

# Production of Dental Filling Composite Material with Improved Mechanical and Antibacterial Properties by ZnO/Chitosan Doped Silica/PMMA Core/Shell Nano Hybrid Structured Inorganic Matrix

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by

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This is to certify that we have read the thesis **Production of Dental Filling Composite Material with Improved Mechanical and Antibacterial Properties by ZnO/Chitosan Doped Silica/PMMA Core/Shell Nano Hybrid Structured Inorganic Matrix** submitted by **Izel OK**, and it has been judged to be successful, in scope and in quality, at the defense exam and accepted by our jury as a MASTER'S THESIS.

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Date: 16.08.2022

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# Production of Dental Filling Composite Material with Improved Mechanical and Antibacterial Properties by ZnO/Chitosan Doped Silica/PMMA Core/Shell Nano Hybrid Structured Inorganic Matrix

## Abstract

Organic-inorganic (hybrid) dental filling composite resins are becoming increasingly popular as a result of their aesthetic quality, good mechanical properties, optimum wear resistance, and ease of use. However, dental composites have relatively high brittleness, low strength, and durability, which limits applications. Dental filling composites containing nanoparticles have been developed in recent years to address the drawbacks of dental composites. These composites have high filling percentages and provide high strength and durability to the resin. However, the characteristics of the dental filling composites are greatly influenced by the distribution of nano-sized filler particles in the polymeric matrix. Coating the nanoparticles with polymer is one of the studies being conducted to make the dispersion homogeneous and stable. One of the most common among these studies is the Si/PMMA core/shell structure. In this structure, silica is preferred due to its physical and chemical properties such as good mechanical strength and non-toxicity, while PMMA is preferred due to its biocompatibility with the oral environment, aesthetics, and low cost. In addition to the studies on improving the mechanical properties of dental composites, ZnO/CS core/shell composites are preferred to improve both the biocompatibility and antibacterial properties of dental composites. In the thesis study, Si-NPs, ZnO NPs and their Si/PMMA, and ZnO/CS core-shell nanostructures were successfully synthesized,

and characterized. The synthesized Si/PMMA and ZnO/CS core/shell nanostructures were added to the commercial Bis-GMA/TEGDMA (50%/50%) dental filling composite as an inorganic filling material at a rate of 0.1 wt% to 1.0 wt% and a novel hybrid dental filling composite was produced. The mechanical and antibacterial properties of the novel dental filling composite were investigated. According to the three-point bending mechanical test of the Bis-GMA/TEGDMA dental composite reinforced with synthesized Si/PMMA core/shell structures at ratios of 0.1%, 0.25, and 0.5% by weight, 0.1wt% doped Bis-GMA/TEGDMA-Si/PMMA dental composite structure had the highest values in terms of flexural strength (66.73 MPa) and flexural modulus (1091.82 MPa). Si/PMMA additive ratio was accepted as 0.1% in all dental composite studies carried out after that. According to the three-point bending mechanical test of the Bis-GMA/TEGDMA-Si/PMMA dental composite reinforced with synthesized ZnO/CS core/shell structures at 0.1%, 0.25%, 0.5%, and 1.0% by weight, 0.5wt% ZnO/CS doped Bis-GMA/TEGDMA-Si/PMMA/ZnO/CS dental composite had the highest flexural strength (58.05 MPa) and flexural modulus (1077.75 MPa). In addition to the mechanical evaluation, Bis-GMA/TEGDMA-Si/PMMA/ZnO/CS dental composite that contains 0.1wt% Si/PMMA and 0.5wt% ZnO/CS, showed the highest antibacterial effect with an effect of 94.83% in antibacterial tests performed against *S. aureus* bacteria strain. In conclusion, Bis-GMA/TEGDMA-Si/PMMA/ZnO/CS composite that contains 0.1wt% Si/PMMA and 0.5wt% ZnO/CS presented the best results for both improved mechanical properties and antibacterial effect. It is believed that with the introduction of such new generation dental filling composites in clinical applications, they may reduce chewing-induced fractures and cracks, and prevent decay due to their strong antibacterial properties, and thus, dental filling composites may become more user-friendly.

**Keywords:** Dental Composite Resin, Hybrid Composite, Polymethylmethacrylate (PMMA), Silica Nanoparticle, Si/PMMA Core/Shell, Zinc Oxide, Chitosan, ZnO/CS Core/Shell, Mechanical Properties, Antibacterial Properties

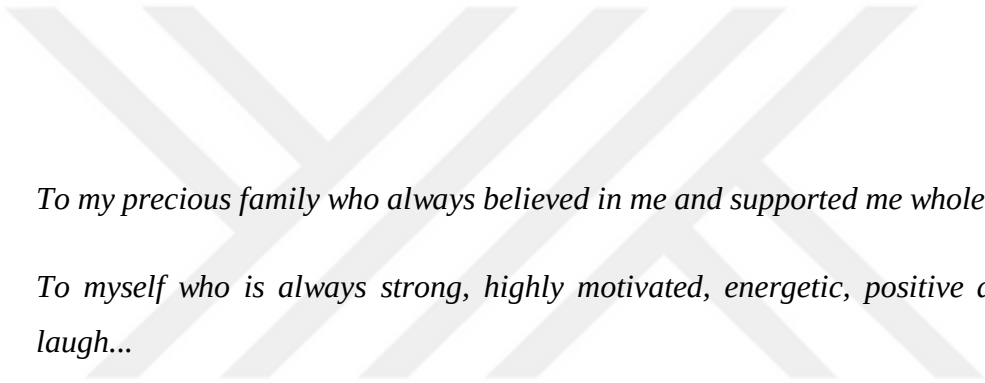
# ZnO/Kitosan Katkılı Silika/PMMA Çekirdek/Kabuk Nano Hibrit Yapılı İnorganik Matris ile Mekanik ve Antibakteriyel Özellikleri Geliştirilmiş Dental Dolgu Kompozit Malzemesi Üretilmesi

## ÖZ

Organik-inorganik (hibrit) dental dolgu kompozitleri, sahip oldukları estetik kalite, iyi mekanik özellikler, optimum aşınma direnci ve kolay kullanım gibi avantajları nedeniyle her geçen gün popülerlik kazanmaktadır. Bununla birlikte, dental dolgu kompozitleri uygulamaları sınırlandıran, nispeten yüksek kırılma kırılma, düşük mukavemete ve dayanıklılığa sahiptir. Dental kompozitlerin sahip oldukları dezavantajlara çözüm geliştirebilmek amacıyla son yıllarda nanoparçacıklar içeren dental restoratif kompozitlerin geliştirilmektedir. Bu kompozitler, yüksek doldurma yüzdelere sahip olup aynı zamanda reçineye yüksek mukavemet ve dayanıklılık gibi özellikler kazandırmaktadır. Bununla birlikte, yukarıda bahsedilen karakteristik özellikler polimerik matristeki nano boyutlu dolgu parçacıklarının dağılımından büyük ölçüde etkilenmektedir. Dağılımı homojen ve kararlı bir hale getirmek için yapılan çalışmalardan biri de nanoparçacıkların etrafının polimerle kaplanmasıdır. Bu çalışmalar arasında en yaygın olanlarından biri de Si/PMMA çekirdek/kabuk yapısıdır. Bu yapıda silika, iyi mekanik mukavemet ve toksik olmama gibi fiziksel ve kimyasal özelliklerinden dolayı, PMMA ise oral ortamla biyouyumluluğu, estetik ve düşük maliyetli olması açısından tercih edilmektedir. Dental kompozitlerin mekanik özelliklerinin geliştirilmesi çalışmalarına ek olarak dental kompozitlerin hem biyouyumluluğunun hem de antibakteriyel özelliklerinin iyileştirilmesi amacıyla

ZnO/CS çekirdek/kabuk yapıları tercih edilmektedir. Tez çalışmasında Si-NP'ler, ZnO NP'ler ve bunların Si/PMMA ve ZnO/CS çekirdek-kabuk nanoyapıları başarıyla sentezlenmiş ve karakterize edilmiştir. Sentezlenen Si/PMMA ve ZnO/CS çekirdek/kabuk nanoyapıları ticari Bis-GMA/TEGDMA (%50/%50) dental dolgu kompozitine inorganik dolgu malzemesi olarak ağırlıkça %0,1-%1,0 oranında eklenmiş ve yeni bir hibrit diş dolgu kompoziti elde edilmiştir. Yeni diş dolgu kompozitinin mekanik ve antibakteriyel özellikleri araştırılmıştır. Sentezlenmiş Si/PMMA çekirdek/kabuk yapılarının, ağırlıkça %0,1, %0,25 ve %0,5 oranlarında katkılanmasıyla güçlendirilmiş Bis-GMA/TEGDMA diş kompozitinin üç nokta eğme mekanik testine göre, %0,1 Si/PMMA katkılı Bis-GMA/TEGDMA-Si/PMMA dental kompozit yapısı eğme mukavemeti (66.73 MPa) ve eğme modülü (1091.82 MPa) açısından en yüksek değerlere sahip olmuştur. Sonrasında gerçekleştirilen tüm dental kompozit çalışmalarında Si/PMMA katkı oranı %0,1 olarak kabul edilmiştir. Sentezlenmiş ZnO/CS çekirdek/kabuk yapılarının, ağırlıkça %0,1, %0,25, %0,5 ve %1,0 oranlarında katkılanmasıyla güçlendirilmiş Bis-GMA/TEGDMA-Si/PMMA diş kompozitinin üç nokta eğme mekanik testine göre %0,5 ZnO/CS katkılı Bis-GMA/TEGDMA-Si/PMMA/ZnO/CS dental kompozit en yüksek eğme mukavemetine (58.05 MPa) ve eğme modülüne (1077.75 MPa) sahip olmuştur. Mekanik testlere ek olarak *S. aureus* bakterisine karşı gerçekleştirilen antibakteriyel testlerde, ağırlıkça %0,1 Si/PMMA ve %0,5 ZnO/CS içeren Bis-GMA/TEGDMA-Si/PMMA/ZnO/CS dental kompozit, *S. aureus*'a karşı %94,83 lik etki ile en yüksek antibakteriyel etkiyi göstermiştir. Sonuç olarak, ağırlıkça %0,1 Si/PMMA ve ağırlıkça %0,5 ZnO/CS içeren Bis-GMA/TEGDMA-Si/PMMA/ZnO/CS kompoziti hem geliştirilmiş mekanik özellikler hem de gelişmiş antibakteriyel etki açısından en iyi sonuçları vermiştir. Bu tür yeni nesil diş dolgu kompozitlerinin klinik uygulamalara girmesiyle çiğneme kaynaklı kırık ve çatlakları azaltabileceği, güçlü antibakteriyel özelliklerinden dolayı çürümeyi önleyebileceği ve böylece diş dolgu kompozitlerinin daha kullanıcı dostu hale gelebileceği düşünülmektedir.

**Anahtar Kelimeler:** Dental Kompozit Resin, Hibrit Kompozit, Polimetilmetakrilat (PMMA), Silika Nanoparçacık, Si/PMMA Çekirdek/Kabuk Çinko Oksit, Kitosan, ZnO/CS Çekirdek/Kabuk Mekanik Özellikler, Antibakteriyel Özellikler



*To my precious family who always believed in me and supported me wholeheartedly...*

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# List of Abbreviations

|                                  |                                        |
|----------------------------------|----------------------------------------|
| AgNPs                            | Silver NP                              |
| Al <sub>2</sub> O <sub>3</sub>   | Alumina                                |
| APTES                            | 3-aminopropyl)triethoxysilane          |
| Bis-EMA                          | Ethoxylated bisphenol-A-dimethacrylate |
| Bis-GMA                          | Bisphenol A glycerolate dimethacrylate |
| Ca                               | Calcium                                |
| CaCl <sub>2</sub>                | Calcium chloride                       |
| CBZ                              | Carbamazepine                          |
| CS                               | Chitosan                               |
| CTAB                             | Hexadecyltrimethylammonium bromide     |
| CQ                               | Camphorquinone                         |
| C <sub>2</sub> H <sub>5</sub> OH | Ethanol                                |
| DA                               | Deacetylation                          |
| DC                               | Degree of conversion                   |
| DD                               | Degree of deacetylation                |
| dECM                             | Decellularized extracellular matrix    |
| DLS                              | Dynamic light scattering               |
| DNA                              | Deoxyribonucleic acid                  |
| D3MA                             | Decandiol dimethacrylate               |
| EHCs                             | Ethylamine hydroxyethyl                |
| E.coli                           | Escherichia coli                       |
| FA                               | Folic acid                             |

|                                              |                                                           |
|----------------------------------------------|-----------------------------------------------------------|
| FBI-OFET                                     | Functional bio-interlayer organic field-effect transistor |
| FT-IR                                        | Fourier transform infrared spectrometer                   |
| GO                                           | Graphene oxide                                            |
| GT                                           | Green tea                                                 |
| HA                                           | Hydroxyapatite                                            |
| HBP                                          | Hyperbranched polymer                                     |
| HO•                                          | Hydroxyl radical                                          |
| H <sub>2</sub> O <sub>2</sub>                | Hydrogen peroxide                                         |
| KOH                                          | Potassium hydroxide                                       |
| K <sub>2</sub> S <sub>2</sub> O <sub>8</sub> | Potassium persulfate                                      |
| LA                                           | Laser ablation technique                                  |
| LDPE                                         | Low density polyethylene matrix                           |
| LOD                                          | Limit of detection                                        |
| LPS                                          | Lipopolysaccharides                                       |
| MC                                           | Mineralized collagen                                      |
| MgO                                          | Magnesia                                                  |
| M/M                                          | Monomer/monomer                                           |
| MMA                                          | Methyl methacrylate                                       |
| MPTS                                         | 3-(trimethoxysilyl)propyl methacrylate                    |
| MSN                                          | Mesoporous silica NP                                      |
| MW                                           | Molecular Weight                                          |
| Na                                           | Sodium                                                    |
| NaOH                                         | Sodium hydroxide                                          |
| NH <sub>3</sub>                              | Ammonia                                                   |
| NH <sub>4</sub> OH                           | Ammonia solution                                          |
| NP                                           | Nanoparticles                                             |
| NR                                           | Natural rubber                                            |

|                  |                                   |
|------------------|-----------------------------------|
| O <sub>2</sub>   | Oxygen                            |
| O <sup>-2</sup>  | Superoxide anion                  |
| P                | Phosphorus                        |
| PE               | Polyethylene                      |
| PCL              | Polycaprolactone                  |
| PGA              | Poly(glycolic acid)               |
| PLA              | Poly(lactic acid)                 |
| PLGA             | Poly(lactic-co-glycolic acid)     |
| PMMA             | Polymethyl methacrylate           |
| PP               | Polypropylene                     |
| PVA              | Polyvinyl alcohol                 |
| PVD              | Physical vapor deposition         |
| P/M              | Polymer/monomer                   |
| P/P              | Polymer/polymer                   |
| RNA              | Ribonucleic acid                  |
| ROS              | Reactive oxygen species           |
| SA               | Streptavidin                      |
| SEM              | Scanning electron microscope      |
| Si-NP            | Silica Nanoparticles              |
| S. aureus        | Staphylococcus aureus             |
| TCP              | Tricalcium phosphate              |
| TEGDMA           | Triethylene glycol dimethacrylate |
| TEOS             | Tetraethylorthosilicate           |
| Tet              | Tetradrine                        |
| TGA              | Thermogravimetric analysis        |
| TiO <sub>2</sub> | Titanium dioxide                  |
| UTMA             | Urethane tetramethacrylate        |

|                                                                |                                  |
|----------------------------------------------------------------|----------------------------------|
| UV-vis                                                         | UV-visible spectrophotometer     |
| ZnO                                                            | Zinc oxide                       |
| $\text{Zn}(\text{CH}_3\text{COO})_2 \cdot 2\text{H}_2\text{O}$ | Zinc acetate dehydrate           |
| $\text{ZrO}_2$                                                 | Zirconia                         |
| XPS                                                            | X-ray photoelectron spectroscopy |
| XRD                                                            | X-ray diffraction                |
| 1-D                                                            | One-dimensional                  |
| 2-D                                                            | Two-dimensional                  |
| 3-D                                                            | Three-dimensional                |
| 4EDMAB                                                         | Ethyl 4-dimethylaminobenzoate    |
| $\zeta$ -potential                                             | Zeta potential                   |

# List of Symbols

atm                      Atmospheric pressure

cm<sup>3</sup>                      Cubic centimetre

kDa                      kiloDalton

nm                      Nanometer

%                      Percent

°C                      Centigrade degrees

# Chapter 1

## 1 Introduction

The applications of resin composite materials in medical fields have increased due to their aesthetic appearance as well as their good physical and mechanical properties. Organic-inorganic (hybrid) dental restorative composite resins, which are increasingly popular in dental applications, particularly as restorative and filling materials, are gaining traction in comparison to metallic dental amalgams due to their aesthetic quality, preferred mechanical properties, and optimum wear resistance [1]. The mechanical properties of dental resin composites have been reported in studies to be significantly affected by the particle morphology (shape), size range, and volume content of the filler in their structure [1,2]. Composites used in the dental restoration are structurally composed of three basic components. These; i) inorganic filler material of various types, shapes, and sizes, ii) an organic matrix supporting the inorganic filler material, iii) a silane coupling agent that binds organic and inorganic components together. Studies have shown that the mechanical properties and aesthetic appearance of resin composites are affected by the components and microstructures they contain [1,2]. The interphase, which is composed of silane-derived coupling agents, is used to strengthen the adhesion between different organic matrix and inorganic filler phases and provides a bond that significantly affects the overall performance of the composite. The interphase also influences the degree of aggregation of the particles used as the filler, as well as the transfer of stress between the stronger filler phase and the weaker matrix phase [3]. In addition to silane coupling agents, various new monomers such as ring-opening monomers, liquid crystalline monomers, branched and dendritic monomers have been developed in recent years for use as an organic matrix [4]. Bisphenol A glycerolate dimethacrylate (Bis-GMA) is the most commonly used base monomer as an organic matrix material. Despite its high reactivity, Bis-GMA is a

highly viscous material due to the presence of hydroxyl (-OH) groups in its structure and the  $\pi$ - $\pi$  interactions imparted by aromatic rings. Therefore, it is combined with low viscosity monomers such as triethylene glycol dimethacrylate (TEGDMA) [5]. However, due to reasons such as complex synthesis processes due to monomer systems in organic matrix studies, and difficulties in photosensitivity and storage stages, studies in the field of dental composites have mostly focused on inorganic filling materials [1,4]. With the studies on filling materials, different types of composites have started to be developed, including macro-dimensional filled composites (traditionally used), micro-dimensional filled composites, hybrid, micro-hybrid, and nano-sized filled composites [6]. Along with nanotechnological studies, the development of dental restorative composites containing nanoparticles (NPs) has gained prominence. These composites are distinguished from micro-sized filled composites with their filling percentages and their unique properties. In addition, the incorporation of NPs in dental composites improves resin properties such as abrasion resistance, high transparency, and strength [2,7]. However, the distribution of nano-sized filler particles in the polymeric matrix has a significant impact on the above-mentioned properties. Clustered structures are formed due to the very high surface area of the NPs and the surface charges of the inorganic NPs, so the control of their distribution in the polymeric matrix is difficult at high filler amounts. These clustered structures can serve as stress concentration points and causes material degradation. In order to regulate the distribution of inorganic NPs in the polymeric matrix and to increase the interfacial adhesion between the particle and the polymer, synthesis routes have been developed in which the inorganic particles are surrounded by the polymer (hybrid structures-shell/core structure).

Silicon dioxide ( $\text{SiO}_2$ ), also known as silica (Si), is one of the most widely used inorganic fillers in the preparation of dental composites, as well as in the polymer and rubber industry, due to its physical and chemical properties such as good mechanical strength and non-toxicity [2,8]. Besides silica NPs (Si-NPs), polymethyl methacrylate (PMMA) is one of the most used materials in dental applications due to its biocompatibility, low-cost, being aesthetics, resistant to exposure to direct sunlight and has characteristic properties that do not change much under the influence of UV radiation. However, as a disadvantage, PMMA lacks mechanical strength and may break in situations such as irregular chewing movements [9,10]. Si/PMMA hybrid

nanocomposite structures, which are being developed in order to find solutions to possible disadvantages, have recently attracted great attention due to their ability to protect against corrosion in an efficient and environmentally compatible manner. This corrosion protection created by Si/PMMA hybrid nanocomposites is achieved by supporting the covalent bond between PMMA and SiO<sub>2</sub> nodes with silane binding agents, and the tightly packed nanostructure acting as an effective corrosion barrier against aggressive agents [11]. In addition to research into improving the mechanical properties of dental composites, chitosan (CS), zinc oxide nanoparticles (ZnO NPs), or ZnO/CS nanocomposites have been developed to improve both the biocompatibility and antibacterial properties of PMMA structured dental composite resins [10]. CS, a naturally occurring and linear cationic polysaccharide, is obtained by deacetylation of chitin. Due to its high biocompatibility, easy biodegradability, low immunogenicity, antimicrobial activities, non-toxicity, susceptibility to chemical modifications and relatively low cost, it has widespread applications in drug delivery systems, tissue engineering, cosmetics industry and food industry [12–14]. However, organic antimicrobial agents like CS are generally not very stable, and therefore, the production of composites of CS with inorganic metal oxides have taken attention as they increase the stability and antibacterial activity of the composites. Among the metal oxides, ZnO has attracted great interest in many fields, especially in cosmetics and medical fields, due to its good photocatalytic activity, high stability, non-toxicity and antibacterial properties [12,15]. ZnO NPs have also been found to have excellent pharmaceutical potential against pathogenic bacteria as well as deadly diseases such as cancer and diabetes [16]. ZnO particle size and concentration have a strong effect on the amount of Gram-positive and Gram-negative bacteria. Generally smaller particles have a better antibacterial activity. On the other hand, due to their small size, ZnO NPs often show aggregation property. Thus, surfactants and polymers are frequently used as stabilizers in aqueous media to prevent particle aggregation [17]. Another method for preventing aggregation is to combine ZnO NPs with different materials to form composite structures. Recent studies on ZnO/CS composites have shown that the antibacterial and antibiofilm properties of ZnO NPs are significantly improved as a result of their synergistic reactions with CS [12,15,18].

Therefore, in the thesis study, we addressed to improve the mechanical and antibacterial properties of Bis-GMA/TEGDMA dental composite by adding the

Si/PMMA core/shell and ZnO/CS core/shell structures. The addition of Si/PMMA core/shell hybrid nanostructure and ZnO/CS nanostructure to Bis-GMA/TEGDMA dental composite improved not only the mechanical properties but also the antibacterial properties of the dental filling composite. It has been thought that the final composite structure obtained will reduce the dental applications that need to be renewed continuously and contribute to the use of longer-lasting dental fillings. It has been believed that the thesis will contribute to the studies on dental filling materials. The production stages of the dental filling composite material produced in the thesis study and the representation of the final material are given in Figure 1.1.

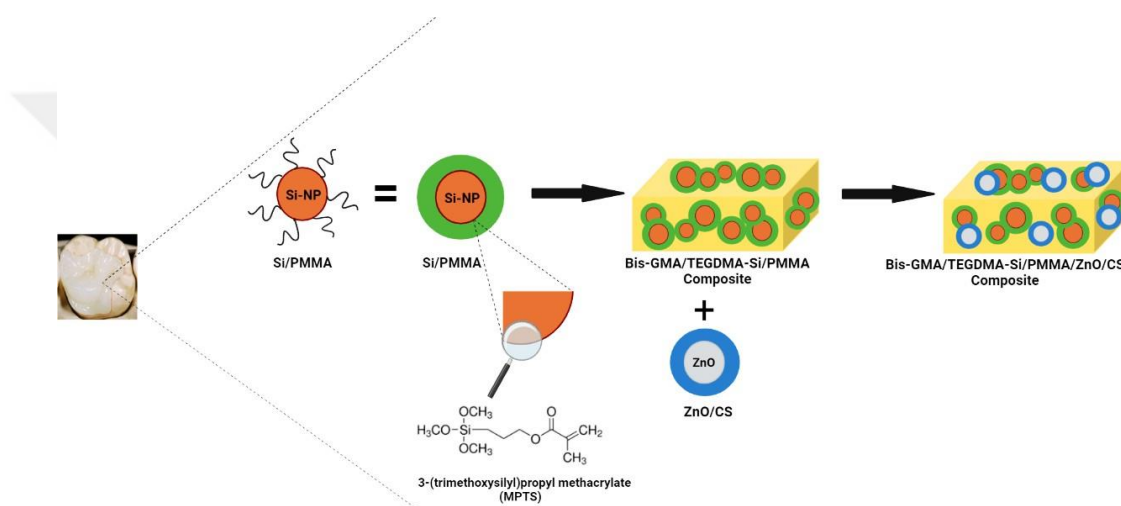


Figure 1.1: Representative illustration of the obtained Bis-GMA/TEGDMA-Si/PMMA/ZnO/CS dental filling composite

# Chapter 2

## 2 Literature Research

### 2.1 Biomaterials

Multicellular organisms are complex structures composed of billions of cells that form organs and tissues. Although these organs and tissues function regularly in a healthy organism, when they are damaged due to various reasons such as disease, genetic or environmental factors, the working order of the organs is disrupted and they are unable to perform their functions properly. In these cases, biomaterials have been started to be produced as an alternative solution to guide, support, replace or restore living tissues that have lost their function as illustrated in Figure 2.1. The most striking features of these materials are that they can be used in a biological environment without harming the organs or tissues with which they interact [19–21].

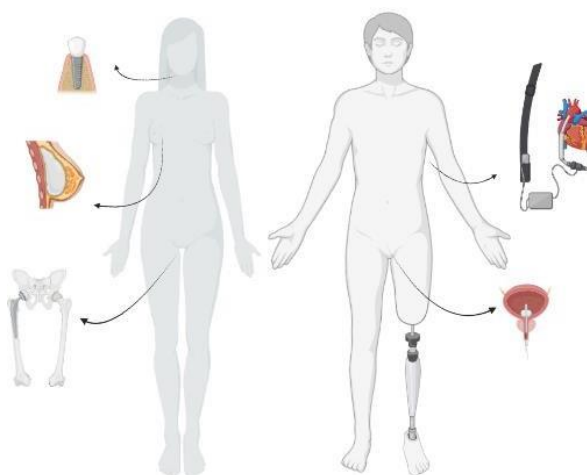


Figure 2.1: Various applications of biomaterials

### 2.1.1 History of Biomaterials

Although the term biomaterial has only been started to be used recently, the applications of biomaterials go back to ancient civilizations. According to reports, Egyptian mummies have been found with artificial eyes, ivory teeth, and metal noses, while the Chinese and Indian civilizations used waxes, glues, and tissues to repair missing or defective parts of the body. Copper and bronze were other materials used at that time [19]. Over time, copper was replaced by gold. For example, gold was used in dentistry 2,000 years ago by the Chinese, Aztecs, Romans, and Greeks [22]. Aside from these, the first glass eye was produced in 1832 by the Ludwig Müller-Uri glass blower in Lauscha, Germany [23]. Over the centuries, advances in synthetic materials, surgical techniques, biocompatibility and the development of new sterilization methods have resulted in new biomaterials and application areas. Especially the development of new sterilization methods has accelerated the inventions in this field. Traditionally, surgical and implantation applications were not as successful as desired due to inadequate sterilization conditions. After the importance of sterilization was emphasized by Joseph Lister in the 1870s, the success rate in the application of biomaterials increased [21].

In the early 1900s, new biomaterials that we know and use today were discovered and started to be used in a variety of applications. For example, cobalt-chromium (Co-Cr) alloy, also known as vitallium, was introduced in 1936 due to its superior strength and improved corrosion resistance. Following this, tantalum and titanium gained popularity for surgical applications. Also, it was discovered during World War II that PMMA did not cause adverse chronic reactions in the body and could be used in medical applications [24]. After that, Sir John Charnley designed the first total hip implant with ball-head femoral stem and Teflon cups. In 1958, Furman and Robinson performed the first successful cardiac stimulation. Following these developments, polymeric biomaterials, ceramics, and composites have become increasingly popular [21,24]. As a result, with the development of technology, a wide variety of biomaterials have been produced to be used for different purposes such as bone plates, joint replacements, vascular grafts, heart valves, intraocular lenses, dental implants, biosensors, artificial hearts, blood tubes, etc [19].

### 2.1.2 Properties of Biomaterials

Biomaterials are used in medical applications because they have unique physical, mechanical, chemical, and biological properties. The properties of biomaterials vary depending on their type and application. Biomaterials [21,23,24];

- Should be biocompatible and should not cause any rejection, inflammation or immune response in the host organ or tissue so that there is no undesirable effect between the tissues or organs with which they interact.
- Should not be toxic, carcinogenic or allergic etc.
- Should have appropriate mechanical properties (pressure, tension, shear, impact) to ensure a correct application performance.
- Should have appropriate physical and chemical properties (e.g. density, form, porosity, surface roughness topography, hydrophilic or hydrophobic, with similar functional groups) to maintain the functions of the tissue to which they are replaced or implanted.
- Should not be corrosive to prevent any corrosion in the pH and temperature conditions under which they will be used.
- Should have a modifiable surface to improve tissue adhesion.
- Should either degrade or be stable for life, depending on the intended use.
- Should be bioinert to avoid undesirable chemical reactions with tissue.
- Should be sterilizable, economical, accessible, and easy to produce.

### 2.1.3 Biocompatibility

Biocompatibility is the ability of a material to function without causing any local or systemic adverse reactions with the tissues and organs with which it interacts in the biological environment [25–27]. As shown in Figure 2.2, the concepts of the host, material, and material function are interconnected and play an important role in biocompatibility. Biocompatibility is a dynamic property that is determined not only by the material itself but also by the way the material interacts with its surroundings. Because the body's response may change over time due to various factors such as corrosion, fatigue, disease, and aging, biocompatibility may be affected [25,28].

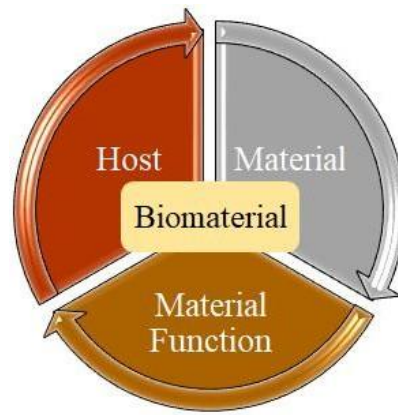


Figure 2.2: The concepts of biocompatibility in terms of host, material, and material function

Biocompatibility is one of the most important properties of a biomaterial since it increases the acceptability of inanimate substances in a living body. On the other hand, it should be noted that biocompatibility depends on the application site. For example, certain materials may be biocompatible in bone replacement, while the same material may not be biocompatible in direct blood contact applications [20].

#### 2.1.4 Classification of Biomaterials

Biomaterials are classified into natural materials, metals, ceramics, polymers, and composites based on their structure and properties as can be seen in Figure 2.3.

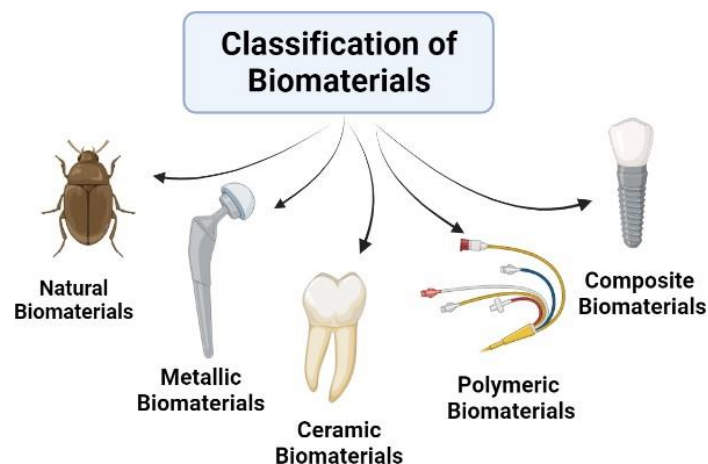


Figure 2.3: Classification of biomaterials

#### 2.1.4.1 Natural Biomaterials

Natural biomaterials are biological polymers (biopolymers) and decellularized tissues that are synthesized from biological systems (microorganisms, animals, and plants) or biological starting materials (e.g. corn, sugar, starch, etc.) [29]. Due to their biocompatibility, natural bioactivity including cell proliferation and adhesion, non-toxicity, chemical and mechanical properties similar to tissues, and low cost, these materials are highly demanded in biological applications, including tissue engineering, wound dressings, and drug delivery [21,30]. Decellularized tissues are similar to the original tissue due to their natural geometry and structure and can be easily integrated into biological systems. Some examples of biopolymers are cellulose, chitin, chitosan (CS), alginate, dextran, collagen, gelatin, fibrinogen, silk, and decellularized extracellular matrix (dECM) [30]. These materials are also biodegradable because they are derived from biological resources. This feature can be evaluated as an advantage or a disadvantage according to the types of biomaterial applications [21]. On the other hand, these materials can be applied not only as a single component, but also in combination with synthetic polymers.

- Chitosan (CS)

Chitin, a linear polysaccharide of  $\beta$ -(1–4)-2-acetamido-2-deoxy-D-glucose, is the second most abundant natural polymer on Earth after cellulose. It is commonly found in fungal and yeast cell walls, and exoskeletons of arthropods. As a structural component, it provides support and protection to organisms. Although chitin is a biocompatible, bioactive, biodegradable polymer with high mechanical strength, its solubility is poor, which makes its use limited. Therefore, CS, the main derivative of chitin, has been remarkably popular compared to chitin. CS is a linear copolymer that is derived from chitin through a deacetylation process to obtain a linear structure of glucosamine and N-acetylglucosamine linked in a  $\beta$ -1,4 manner as seen in Figure 2.4 [31,32].

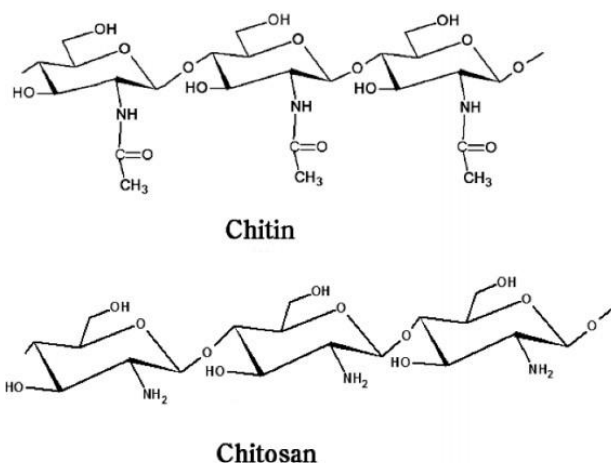


Figure 2.4: Chemical structure of chitin and CS [33]

Chemical deacetylation is a method of obtaining CS, which is soluble in dilute acid in minimum time and does not decompose, by removing the acetyl group in chitin. This process involves treating chitin with concentrated sodium hydroxide (NaOH) or a potassium hydroxide (KOH) solution (40-50%) at elevated temperatures, usually above 80 °C, for 30 minutes or longer to remove some or all of the acetyl groups from the polymer. Typical commercial CS has a degree of deacetylation (DD) of about 85% (85% DD) [34,35]. Temperature and time of deacetylation, alkali concentration, atmosphere (air or nitrogen), chitin/alkaline solution ratio, chitin density and particle size are some of the factors that affect the degree of deacetylation [35]. After deacetylation, CS is composed of acetylated and deacetylated units as shown in Figure 2.5. The ratio between these two units is regarded as the degree of deacetylation. Acetylated units, N-acetyl glucosamine contribute to bond hydrogen bonds and hydrophobic interactions, while the amino group of deacetylated units, glucosamine, plays a role to be ionized, which gives cationic polyelectrolyte properties to CS [36]. When the degree of deacetylation of CS reaches about 50%, it becomes soluble in aqueous acidic medium. When CS dissolves in acidic medium, the amino groups in the chain are protonated and the polymer becomes cationic, allowing it to interact with a variety of molecules [34].

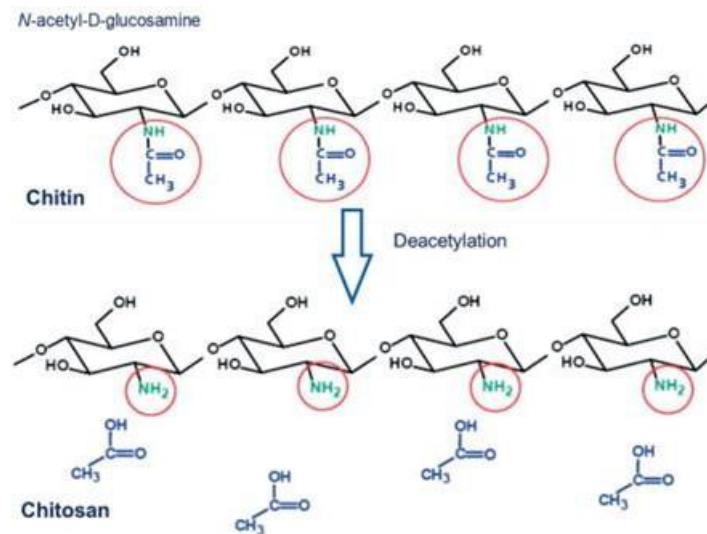


Figure 2.5: The structure of chitin and CS after deacetylation process of chitin [37]

Owing to the cationic groups in its structure, CS has inherent antimicrobial properties against bacteria, yeast, molds, and fungi [38]. In conclusion, biocompatibility, biodegradability, non-toxicity, antibacterial and other properties of CS make it a good candidate for use in various fields [36]. Although the antimicrobial mechanism of CS has not been fully defined, several hypotheses have been proposed as shown in Figure 2.6. These are:

- CS is positively charged due to its cationic amino groups and interacts with the negatively charged bacterial cell wall, damaging its intracellular components and causing the death of the bacteria [39].
- CS can enter the nucleus of the cell and bind with deoxyribonucleic acid (DNA), blocking the transcription of ribonucleic acid (RNA) from DNA and thus inhibiting protein synthesis [38].
- Various metal ions, including  $K^+$ ,  $Na^+$ ,  $Ca^{2+}$ ,  $Fe^{2+}$ ,  $Mg^{2+}$ ,  $Ni^{2+}$ ,  $Co^{2+}$ ,  $Cu^{2+}$  and  $Zn^{2+}$  are vital for cell wall stability as they are associated with the molecules of the bacterial cell wall. CS acts as a chelating agent of such metal ions and inhibits microbial growth [40].

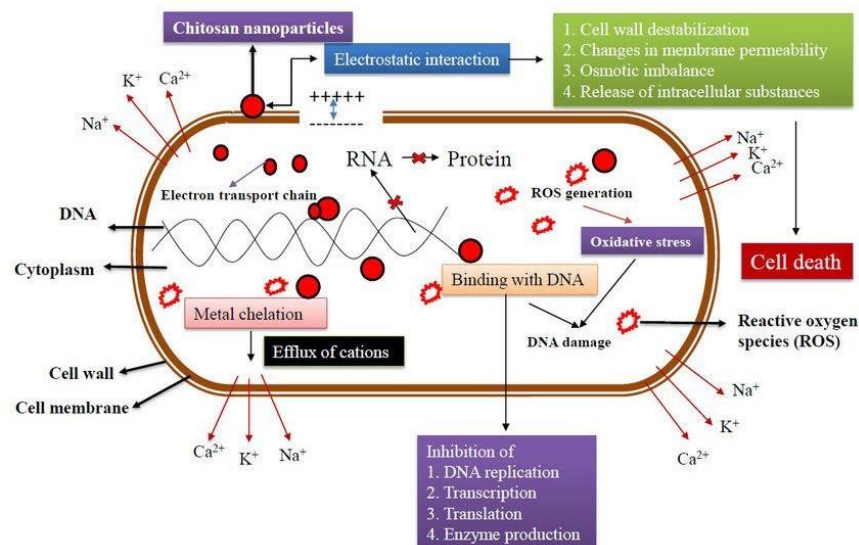


Figure 2.6: Antibacterial mechanisms of CS NPs [40]

There are many studies in the literature that highlight the antimicrobial activity of CS when used alone or in combination with other materials or NPs. For example, in a green synthesis study, CS /polyvinyl alcohol (CS/PVA) was blended with different amount of *Hibiscus Sabdariffa* L. extract to enhance the antimicrobial activity of (CS/PVA). The obtained results indicated that the complex showed great antimicrobial activity [41]. Although the certainty of the antimicrobial activity of CS is known, there are several factors that affect this activity. These are;

*Size and Concentration of CS:* Reducing the size and increasing the surface area increases the antimicrobial activity. Therefore, according to the literature, CS NPs have more antibacterial effect than CS solutions. Because CS NPs have more positively charged amino groups, which, as mentioned above, promote stronger binding to the negatively charged surface of microorganisms [42,43]. On the other hand, with increasing CS concentration, positively charged amino groups can coat the cell surface and prevent leakage of intracellular components [44] For example, Reesha et al. prepared an antimicrobial packaging material by homogeneously embedding 1.0%, 3.0% and 5.0% chitosan (w/w) in a low-density polyethylene matrix (LDPE). The study revealed that 3.0% LDPE/CS films displayed better physical and antimicrobial properties compared to other films used in the study [45].

*Molecular Weight (MW) and Deacetylation (DA) of CS:* Microorganism activity of CS and CS-NPs is also affected by MW and DA. According to the literature, increased CS

MW leads to decreased CS activity. For example, in a study, CS NPs prepared at various MWs were evaluated in terms of size distribution and Z-potential. The results showed that as the MW of the CS decreased, the size of the CS NPs decreased and the zeta potential increased. It was also discovered that smaller CS NPs and higher zeta potential showed higher antibacterial activity against *Streptococcus pneumoniae* [46,47]. On the other hand, CS with higher DA has stronger antibacterial activity due to its higher positive charge. For example, in one study, g-chitosan (81.56%) with a higher DD showed higher antibacterial activity than e-chitosan (62.71%) [48].

*Hydrophilic/Hydrophobic Characteristic of Chitosan:* Due to glucosamine's ionizing ability, CS dissolves easily in acid solutions with pH less than 6.3, such as dilute nitric acid, acetic acid, formic acid, hydrochloric acid, phosphoric acid, carbonic acid, and perchloric acid. On the other hand, it is insoluble in water and slightly soluble in common organic solvents such as dimethylformamide, dimethyl sulfoxide, and dinitrogen tetroxide. Moreover, the solubility of CS depends on the degree of N-acetylation (DA) and Mw. Generally, antimicrobial agents require an aqueous medium for their activity as completely dry samples are unable to easily release the energy required to initiate the interaction, which is stored in chemical bonds. Therefore, hydrophilicity and hydrophobicity are other factors that affect the antibacterial properties of CS. Since CS is insoluble in water, chemical modifications can improve the water solubility of CS and its derivatives and expanding their applications [35,36,39,49,50]. For example, Xie et al. prepared ethylamine hydroxyethyl CS (EHCs) to improve the water solubility of CS. In the study, they discovered that EHCs reduce intermolecular interactions like van der Waals forces and thus increase water solubility. In addition, the derivative inhibited the growth of *E. coli* [51].

*pH:* pH is another factor on the antimicrobial activity of CS. Since CS dissolves in acidic conditions, it shows antibacterial activity, while the antimicrobial activity decreases as the pH value increases [50]. On the other hand, it is incorrect to claim that chitosan and its derivatives completely lose their antimicrobial activities when exposed to neutral conditions [52].

*Microorganism Type:* The antimicrobial properties of CS vary according to the species it affects. For example, the activity differs due to differences in cell membrane structures of Gram-positive and Gram-negative bacteria. As seen in Figure 2.7, the cell

membrane surface of Gram-positive bacteria consists of peptidoglycans, teichoic acid and lipoteichoic acids. However, the cell surface of Gram-negative bacteria has an extra outer membrane containing lipopolysaccharides (LPS), which provides the bacteria with hydrophilic surface properties and acts as a barrier against macromolecules and hydrophobic toxins [53,54].

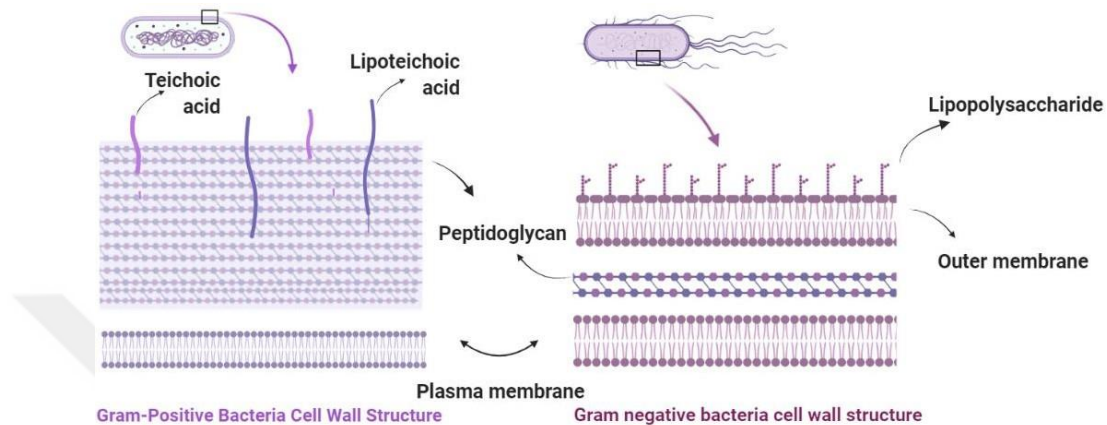


Figure 2.7: Cell wall structures of Gram-positive and Gram-negative bacteria

CS-based nanomaterials are of great interest in a wide range of biomedical fields due to their versatility, biocompatibility, and unique chemical properties such as biodegradability and non-toxicity.

*Wound dressing:* Skin wounds are an important problem that can lead to serious complications. Infections caused by bacterial formation in the wound area are one of the most common problems encountered during the wound healing process. In recent years, dressings with antimicrobial properties have been developed to address this issue and provide an effective wound healing process. CS is widely used as a wound dressing in different structures such as hydrogel, sponge, membrane, film, due to its biodegradable, biocompatible, antimicrobial, and hemostatic properties. CS can be used alone in these antimicrobial dressings, but it can also be combined with other ingredients [54]. For example, Adeli et al. produced PVA/Chitosan/Starch nanofiber mats using the electrospinning method for wound dressing application. Antibacterial activity and cytotoxicity as well as improved mechanical properties make it a notable candidate for dressing application [55].

*Tissue Engineering for Bone Regeneration:* Tissue engineering has been developed as an alternative to traditional treatments such as organ transplantation and artificial

implants. For tissue engineering applications, many different types of biomaterials have been developed, including hydrogels, bioceramics, and composites. CS-based biomaterials are also attracting attention, due to the desirable biological properties of CS [56]. In a recent study, scaffolds for bone regeneration were prepared with poly-epsilon-caprolactone (50 kDa) containing CS and vancomycin. The obtained scaffolds exhibited suitable properties in terms of morphological, vancomycin release and biological properties [57].

*Drug Delivery Carrier:* Due to its biological properties, CS is also employed in drug delivery systems. CS can be used in variety of forms including NPs, microspheres, capsules, tablets, films, depending on the function and applications of the carrier. For example, in a study in which CS has been extensively researched in brain drug delivery, carbamazepine (CBZ) was transported by carboxymethyl chitosan NPs. The main objective was to facilitate the passage of the drug through the blood-brain barrier, thereby increasing the brain drug concentration and treatment efficacy [58].

#### 2.1.4.2 Metallic Biomaterials

Metallic biomaterials, mostly stainless steels, titanium (Ti) and its alloys, and cobalt-based (Co) alloys are valued due to properties such as reduced deformation under load, long-term shape retention, having good mechanical and high tensile strength, having resistance wear, being bioinert, being low cost and presenting easy production features [21,23,59]. Metals can be used as a single component or in alloy forms. The main purpose here is to improve the properties of the metal to make it less brittle, harder, and more resistant to corrosion. In this context, stainless steel, which contains iron (Fe) mixed with chromium (Cr), nickel (Ni), molybdenum (Mo), and carbon (C), is considered one of the best-known metal alloys. Other examples of metals used for medical applications include Fe, Cr, Co and its alloys, Ni, Ti and its alloys, Ta (tantalum), and zinc (Zn) [21].

Metallic biomaterials are generally used for applications such as hip joint replacement, knee joint replacement, dental implants and cardiovascular devices, surgical instruments, etc [19]. Metallic implants have a higher elastic modulus than natural bone. For example, stainless steel and Co-Cr alloys are approximately 10 times stronger than natural bone, while Ti and its alloys are approximately 5 times stronger.

On the other hand, although metallic biomaterials are chosen for hard tissue applications, incompatibilities can arise between the implant and the tissue [24]. Also, corrosion is a common issue for metals and alloys in wet and/or salty environments such as in vivo. Because metal atoms spontaneously react with oxygen, hydrogen protons and ionic salts in these environments over time, metallic implants are exposed to corrosion over time [60].

- ZnO Nanoparticles (ZnO NPs)

ZnO NPs are considered one of the most important metal oxide NPs. These materials have remarkable chemical and physical properties including biocompatibility, biosafety, low toxicity, low-cost synthesis, antimicrobial activity, high electron transfer rates, improved analytical performance, and photocatalytic activity. In addition, ZnO is a semiconductor material with a wide direct band gap (3.37 eV). It has high mechanical and thermal stability and has a high exciton energy (60 meV) at room temperature. Due to these properties, it is widely used in various fields such as electronics, optoelectronics, photocatalysis, biosensors and biomedicine [61–68].

ZnO has three crystal structures, as shown in Figure 2.8;

- hexagonal wurtzite,
- cubic rock salt,
- cubic zinc blende.

Among them, rock salt can be formed at relatively high pressures of about 10GPa. However, cubic substrates can be utilized to synthesize the zinc blende structure. Wurtzite ZnO has a hexagonal structure with lattice parameters  $a = 0.3296$  and  $c = 0.52065$  nm, which is the most common structure as it is most stable at ambient conditions. [69].

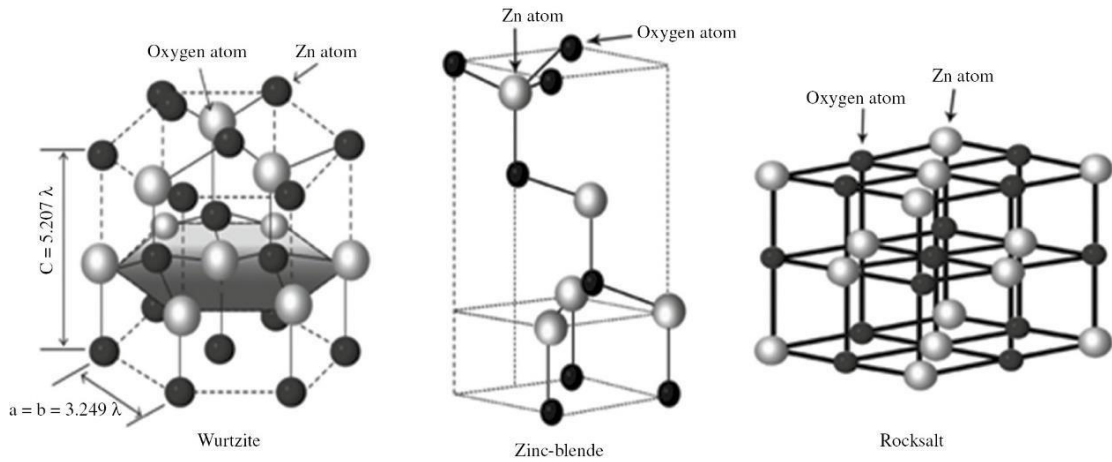


Figure 2.8: Structures of ZnO crystals [70]

ZnO NPs can be classified into various structures depending on their synthesis conditions and application areas, and they have potential value in a wide range of nanotechnology fields. ZnO can be found in one-dimensional (1-D), two-dimensional (2-D), and three-dimensional (3-D) structures. The 1-D structure group includes nanorods, - needles, - wires, - coils, - strips, - pipes, - springs, - rings, - belts, and - combs, while the 2-D structure group consists of nanoplate/nanosheets and nanopellets. Finally, the group of 3-D structures includes flowers, dandelions, snowflakes, etc [71].

Furthermore, due to their unique antibacterial and antifungal properties, nano-sized ZnO structures are used as antimicrobial agents. In addition to these properties, ZnO-NPs also have high catalytic and high photochemical activities. ZnO has high optical absorption in the UVA (315–400 nm) and UVB (280–315 nm) regions, which enables ZnO to be used as a UV protector in cosmetics [72].

- Synthesis of ZnO NPs

Various ZnO nanostructures have been produced using a variety of methods classified as physical, chemical, and biological (green) synthesis techniques.

#### a) Physical Methods

Physical methods include high energy ball milling, arc plasma, thermal evaporation, physical vapor deposition, ultrasonic irradiation, and laser ablation. In most

physical/mechanical processes, the production rates of ZnO NPs are very high, making them ideal for performing industrial processes [73].

*High energy ball milling:* This method uses hard balls in a high energy shaker mill to produce fine metal NPs. In this technique, powdered material placed inside a ball mill is subjected to high energy collision from balls, producing large quantities of material [74] .

*Laser ablation technique (LA):* In this technique, a laser beam is used to remove metallic ions from a solid or liquid surface. While the advantages of this method make it a sustainable, easy-to-use, and efficient laboratory method, an important problem with LA is the control of NP size and size distribution [75,76].

*Arc plasma:* It is the conversion of bulk materials into nanomaterials by condensation and evaporation.

*Thermal evaporation technique:* In this technique, atoms, clusters of atoms or molecules are removed as a stream of steam from a metal crucible by heating the crucible or filament or by passing a current through it [77].

*Physical vapor deposition (PVD):* In this method, a vapor stream is created and then condensed into a thin film on a substrate in a vacuum chamber [77].

## b) Chemical Methods

Chemical synthesis methods include sol-gel, hydrothermal and solvothermal, co-precipitation, chemical vapor deposition and microemulsion processes.

*Sol-gel techniques:* Sol-gel is a technique used in materials chemistry to produce small NPs and it is mostly used for the production of metal oxide NPs. In this technique, initially monomers or starting materials (for example chlorides or metal alkoxides) are dispersed in a liquid, which is a colloidal solution, forming the sol. This sol is then deposited by spraying, spinning, gelling, precipitation or coating. Finally, the NPs in the sol are polymerized and the remaining organic and inorganic components are heat treated to form amorphous or crystalline coatings, yielding the final product as seen in Figure 2.9 [78].

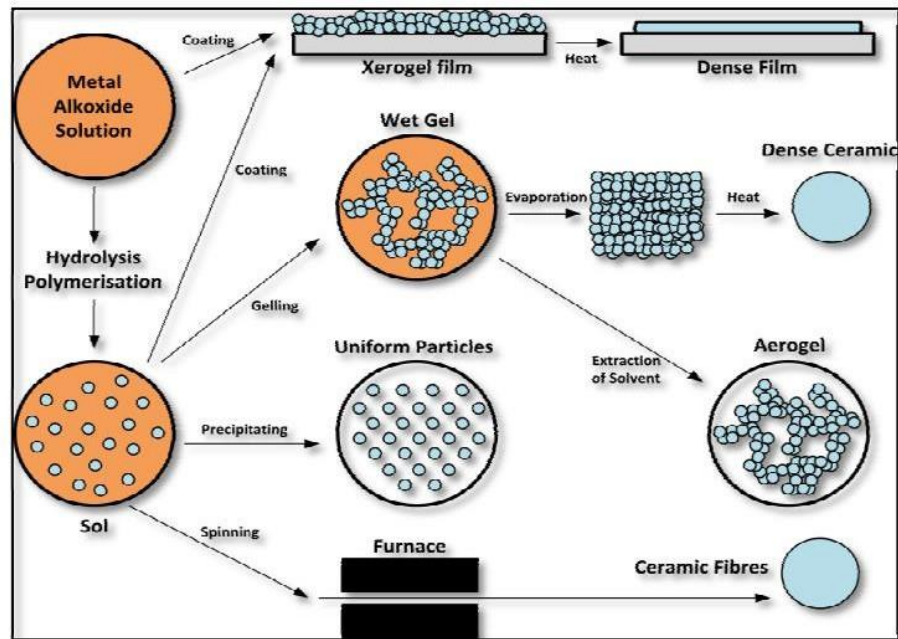


Figure 2.9: Steps of sol-gel method [78]

Sol-gel processes have many advantages, such as control over precursor solutions, susceptibility to modifications that allow coupling or encapsulation with various functional groups, adaptive microstructure, and simplicity. However, some produced samples also have some disadvantages such as high permeability, low abrasion resistance, poor bonding [79,80]. Hasnidawani et al synthesized ZnO NPs in range of 81-84 nm through sol gel method using zinc acetate dehydrate ( $\text{Zn}(\text{CH}_3\text{COO})_2 \cdot 2\text{H}_2\text{O}$ ) as a precursor and ethanol ( $\text{CH}_2\text{COOH}$ ) was used as solvent, NaOH and distilled water were used as medium [81].

*Hydrothermal and Solvothermal Techniques:* Hydrothermal processes are methods carried out in a closed reaction vessel called autoclaves under high pressure (1-10,000 atm) and high temperature (100-1000°C). In this method, insoluble or slightly soluble substances at room temperature are dissolved under high pressure and temperature [82]. The type of method varies with the solvent used. For example, if water is used as a solvent, the process is called hydrothermal technique, if organic solvents are used, the process is called solvothermal technique [83]. Hydrothermal techniques, which have advantages such as reaction performance and low energy consumption in liquid media, provide the obtaining of nanomaterials with crystallinity, crystalline phase, morphology, and size control [83]. However, it has drawbacks such as the possibility

of slow reaction kinetics, the inability to keep the reactor open, and possible safety issues [80].

*Co-precipitation Techniques:* This method generates NP by simultaneously nucleating, growing, coarsening, and agglomerating small nuclei. Although this method has advantages such as simplicity, the absence of high temperatures and energy efficiency, it also has disadvantages such as time consumption, difficulty in reproducibility, and the possibility of impurities precipitating with the product [84].

*Microemulsion technique:* In this method, during the collision of water droplets in a microemulsion medium, a rapid exchange of reactants occurs. A precipitation reaction occurs in the nanodroplets as a result of this change, resulting in nucleation growth followed by coagulation of the primary particles. As a result, NPs are stabilized by the presence of a surfactant. This method is easy to prepare, has thermodynamic stability, and generally produces NPs with a crystalline structure and high specific surface area. On the other hand, it is easily affected by external factors such as temperature, pH, and requires the use of high concentrations of surfactants [84].

*Chemical vapor deposition:* It is a method used to create high-quality, high-performance solid materials. In this method, one or more volatile precursors are used to produce the desired residue on the water surface (substrate). Frequently, volatile by-products are also obtained, which are removed from the reaction chamber by the gas stream. This process is commonly used in the semiconductor industry to produce thin films [84].

*Biological (green) synthesis:* Plants, bacteria, fungi, algae, and other organisms are used in the biosynthesis of NPs. The advantages of this approach are that biosynthesized NPs have higher catalytic activity, are environmentally friendly, are more biocompatible, can be used directly in living systems, and use less toxic chemicals during the process [85,86].

- Biomedical Applications of ZnO

Zn is one of the most common trace elements found in the body and involved in metabolism. This metal plays an important role in growth and development, protein

and nucleic acid metabolism, hematopoiesis and neurogenesis by acting as a signaling factor [16,87,88].

ZnO NPs aid in the absorption of Zn elements by the body. Furthermore, owing to their valuable properties such as biocompatibility, biosafety, low toxicity, environmental friendliness, low-cost synthesis, and antimicrobial activity, ZnO NPs are frequently used in biomedical applications such as anticancer, antibacterial, antioxidant, antidiabetic, and anti-inflammatory activities, as well as drug delivery and bioimaging applications [16].

*Drug Delivery:* ZnO-NPs are prone to surface modifications due to the significant number of -OH groups on their surface, and they are used to deliver different therapeutic molecules such as drugs, nucleic acids, etc [89]. Because ZnO NPs are small enough to pass through smaller capillaries, they are absorbed by cells and allow drugs to accumulate in target areas effectively and be released in the targeted area for a long time due to their encapsulation capabilities [90,91]. In a study, titanium dioxide ( $\text{TiO}_2$ ) and ZnO core-shell nanostructure ( $\text{TiO}_2@\text{ZnO}$ ) and  $\text{TiO}_2@$  mesoporous ZnO-graphene oxide ( $\text{TiO}_2@\text{ZnO-GO}$ ) hybrid nanocomposites were synthesized as a pH-sensitive carrier for colon-targeted drug delivery and curcumin was loaded to the nanostructures. The nanocarriers showed pH-dependent drug release behaviors and curcumin released from  $\text{TiO}_2@\text{ZnO-GO}$  in neutral pH was higher than in an acidic medium, due to the presence of a large amount of carboxylic acid group GO nanosheet. Furthermore, curcumin-loaded NCs also demonstrated anticancer properties [92].

*Wound healing:* Because there is an increasing number of bacteria generating resistance to antibiotic, biocompatible and antibacterial NPs are used in wound healing. ZnO NPs have been successfully used in wound healing applications due to their potent antimicrobial properties and key roles of Zn in cell regeneration matrix remodeling [93]. As an example, 3D porous films based on CS/pectin/ZnO (NPs) were prepared for wound healing application. The results showed that the size and porosity decreased with increasing of the content of ZnO NPs. Its mechanical strength was improved and exhibited controlled swelling. Also, the structure demonstrated antimicrobial activity against the *E. coli*, *S. aureus* and fungi (*A. niger*) [94].

*Antibacterial activity:* Each year, diseases caused by bacterial infections kill hundreds of people. Especially antibiotic resistance complicates the treatment of infectious diseases. In recent years, with increasing nanotechnological studies, alternative solutions have been developed against pathogenic bacteria [89]. In this context, ZnO NPs and their combinations are preferred for the effective treatment of pathogenic bacterial diseases. The antimicrobial activity of ZnO NPs is dependent on the several mechanisms including the release of antimicrobial ions, damaging the bacterial cell by the interaction of NPs with microorganisms, and ability to induce a significant increase in reactive oxygen species (ROS) in cell lines [95,96]. ROS, which include superoxide anion ( $O^{2-}$ ), hydrogen peroxide ( $H_2O_2$ ), and hydroxyl radical ( $HO\bullet$ ), are radical and non-radical oxygen species formed by the partial reduction of oxygen ( $O_2$ ). ROS are generated during mitochondrial oxidative metabolism as well as during the cellular response to xenobiotics, cytokines, and bacteria [97].

The generation of ROS process can be explained like:

- The electron and hole interact with water ( $H_2O$ ) to form  $\bullet OH$  and  $H^+$ .
- $O_2$  molecules produce superoxide anion ( $\bullet O^{2-}$ ), which reacts with  $H^+$  to produce  $HO\bullet$
- $HO\bullet$  reacts with  $H^+$  giving  $H_2O_2$  molecules.

As a result, the final molecule is prepared to enter the membrane and damage or kill the bacteria [98]. Superoxides and hydroxyl radicals cannot pass through the bacterial cell membrane due to their negative charge, whereas  $H_2O_2$  molecules pass through the bacterial cell wall, damaging the cell and causing cell death [99].

The release of  $Zn^{2+}$  ions by ZnO is another mechanism for antibacterial action of ZnO-NPs because the released  $Zn^{2+}$  inhibits active transport and disrupts of the enzyme system. In this process,  $Zn^{2+}$  ions are released by ZnO, the microorganism absorbs these  $Zn^{2+}$  ions, and these ions interact with the thiol group of respiratory enzymes. This causes ROS to form, and then respiratory enzymes are inhibited. As a result, ROS damage microorganism membranes, DNA and mitochondria, causing the microorganism to die [100,101]. In general, ZnO NPs have been tested for antimicrobial activity against Gram-positive bacteria such as *Bacillus subtilis* and *S. aureus*, which have shown sensitivity to these nanoparticles, and some Gram-negative

bacteria such as *Pseudomonas aeruginosa*, *Campylobacter jejuni* and *E. coli*. Because of structural differences as cited before in the bacterial membrane, Gram-negative bacteria are more resistant to ROS than Gram-positive bacteria, as the outer membrane reduces the absorption of ROS into the cell, causing Gram-negative bacteria to be more resistant to ROS [53].

Another explanation for antibacterial activity is the polarity of the cell membrane. Polarity promotes negatively charged free radicals like hydroxyl radicals, superoxide, and peroxide ions to penetrate to cells, causing cell damage and death [95]. In addition, the antimicrobial activity of ZnO NP is also affected by the size, surface area, morphology, concentration of the NPs, and its synergistic effect with other antimicrobial agents [96]. These antibacterial mechanisms are summarized in Figure 2.10.

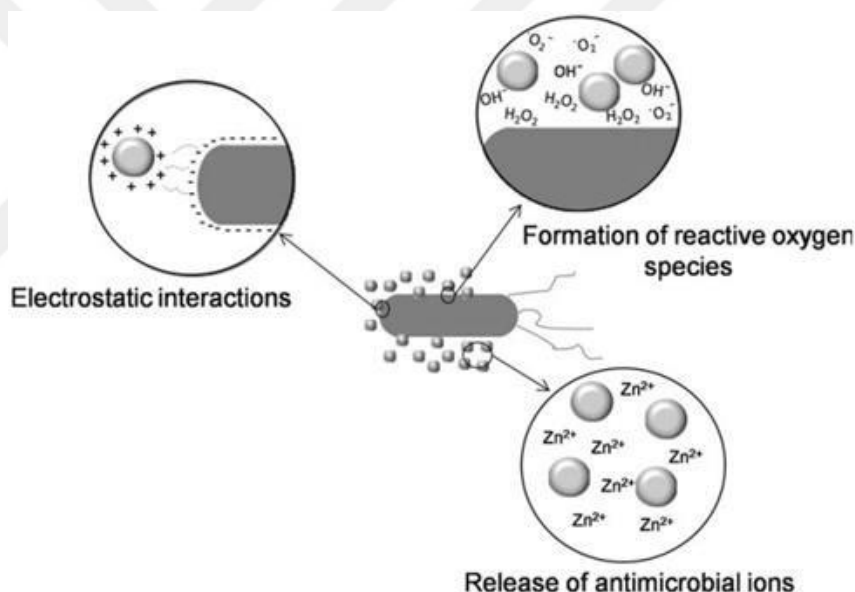


Figure 2.10: The antibacterial mechanisms of ZnO NPs [96]

As an example of eco-friendly green synthesis, silver (Ag)-doped ZnO NPs were synthesized using the aqueous extract of *Azadirachta indica* (Neem) leaves as the reducing agent. The composite showed good antibacterial and antidiabetic activity. Moreover, Ag-ZnO based chitosan film employed on rat exhibited wound treatment efficacy [102]. Also, CS/PVA blend films doped with ZnO NPs had higher the antimicrobial efficacy as compared to pure Cs/PVA film as well as thermal stability and tensile properties [103].

*Biosensors:* Biosensors are analytical devices that detect and process biologically available data with high sensitivity and accuracy, producing measurable signals in the process. The use of ZnO-NPs for various biosensing applications has grown in recent years. In a study, electrosynthesized ZnO NPs agents were developed to improve functional bio-interlayer organic field-effect transistor (FBI-OFET) biosensors stability. Following that, streptavidin (SA) was used as the capturing biomolecule to detect biotin and the ZnO NPs were integrated into the FBI-OFET template. After the study, the researchers observed that the ZnO-FBI-OFET biosensors exhibited longer shelf life compare to the bare biosensor. Moreover, the ZnO NPs increased the sensing performances [104].

#### 2.1.4.3 Ceramic Biomaterials

Ceramics are called bio ceramics when they are used in medical applications, due to their composition and similar properties to bone tissue. Ceramics are inorganic materials usually formed by a combination of ionic and covalent bonds [105]. Because ionic bonds are strong and versatile, ceramics have higher melting temperatures than metals and polymers with metallic or covalent bonds. They also lack free electrons. Therefore, they have low electrical and thermal conductivity [21].

These materials are non-toxicity and non-immunogenicity and they exhibit properties such as high strength, resistance to abrasion and acidic and alkaline chemical attack, thermal and chemical stability, biocompatibility, and easy availability. As a result, they are preferred, particularly in hard tissue applications [24]. The most preferred ceramic materials are SiO<sub>2</sub>, alumina (Al<sub>2</sub>O<sub>3</sub>), zirconia (ZrO<sub>2</sub>) and magnesia (MgO), hydroxyapatite (HA), tricalcium phosphate (TCP) [105]. Ceramics are now extensively used in dentistry and production of orthopedic implants owing to their good resistance properties

- Silica Nanoparticles (Si-NPs)

Silicon dioxide (SiO<sub>2</sub>), also known as silica (Si), is a chemical formed by the reaction of silicon (Si) and O<sub>2</sub>, the two most abundant elements on the earth. The SiO<sub>4</sub> tetrahedron is the fundamental building block of Si and silicate structures. As seen in Figure 2.11, this structure has a silicon ion at the center and four oxygen atoms at each

of its four corners. Because the oxygen ion is much larger than the  $\text{Si}^{4+}$  ion, the four oxygens of a  $\text{SiO}_4$  unit are in mutual contact and the silicon ion appears in a tetrahedral hole. Si can be natural or synthetic, crystalline or amorphous. Sea sand is the best example of Si, which is found in nature [106].

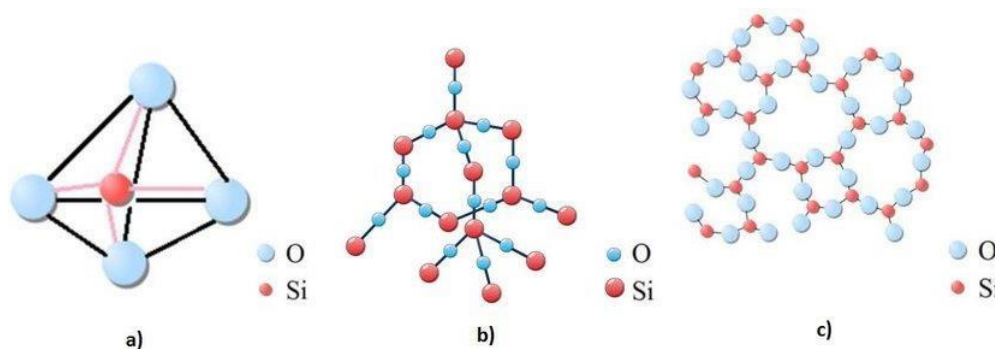


Figure 2.11:  $\text{SiO}_2$  chemical structure of (a) tetrahedral basic unit, (b) quartz like silica crystal and (c) amorphous network [107]

Si-NPs are gaining popularity in nanotechnological applications. Monodisperse Si-NPs with specific particle size (50-250 nm) and porosity are used as alternatives in biomedical applications such as controlled release applications and drug delivery, as amorphous Si-NPs are biologically inert, biocompatible, chemically and mechanically resistant [108]. Silica NPs can be produced in various sizes, shapes, and morphologies by changing the reaction parameters such as pH, temperature, and chemical structure of surfactants formed during synthesis. In this thesis, the Si-NPs are synthesized by the Stöber method, and the synthesis method will be detailed in the under the title named “Synthesis of Silica Nanoparticles” On the other hand, different types of Si-NPs have been reported in the literature, including mesoporous silica NP (MSN) with porous structure [109], spherical or rod-shaped silica NP [110], and core/shell silica NPs [111].

- Synthesis of Silica Nanoparticles

Si-NPs can be synthesized with various shapes, morphology, and physicochemical properties in the size range of 10-500 nm. The size and shape of the synthesized Si-NPs can be altered by the addition of additives such as electrolytes, surfactants, and organic acids, and the release rates can be controlled, especially in drug delivery systems. The Stöber process is the most widely used method for the synthesis of Si-NPs [108,112].

*The Stöber Method:* The Stöber method is a sol-gel reaction in which ammonia ( $\text{NH}_3$ ) is used as the catalysis, hydrolysis of tetraethylorthosilicate (TEOS) as Si source, occurs in the presence of water and low molecular weight alcohols such as  $\text{C}_2\text{H}_5\text{OH}$ , followed by condensation as can be seen in Figure 2.12 [113]. Stöber et al. synthesized spherical and monodisperse Si-NPs ranging from less than  $0.05\ \mu$ , to  $2\ \mu$  in diameter from aqueous alcohol solutions of silicon alkoxides using the sol-gel method for the first time in 1968. The study discovered that the particle size and reaction rate were affected by the type of alcohol. The particles prepared in methanol solutions had the smallest dimensions and the reaction was fastest, while the particles prepared in n-butanol had the largest dimensions and the reaction was slowest. It was also stated that the type of silicon alkoxide had an effect on the reaction rate and particle size [114]. Hydrolysis and condensation are the chemical reactions which are involved in the formation of Si-NPs, as illustrated below [113].



The reaction rate for hydrolysis depends on the pH, the amount of water in the synthesis solution, and the type of alkyl group. Generally, longer chain alkyl groups slow down the rate of hydrolysis. Similarly, the steric effects of larger alkyl groups on neighboring species inhibit condensation [115].

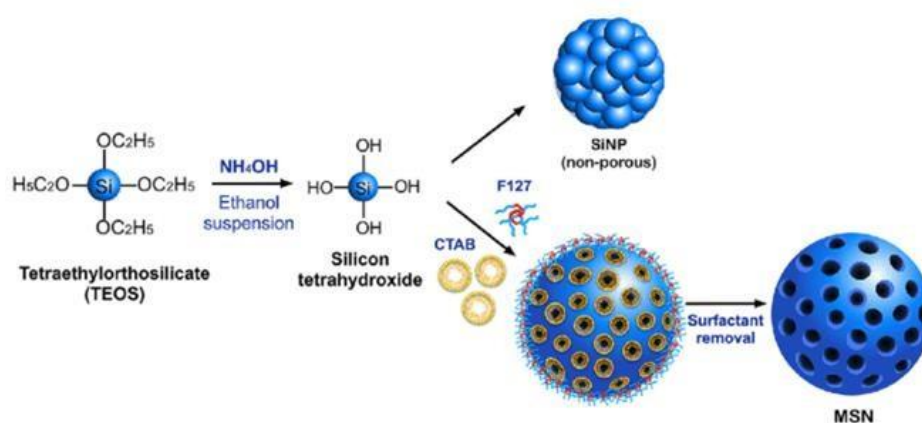


Figure 2.12: Stöber Method to synthesize of Si-NPs [116]

The hydrolysis reaction is the first step in the Stöber synthesis. In general, hydrolysis of TEOS, the most common Si precursor, occurs in this reaction. Here, the nucleophilic substitution of the ethoxy group ( $\text{Si}-\text{OR}$ ) with silanol groups ( $-\text{Si}-\text{OH}$ ) occurs via a

penta-complexation transition state. Hydrolysis is usually performed with water, but in some cases, an acid or base may be used.  $\text{NH}_3$  is commonly used as a catalyst in the Stöber synthesis to accelerate the rate of hydrolysis and condensation. Because  $\text{OH}^-$  ions in ammonia solution ( $\text{NH}_4\text{OH}$ ) are more effective nucleophiles than  $\text{H}_2\text{O}$  molecules, the rate of hydrolysis increases with  $\text{NH}_3$  concentration. The first hydrolysis step is slower and determines the reaction rate. Then, the rate increases as more ethoxy groups are converted to silanol groups due to the increase of the positive charge of the silicon atoms and the reduction of the steric hindrance by replacing the ethoxy groups with  $\text{OH}^-$  nucleophiles [113,117]. After the hydrolysis reaction, the condensation reaction occurs in which the silanol groups ( $-\text{OH}$ ) of the silica monomers or oligomers condense to form a siloxane ( $-\text{O}-\text{Si}$ ) bond. Like the hydrolysis reaction, the nucleophiles are displaced in this reaction as well, but the rate of condensation is much faster than the rate of hydrolysis. Silanol groups are more easily deprotonated than water molecules, increasing the positive charge density on the silicon atom and allowing for a more favorable nucleophilic attack. Therefore, silanol monomers prefer to bind to larger siloxane network aggregates rather than other monomers or small oligomers [113,117].

- Surface Modification of Si-NPs

The size and homogeneity of Si-NPs affect the quality in the application area. Because Si-NPs are prone to surface modifications, their surface can be modified using a variety of methods such as covalent bonding of complexes, co-densification, physical adsorption of polymers, and surface grafting [118]. This improves the colloidal stability of the NPs and prevents aggregation. In addition, increasing the interaction between the reacting materials and the NPs, improving the biocompatibility, and ensuring the target-specific distribution are among the other objectives of the surface modifications [119].

Generally, two methods are used mostly in modification processes. One of them is the hydrolysis of silicon atoms in siloxane bonds ( $\equiv\text{SiO}-\text{Si}\equiv$ ). The other method is the more common and conventional method that involves the use of different types of organosilanes containing active chloro-, methoxy-, ethoxy- or amino- groups chemically associated with the surface of the carrier [120]. High silanol groups ( $\text{Si}-$

OH), which are commonly found on the surface of Si-NPs and are accepted as attachment sites for surface probes, play a role in the organosilane method. During the reactions, co-condensation or post-synthetic grafting occurs on the surface of silanol groups of various functional silanes such as 3-(trimethoxysilyl)propyl methacrylate (MPTS) or 3-Aminopropyltriethoxysilane (APTES). The primary distinction between these two processes is that in post-synthetic grafting, the conjugation of functional groups mostly occurs on the NP surface, whereas co-condensation requires the presence of modified functional groups even within the pores of the NPs [116].

- Biomedical Applications of Si-NPs

Si-NPs, which can be synthesized in a variety of sizes, shapes, and morphologies, are used in many fields including biomedicine, chemistry, medicine, sensing, and are especially preferred in biomedical applications such as drug delivery systems, biosensors, and dental applications. Si-NPs, which are preferred in drug delivery systems due to their versatility such as biocompatibility, variable morphology, and size, come to the fore, especially in cancer and antimicrobial studies. For example, MSNs containing folic acid (MSN-COOH-Tet-HBP-FA) have been developed for the delivery of tetradrine (Tet), a type of antitumor drug. Carboxyl surface modification of folic acid improved the drug loading capacity of NPs. ANPs also became more stable as a result of hyperbranched polymer (HBP). A pH-sensitive drug delivery system based on folic acid-targeted HBP-modified MSN for cancer therapy [121]. An example of antimicrobial applications is a study investigating the antimicrobial photodynamic effects of free curcumin and curcumin Si-NP as photosensitizers on *P. aeruginosa* and *S. aureus* planktonic and biofilm forms. The study concluded that the composite structure inhibited bacterial biofilm growth, had no significant cytotoxic effect on human normal fibroblasts and showed wound healing properties. Antimicrobial photodynamic and wound healing activity of curcumin encapsulated in Si-NPs [122]. Another area of use for Si-NPs is sensors. In one study, a Si-NP functionalized electrochemical sensor was developed to detect *E. coli*. In the study, it was noted that the sensor detected *E. coli* within 5 minutes, and the best limit of detection (LOD) could reach  $10^3$  CFU/mL in just 30 minutes [123]. In a study on the use of Si-NPs in dental applications, Ag@SiO<sub>2</sub> NPs were used. This composite was formed by coating silver NP (AgNPs) synthesized from green tea (GT) extract with

Si-NPs. In the study, the cytotoxicity of the composite in dental pulp fibroblasts and its effect against *Streptococcus mutans* were evaluated. As a result, the composite structure was found to have a 44 % reduction in *S. mutans* biofilm formation and no cytotoxic effect. Biogenic synthesis and antimicrobial activity of silica-coated silver nanoparticles for esthetic dental applications [124]. In another study, novel nanoporous Si-NPs were synthesized and introduced into PMMA-based resins to improve their mechanical properties. At the end of the study, it was stated that the homogeneously dispersed Si-NPs in the PMMA matrix provided mechanical support [125].

#### 2.1.4.4 Polymeric Biomaterials

Polymers are large molecules (macromolecules) formed by the repetition of small and simple chemical units, also known as monomers [60]. Polymers with biocompatibility properties have been used as polymeric biomaterials, also known as biomaterials, in medicine for many years [19,24]. Polymeric biomaterials are used extensively in biomedical applications such as surgical instruments, implantable devices, catheters, vascular grafts, soft tissue replacement, wound dressing, drug delivery systems, and dental implantation. Because these polymers enable the creation of suitable materials for medical applications, thanks to their changeable physical and chemical structures and controllable morphologies with various functional groups [126].

Polymeric biomaterials can be classified based on their structures, types of synthesis reactions, functional properties, and applications. For example, polymeric biomaterials are divided into two as either natural or synthetic materials. While the most widely known polymers of natural origin are natural rubber (NR), CS, collagen, alginate, proteins, and sugarcane-based materials, the most well-known synthetic materials are PMMA, polyethylene (PE), polypropylene (PP), poly(lactic acid) (PLA), poly(glycolic acid) (PGA) and poly(lactic-co-glycolic acid) (PLGA), Polycaprolactone (PCL) poly(vinyl chloride), polyurethane, etc [23,24,126]. According to the type and requirements of the biomedical applications, they are further classified as biodegradable and non-biodegradable polymers. While natural polymeric biomaterials are generally biodegradable, synthetic polymers might be both non-biodegradable and biodegradable polymers. For example, biodegradable polymers include PLGA, PLA, PGA, and PCL, whereas non-biodegradable polymers include

PMMA, PE, and PP [24]. Another type of classification is the polymerization synthesis reaction. Polymers are produced using two types of polymerization methods: addition (chain growth) polymerization and condensation polymerization. Addition polymerization occurs by adding monomer molecules to each other without the formation of any by-products (water, methanol, etc.) [127]. If the polymer formed by this method consists of the same monomers, it is referred to as homopolymer, if it consists of two or more different monomer types, it is referred to as heteropolymer or copolymer. For example, polylactic acid (PLA) is a homopolymer because it only contains lactic acid monomer residues, whereas poly(lactic acid-co-glycolic acid) (PLGA) is a copolymer because it contains lactic acid and glycolic acid as two types of repeating monomers [24,60]. On the other hand, in condensation polymerization, when monomer molecules combine to form a polymer chain, by-product (water, methanol, etc.) formation occurs at the end of the reaction. Basically, the molecular structures of polymers are classified as linear, branched, and cross-linked. A linear polymer has a long chain, a branched polymer has many branches that are covalently bonded to the main chain, and a cross-linked polymer has a three-dimensional molecular structure [24]. These molecular structures, morphology, composition, molecular weight, and crystallinity are factors that affect the properties of a polymer.

- Polymethyl Methacrylate (PMMA)

PMMA is an acrylic, synthetic polymer which has an aesthetic structure, the allows to change its physical and chemical structure, is optically transparent, has a glass transition temperature range of 100 °C to 130 °C, and has a density of 1.20 g/cm<sup>3</sup> at room temperature. PMMA, which can withstand the temperature range of 70°C - 100°C, also has reasonable resistance to chemicals and is unaffected by the aqueous solution of most laboratory chemicals. However, it should be noted that PMMA has low resistance to chlorinated and aromatic hydrocarbons, esters, or ketones. On the other hand, despite its high thermal stability, PMMA has a low thermal conductivity ( $5.7 \times 10^{-4} \text{ }^{\circ}\text{C cm}^{-1}$ ). Mechanically, PMMA does not break at rupture, because it has a high Young's Modulus and low elongation at break [128,129]. On the other hand, although PMMA has disadvantages such as poor water absorption and bending strength, there are various alternatives to overcome these problems such as different modification techniques or generating composites with carbon-based or different

inorganic materials [128]. The structures of MMA and PMMA have been illustrated in Figure 2.13.

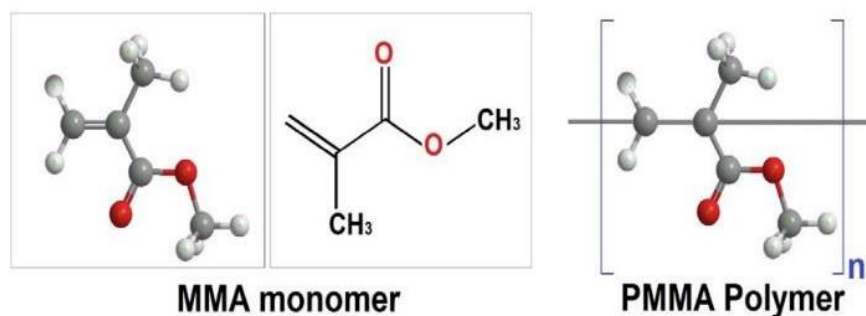


Figure 2.13: Structure of MMA and PMMA [130]

Solubility is typically defined as the uniform distribution of a solute (solid, liquid, or gas) in a solvent. Solubility is affected by temperature, physical and chemical properties of interacting materials. In general, materials with similar structures have the ability to dissolve each other, and as the polarity of the material increases, its interaction with other materials also increments, resulting in a solubility boost [129]. PMMA, which is an organic polymer, dissolves better in organic solutes such as ketones and esters compared to water. On the other hand, even if PMMA does not react chemically with water, increased interaction time with water may cause dimensional changes or cracks due to cyclic water absorption and drying [128].

PMMA was first reported by Redtenbacher in 1843 [131]. In the following years, one of the most notable developments was the work carried out by Otto Rohm at the beginning of the 20th century [132]. Nowadays, PMMA is one of the most preferred materials in the biomedical field, especially in dental and bone applications.

- **Synthesis of Polymethyl Methacrylate**

Grafting and cross-linking are two important bonding methods that occur depending on the reaction conditions during polymer synthesis. Grafting is the process of producing macromolecules called copolymers, which are formed by the covalent bonding of two different monomers or polymers. In this case, one of the polymers is called the stem polymer (backbone). Crosslinking, on the other hand, is the multidirectional interaction of monomers or polymers that leads to chain elongation and the formation of networks. Depending on the type of material reacting, there are

three mechanical approaches to grafting and crosslinking: polymer/polymer (P/P) approach, polymer/monomer (P/M) approach, and monomer/monomer (M/M) approach [133].

In the polymer/polymer synthesis approach, two different polymer types form a copolymer with covalent and other cross-links (hydrogen bonds and other intermolecular bonds), while in the polymer/monomer synthesis approach, a reaction initiator must be present in the environment for a polymer and monomer to react. This approach is the most widely used method for PMMA synthesis. Furthermore, in the monomer/monomer synthesis approach, the two monomers must be different or multifunctional in order for the polymer synthesized using only monomers to be a copolymer. Otherwise, a homopolymer can be synthesized rather than a copolymer. This mechanism is similar to the conventional polymerization mechanism, but different end products are formed by crosslinking, branching, and block copolymer formation [129,133].

- **Biomedical Applications of Polymethyl Methacrylate**

Owing to its biocompatibility, ease of preparation, physical and chemical properties that allow surface modifications, low cost, and high fracture resistance, PMMA is mostly used as supporting material in dental and orthopedic applications in the biomedical field. PMMA is preferred for the preparation of bone cement in orthopedics, while in the dental field, PMMA is used to produce dental composite resin, denture bases, impression trays and printed or ground molds, etc. However, PMMA, which still needs to be developed in terms of mechanical strength, is generally used in various composite structures with different materials. As an example of bone cement application, a bioactive composite bone cement has been developed for the treatment of osteoporotic vertebral compression fractures by incorporating mineralized collagen (MC) into PMMA bone cement (MC-PMMA). The study concluded that MC-PMMA bone cement exhibited lower compressive strength and compressive modulus than PMMA bone cement, facilitated cell proliferation and differentiation, and showed good adaptive mechanical properties and biocompatibility [134]. In another study, Si/PMMA core/shell structure was used in a dental filling material known as bis glycidyl dimethacrylate (Bis-GMA)/triethylene glycol

dimethacrylate (TEGDMA), which is a common dental composite. According to the study, the properties of the filler material are affected by the filler content and the amount of PMMA. In addition, it was emphasized that the PMMA-added composite had a higher modulus than the PMMA-free, only silica-containing dental composites, but if the PMMA amount was further increased, the modulus of the composite decreased [2].

#### 2.1.4.5 Composite Biomaterials

Composites are materials formed by combining at least two different components and have superior performance compared to their raw materials because the components used to obtain a composite material maintain their physical, chemical, and structural properties after the compounding process. As a result, composite materials have improved properties compared to single-phase materials that can offer limited properties, and the demands for these materials are increasing day by day [21,24]. A composite usually consists of two phases, a continuous phase called matrix and a discontinuous phase called reinforcement. The matrix acts as a bridge, distributing the stress between the reinforcement materials and providing protection against external factors [24]. The reinforcement phase is responsible for supporting and functionalizing the matrix. Therefore, it is generally harder and more resistant than the matrix [19,23]. Besides these two phases, some composites might have an interphase to increase the interaction between matrix and reinforcement phases as shown in Figure 2.14. The interphase is especially important for short fiber or particle reinforced composites because the interphase acts as a bridge to transfer the stresses applied to the composite from the matrix to the filler [135].

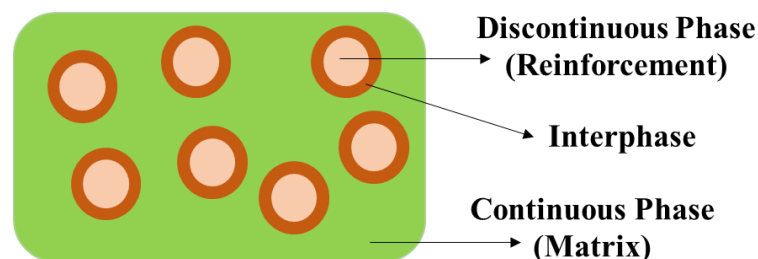


Figure 2.14: The structure of a composite

For example, hard fillers such as rubber particles are combined with brittle matrices to increase impact strength. In addition, the reinforcement phase may also consist of particles such as carbon or polymer fibers, ceramics, or silica embedded in the matrix, often in various combinations [24]. Figure 2.15 shows the different variety of composites based on the reinforcement phase.

Molecular weight and particle size of the matrix phase, properties of interphase, types, shape, size and distribution of the reinforcement phase are the parameters that affect the physical and chemical properties of a composite [19,24,135].

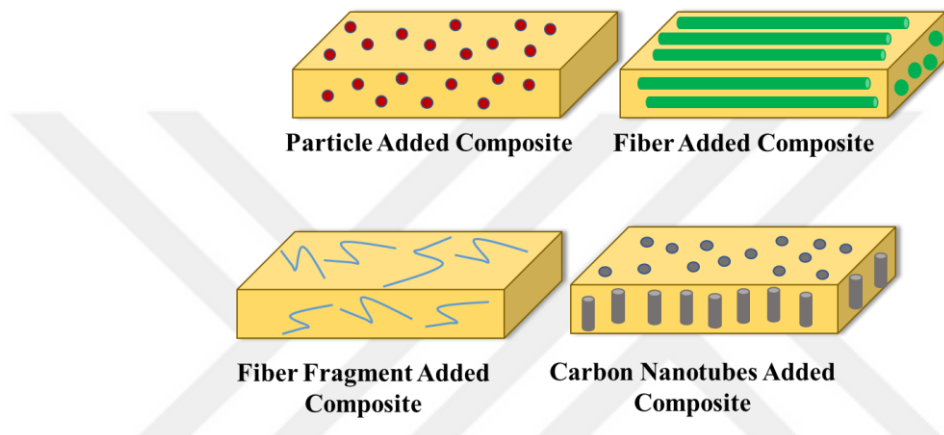


Figure 2.15: Different variety of composites based on the reinforcement phase

Composite materials are frequently preferred in orthopedic implants such as dental filling composites, reinforced bone cement, catheters, total joint replacements, spine rods, discs, plates, screws and ligaments [19,136]. PMMA was used as a matrix in a study of PMMA/GO and PMMA/GO/TiO<sub>2</sub> nanocomposites used as dental materials, while GO and TiO<sub>2</sub> were preferred as fillers. According to the study, the composite containing GO performed better [137].

- **Dental Filling Composites**

In recent years, as an alternative to amalgam materials used in dental applications, more biocompatible, physically improved, and more aesthetic filling materials have been required, following that the concept of adhesive restorative materials has emerged. These materials are diversified as resin composite restorative materials, polyacid modified resin composites (compomers) and glass ionomer (polyalkenoate) cements [138].

Resin composites are materials that basically consist of an organic polymeric matrix, inorganic fillers and a silane coupling agent that binds the first two components together, as can be seen in Figure 2.16. The mechanical properties of a composite material are affected by the distribution, types, sizes, physical and chemical properties of the components in the composite [1].

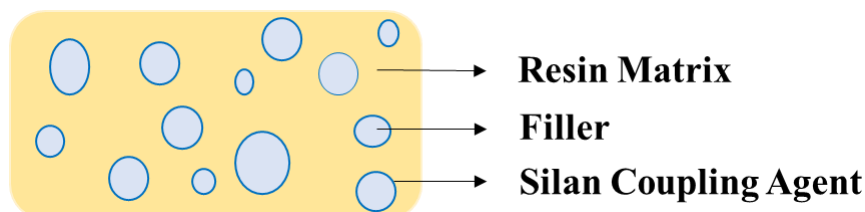


Figure 2.16: Composition of resin composite

*Resin Matrix:* The first known composite materials in tooth color were silicate cements. However, these materials are no longer used because of their high solubility and pulp toxicity. PMMA-based materials were the first resin-based materials developed as an alternative and are still used today. However, PMMA is generally produced in composite structures with other materials because of its inadequacy in terms of adhesion and mechanical strength [138]. In this context, new resin monomers consisting of bisphenol A glycerolate dimethacrylate (Bis-GMA) (2,2-bis[4-(2-hydroxy-3-methacryloxypropoxy)]) developed by Bowen in 1962 has formed the basis of today's restorative resin materials and it is still one of the most widely used resin matrices [139,140]. Triethyleneglycol dimethacrylate (TEGDMA), urethane dimethacrylate (UDMA), ethoxylated bisphenol-A-dimethacrylate (Bis-EMA), decandiol dimethacrylate (D3MA), bis(methacryloyloxymethyl) tricyclodecane, and urethane tetramethacrylate (UTMA) are other base monomers used in current commercial composites. Since there are hydrogen bond interactions between hydroxyl (-OH) groups and monomer molecules, Bis-GMA has a very high viscosity. As a result, it is frequently diluted with another liquid monomer to reduce viscosity and facilitate to use it conveniently [141]. In this context, TEGDMA is the preferred diluent monomer for Bis-GMA and UDMA based composites as it is less viscous and has excellent copolymerization properties [142,143]. In order to overcome the physical and chemical disadvantages of composites, modifications of monomers have been carried out using ring-opening monomers such as spiro-orthocarbonates or organically

modified ceramics, also called ormocer, resulting in a reduction in polymerization shrinkage [144].

*Inorganic fillers:* Inorganic fillers are materials that fill the gap between the composite matrix and help to determine the physical properties of the composite, such as strength and modulus. The type, size range, morphology, and volume of the filling materials affect the mechanical properties of the composites. In addition, fillers improve the aesthetic properties of the dental composite and decrease the thermal expansion coefficient, water sorption, and polymerization shrinkage. SiO<sub>2</sub> was the first filler used in dental resin composites. Because SiO<sub>2</sub> has a lower refractive index than the conventional Bis-GMA/TEGDMA resin matrix, it is more opaque than other fillers used in such a resin system. In addition to SiO<sub>2</sub>, glass fillers containing calcium (Ca), sodium (Na), or phosphorus (P) oxides have also been used as fillers in resin composites [143].

The dimensions of the filler material have a significant impact on the mechanical properties of the composite. The smaller the particle size, the more likely it is to fill the gaps between the fillers, and the filler loading will be maximized. As the particle size decreases, the hydrogen bond formed with the resin matrix increases with the increased surface area. This increases the viscosity of the resin composite and therefore its resistance to mixing [143,145]. Filler size is also considered another classification parameter. Accordingly, composites are classified as macro-filled (10 to 50 µm), micro-filled (40 to 50 nm), hybrid filled (10 to 50 µm + 40 nm), micro-hybrid (0.6 to 1 µm and 40 nm), and nano-filled (1 to 100 nm) as depicted in Figure 2.17. Resins with good abrasion resistance and strength have been produced, particularly with the development of nano-sized fillers [146,147].

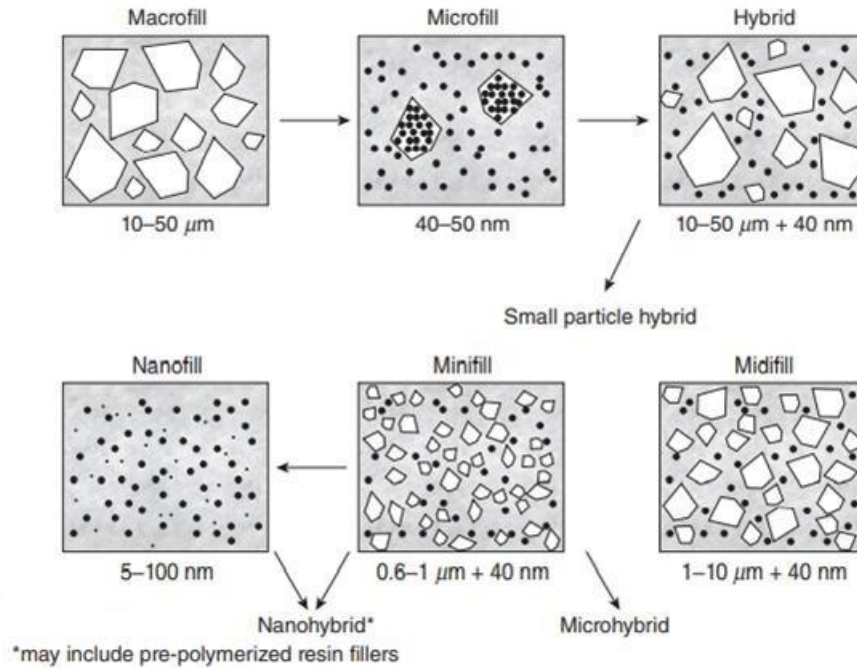
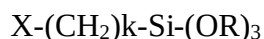


Figure 2.17: Classification of resin composites depending on the size of fillers [138]

Dental filling materials containing nano-filled composites are gaining popularity every single day. Nanoscale materials fill the gaps between the matrix thanks to their small size, provide high filler loading and increase bonding due to their large surface areas, allowing for more advanced mechanical and chemical composite production. An example of nano-sized filler composites is a combination of nanometric particles and nanoclusters developed by Filtek Supreme. The primary fillers are chemically synthesized spherical silica with dimensions of 20 nm or 75 nm, and the other component is  $\text{SiO}_2$  nanoclusters, approximately 0.6  $\mu\text{m}$  in size [6,148]. A new filling material consisting of spherical silica and zirconia has been developed by Tokuyama Dental Corp for Estelite  $\Sigma$  (Estelite) using the sol-gel method. The average particle size of this developed filling material is 0.2  $\mu\text{m}$  and has a narrow range between 0.1 and 0.3  $\mu\text{m}$ , therefore this material is called submicron composite [6]. Furthermore, antimicrobial nanoparticle-containing composites exhibit antimicrobial properties. Although different theories have been developed about how NPs inhibit the growth of bacteria in the oral cavity, the most accepted theory is the oxidative stress theory, which was also discussed in the previous chapters [143,149].

**Silane coupling agents:** Silane coupling agents are substances that form strong covalent bonds between inorganic fillers and organic matrix. Silanized fillers have higher tensile strength than non-silanized fillers because the strengthening of covalent

bonds increases the mechanical strength of the composite. Unsilanized filler particles absorb significantly more water than silanized filler particles [142,143]. An organofunctional silane coupling agent contains two functional groups at the ends of its molecular backbone, as seen below.



In this formula, X represents an organofunctional (e.g. methacrylate, acrylate, isocyanato, epoxy) group, while R is a hydrolyzable alkoxy (methoxy, ethoxy) group.  $-(\text{CH}_2)_k-$  is the group that connects the organofunctional group and the Si atom [150]. The alkyl group is hydrolyzed to silanol to react with the mineral surface of the filler and binds to the hydroxyl groups of the  $\text{SiO}_2$  particles, while the organofunctional group copolymerizes with the resin matrix. The most widely used silane agent is 3-(trimethoxysilyl)propyl methacrylate (MPTS), as can be depicted in Figure 2.18. The thickness of the silane layer has a significant effect on the efficiency of the silanization process. As the silane thickness increases, its layers become more uneven and physisorbed on the surface of the fillers, increasing the risk of weak bond formation [143].

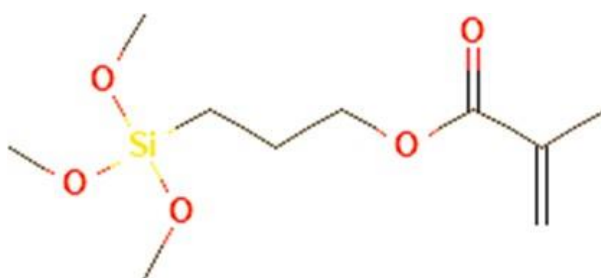


Figure 2.18: The chemical structure of 3-(trimethoxysilyl)propyl methacrylate (MPTS) [151]

- Physical properties

It has been previously stated that the particle size of the fillers has effects on the physical, chemical, and biological properties of the resin composite. Curing during polymerization is another factor affecting the quality of the resin. Curing is concerned with determining whether the degree of conversion (DC), which expresses the percentage of conversion from the  $\text{C}=\text{C}$  structure to the  $\text{C}-\text{C}$  structure, is sufficient. High DC is required to improve the physical, mechanical and biological properties of

resin-based composites. Especially, resin composites using nanofillers show good durability, while their amount and distribution in the composite are other parameters influencing the mechanical properties [143]. In one study, Canché-Escamilla et al. added an emulsion polymerized silica/PMMA core/shell structure to a Bis-GMS/TEGDMA dental composite structure and found that it affected the mechanical properties of the composite. The hybrid structure prevented the aggregation of silica NPs and, at the end of the study, the composite obtained using silica/PMMA hybrid NPs (ratio of 78/22) had a higher modulus than the composite obtained with the same amount of Si-NPs [2]. Testing of the mechanical properties of resin materials is generally based on tensile, compressive, and shear stresses. In these tests, dental composites produced with nanocomposites generally show equivalent or higher strength than other types of dimensionally oriented composites [1]. For example, in a study, Beun et al. compared three nano-filled composites, four universal hybrids, and two micro-filled composites. According to the study, the filling ratio increased as the size decreased, and the filler load ratio of nano-filled composite was between 71.9% and 84.1%, for universal hybrid composites was between 71.0% and 79.7%, and in the case of micro-filled composites it was between 51.3% and 54.9%. Overall, nano-filled resin composites outperformed universal and micro-filled composites in terms of elastic modulus, while micro-filled composites had the lowest mechanical properties [152]. Another mechanical property issue is polymerization shrinkage as shown in Figure 2.19, which is one of the most significant drawbacks of resin-based composite restorations. During polymerization, volumetric shrinkage occurs as a result of the conversion of monomers into a tightly packed polymer network. As a result of these stresses, leakage, caspal deviation and enamel cracking may occur. Filler content, DC, and the combination of matrix and monomer all influence polymerization shrinkage. For example, since TEGDMA has a lower molecular weight and is less viscous, they increase the amount of polymerizable C=C which leads to greater shrinkage. Therefore, their concentration in the composite is important. The water absorption ability of the composite is another factor that influences polymerization shrinkage. Expansion occurs in composites with high water absorption, which can prevent contraction. On the other hand, while water absorption reduces polymerization shrinkage, it can also cause deterioration in the mechanical properties and color stability of resin composites. Moreover, thermal stresses add extra strain to the

interphase connection between restoration and tooth structure, in addition to the negative effect of polymerization shrinkage [143].

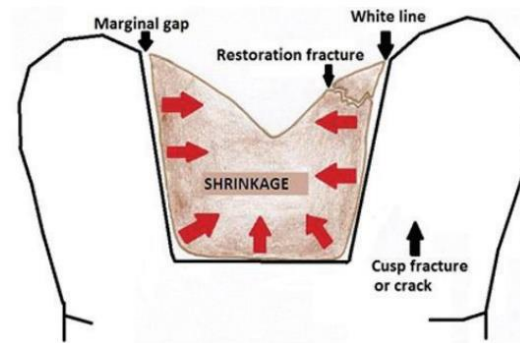


Figure 2.19: Polymerization shrinkage of the dental composite [153]

# Chapter 3

## 3 Materials and Method

Experimental methods carried out in this thesis involve six stages: (i) Si-NPs synthesis, (ii) Si-NPs silanization with MPTS, (iii) Si/PMMA core/shell structure synthesis, (iv) ZnO-NP synthesis, (v) ZnO/CS core/shell structure synthesis, and (vi) obtaining the Bis-GMA/TEGDMA-Si/PMMA/ZnO/CS dental filling composite structure. Experimental studies are summarized in Figure 3.1.

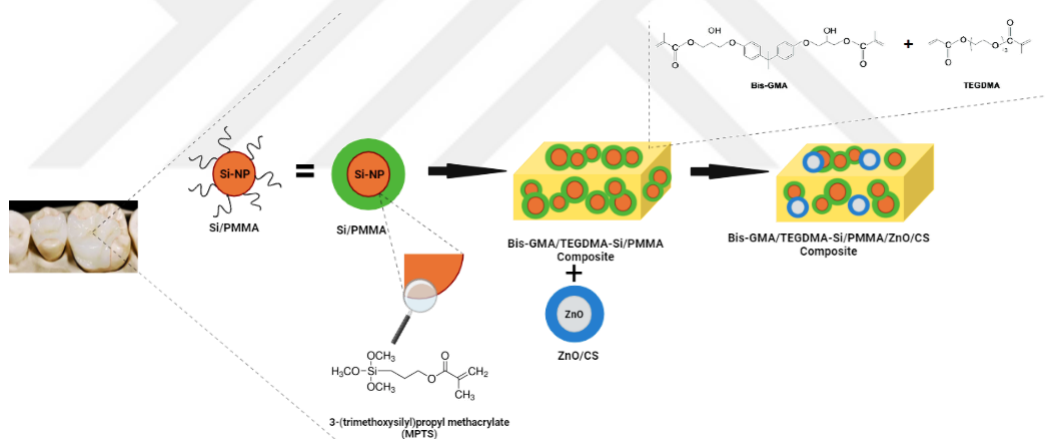


Figure 3.1: Representative illustration of the obtained Bis-GMA/TEGDMA-Si/PMMA/ZnO/CS dental filling composite

### 3.1 Materials

The chemicals and equipments used during the experimental section are summarized below. All the chemicals used were of analytical grade. Distilled water was used throughout the study.

### Synthesis of Si-NPs

- Tetraethyl orthosilicate (TEOS) (Sigma Aldrich)
- Ammonium hydroxide solution (NH<sub>4</sub>OH) (Sigma Aldrich)
- Absolute ethanol (C<sub>2</sub>H<sub>5</sub>OH, >99.7%) (Sigma Aldrich)
- Hot Plate Stirrer (IKA)
- Ultrasonic Bath (Elmasonic S50R)
- Centrifuge (Hettich Zentrifugen Universal 320)

### Synthesis of Si@MPTS-NPs

- Absolute ethanol (C<sub>2</sub>H<sub>5</sub>OH, >99.7%) (Sigma Aldrich)
- 3-(Trimethoxysilyl)propyl methacrylate (MPTS) (Sigma Aldrich)
- Ultrasonic Bath (Elmasonic S50R)
- Centrifuge (Hettich Zentrifugen Universal 320)

### Synthesis of Si/PMMA Core/Shell Structure

- Methyl methacrylate (MMA) (Merck)
- Hexadecyltrimethylammonium bromide (CTAB) (Sigma Aldrich)
- Potassium persulfate (K<sub>2</sub>S<sub>2</sub>O<sub>8</sub>, 99+%) (Acros Organics)
- Calcium chloride (CaCl<sub>2</sub>) (Sigma Aldrich)
- Hot Plate Stirrer (IKA)
- Ultrasonic Bath (Elmasonic S50R)
- Centrifuge (Hettich Zentrifugen Universal 320)

### Synthesis of ZnO NPs

- Zinc acetate dihydrate (Zn(CH<sub>3</sub>COO)<sub>2</sub>·2H<sub>2</sub>O) (Sigma Aldrich)
- Sodium Hydroxide (NaOH) (Sigma Aldrich)
- Hot Plate Stirrer (IKA)
- Ultrasonic Bath (Elmasonic S50R)
- Centrifuge (Hettich Zentrifugen Universal 320)

### Synthesis of ZnO/CS Core/Shell Structure

- Synthesized ZnO NPs
- Chitosan (Low Molecular Weight) (Sigma Aldrich)
- Hot Plate Stirrer (IKA)
- Ultrasonic Bath (Elmasonic S50R)
- Centrifuge (Hettich Zentrifugen Universal 320)

### Preparation of Bis-GMA/TEGDMA Dental Filling Composite

- Bisphenol A glycerolate dimethacrylate (BisGMA) (Sigma Aldrich)
- Triethylene glycol dimethacrylate (TEGDMA) (Sigma Aldrich)
- Ethyl 4-dimethylaminobenzoate (4EDMAB, %99) (Sigma Aldrich)
- Camphorquinone (CQ, %97) (Sigma Aldrich)
- Ultrasonic Bath (Elmasonic S50R)
- UV Curing System (Omnicure Series 2000)

## 3.2 Experimental Section

### 3.2.1 Synthesis of silica nanoparticles

Synthesis of nonporous Si-NPs were carried out by slightly modification of the Stöber procedure [118]. Briefly, 8.6 mL TEOS was added to 200 mL  $C_2H_5OH$ , then 12 mL  $NH_4OH$  was added to mixture dropwise and stirred at room temperature for 12h. Then, the particles were purified by washing with  $C_2H_5OH$  at 7500 rpm for 3x30 min by using ultrasonic bath and centrifuge. The graphical summary of the synthesis of Si-NPs is illustrated in Figure 3.2, synthesized Si-NPs are shown in Figure 3.3 and Figure 3.4.

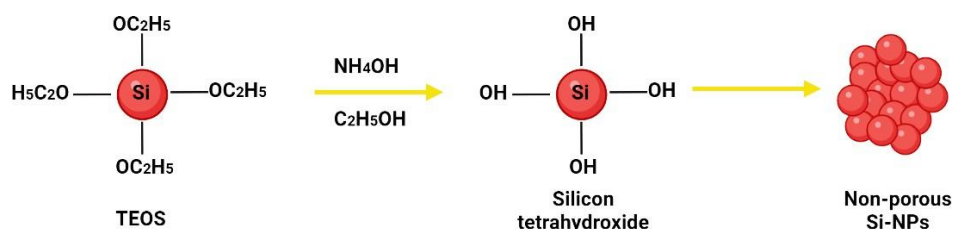


Figure 3.2: The graphical summary of the synthesis of Si-NPs



Figure 3.3: Synthesized Si-NPs before purification



Figure 3.4: Synthesized Si-NPs after purification, inside  $C_2H_5OH$

### 3.2.2 Surface modification of Si-NPs and synthesis of Si@MPTS-NPs

For the surface modification, silanization, of Si-NPs, 100 mg synthesized Si-NPs were dispersed into the 3.3 mL  $C_2H_5OH$  for 45 min. in an ultrasonic bath. Then, 83 mg MTPS as a silane coupling agent was added into the solution and kept dispersed for 2.5 h in the ultrasonic bath and the collected particles were washed with  $C_2H_5OH$  in 7500 rpm for 3x30 min by using ultrasonic bath and centrifuge[154].

### 3.2.3 Synthesis of Si/PMMA core/shell structure

Si/PMMA core/shell structure was synthesized by using emulsion polymerization as ratio of 50/50 of Si/PMMA. Firstly, 220 mg CTAB as a surfactant and 400 mg silanized Si-NPs were mixed in 8 mL distilled water in the reaction vessel under vigorous stirring at 50°C for 2 hours. Then, 120 mg KPS and 400 mg MMA monomer were added into the solution and the solution was kept stirring at 80±0.5°C for 1 hour. At the end of the reaction, the solution was left to cool. After cooling, the white solution was precipitated with the 0.1 M CaCl<sub>2</sub> aqueous solution. After precipitation, the particles were purified by washing with distilled water at 7500 rpm for 3x30 min. by using ultrasonic bath and centrifuge and dried at 60°C overnight [111]. The graphical summary of the synthesis of Si@MPTS-NPs and Si/PMMA core/shell structure are illustrated in Figure 3.5 and the reaction of synthesis of Si/PMMA core/shell structure is shown in Figure 3.6.

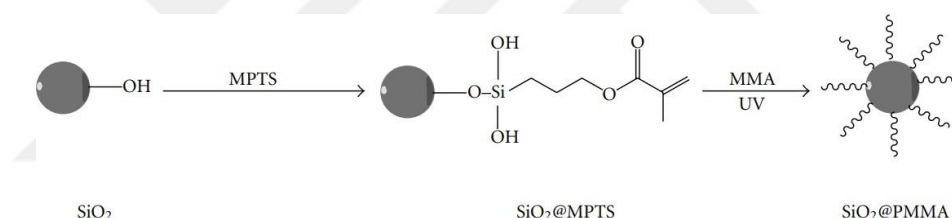


Figure 3.5: The graphical summary of the synthesis of Si@MPTS-NPs and Si/PMMA core/shell structure [154]



Figure 3.6: The reaction of synthesis of Si/PMMA core/shell structure

### 3.2.4 Synthesis of ZnO nanoparticles

After synthesis of Si-NPs, Si@MPTS-NPs and Si/PMMA core/shell structure, synthesis of ZnO NPs was the next step of the experimental section. Synthesis of ZnO NPs was performed by dissolution of 876 mg  $\text{Zn}(\text{CH}_3\text{COO})_2 \cdot 2\text{H}_2\text{O}$  in 200 mL DI at room temperature. Then, 2 M NaOH solution was added into the solution dropwise until the pH reached 11 and the reaction was kept stirring at room temperature for 2 h. After the reaction, the particles were purified by washing with distilled water at 7500 rpm for 3x30 min by using ultrasonic bath and centrifuge and dried at 60°C overnight [155]. The reaction of synthesis of ZnO NPs is shown in Figure 3.7.



Figure 3.7: The reaction of synthesis of ZnO NPs

### 3.2.5 Synthesis of ZnO/CS core/shell structure

The synthesis of ZnO/CS core/shell was performed by dissolving of 100 mg synthesized ZnO NP in 10 mL 1.0% (wt%) CH<sub>3</sub>COOH solution. Then, 100 mg CS (Low MW) was added into the solution. The reaction was maintained in the ultrasonic bath at 40°C for 20 min. Next, 1M NaOH was added dropwise into the mixture until reaching pH 1 and the reaction was kept stirring at 65°C for 3 h. After the reaction ended, the particles were purified by washing with distilled water at 7500 rpm for 3x30 min. by using ultrasonic bath and centrifuge and dried at 60°C overnight [156]. The graphical summary of the synthesis of ZnO/CS core/shell structure is illustrated in Figure 3.8.

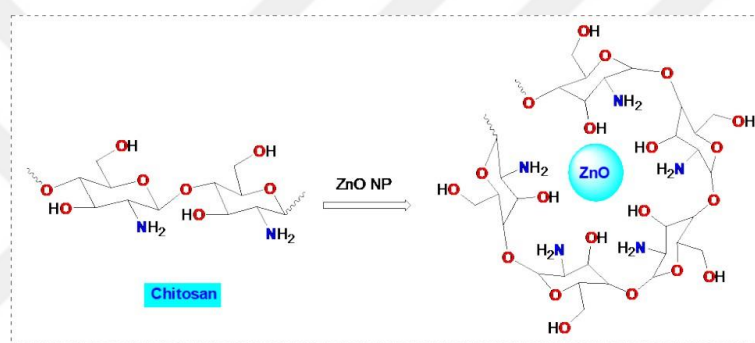


Figure 3.8: The graphical summary of the synthesis of ZnO/CS core/shell structure [157]

### 3.2.6 Preparation of Bis-GMA/TEGDMA dental filling composite

During the preparation of Bis-GMA/TEGDMA dental filling composite, only Bis-GMA/TEGDMA composite structure without Si-NPs doping was prepared at the ratios of 30%/70%, 50%/50% and 60%/40% (w/w). The reason for this approach is to obtain a dental filling composite with the highest mechanical strength and modulus for to use in future experimental studies. The amounts of chemicals used in experimental studies are given in Table 3.1. While preparing the dental filling material, first of all, the highly viscous Bis-GMA was weighed in a determined amount and heated in an ultrasonic bath in a glass petri dish. Meanwhile, TEGDMA and photoinitiators CQ (0.2% wt) and 4EDMAB (0.8% wt) were weighed in the dark and mixed in a glass bottle wrapped in aluminum foil until a homogeneous mixture was obtained. All

materials were kept mixing in an ultrasonic bath at 40°C for 30 minutes. Then the mixture of TEGMA, CQ and 4EDMAB was added to the Bis-GMA in the petri dish and kept stirring at 40°C for 3 hours, covered with aluminum foil. The resulting mixture was poured into a Teflon mold measuring 2 mm x 2 mm x 25 mm (ISO 4049-2000 “Dentistry-Polymer-based restorative materials” standard) and cured under UV light for 120 minutes with density of 7mW/cm<sup>2</sup> and 365 nm. They were then incubated overnight in distilled water in the dark to prepare for three-point bending mechanical tests [2,158]. Dental filling composites prepared by combining Si@MPTS-NP, Si/PMMA core/shell and ZnO/CS core/shell structures were prepared in the same way. Additives were added to the mixture of TEGDMA, CQ and 4EDMAB in the amounts indicated in Table 3.1. The curing process under UV light is shown in Figure 3.9 and the dental composite after curing process is shown in Figure 3.10.

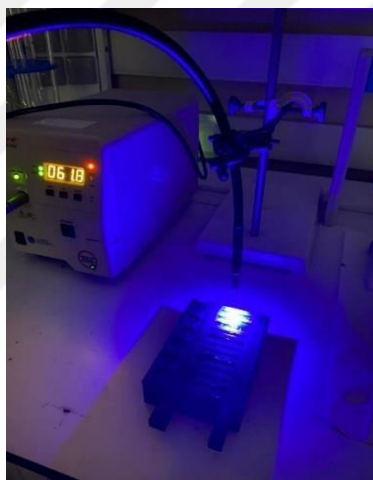


Figure 3.9: The curing process of Bis-GMA/TEGDMA based dental filling composite under UV light



Figure 3.10: The Bis-GMA/TEGDMA based dental composite after curing process

Table 3.1: The composition of Bis-GMA/TEGDMA dental filling composite with and without Si@MPTS-NPs, Si/PMMA and ZnO/CS core/shell structures. The green parts represent the accepted compositions and every next experiment was performed according to previous green part.

| <b>a) Composition of Bis-GMA/TEGDMA Dental Filling Composite (wt%)</b>                                                  |                                                                                                                          |                                                                                                                          |                                                                                                                          |
|-------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------|
| <b>30%70%</b><br><b>Bis-GMA/TEGDMA</b><br>900 mg Bis-GMA<br>2100 mg TEGDMA<br>6 mg CQ<br>24 mg 4EDMAB                   | <b>50%50%</b><br><b>Bis-GMA/TEGDMA</b><br>1500 mg Bis-GMA<br>1500 mg TEGDMA<br>6 mg CQ<br>24 mg 4EDMAB                   | <b>40%60%</b><br><b>Bis-GMA/TEGDMA</b><br>1200 mg Bis-GMA<br>1800 mg TEGDMA<br>6 mg CQ<br>24 mg 4EDMAB                   |                                                                                                                          |
| <b>b) Composition of Bis-GMA/TEGDMA-Si/PMMA Dental Filling Composite (wt%)</b>                                          |                                                                                                                          |                                                                                                                          |                                                                                                                          |
| <b>0.1% Si/PMMA</b><br>1500 mg Bis-GMA<br>1500 mg TEGDMA<br>6 mg CQ<br>24 mg 4EDMAB<br>3.03 mg Si/PMMA                  | <b>0.25% Si/PMMA</b><br>1500 mg Bis-GMA<br>1500 mg TEGDMA<br>6 mg CQ<br>24 mg 4EDMAB<br>7.57 mg Si/PMMA                  | <b>0.5% Si/PMMA</b><br>1500 mg Bis-GMA<br>1500 mg TEGDMA<br>6 mg CQ<br>24 mg 4EDMAB<br>15.15 mg Si/PMMA                  |                                                                                                                          |
| <b>c) Composition of Bis-GMA/TEGDMA-Si@MPTS-NPs Dental Filling Composite (wt%)</b>                                      |                                                                                                                          |                                                                                                                          |                                                                                                                          |
| 1500 mg Bis-GMA<br>1500 mg TEGDMA<br>6 mg CQ<br>24 mg 4EDMAB<br>3.03mg Si@MPTS-NPs                                      |                                                                                                                          |                                                                                                                          |                                                                                                                          |
| <b>d) Composition of Bis-GMA/TEGDMA-Si/PMMA/ZnO/CS Dental Filling Composite (wt%)</b>                                   |                                                                                                                          |                                                                                                                          |                                                                                                                          |
| <b>0.1% ZnO/CS</b><br>1500 mg Bis-GMA<br>1500 mg TEGDMA<br>6 mg CQ<br>24 mg 4EDMAB<br>3.03 mg Si/PMMA<br>3.03 mg ZnO/CS | <b>0.25% ZnO/CS</b><br>1500 mg Bis-GMA<br>1500 mg TEGDMA<br>6 mg CQ<br>24 mg 4EDMAB<br>3.03 mg Si/PMMA<br>7.58 mg ZnO/CS | <b>0.5% ZnO/CS</b><br>1500 mg Bis-GMA<br>1500 mg TEGDMA<br>6 mg CQ<br>24 mg 4EDMAB<br>3.03 mg Si/PMMA<br>15.16 mg ZnO/CS | <b>1.0% ZnO/CS</b><br>1500 mg Bis-GMA<br>1500 mg TEGDMA<br>6 mg CQ<br>24 mg 4EDMAB<br>3.03 mg Si/PMMA<br>30.33 mg ZnO/CS |

### 3.3 Characterizations

NPs used in biotechnological applications must be biocompatible, being in harmony with living systems and not showing negative effects. Therefore, it is very important to characterize these NPs and investigate their properties such as size and surface charge, morphology, crystallinity, absorbance, thermal stability, and antimicrobial

effect. The size of NPs is important for biological applications in NP-based systems. As the particle size decreases, the surface area increases and hence the interaction of the particle with the environment also increases. This situation can have advantages as well as disadvantages according to the application area. For example, the uptake of NPs into the cell is highly dependent on particle size. When NPs are large enough, they can be recognized as foreign substances and swallowed by macrophages, but when they are too small, the control of the NPs might be hard and they can reach organs/tissues other than the target ones, causing undesirable effects. Therefore, the desired size of NPs may vary according to the application area.

The characterizations were performed to determine the particle size, surface charge, the morphology, absorbance, and the qualitative structures of synthesized Si-NP, Si@MPTS-NPs, Si/PMMA core/shell ZnO NPs, and ZnO/CS core/shell structure. Besides them, the mechanical tests and antibacterial tests were performed to determine the mechanical strength and antibacterial properties of Bis-GMA/TEGDMA-based dental filling composites. During the characterizations of the NPs, the hydrodynamic particle sizes and surface charges of the synthesized NPs were determined by dynamic light scattering (DLS) and zeta potential ( $\zeta$ -potential) (*Anton Paar Litesizer 500, Particle Analyzer*). Fourier transform infrared spectrometer (FT-IR) (*Thermo Scientific Nicolet is50 FT-IR*) analysis was employed between 400-4500  $\text{cm}^{-1}$  to determine qualitative structural confirmation of synthesized NPs. The morphology and size of the NPs were determined by scanning electron microscope (SEM) (*Carl Zeiss 300VP*). The absorbance of the NPs was measured by UV-visible spectrophotometer (UV-vis) (*Thermo Scientific Evolution 201 UV-Visible Spectrophotometer*) in the region of 280-600 nm prepared. The thermal stability of the NPs was characterized by using thermogravimetric analysis (TGA) (*TA TGA-SDT Q600*) over the temperature range 30–800°C at a rate of 10°C/min under nitrogen atmosphere. Moreover, for the determination of the elemental structure, X-ray photoelectron spectroscopy (XPS) (*Thermo Scientific K-Alpha*) analysis was performed. ZnO and ZnO/CS NPs were analyzed by using X-ray diffraction (XRD) (*Thermo Scientific ARL X'TRA*) with uses Cu ( $K\alpha$ ) radiation ( $\lambda = 0.15418 \text{ nm}$ ). The XRD data were recorded with a 2°/min scanning speed in the range of  $3 \leq 2\theta \leq 90^\circ$ . Moreover, three-point bending mechanical tests of dental filling composites were carried out using a universal tester (*SHIMADZU AG-IC*) with a cross-head speed of 1 mm/min.

### 3.3.1 Dynamic light scattering (DLS) and zeta potential ( $\zeta$ -potential)

Dynamic light scattering (DLS) is a technique used to measure the particle size of colloidal samples in solution, examine formulation stability, and detect the presence of aggregation or agglomeration. The working principle of DLS analysis is to measure the hydrodynamic size of the particles with light scattered from a laser passing through a colloidal solution and analyze the modulation of the intensity of the scattered light as a function of time. The Brownian motion of particles is related to their hydrodynamic diameters. Using the DLS method, it is possible to measure the particle size distribution in the oligomer and submicron size ranges from about 0.3 nm to 10  $\mu\text{m}$ . The results obtained as a result of the measurement are calculated based on the equation below.

$$D = \frac{kT}{6\pi\eta R_h} \quad (1)$$

In the equation, D is the diffusion coefficient, k is the Boltzmann's constant, T is the temperature,  $\eta$  is the solvent viscosity, and  $R_h$  is the rheodynamic radius of the particle solution [159,160]. While the DLS method is insufficient when used alone to determine particle size, it can provide more precise results about the aggregation state and size of NPs when supplemented with data from imaging devices such as transmission electron microscopy (TEM) or scanning electron microscopy (SEM). The  $\zeta$ -Potential is a method used to determine the surface charge and thus the stability of colloidal NPs in the electrolyte solution. The basic principle of this method is that particles with oppositely charged surfaces (counter ions) are attracted, while particles with a similar charge are repulsed. This determines the interaction of NPs with other substances or their susceptibility to aggregation. The  $\zeta$ -Potential can also provide information about surface modifications or core/shell structures depending on the changes in surface charges [161]. During the characterizations of the NPs, the hydrodynamic particle sizes and surface charges of the synthesized NPs were determined by DLS and  $\zeta$ -Potential (*Anton Paar Litesizer 500, Particle Analyzer*) as shown in Figure 3.11. For the DLS characterization, the NPs were dispersed in double distilled water while for  $\zeta$ -Potential analysis, the NPs were dispersed in PBS solution.

The results of DLS and  $\zeta$ -Potential characterization are discussed in the “Results and Discussion” section.

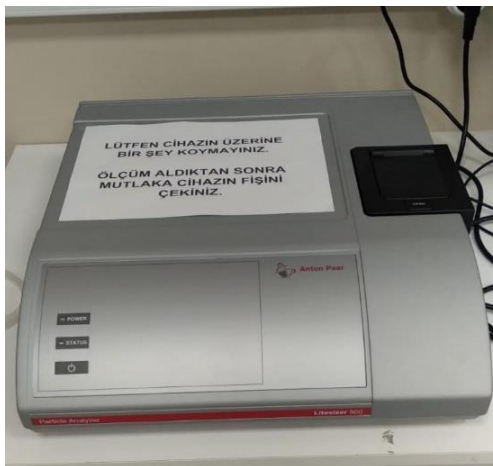


Figure 3.11: Anton Paar Litesizer 500, Particle Analyzer used for DLS and  $\zeta$ -Potential characterizations

### 3.3.2 Fourier transform infrared spectrometer (FT-IR)

Infrared spectroscopy (IR) is an optical technique that enables the identification of compounds by analyzing their chemical structures. Because various functional groups in a molecule absorb IR radiation at different frequencies, the presence of specific chemical functional groups in the molecule can be detected by IR spectroscopy. The spectrum is obtained by passing (reflecting) a beam of IR radiation through the material. The resulting spectrum consists of a plot of absorption, transmission, or reflection intensity versus wavelength or frequency. FT-IR, on the other hand, is one of the most preferred IRs due to its high signal-to-noise ratio, ease of use and relatively low cost [162]. In this thesis, FT-IR (*Thermo Scientific Nicolet iS50 FT-IR*), as can be seen in Figure 3.12, analyses were employed between 400-4500  $\text{cm}^{-1}$  to determine qualitative structural confirmation of synthesized NPs. The results of FT-IR analysis are discussed in the “Results and Discussion” section.



Figure 3.12: Thermo Scientific Nicolet iS50 FT-IR used for FT-IR analysis of synthesized NPs

### 3.3.3 Scanning electron microscope (SEM)

SEM is a common method preferred to observe the micro or nanostructures and morphologies of materials. During SEM imaging, a low-energy electron beam is emitted into the material and scans the surface of the sample. When the beam reaches the material, several different interactions occur at/or near the sample surface, leading to the emission of photons and electrons [162,163]. The morphology and size of the NPs were determined by SEM (*Carl Zeiss 300VP*) which is depicted in Figure 3.13. The results of SEM characterization are discussed in the “Results and Discussion” section.



Figure 3.13: Carl Zeiss 300VP used for SEM characterization of synthesized NPs

### 3.3.4 UV-vis spectroscopy (UV-vis)

UV-vis is a characterization method that measures the absorbance of UV to visible light by a material as a function of the wavelength. The UV region is between 190-350 nm, while the visible region is between 350-800 nm. Absorption occurs during the electronic transition from the ground state to the excited state and its magnitude depends on Beer Lambert's law:

$$A = abc \quad (2)$$

In this equation, A represents absorbance, a is the wavelength dependent absorption coefficient, b represents the path length through the solution in an analytical cell, and c is the molar concentration of the absorbing analyte. The UV-vis method can provide both qualitative and quantitative information about a liquid sample. According to Beer Lambert's law, the UV-vis method can be used for a quantitative analysis since the absorbance of a specific substance in a solution is directly proportional to the concentration of that material [162]. In this thesis, the absorbance of the synthesized NPs was measured by UV-vis (*Thermo Scientific Evolution 201 UV-Visible Spectrophotometer*), as presented in Figure 3.14, in the region of 280-600 nm. The results of UV-vis characterization are discussed in the “Results and Discussion” section.



Figure 3.14: Thermo Scientific Evolution 201 UV-Visible Spectrophotometer used to measure the absorbance of the synthesized NPs

### 3.3.5 Thermogravimetric analysis (TGA)

TGA is a technique used for the measurement of the thermal stability of materials. In this method, the changes in the sample weight with the increase in temperature can be measured. TGA is also used to measure the moisture and volatile content of the sample. The instrument basically consists of two main components: (i) a programmable furnace that controls the heating rate of the sample, and (ii) a highly sensitive balance that is thermally isolated from heat and used to measure weight changes. To maximize the accuracy and precision of weighing, the balance must be thermally isolated. The addition of an IR- spectrometer to the TGA allows for the analysis and identification of gases produced by the decay of the sample. A commercial TGA has a temperature range of  $>1000^{\circ}\text{C}$ , a stability accuracy of  $0.1\text{ }\mu\text{g}$ , and a warming rate that can range from  $0.1$  to  $200^{\circ}\text{C}/\text{min}$  under an atmosphere of room air or other gas [164]. The thermal stability of the NPs was characterized by using TGA (TA TGA-SDT Q600), as depicted in Figure 3.15, over the temperature range  $30\text{--}800^{\circ}\text{C}$  at a rate of  $10^{\circ}\text{C}/\text{min}$  under nitrogen atmosphere. The results of TGA characterization are discussed in the “Results and Discussion” section.



Figure 3.15: TA TGA-SDT Q600 used to measure the thermal stability of the synthesized NPs

### 3.3.6 X-ray photoelectron spectroscopy (XPS)

XPS is a technique for determining the elemental composition, chemical and electronic states of a material. The principle of XPS analysis is to irradiate a sample with X-rays and measure the kinetic energy of electrons emitted from the material under investigation. Surface atoms have characteristic peaks in the XPS spectrum, and the

energy and intensity of these peaks allow identification and measurement of all available surface elements (except hydrogen) [165]. In this thesis XPS (*Thermo Scientific K-Alpha*), as pictured in Figure 3.16, analysis was performed to determine the elemental composition of the synthesized NPs. The results of XPS characterization are discussed in the “Results and Discussion” section.



Figure 3.16: Thermo Scientific K-Alpha XPS device used to determine the elemental composition of the synthesized NPs

### 3.3.7 X-ray diffraction (XRD)

XRD is a technique used to characterize the structure and composition of materials. In the XRD method, a monochromatic X-ray beam is emitted towards the material and the intensity of the refracted beam is measured as a function of the angle of incidence. The intercrystalline spacing ( $d$ ) in the material, is calculated using the formula below.

$$2d = \sin\theta = \lambda \quad (3)$$

where  $\lambda$  is the wavelength of the X-ray used and  $\theta$  is the incident angle. Interatomic plane spacing  $d$  and diffraction angles give various information on material composition and structural properties such as shape and size of the unit cell, width and shifts of diffraction lines, crystallite dimensions and stress [162]. In this thesis ZnO and ZnO/CS NPs were analyzed by using XRD (*Thermo Scientific ARL X'TRA*), as presented in Figure 3.17, with uses Cu ( $K\alpha$ ) radiation ( $\lambda = 0.15418$  nm). The XRD data were recorded with a  $2^\circ/\text{min}$  scanning speed in the range of  $3 \leq 2\theta \leq 90^\circ$ . The results of XRD characterization are discussed in the “Results and Discussion” section.



Figure 3.17: Thermo Scientific ARL X'TRA used to analyze of ZnO and ZnO/CS NPs

### 3.3.8 Three-point bending mechanical test

The three-point bending test is a mechanical test used to measure the Young's modulus of a beam-shaped material. A three-point bending test was performed to determine the mechanical properties of dental composites. For the test, Teflon molds of  $2\text{ mm} \times 2\text{ mm} \times 25\text{ mm}$  prepared according to the ISO 4049-2000 “Dentistry-Polymer-based restorative materials” standard were used. After the reaction, the composite materials poured into these molds were cured under UV light. In order to determine the bending properties of the samples, mechanical tests were carried out with at least three samples for each dental composite sample. Before testing, the samples were kept overnight in the dark in distilled water. The tests were carried out using a universal tester (*SHIMADZU AG-IC*) with a cross-head speed of 1 mm/min. Figure 3.18 shows the three-point bending test, and Figure 3.19 shows the composite parts obtained after the three-point bending test.



Figure 3.18: SHIMADZU AG-IC used to perform three-point bending test of dental filling composites



Figure 3.19: The dental filling composite parts obtained after the three-point bending test

### 3.3.9 *In vitro* antibacterial activity assays

*In vitro* antibacterial activity assays were performed by using *S. aureus* bacteria strain. Bacterial suspensions were taken from at  $-80^{\circ}\text{C}$  stored stock. Subsequently, the bacterial strains were cultivated in tryptic soy agar (TSA) medium and incubated at  $37^{\circ}\text{C}$  overnight. After the incubation, a single colony was isolated, seeded in tryptic soy broth (TSB) and incubated at  $37^{\circ}\text{C}$  overnight followed centrifugation performed. Centrifuged bacteria were suspended in phosphate buffer saline (PBS) made ready for application to be about  $10^8$  CFU/mL (600 nm: 0.6-0.8 optical density). Each application was prepared with diluted to  $10^6$  CFU/mL. Materials and bacteria solution were combined and incubated at  $37^{\circ}\text{C}$  overnight. Then colony counting method was applied to all groups [166]. Figure 3.20

shows the tubes which involve dental filling composites during the *in vitro* antibacterial activity assays.

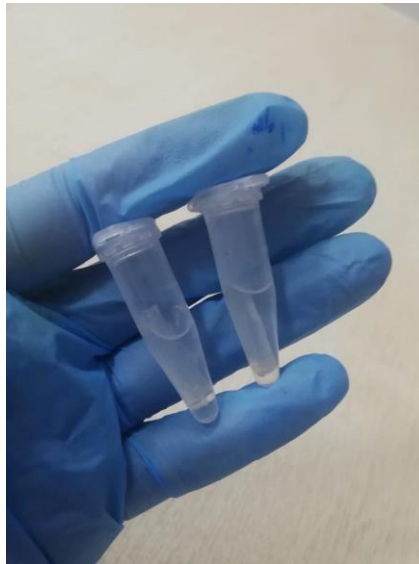


Figure 3.20: Dental filling composites during the *in vitro* antibacterial activity assays against *S. aureus* bacteria strain

## Chapter 4

# 4 Results and Discussion

### 4.1 Dynamic light scattering (DLS) and zeta potential ( $\zeta$ -potential)

DLS and  $\zeta$ -potential analyses belonging to Si-NPs, Si@MPTS-NPs, and Si/PMMA core/shell structure are illustrated in Figure 4.1, while DLS and  $\zeta$ -potential analyses belonging to ZnO NPs and ZnO/CS core/shell structure are illustrated in Figure 4.2. According to DLS and  $\zeta$ -potential analyses of Si-NPs, Si@MPTS-NPs, Si/PMMA core/shell, the hydrodynamic diameter of nonporous Si-NPs and Si@MPTS were around 100 nm [118] and  $\zeta$ -potential values of these NPs were negative (-) and the surface charges of the Si-NPs were approached to neutral after silanization, after pH 7. Despite the fact that the size of these NPs was not significantly altered after silanization, alterations in  $\zeta$ -potential values demonstrate the successful synthesis of the reaction and the presence of the interaction between Si-NPs and MPTS silane agents. On the other hand, since the thickness of the silane layer has a significant effect on the efficiency of the silanization process, and as the silane thickness increases the layers become more irregular and increase the risk of weak bonds by physisorption on the surface of the fillers, the fact that the particle size does not increase much is interpreted as better in terms of the quality of the particles formation [143]. The NPs size of Si/PMMA core/shell structure was obtained at around 250 nm. In terms of the surface charges which were measured at different pH values to observe the changes of the synthesized NPs, the surface charges of Si-NPs and Si@MPTS-NPs were negative (-), whereas the surface charge of Si/PMMA core/shell was positive (+). This is because of the employment of cationic CTAB surfactant as a polymerization reaction surfactant [167]. Because CTAB is a cationic surfactant having (+) charges on the

surface and Si@MPTS-NPs have (-) charges, during the polymerization reaction, the interaction between them was higher.

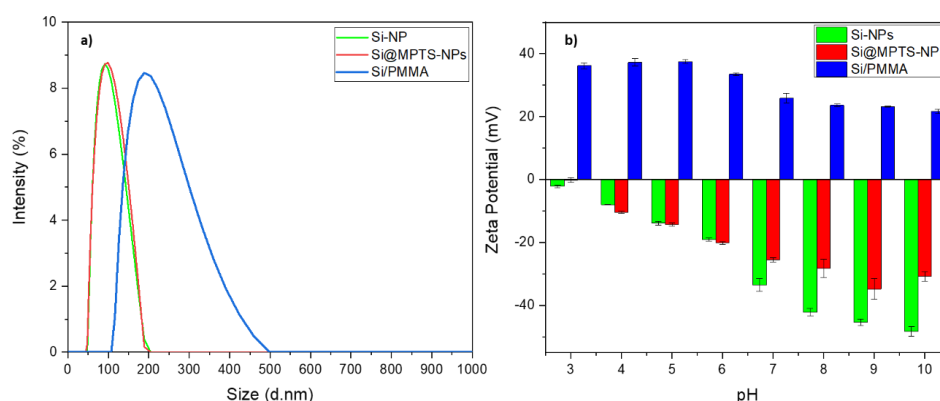


Figure 4.1: (a) the hydrodynamic size and (b)  $\zeta$ -potential of Si-NPs, Si@MPTS-NPs and Si/PMMA core/shell structure

The hydrodynamic diameter of ZnO NPs was around 160 nm whereas the hydrodynamic diameter of ZnO/CS core/shell structure was around 270 nm [156]. The  $\zeta$ -potential of ZnO was (+) at pH 7 and it has continued until pH 10, which is the isoelectric point of ZnO, and then the  $\zeta$ -potential turned (-) at pH 11 and pH 12 [168]. After the core/shell reaction the  $\zeta$ -potential of ZnO/CS decreased since  $\zeta$ -potential of CS is lower than  $\zeta$ -potential of ZnO NPs. This is another evidence for the formation of ZnO/CS core/shell structure [169].

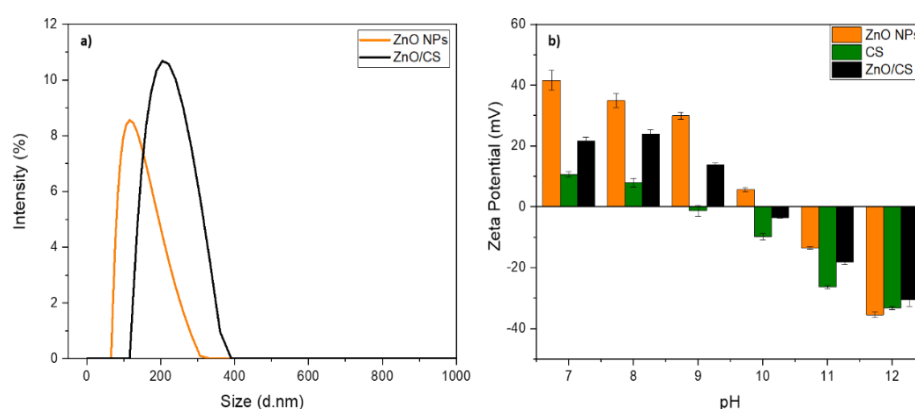


Figure 4.2: (a) the hydrodynamic size and (b)  $\zeta$ -potential of ZnO-NPs and ZnO/CS core/shell structure

## 4.2 Fourier transform infrared spectrometer (FT-IR)

The FT-IR spectra of Si-NPs, Si@MPTS-NPs and Si/PMMA core/shell structure were presented in Figure 4.3 to confirm the qualitative structural confirmation of synthesized NPs. The peak at  $3340\text{ cm}^{-1}$  represents the -OH bonds which is common in the three of the structures. The adsorption band around  $2930\text{ cm}^{-1}$  refers to the stretching vibrations of the C-H bond in the alkyl group of MPTS and MMA which is also considered characteristic peaks of MMA bonds [111,170]. The peaks at  $1734\text{ cm}^{-1}$  and  $1645\text{ cm}^{-1}$  correspond to the stretching vibrations of the C=O and C=C bonds for MPTS and MMA [170,171]. The bonds at  $1053\text{ cm}^{-1}$  are attributed to characteristic Si-O bonds which are found in three of the samples. The peaks at  $787\text{ cm}^{-1}$  and  $580\text{ cm}^{-1}$  refer to C=C bonds as common bonds in all samples.

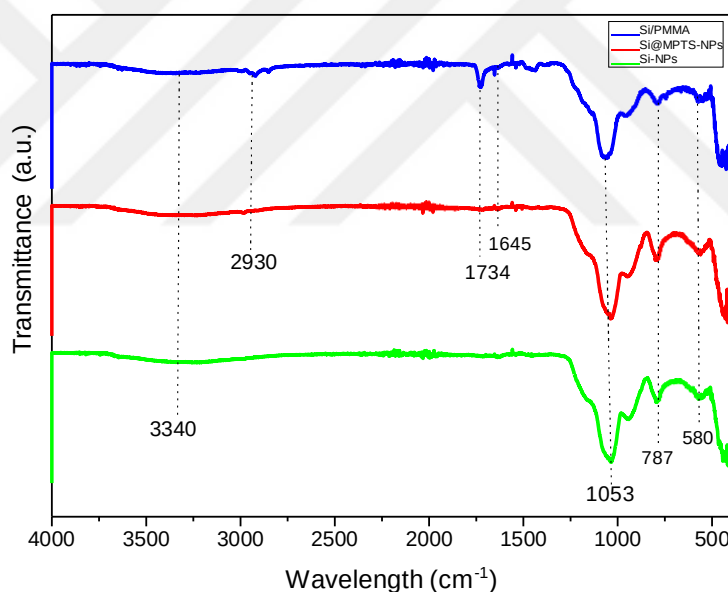


Figure 4.3: FT-IR spectra of Si-NPs, Si@MPTS-NPs and Si/PMMA core/shell structure

FT-IR spectra ZnO NPs, and ZnO/CS structures were demonstrated in Figure 4.4. The peak at  $3378\text{ cm}^{-1}$  represents the O-H stretching which is common in ZnO NP and ZnO/CS core/shell structure. The peak at  $2936\text{ cm}^{-1}$  corresponds to C-H stretching vibration which is another common peak for two samples. The peaks at  $1654\text{ cm}^{-1}$  attributed to C=O stretching vibration as common peaks. The absorption at  $892\text{ cm}^{-1}$  is due to the formation of tetrahedral coordination of Zn. Besides them, as a

characteristic peak of CS, ZnO/CS core/shell structure has a peak at  $1036\text{ cm}^{-1}$  which corresponds to C–O–C bridge stretching [172,173].

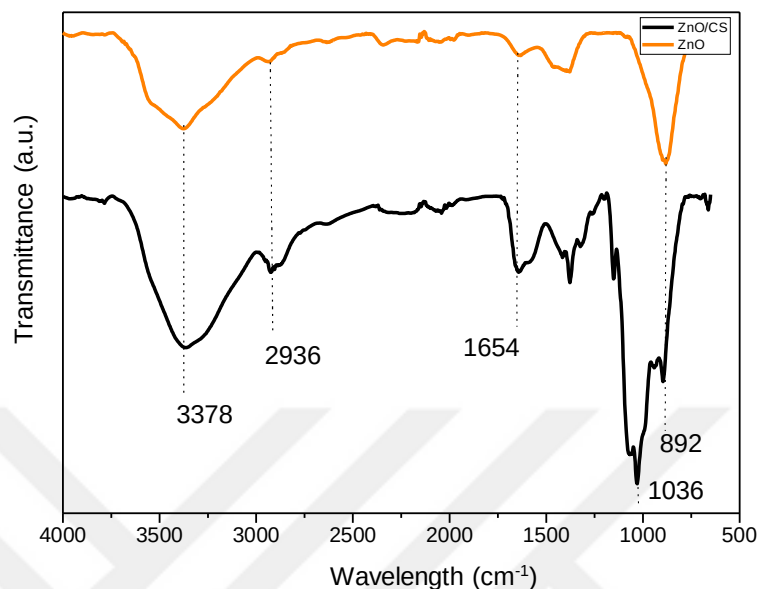


Figure 4.4: FT-IR spectra of ZnO NP and ZnO/CS core/shell structure

### 4.3 Scanning electron microscope (SEM)

The SEM images of Si-NPs, Si@MPTS-NPs, and Si/PMMA core/shell structure depicted in Figure 4.5, the SEM images of ZnO NPs and ZnO/CS core/shell structure depicted in Figure 4.6 and SEM images of Bis-GMA/TEGDMA dental filling composite, Bis-GMA/TEGDMA-Si@MPTS dental filling composite, Bis-GMA/TEGDMA-Si/PMMA dental filling composite, and Bis-GMA/TEGDMA-Si/PMMA/ZnO/CS dental filling composite depicted in Figure 4.7 demonstrated the morphologies of the NPs and composites. According to the SEM image, the NPs based on Si are monodisperse uniform-sized, and have well-shaped spherical structures. These results also supported the DLS analysis in terms of monodispersity. Moreover, bare Bis-GMA/TEGDMA dental filling composite has a smoother surface while after the addition of NPs to the composite, the surfaces have been rougher [2,118,174].

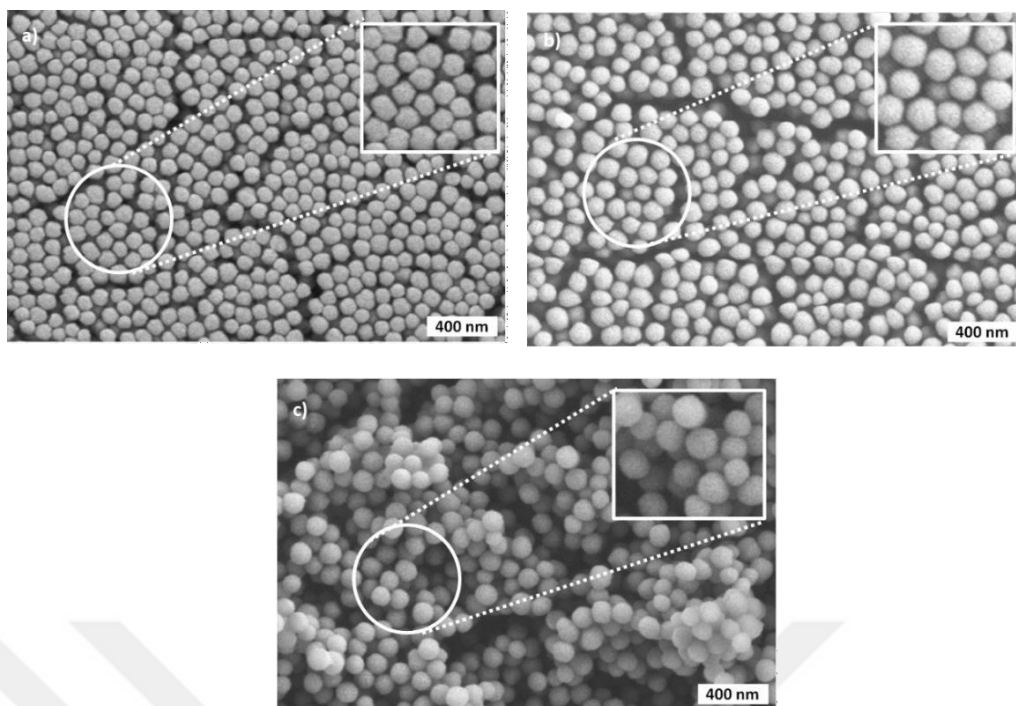


Figure 4.5: SEM images of (a) Si-NPs, (b) Si@MPTS-NPs and (c) Si/PMMA core/shell structure

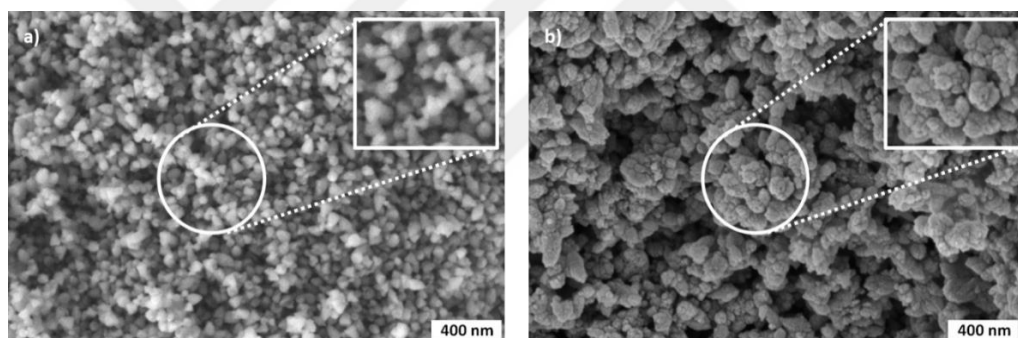


Figure 4.6: SEM images of (a) ZnO NPs, (b) ZnO/CS core/shell structure

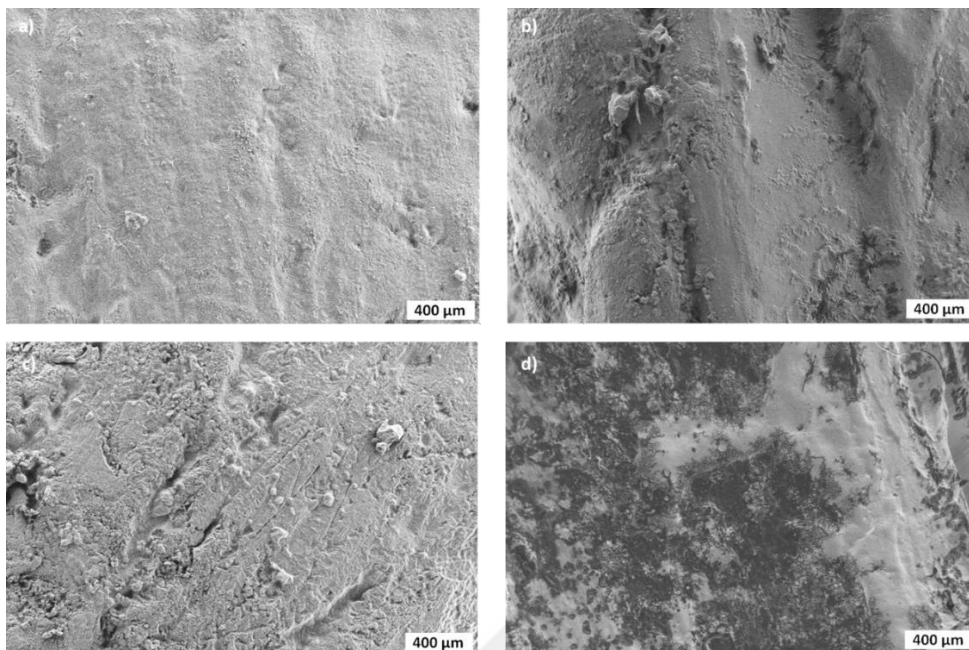


Figure 4.7: SEM images of (a) Bis-GMA/TEGDMA dental filling composite, (b) Bis-GMA/TEGDMA-Si@MPTS dental filling composite (b) Bis-GMA/TEGDMA-Si/PMMA dental filling composite, (d) Bis-GMA/TEGDMA-Si/PMMA/ZnO/CS dental filling composite

#### 4.4 UV-vis spectroscopy (UV-vis)

The UV-Vis spectra of the pristine Si-NPs, Si@MPTS-NPs and Si/PMMA core/shell structure were studied and were presented in Figure 4.8. According to the UV-Vis spectra absorption peaks of the Si-based NPs were 286 nm [175] . The UV-Vis spectra of the ZnO NPs and ZnO/CS core/shell structure were studied and were presented in Figure 4.9. Absorption peak of the ZnO/CS core-shell structure observed at 363 nm [156].

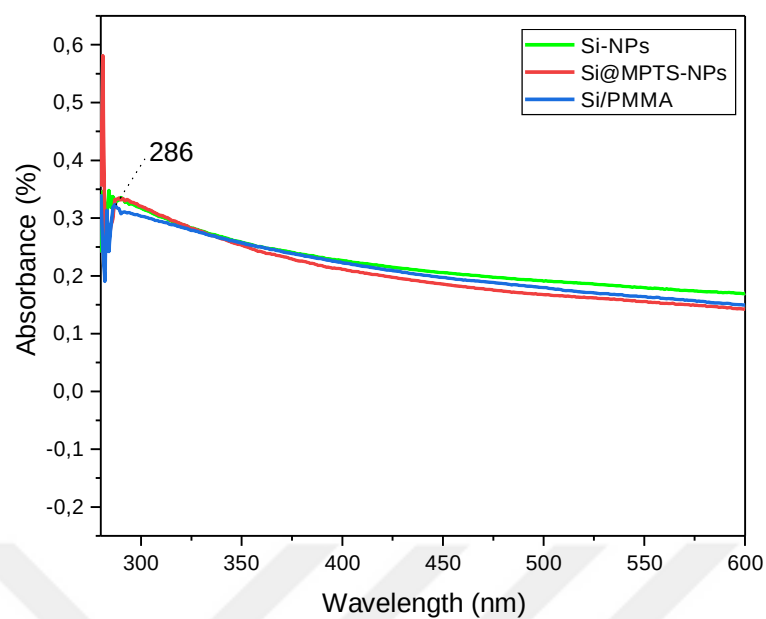


Figure 4.8: UV-Visible analysis of Si-NPs, Si@MPTS-NPs and Si/PMMA core/shell structure

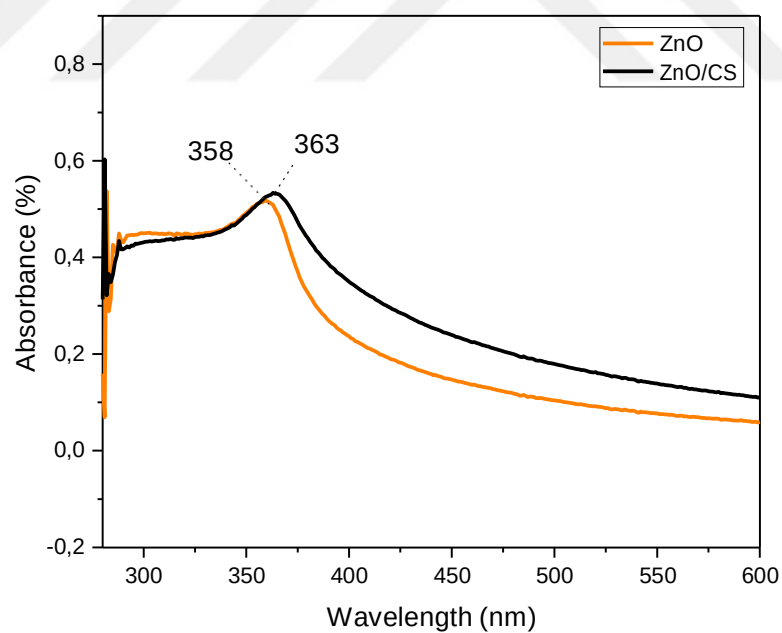


Figure 4.9: UV-Visible analysis of ZnO NPs and ZnO/CS core/shell structure

## 4.5 Thermogravimetric analysis (TGA)

According to TGA analyses of Si-NPs-based structure, in the graphs, there was approximately 5-10% decomposition of Si-NPs and Si@MPTS-NPs at 122°C, and Si/PMMA at 156°C and 186°C as can be seen in Figure 4.10. It could be possible because of the moisture in the materials. Any great decomposition was not observed at increasing temperature, for Si-NPs and Si@MPTS-NPs, which indicates that the structures were homogeneous. At 563°C, a weight loss of about 5% was recorded in Si@MPTS-NPs. High temperature is required to decompose and evaporate the organic content of Si@MPTS-NPs. This indicates that the silane-coupling agent is strongly attached to the surface of the Si-NPs and a covalent bond can be expected [154,176]. Regarding the decomposition of Si/PMMA core/shell structure, a great decomposition of about 35% occurred between 265°C and 410°C. This is considered as the decomposition temperature of PMMA. However, the structure was not completely degraded due to the presence of silica in the structure [111,154].

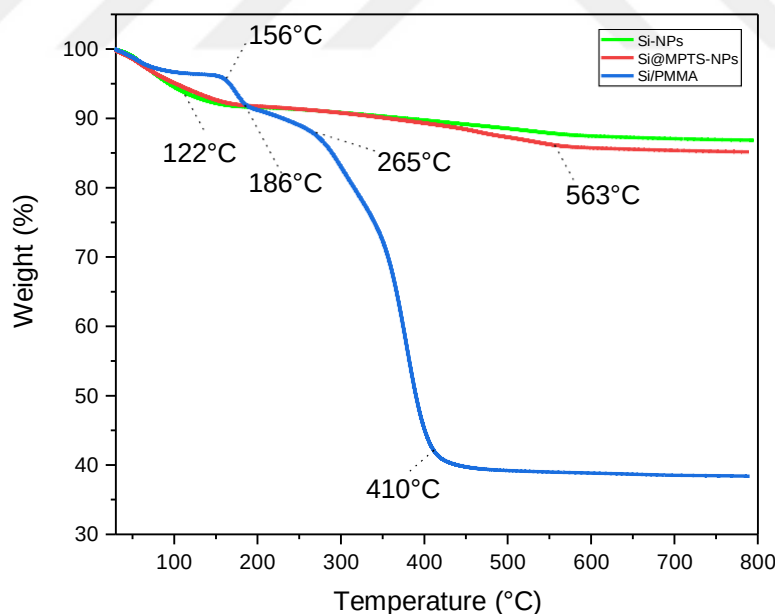


Figure 4.10: TGA analyses of Si-NPs, Si@MPTS-NPs, and Si/PMMA core/shell structure

Regarding TGA analyses of ZnO NPs and ZnO/CS core/shell structure as seen in Figure 4.11, the weight loss of ZnO NPs was less than 5% and the weight remained

constant up to 800°C. Because ZnO NPs presented a homogeneous structure, a much higher temperature was required for the complete dissociation of these NPs. The 5% degradation that occurs was due to the removal of moisture from the surface of the materials. On the other hand, 5% decomposition for ZnO/CS at 100°C was the removal of moisture from the surface as well. The main degradation for this structure started at 263°C and continued up to 600°C and the total weight loss was recorded as around 20% in this range. After 600°C the weight loss of ZnO/CS continued but was not as much as before and was reported as about 5% [173,174].

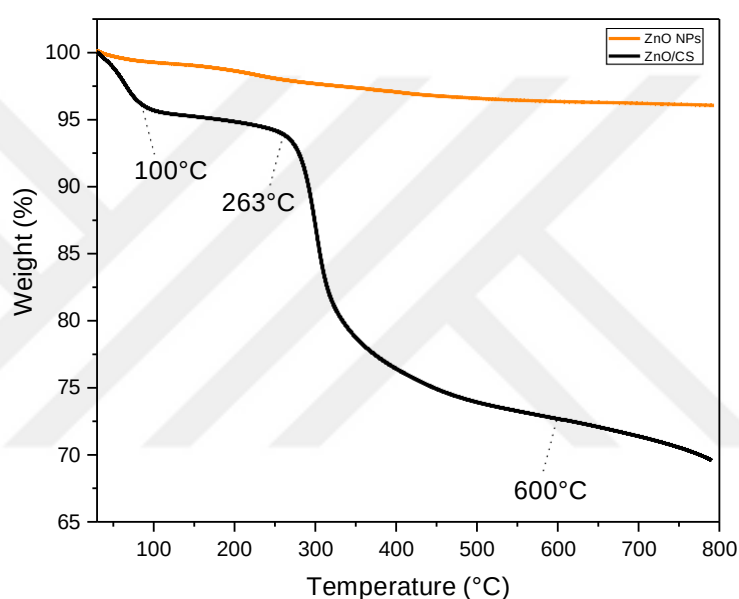


Figure 4.11: TGA analyses of ZnO NPs and ZnO/CS core/shell structure

Moreover, the TGA curves for dental filling composites as bare and prepared with Si@MPTS-NPs, Si/PMMA, and Si/PMMA/ZnO/CS fillers can be seen in Figure 4.12. All samples degraded at the end of the process as the polymeric matrix of the composite was degraded. Initial degradation of all composites continued up to 270°C with 10% weight loss related to the removal of the water on the surface of the composite. On the other hand, the main degradation of all samples started at approximately 270°C and continued to 464°C and 480°C with 90% weight loss. The cause of the degradation was the breaking of bonds in the main chain of the network. The presence of the ZnO/CS core/shell in the dental composite also affected the thermal behavior of the Bis-GMA/TEGDMA matrix. Because, as mentioned above,

the degradation of ZnO/CS core/shell continued after around 600°C, and the weight loss of the dental composite with ZnO/CS continued up to 700°C [2].

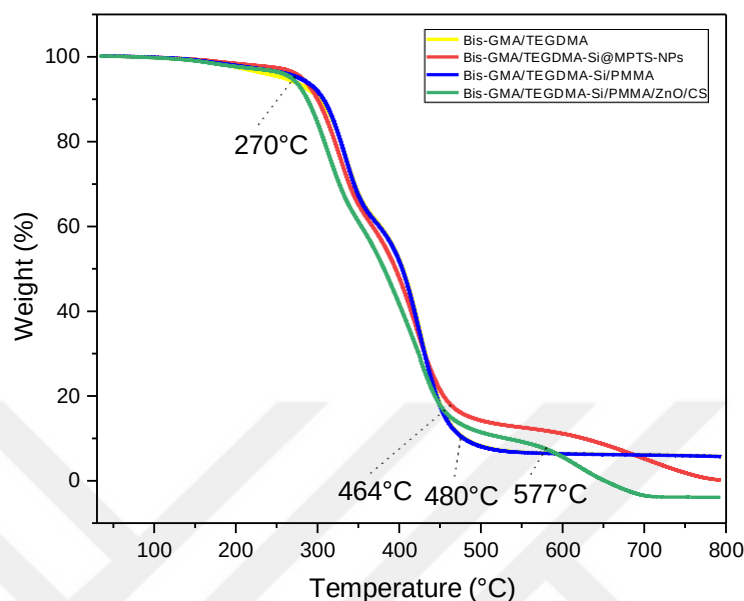


Figure 4.12: TGA analyses for Bis-GMA/TEGDMA, Bis-GMA/TEGDMA-Si@MPTS-NPs, Bis-GMA/TEGDMA-Si/PMMA, and Bis-GMA/TEGDMA-Si/PMMA/ZnO/CS dental filling composites

## 4.6 X-ray photoelectron spectroscopy (XPS)

The wide scan XPS spectra of Si-NPs (a), Si@MPTS-NPs (b), and Si/PMMA core/shell (c) are depicted in Figure 4.13. As can be seen in the graphics, the wide scan XPS spectra of Si/PMMA had the strongest signal appearing, which corresponds to the C–C/C–H aliphatic hydrocarbon at a binding energy of 285 eV. This peak was larger than the C1s signal intensity of Si-NPs and Si@MPTS-NPs and represented the presence of PMMA on the surface of silica. Regarding O1s signal at a binding energy of 533 eV, the O1s signals of Si-NPs and Si@MPTS-NPs are owing to the Si–O bond. This O1s signal of the Si/PMMA was weaker and it corresponded to the carbonyl oxygen (O=C) of MMA. The signal of Si2p of Si-NPs and Si@MPTS-NPs at a binding energy of 103 eV was much stronger than Si/PMMA because Si-NPs were core and they were encapsulated with PMMA shell [154].

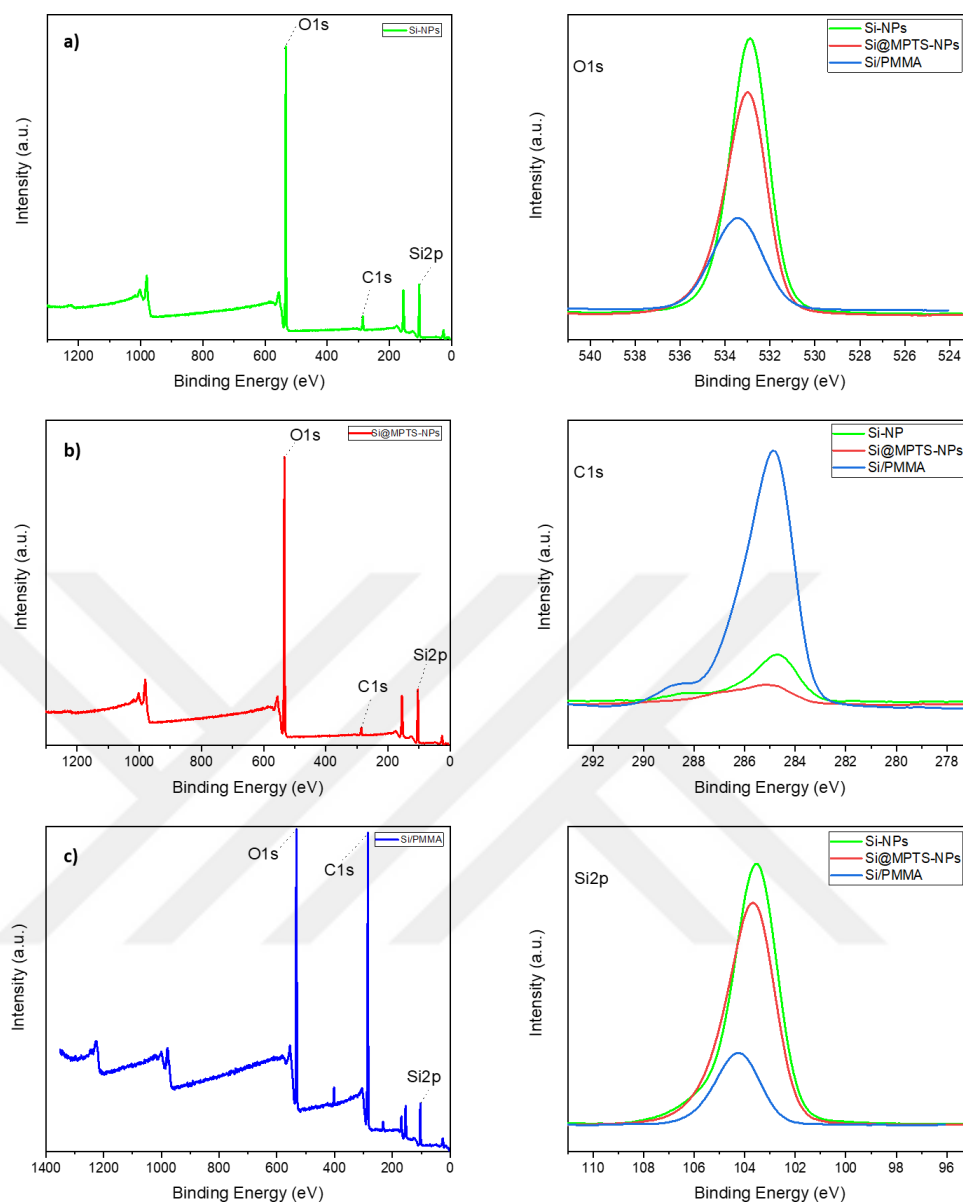


Figure 4.13: XPS spectra of (a) Si-NPs, (b) Si@MPTS-NPs, and (c) Si/PMMA core/shell structure

Moreover, the wide scan XPS spectra of ZnO NPs (a) and ZnO/CS core/shell (b) are depicted in Figure 4.14. The two peaks located at 1022 eV and 1045 eV correspond to the binding energies of Zn2p<sub>3/2</sub> and Zn 2p<sub>1/2</sub> lines, respectively. A single peak of O1s spectrum located at 532 eV can be attributed to O<sup>+2</sup> ions in the ZnO. The C1s spectrum was located at 285 eV and 286 eV [177].

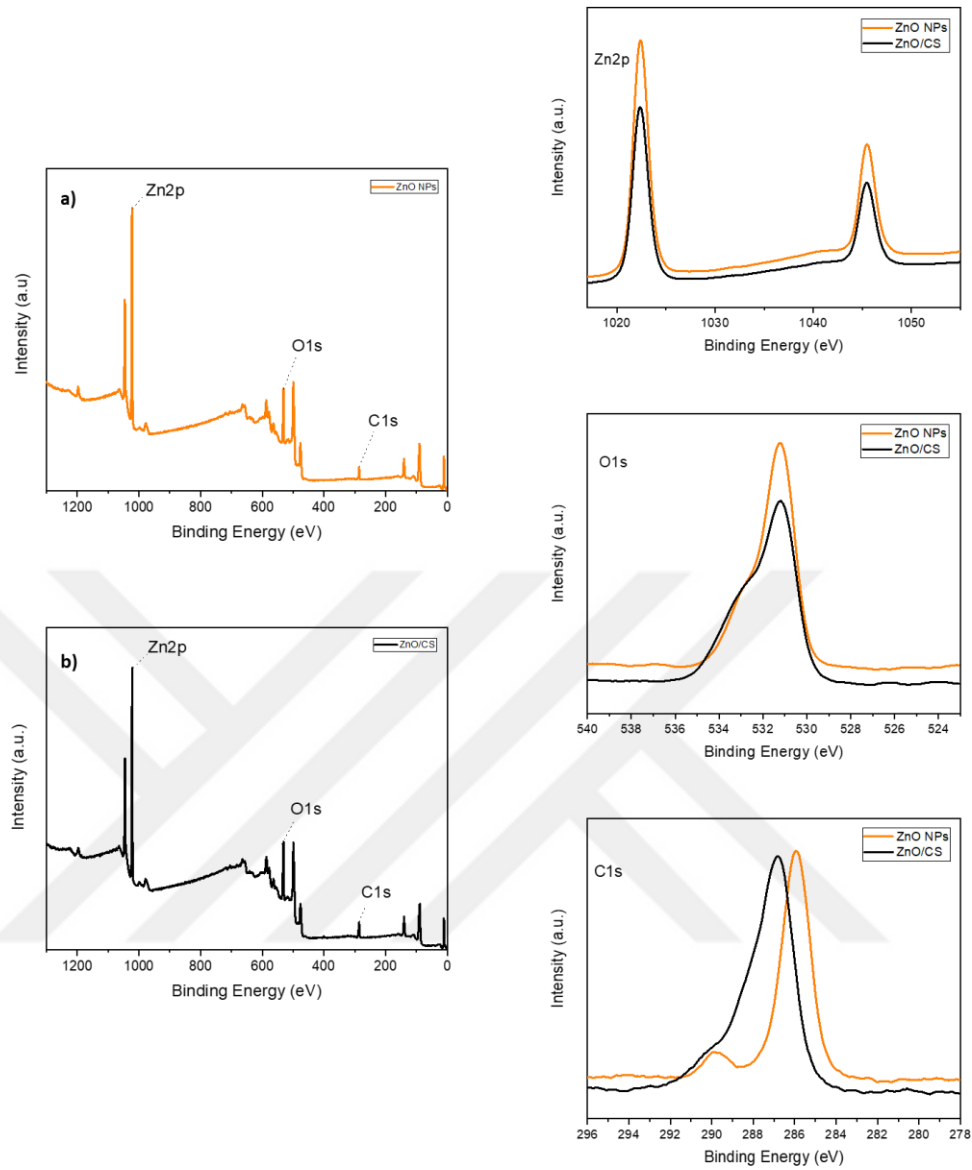


Figure 4.14: XPS spectra of (a) ZnO NPs and (b) ZnO/CS core/shell structure

## 4.7 X-ray diffraction (XRD)

The XRD profiles of ZnO NPs and ZnO/CS core/shell are shown in Figure 4.15. ZnO NPs exhibited strong diffraction peaks at  $31.92^\circ$  (1 0 0),  $34.52^\circ$  (0 0 2),  $36.42^\circ$  (1 0 1),  $47.56^\circ$  (1 0 2),  $56.52^\circ$  (1 1 0),  $62.88^\circ$  (1 0 3) and  $67.98^\circ$  (1 1 2) corresponding to the hexagonal wurtzite structure of ZnO. In addition, the XRD pattern of ZnO/CS core/shell structure showed the main peaks corresponding to ZnO NPs. On the other hand, the intensity of the corresponding peaks of the ZnO/CS core/shell structure was lower compared to the pure ZnO NPs. This is thought to be due to interactions between

ZnO and CS. The crystal structure of ZnO remained intact even after interaction with CS, and the appearance of two sets of diffraction peaks corresponding to ZnO and CS indicated the successful formation of the ZnO/CS core/shell structure. In addition, the absence of peaks corresponding to Zn in the medium indicated that the synthesized ZnO NPs and ZnO/CS core/shell structure were of high purity [156,178]. The characteristic peak of CS [179] is shown by (a) , and the peaks indicated by (b) are the peaks of the ZnO structure.

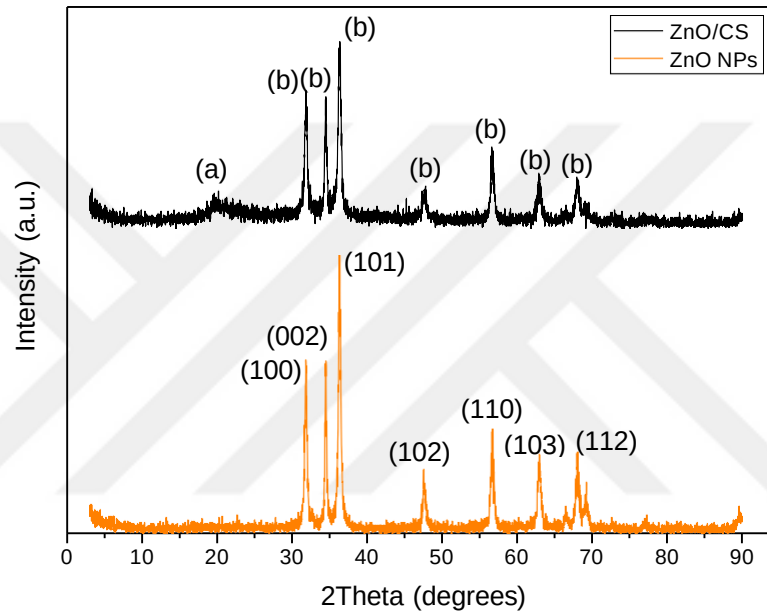


Figure 4.15: XRD spectra of ZnO NPs and ZnO/CS core/shell structure

## 4.8 Three-point bending mechanical test

There are several studies in the literature regarding Bis-GMA/TEGDMA ratios in Bis-GMA/TEGDMA dental composite preparation. For example, Canché-Escamilla et al. [2] used the Bis-GMA/TEGDMA ratio as 40%/60% (w/w), while Amiri et al. [180] used this ratio as 50%/50%. Therefore, in order to compare the effect of the Bis-GMA/TEGDMA ratio on the mechanical properties of the dental composite, a three-point bending test was first performed with the pure Bis-GMA/TEGDMA dental composite material prepared at 30/70%, 40/60% and 50/50% by weight. Si-NP-based or ZnO NP-based materials were not used as additive materials in these composites. As a result of the three-point bending test, both the average flexural strength of 39.80

MPa and the average flexural modulus of 692.07 MPa, the highest mechanical values were obtained in the Bis-GMA/TEGDMA dental composite structure with 50%/50% as can be seen in Table 4.1 and Figure 4.16. The reason for this is thought to decrease the durability of the composite with the decrease of the highly viscous Bis-GMA material. The Bis-GMA/TEGDMA ratio was calculated as 50%/50% weight for all dental composite structures prepared in the next experimental stages of the thesis.

Table 4.1: The average results of three-bending test of pristine Bis-GMA/TEGDMA dental filling composite in terms of flexural strength and flexural modulus

| Flexural Strength (MPa) |         |         |         |
|-------------------------|---------|---------|---------|
| Bis-GMA/TEGDMA (wt%)    | 30%/70% | 40%/60% | 50%/50% |
|                         | 29.85   | 31.39   | 39.80   |
| Flexural Modulus (MPa)  |         |         |         |
| Bis-GMA/TEGDMA (wt%)    | 30%/70% | 40%/60% | 50%/50% |
|                         | 608.67  | 514.50  | 692.07  |

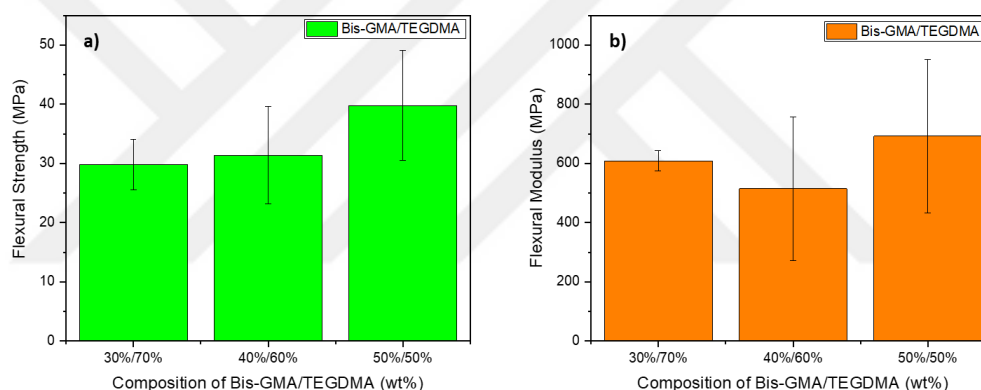


Figure 4.16: The average results of three-bending test of pristine Bis-GMA/TEGDMA dental filling composite in terms of flexural strength and flexural modulus

Later, the Bis-GMA/TEGDMA dental filling composites were prepared at 0.1% Si/PMMA, 0.25% Si/PMMA, and 0.5% Si/PMMA weight ratios. As a result of the three-point bending test, both the average flexural strength of 66.73 MPa and the average flexural modulus of 1091.82 MPa, the highest mechanical values were obtained in the Bis-GMA/TEGDMA dental composite structure with 0.1% Si/PMMA as can be seen in Table 4.2 and Figure 4.17. The Bis-GMA/TEGDMA-Si/PMMA was calculated as 50%50% Bis-GMA/TEGDMA and 0.1% Si/PMMA weight for all dental composite structures prepared in the next experimental stages.

Table 4.2: The average results of three-bending test of Bis-GMA/TEGDMA dental filling composite at 0.1% Si/PMMA, 0.25% Si/PMMA, and 0.5% Si/PMMA ratio in terms of flexural strength and flexural modulus

| Flexural Strength (MPa)      |              |               |              |
|------------------------------|--------------|---------------|--------------|
| Bis-GMA/TEGDMA-Si/PMMA (wt%) | 0.1% Si/PMMA | 0.25% Si/PMMA | 0.5% Si/PMMA |
|                              | 66.73        | 44.25         | 44.86        |
| Flexural Modulus (MPa)       |              |               |              |
| Bis-GMA/TEGDMA-Si/PMMA (wt%) | 0.1% Si/PMMA | 0.25% Si/PMMA | 0.5% Si/PMMA |
|                              | 1091.82      | 980.77        | 983.18       |

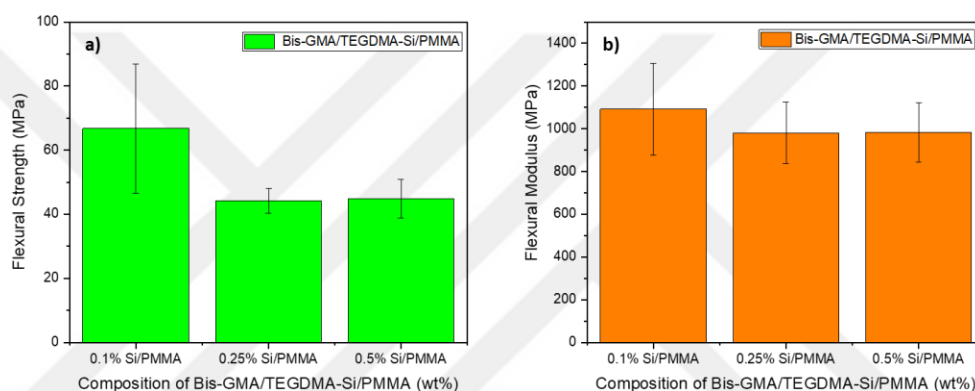


Figure 4.17: The average results of three-bending test of Bis-GMA/TEGDMA dental filling composite at 0.1% Si/PMMA, 0.25% Si/PMMA, and 0.5% Si/PMMA ratio in terms of flexural strength and flexural modulus

After that, the Bis-GMA/TEGDMA-Si/PMMA dental filling composites were prepared at weight ratios of 0.1% ZnO/CS, 0.25% ZnO/C, 0.5% ZnO/CS, and 1.0% ZnO/CS. As a result of the three-point bending test, both the average flexural strength of 58.05 MPa and the average flexural modulus of 1077.75 MPa, the highest mechanical values were obtained in the Bis-GMA/TEGDMA-Si/PMMA dental composite structure with 0.5% ZnO/CS as can be seen in Table 4.3 and Figure 4.18. Both flexural strength and flexural modulus increased by increasing of ZnO/CS core/shell additives until 0.5% ratio. And then the mechanical properties decreased as a result of increasing the additive ratio.

Table 4.3: The average results of three-bending test of Bis-GMA/TEGDMA-Si/PMMA dental filling composite at 0.1% ZnO/CS, 0.25% ZnO/C, 0.5% ZnO/CS, and 1.0% ZnO/CS ratio in terms of flexural strength and flexural modulus

| Flexural Strength (MPa)             |                             |                              |                             |                             |
|-------------------------------------|-----------------------------|------------------------------|-----------------------------|-----------------------------|
| Bis-GMA/TEGDMA-Si/PMMA/ZnO/CS (wt%) | 0.1% Si/PMMA<br>0.1% ZnO/CS | 0.1% Si/PMMA<br>0.25% ZnO/CS | 0.1% Si/PMMA<br>0.5% ZnO/CS | 0.1% Si/PMMA<br>1.0% ZnO/CS |
|                                     | 32.36                       | 40.31                        | 58.05                       | 28.18                       |
| Flexural Modulus (MPa)              |                             |                              |                             |                             |
| Bis-GMA/TEGDMA-Si/PMMA/ZnO/CS (wt%) | 0.1% Si/PMMA<br>0.1% ZnO/CS | 0.1% Si/PMMA<br>0.25% ZnO/CS | 0.1% Si/PMMA<br>0.5% ZnO/CS | 0.1% Si/PMMA<br>1.0% ZnO/CS |
|                                     | 538.83                      | 772.14                       | 1077.75                     | 810.89                      |

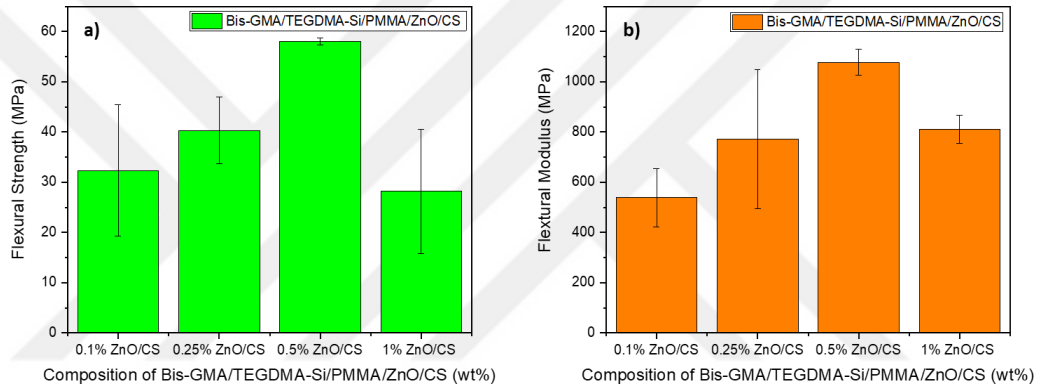


Figure 4.18: The average results of three-bending test of Bis-GMA/TEGDMA-Si/PMMA dental filling composite at 0.1% ZnO/CS, 0.25% ZnO/C, 0.5% ZnO/CS, and 1.0% ZnO/CS ratio in terms of flexural strength and flexural modulus

Finally, the contributions of the Si/PMMA core/shell structure and the ZnO/CS core/shell structure to the mechanical properties of dental composite were evaluated. For this evaluation, pristine Bis-GMA/TEGDMA structure without additives, Bis-GMA/TEGDMA-Si/PMMA with 0.1% Si/PMMA and Bis-GMA/TEGDMA-Si/PMMA/ZnO/CS with 0.1% Si/PMMA and 0.5% ZnO/CS structures were compared mechanically as seen in Table 4.4 and Figure 4.19. According to the three-point bending test results in terms of flexural strength, the Bis-GMA/TEGDMA-Si/PMMA composite showed the best performance with 67.66% increasing compare to the pristine Bis-GMA/TEGDMA composite. The presence of ZnO/CS structure decreased the flexural strength of the composite with 14.95% decreasing compares to Bis-GMA/TEGDMA-Si/PMMA, but there was 45.85% increasing in flexural strength compared to the pristine Bis-GMA/TEGDMA composite. On the other hand,

according to the three-point bending test results in terms of flexural modulus, Bis-GMA/TEGDMA-Si/PMMA composite showed 57.76% increasing compare to the pristine Bis-GMA/TEGDMA composite. The presence of ZnO/CS structure decreased the flexural modulus of the composite with 1.31% decreasing compare to Bis-GMA/TEGDMA-Si/PMMA composite, but there was 55.72% increase in flexural modulus compared to the pristine Bis-GMA/TEGDMA composite.

Table 4.4: The average results of three-bending test of pristine Bis-GMA/TEGDMA, Bis-GMA/TEGDMA-Si/PMMA, and Bis-GMA/TEGDMA-Si/PMMA/ZnO/CS

| Flexural Strength (MPa)         |                                      |                                                     |
|---------------------------------|--------------------------------------|-----------------------------------------------------|
| Bis-GMA/TEGDMA<br>50%/50% (wt%) | Bis-GMA/TEGDMA<br>0.1% Si/PMMA (wt%) | Bis-GMA/TEGDMA<br>0.1% Si/PMMA 0.5%<br>ZnO/CS (wt%) |
| 39.80                           | 66.73                                | 58.05                                               |
| Flexural Modulus (MPa)          |                                      |                                                     |
| Bis-GMA/TEGDMA<br>50%/50% (wt%) | Bis-GMA/TEGDMA<br>0.1% Si/PMMA (wt%) | Bis-GMA/TEGDMA<br>0.1% Si/PMMA 0.5%<br>ZnO/CS (wt%) |
| 692.07                          | 1091.82                              | 1077.75                                             |

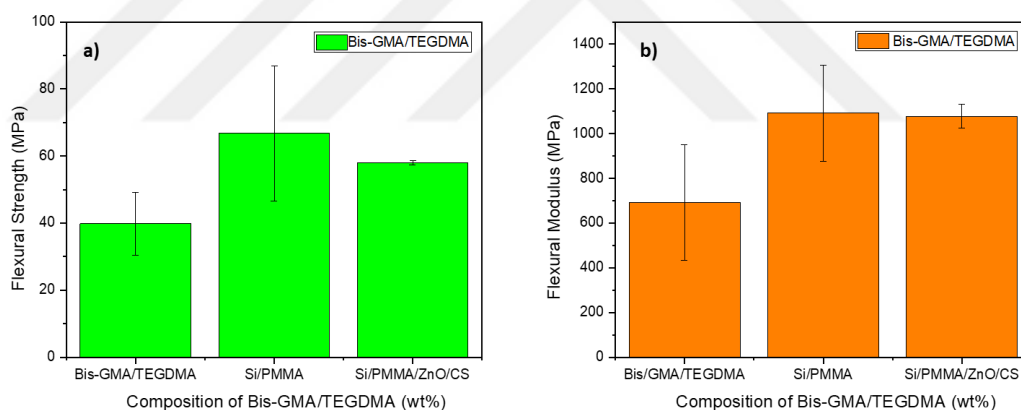


Figure 4.19: The average results of three-bending test of pristine Bis-GMA/TEGDMA, Bis-GMA/TEGDMA-Si/PMMA, and Bis-GMA/TEGDMA-Si/PMMA/ZnO/CS

## 4.9 *In vitro* antibacterial activity assays

*In vitro* antibacterial activity assays of Si-NPs, Si/PMMA core/shell structure, ZnO NPs, and ZnO/CS core/shell structure against *S. aureus* strain are shown in Figure 4.20. During the antibacterial test, powder NPs have prepared in 0.25mg/mL concentrations, they have performed the *S. aureus* strain. Both Si-NPs and Si/PMMA core/shell structures have shown antibacterial activity against *S. aureus* bacteria strain

at 67.31% and 84.62%, respectively. Even though the antibacterial properties of Si-NPs are not common in the literature, Agnihotri et al. reported that Si-NPs have slightly antibacterial activity against *S. aureus* bacteria [181]. In addition, Si/PMMA core/shell structure has shown antibacterial activity and the results are similar to Sodagar et al' study. In their study, they evaluated the antibacterial effect of PMMA with SiO<sub>2</sub> NPs and TiO<sub>2</sub> NPs against Gram-positive bacteria and they indicated that Si/PMMA structure has 51% antibacterial activity against *Streptococcus mutans* and 67% antibacterial activity against *Lactobacillus acidophilus* after 90 mins incubation period [182]. In this thesis study, antibacterial activity of Si-NPs and Si/PMMA core/shell structures resulted from the differences of surface charge of NPs and cell membrane charge of *S. aureus*. Since the surface charge of Si-NPs is around -30mV and the surface charge of Si/PMMA core/shell structure is around +30mV at pH 7, while the surface charge of the cell membrane of *S. aureus* is (-), there have been surface charge interactions between the cell membrane and NPs. This interaction has been higher between Si/PMMA and *S. aureus* resulting in higher antibacterial activity.

Apart from the antibacterial activity of Si-based NPs, as can be seen in Figure 4.20 (b), ZnO NPs and ZnO/CS core/shell structure had considerably antibacterial activity against *S. aureus* at 98.1% and 92.31%, respectively. In the literature, there are various studies reporting the antibacterial activity of ZnO NPs and ZnO/CS core/shell structure. For example, Yusof et al. performed a zone inhibition test and they reported that ZnO NPs had 16 mm zone diameter against *S. aureus* [183]. In another study, ZnO/CS nanostructure had 42 mm zone inhibition at 20ml/0.05wt% concentration whereas it had 40.33 mm zone inhibition at 60ml/0.05wt% concentration against *S. aureus* [184]. Li et al. reported that the inhibition zone diameter increased against *S. aureus* by increased ZnO NPs content in the ZnO/CS nanostructure [185]. In this thesis study, this antibacterial activity resulted from the differences in surface charge of NPs and cell membrane charge of *S. aureus*. Since the surface charge of ZnO NPs was +40 mV and the surface charge of ZnO/CS core/shell structure was +20mV, ZnO NPs demonstrated more antibacterial activity than ZnO/CS core/shell structures against *S. aureus*.

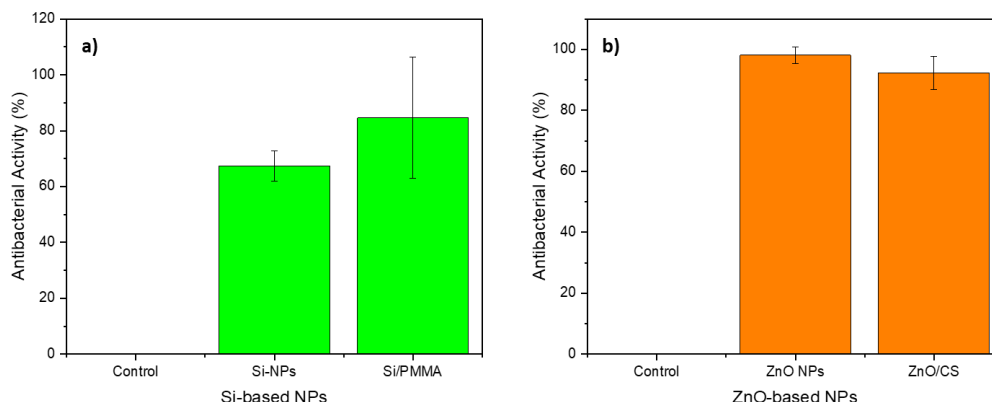


Figure 4.20: Antibacterial test results of (a) Si-NPs and Si/PMMA core/shell structure, (b) ZnO NPs and ZnO/CS core/shell structure against *S. aureus* strain

Moreover, antibacterial activity of dental filling composite was given in Figure 4.21. In comparison with the control group, Bis-GMA/TEGDMA dental composite showed a slightly antibacterial effect at 31.71% against *S. aureus*. De Mori et al. reported that PMMA-only cements showed low antibacterial effect against *S. aureus* [186]. Since this value was low, it might be because of environmental effect or effect of MMA and it can be ignorable. In addition, since Si/PMMA core/shell structure had antibacterial effect, Bis-GMA/TEGDMA-Si/PMMA dental composite showed an antibacterial effect of 55.92% against *S. aureus* strain. Bis-GMA/TEGDMA-Si/PMMA/ZnO/CS dental composite showed the highest antibacterial effect with an effect of 94.83% against *S. aureus* strain. According to the antibacterial results, it was observed that the antibacterial properties of the dental composite increased even more due to the antibacterial properties of the ZnO/CS core/shell structure.

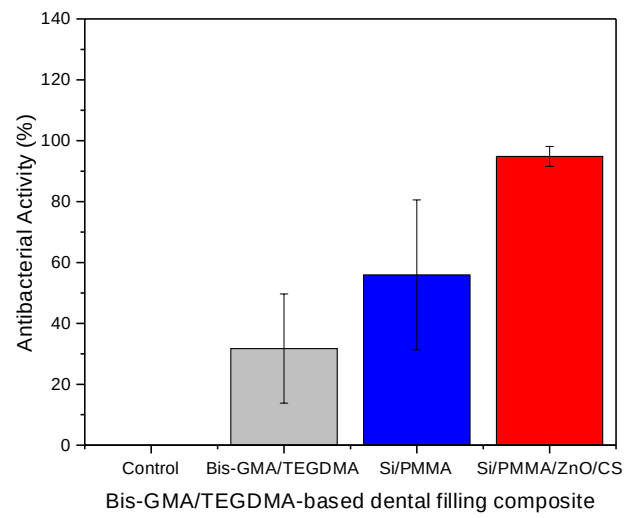


Figure 4.21: Antibacterial test results of Bis-GMA/TEGDMA dental filling composite, Bis-GMA/TEGDMA-Si/PMMA dental filling composite, and Bis-GMA/TEGDMA-Si/PMMA/ZnO/CS dental filling composite against *S. aureus* strain

# Chapter 5

## 5 Conclusion

Within the scope of the thesis study, Si-NPs, ZnO NPs and their Si/PMMA, and ZnO/CS core-shell nanostructures were successfully synthesized, and characterized. The synthesized Si/PMMA and ZnO/CS core/shell nanostructures were added to the commercial Bis-GMA/TEGDMA (50%/50%) dental filling composite as an inorganic filling material at a rate of 0.1 wt% to 1.0 wt% and a novel hybrid dental filling composite was produced. The mechanical and antibacterial properties of the novel dental filling composite were investigated. In this context, briefly the following results were obtained.

- ✓ The synthesized Si/PMMA core/shell nanostructures were added to commercial Bis-GMA/TEGDMA (50%/50%) dental filling composite at 0.1 wt%, 0.25wt%, and 0.5wt%. The mechanical properties of the obtained novel composites were evaluated by a three-point bending mechanical test. According to the result, Bis-GMA/TEGDMA dental composite with 0.1% Si/PMMA additive presented the highest values in terms of flexural strength (66.73 MPa) and flexural modulus (1091.82 MPa). Si/PMMA additive ratio was accepted as 0.1% in all dental composite studies carried out after that.
- ✓ ZnO/CS core/shell structure was combined with the Bis-GMA/TEGDMA-Si/PMMA dental composite at the ratios of 0.1%, 0.25%, 0.5%, and 1.0% by weight, and three-point bending mechanical tests were performed. The results showed the highest values in terms of flexural strength (58.05 MPa) and flexural modulus (1077.75MPa) with the Bis-GMA/TEGDMA-Si/PMMA dental composite that contain 0.1% Si/PMMA and 0.5% ZnO/CS additive.
- ✓ In addition to the mechanical evaluation, Bis-GMA/TEGDMA-Si/PMMA/ZnO/CS dental composite that contain 0.1% Si/PMMA and 0.5%

ZnO/CS, showed the highest antibacterial effect with an effect of 94.83% bacterial death in antibacterial tests performed against *S. aureus*.

In conclusion, Bis-GMA/TEGDMA-Si/PMMA/ZnO/CS composite that contain 0.1 wt% Si/PMMA and 0.5 wt% ZnO/CS presented the best results for both improved mechanical properties and antibacterial effect. It is believed that with the introduction of such new generation dental filling composites in the clinical applications, they may reduce chewing-induced fractures and cracks, prevent decay due to their strong antibacterial properties, and thus, dental filling composites may become more user-friendly.



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2019–2022 Izmir Kâtip Çelebi University, Department of Nanoscience and Nanotechnology

## Work Experience:

2015–2016 Coordinator of BioForm III Conference by IEEE YTU Student Branch  
2016–2017 Corporate Relations Coordinator at IAESTE YTU Local Committee  
2017–2017 Virology Laboratory Intern in University of Tartu/ Estonia 2017–  
2018 Student Assistant in Yıldız Technical University  
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2018 Management Intern in Viking Chemistry and Cosmetics Company  
2018-2018 Tissue Engineering Laboratory Intern in İzmir Institute of Technology  
2018–2019 Microbiology Laboratory Intern in West Pomeranian University of Technology in Szczecin/Poland (IAESTE International Exchange Internship Program)  
2019–2019 Molkim Industrial Products Company- R&D Engineer  
2021–2022 Ege University- R&D Engineer  
2022–2022 Universidad Complutense de Madrid: Scientific Researcher (Erasmus + Internship Mobility Program)

## Publications:

1. Preparation and Characterization of Chitosan-Based Hydrogels Reinforced by Mesoporous Silica Nanoparticles (MSN) - presented at 4th International Students Science Congress, 18-19 Sep 2020, Izmir Katip Celebi University

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5. Book chapter named "Biyosinyaller ve Biyomedikal Algılayıcılar"-Submitted
6. Book chapter named "Graphene-Metal Oxide-Based Nanocomposite for Electrochemical Sensors"-Submitted

