SYNTHESIS OF CHITOSAN THIN FILMS FOR BIOMEDICAL APPLICATIONS

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ABSTRACT

Master's Thesis

SYNTHESIS OF CHITOSAN THIN FILMS FOR BIOMEDICAL APPLICATIONS

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Chitin is a biopolymer that is as abundant on Earth as cellulose. It is present in the shells of some sea-dwelling organisms as well as in the structural wall of fungi. Chitosan is a polysaccharide that produced by deacetylating chitin, this process is the elimination of acetyl moiety from its composition. The free amino groups in its molecular structure impart crucial and functional properties to chitosan, such as biocompatibility, biodegradability. Additionally, it exhibits antibacterial activity due to it is ability to kill bacterial cell membranes and inhibit microbial growth, showing a broad spectrum of effects against very types of bacteria. Thanks to these properties, chitosan finds applications in some bacterias, including preventing infections that occur on the surfaces of implantable medical devices—often leading to patient fatalities—as well as in wound dressings. Chitosan can be coated onto surfaces using various methods. The present study focuses on producing nanometer scale thin films of chitosan on multiple substrates via Plasma-Enhanced Chemical Vapor Deposition (PECVD) method. The produced surfaces were characterized using FTIR, XPS, SEM analytical techniques. For antibacterial activity analysis, bacterial inhibition zone tests were performed on *Staphylococcus aureus* and *Escherichia coli*, which exhibit activity in different tissues and on implant surfaces in the human body and are extremely challenging to inhibit from surfaces. In addition, thin film coating was applied to some surfaces of a certain size and colony counts were made on these two bacteria on the 1st day, 4th day and 7th day.

January 2025, 49 pages

Key Words: Chitosan, plasma polymerization, thin film, plasma-surface modification, antimicrobial

ÖZET

Yüksek Lisans Tezi

BİYOMEDİKAL UYGULAMALAR İÇİN KİTOSAN İNCE FİLM SENTEZİ

Gözdenur DURSUN

Ankara Üniversitesi
Fen Bilimleri Enstitüsü
Biyomedikal Mühendisliği Anabilim Dalı

Danışman: Prof. Dr. Hilal GÖKTAŞ

Kitin yeryüzünde selüloz kadar bol bulunan bir biyopolimerdir. Denizde yaşamını sürdüren bazı organizmaların kabuklarında ve mantarların yapısal duvarında bulunur. Kitosan, kitinin deasetilasyonu ile elde edilen bir polisakkarittir; bu işlem, asetil kısmının bileşimden çıkarılmasıdır. Moleküler yapısında yer alan serbest amino grupları, kitosana biyouyumluluk, biyobozunurluk gibi son derece önemli ve işlevsel özellikler kazandırmaktadır. Bunun yanı sıra, bakteriyel hücre zarını bozma ve mikrobiyal büyümeyi engelleme yeteneğinden dolayısıyla antibakteriyel aktivitesi mevcuttur, çeşitli bakteri türlerine karşı geniş bir etki spektrumu sergilemektedir. Kitosan tüm bu özellikleri sayesinde, başta implante edilebilir tıbbi cihaz yüzeyinde meydana gelen ve hastaların ölümüne sebep olan enfeksiyon oluşumunun önüne geçmesine ek olarak, yara örtüleri gibi kullanım alanına sahiptir. Kitosan, çeşitli yöntemler yardımıyla yüzeylere kaplanabilmektedir. Yürütülen çalışmada, kitosanın PDKBB (Plazma Destekli Kimyasal Buhar Biriktirme) yöntemi ile çeşitli substratlar üzerinde nanometre düzeyde kitosan ince film üretimine odaklanılmıştır. Üretilen yüzeyler, FTIR, XPS, SEM analiz yöntemleri kullanılarak karakterize edilmiştir. Antibakteriyel aktivite analizi için, insan vücudunda farklı dokularda ve implant yüzeylerinde aktivite gösteren ve yüzeyden inhibisyonları son derece zor olan *Staphylococcus aureus ve Escherichia coli* bakterileri üzerinde bakteri inhibisyon bölge testleri gerçekleştirilmiştir. Ayrıca belirli büyüklükteki bazı yüzeylere ince film kaplama uygulanmış ve bu iki bakteri üzerinden 1. gün, 4. gün ve 7. gün koloni sayımı yapılmıştır.

Ocak 2025, 49 sayfa

Anahtar Kelimeler: Kitosan, plazma polimerizasyonu, ince film, plazma yüzey modifikasyonu, antimikrobiyal

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Gözdenur DURSUN
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SYMBOLS AND ABBREVIATIONS

°C	Degree Celsius
cm	Centimeter
CVD	Chemical Vapor Deposition
DC	Direct Current
DJ	Double J
FTIR	Fourier Transform Infrared Spectroscopy
FWHM	Full-width half maxima
g	Gram
h	hour
H_2O_2	Hydrogen peroxide
kDa	Kilodalton
LMW	Low Molecular Weight
McF	Mac Farland
mL	Milliliter
MW	Molecular Weight
NaOH	Sodium hydroxide
nm	Nanometer
PBS	Phosphate Buffered Saline
PECVD	Plasma-Enhanced Chemical Vapor Deposition
PVD	Physical Vapor Deposition
RF	Radio Frequency
rpm	Revolutions per Minute
SEM	Scanning electron microscopy
SFE	Surface Free Energy
TSA	Tryptic Soy Agar
TSB	Trypticase Soy Broth
µL	Microliter
µm	Micrometer
XPS	X-Ray photoelectron spectroscopy

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1. INTRODUCTION

As a biopolymer found in nature, chitosan is derived from chitin using the deacetylation method. This chitin serves as a fundamental building block of the tough outer coverings in various sea-dwelling animals, including crabs and shrimps (Bhardwaj and Kundu, 2010). In addition, the fungal cell wall contain chitin (Morin-Crini et al., 2019). The process of converting acetylamino (-NH-CO-CH3) groups on chitin to amino (-NH2) groups is named deacetylation process (Annu and Ahmed, 2019).

Its properties depend on the deacetylation rate. Chitosan is offered in a variety of molecular weights, which vary from high to low. There are various forms of chitosan such as film, gel, hydrogel, fiber, solution. In these thesis studies, chitosan, which has a low molecular weight, is used. Chitosan’s molecular weight which falls from 50 to 2000 kDa, is determined by the raw material used and the method employed during its preparation (Mourya et al., 2011). The viscosity of chitosan is also crucial parameter that determines its usability in the commercial applications. There is a direct relationship between viscosity and chitosan’s molecular mass, The viscosity of chitosan rises as its molecular weight grows. For this reason, chitosan having a low molecular weight was preferred in this thesis. In the CVD device, powder chitosan must have low viscosity in order to evaporate more easily. Therefore, low molecular weight chitosan was used (Al Sagheer et al., 2009; jeon et al., 2001; Charles et al., 2014).

Chitosan possesses numerous biological, chemical, and physical properties, along with various advantages. In the context of biomedical applications, the biological properties it possesses are particularly important. From a biological perspective, its antibacterial properties, antimicrobial properties, prevention of blood clotting, non-toxicity, acceleration of wound healing, biocompatibility, antifungal properties, biodegradability and non-allergenicity are the features that make chitosan unique in biomedical applications (Engkagul et al., 2017).

Additionally, it exhibits antibacterial activity due to it is ability to kill bacterial cell membranes and inhibit microbial growth. Thanks to these properties, chitosan finds applications in some bacterium, including preventing infections that occur on the surfaces of implantable medical devices.

Although there are advantages to the use of chitosan in biomedical fields in this thesis, it is a material used in many areas. Apart from its use in health areas such as dentistry, drug distribution and cancer treatment, Besides, it is applied in water purification and in increasing the longevity of food products (Engkagul et al., 2017)

Although chitosan is used in various fields, thin film studies are quite limited. Thin films have a vital role in biomedical applications. In the thesis, chitosan is used as a precursor in thin film coatings, employing a method that has not been previously applied to this material. The Plasma Enhanced Chemical Vapor Deposition (PECVD) technique was used to produce chitosan thin films on various surfaces. PECVD offers several advantages, such as operating at low temperatures, having a high deposition rate, utilizing both organic and inorganic materials/monomers as precursors, and enabling the deposited films to exhibit unique chemical properties (Tüfekçi, 2023). Briefly, the purpose of this thesis is to inhibit bacteria on surfaces in the biomedical field by producing chitosan thin films with PECVD and to demonstrate that chitosan is an important material from a biomedical perspective.

2. LITERATURE REVIEW

2.1 Chitosan

Chitin is a biopolymer that is as abundant on Earth as cellulose. This chitin serves as a fundamental building block of the tough outer coverings in various sea-dwelling animals, including crabs and shrimps (Hudson and Jenkins, 2001). Chitosan is biopolymer that obtained as a result of deacetylation of chitin (Hudson and Jenkins, 2001). The process of converting acetylamino (-NH-CO-CH3) groups on chitin to amino (-NH2) groups is named deacetylation process (Annu and Ahmed, 2019). In recently, mushrooms have been preferred more frequently for the production of chitosan with higher purity and better quality. Chitosan is more advantageous than chitin. It is due to the best amino groups that can undergo many chemical reactions in its structure.

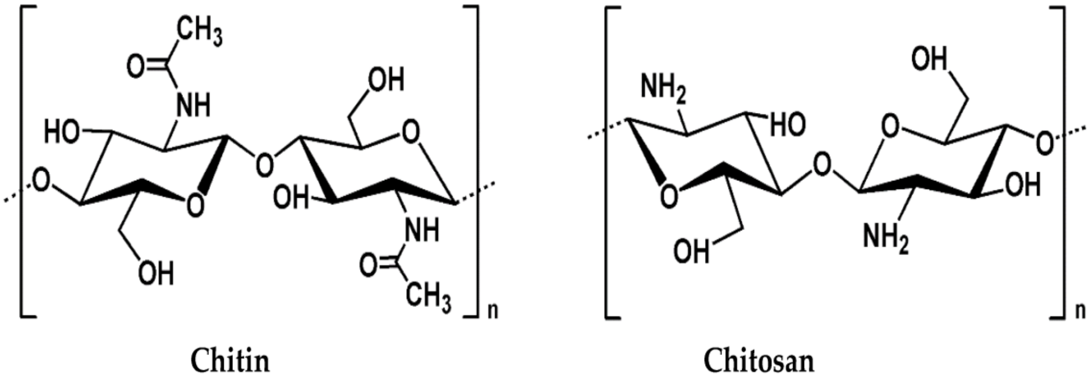

Figure 2.1 Chitin and chitosan chemical structure (Peker et al., 2006)

There are some steps to follow to obtain chitosan from chitin. The process of chitin isolation involves removing other components from the shell and it is performed for this can be grouped under four main steps:1. Preparation of shells, 2. Removal of minerals is called demineralization process, 3. Removal of proteins is called deproteinization process, 4. Removal of pigments is called decolorization process (Al Sagheer et al., 2009). In more detail, first the shells of the crustacean are removed, washed and dried in the oven. The deproteinization method is applied to the dried shells. At this stage, the shells are kept in NaOH solution and dried. The demineralization method is applied to the deproteinized and dried shells. At this stage, they are kept in HCL solution and dried. Then the decolorization method is applied. They are kept in H_2O_2 and HCL solutions and dried. Then the obtained chitin is deacetylated in NaOH solution. And as a result, the material called chitosan is obtained. These steps are presented in the figure 2.2

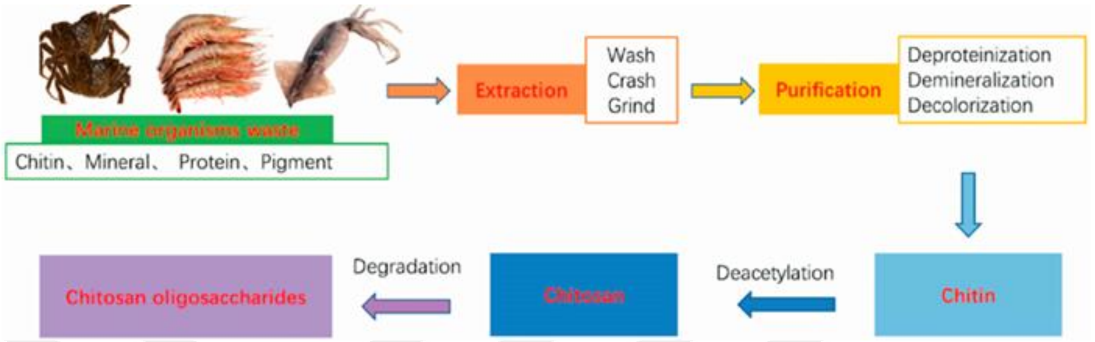

Figure 2.2 Steps of obtaining chitosan from chitin (Li et al., 2020)

Chitosan is mostly found in powder form. Chitosan, due to its cationic nature, can be dissolve in some environments with pH<6 (Demir and Seventekin, 2009). While it is not soluble in water, it readily dissolves in solutions that are acidic, including diluted acetic acid and formic acid. Thanks to this feature, we can see various applications of chitosan in many different areas. This feature increases the number of studies conducted on chitosan. The fact that chitosan has rich biological, chemical and physical properties makes it a unique material that can be used in many studies.

Chitosan’s molecular weight, which falls from 50 to 2000 kDa, is determined by the raw material used and the method employed during its preparation (Mourya et al., 2011). The viscosity property also determines the usability of chitosan in the commercial field, it is one of the vital parameters that determine. There is a direct relationship between viscosity and chitosan’s molecular mass, The viscosity of chitosan rises as its molecular weight grows. For this reason, chitosan having a low molecular weight was preferred in this thesis. Because in the CVD device, powder chitosan must have less viscosity in order to evaporate more easily. Therefore, low molecular weight chitosan was used.

Chitosan possesses numerous biological, chemical, and physical properties. It has some important biological properties such as antibacterial, biodegradability, antimicrobial, antioxidant, biocompatibility, antifungal, and anticoalugant. In addition, it has chemical and physical properties with changing according to molecular weight and deacetylation rate. Considering all these features, Low molecular weight chitosan was considered to be more suitable for biological and chemical uses, along with a wider array of applications. Additionally, most chitosan exhibits a degree of deacetylation greater than 85%, and their molecular weights span from 50 to 200 kDa (Mourya et al., 2011). Chitosan's molecular weight differs according to the method of production. Chitosan of a certain molecular weight of 100 kDa or lower is categorized as low molecular weight, while those with a molecular weight of 1000 kDa or higher are considered high molecular weight (Santoso et al., 2020).

Table 2.1 The role of molecular weight and deacetylation rate in determining the characteristics of chitosan (Dash et al., 2011)

Property	**Impact of increasing deacetylation rate**	**The MW of the effect of the increase**
Solubility	Rises	Reduces
Viscosity	Rises	Rises
Biodegradability	Reduces	Reduces
Biocompatibility	Rises	-
Antimicrobial property	Rises	Reduces
Antibacterial property	Rises	Reduces
Antioxidant property	Rises	Reduces

Chitosan finds wide usage in various applications, such as wastewater treatment and nutrition preservation, where it helps increase shelf life. Its biological properties are especially significant in the biomedical and health domains.

It has been established that chitosan films with low acetylation levels support keratinocyte proliferation in the wound during wound healing, thus contributing to epidermis regeneration, and therefore are a very suitable material for use as a wound dressing. There are some specific polar groups in chitosan such as -OH, -COOH, and $-NH_2$ that these can be used as moisture retention. Additionally, it is used in dentistry, cancer treatment, gene therapy, drug delivery, film forming and several applications.

2.2 Antibacterial Mechanism of Chitosan

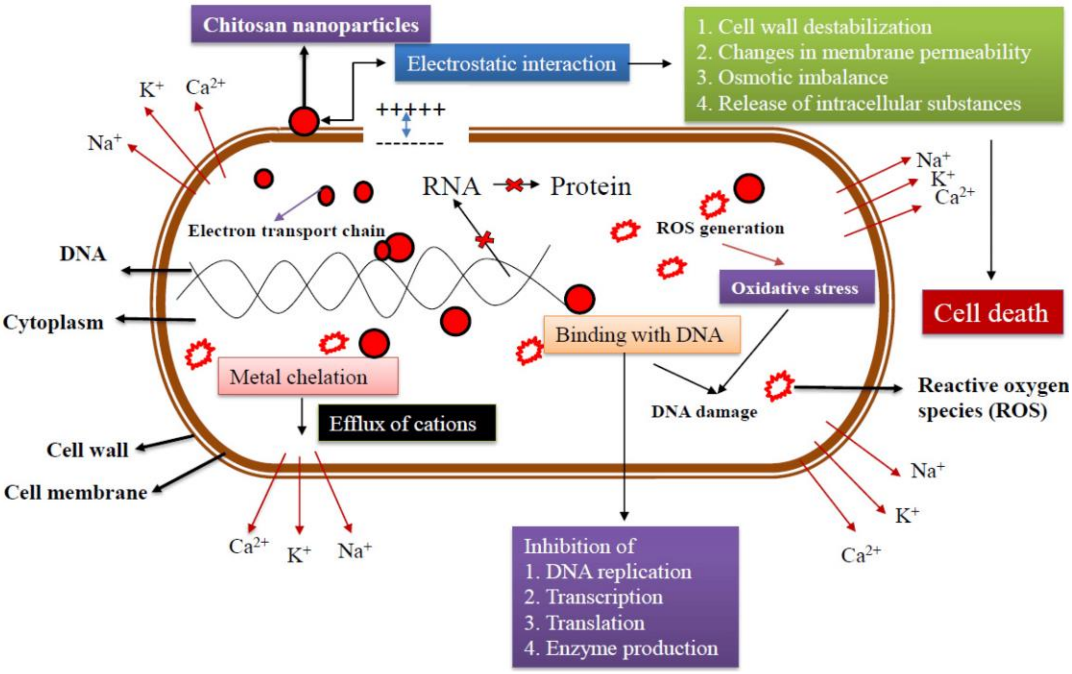

Figure 2.3 Chitosan's mode of antibacterial action (Chandrasekaran et al.,2020)

Chitosan is an antibacterial material. The antibacterial mechanism is given below. Chitosan in its dissolved form contains an amino group (NH_3+) that binds to the anions of cell walls of bacterial organisms, leading to bacterial death. The antibacterial effect increases with concentration, indicating a direct relationship. Chitosan with varying molecular weights can inhibit bacterial growth and has a significant effect on bacteria such as *E. coli* and *S. aureus* (Chandrasekaran et al.,2020). Chitosan’s antibacterial action can be divided into two mechanisms. One involves the adsorption of chitosan on the cell surface, forming a polymer layer. The membrane hinders the nutrient uptake by the cells, playing a role in bacteriostasis and decontamination, which prevents bacteria from feeding and causes them to die (Cava et al, 2010). In the second mechanism, by osmosis, chitosan enters the cells, attaches to the anionic cytoplasm, triggering flocculation and disturbing the cells physiological functions, leading to bacterial death (Cava et al, 2010). Chitosan's molecular weight modifies bacterial behavior, but the antibacterial mechanism primarily arises from the NH3+ group with its cationic charge, which interacts with anionic charge on the cell walls of bacterial organisms (Cava et al, 2010; Ivask et al., 2013; Birsoy et al., 2015).

2.3 *Staphylococcus aureus* & *Escherichia coli* Bacterias

To assess the antibacterial properties of chitosan, it is important to know its behavior with different types of bacteria. Given the structural distinctions based on their gram types, both negative and positive, both types must be tested through experiments. For this reason, *E. coli* and *S. aureus* were selected for the experiments. Chitosan demonstrates antibacterial activity according to gram classification of bacteria, it has greater antibacterial activity on positive than negative ones, which is mainly due to the differences in their cell wall structures. The Gram-positive types bacterium has an outer membrane outside their walls, while negative one does not (Neuhaus, 2003). Therefore, gram-positive bacterium has thicker cell walls, which contribute to the strength and integrity of the cell (Neuhaus, 2003). *S. aureus* can result in various infections that impact the skin, soft tissues, endovascular sites, and internal organs. *E. coli* is the dominant commensal inhabitant of the gastrointestinal tracts in humans and organisms have warm-blooded, it is recognized as one of the most crucial pathogens (Yıldırım et al, 2016).

Table 2.2 The bacteria that were tested

Bacteria Name	**Gram Type**	**ATCC number**
Staphylococcus aureus	Gram-Negative	ATCC 25922
Escherichia coli	Gram-Positive	ATCC 25923

2.4 Chemical Vapor Deposition (CVD)

CVD (Chemical Vapor Deposition), is a thin film coating technology. This method is a process that utilizes chemicals to generate a thin material layer on a surface. CVD allows the coating of various materials, including metals, ceramics, and polymers. With this method, even complex structures can be coated homogeneously. CVD involves depositing solid materials from a vapor phase through chemical reactions near a heated substrate, forming thin films, powders (Carlsson and Martin, 2010). A significant advantage of CVD is its capacity to generate uniform coatings, which is why it is frequently used in thin film synthesis (Carlsson and Martin, 2010).

2.5 Plasma Polymerization

Plasma is the 4th state of matter and the state in which monomers react in the plasma environment and form thin films is called plasma polymerization. Plasma provides the polymerization of monomers with energy. In this study, plasma polymerization was used. In other words, this method is called PECVD is that stand for plasma-enhanced chemical vapor deposition. Both CVD and PECVD are significant as thin film synthesis and have some differences in their processes.

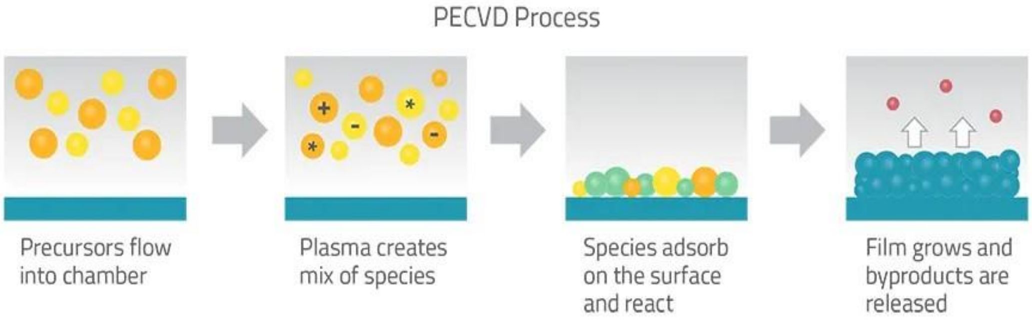

Figure 2.4 PEVCD process (Dennis Hausmann, 2018)

PECVD provides faster deposition, smoother surfaces and superior edge coating by performing chemical reactions with the help of plasma at low temperatures. So, it is often preferred in sensitive and high-quality applications. CVD, on the other hand, operates based on chemical processes that occur at high temperatures and is especially used in some applications that are not sensitive to temperature. Both methods are selected according to the requirements of the application. In briefly, PECVD and CVD are both important thin film deposition techniques but serve different purposes. PECVD is ideal for temperature-sensitive applications due to its plasma-assisted, low-temperature process, offering faster deposition and better uniformity (Hamedani et al., 2016). PECVD is used in this study is that because it produces a very thin, smooth film. Plasma polymerization can occur at low temperatures, which is another significant advantage. Materials that evaporate at high temperatures at room temperature can evaporate at lower temperatures under pressure. PECVD is suitable for lower temperature processing, faster deposition, and better film uniformity, while CVD is more suitable for applications requiring high temperatures and better crystalline films (Ryu and Kim, 2018).

2.6 Surface Characterization Techniques

2.6.1 Fourier transform infrared (FTIR) spectroscopy

FTIR is an analysis technique that stands for Fourier Transform Infrared Spectroscopy. It is used to have the spectrum of absorption of material. The material can be solid, liquid or gas. It examines the molecular vibration of the material. The working principle of FTIR can be summarized as follows: when light is directed onto the sample, the bonds in the molecule vibrate and the bonds in the molecule absorb light of different wavelengths (Smith, 2011). All of the data obtained is collected as a given signal and the Fourier transform is performed to make this data easier to read. When the obtained spectrum is analysed, FTIR gives all the functional groups of the sample taken and also helps gather information regarding the molecular structure and chemical composition of the sample. It can be used many areas.

2.6.2 X-Ray photoelectron spectroscopy (XPS)

XPS is one of the quantitative methods that chemical structure of elements can be determined. Each molecule and compound composed of numerous elements and chemical bonds. While the XPS analysis gives the general survey of what the elements in the sample are, it also gives the areas of the peaks of the individual bonds of these elements and gives information about the percentage of these elements in the sample. According to the working principle, X-rays coming to the surface cause photoelectric emission from the atoms on the surface. The computation of the motion-related energy of these photoelectrons is executed and this computation is essential for determining the binding energy of the electrons, offering insights into the elements and their chemical states in the sample (Huang and Zhang, 2016). The resulting spectrum is in the form of peaks. As a result of XPS analysis, it is clearly seen which elements are in the material. It is also possible to know which element is in which proportion.

2.6.3 Scanning electron microscopy (SEM)

One of the most commonly used imaging techniques is SEM (Scanning Electron Microscopy), which scans the surface of a sample to produces high-resolution images by focusing the electron beam (Goldstein et al., 2017). The interaction involving the beam of electron and the surface atoms of the substance results in the emission of different signals. The signals collide and an image is formed. Briefly, SEM image gives information about the morphological structure of a surface and shows the structure, roughness, cracks on the surface and distribution on the surface of the surface. The morphology of coated surfaces can be evaluated by looking at SEM images.

2.6.4 Thermogravity analysis (TGA)

TGA analysis, also known as thermogravimetric analysis, produces a thermal curve (thermogram) that displays the sample's mass change in relation to temperature or time. This technique is used to quantitatively assess sample’s mass, from as low as 1 mg to as high as several grams, with the furnace temperature rising up to 1600°C, either with a constant or fluctuating gas flow (Chong et al. 2019).

3. MATERIALS AND METHOD

3.1 Material

Table 3.1 Materials employed, brands and their targeted usega during thin film sample preparation

	Material	**Brand/Company**	**Targeted Usega**
Thin Film Sample Preparation	Gutta percha	Sure Dent Corporation	Substrate for inhibition zone analysis
	Chitosan (LMW) (448869)	Sigma-Aldrich	Coating monomer
	Glass slide	ISOLAB	Sample surface for thin film fabrication, used for XPS analysis
	Silicon wafer	SIEGERT WAFER	Sample surface for thin film fabrication, used for FTIR and SEM analysis
	70% Ethanol	ISOLAB	Sterilization in sample preparation
	Spatula set	ISOLAB	Monomer conveyance from bottle to glass tube
	Fireproof tape	Boss Tape	Pasting the samples to the CVD tray
	Forceps	ISOLAB	Placing of samples
	Filter Paper	ISOLAB	Measure the amount of monomer on a precision balance
	Petri dishes	ISOLAB	Protecting the sample from contamination
	Gloves	Dolphin	Protection the hands from the chemicals and prevention the hand from touching the sample

Table 3.2 Devices employed, brands and their targeted usega during thin film coating

	Device	**Brand/Company**	**Targeted Usega**
Thin Film Coating	Chemical Vapor Deposition (CVD)	Kenar Engineering	Manufacturing chitosan thin films on sample surfaces
	RF Plasma Source	RFPT Co., Ltd	Manufacturing chitosan thin films on sample surfaces
	Cold trap	CLS CLCT -40	Prevention any chemicals from leaking into the vacuum pump.
	Vacuum Gage	Vacuubrand VAP5	Showing reactor pressure
	Vacuum Glass Desiccator	ISOLAB	Protection of thin film fabricated samples from any contamination
	Vacuum Drying Oven	ILDAM	Protection of thin film fabricated samples from any contamination
	Precision Balance	Weightlab instruments	Adjusting the sufficient amount of chitosan for thin film fabrication

Table 3.3 Materials / Devices employed, brands and their targeted usega during microbiology experiments and analysis

	Material & Device	**Brand/Company**	**Targeted Usega**
Microbiology Experiments and Analysis	*Staphylococcus aureus* (ATCC 25923) Standard strain	American Type Culture Collection	Examination of antibacterial effect of chitosan thin films
	Escherichia coli (ATCC 25922) Standard strain	American Type Culture Collection	Examination of antibacterial effect of chitosan thin films
	McFarland Densitometer	BIOMERIEUX/ DensiCHEK plus	Bacterial suspension adjustment
	Test tubes	BIOSAN	Storage of bacterial suspension for concentration adjustment
	Tryptic Soy Broth	Merck 105459.0500 Tryptic Soy Broth for Microbiology	Bacterial broth used during sample storage in different incubation periods
	Tryptic Soy Agar	Merck 105458.0500 Tryptic Soy Agar for Microbiology	Bacterial agar used for inoculating bacterial suspension for the analysis of bacterial viability
	Centrifuge Tubes (2 ml)	ISOLAB	Preparation of TSB concentration
	Centrifuge Tubes (50 ml)	ISOLAB	Storage TSB and PBS for dilutions

Table 3.3 Materials / Devices employed, brands and their targeted usega during microbiology experiments and analysis (continued)

Microbiology Experiments and Analysis	Vortex	Fine Vortex (FINERPCR)	Used for both preparing homogenized bacterial suspension preparation also homogenized agar surface
	Incubator	Memmert (IN110, $+5^{o}/+80^{o}$)	Creation for a safe environment for bacterial growth on agar surfaces
	Precision Balance	BEL	Adjustment the amount of powder when preparing TSA and TSB solutions
	Magnetic Stirrer	Heidolph	For homogeneous mixing when preparing TSA and TSB solutions
	Erlenmeyer Flask (1000 ml & 500 ml)	S & H Labware Boro 3.3	Used for preparation of TSA and TSB solutions
	Graduated Cylinder (1000 ml)	S & H Labware Boro 3.3	Use for Preparation of TSA and TSB solutions
	Micropipettes	ISOLAB	Liquid measurement for bacterial suspension preparation and agar
	Micropipette tips	ISOLAB	Liquid transformation between centrifuge tubes and agar

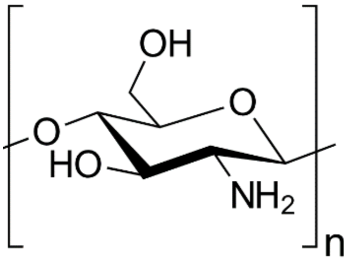

Figure 3.1 Chemical structure of chitosan monomer (Peker et al., 2006)

3.2 Methods

Thin film fabrication and microbiological studies were conducted in different places. Firstly, all the sample preparations, thin film fabrications and sample storage parts were completed at Ankara University Biomedical Engineering Department. Antibacterial analysis and inhibition zone analysis were completed at TOBB Education and Technology University.

3.2.1 Sample preparation

In the beginning of each synthesis, pre-cut glass slides and silicon wafers were placed into petri dished and 70% ethanol solution was filled until all the sample surfaces were covered and soaked for 10 minutes. Then from edges, excess ethanol droplets were removed by gentle tapping the sample edges into filter papers. Then placed into another petri dish for full drying. Following the drying, samples were transferred onto sample holder which was previously sterilized. Samples were secured with heat-resistant tape and transferred into reactor chamber in an up-side down position for better coating.

3.2.2 Sample positioning in CVD

After sample preparation, sample surfaces were placed upside down in plasma.

Figure 3.2 Position of surfaces in plasma chamber

3.2.3 Thin film production

Figure 3.3 General perspective of CVD

In Figure 3.3 General perspective of CVD device with plasma source on the right was photographed in real-time. During usage of CVD device, internal environment preserved its low-pressure state and therefore contamination free condition. Low pressure levels were ensured with vacuum pump. Chitosan was sent through from outside into reactor chamber, powder was placed into glass tubes and heated separately from the reactor.

When chitosan was heated up to its optimum temperature, it was sublimated through the reactor pipes and reached inside the reactor. Monomer vapor then was able to react with glass slide, silicon wafer and gutta percha sample. When the placing was done and reactor was securely closed, vacuum pump was switched

Subsequent to sample and monomer placing preparation, reactor was closed and vacuum pump switched on. Throughout the experiments, pressure level tried to be limited between 60-80 m Torr levels. During pressure drop, reactor wall was heated up 50°C to prevent sublimated chitosan vapor from adhering. During low pressure levels, monomers can evaporate and sublimate at lower temperatures differently from room pressure conditions. Additionally, sample surfaces were preserved from any kind of contaminants since no air trapping was allowed due to vacuum pressure.

At the beginning of vacuum pressure drop, chitosan glass tubes valve was open to lower the pressure of chitosan monomer tube and make it the same as the reactor chamber. As mentioned on the previous paragraph, due to lower pressure in the monomer tube, monomers tend to evaporate or sublimate at lower heating degrees. When pressure drop stabilized around 60 to 80 m Torr levels, plasma was switched on at 5W and samples were exposed to plasma for 1 minute. With this way, sample surfaces were activated ions and made them more retainer for ionized monomer gas.

When plasma exposure was done, chitosan was heated until it was sublimated. For controlled thin film thickness, sublimated gas pressure was controlled during experiment.

When optimum monomer pressure was obtained, plasma source was switched on once again. This time it was for the excitation and ionization of chitosan molecules, so that when they’ve energised their high energy state can stimulate their adherence characteristic. Highly energised chitosan molecules tend to lower their energy state and become more stable. To achieve that they've hold on sample surfaces When 1 hour of plasma exposure was completed, plasma was switched off and remaining chitosan vapor was let to adhere as much as they can for 10 minutes. Then when the times was run out vacuum pump was switched off and samples were taken out from the reactor chamber. Samples were transferred from CVD reactor to petri dishes it was placed into either desiccator or vacuumed drying oven to preserve cleanness of samples.

3.2.4 Antibacterial analyses studies

3.2.4.1 Bacterial colony analysis

The antibacterial characteristic of chitosan coated thin films were analysed with proposal *E. coli* and *S. aureus.* Adherence characteristics of bacteria was compared with reference bacterial inoculation results.

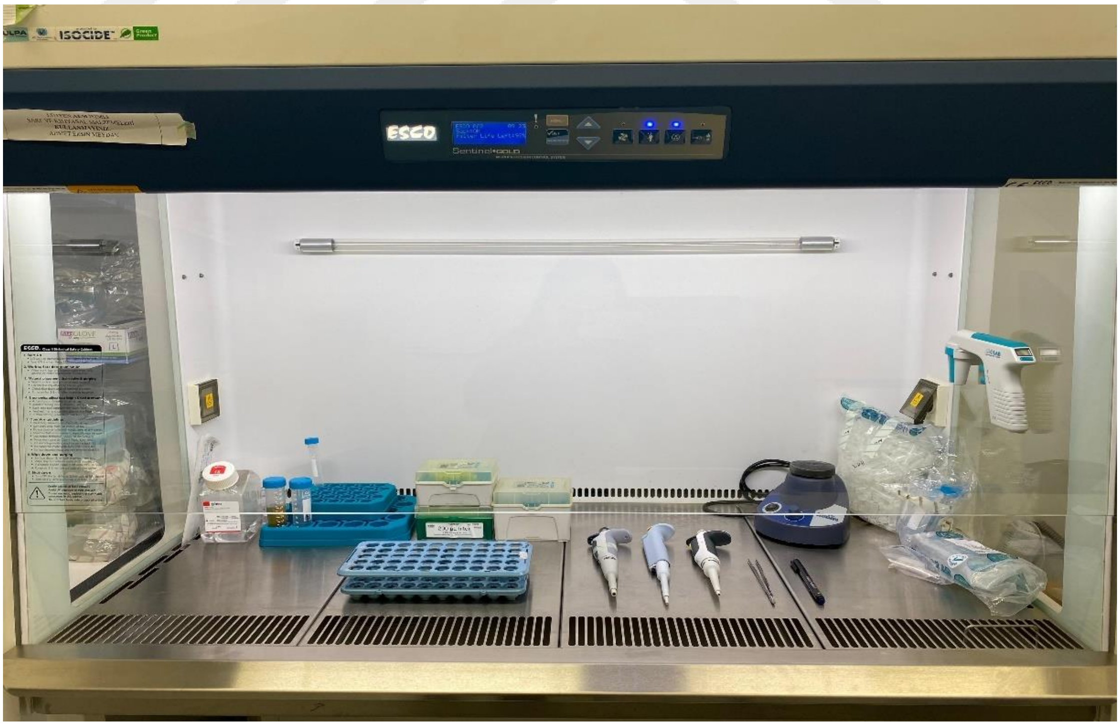

Figure 3.4 Bacterial studies were performed in the biosafety cabinet

As mentioned previously, chitosan coated samples were examined against *E. coli* and *S. aureus.* Inoculation studies were performed in 3 different days which were 1st, 4th, and 7th day. For each day 4 glass slide samples were used therefore results were had a control set to conclude whether the chitosan thin films were effective against bacterial colonization or not. Additionally, for the comparison reference surfaces were also inoculated in the same procedure. Before performing inoculation, both broth and agar feedlots were prepared.

Both, Tryptic Soy Broth and Tryptic Soy Agar were purchased in powder form. For Tryptic Soy Broth, 15 g of powder was weighed in precision balance and transferred into Erlenmeyer flask. Next, 500 mL of distilled water was accurately measured using a graduated cylinder and added to the Erlenmeyer flask. In order to mix powder and water magnetic stirrer was used at 720 rpm at room temperature until it was completely homogenised from view-sight. Similarly, for Tryptic Soy Agar solution was prepared into a different Erlenmeyer flask with 40 g of powder and 1 L of distilled water. The same mixing steps were also repeated for Tryptic Soy Agar. Following the preparations, Erlenmeyer flasks were covered with gauze patches containing cottons and additionally aluminium foil were covered the outer layer for the usage of autoclave tapes.

Cotton balls and gauze patches helps to prevent vaporization of feedlots and contamination of them due to possible mixing of water vapor trapped inside the autoclave. Sterilization was completed at 120^{o}C for 2 hours.

After autoclave have been completed, for the agar preparations the area that was worked was sterilized with Bunsen Burner about 30 cm circle and two of them used at once. After 15 minutes of sterilization, agar solutions were poured into gamma sterile petri dishes about one fourth of its whole depth. After each pouring dishes were placed onto a pre-sterilized and dry surface to let the agar to be completely solidify.

When all the petri dishes were controlled for the solidification, they were wrapped in a clean package and sealed. Then placed into refrigerator at +4^{o}C, when their production dates were written on the package.

Following the feedlot preparations, bacterial passages were prepared for inoculations. In this step before freezing *E. coli* and *S. aureus* suspensions were placed into cell holders and placed into the biosafety cabinet. Each bacterium was let to completely defrost, then with the help of a swap/inoculation stick a small amount was taken a swapped on the whole surface. This step was repeated for each bacterium on a new agar surface. This was considered as the first passage, in order to be used it should be second passage. So, the first passage was then placed into the incubator for 24 hours. Then the next day proliferated bacteria from the first one was transferred onto a new agar. And again, placed into the incubator. After the 24 hours of incubation bacteria became usable for inoculation studies. In this step, 3 mL of fresh TSB solution was accurately measured using a micropipette and added to a glass vial. Then a new swab used for the addition of each bacterium into different fresh TSB mediums. After that, concentrations were checked with McFarland densitometry. Until it was stabilized at 0.5 Mc F level which is equal to 1.5 x 10^8 CFU/mL, few adjustments were repeated for each bacterium. Then in order to count colonies precisely, suspension was diluted as 1:100 ratio. which is equal to 1.5 x 10^6 CFU/mL (Solovchuk et al., 2023).

For the sample inoculation, each sample was added into 15 mL centrifuge tubes separately and filled until their top part with homogenized TSB dilution wit micropipette. Then samples were securely closed with the capes and placed into the incubator for different periods of 1st, 4th, and 7th day. In incubator, reactor humidity was preserved with addition of distilled water inside the reactor.

In each following incubation day, firstly fresh agars were taken out the fridge and placed onto sterilized table in an upside-down position tis way any possible water droplet slipping into the agar have been prevented. Then centrifuge tubes were filled with TSB and PBS solutions. PBS was used for the analysis of bacteria that couldn’t hold onto sample surfaces yet managed to live in the broth. TSB was used to analyse bacterial colonies that have adhered onto the sample surface (Nedwell, 1999).

For each period, there were different dilutions were made in order to properly count the bacterial colonies formed on sample surfaces and broth environment. Dilutions were changed from 10^1, 10^3, 10^5, 10^7, 10^9, 10^{11}, 10^{13} depending on the results of the first day results, dilutions were prepared. In each agar, 4 dilutions were inoculated to make sure that the precise counting of colonies was achieved.

In each inoculation period, samples were taken out the incubator and firstly was washed with PBS to remove all the bacteria that couldn’t hold on the sample surface. Then it was gently washed then transferred into fresh TSB broth for the detection of surface adhered bacteria. With this distinction, antimicrobial characteristics of the chitosan thin films were analysed.

For dilution preparations, firstly for each sample 1 mL TSB and 1 mL PBS solutions were added into sterile centrifuge tubes. Then for the first dilution 900 μL TSB was added all samples. Then remaining dilution tubes were filled with 990 μL TSB since from the first dilution 10 μL of bacterial suspension was transferred one by one. Only for the first 900 μL TSB 100 μL TSB or PBS was transferred from. During each transfer, centrifuge tubes were vortexed and clean, sterile pipette tips were used.

Following the preparation of dilution, it was time for the agar inoculation. For this, agar plates were placed firstly into the biosafety cabinet, then the last four dilutions were chosen for the inoculation. Before each inoculation, centrifuge tubes were vortexed and 100 μL of the suspension was added on the area where each dilution was marked. In each, addition pipette tipes were certainly changed.

After completing all the inoculations agars were placed into incubator for 24 hours for bacterial colonization and surface stabilizations.

Following 24 hours, the agar plates were removed from the incubator, and the colonies were counted without magnification. All the countable colony numbers were notes according to their dilution rates, example figure is presented in figure 3.5.

A total of 1st day, 4th day and 7th day colonies were counted. In order to count the 4th and 7th day colonies, dilution transfers and bacterial cultivations were done in the same way for each 24 hours before. In short, Bacteria were cultured in petri dishes by preparing a dilution again and counted on the specified days. Petri dishes used for bacterial counting are not used on other days.

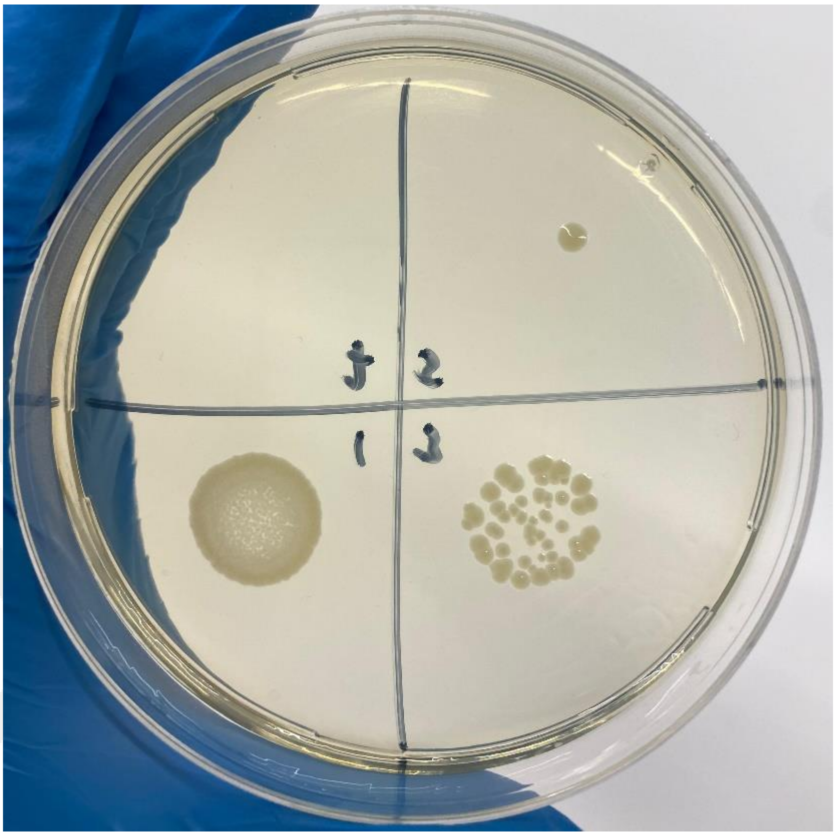

Figure 3.5 Bacteria colony on petri dish

3.2.4.2 Zone of inhibition test

During microbiology analysis, simultaneously zone of inhibition test have been conducted. Similar to the first bacterial incubation, for *E. coli* and *S. aureus* bacteria 1.5x10^6 CFU/mL suspensions were prepared just like following the previous section. Then on a fresh agar, 500 µL suspension of each bacterium added on different agar plates. Then for proper bacterial inoculation a swab has been used for swapping the suspensions homogenously. Then gutta perchas were placed on the homogenously inoculated agars and placed into incubator for each period of time. In each period (1st, 3rd, 4th and 7th day) surface photos were taken. At the same time, reference bacteria were incubated and photographed at the relevant times.

4. RESULTS AND DISCUSSION

4.1 TGA Analysis

Many experiments have been conducted on glass and silicon wafer surfaces with CVD device. Chitosan is a material with a high evaporation temperature at room temperature. The aim is to produce thin films by evaporating at lower temperatures with a CVD device. Chitosan powder monomer was placed in glass tubes and the valve was opened and the experiment was started when the pressure dropped. In all experiments, all variables except one were kept constant. The variable that changed was the temperature value. As a result of many experiments, it was thought that I could evaporate chitosan between 120-200^{o}C. Although the initial pressures, the amount of chitosan placed in the glass tube, the position of the samples in the CVD device, and the duration of the experiment were kept equal, some differences were observed.

As an outcome of the experiments was conducted at different temperatures of 120^{o}C, 160^{o}C, 180^{o}C and 200^{o}C, it was determined that the colour changes of the chitosan remaining in the glass tube were different. The rise in pressure while increasing the temperatures during the experiment indicates that the monomer entered the CVD device. However, at some temperature values, when the desired temperature was reached and the experiment was started, no increase in pressure was observed. In the experiments conducted at 120^{o}C, 180^{o}C and 200^{o}C, no increase in pressure was observed. This indicates that chitosan monomer did not enter. In fact, when FTIR analysis was performed on thin films made at this temperature as a result of the experiment, it was understood that their structures were different from chitosan. However, in the experiment conducted at 160^{o}C, an increase in pressure was observed, and peaks similar to those of chitosan monomer were also obtained in the FTIR analysis. In other words, since the best result was obtained at 160^{o}C, the temperature was optimized to 160^{o}C for all subsequent experiments.

The colour changes of the chitosan monomer remaining in the glass tube at the end of the experiment carried out at different temperatures are presented in the figure 4.1

Figure 4.1 Colour changes of the chitosan remaining in the tube from experiments conducted at different temperatures

When the FTIR analysis of these different coloured chitosan remaining after the experiment was performed, it was determined that their structures differed from the chitosan monomer. In other words, it was understood that the structure of the chitosan monomer was deteriorated at these temperatures. While the powders heated at 120^{o}C and 160^{o}C still resembled chitosan monomer, the powders at 180^{o}C and 200^{o}C burned. For this reason, TGA analysis was performed on these powders and monomer. It was performed to determine the temperature to be used in the experiments.

TGA is an analyse method that gives the loss of mass of a substance over time by increasing it to a certain temperature, and its graph is given as temperature-mass. Even though 120°C appears to be the closest to the monomer in the TGA analysis, there is no increase in pressure, that is, no chitosan monomer entry into the CVD device, in the experiments performed at 120°C. Therefore, the temperature was optimized at 160°C. In fact, in the FTIR method performed on the substrates, it was seen that there was no coating at 120°C. But at the end of the experiment at 160°C, there is a coating on the silicon wafer that is close to blue.

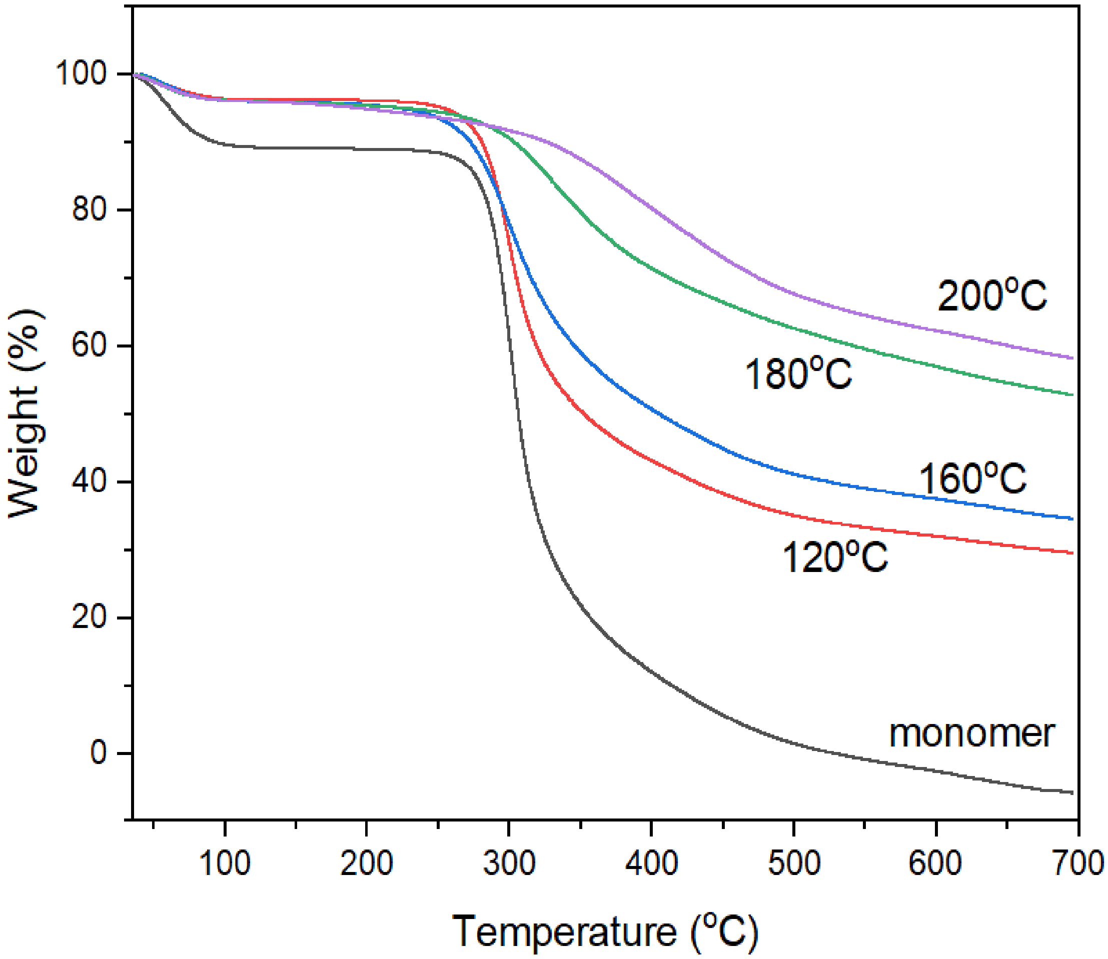

Figure 4.2 TGA result of different temperature values

4.2 FTIR Analysis and XPS Analysis

FTIR and XPS method were completed to analyse whether the bonds and elements of the chitosan monomer's molecular structure were found in the produced chitosan thin film.

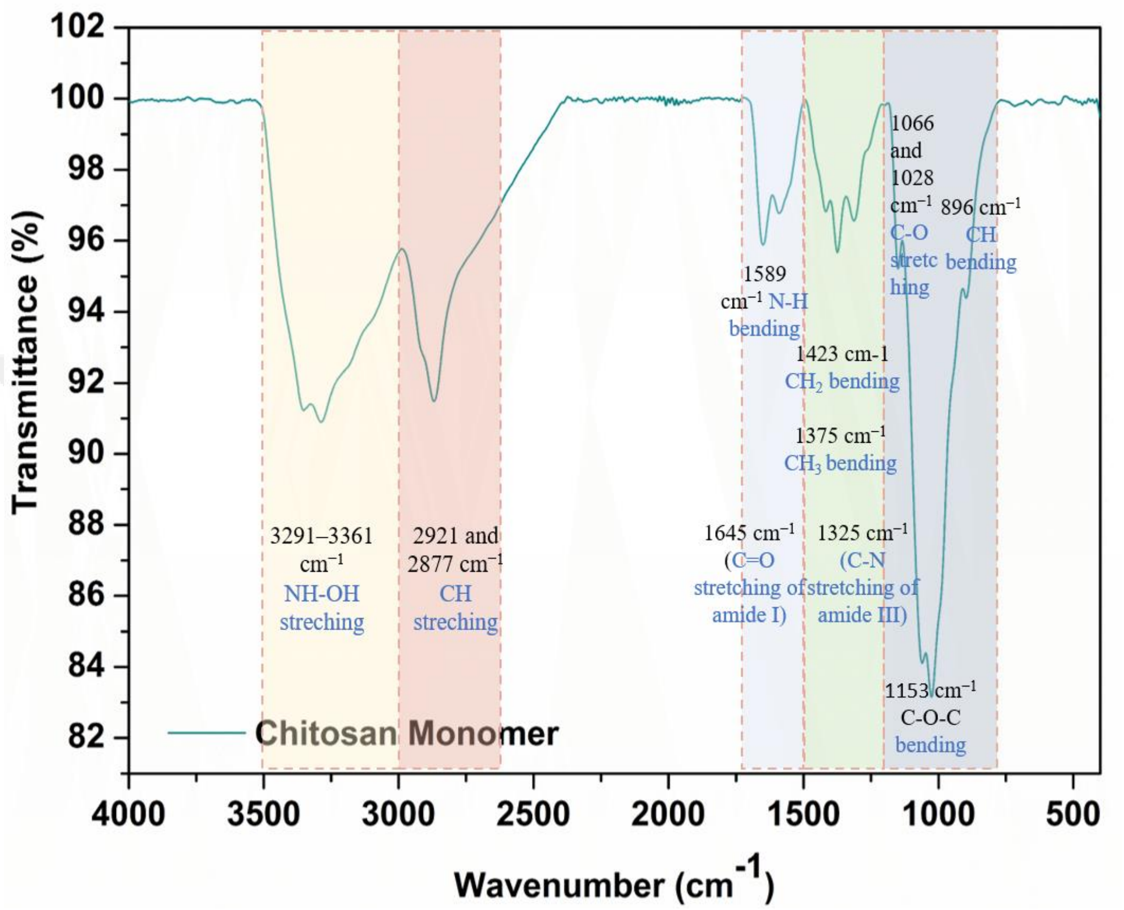

Figure 4.3 FTIR result of chitosan monomer

In Figure 4.3, FTIR result of chitosan monomer is given. The N-H and O-H stretching are represented by a band in the range of 3291–3361 cm^{-1}. C-H symmetric and asymmetric stretching can be attributed to the bands around 2921 and 2877 cm^{-1}. Residual N-acetyl groups were identified by the bands observed at around 1645 cm^{-1} (C=O stretching of amide I) and 1325 cm^{-1} (C-N stretching) (Silva et al., 2010). The N-H bending of the primary amine is indicated by a band at 1589 cm^{-1} (Silva et al., 2010). Additionally, the CH2 bending and CH3 symmetrical deformations are supported by bands around 1423 and 1375 cm^{-1} (Walkers and Oliver, 2004). At 1153 cm^{-1}, the absorption band is linked to the asymmetric stretching of the C-O-C bridge. The bands at 1066 and 1028 cm^{-1} are related to C-O stretching, and the band at 896 cm^{-1} corresponds to the CH bending vibration (Walkers and Oliver, 2004).

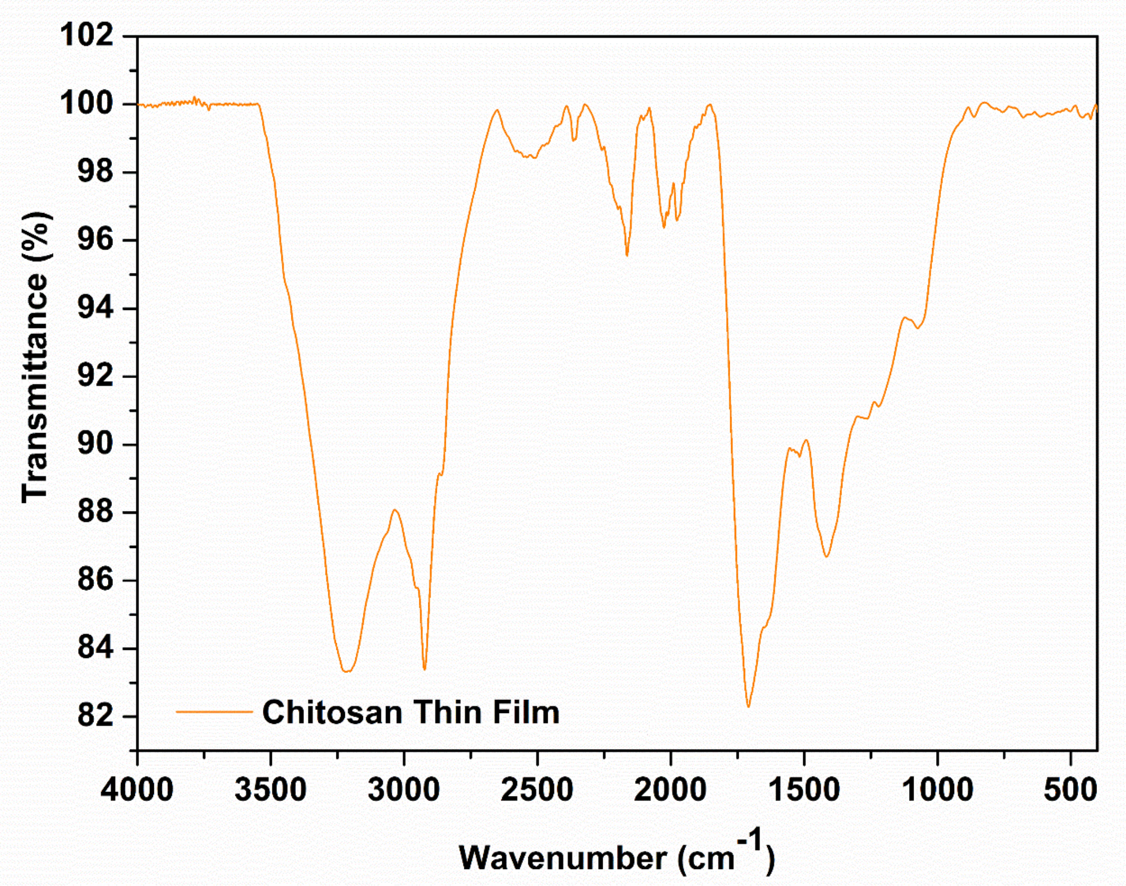

Figure 4.4 FTIR result of chitosan thin film on silicon wafer

In figure 4.4, FTIR result of chitosan thin film is given. C=O bond was formed at 1700 cm^{-1} due to plasma polymerization.

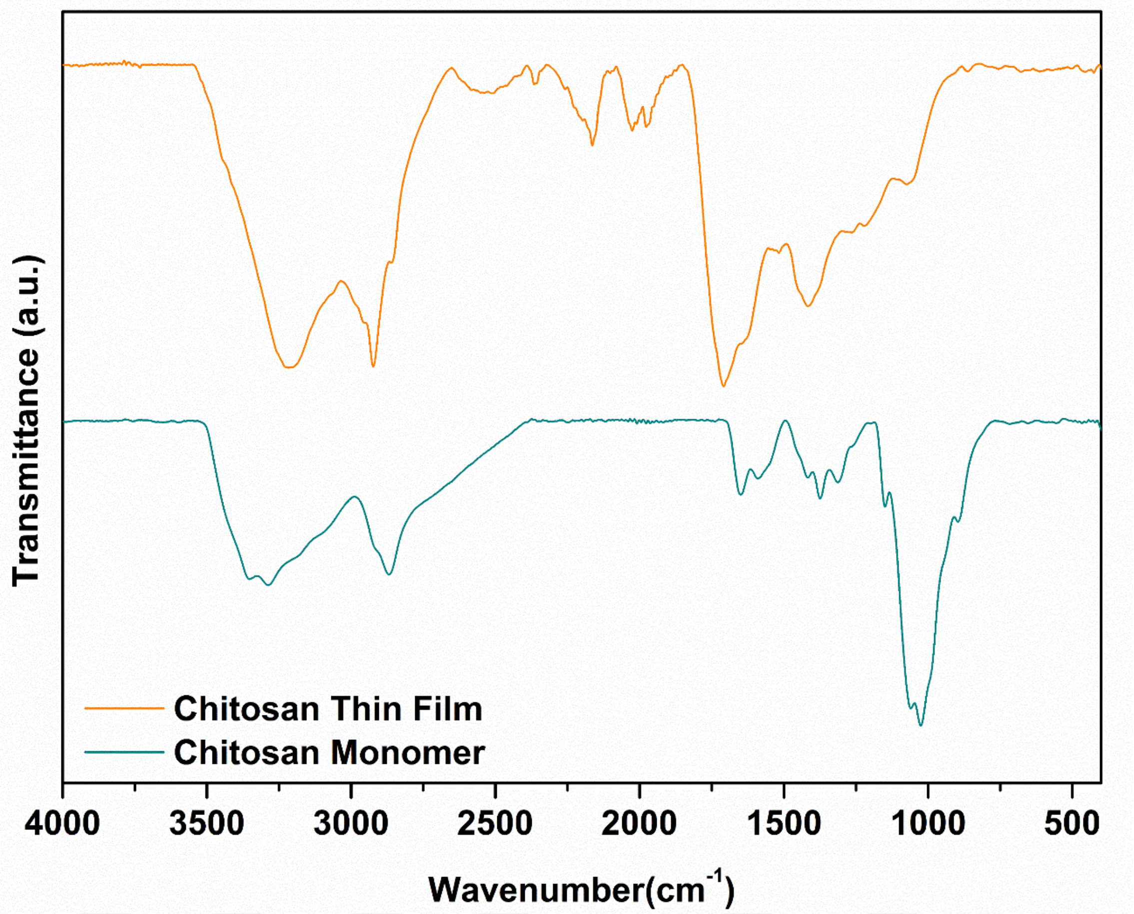

Figure 4.5 Comparison of FTIR results of chitosan monomer and chitosan thin film

In the figure 4.5 FTIR comparison of chitosan monomer and chitosan thin film is given. The important OH peak and NH peak which determine the properties of chitosan are clearly the same. The two spectra are almost the same, only there is a C=O peak at 1700 caused by plasma polymerization. In the thin film, all the distinctive peaks of monomer chitosan are present.

In following figures 4.6 & 4.7 & 4.8 & 4.9 demonstration of XPS C1s, N1s and O1s deconvolution spectrum of plasma polymerized chitosan thin film and general survey spectra of chitosan thin film is given. In the general survey, as expected, only the elements C, N, and O are found in the structure of chitosan. The detailed information about peak values corresponding to each chemical bond, their strength, the area under each peak and equivalent full-width half maxima (FWHM) values were presented in table 4.1, table 4.2 and table 4.3.

Analysis of C1s spectrum demonstrated five distinct peak configuration and presence of each peak matched with the peaks seen on the FTIR spectrum. When corresponding energy bands of C1s compared with literature C-C / C-H bond correspond to 284,8 eV, C-O-H bond correspond to 286,2 eV, C-O-C bond correspond to 286,8 eV, C-N-H_2 bond correspond to 288.08 eV and finally C-N^+-H bond correspond to 289,09 eV that these values were similar all supported by earlier studies (Bhatt et al., 2012; Valinataj Omran et al., 2019). For N, there is a shift of about 1 eV between the bond between N and the N^+. Similarly, Analysis of O1s spectra demonstrated three distinct peak configuration and presence of each peak matched with the peaks seen on the FT-IR spectrum. when O1s spectra examined C=O bond seemed to appear at 532,093 eV, C-O-H bond at 532,868 eV and C-O-C bond at 532,7 eV all were supported by findings of previous studies (Truica-Marasescu and Wertheimer, 2008). Finally, Analysis of N1s spectra demonstrated two distinct peak configuration and presence of each peak matched with the peaks seen on the FT-IR spectrum when N1s spectra examined C-N-H_2 bond correspond to 400,06 eV and C-N^+-H bond correspond to 401,49, so there is a shift of about 1,43 eV the bond between N and the N^+. These results were as expected.

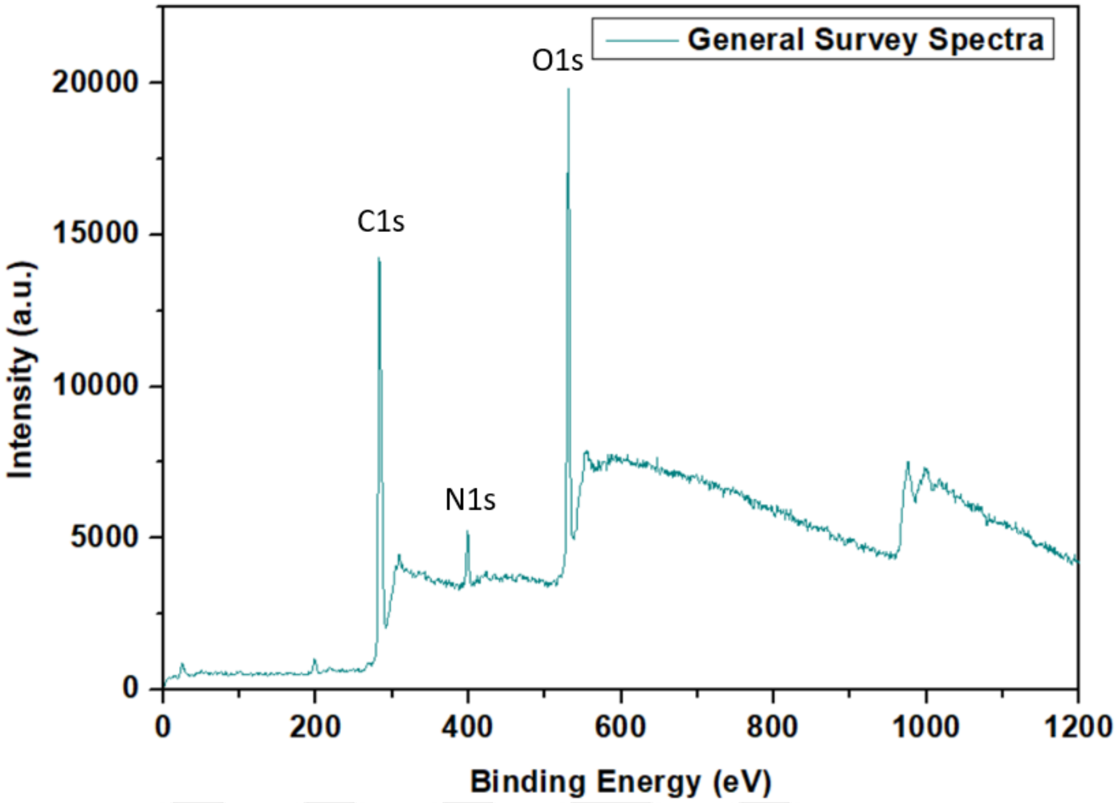

Figure 4.6 XPS General survey spectrum of chitosan thin film

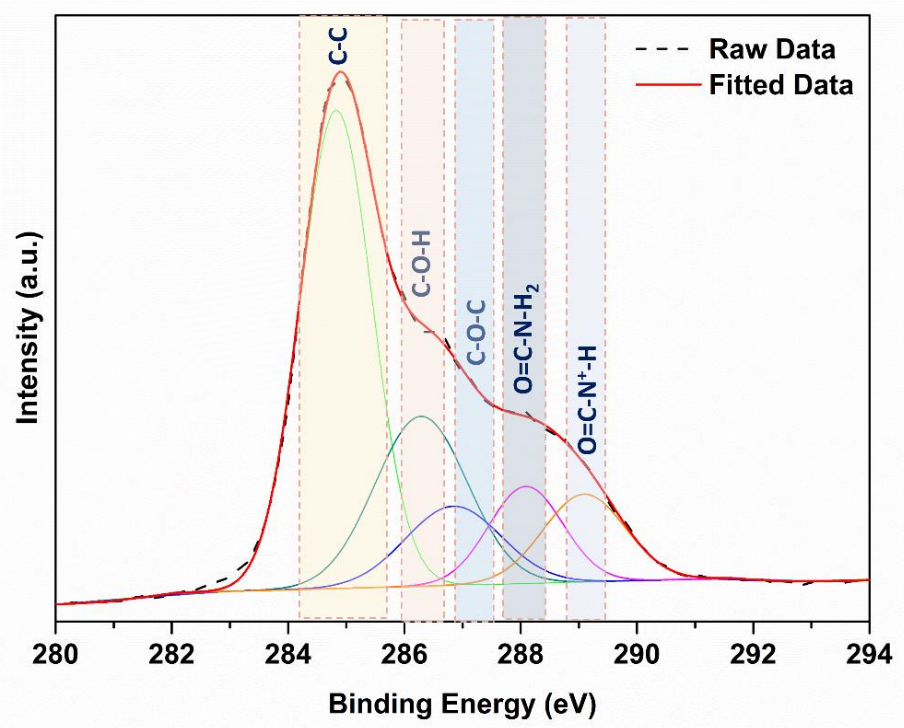

Figure 4.7 Chitosan’s high-resolution C1s spectrum following deconvolution

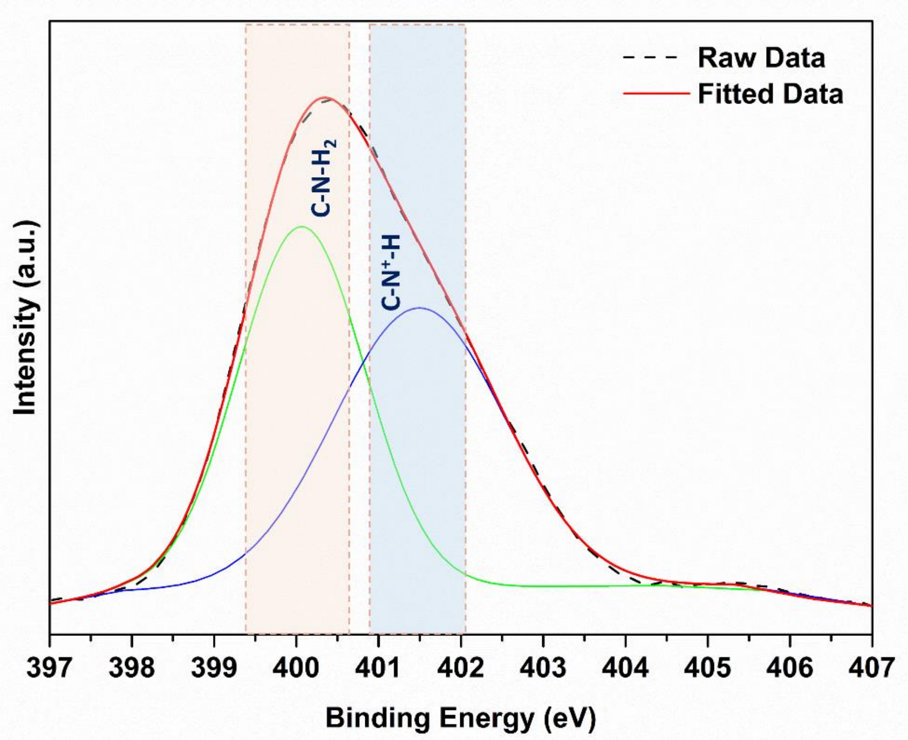

Figure 4.8 Chitosan’s high-resolution N1s spectrum following deconvolution

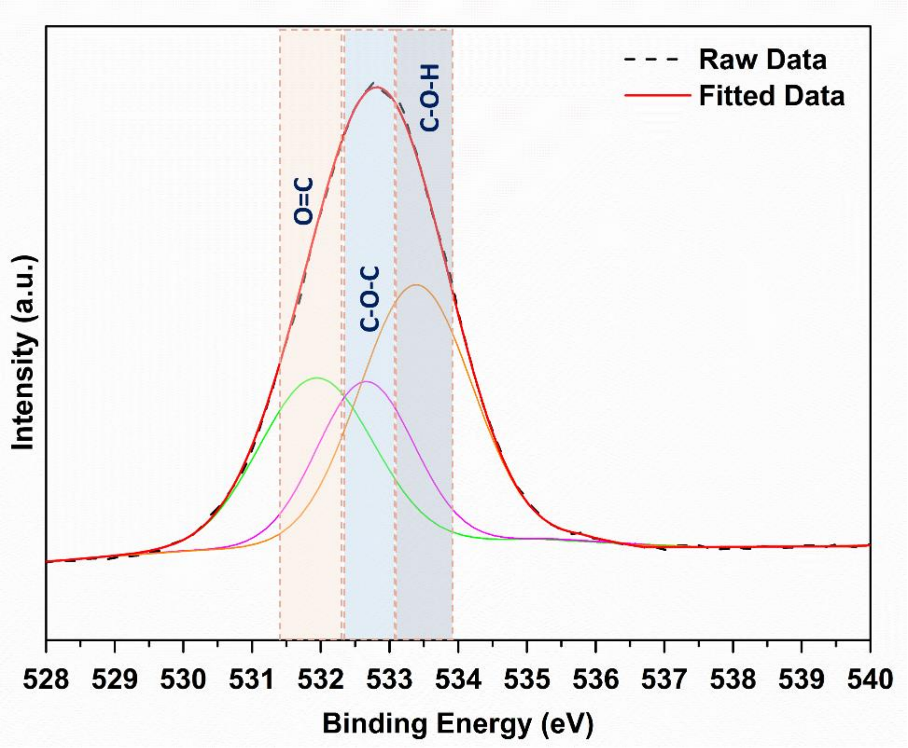

Figure 4.9 Chitosan’s high-resolution O1s spectrum following deconvolution

Table 4.1 C1s XPS spectrum of chitosan (R^2=0,9995)

Area Fit	**Area FitTP**	**Center Max**	**FWHM**	**Chemical Bonds**
10950,98	47,59	284,82	1,44	C-C / C-H
5019,10	21,81	286,28	1,86	C-O-H
2423,69	10,53	286,84	1,93	C-O-C
2300,88	10,00	288,08	1,50	$C-N-H_2$
2312,20	10,05	289,09	1,66	$C-N^+-H$

Table 4.2 O1s XPS spectrum of chitosan (R^2=0,9996)

Area Fit	**Area FitTP**	**Center Max**	**FWHM**	**Chemical Bonds**
6256,42	29,96	531,93	1,94	C=O
5145,02	24,64	532,65	1,65	C-O-C
9476,58	45,39	533,38	1,92	C-O-H

Table 4.3 N1s XPS spectrum of chitosan (R^2=0,9995)

Area Fit	**Area FitTP**	**Center Max**	**FWHM**	**Chemical Bonds**
1198,33	48,96	400,06	1,81	$C-N-H_2$
1248,93	51,03	401,42	2,44	$C-N^+-H$

4.3 SEM Images

SEM image offers details about the morphological structure of a surface and shows the structure, roughness, cracks on the surface and distribution on the surface of the surface. The morphology of coated surfaces can be evaluated by looking at SEM images. So, for this study, SEM images were taken from the surface of chitosan thin films produced with PECVD method. These images are as in figure 4.10 a and 4.10 b below. Figure 4.10 a has a homogeneous and uniform coating. However, figure 4.10 b shows that a cracked coating occurs due to stress.

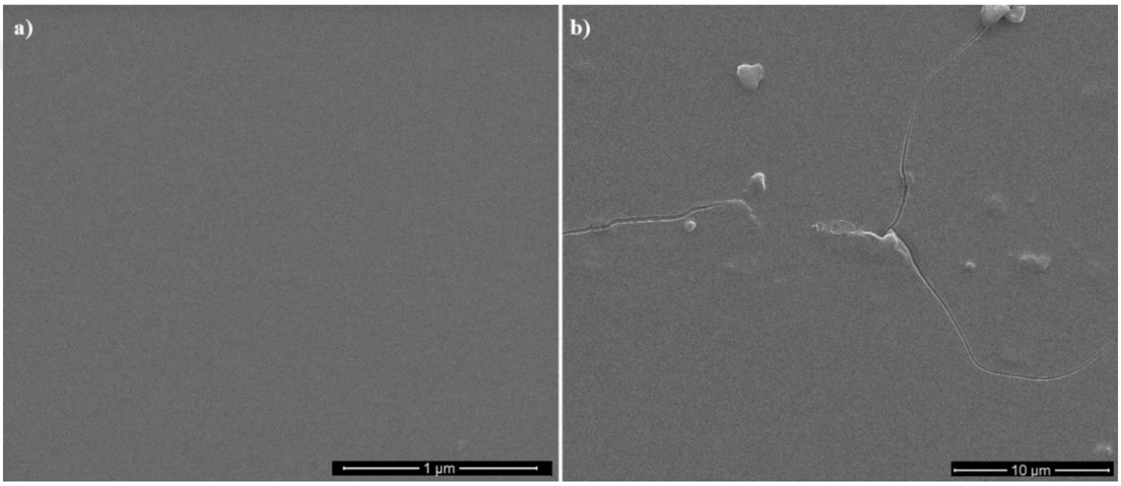

Figure 4.10 Sem images of chitosan thin film

4.4 Antibacterial Analysis Results

What was done in microbiology studies was mentioned in the method section. Studies were carried out with 2 bacteria in microbiology that these are *E. coli S. aureus*. Additionally, 2 distinct studies were conducted in this section: zone of inhibition test and bacterial colony counting.

4.4.1 Zone of inhibition test results

For analysing the antibacterial activity of the produced chitosan thin films, tests for zone of inhibition were conducted. Firstly, *E. coli* was analysed on gutta perchas for coated and uncoated samples. As shown in Figure 4.11, there was no removal of bacteria on the surfaces for uncoated surfaces. When chitosan-coated surfaces were examined, it was seen that it eliminated the surrounding bacteria and there was no bacterial growth. Similarly, Photos were taken on 1st day, 3rd day, 4th day, and 7th day for both samples. As shown in Figure 4.12 *E. coli* bacteria eliminated around by the coated gutta percha. As a result, it was proven that chitosan has the ability to inhibit *E. coli*.

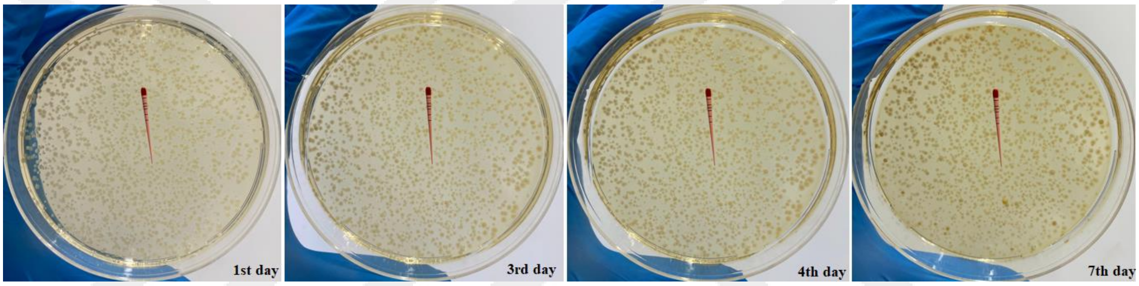

Figure 4.11 *E. coli* for 1st day, 3rd day, 4th day and 7th day on uncoated reference samples

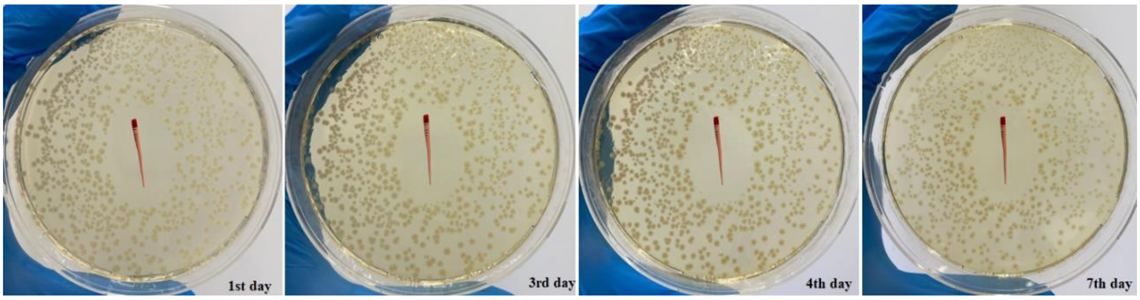

Figure 4.12 *E. coli* for 1st day, 3rd day, 4th day and 7th day chitosan coated samples

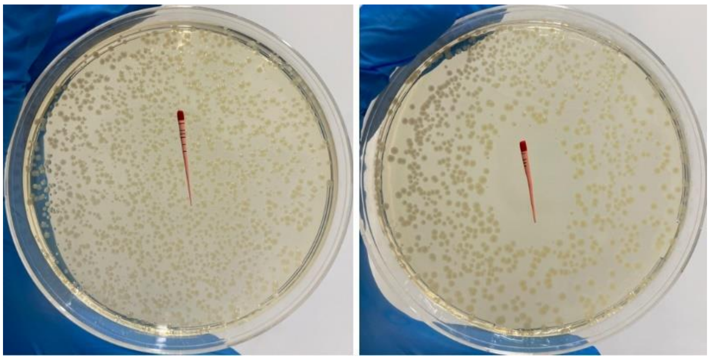

Figure 4.13 Uncoated references and coated samples for *E. coli* (1st day)

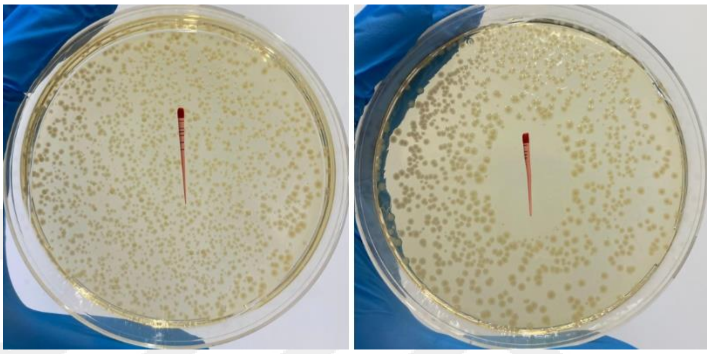

Figure 4.14 Uncoated references and coated samples for *E. coli* (3rd day)

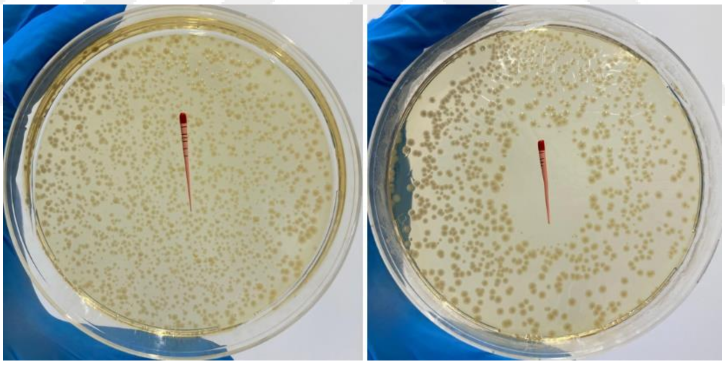

Figure 4.15 Uncoated references and coated samples for *E. coli* (4th day)

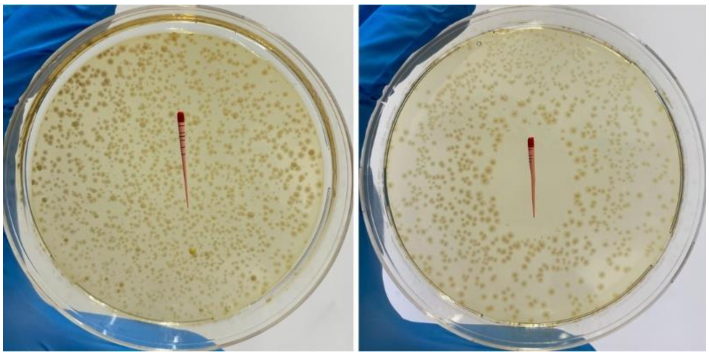

Figure 4.16 Uncoated references and coated samples for *E. coli* (7th day)

Secondly, Inhibition zone test was performed for *S. aureus* bacteria also. Uncoated surfaces and coated surfaces were incubated with *S. aureus* bacteria. As shown in Figure 4.17, similar to *E. coli* bacteria, no inhibition was observed in uncoated surfaces. However, chitosan-coated surfaces were examined, it eliminated the bacteria and there was no bacterial growth in the immediate vicinity. Similarly, photographs were taken on both the uncoated surface and the chitosan coated surface on 1st day, 3rd day, 4th day and 7th day. In Figure 4.18, the *S. aureus* bacteria in the surroundings were eliminated by the coated surface as seen. As a result, it was proven that chitosan has an inhibitory ability. In addition, it showed many times more efficient on *S. aureus* bacteria than on *E. coli*. As a result, it was proven that chitosan has the ability to inhibit.

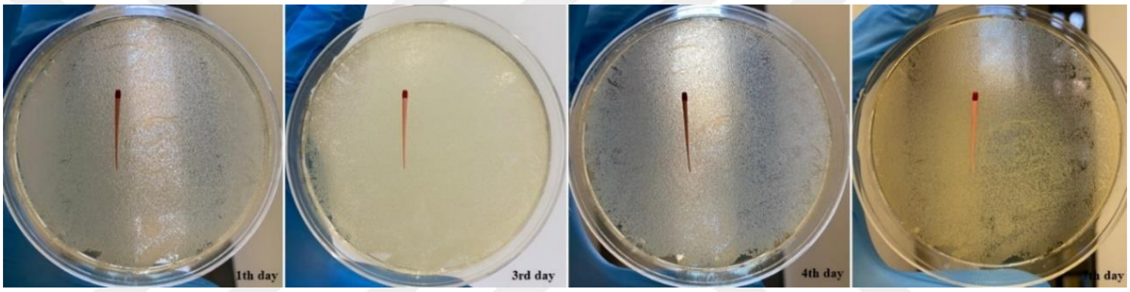

Figure 4.17 *S. aureus* for 1st day, 3rd day, 4th day and 7th day on uncoated samples

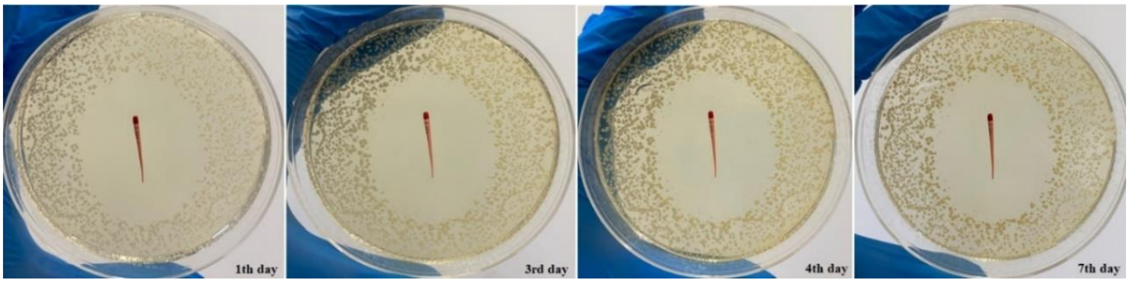

Figure 4.18 *S.* 1st day, 3rd day, 4th day and 7th day on chitosan coated samples

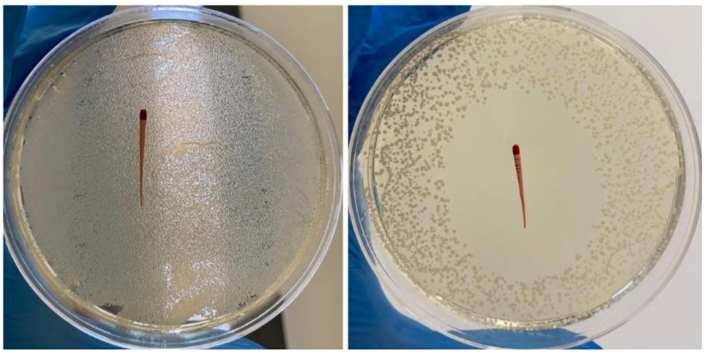

Figure 4.19 Uncoated references and coated samples for *S. aureus* (1st day)

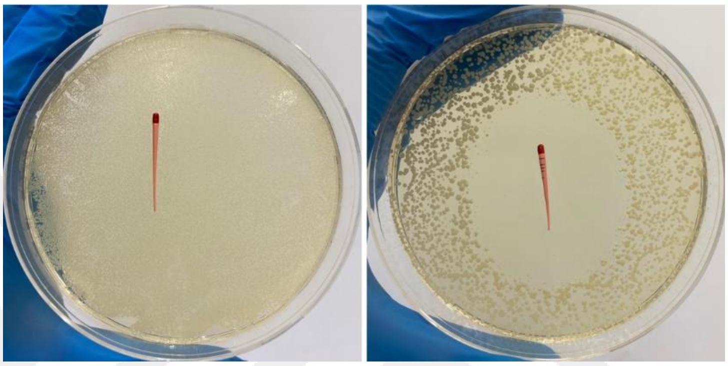

Figure 4.20 Uncoated references and coated samples for *S. aureus* (3rd day)

Figure 4.21 Uncoated references and coated samples for *S. aureus* (4th day)

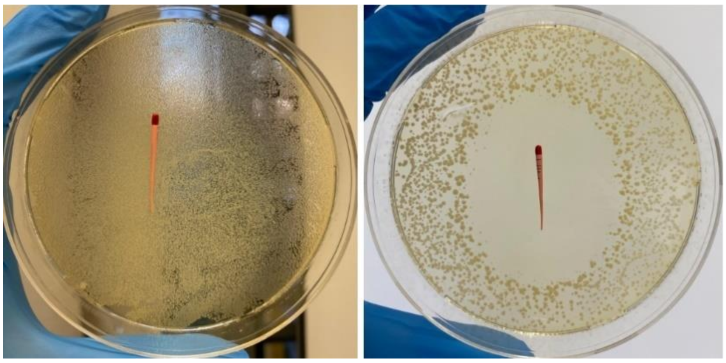

Figure 4.22 Uncoated references and coated samples for *S. aureus* (7th day)

4.4.2 Bacterial colony counting results

In this thesis study, colony counts were carried out for a specific time sequences and the adhesion & non-adhesion tendencies of bacteria were analysed. It was analysed whether the thin films have any antibacterial effect on the bacteria on the 1st day, 3rd day, 4th day and 7th day or not. Statistical analyses were performed with ONE-WAY ANOVA. Figure 4.23 shows the concentration of adsorbed *E. coli* on the surface of chitosan coated samples and uncoated samples on three different days.

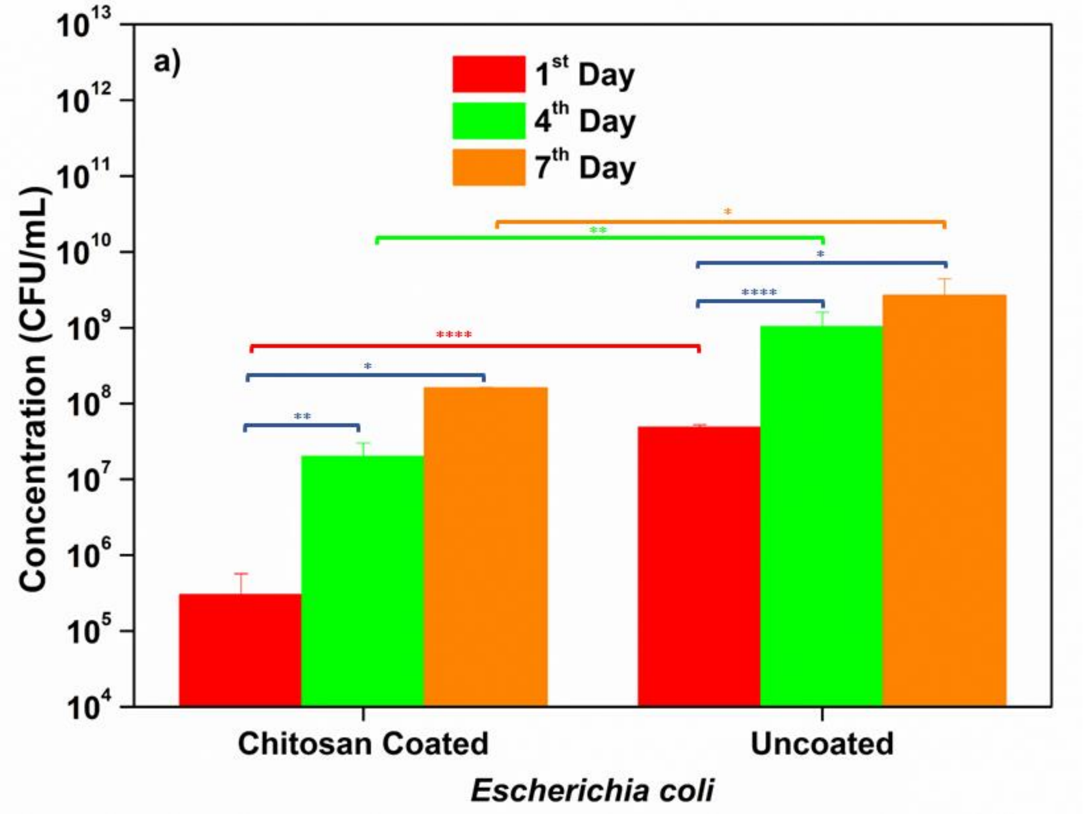

Figure 4.23 Column chart of the comparison of *E. coli* bacteria's tendency to adhere to chitosan-coated and uncoated surfaces at different time periods (Red: 1st day, Green: 4th day, Orange: 7th day). p<0,05: *, p<0,01: ** p<0,005: ***, p<0,001: ****

As shown in Figure 4.23, Chitosan coated surfaces prevented *E. coli* bacteria from adhering to the surface for 1st day, 4th day and 7th day compared to uncoated ones. There is a significant inhibition of bacterial colony, according to percentages. For the first day, Chitosan thin film was able to inhibit 99.43% of *E. coli*. On the 4th day inhibition rate was approximately 98.5%, in the 7th day total inhibition rate was 93.99%. These results proof that the chitosan coated surface has an antibacterial action on *E. coli* bacteria.

Figure 4.24 shows the concentration of non-adsorbed *E. coli* on the surface of the chitosan coated sample, and the reference uncoated samples for three different days.

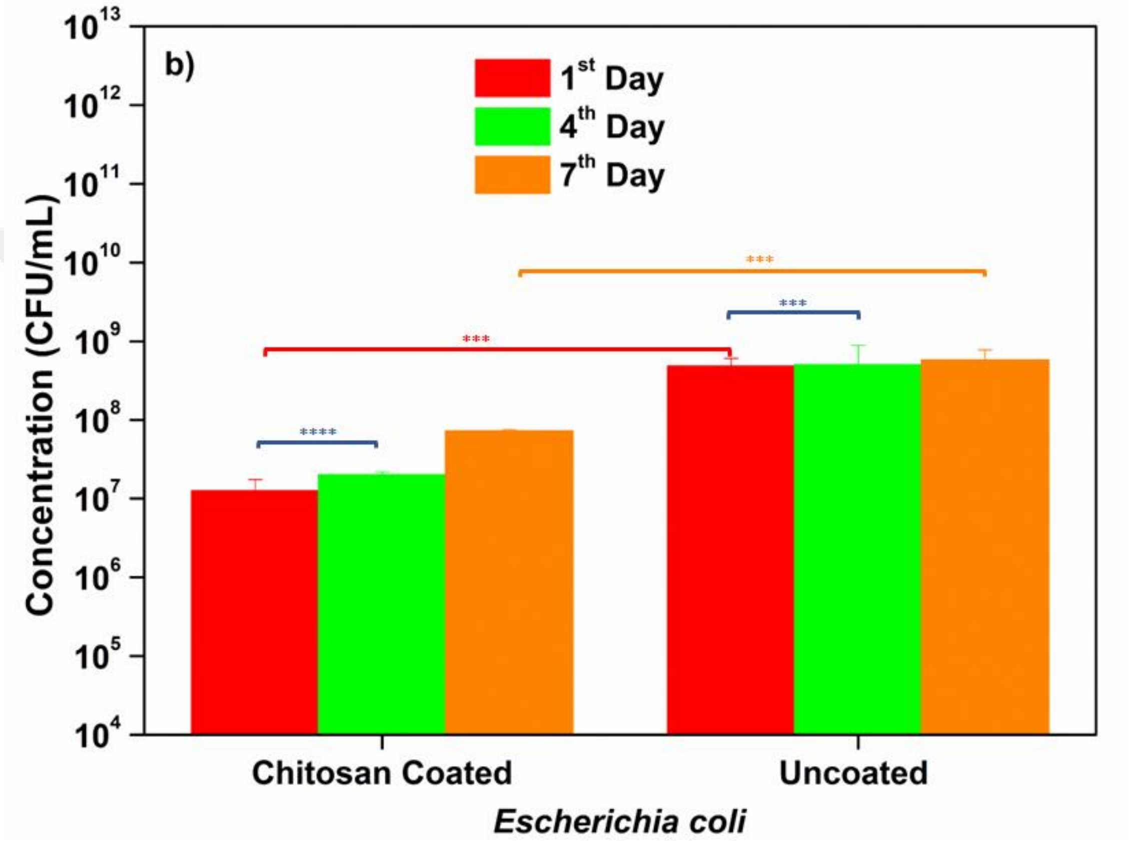

Figure 4.24 Column chart of the comparison of *E. coli* bacteria's tendency to non-adhere to chitosan-coated and uncoated surfaces at different time periods (Red: 1st day, Green: 4th day, Orange: 7th day). p<0,005: ***, p<0,001: ****

As shown in figure 4.24, chitosan films prevent *E. coli* bacteria adhesion to the surface for 1st day, 4th day and 7th day. When colonies of bacteria that cannot adhere to the surface were examined, the situation is the same. Statistical analysis indicated that there was inhibition of bacterial colony even when the bacteria wasn't able to hold on surfaces, this conclusion was supported with the bacterial inhibition percentages. In the first day inhibition was around 96.54% of bacteria. On the 4th day and 7th day inhibition rate is

96.03% and 89.1% respectively. Chitosan thin film inhibited bacteria from surfaces and environment. It demonstrates that the chitosan-coated surface has an inhibitory effect on E. coli bacteria.

Figure 4.25 shows the concentration of adsorbed *S. aureus* on the surface of the chitosan coated sample, and the reference uncoated samples for three different days.

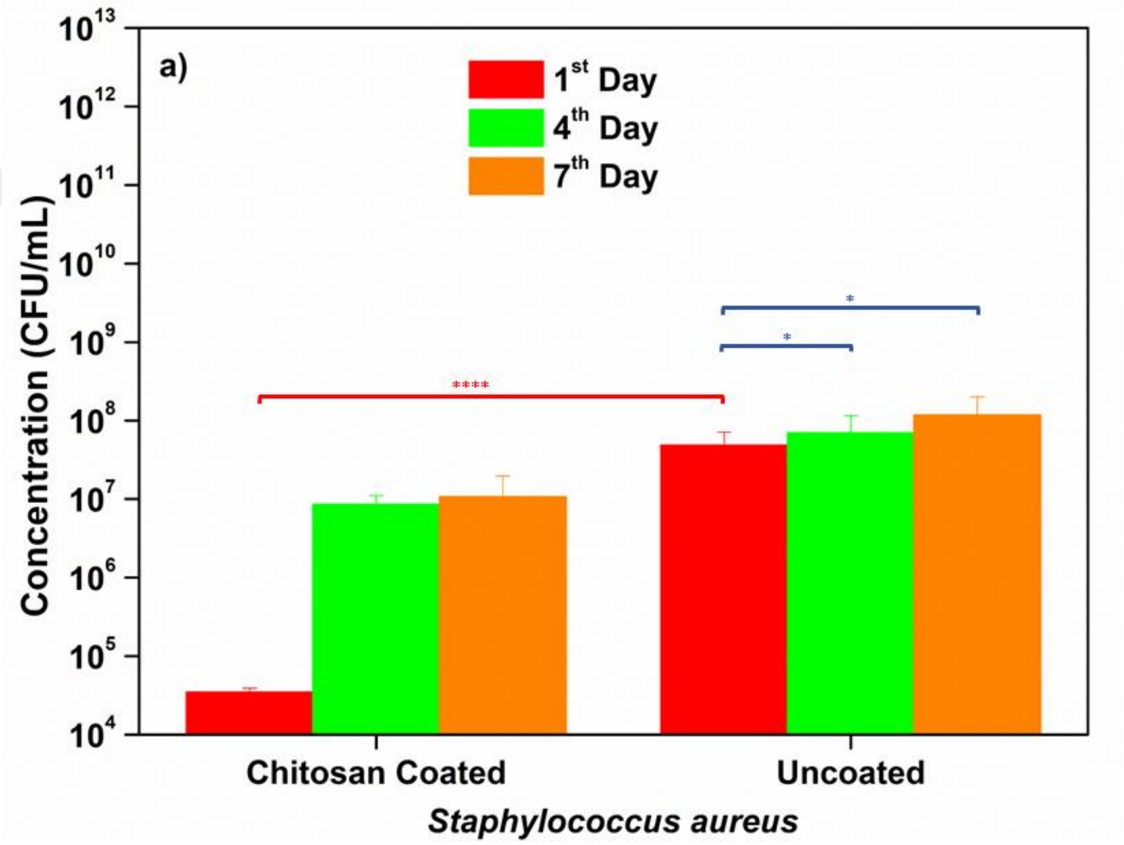

Figure 4.25 Column chart of the comparison of *S. aureus* bacteria's tendency to adhere to chitosan-coated and uncoated surfaces at different time periods (Red: 1st day, Green: 4th day, Orange: 7th day). $p<0,05$: *, $p<0,001$: ****

As shown in figure 4.25, Chitosan coated samples prevented *S. aureus* bacteria from adhering to the surface for 1st day, 4th day and 7th day compared to control, uncoated surfaces. Statistical analysis shown that there was a significant inhibition of bacterial colony, this conclusion was supported with the inhibition percentages such as for the first day comparisons the composite activity of chitosan was able to eradicate 99.81% of bacteria. On the 4th day inhibition rate scales up to 82.34%, at the end of the first week the 7th day incubation total inhibition rate was 90.98%. This result reveals that the chitosan coated surface produced by PECVD technique has an antibacterial action on *E. coli* bacteria. Figure 4.26 shows the concentration of non-adsorbed *S. aureus* on the chitosan coated samples and the reference uncoated samples for three different days.

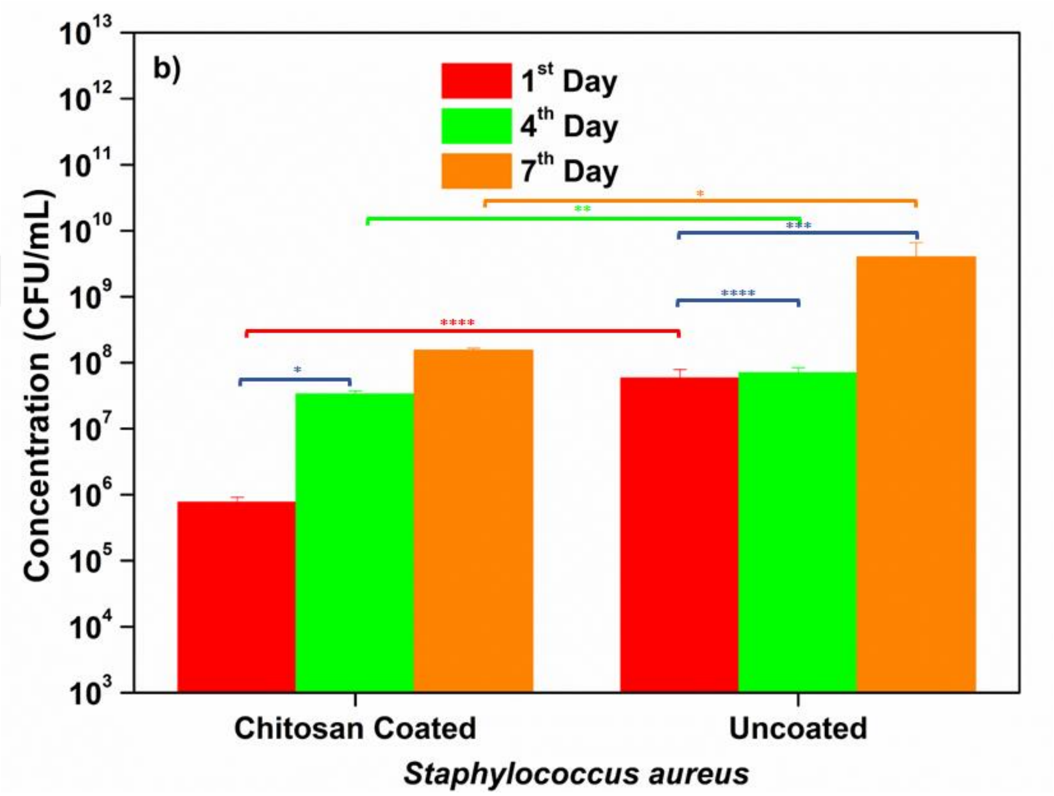

Figure 4.26 Column chart of the comparison of *S. aureus* bacteria's tendency to non-adhere to chitosan-coated and uncoated surfaces at different time periods (Red: 1st day, Green: 4th day, Orange: 7th day). p<0,05: *, p<0,01: ** p<0,005: ***, p<0,001: ****

As shown in figure 4.26, Chitosan coated samples prevent *S. aureus* bacteria adherence to the surface for 1st day, 4st day and 7th day. It is seen that *S. aureus* bacteria cannot adhere to the surface of the chitosan coated sample. There is an inhibition of bacterial colony when the bacteria were not able to adhere on surfaces, this conclusion was supported with the bacterial growth rates. The activity of chitosan was able to eradicate 98.60%, 98.57%, 96.16% for the 1st, 4th and 7th days. These proved that chitosan thin film was not only inhibiting bacteria from surfaces but also from the environment. This reveals that the chitosan coated surface has antibacterial/antimicrobial effect on *S. aureus* bacteria.

5. CONCLUSION

The central objective of this thesis was to produce chitosan thin films by PECVD method and to inhibit related bacteria by using these thin films in the biomedical field. All studies carried out in this direction are explained in the relevant sections of the thesis and shown with relevant graphics.

The purpose of using the PECVD device was to evaporate chitosan at low temperature and the purpose was achieved. With the plasma polymerization method, chitosan was evaporated at the desired temperature and the coating was obtained.

Analysis methods such as FTIR, XPS, SEM were used to analyse the results. In addition, the degree to which chitosan would provide the most appropriate optimization was analysed by TGA. Later, microbiology studies were carried out on the thin films produced and it was shown how effective they were on the two bacteria mentioned.

The characterization of synthesized thin film revealed that:

-The FTIR results of chitosan monomer and the FTIR results of the produced thin film were consistent.

- SEM images show a homogeneous coating on the substrates.

- The XPS data indicated the existence of C, H, and N atoms, along with their associated bonding arrangements and weights, closely resembling the molecular structure of chitosan.

- In microbiology studies, firstly, the results were better than expected. It was known as a result of the research that gram-positive bacterium were more resistant than gram-negative ones. The two bacteria were photographed on the 1st day, 3rd day, 4th day and 7th day and inhibition were observed around the chitosan-coated gutta percha in both. And according to the obtained diffusion tests, *S. aureus* bacterium showed better results than *E. coli*. While there was inhibition in a small area in *E. coli*, there was a considerable amount of inhibition in *S. aureus* bacteria. The results were much more efficient than expected. And the results were obtained in accordance with the research.

Secondly, when we look at the bacterial colony count data, chitosan coated surfaces inhibited significantly more bacteria than uncoated surfaces. Bacteria were counted on the 1st day, 4th day and 7th day. The inhibition rate was obtained by taking the average of these data and the proportion between them. The inhibition rate of coated surfaces is over 90% compared to uncoated ones.

Considering all the analysis and data, the expected results have been obtained. Considering the many properties of chitosan, the variety of its properties according to its molecular weight and the large number of areas of use, and the proof of its antibacterial effect in this thesis, chitosan may become a frequently used material in the future and is a material suitable for use in different biomedical applications.

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