supposed to have more excellent osseointegration compared to smooth surfaces, however; study results have been inconsistent. As a result, it is unclear if numerous procedures offer superior predicted outcomes (A. Barfeie, 2015).

Chemical and physical treatments are the two main approaches to enhance surfaces by modifying or altering the characteristics of dental implants to achieve

desirable biocompatibility, as well as favorable corrosion resistance and good osseointegration, which are explained below:

### 1.5.1 Chemical Treatment

Through chemical treatment, the process of modifying or changing the morphology and condition of the implant by either chemical reaction or chemical adsorption amidst both the modifier surface and the titanium surface is obtained (Pietro Mandracci, 2016).

The most used methods in chemical treatment are as follows:

#### 1.5.1.1 Anodization

This electrochemical method has the potential to enhance biocompatibility, cell adhesion, osteoblast proliferation, and blood-clot structure through titanium surface oxidation. Due to the anodization method, in the form of anatase, the expanding oxide layer of titanium dioxide that produces microporosity has been plunged into the implant with acids like  $\text{HNO}_3$ ,  $\text{H}_3\text{PO}_4$ , and  $\text{H}_2\text{SO}_4$  even though a flow is put in. The most significant titanium dioxide ( $\text{TiO}_2$ ) stages have also been identified as rutile and anatase. Specifically, the element within the anatase stage is produced by four titanium dioxide ( $\text{TiO}_2$ ) components that O atoms are bonded to 3 titanium atoms and placed in the same line to produce a complicated shape (Bourikas, Kordulis, & Lycourghiotis, 2014). According to Zhihao Gong et al., anodizing dental implants have been shown to promote blood-clot retention (Zhihao Gong, 2019). Finally, anodizing dental implants can improve osseointegration as well as nano-roughness (Changjiang Pan, 2021).

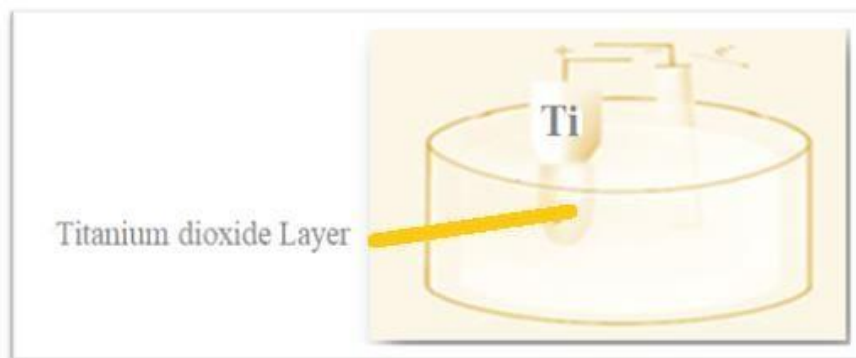


Figure 7: Anodization Illustration (Francesca Accioni, 2022).

### 1.5.1.2 Acid-Etching

The acid-etching technique uses corrosive acids such as  $\text{HNO}_3$ ,  $\text{HF}$ ,  $\text{H}_2\text{SO}_4$ ,  $\text{H}_3\text{PO}_4$ , and  $\text{HCL}$  to induce micro-roughness on the titanium surface via micro-well configuration. Acid-etching erosion often occurs after sandblasting (SLA). Moreover, the entire procedure of acid-etching erosion is assumed to be the source of surface modification, referred to as large grit acid etching and sandblasting (Anil, Anand, Alghamdi, & Janse, 2011).

During long-term clinical trials, Osseotite® achieved a more than 96% performance. As a result of increasing roughness and osteogenic reactions owing to adhesion, differentiation, and osteogenic cell proliferation, titanium surfaces subjected to the acid-etching method have shown favorable outcomes (Velasco-Ortega, et al., 2016) (Sebastian Blatt, 2018). Nevertheless, there is a significant variation due to the biocompatibility, corrosion resistance, and mechanical properties, with either under-etching or over-etching of the surface during an unregulated procedure identified in some instances (Pankaj Chauhan, 2021).



Figure 8: Acid-etching Illustration (Francesca Accioni, 2022).

### 1.5.2 Physical Treatment

Excellent biocompatibility and the potential for good osseointegration are achieved with the use of titanium in the field of dental implants. There are some trending methods to enhance the surface roughness and composition due to the osseointegration rate between the titanium surface and live bone. Physical treatment is one of the approaches to modifying or altering the surface roughness and morphology of titanium implants to achieve a satisfying osseointegration pathway.

The most used methods in physical treatment are as follows:

### 1.5.2.1 Sandblasting

According to Cao et al.'s study (Lei Cao, 2018), sandblasting can eliminate either plastic deformation layers or contaminants to achieve a specific surface that is available to be modified topographically. Through sandblasting with an airflow of accelerated particles and under appropriate pressure, various particles, such as hydroxyapatite (HA), silicon dioxide ( $\text{SiO}_2$ ), as well as aluminum dioxide ( $\text{Al}_2\text{O}_3$ ), and titanium dioxide ( $\text{TiO}_2$ ), would cause micro-roughness to the surface of the implant. In addition, the benefits of wettability and topography on the surface of the titanium have emerged to promote cell adhesion, differentiation, and proliferation from sandblasting method (Frank P. Nothdurft, Cenk Aktas, & Yasmin Mehraein, 2014). The absorbable sandblasting media in the titanium implant treatment showed superior performance without any visible marginal bone loss during a 4-year medical monitoring trial.

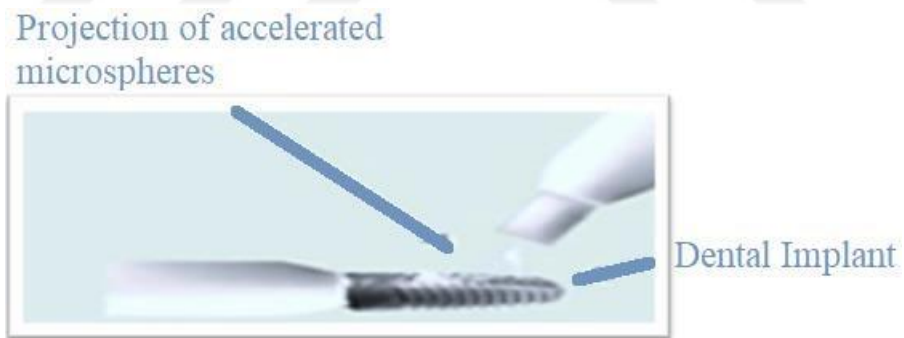


Figure 9: Sandblasting Illustration (Francesca Accioni, 2022).

### 1.5.2.2 Plasma Spray

Due to the extra layer of OH groups, the plasma-spraying technique is combined with sandblasting to increase protein absorption and biocompatibility. In other words, the plasma-spraying technique modifies or changes the implant's surface by projecting titanium particles at high temperatures into a plasma torch and shapes or forms the film via Ti-OH residues at about  $30\ \mu\text{m}$  thickness (Pietro Mandracci, 2016).

Md Mirazul MahmudAbir et al. have recently shown that plasma spraying has the potential for both characteristics of antibacterial enhancement as well as bacterial adhesion. Antibacterial properties have improved as the number of Magneli phases has increased. In addition, bacterial adhesion has improved since the resulting submicron-sized particles have hydrophilic characteristics (Md Mirazul MahmudAbir, 2022).



Figure 10: Plasma Spray Illustration (Francesca Accioni, 2022).

### 1.5.2.3 Laser-Etching

The use of a laser annealing machine to remove tiny materials is generally referred to as laser etching or laser ablation. Laser etching can affect nano topography surfaces, while other methods such as sandblasting can help develop both micro and macro roughness surfaces. This method requires the use of laser sources to cause the metal to dissolve in place due to the heat generated by absorbing the radiation (Anil Kurella, 2005). Therefore, micro-matching is produced due to the emergence of micro-channels contributing to quicker bone amending (Nevins, et al., 2010). Due to the maximum benefits of optimizing both available frequencies and energy density, laser-etching can adjust the wavelength. Furthermore, to achieve microstructures with corrosion resistance and improved hardness, the source may be pulsed, and the time of reaction controlled (Saad M. Al-Zubaidi, 2020). For example, according to the SLActive® research study conducted by the Straumann Institute in Basel, Switzerland, a final manufacturing step in which the implant was rinsed with a stream of nitrogen to protect it from the air and then immersed in a NaCl solution for storage, produced a nano-roughened surface through blasting and acid etching. Increased hydrophilicity throughout the biological reaction and cell recruitment and the long-term

effectiveness of about 91.7% faster bone mending are the outstanding advantages of laser-etching technology (Davide Farronato, 2014).

As another example of taking advantage of using this technique, on top of commercially pure titanium, Boutinguiza et al. (M. Boutinguiza, 2018) utilized it to deposit silver nanoparticles.

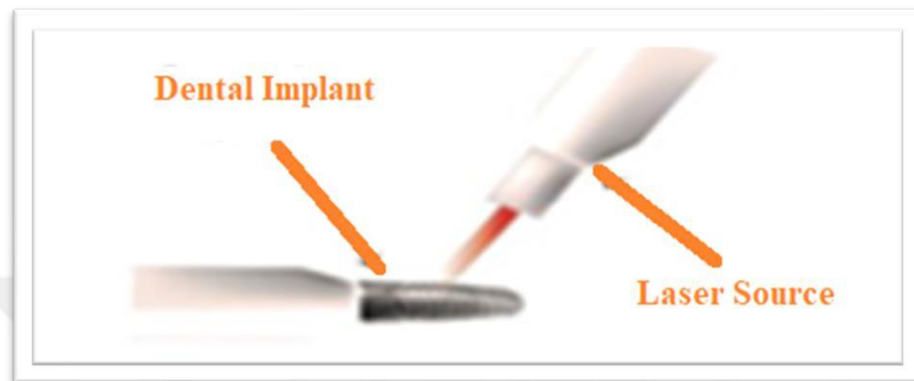


Figure 11: Laser-etching Illustration (Francesca Accioni, 2022).

## 1.6 Bacteria

Bacteria are found in virtually every environment on Earth, including soil, water, air, and the bodies of plants and animals. They are the most abundant organisms on the planet and play a critical role in various ecosystems and human life. Bacteria are tiny organisms that typically vary from 1 to 6  $\mu\text{m}$  in length and 0.5 to 1  $\mu\text{m}$  in diameter. Bacteria's DNA floats abundantly throughout bacterial cells. It consists of the nucleoid, a twisted, thread-like material, and some bacteria have plasmids, which are circular pieces of DNA (Musarrat, 2006). Bacteria are prokaryotes that have nuclei and other membrane-bound organelles. In addition, their structure has been organized as a distinct internal in a single-celled form (Kahate, 2022). Bacteria can carry out a wide range of metabolic processes, including photosynthesis, nitrogen fixation, fermentation, and respiration. Although many bacteria are beneficial and necessary for life, some bacteria can cause disease in humans and other organisms. Common examples of pathogenic bacteria include *Streptococcus*, *Escherichia coli*, *Salmonella*, and *Staphylococcus*. These bacteria are the potential to cause serious diseases as a result of infections and food poisoning. For example, *Streptococcus* can cause a variety of human illnesses, ranging from mild infections such as strep throat to more serious and

potentially life-threatening conditions such as pneumonia, sepsis, and meningitis (Georgiev, 2009). Bacteria can be mistaken for archaea, which are both prokaryotes and single-celled organisms (CARL R. WOESE, 1990). The materials that archaea use to build their cell walls and their metabolic processes are the notable differences between bacteria and archaea. The cell walls of bacteria are made of peptidoglycan, while the cell walls of archaea are made of different materials, including pseudo-murein, glycoproteins, or S-layer proteins. (Schleifer, 2008).

Due to the basic shapes of bacteria, they are divided into five fundamental types that are listed as follows (Young, 2006):

- *Spherical (cocci)*
- *Rod (bacilli)*
- *Spiral (spirilla)*
- *Comma Shaped (vibrio)*
- *Corkscrew (spirochaetes)*

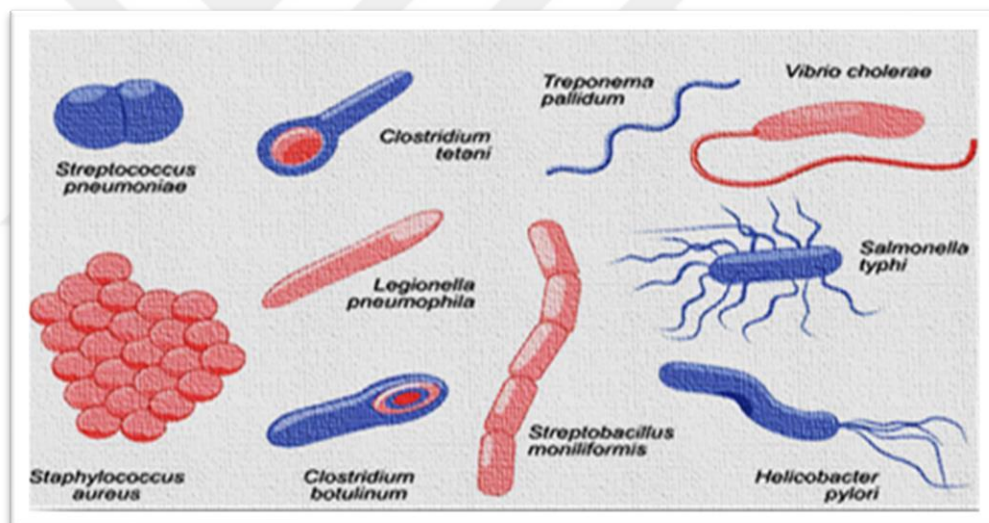


Figure 12: Various Forms of Bacteria (Society, 2022).

As shown above (Fig. 12), various bacteria shapes and/or forms exist as single cells and in clusters, chains, and pairs.

Gram-stain is a test to categorize bacteria as both gram-positive and gram-negative, which are divided based on the structure of their cell walls. Gram-positive bacteria lack an outer membrane, whereas gram-negative bacteria have an outer membrane. For instance, *Streptococcus pneumonia* is a gram-positive bacterium, whereas *Escherichia coli* and *Vibrio cholerae* are gram-negative bacteria (Post, 2019).

According to the presence of oxygen to survive and reproduce, bacteria can be divided into three types, which are called aerobic, anaerobic, and facultative anaerobe (Lucas Pedraz, 2019). Aerobic bacteria can only survive and reproduce in the presence of oxygen, whereas anaerobic bacteria can only survive and grow in the absence of oxygen. Besides, facultative anaerobe is an exceptional type that prefers to survive and grow in the presence of oxygen but can continue growing without oxygen (D.J. Bradshaw, 1997).

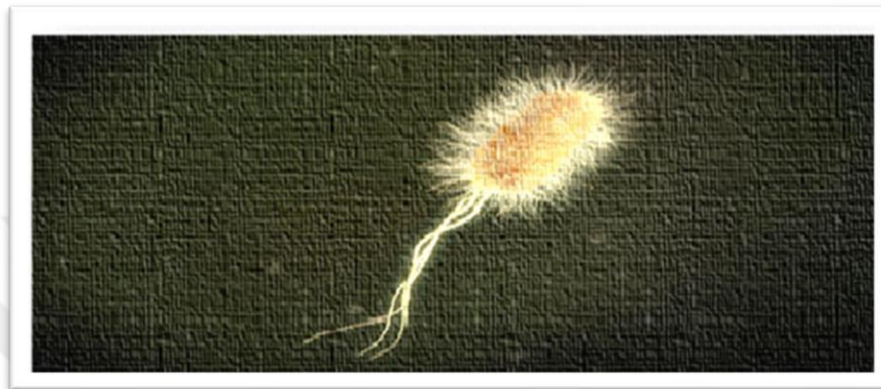


Figure 13: 3D Schematic of *Escherichia Coli* (Society, 2022).

### 1.6.1 The Role of Bacteria in Oral Cavity

Bacteria play an important role in the oral cavity, both in health and disease. In a healthy oral cavity, a balanced microbial community helps maintain the health of the teeth and gums. However, when the balance of bacteria in the oral cavity is disturbed, it can lead to oral diseases such as dental caries (tooth decay) and periodontal disease (gum disease). This can be caused by factors such as a lack of oral hygiene, a diet high in sugar and carbohydrates, and certain medications (Asma Gasmi Benahmed, 2021) (Anne Marie Lynge Pedersen, 2019). Specific types of bacteria, such as *Streptococcus mutans*, are known to be particularly detrimental in the oral cavity. These bacteria produce acid that can erode tooth enamel and lead to tooth decay. Other bacteria, such as *Porphyromonas gingivalis* and *Enterococcus faecalis*, have been linked to the development of periodontal disease (Marzena Kucia, 2020).

The oral cavity is a vital part of the human body and plays an essential role as a mini ecosystem in the body. Determining an individual's oral health depends on its healthy and good indigenous microflora, the surface of the teeth and gums,

and the oral cavity linings. These indigenous microorganisms are included in various types of bacteria, viruses, and fungi, of which more than 700 species have been found following the developments in both genetic and microbiological methods, and still many continue to be discovered (Dr. J.M. Albandar, 1999).

Microorganisms such as those in the oral cavity are typically found on the surface tissues of all humans. Their variety and number vary with their hygiene levels, age, and nutrition (Neetu Sharma, 2018). Furthermore, such microorganisms can be caused by a wide range of systematic infections, such as bacterial endocarditis, pediatric osteomyelitis, preterm low birth weight, cardiovascular disease, as well as bacterial respiratory pneumonia (Ploeg, 2005). However, different bacterial species in the normal oral cavity are noticeable, such as *Veillonella*, *Corynebacterium*, *Staphylococcus*, *Enterococcus*, *Bacteroids*, *Lactococcus*, *Lactobacillus*, and the genus *Streptococcus* (Qian-Qian Wang, 2012).

### **1.6.2 The Emergence of Bacteria in Implant Failure**

Implant failure can occur for a variety of reasons, one of which is the emergence of bacteria in the implant site. Bacteria can colonize the implant surface, form a biofilm, and cause infection leading to implant failure. The presence of bacteria in implant failure can be due to a variety of factors, including abundant emergence of bacterial adhesion around implants, poor surgical technique, inadequate sterilization of implant surfaces, contamination during implantation, compromised immune systems, and systemic infections (Carla Renata Arciola, 2018). It is highly important to note that the types of bacteria that can cause implant failure may vary depending on the location and type of implant (Marc Quirynen, 2002).

Several types of bacteria that can potentially damage implants in the oral cavity are summarized as follows (Bakken, 2015):

- ***Streptococcus mutans***: This bacterium is one of the primary culprits in the development of dental caries and can also form biofilms on implant surfaces.
- ***Porphyromonas gingivalis***: This bacterium is associated with periodontitis, a condition that can cause implant failure by destroying the bone and soft tissue surrounding the implant.

- ***Aggregatibacter actinomycetemcomitans***: This bacterium is also associated with periodontitis and has been found to colonize implant surfaces and form biofilms.
- ***Staphylococcus aureus***: This bacterium is commonly found on the skin and can cause infection if it enters the bloodstream and reaches the implant site.
- ***Enterococcus faecalis***: This bacterium is commonly found in the oral cavity and has been associated with persistent infections of dental implants.

A detrimental chronic condition affecting soft and hard tissues around dental implants and consequently leading to implant failure is peri-implantitis (Frank Schwarz, 2016). Smoking, insufficient oral hygiene, and periodontitis have all been related to peri-implant ailments (Meyle, 2008). Both increased bacterial adhesion and poor osseointegration between the bone and the implant can be caused by micro-mobility due to peri-implant bone loss (Shriya Nimbalkar, 2020). Generally, dental implants have outstanding long-term survival rates, but simultaneously, implant failures occur in a minority of cases. Statistically, primary implant failure occurs in 1– 2% of patients during the first few months, while secondary implant failure, commonly caused by peri-implantitis, occurs in about 5% of cases after several years of excellent osseointegration results (Ralf Smeets A. H., 2014). The most common bacterial species in peri-implantitis areas are *E. saphenum*, *E. brachy*, *Filifactor alocis*, *Eubacterium nodatum*, *Fusobacterium nucleatum*, *Centipeda periodontii*, *Slackia exigua*, *Parascardovia denticolens*, *Prevotella intermedia*, *Parvimonas Micra*, and *Porphyromonas gingivalis* (Tamura N, 2013). However, when comparing periodontitis-affected teeth, larger numbers of *Treponema denticola*, *Prevotella intermedia*, *Tannerella forsythia*, *Porphyromonas gingivalis*, and *Aggregatibacter actinomycetemcomitans* occur in peri-implantitis (Priscila Ladeira Casado, 2011).

### 1.6.3 The Role of Enterococci Bacteria Strain in Dentistry

*Enterococci* are a group of bacteria commonly found in the human gastrointestinal tract and female genital tract. In dentistry, *Enterococcus* bacteria are associated with several important roles, including (Charles H. Stuart, 2006):

- **Root canal infections:** *Enterococci* are often associated with persistent infections in the root canals of teeth, which can lead to serious complications such as abscesses and bone loss.
- **Dental caries:** *Enterococci* are present in dental caries (tooth decay) lesions. The bacteria produce acid that can dissolve tooth enamel, leading to tooth decay.
- **Periodontal disease:** *Enterococci* have been implicated in periodontal disease, a chronic inflammatory condition that affects the tissues that support the teeth.
- **Antibiotic resistance:** *Enterococci* can transfer antibiotic-resistance genes, which can lead to the development of drug-resistant strains.
- **Probiotics:** *Enterococci* have been used as probiotics to promote oral health. Certain *enterococci* strains have antimicrobial activity against other detrimental oral bacteria, such as *Streptococcus mutants* associated with dental caries.

Consequently, *Enterococci* can play both beneficial and detrimental roles in dentistry. While certain strains of *Enterococci* have probiotic potential, others can cause serious dental infections and contribute to the development of antibiotic resistance. *Enterococci* bacteria are facultative anaerobes, gram-positive and nonsporulating, with a low GC that nowadays are globally among the most common multidrug-resistant hospital infections (Phillips, 2009). *Enterococci* can cause a wide range of diseases, such as wound infections, surgical infections, urinary tract, endocarditis, and sepsis (Seema Sood, 2008). Even though the genus *Enterococcus* contains over 40 biologically distinct species, two of them, *E. faecalis* and *E. faecium*, can cause more than 90% of enterococcal infections (Daria Van Tyne, 2013). Among *Enterococci* strains, *Enterococcus faecalis* has mostly been related to a broad range of ailments such as unsuccessful root canal therapy, long-term periodontitis (T. E. Rams, 1992), and even caries lesions (I. G.

CHESTNUTT, T. W. MACFARLANE, and K. W. STEPHEN, 1994). *Enterococcus faecalis* (EF) is a bacteria strain commonly found in the human gastrointestinal tract and is known to cause infections in various parts of the body, including the urinary tract and bloodstream. Moreover, *E. faecalis* can be found in the oral cavity and its detrimental impact can cause dental implant infection due to peri-implant disease. *E. Faecalis* (EF) can colonize the implant surface and form biofilms, which are communities of bacteria embedded in a matrix of extracellular polymeric substances (EPS). Biofilms protect bacteria from host defenses and antimicrobial agents, making them resistant to conventional antibiotic therapy (Delphine Dufour, 2012).

Razavi. et al. (A. Razavi, 2007) suggested that *Enterococci* colonization in the healthy oral cavity is transient, but at the same time, there is a possibility that *Enterococci*'s primary source of root canal infection is related to foodborne.

One of the most common causes of primary root canal infections in teeth with coronal leakage is *E. faecalis*, howbeit not in large numbers (Brenda P. F. A. Gomes, 2006). *Enterococci* can persist in root canal systems in monocultures and mixed cultures (LARS FABRICIUS, 1982), and once established, they are difficult to eradicate (B, ENGSTROM, 1964). Therefore, *Enterococcus faecalis* can play a role in causing peri-implantitis, a destructive inflammatory disease that affects the soft and hard tissues around dental implants. To clarify, the most common causes of periodontal diseases in the early months of placing dental implants are gingivitis, periodontitis, and chronic periodontitis (Bruce L Pihlstrom, 2005).

Long-term (chronic) periodontitis caused by *E. faecalis* can lead to peri-implantitis, which is one of the main causes of implant failure (Subramani & Wismeijer, 2012).

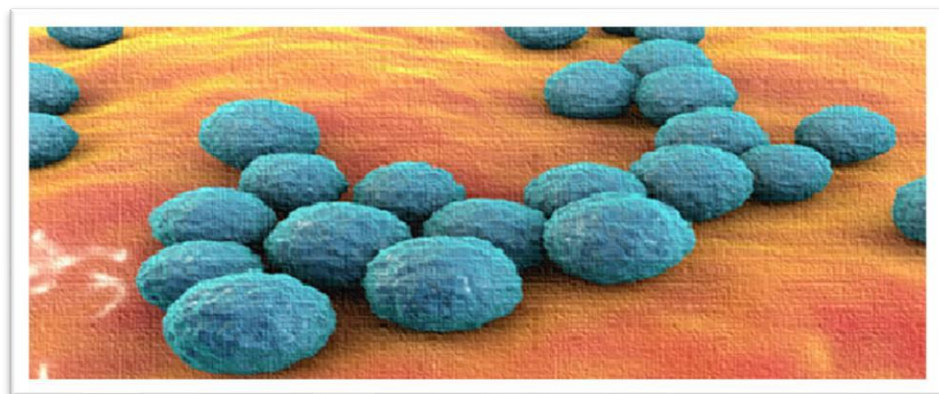


Figure 14: *Enterococcus Faecalis* Illustration (Staff, istockphoto, 2017).

## 1.7 The Role of Honeycomb Structures in Biomedical Engineering

As the name implies, the honeycomb structure is designed in the shape of a beehive. Honeycomb structures are widely used in several fields, such as chemical engineering, mechanical engineering, nanofabrication, and architecture. Honeycomb structures are a common pattern in nature and have been widely used in various engineering fields, including biomedical engineering (Caigui Jiang, 2014).

The characteristic of the general honeycomb structure in the biomedical engineering field can be summarized as follows (BABU, 2020) (Safaei, 2022):

- **High strength and stiffness:** Honeycomb structures are known for their high strength-to-weight ratio, which makes them ideal for applications where strength and stiffness are crucial. In biomedical engineering, honeycomb structures are often used in implants and prosthetics, where they need to withstand the mechanical stresses of the body.
- **Lightweight:** Due to the geometric pattern of the honeycomb structure, it has a high strength-to-weight ratio, which makes it an ideal material for biomedical implants and devices that need to be lightweight.
- **Good thermal insulation:** Honeycomb structures have good thermal insulation properties, which makes them useful in applications where thermal stability is important. In biomedical engineering, honeycomb structures are often used in devices that need to maintain a constant temperature, such as incubators and drug delivery systems.
- **Biocompatibility:** Honeycomb structures can be made from various biocompatible materials, meaning they are not harmful to living tissues. This makes them ideal for use in biomedical implants and prosthetics.
- **High surface area:** The honeycomb structure has a high surface area-to-volume ratio, which makes it ideal for applications where surface interactions are important. In biomedical engineering,

honeycomb structures are often used in tissue engineering applications, where cells need to interact with the surface of the scaffold to form new tissues.

In addition, the honeycomb structure is a versatile and useful pattern in the biomedical engineering field, with many applications across a range of devices and systems, especially in the application of tissue engineering as well as regenerative medicine (Zhang, 2015). The main reason behind it is because of its mechanical performance as well as specific energy absorption (Gibson, 1997). However, understanding the unique properties of honeycomb structures has become the most challenging area due to their materials, scales, and structures. In other words, these textural outlooks will play an important role in designing and fabricating honeycomb structures in various ranges, including macro, micro, and nanoscale. For instance, nanohoneycomb structures have recently been developed for biocatalysis and bio-adsorption (Wang, 2019). As shown in Figure 15, various types of inside cells in honeycomb structures are used for different purposes in several fields, including biomedical engineering (Zhonggang WANG, 2016). As illustrated below (Fig. 15), the different types or shapes of honeycomb structures from (a) to (f) are:

- (a) general structure
- (b) triangular structure
- (c) double hexagonal structure
- (d) inside the circle of the structure
- (e) full double hexagonal structure
- (f) full inside of the circular structure

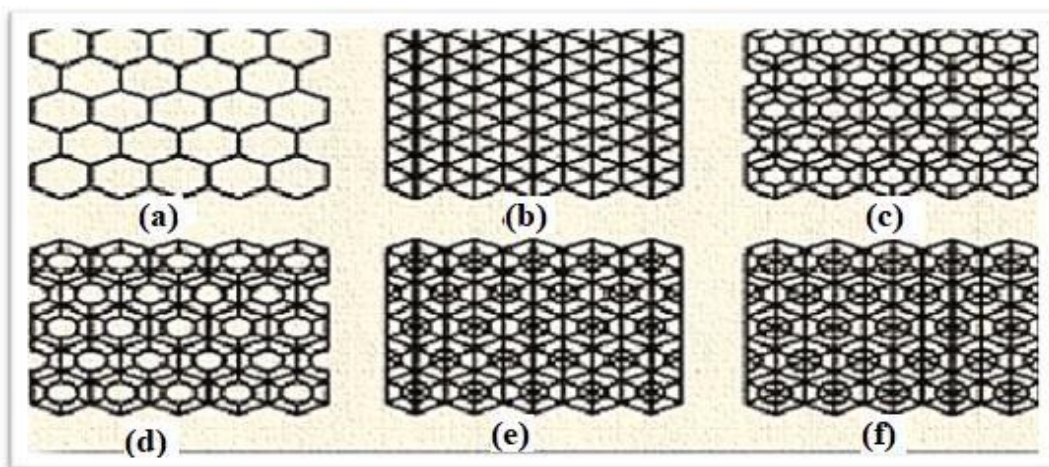


Figure 15: Types of Inside Cells in the Honeycomb Structure (Zhonggang WANG, 2016).

This study compared four different surface patterns: honeycomb, plain, line type 1, and line type 2, to determine their effect on minimizing bacterial adhesion to titanium plates. The research aimed to investigate the potential variations in bacterial adhesion between different surface patterns. Additionally, considering the distinctive properties and diverse applications of the honeycomb structure in biomedical engineering, this study hypothesized that the honeycomb pattern could decrease the number of bacterial colonies on titanium plates.



## Chapter 2

### Materials and Methods

In this study, the effectiveness of various titanium surface patterns in combating *Enterococcus Faecalis* (EF), a type of bacteria with antimicrobial properties, was investigated. The preparation of titanium samples took place at the Laboratories of Boğaziçi University Center for Life Sciences and Technologies during the pre-experimental phase. The main experiments were done in Biophotonics and Tissue Laboratories of Boğaziçi University Biomedical Engineering Institute.

#### 2.1 Preparation of Patterns

This study aimed to assess the antimicrobial properties of different surface patterns, including the honeycomb pattern, which is a widely recognized natural design. The honeycomb pattern was specifically chosen for testing due to its unique structural characteristics. Given the increasing interest in the biomedical engineering field, the honeycomb design holds promise for minimizing bacterial adhesion on titanium plates compared to other pattern shapes.

To create the samples, the titanium plates with 0.50 mm height, were cut into circular shapes with 10,95 mm diameter using a laser-cutting technique. This method involved utilizing a laser beam to precisely shape and cut the metal according to the desired pattern designs. Four distinct pattern groups were generated, each featuring a unique design and material composition: Plain Pattern, Line Type 1 Pattern, Line Type 2 Pattern, and Honeycomb Pattern.

The structure and shape of the experimental pattern groups shown in Figure 16 are briefly defined below:

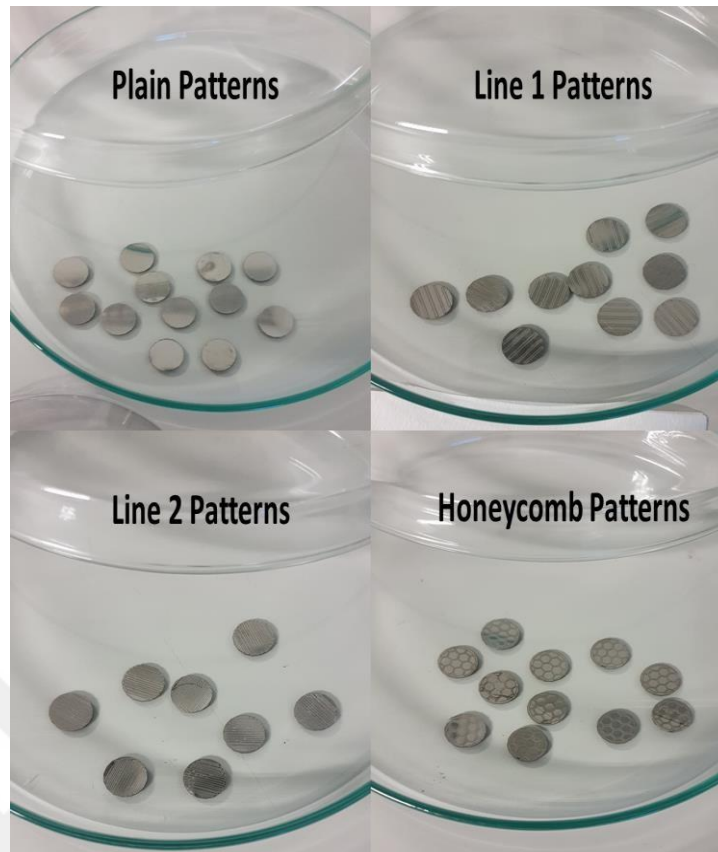
Group 1. Plain Pattern: The surface had no pattern, and its material base was laser-manufactured metal.

Group 2. Line Type 1 Pattern: The surface had low-density lines, and its material base was laser-manufactured metal. The distance between the two lines was 0.300 mm.

Group 3. Line Type 2 Pattern: The surface had much denser lines, and its material base was laser-manufactured metal. The distance between the two lines was 0.162 mm. Group 4. Honeycomb Pattern: This group exhibited a surface resembling a bee's nest, characterized by a closely arranged series of hexagonal shapes. The length of each honeycomb was 0.260 mm and the distance between two neighbor-honeycomb was 0.031 mm. The material base of this pattern was constructed using laser-manufactured metal.

The patterns were created by the manufacturer using metallic material and were designed to have equal dimensions per the specifications provided by the article's researchers. In addition, to maintain consistency and accuracy in the experiment, all pattern groups were crafted from the same laser-manufactured metal material. Subsequently, the surfaces were thoroughly cleaned using deionized water and subjected to sterilization using an autoclave.

As shown below (Fig. 16), the four groups of samples were separated into four different Petri dishes, and these four types of sample shapes were shown in the original photos. For plain (a), there was no specific shape on its surface, while for the other patterns, the differences in the shapes on their surfaces were recognizable. The surface of line 1 (b) was made of low-density lines, while on the surface of line 2 (c), the lines were much denser than line 1, and the surface shape of the honeycomb group (d) was designed as a honeycomb.



*Figure 16: Illustration of Four Different Groups of Patterns Used in the Study.*

As shown above (Fig. 16), each titanium sample group was placed in glass plates group by group, and the plates were wrapped in aluminum foil to ensure that they would not be contaminated, and then the plates were sterilized in the autoclave (OT 40L, NÜVE/Turkey) for 2 hours at 121°C. After sterilization, each plate was placed in the biosafety class II cabinet.

## **2.2 Preparation of Broth**

BHI (Brain Heart Infusion) broth is a liquid bacterial culture medium commonly employed in microbiology to facilitate the growth of various microorganisms. It comprises nutrient-rich components that promote bacterial growth. In this study, Brain Heart Broth (Brain Heart Broth Merck-64271, Darmstadt, Germany) was utilized as a culture medium to cultivate and culture the bacteria *Enterococcus faecalis* (EF). This widely used medium provides essential nutrients necessary for bacterial growth.

Firstly, according to the manufacturer's instructions, Brain Heart Infusion (BHI) broth was used by adding 14.8 grams of broth and 400 mL of distilled water. After that, the contents of the flask or bottle were thoroughly mixed by shaking or swirling until the powder was completely dissolved and the solution was sterilized in an autoclave at 121°C for 2 hours.

Phosphate Buffered Saline was prepared using PBS tablets (Phosphate Buffered Saline Solution Tablets, AMRESCO, US) with the addition of 100 ml of distilled water, and the samples were autoclaved for 2 hours.

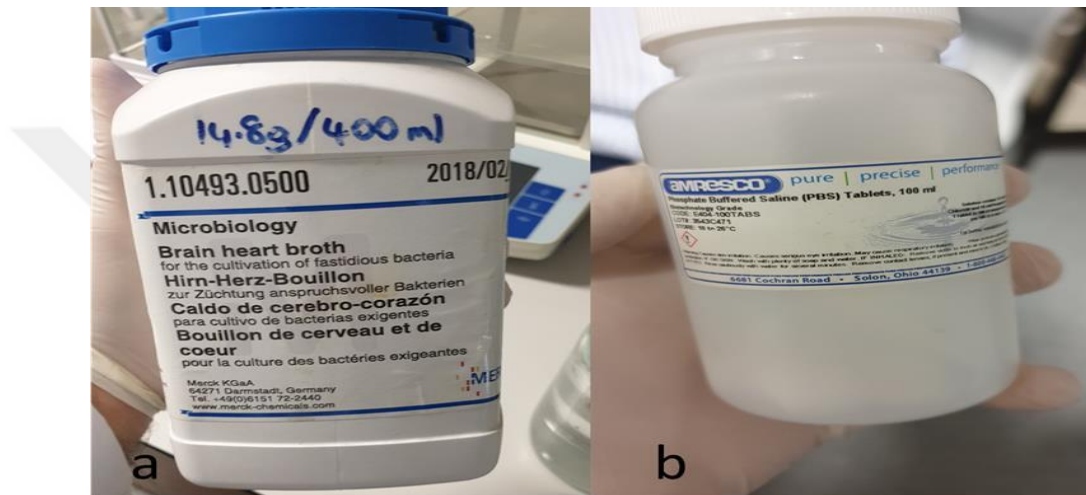


Figure 17: (a): Brain Hearth Broth (b): Phosphate Buffered Saline

### 2.3 Bacterial Culture with Broth

The experimental setup consisted of introducing the bacterial strain onto the titanium plates followed by incubation in controlled conditions.

The bacterial strain used in this study was *Enterococcus faecalis* (EF) (ATSCC 29). After removing the bacteria from the freezer to melt the bacteria, the glycerol stocks were opened under a Class II biosafety cabinet (ESCO Scientific - Airstream® Class II, Biosafety Cabinet, Model AC2-4E, Singapore). After melting, 20 to 25 mL of broth was added to the two sterilized falcon tubes in the Biosafety Class II cabinet, fully decontaminated with 70% alcohol. *Enterococcus faecalis* was added to the two separate broth-filled falcon tubes. The falcon tubes were placed in the incubator (MEMMERT, Germany) at 37°C to allow the bacteria in the broth to multiply in a suitable environment. After 72 hours of keeping the

broth in the incubator, observation of the bacteria confirmed that *Enterococcus faecalis* had grown abundantly.

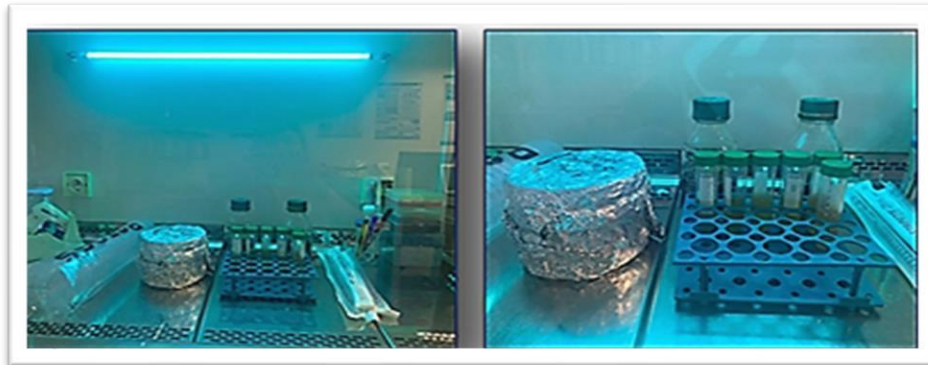


Figure 18: Samples under the UV Light.

## 2.4 Inoculation of Bacteria

After removing the bacteria in Petri dishes (fully wrapped) from the incubator, the four samples (plain, honeycomb, line 1, and line 2) were filled with broth samples containing *E. faecalis* bacteria under the Biosafety Class II cabinet. Each sample received 10 mL of the broth sample. After soaking the samples in bacterial broth, they were all returned to the incubator at 37°C for 72 hours.

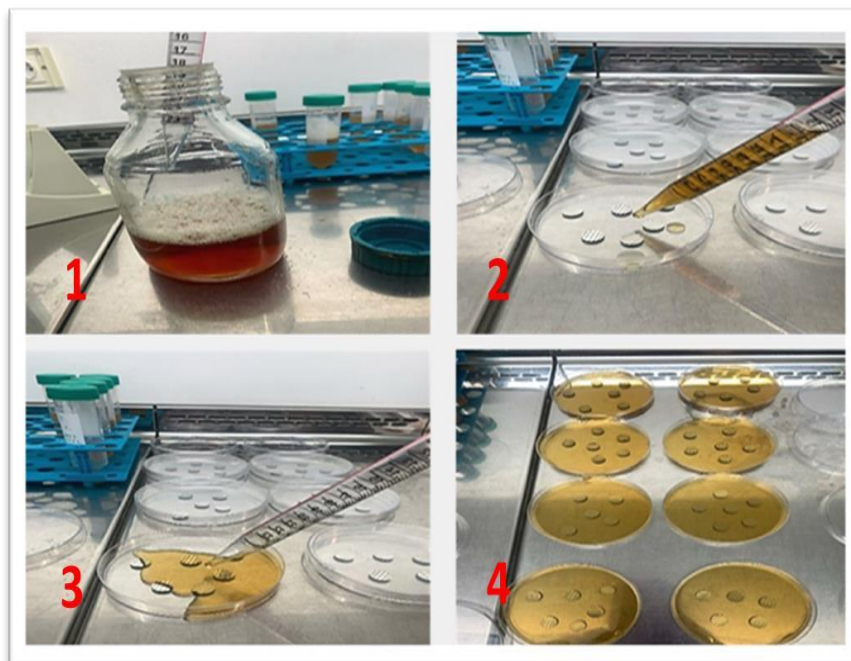


Figure 19: Procedures of Bacteria Inoculation.

## 2.5 Wash the Samples with PBS

Using the Biosafety Class II cabinet, the broth was poured into Falcon tubes and the samples were washed with PBS. After sterilizing the Falcon tubes under UV light for 1 hour, 10 mL of the broth containing *E. faecalis* bacteria was added to each tube. Each sample was washed with 10 mL of PBS. After cleaning, the samples were placed in different Falcon tubes and the samples were returned to the incubator at 37°C. After 72 hours, the samples were renewed by adding another 10 mL of broth containing *E. faecalis* and returned to the incubator at 37°C.

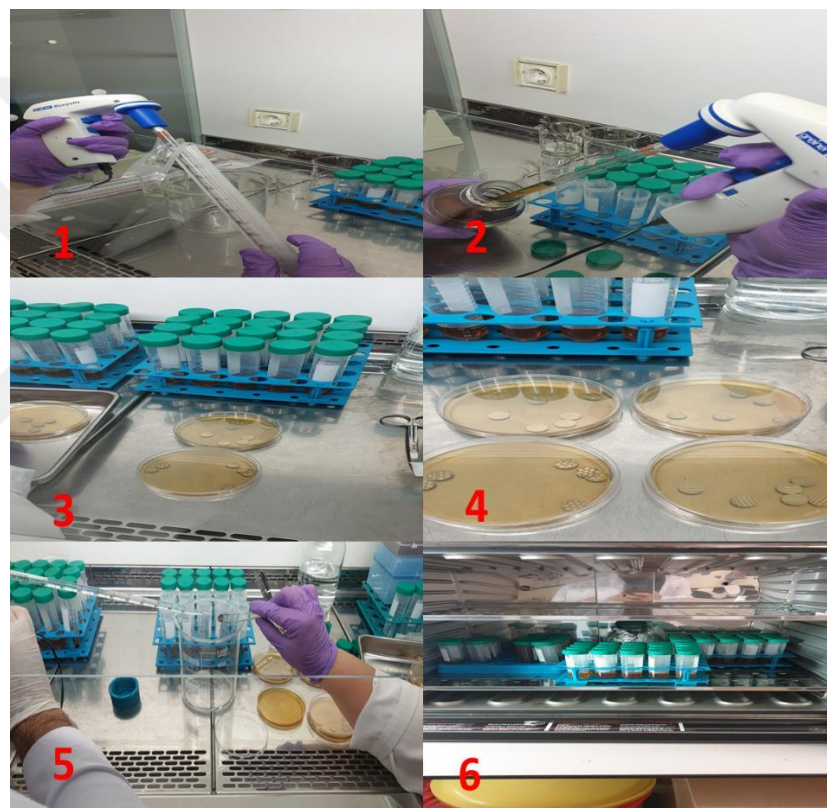


Figure 20: Washing Samples Procedures

## 2.6 Preparation of Agar

67.2 grams of agar (Brain Heart Infusion Agar-manufactured by ACUMEDIA Lansing, Michigan, United States) were measured and added to three beakers filled with 1300 ml of distilled water. The samples were autoclaved for two hours or until the temperature reached 121°C. Under the class II biosafety cabinet conditions, 20 mL of agar was poured into Petri dishes. After the samples were

gelatinized in the Petri dishes, they were inverted before being stored in the refrigerator.

## 2.7 Preparation of PBS

Phosphate-buffered saline was prepared by adding PBS tablets to 100 ml of distilled water. The Samples were autoclaved for 2 Hours.

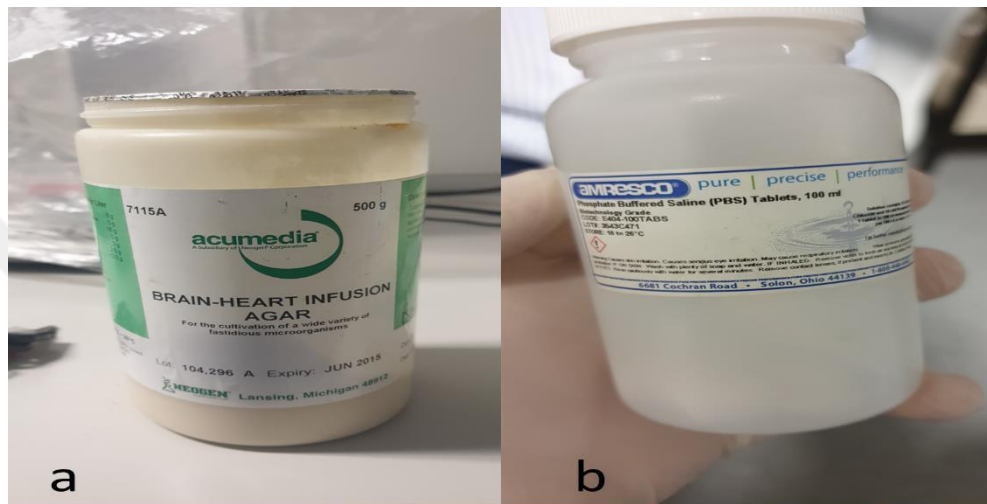


Figure 21: (a): Brain-Heart Infusion Agar (b): Phosphate Buffered Saline

## 2.8 Spreading Bacteria on Agar

Bacterial samples (*E. faecalis*) were spread on agar from four separate groups according to the steps below:

Group Number 1: Honeycomb

Group Number 2: Plain

Group Number 3: Line 1

Group Number 4: Line 2

1. 180  $\mu$ L of PBS, measured with a micropipette (BIOBASE, China), was added to each of the 96 wells in rows B through G. The reason for using PBS was to provide bacteria with conditions in which they could neither reproduce nor die. Row A was used to add the original sample, and the last row, indicated by the letter H, was used as a trash can during dilution.

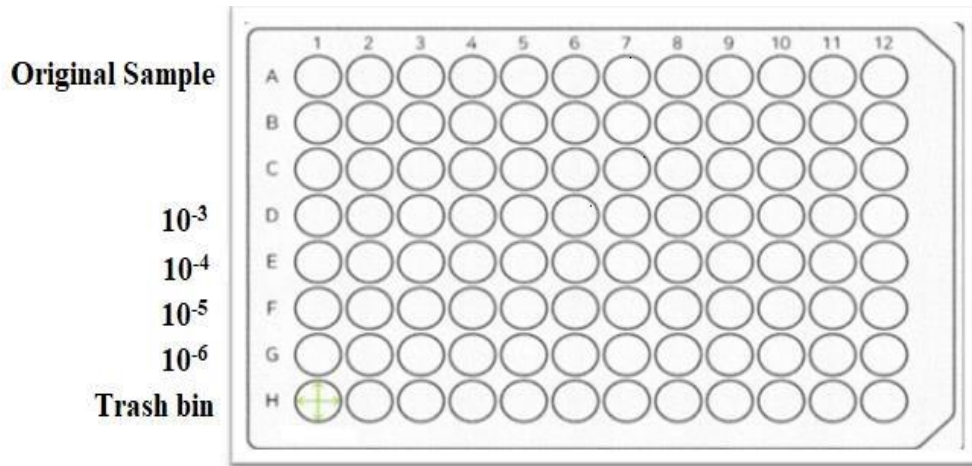


Figure 22: An example of the 96-well plate.

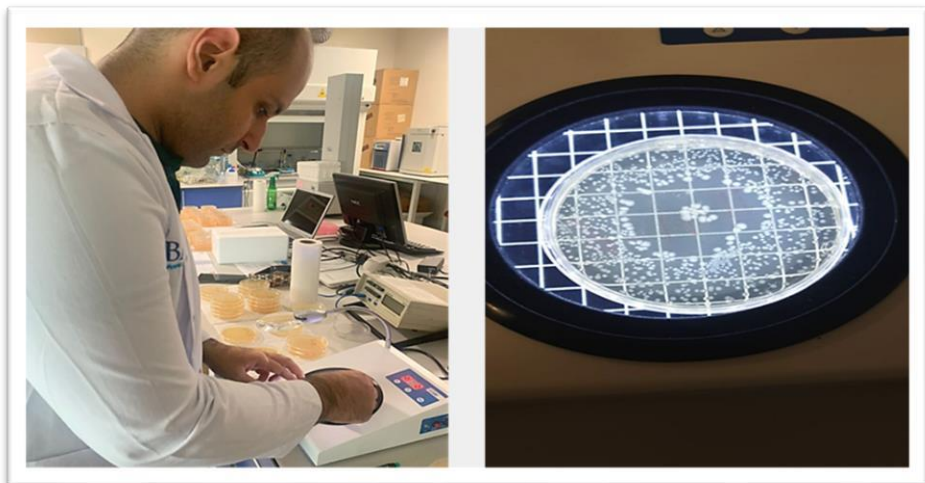
2. The first sample of the first group, Honeycomb, was vortexed for 5 seconds to ensure uniformity of the sample. The micropipette was adjusted to 100  $\mu\text{L}$ , removed from the first sample of the Honeycomb group, and added to the second row of 96 wells, the B row.
3. To dilute the bacteria with PBS, the micropipette was adjusted to 20  $\mu\text{L}$ , and a 20  $\mu\text{L}$  sample was withdrawn. The original sample was emptied and refilled 10 times on the PBS surface of row B without bubbling. The last filled sample was taken to the next row, which was C. Similarly, it was taken to row G, and the last sample obtained during the dilution process was added to row H, which was used as waste to ensure that the dilution process was completed effectively.
4. The micropipette was initially adjusted to 30  $\mu\text{L}$ , and the diluted lines D to G ( $10^{-3}$ ,  $10^{-4}$ ,  $10^{-5}$ ,  $10^{-6}$ ) were used to collect the sample and spread on the agar using a Petriturn-M Petri dish rotary table (Schuett biotech, Germany) to ensure that the bacteria spread evenly over the entire surface of the agar.
5. All samples were incubated at  $37^{\circ}\text{C}$  to provide a suitable environment for bacteria to colonize.



*Figure 23: Prepared BHI Agar Plates are under the UV Light.*

## **2.9 Counting Bacterial Colonies**

After spreading bacteria on agar, as explained in the materials and methods part, the samples were taken from the incubator after 48 hours and arranged group by group on the tissue papers prepared on the bench. The bacterial Colony Counter (YUCHENGTECH, China) was used to count the bacteria's colonization. The samples were placed under the light of the colony counter machine one by one, and the red pen was used to dot each colony of the samples to be counted precisely.



*Figure 24: Bacteria's Colony Counting.*

## Chapter 3

### Results and Data Analysis

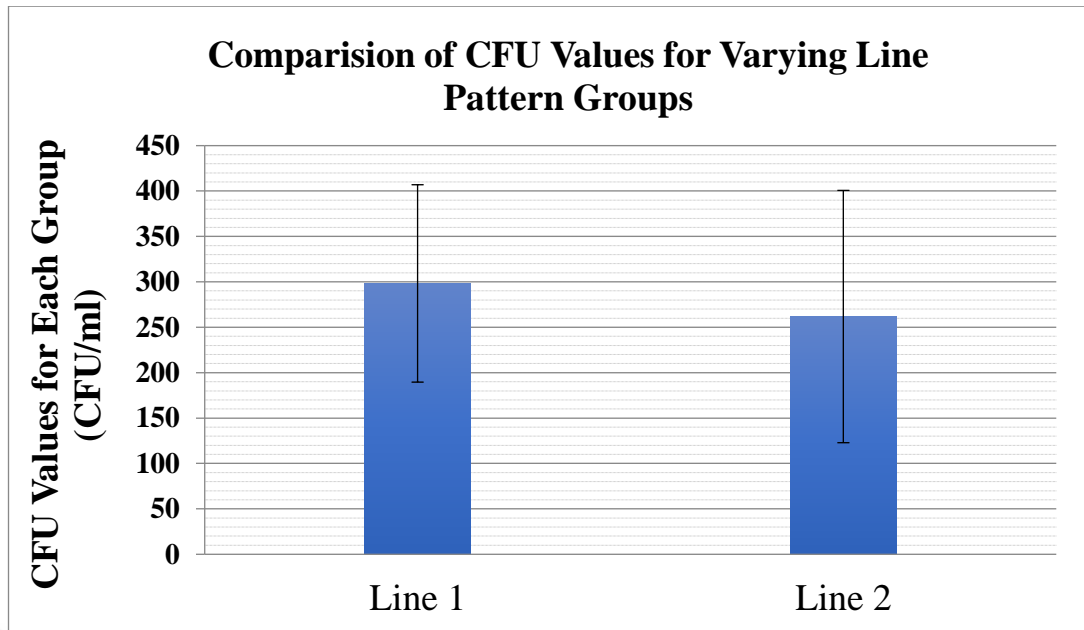
The statistical analyzes were completed by the student t-test. ( $p \leq 0.05$ , student t-test).

The experiments in this thesis were performed in two parts: Preliminary Experiments and Main Experiments. In preliminary experiments, Line Type 1 & Line Type 2 Groups were compared, and using their results Plain, Line Type 1 & Honeycomb-patterned Groups were calculated.

#### 3.1 Experimental Procedures of the Data: Comparing Line Type 1 & Line Type 2 Groups

In the preliminary study, the line type 1 group (with fewer density of line width on its surface) and line type 2 group (with denser line width on its surface) were considered to compare whether there was any significant difference between their CFU values which stand as colony-forming units of the bacteria. The result demonstrated that there was no significant difference between line pattern groups in their CFU values to show any notable ability to minimize bacterial colonization on titanium surfaces.

The figure below shows no significant difference between Line 1 and Line 2 patterns based on their CFU values ( $p \leq 0.05$ , student t-test). As a result, Line 1 was selected for comparison along with the Honeycomb and Plain pattern groups for the next step of the experiment.



*Figure 25: Comparing Line Type 1 and Line Type 2 Groups: There was no significant difference between different types of line patterns based on their CFU values ( $p \leq 0.05$ , student t-test).*

### **3.2 Experimental Procedures of the Data: Comparing Plain, Line Type 1 & Honeycomb Pattern Groups**

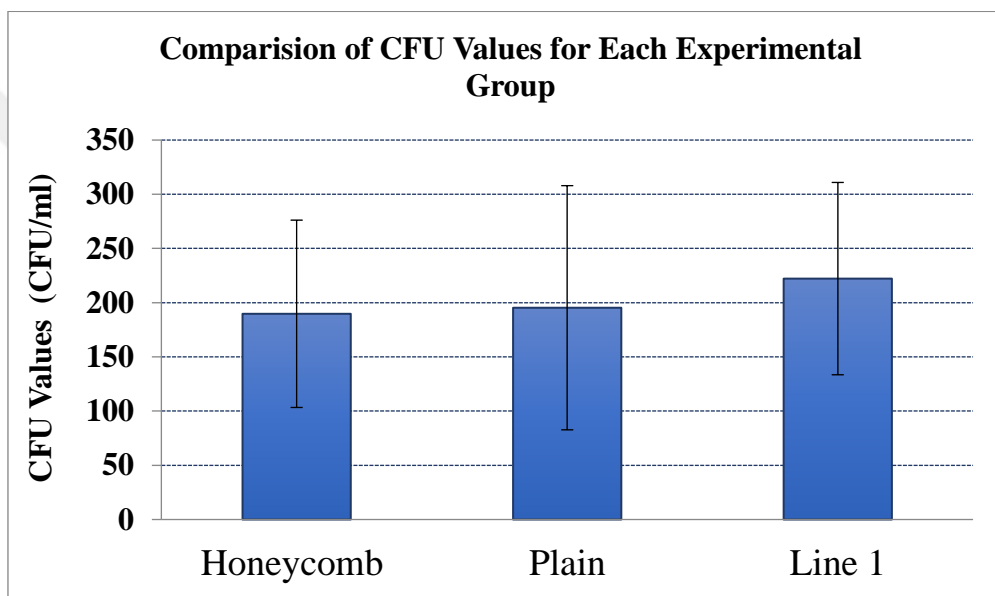
In the main part of the experiments, the dilution  $10^{-6}$  with its number of bacterial colonies was selected for all samples, and the averages and standard deviation were required to compare CFU values in all pattern groups. Based on the obtained data, the average number of CFU values for honeycomb patterns was  $189.75 \pm 82.73$ , the average number of CFU values for the plain patterns group was  $195.33 \pm 106.19$ , and the average number of CFU values for the line 1 patterns group was  $222.2 \pm 79.34$ .

In the experiment's next step, the statistical analyzes were completed by the student t-test. ( $p \leq 0.05$ , student t-test). All types of patterns were compared to the plain type of sample group to ensure whether there was any significant difference between their CFU values.

Consequently, the statistical analyses indicated that none of the final numbers of CFU values was smaller than **0.05** to demonstrate a significant difference between each experimental group to prove less bacterial adhesion

capacity of honeycomb pattern groups compared to the plain and line 1 pattern groups on titanium plate surfaces.

The figure below shows no significant difference between varying patterns based on their CFU values ( $p \leq 0.05$ , *student t-test*). To clarify, despite the slight variation in the mean value depicted in the graph below, the disparity in bacterial adhesion between the honeycomb group and other groups is not substantial enough to support the notion of utilizing honeycomb patterns as the primary solution for achieving a permanent and effective reduction in bacterial adhesion on titanium and its alloy surfaces.



*Figure 26: Comparing Plain, Line Type 1 & Honeycomb Pattern Groups: CFU Values for Each Experimental Group are calculated and analyzed and there was no significant difference between varying patterns based on their CFU values ( $p \leq 0.05$ , *student t-test*).*

## Chapter 4

### Discussion

Bacteria play a crucial role in various fields of dentistry for several reasons. Today, experts are exploring ways to effectively use bacteria in their research to advance dentistry and enhance patient care in areas such as implantation. However, it is important to note that bacteria can be infectious in the oral cavity and can also contribute to respiratory infections. For instance, inhaling periodontopathic bacteria from the mouth can result in aspiration pneumonia. In addition, the teeth can act as a breeding ground for respiratory pathogens, leading to nosocomial pneumonia. Studies have shown that dental plaques of patients in critical healthcare facilities can colonize common respiratory infections (Scannapieco, 1999).

The optimum surface of implants becomes increasingly vital in the optimal osseointegration procedure (Stefanie Kligman, 2021). Recently, there has been significant interest in creating implant surface engineering designs that enhance the osseointegration of titanium implants while reducing bacterial adhesion. One example of such an innovation is the development of zirconia implants that feature a white-colored surface, which has gained considerable attention (F. Rupp, 2017). In contrast, non-metallic surfaces require specific and distinct modification procedures to improve osseointegration. Generally, modifying the surface characteristics of an implant - such as its chemical composition, surface roughness, and free surface energy - can accelerate the healing process, enhance osseointegration, and reduce bacterial adhesion (Heng Dong, 2020) (Amoroso Pier-Francesco, 2006). In this study, surface modifications were compared to examine the effect of patterns.

Dental implantation commonly uses titanium and its alloys due to their extensive research on osseointegration. Numerous studies have verified the high success rate of osseointegration in titanium implants, leading to favorable outcomes. Despite their excellent osseointegration capacity, statistical studies have shown that poor osseointegration can sometimes be a significant problem. To achieve optimal biocompatibility and osseointegration, new procedures and techniques have emerged to modify and/or alter the surface of titanium implants by enhancing surface roughness and morphology. Anodizing, a standard chemical

treatment for surface modification, is one example that can improve the nano-roughness and osseointegration of titanium implant surfaces (Changjiang Pan, 2021).

Serious drawbacks that negatively affect titanium implants' overall performance are periodontitis, peri-implantitis, and marginal bone resorption (Pejeva E1, 2018). Biofilm is a complex community of microorganisms that can attach to various surfaces and exist in a self-produced matrix of extracellular polymeric substances (EPS). This community of microorganisms is commonly found in the oral cavity and can be observed on tooth surfaces, mucosal surfaces, and dental implants. Biofilm formation in the oral cavity is a natural process that occurs when bacteria adhere to the tooth surface and produce a slimy film that serves as a protective shield. This slimy film, commonly called dental plaque, can accumulate on the teeth, causing tooth decay, gum disease, and eventually tooth loss. Furthermore, when biofilm forms and/or alters dental implants, it can cause implant failure. Implant failure occurs when the implant becomes loose or falls out, which can be attributed to biofilm on the implant surface. This biofilm can cause inflammation, which can lead to bone loss around the implant, ultimately resulting in implant failure. Therefore, it is crucial to monitor and manage biofilm formation in the oral cavity, particularly in patients who have received dental implants, to prevent implant failure and ensure the longevity of the implant (Raphael C. Costa, 2022). Various factors can harm the oral environment, including poor diet, inappropriate lifestyle, smoking, regular alcohol consumption, and poor oral hygiene. These factors have the potential to alter the characteristics of biofilm and bacterial accumulation, which can provide an infectious environment for oral microflora. Oral microflora, also known as oral microbiomes, are microorganisms that grow and reproduce in the oral cavity (MF Zarco, 2011). Therefore, the formation of dental plaque and subsequent pathogenic biofilms can lead to the development of periodontitis and peri-implantitis. These are polymicrobial inflammatory ailments that can lead to the destruction of both teeth and implants (Muhammad Waqar Hussain1, 2018). Periodontitis is a severe gum disease that can provide a suitable environment for bacteria to grow and reproduce, potentially leading to tooth loss. Moreover, peri-implantitis occurs in a specific part of a tooth or implant and has destructive impacts on their proper functionality. Both

periodontitis and periimplantitis are caused by chronic inflammation resulting from bacterial infection.

Marginal bone resorption, also known as alveolar bone resorption, is characterized by the loss of bone tissue surrounding and supporting the teeth. This bone loss occurs in response to chronic inflammation resulting from periodontal disease, a bacterial infection that affects the gums and other structures that support the teeth. Marginal bone resorption can lead to several dental problems, including loose teeth, tooth loss, and changes in the appearance of the face and jaw. In severe cases, it can also affect a person's eating and speaking ability (JANET S. KINNEY, 2007).

Therefore, the presence of bacteria in the oral cavity can have detrimental effects on both natural teeth and dental implants. In periodontal practice, specialists are continuously exploring new strategies to reduce the risk of diseases in the oral cavity. Despite the widespread use of titanium and its alloys for dental implants for over a decade, peri-implant disease has been revealed in 16% of patients after 9 to 14 years of implantation (Ralf Burgers, 2010). This suggests that there may be insufficient research on bacterial adhesion on the surface of titanium implants, leading to treatment suggestions that rely more on first-hand examinations rather than defensible study outcomes (Afya Sahib Diab Al-Radha, 2011). As a result, there is a growing need for in-depth research on the effects of bacterial adhesion on titanium implants to develop more effective preventive and treatment strategies for the peri-implant disease that can help to identify the factors that contribute to bacterial adhesion and develop interventions to prevent or reduce the adhesion of infectious bacteria on the surface of dental implants. This, in turn, can improve the long-term success of dental implants and reduce the risk of peri-implant diseases. Therefore, researchers and dental professionals need to collaborate and prioritize research efforts to better understand bacterial adhesion on dental implants and its role in the development of peri-implant disease. This can lead to the development of evidence-based treatment strategies that can improve oral health outcomes for patients with dental implants.

Gibson's study (Gibson, 1997) demonstrates honeycomb structures' distinctive mechanical properties and exceptional energy absorption capabilities, positioning them as an ideal material for multiple applications. Recent developments in biomedical engineering have highlighted the many advantages of

using honeycomb structures in this field due to their biocompatibility, high surface area, lightweight, robustness, and stiffness, as well as their effective thermal insulation properties.

The use of honeycomb structures in biomedical applications, such as titanium and its alloy surfaces, zirconium implant surfaces, and scaffolds, has proven to be beneficial in creating a conducive environment for the overall performance of restorative dentistry, tissue engineering, and regenerative medicine (Sarah Sundelacruz, 2009). The honeycomb structure has attracted significant interest in the fields of tissue engineering and regenerative medicine, owing to its distinctive mechanical performance and unique properties. Additionally, its various patterns such as general structures, triangular structures, double hexagonal structures, and full double structures are currently trending in diverse fields including chemical and mechanical engineering, nanofabrication, and architecture. Besides, these unique and varied features of honeycomb structures can have potential effects in the field of dental implantation.

This current study builds on previous research that has shown honeycomb structures' effectiveness in various biomedical applications. The study aimed to compare the effectiveness of honeycomb patterns with other pattern shapes to evaluate whether the unique geometrical structure of honeycomb patterns could be a viable long-term solution for reducing bacterial adhesion. Bacterial adhesion to titanium and its alloys is a major concern in various fields, including biomedical engineering, dental implants, and orthopedics. The degree of bacterial adhesion on these surfaces can be influenced by several factors, including surface roughness, chemistry, and topography (S. Ferraris, 2016). Higher surface roughness has been found to lead to increased bacterial adhesion, while lower roughness levels are associated with reduced bacterial adhesion. In addition, the presence of functional groups such as hydroxyl, carboxyl, or amine groups can also lessen bacterial adhesion capacity by modifying the surface properties of the implant (Denisa Alexandra Florea, 2019). Statistical studies have indicated that surface topography is another critical factor in bacterial attachment, with overly smooth or rough surfaces leading to greater bacterial attachment. To combat this, new surface modification techniques such as sandblasting have emerged to moderate surface topography and decrease bacterial adhesion on titanium and its alloy implants (Chengcheng Yin, 2016).

The purpose of this study was to compare the honeycomb pattern with other laser-manufactured patterns, including plain, line 1, and line 2, all with the same plate thickness and metallic materials, to evaluate the potential of the honeycomb pattern to minimize bacterial adhesion on titanium surfaces. The study hypothesis focuses specifically on the energy absorption and mechanical performance as well as the unique geometrical shape of the honeycomb pattern and whether it can significantly reduce bacterial colonization compared to the other pattern groups. If the results indicate a significant reduction in bacterial adhesion on the honeycomb pattern surfaces, further investigations such as surface coatings using modification techniques such as sandblasting (SLA) can be explored to improve the surface roughness, chemistry, and topography on titanium implants made of a structure of honeycomb pattern surfaces.

To assess the capacity of bacterial adhesion on various surface patterns, *Enterococcus Faecalis* was selected as the subject of the study. *E. faecalis* is a gram-positive, facultative anaerobe, and non-sporulating bacteria with low GC content that can create infectious environments and cause oral diseases such as peri-implantitis in the oral cavity. Peri-implantitis is a bacterial infection that occurs when bacteria accumulate on the surface of dental implants, leading to inflammation and damage to surrounding tissues. One of the identified bacteria involved in the development of periimplantitis is *Enterococcus faecalis*. It has been established that this bacterium can attach to dental implant surfaces and form biofilms, which are bacterial communities that are resistant to the body's immune system and antibiotics. The appearance of *Enterococcus faecalis* in dental implant sites can trigger the onset of peri-implantitis, which can result in tooth loss, implant failure, and the necessity for implant removal (Daria Van Tyne, 2013).

To establish an appropriate environment that could minimize the number of bacteria on titanium plate surfaces, this research study utilized a range of patterns or structures on titanium plates. The patterns used included plain patterns (with no shapes on the surface), line patterns (with a few and more lines on the surface), and the honeycomb pattern (with the shape of a beehive on the surface). The goal was to determine which of these patterns could reduce the adhesion capacity of *E. faecalis* (EF) bacteria on titanium surfaces more effectively than the other patterns.

The preliminary examination demonstrated an insignificant difference in the CFU values in line 1 and line 2 pattern groups to show any remarkable reduction in bacterial adhesion on titanium plates. Therefore, line 1 was considered for further experiments along with honeycomb and plain pattern groups. Statistical analysis showed that the honeycomb group's average number of bacterial colonies demonstrates the lowest number, but it is not significantly different from the plain and line 1 pattern groups.

As it has been demonstrated in the results part, the number of CFU values of all pattern groups could not indicate a significant difference in decreasing bacterial adhesion capacity on titanium plates. In other words, the general structure of a honeycomb pattern has the potential to minimize the number of bacterial colonies on titanium plates better than other pattern groups. However, the difference in the bacterial adhesion capacity on the surfaces of titanium plates in each group was not significant to be considered the general structure of a honeycomb pattern as a main material to improve titanium and its alloy surfaces by reducing the number of bacterial colonies. As a result, further techniques and experiments needed to be examined and analyzed to enhance the overall performance of titanium and its alloy surfaces in various areas of biomedical engineering, particularly in the field of restorative dentistry.

## Chapter 5

### Summary and Conclusion

Over the past few years, significant progress has been made in the field of dental implant research. Studies have shown that modifying the surface characteristics of dental implants can reduce the number of bacteria that adhere to the implant, thereby preventing the formation of infectious biofilms. In addition, the use of titanium and its alloys in dental implantation has been found to promote favorable osseointegration, corrosion resistance, formability, and machinability. With these benefits in mind, researchers are now exploring ways to further minimize bacterial adhesion on titanium implants while maintaining their anti-toxic properties, corrosion resistance, and biocompatibility. Despite the ongoing advances in manufacturing techniques for titanium dental implants, some challenges still need to be addressed. The peri-implant disease can result in severe infections that affect a specific part of the implant. This condition can cause bone loss and inflammation in the soft tissues, both of which can damage the proper function of the dental implant. To address such issues, new surface modification techniques have been developed. Physical treatments include sandblasting, laser-etching, and plasma spraying as well as chemical treatments such as acid-etching and anodizing, each with unique procedures for enhancing dental implant technology. In addition, various structures of honeycomb patterns have received significant attention in the field of biomedical engineering due to their unique properties, variable geometrical shapes, mechanical performance, as well as specific energy absorption which can be used to perform any possible biostatistical tests to detect preferences in the use of dental implantation. By leveraging such discoveries, researchers can improve the overall performance of titanium and its alloy surfaces for various applications, including restorative dentistry.

As the honeycomb pattern may have the potential to have a smaller number of bacterial colonies on titanium plates, it can be a promising approach to minimize bacterial adhesion on the surface of titanium plates. These findings provide valuable insights into the selection and effectiveness of surface patterns in reducing bacterial adhesion on titanium plates.

Further research is necessary to explore alternative strategies and patterns that can more effectively address this challenge and enhance the long-term success of dental and medical prostheses by setting up further surface modification techniques such as sandblasting (SLA) on honeycomb structures made of titanium-based materials to significantly decrease the number of bacterial colonies on different hexagonal pattern shapes known as honeycomb structures.



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