Effectiveness of Se/ZnO NPs in enhancing antibacterial activity of resin based dental composite

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ABSTRACT

Biofilm formation in the resin-composite interface is a major challenge of resin based dental composites that may lead to secondary caries formation. The use of nanoparticles (NPs) to enhance the antibacterial properties of resin composites are highly desired. The present study focused on effectiveness of Se doped ZnO (Se/ZnO) NPs as an antibacterial nanofiller and its impact of mechanical properties of the resin composites. Pristine and Se/ZnO NPs were synthesized by the mechanochemical method, and confirmed through UV-Vis Spectroscopy, FTIR (Fourier Transform Infrared) analysis, X-ray Diffraction (XRD) crystallography, Scanning Electron Microscopy (SEM), Energy Dispersive Spectroscopy (EDS), and Zeta analysis. The resin composites were then modified by varying concentrations of pristine and Se/ZnO NPs. For the antibacterial analysis, a single specie (*S. mutans* and *E. faecalis*) and a saliva microcosm model was utilized. Hemolytic assay and compressive strength tests were also performed to test the cytotoxicity and mechanical strength of modified composite resin.

1% Se/ZnO NPs when incorporated in composite resin showed higher antibacterial activity, higher biocompatibility, and higher mechanical strength when compared to composites with 1% ZnO NPs. Mechanical strength was unaffected by addition of Se/ZnO NPs. Furthermore, the hemolytic activity was also within the safe limit. Se/ZnO NPs can be used as efficient antibacterial nanofiller for resin composites and are effective in preventing secondary caries formation.

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CHAPTER 1

1. INTRODUCTION

Secondary carries are almost like the primary carries in appearance and usually present on the gingival side of the tooth (Mjör & Toffentti, 2000). Carries formation on the interface of the restorative material is usually because of the occurrence of gaps between the tooth and the restorative material (van de Sande et al., 2014). Failure of restorative material because of the occurrence of secondary carries leads to the replacement of restorative material which is very costly (Mjör & Toffentti, 2000). The estimated annual cost of restorative materials in 2005 in the US was $46.2 billion and it was expected to reach $49.7 billion which is a huge cost (Beazoglou et al., 2007). Affecting 35% of the global population (Bhadila et al., 2020).

For such purposes composite resin has gained some advancement and popularity in recent years due to its physical and mechanochemical properties (Dias et al., 2019). That means the restorative material used should be aesthetically appealing and should have good mechanical and chemical binding properties with the enamel and dentin. Different composites have their specific pros and cons (Hamouda & Elkader, 2012). Gap formation is one of the major concerns faced by the composite-based filling which allows the cariogenic biofilm to enter the interface of dentine and composite resin eventually resulting in the formation of secondary carries (Maske et al., 2019). Secondary carries limit the life of composite resin which needs immediate replacement (Han et al., 2021). Biofilm accumulation tendency in composite resin is relatively higher than in other restorative materials, which increases the demand for some antibiotic-induced composites to completely inhibit or lower the biofilm accumulation rate (Arun et al., 2020) (Han et al., 2021). Bacteria responsible for biofilm formation produce acid on the surface of a tooth by the process of carbohydrate fermentation. Acid production causes the demineralization of the calcified tissues of the tooth, ruining the tooth structure (Han et al., 2021) (Larsen & Fiehn., 2017) (Cocco et al., 2015).

The field of biomaterials is trying to find the solutions to such problems faced by individuals. There are many proposed strategies that are being practiced to overcome this problem (van de Sande et al., 2014). One of them is to incorporate some antibiotics in the dental restorative

materials which will reduce the adherence of pathogens on the surface of these materials which are involved in the formation of biofilm (Nedeljkovic et al., 2020).

The idea of incorporating antibiotics in the dental restorative materials specifically in the composite resin was to overcome the problems faced by biofilm accumulation because the inhibition of acid enforced demineralization can enhance the life of composite resin (Tarle et al., 2019) (Cocco et al., 2015). Hence, the chance of occurrence of recurrent carries will eventually be reduced (Kattan et al., 2015a). The antibacterial properties of many metal oxides including silver, gold, titanium, and zinc have been tested. For this purpose, the field of nanotechnology has played a very important role. The incorporation of these metal oxides in form of nanoparticles has been proven the best approach so far. (Lee et al., 2020) (Elumalai & Velmurugan, 2015).

Incorporated metals that alter the physical and mechanical properties of composite resin are not appreciated (Bayne et al., 2019). Silver metal shows higher antibacterial activity without altering the significant mechanical properties, but it imparts color to the composite resin changing its physical properties which is why it is usually restricted to the posterior dental fillings (Cocco et al., 2015) (Kattan et al., 2015b). Silver metal also releases its ions in the environment exhibiting its antibacterial properties, but the release of these ions can be toxic to human health also making it less promising in long-lasting antibacterial activity. It is observed that the release of ions can also alter the mechanical properties of the composite resin. (Han et al., 2021) (Arun et al., 2020) (Cocco et al., 2015). Expecting to increase the life of composite resin the ideal choice of nanosized metal oxide particles will be the one that will restore or enhance its physical, chemical, and mechanical properties (Bayne et al., 2019) (Aydin Sevinç & Hanley, 2010).

ZnO is expected to have such properties along with antibacterial properties which will make it more favorable to use in nanotechnology to overcome the problem of secondary carries (Lee et al., 2020) (Han et al., 2021). Incorporating ZnO nanoparticles in commercially available composite resin, exhibit antibacterial properties against oral bacterial that involves in biofilm accumulation, by releasing Zn^{+2} ions in the interface of the tooth and composite resin after the gap formation by the polymerization of composite resin which will inhibit the biofilm accumulation in the interface, thus preventing the secondary carries formation (Aydin Sevinç & Hanley, 2010).

The ion shedding ability of ZnO-NPs does not allow them to undergo prolonged antibacterial activity (Arun et al., 2020) (Moradpoor et al., 2021). These Zn^{+2} ions released by composite are not toxic to human cells but show higher toxicity against the bacterial cells (Moradpoor et al., 2021) Hence, the doping of another metal on these ZnO-NPs is required to make them long-lasting and promising antibacterial agents. The doping technique is specifically used to enhance the biological activities of any agent. In the case of ZnO-NPs doping of different metals on it exhibits its long-term activity and stability. In this study, selenium metal is selected as a doping agent. Because the antibacterial properties of Se-NPs in an antibiofilm study are observed against *S. mutans* which is one of the oral bacteria involved in dental infection. (Haris & Khan, 2017). Selenium metal along with ZnO showed enhanced antibacterial activity by oxidative stress by releasing reactive oxygen species in the environment disrupting the bacterial cell wall causing the release of cellular organelle in the environment and eventually the breakdown of protein, nucleic acid, and enzymes. They have also shown induced apoptosis in the bacterial cell by the Se-based nanoparticles (Nair et al., 2011).

This study was designed to synthesize and characterize Se doped ZnO nanoparticles (Se/ZnO NPs) and to incorporate them in composite resin to enhance the stability and prolonged antibacterial activity against oral microorganisms through the in vitro antibacterial and antibiofilm models. hemolysis and mechanical strength of Se/ZnO NPs was also tested.

1.1. Objectives

Objectives of this research are

Synthesis of Se doped ZnO NPs.

Characterization of prepared Se/ZnO NPs to confirm the synthesis.

Composite resin modification by incorporating the prepared Se/ZnO NPs.

Antibacterial activity evaluation of Se-doped ZnO NPs against oral bacterial strains.

Mechanical properties evaluation of Se-doped ZnO modified resin based dental composites.

Biocompatibility testing of modified composite resin.

CHAPTER 2

2. LITERATURE REVIEW

2.1. Primary caries

The term “dental caries” was derived from Latin word “Caris” means rotting and was first used in 1634. Due to the limited information available on the disease occurrence and cause of the disease it was used to describe the hole in tooth earlier. In 5000 BC it was believed that these holes in teeth caused by some “tooth worm”. Later, the studies found that tooth decay is the common cause of caries formation. Damage to the “enamel” which is the outer most protective layer of tooth led to the formation of dental caries. Enamel is damaged because of the presence of acid that is produced by the oral bacteria. Lactic acid is produced and cause damage to the first protective layer of tooth. Once the enamel is damaged the tooth is more prone to the damage. The untreated damage led to the formation of dental caries which initially starts with discomfort that is sensitivity, irritation, bacterial infection and eventually the tooth loss (Conrads, 2018).

“Primary caries” can be defined as the most common prevalent conditions affecting people worldwide and can strike anyone at any time. Dental caries are the acidic metabolic byproduct of microbial fermentation of food ingredients that cause the localized damage to the delicate dental tissues (Selwitz et al., 2007). These localized damages grow over time as a result of highly complex interaction between the microorganisms that produce acid, simple carbohydrates, and multiple host components including teeth and saliva. This condition affects both the crowns and the roots of teeth, and it can also begin as a serious case of tooth decay in newborns and in the toddlers' primary teeth in the first few months of life. The combination of biological, psychological, and socioeconomic factors has historically been connected to this widespread infectious disease. It affects around 40–50% of people in developed countries (Vergnes et al., 2012).

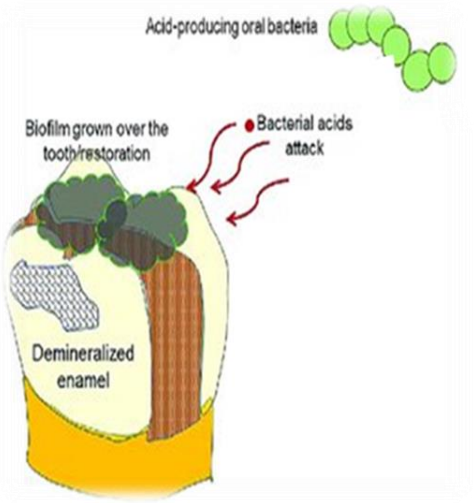

Figure 1: Tooth decaying as a result of biofilm formation

2.1.1. Pathophysiology

Acid-producing bacteria, a precursor that the pathogens can use, and a range of host components, including teeth and saliva, work together to generate dental caries. Dental caries develops when the physiological equilibrium between oral bacterial biofilms and dental nutrition is interrupted. On teeth, there are biofilm communities of bacteria that are trapped in the polysaccharide, enzyme, and DNA matrix material that was released by the cells. This organic matrix gives the bacteria better antibiotic resistance while shielding them from host defenses, infections, and evaporation(Selwitz et al., 2007). The formation of caries lesions is influenced by the presence of fermentable carbohydrates, as well as other environmental factors, microbes, and host factors. The two most significant acidogenic bacteria to date are Streptococcus mutans and Enterococcus faecalis. The faecalis participate as boosters, with the mutans acting as sort of initiators. Certain specific environmental factors, such as the presence of dietary carbohydrates that can be fermented and a lack of oxygen, act as stimulants for microbial activity. A weak 12 redox potential and exposure to reduced carbohydrates, especially sucrose, are two environmental conditions that promote the growth and metabolism of these organisms. Fermentation and acid production happen quickly when oxygen levels are low and sugar levels are high (Conrads, 2018). The most important mediator of caries is an acid result of plaque biofilm metabolism. At first, plaque acid destroys the calcium hydroxyapatite particles on crystalline surfaces and prisms rich in carbonate. Despite the lesion front moving quickly, the lesions interface appears to remain

intact mostly due to reprecipitation of dissolved crystals helped by higher fluoride surface concentrations (Robert et al., 2007).

2.2. Stages of caries progression

The development of dental plaque is crucial to tooth decay. Plaque is a white, sticky film that develops on the surfaces of the teeth. This material is composed of saliva, food particles, and microbes. If you don't brush your teeth frequently, plaque may accumulate on them. Tartar can develop because of it’s hardening over time. Tartar could help keep bacteria around longer, making it more difficult to get rid of them. In general, tooth decay occurs in five stages.

Stage 1: Dental demineralization

Enamel is a type of tissue that often serves as the teeth's outer layer. Enamel, the toughest tissue in our bodies, is primarily made of another mineral. Unfortunately, the enamel tends to lose such minerals when teeth are exposed to the acids produced by plaque bacteria. If it occurs, there can be a white spot on one of the teeth. The earliest indication of tooth decay is the loss of minerals in this area.

Stage 2: Enamel degradation

If this dental caries was allowed to worsen, the enamel would continue to deteriorate. Over time, a white spot may darken and take on a brownish hue. Dental caries, or tiny holes in the teeth, can develop as the enamel deteriorates. A dentist will then fill the cavities.

Stage 3: Dentin degradation

In fact, the layer under the enamel is called dentin. Due to its lesser weight than enamel, it is more susceptible to acid erosion. As a result, once tooth decay has reached the dentin, it advances more swiftly. Additionally, the dentin contains tubes that join the fibres of the tooth. Patients may experience sensitivity when the dentin is affected by dental caries as a result. When consuming hot, cold, or soft drinks or food, this is most apparent.

Stage 4: Pulp decaying

A tooth's pulp is only its deepest surface. It contains the nerves and blood vessels that keep these teeth healthy. The nerves in the pulp provide teeth their sense of touch. 14 This pulp irritates when it is damaged, which causes it to start swelling. Stress on the nerves occurs from the supporting tissues' inability to expand to counteract the swelling. This might make you uncomfortable.

Stage 5: Dental Abscess

As tooth decay advances, bacteria can enter the pulp and cause an infection. An abscess is a pus-filled pocket that develops at the root of a tooth because of heightened dental inflammation.

Pain from abscessed teeth may radiate to the jaw and be terrible. Possible symptoms include fever, swollen lymph nodes in the neck, and swelling of the gums, cheeks, or jaw. Because the infection could spread to the jaw bones and eventually to other areas of the head and neck, the tooth abscess needs to be treated right away. Additionally, the injured tooth may need to be extracted in some cases (Carrasco, 2022).

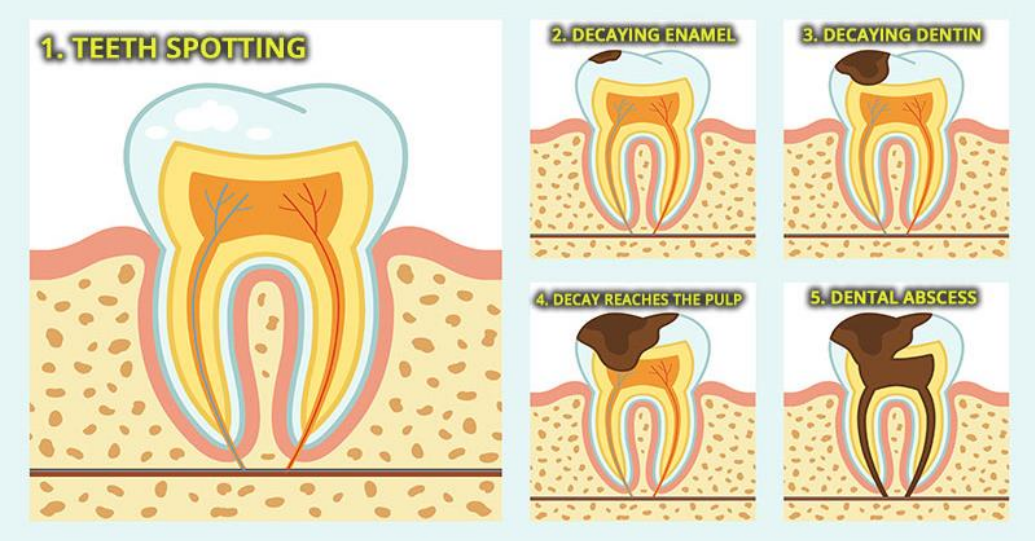

Figure 2: Dental carries progression (D.D.S, 2020)

2.3. Biofilm formation and Secondary Caries

Secondary carries are almost like the primary carries in appearance and usually present on the gingival side of the tooth (Mjör & Toffentti, 2000). The development of tiny fracture spots between fillings and tooth tissue, which enable saliva entry, results in secondary caries. Secondary caries results from cariogenic bacteria in saliva attacking tooth tissues and surface when the environment of the micro fissures is more favourabl (Feng, 2014). Microbe biofilms adhere to all tissue surfaces in the mouth cavity. These bacterial biofilms are what lead to the decay of specific tooth structures. Additionally, they affect how long dental restorations last (Engel et al., 2020). After the filling had been in place for a while, secondary caries started to grow on the tooth as a result of biofilm formation. Additionally, this is the main reason why dental restorative materials fail. Regardless of the filler substance used, they cannot entirely be avoided. The percentage of secondary caries is significant after dental fillings. The primary issue here is secondary caries, which is caused by a highly complex interaction between damaged soft 19 tissue, underlying biofilms that frequently preserve the microbial conditions that ultimately caused the primitive lesion, and orthodontic biomaterials that may help, or even make things worse (Brambilla & Ionescu, 2021).

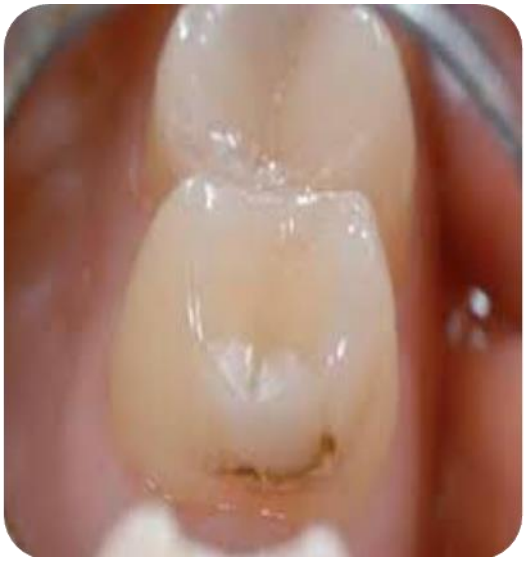

Figure 3: Secondary caries appearance

2.3.1. Epidemiology

The diagnostic standards can change from study to study, making it difficult to compare the prevalence of dental caries around the world. The frequency and severity of adult caries have, however, declined in many wealthy countries over the past few decades. Additionally, the rate at which illnesses advance slows down as people age. The interproximal and smooth parts of permanent teeth have seen a greater reduction in caries than the decaying or occlusal areas. In different demographic groups, the prevalence and severity of dental caries may have moderately increased or maintained (Pitts, 2004; Selwitz et al., 2007; *Visual and Visuo-Tactile Detection of Dental Caries - A.I. Ismail, 2004*, n.d.). Five times more common than asthma, caries is one of the most common chronic paediatric disorders in the country. Since more people are maintaining their teeth longer, dental caries has become more prevalent among the elderly both locally and globally (Guiguimde et al., 2014). In elderly individuals, new caries production may be comparable to or larger than in younger individuals. The average American loses five teeth by the age of 35 and has an additional 11 teeth that are damaged by caries, making this the most common dental condition in the US and one of the main causes of tooth loss in young adults (Anderson, 2002).

Risk factors

Although most risk factors appear to be dynamic, a person's risk for caries may change over time. Enamel degradation is caused by a few physiological and chemical factors, including insufficient salivary flow and composition, numerous microbial infections, insufficient fluoride exposure, gingival recession, immunological elements, the need for specialized medical care, and hereditary factors (Featherstone et al., 2003).

2.4. Available treatments

By having routine dental exams, you can find cavities and other oral problems before they worsen or start to cause troublesome symptoms. The greater the chance of reversing and avoiding tooth deterioration in its early stages, the sooner people seek treatment. A cavity won't need much therapy if it is treated before it becomes more irritating. Cavity therapy is often based on the severity of the cavity and the situation. Dental fillings are the most often utilized treatment option. Dental fillings, sometimes referred to as restorations, become the main treatment choice when a decay has progressed past its initial stage. These fillings are made of a variety of

materials, including some porcelain fillings, some tooth-colored resin composites, and a combination of elements known as dental amalgam (Zero et al., 2009).

2.4.1. Dental fillings

Fillings are frequently used to repair teeth, such as those that are broken or shattered, that have already been worn down by usage. To fill the area where the rotting material was extracted, the dentist would first remove the decaying portion of the tooth. Dental filling materials come in a wide range today. There are several materials available for filling teeth, including gold, porcelain, silver amalgam (mercury coupled with metal, lead, zinc, and copper), tooth-colored polymers, and resin composites. Another material made up of glass particles is glass ionomer. The use of this compound is analogous to that of composite resin fillings. The location and severity of the degradation, as well as the cost of the filling product, decide the type of filling that is appropriate (Bhaduri & Bhaduri, 2009).

2.4.2. Pros and Cons of various dental fillings

- **Gold fillings**

Gold fillings are significantly more expensive yet endure more than 10 years and do not corrode. They cost almost ten times as much as standard dental fillings. If they come into contact with silver, they can provide an electric shock to the lips. This is due to saliva's ability to transmit current between the two metals in the mouth cavity, which results in a sudden shock (Hanson & Pleva, 1991).

- **Amalgams fillings**

Amalgam fillings have been in use for more than a century and are stronger and more long-lasting than gold fillings. However, they have a few flaws, like the fact that they don't adhere to teeth well, requiring a full cavity to be cut by the dentist to keep them in place. While amalgam fillings are less expensive overall than gold fillings, they also discolor tooth structure, giving it a greyish appearance. Additionally, it increases teeth's sensitivity to both hot and cold food. Since amalgam fillings include mercury, many patients develop adverse reactions to it (Rangreez & Mobin, 2019).

- **Fillings made of porcelain**

The most frequent fillings are made of porcelain, which resists stains better than resin composite fillings. This material might last longer than ten years and cost as much as gold. More abrasion

resistance than any other dental filling is provided by porcelain or ceramic fillings. These fillings have the disadvantage of being brittle and are typically utilized in large cavities to prevent fracture (Kelly & Benetti, 2011).

- **Ionomer Glass fillings**

It is made of a specific kind of glass and acrylics. The most frequent applications for this substance are fillings that extend below the gum line. Glass ionomers emit fluoride, which may help shield teeth from further deterioration. However, this material is more susceptible to stress and breakage and is weaker than resin composites (Sidhu & Nicholson, 2016).

Figure 4: Types of dental fillings

2.4.3. Resin Based-Dental Composite materials

Dental composites, also referred to as resin-based composite materials, are synthetic polymers that combine a mineral- or resin-based filler-particle-and-short fibre mixture with a polymeric substrate via coupling agents. Similar to dental amalgam, this essentially serves to replace tooth structure that has been lost due to trauma, caries, or other illnesses. Additionally, composites can be used to adhere dentures and crowns, among other things. As amalgam is phased out in dentistry, composite materials have emerged as one of the most often used cosmetic restoration options. It closely resembles the appearance of teeth and is made of a mixture of plastic and powdered glass. Resin composite fillings are used to change the colour of the teeth, restore decayed teeth, heal fractured teeth, close gaps between teeth, and level out the teeth's look. The dentist will prepare the resin and apply it on the tooth. Using a certain curing light, each layer is made harder. The dentist will shape the composite to fit within the tooth when it has dried and hardened. The composite material is then polished and smoothed to prevent stains and early wear (Xu et al., 2019)

Types of dental composites

Types There are two types of dental composites

i. Self-cure (chemically activated) resins.
ii. Light-cure (photochemically activated) resins.

- **Self-cure resins**

The self-cure resins are delivered as two pastes that must be combined to begin polymerization. Air may indeed be absorbed into the mixture during blending, degrading the material, and thus the operator does not have control over the working period after mixing.

- **Light-cure resins**

The photosensitive activator system and a source of light are used to activate the light-cure resin, which comes as a single paste. It doesn't require mixing, making it tougher and less damaging, and it has a completely modifiable working interval (Xu et al., 2019).

2.4.4. Chemical composition of dental composites

Inorganic filler particles are coated in silane and dimethacrylate resin, typically bisglycidil methacrylate (BISGMA) or urethane dimethacrylate (UDMA), to create dental composites. A portion of a lower-molecular-weight monomer, like triethyleneglycol dimethacrylate (TEGDMA), may be added to lessen viscosity. Filler materials like barium silicate glass, quartz, or zirconium silicate are frequently combined with 5% to 10% of extremely small (0.04-m) colloidal silica particles. In modern dental composite materials, quartz or porcelain particles are dispersed throughout a photopolymerizable synthetic resin matrix. In order to make the resulting glass radiopaque to x-rays, the polymer elements are combined with a sharply split inorganic substance, such as barium aluminosilicate glass or another crystal composition that has a suitable quantity of radiopaque oxide (Hervás García et al., 2006). Dental composites made with resin fall into two groups based on its handling and filler content. Viscous and having a range of filler sizes, lightweight composites are strong. There are several flowable composites, such as the HEMA monomer, that can reduce viscosity without reducing the filler loading (Pratap et al., 2019).

2.4.5. Constraints

Due to their close resemblance to real teeth in terms of colour and look, dental composite resins are more aesthetically pleasing. Additionally, since they require less drilling, less tooth enamel must be removed. Unlike other materials, they harden quickly and create a solid bond with the tooth that keeps it from shattering. Finally, they can be repaired if they are damaged. However, the development of biofilm over restorative materials that results in secondary caries is a problem that is seen as the failure of the restoration. Due to this issue, nanoparticle modification of composite resins is favoured (Erickson, 2017).

2.5. Nanotechnology in dental treatments

New dental restorative materials with improved properties and anticaries potential are being created using nanotechnology. Dental resin composites contain nanoparticles as efficient caries control measures. They have a wide variety of uses in both research and innovation, particularly in the creation of innovative materials. They are typically designed with distinctive qualities that primarily attract material scientists and biologists. Nanomaterials have a lot of potential for limiting demineralization, re-mineralizing the tooth structure, and minimizing the growth of biofilms as well as for battling bacteria that cause caries (Melo et al., 2013). The goal of restorative dentistry is to diagnose and cure diseases that damage the tooth and the tissues that are connected to it. Advanced operations are necessary for restoring dental function, enhancing aesthetics, and replacing damaged tooth structures. The creation of biodegradable and mostly harmless resin composites has tremendously benefited from nanotechnology. Dental composite materials consisting of resin have advanced significantly in recent years. Surface hardness and mechanical properties like minimum polymerization shrinkage and strong abrasion resistance can be improved by adding nanoparticles to resin composites (Barot et al., 2021).

2.5.1. Modification of dental composite resin into nanofillers for treatment of Secondary Caries

Multiple strategies are proposed to overcome the problem of secondary caries (van de Sande et al., 2014). One of them is to incorporate antibiotics in the dental restorative materials which will reduce the adherence of pathogens on the surface of these materials which are involved in the formation of biofilm (Nedeljkovic et al., 2015).

These antibiotics limits biofilm accumulation, hence controlling the acid enforced demineralization, that eventually enhances the life of composite resin (Cocco et al., 2015; Tarle et al., 2019). Hence, the chance of occurrence of secondary carries will eventually be reduced (Kattan et al., 2015a). For this purpose, the field of nanotechnology has played a very important role (Elumalai & Velmurugan, 2015; Lee et al., 2020).

2.5.2. Metal oxide based nanofillers

The in-corporation of metal oxides in THE form of NPs has been proven the best approach so far (Elumalai & Velmurugan, 2015; Lee et al., 2020). Multiple antibacterial nanoparticles of many metal oxides including silver (Ag), gold (Au), titanium (Ti), and zinc (Zn) have been tested.

Zinc Oxide (ZnO) NPs has been reported as an effective antibacterial metal oxide NPs (Han et al., 2021; Lee et al., 2020). It has been reported as an antibacterial nanofiller for dental composites to prevent secondary caries formation(Han et al., 2021; Lee et al., 2020). Incorporating ZnO NPs in commercially available composite resin, exhibit antibacterial properties against oral bacterial that involves in biofilm accumulation, by releasing Zinc ions (Zn+2) in the interface of the tooth and composite resin after the gap formation by the polymerization of composite resin which will inhibit the biofilm accumulation in the interface, thus preventing the secondary carries (Aydin Sevinç & Hanley, 2010).

2.5.3. Limitations of metal oxide NPs incorporated composite resin

It is reported that ZnO NPs have limited antibacterial activity at lower concentrations, whereas increasing the concentration deteriorates the mechanical properties of the composites (Arun et al., 2020; Moradpoor et al., 2021). Hence, the doping of another element on these ZnO NPs is required to enhance the antibacterial potential of the NPs. The doping technique is specifically used to enhance the biological activities of any agent. In the case of ZnO-NPs doping of different metals on it exhibit its long-term activity and stability (Nair et al., 2011)

2.5.4. Doping of metalloid (Se) in ZnO nanoparticles

Selenium metalloid (Se) is a metalloid with high antibacterial potential (Ghaderi et al., 2022). Se metal was selected based on its antioxidant properties, antiviral properties, mainly due to antibacterial properties. Se is a metalloid with nutritional properties in human diet in small

amounts which makes it more biocompatible to human cells. Se is also an anti-inflammatory agent which helps in wound healing or speeding up the process.

Figure 5: Properties of Se metalloid

2.5.5. Reactive Oxygen Species and doping

ZnO nanoparticles have a specific antibacterial impact on microorganisms, just like silver nanoparticles do. Gram-positive and gram-negative bacteria can both be sterilized by ZnO nanoparticles. ZnO nanoparticles have only minor effects on human cells while being selectively harmful to microorganisms. Reactive oxygen species and the alteration of cell membrane activity are connected to its process. Microorganism growth can be inhibited by active chemicals like H_2O_2 produced by ZnO nanoparticles. The penetration of Zn^{2+} into cell medium, which lowers the formation of biofilms by inhibiting the active transport and metabolism of carbohydrates, and the replacement of magnesium to disrupt the enzyme system which is essential for the enzyme activity of oral biofilms is another potential antibacterial mechanism of ZnO. It has a more effective sterilizing impact on streptococci than silver nanoparticles. The correct nanofiller content, however, is crucial, and 10% ZnO nanoparticles used in dental composite resin can efficiently kill bacteria, according to studies. Further study is needed to concentrate on

maximizing antibacterial activity by managing the quantity of dental composite resins in order to maintain original mechanical properties (Liu et al., 2021).

Doping enhances the antibacterial activity by enhancing the release of ROS. Two metals will act on enhanced release of the ROS side by side. The doped NPs attack the cell by three different ways; by releasing the ROS, by altering the membrane permeability to inhibit the enzyme activity and cellular metabolic activities, by releasing metal ions in the environment that will affect the pH of the membrane and enter the cell and induce apoptosis (Ghaderi et al., 2022).

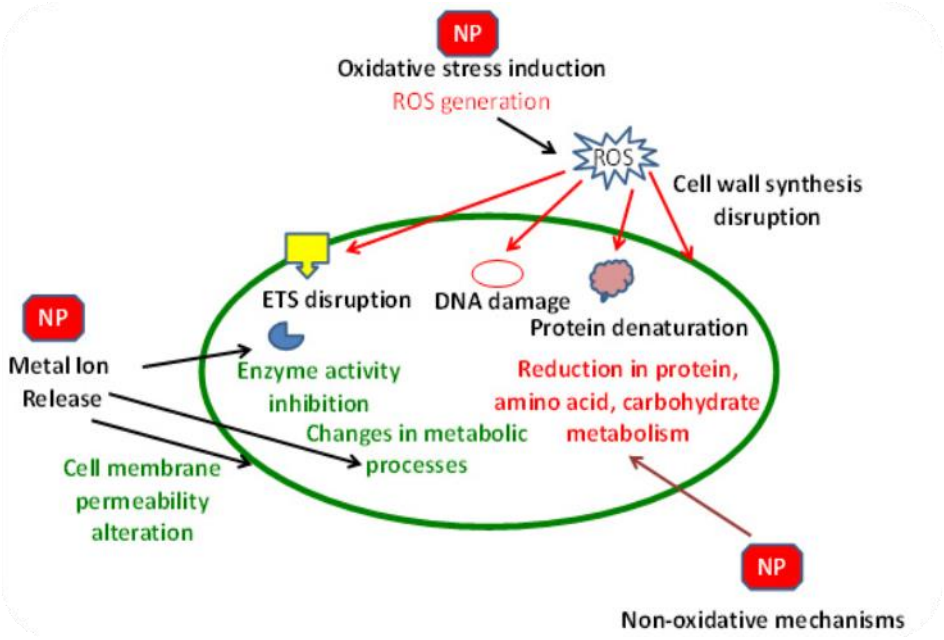

Figure 6: Bacterial cell interaction with NPs

CHAPTER 3

3. MATERIALS AND METHODS

Zinc aetate dihydride and selenium powder used in this research were purchased from Sigma Aldrich. Oxalic acid was purchased from local market.

3.1. Synthesis of simple ZnO NPs and Se/ZnO NPs:

Desired NPs were synthesized by the mechanochemical reaction. Simple ZnO NPs were first prepared by manually mixing 25mmol or 5.48g of zinc acetate dihydrate (Zn $(CH_3COO)_2 \cdot 2H_2O$) and 42 mmol 3.78g of oxalic acid ($C_2H_2O_4 \cdot 2H_2O$) in pestle and mortar at room temperature. The mixing and grinding continued until the smell of acetic acid stops, indicating the completion of the reaction. It takes almost 30 minutes to complete the reaction and the product formed was hydrated zinc oxalate (ZnC_2O_4) (Taha et al., 2019). The precursors were washed with ethanol multiple times, each step involved manual shaking and 10 minutes of sonication. Afterward, the NPs were dried in an oven at 40 °C for 12 hours. Dried NPs were annealed at 450°C for 30 minutes in a temperature-controlled furnace to obtain fine crystalline simple ZnO NPs.

Similarly, Se/ZnO NPs were obtained by adding 0.5 mmol of Se powder (0.04g) was added to the mixture of 5.48g of zinc acetate dihydrate (Zn $(CH_3COO)_2 \cdot \cdot \cdot 2H_2O$) and 3.78g of oxalic acid ($C_2H_2O_4 \cdot 2H_2O$) in pestle and mortar and grinding was done at room temperature. The same procedure was followed to obtain Se/ZnO NPs.

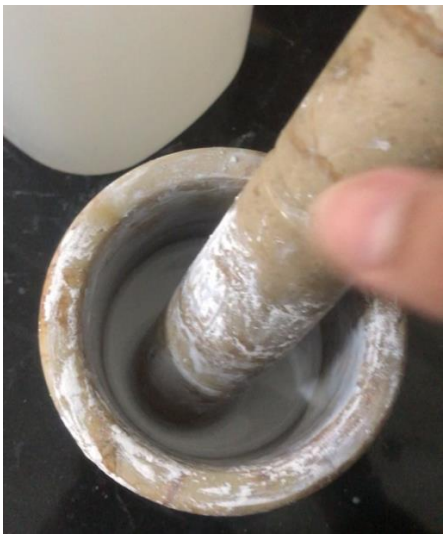

Figure 7: NPs preparation by mechanochemical reaction

3.2. Characterization:

The synthesized simple ZnO NPs and Se/ZnO NPs were confirmed by several characterization techniques based on their charge, size, elemental vibrations, elemental composition present in them and their crystal structure. All these confirmations tests were done and confirm the successful synthesis of our desired nanoparticles.

3.2.1. UV-Vis Spectrometry

The first confirmation was done by UV-Vis spectrophotometry from UV-2800 BMS Biotechnology Medical Services, Madrid, Spain spectrophotometer. Sample containing cuvette was placed in front of the UV lamp in UV-Vis spectrometer. The lamp throws light rays of different wavelengths on the sample and the absorbance at different wavelengths is measured. The lambda maxima are the maximum absorption of the sample and is directly proportional to the concentration of the sample mixture in the cuvette. It is also known as molar absorptivity and used to compare the spectra of various chemicals.

3.2.2. FTIR analysis

Second confirmation was done by FTIR from Bruker FTIR Spectrometer ALPHA ll (Westborough, MA, USA). Fourier Transform Infrared shows the normal stretching and bending of molecular vibrations among the molecules of the fabricated nanoparticles. Metal-oxide

nanoparticles shows the stretching on the lower IR region shows the weak bonding among the molecules.

3.2.3. XRD analysis:

X-Ray Diffraction analysis tells us about the crystalline structure of the molecule and the effect of doping on their crystalline structure. It confirms the microstructure of the fabricated molecule.

3.2.4. Zeta potential:

The net charge on fabricated nanoparticles was determined by the zeta analysis. Their charge also defines their stability because of the intermolecular forces present between them. The zeta potential was analyzed by Malvern Zeta sizer (Malvern).

3.2.5. SEM analysis:

Then SEM was performed with SEM VEG 3 LMU (Tescan, Czech Republic). The size and morphology of our fabricated nanoparticles was determined by the SEM analysis. This analysis was conducted by preparing the glass slides containing the fabricated samples of nanoparticles and coating them with gold (30nm) to induce the conduction into the sample under examination. The Sem images show the physical dispersion of the nanoparticles and their different size in nano scale.

3.2.6. EDX analysis:

Then EDX was performed with SEM VEG 3 LMU (Tescan, Czech Republic). This analysis shows the graphical representation of the elemental composition of the fabricated nanoparticles.

3.3. Preparation of composite resin discs:

Composite resin named Nexcomp-META BIOMED was used in this study to incorporate nanoparticles. This composite resin is composed of Bis-GMA, UDMA, Bis-EMA, and organic polymers. Prepared NPs in different percentages (1%, 2.5%, 5%) per weight (0.1g) of the composite discs were manually mixed in the composite resin. Composite discs were prepared by using plastic molds (2 mm h×4 mm d). After the preparation of the discs, the discs were washed with deionized water and vortexed for a few minutes for depolymerization. These discs were cured and placed under the blue UV light having an intensity of 400 mV/cm2 and a wavelength of 430-480 nm for almost 4 hours. Composite resin discs were prepared to perform an

antibacterial activity, antibiofilm activity, hemolysis test, and mechanical testing of prepared bioactive composite resin.

Figure 8: Washing of composite resin discs

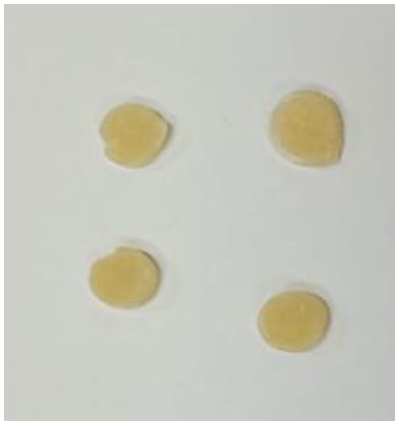

Figure 9: Prepared discs of modified composite resin

3.4. Bacterial strain isolation:

The two main clinical bacterial isolates of *S. mutans* and E. *Faecalis* were utilized to perform the study. To isolate these strains from the fresh salivary sample, saliva was collected in sterilized containers from 10 volunteers. These volunteers neither brushed their teeth nor have eaten anything for at least 10 hours. They also did not have any dental treatments before. These salivary samples were mixed, and different dilutions were made by mixing this sample in sterilized distilled water. These dilutions were spread on Tryptic Soy Agar (TSA) plates and incubated at 37 ^{o} C for 24 hours. Different colored colonies were grown on these incubated TSA

plates. The colonies were picked based on their color and morphological differences and streaked on TSA plates (Li et al., 2014).

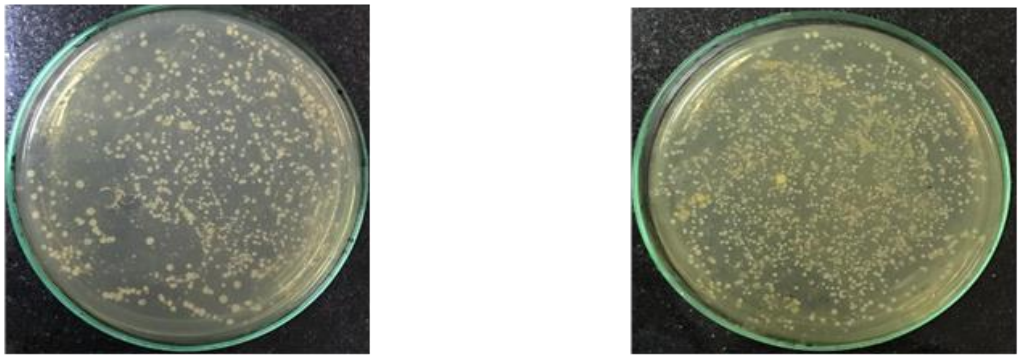

Figure 10: Colonies of saliva sample grown on TSA plates

After 24 hours, colonies grown on TSA plates were picked and further streaking was done on selective media (Blood Agar plates) for our desired bacterial strain.

After isolation of the desired bacterial strains the inoculum was prepared for the further testing models of antibacterial assay.

Figure 11: Bacterial strains grown in liquid TSB media

3.5. In vitro Testing

For antibacterial testing, two models were performed.

2.5.6. 3.5.1. Antibacterial assay

Single specie model:

S. mutans and *E. faecalis* broth cultures were started by inoculating 5 mL of TSBS (1% sucrose) with colonies from the blood agar plates and incubated for 24 hours in a 5% CO_2 incubator. From the overnight *S. mutans* and *E. faecalis* TSBS preculture, a culture was started by inoculating 5 ml of TSBS broth with 200 µl of preculture. After 3 (*S. mutans*) and 3.5 hours (*E. faecalis*) respectively, when the OD of culture reaches 1, a 600 µ culture was diluted through serial dilutions. Then, 500 µL of 10^{-5} diluted cultures of *S. mutans* and *E. faecalis* were added to sterile Eppendorf tubes (Li et al., 2014). The bacteria were incubated with the composite specimens for 6 h in a 5% CO2 incubator. After 6 hours the Eppendorf tubes were removed from the incubator and vortexed. 50µL sample from each Eppendorf was taken and spread on TSA+S plates and incubated for 18-24 hours.

Figure 12: Serial dilutions of bacterial inoculum for single specie model

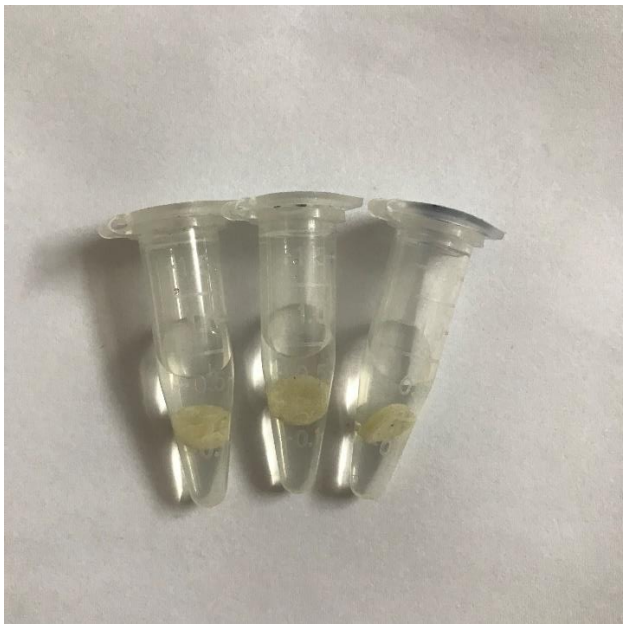

Figure 13: Bacterial inoculum containing modified composite resin discs

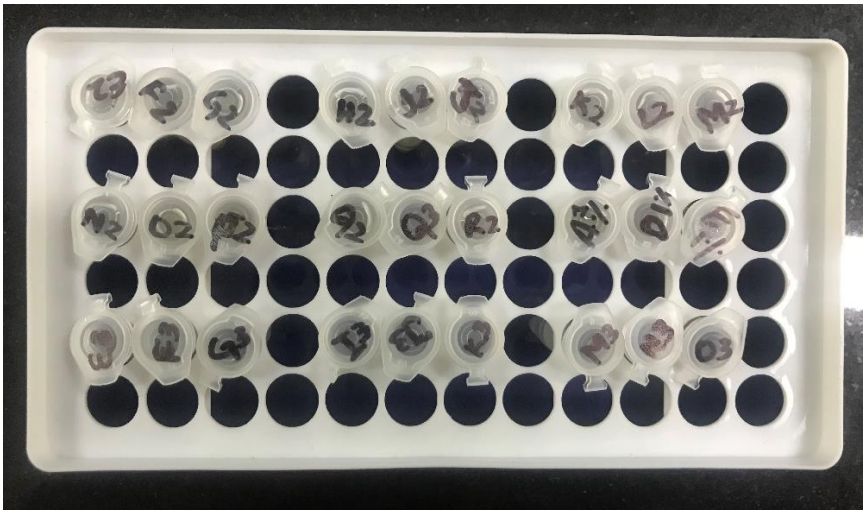

Figure 14: Single specie model ready for incubation for 6 hours

Microcosm model:

Saliva samples from 10 volunteers were collected. It was then diluted with sterile glycerol to the concentration of 30% and stored at -80°C. 50 µl of saliva inoculum was added to 10ml fresh TSBS (1% sucrose) and incubated overnight (Li et al., 2014). 200µl of preculture was added in

5ml of fresh TSBS and the OD obtained 1 at 600nm, it was then serially diluted till 10^{-5}. 500µl of it was added to each Eppendorf with composite discs present in them and placed in an incubator for 6 hours. After 6 hours the Eppendorf were removed from the incubator and vortexed. 50µL sample from each Eppendorf was taken and spread on TSAS (1% sucrose) plates and incubated for 18-24 hours.

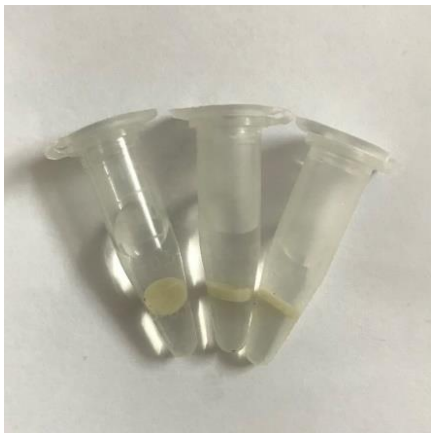

Figure 15: Bacterial samples containing composite resin discs for microcosm model

Colony Forming Units (CFU)

The CFU were counted for further interpretation of the results. Colonies obtained on plates were counted. CFU was calculated using the formula:

$$\frac{\text{No. of colonies} \times \text{Dillution factor}}{\text{Sample poured in each plate in ml}}$$

The dilution factor used was 10^{-5} and the sample spread in each plate was 0.05ml. CFU in each ml was obtained and plotted in the nested graph.

3.6. Mechanical testing

For testing the mechanical strength of prepared composite discs, they were placed in saliva for 24 hours after curing with UV light. Universal Instron Testing Machine was used to determine the compressive strength of the composite discs. A universal testing machine (Instron universal testing machine model static) at a crosshead speed of 0.5 cm/min and load cell of load cell 5KN were used to measure the compressive strength (Aleem et al., 2018; Dias et al., 2019). Specimens were placed vertically on the base of the machine and the

compressive load was applied until fracture. The unit of measured strength was MPa and the compressive strength was calculated by using this formula

Compressive strength = Load X10 (N)

Area (mm2)

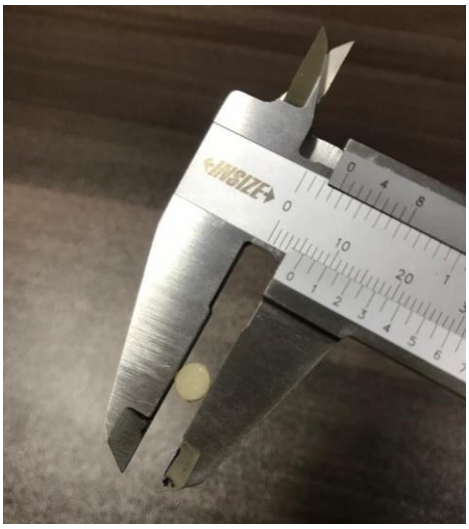

Figure 16: Measuring the diameter of composite disc

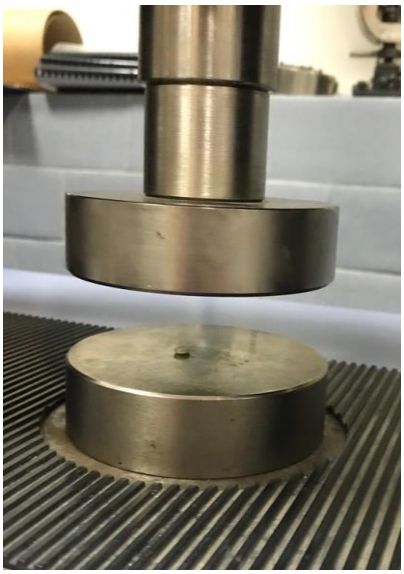

Figure 17: Mechanical testing of sample disc

3.7. Hemolytic Assay:

To test the hemolytic of prepared NPs human blood sample was diluted with PBS in equal amounts and centrifuged for 10 minutes at 13000 rpm to isolate the RBCs. This procedure was repeated twice. The isolated RBCs were diluted with PBS with concentration of 1:2. 10 ml PBS in each test tube containing 200 ul of diluted blood sample and composite discs was incubated for 2 hours. Triton X (10ml) diluted with blood (200ul) was taken as negative control. PBS solution (10ml) with blood sample (200ul) was taken as positive control (Thom et al., 2003). Two approaches were used to analyze the hemolytic activity qualitative analysis and quantitative analysis. After incubation all samples were centrifuged for 5 minutes at 5000 rpm (Taha et al., 2019)

$$\% \text{ Hemolysis} = \frac{(\text{O. D. test sample}) - (\text{O. D. negative control})}{(\text{O. D. posiitive control}) - (\text{O. D. negative control})} \times 100$$

Figure 18: Samples placed in shaking water bath for incubation

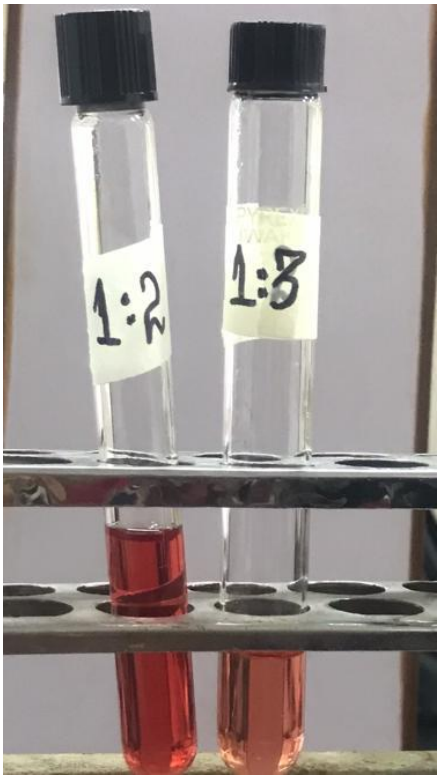

Figure 19: Blood dilution in PBS solution.

CHAPTER 4

4. RESULTS AND DISCUSSION:

Bare ZnO nanoparticles and Se dopped ZnO were successfully obtained in white powder form by mechanochemical method or solid-state reaction and confirmed by different characterization techniques.

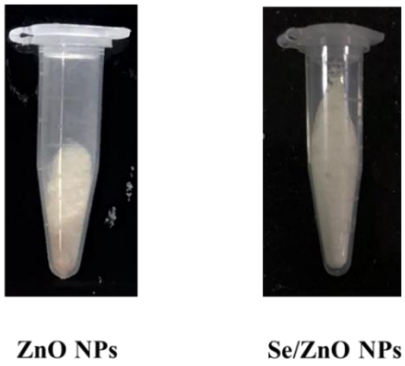

Figure 20: Simple ZnO and Se/ZnO NPs

4.1. UV-Vis spectroscopy:

Obtaining UV-Vis spectra of prepared ZnO NPs and Se/ZnO NPs within the range of 300nm to 400nm. A sharp peak of bare ZnO NPs was obtained at the absorbance of 2 at the wavelength of 361 nm. As a result of doping a blue shift of the sharp peak was observed at the wavelength of 352 nm confirming that the desired nanoparticles have been prepared successfully and the doping of the selenium metal is done successfully.

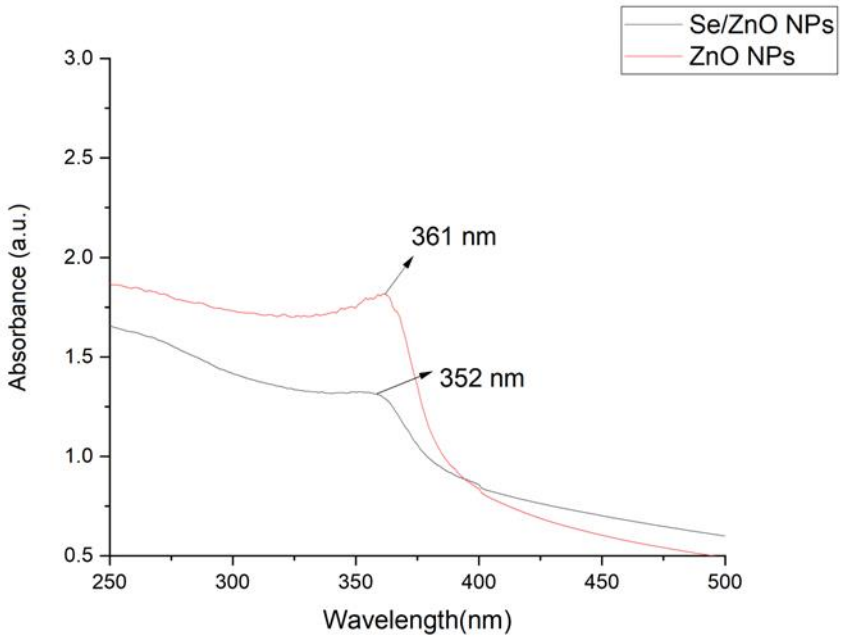

Figure 21:UV-Vis spectra of ZnO NPs and Se/ZnO NPs

4.2. XRD crystallography:

The XRD pattern of pristine ZnO NPs and Se/ZnO was obtained, and the size was calculated considering the 2-theta angle of the highest peak (Figure 20). The theta angles obtained were correspondent to the peaks of JCPDS 36-1451 at 2 theta angles of 31.06°, 33.83°, 35.69°, 47°, 56°, 62.3° and 67.38° in correspondence to their reflections from the (100), (002), (101), (102), (110), (103) and (200) of wurtzite crystallite of ZnO [21]. Hence, we predict that the peaks obtained are due to the crystalline structure of our ZnO NPs. The concordant peaks of Se/ZnO broadened with respect to the ZnO NPs peaks, depicts that the crystalline nature of ZnO undergo distortion due to the doping of Se metalloid in ZnO. Lower intensity also shows the reduction in the ZnO wurtzite phase crystallinity due to Se doping.

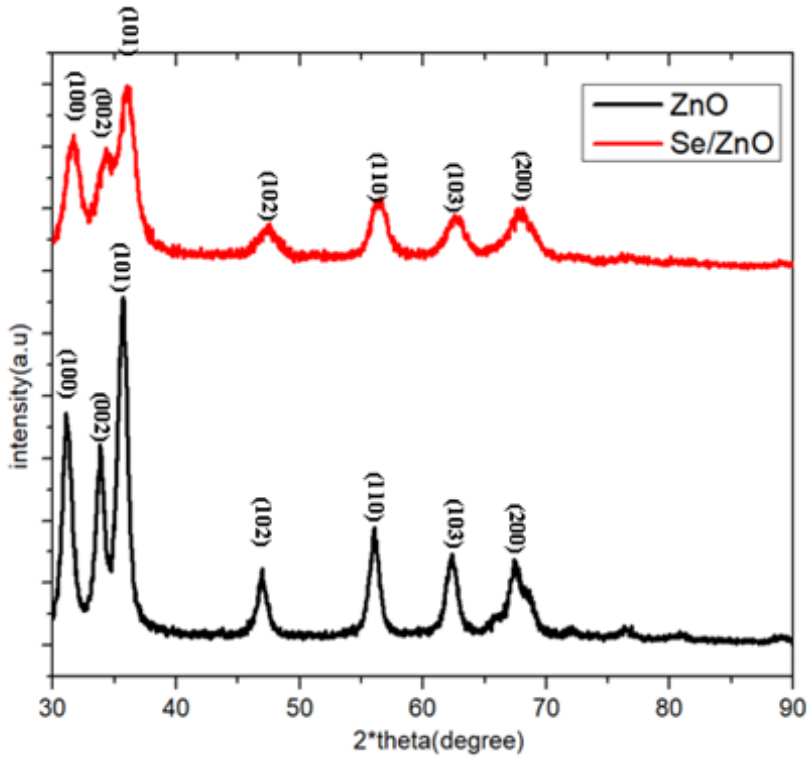

Figure 22: XRD analysis of ZnO NPs and Se/ZnO NPs

The crystallite size was calculated using Scherer equation.

$$D = \frac{k\lambda}{\beta hkl \cos \theta}$$

Where K is the constant 0.90. λ is the wavelength of incidence X-ray that is 0.15406 nm. βhkl is the (FWHM) peak width at half maximum and cos θ is the peak position.

Sr. no	Nanoparticle	2theeta (degree)	FWHM	d	Size (nm)
1.	Pristine ZnO	35.63	0.19	2.51	42.35
2.	Se/ZnO	36.50	0.29	2.54	28.64

Table 1. Crystallite size of pristine ZnO and Se/ZnO

4.3. FTIR spectroscopy:

The Fourier Transform infrared spectra for two samples i.e., simple ZnO NPs and Se/ZnO NPs was obtained between the range of 550 to 4000 cm^{-1}. This spectrum shows the normal stretching and bending of molecular vibration. The broad peak stretching at the range of 3445 cm^{-1} was shifted to 3421 cm-1 was referred to as the molecular vibration of O-H group gives us a clue of se doping. Another absorption band of carboxy group C=O group of ZnO NPs at 1425 cm^{-1} was shifted to 1397 cm^{-1} in the case of doped Se/ZnO NPs, confirming the doping is done successfully. The molecular vibrational shift of metal oxide was observed at lower IR region of the spectra which is exactly below the rage of 600 cm^{-1} (Taha et al., 2019). These shifts in the molecular vibrations of the spectrum depicts that the Se metal is successfully doped in ZnO NPs. The shift in the lower IR region of the spectrum indicated that the bond becomes weak upon doping of Se in ZnO NPs.

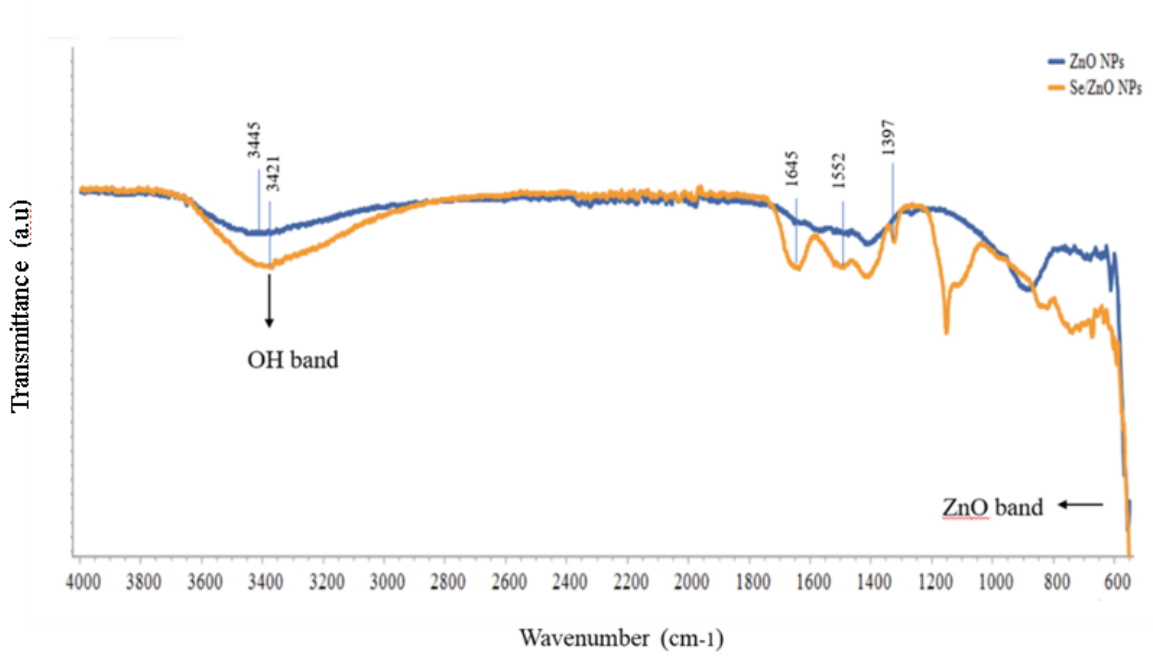

Figure 23: FTIR spectrum of simple ZnO NPs and Se/ZnO NPs

4.4. SEM analysis:

The Sem analysis of the ZnO NPs and doped Se/ZnO NPs confirms that the prepared samples are in nano range and are in very small sizes. The SEM of ZnO NPs shows that the particle's diameter range is between 14.42 and 40 nm. In the case of Se/ZnO doped particles size reduced in size and the diameter ranges from 14.42 nm to 28 nm. These images shows that the doping of Se metal in ZnO nanoparticle results in the size reduction of the nanoparticles. SEM images also shows that the nanoparticles are completely segregated, and no agglomeration occur. Studies shows that the smaller the size of the particle easier for it to penetrate the cell of the bacteria.

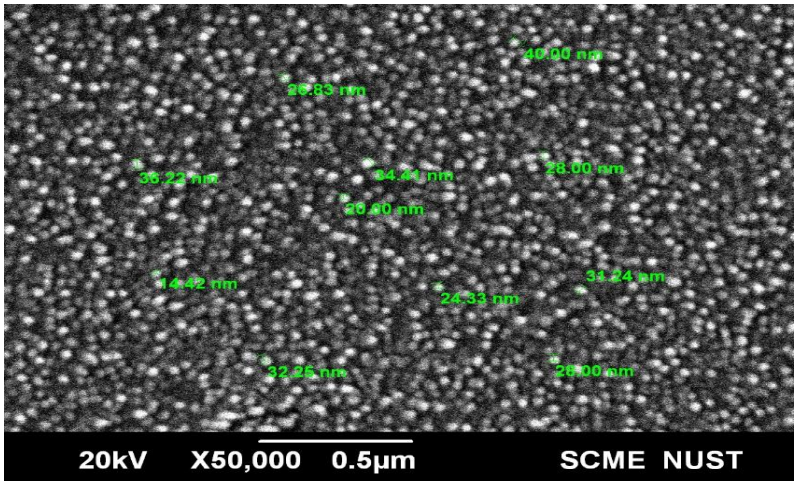

Figure 24:SEM image of simple ZnO NPs

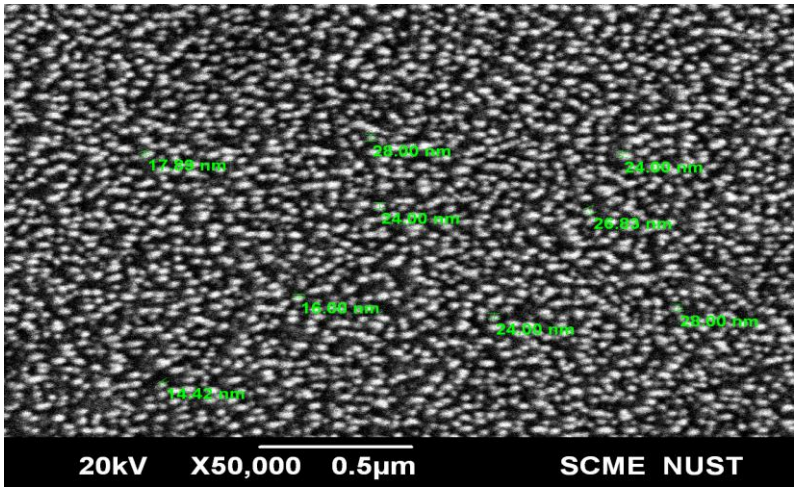

Figure 25: SEM image of Se/ZnO NPs

4.5. EDX:

The EDS analysis of the prepared samples was done to determine the elemental composition present in the prepared samples of nanoparticles. The strong peaks of Zn and O in the EDS analysis of ZnO NPs (fig:20) shows the presence of these elements in the prepared samples of nanoparticles. Whereas the significant peaks of Se, Zn and O in the EDS analysis of Se/ZnO NPs (fig:21) shows the presence of these elements in the prepared samples. And the reduction of Zn metal in these nanoparticles indicate that the Se doping is done successfully in the ZnO nanoparticles.

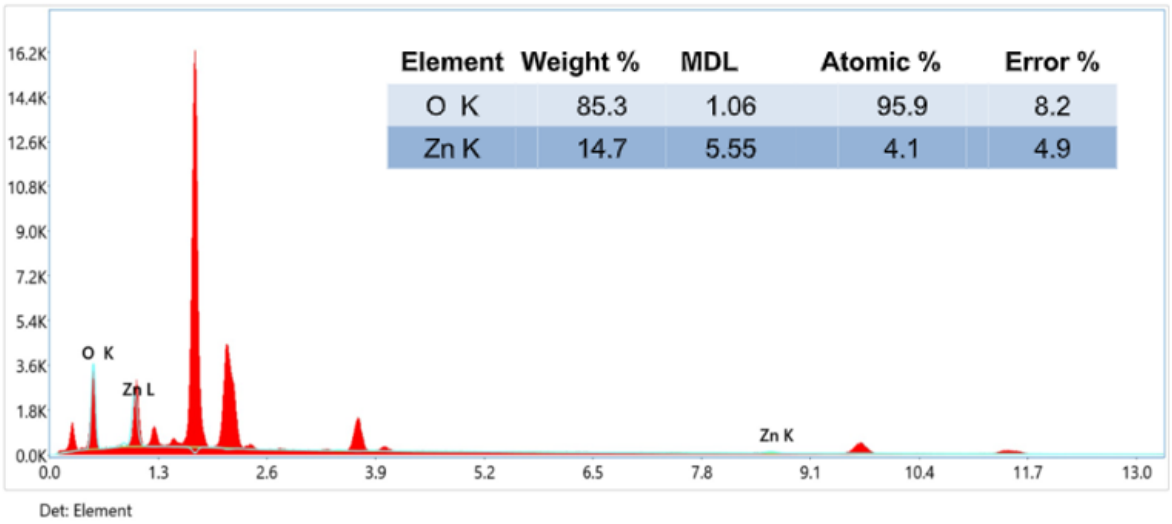

Figure 26: EDX of simple ZnO nanoparticles

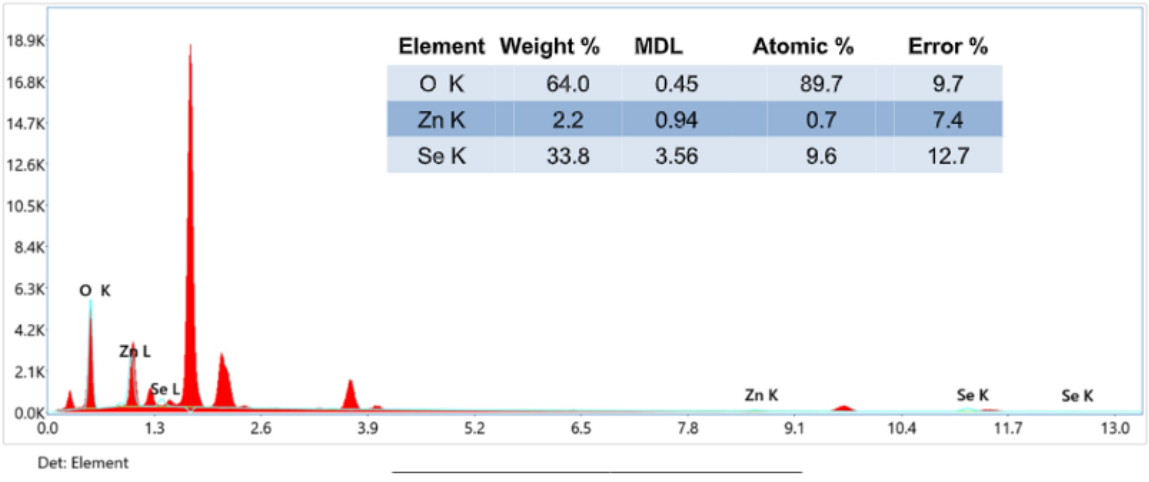

Figure 27: EDX analysis of Se/ZnO NPs

4.6. Zeta potential:

Zeta analysis was done to analyze the charge potential of prepared nanoparticles. The zeta potential of each nanoparticle of ZnO sample was obtained as +16.1 mV. Similarly, the zeta potential of the nanoparticles of Se/ZnO sample was obtained as +18.5mV. Nanoparticles are stable because the zeta potential was higher than +15mV. They are supposed to be stable because of the higher charges their repulsion forces does not allow them to come closer and there will be less chances of aggregation (Krishnakumar & Elansezhian, 2022). Both our bacteria are gram positive that usually poses negative charge on their cell wall. These electrostatic forces between the bacterial cell wall and the nanoparticles are more favorable for enhancing the antibacterial activity.

4.7. Isolation of bacterial strains:

Bacterial strains required for the experimental procedure were isolated successfully from human saliva sample on TSA media plates and confirmed the strains by growing them on blood agar plates. On blood agar media *S. mutans* growth was confirmed by the appearance of darker colonies (Figure: 7). The isolation of *E. faecalis* was confirmed by slightly lighter colored colonies than *S. mutans*. (Figure: 8). Both the species undergo the lysis of erythrocytes on blood agar plates. Because these bacteria may produce some toxins that effect or destroy the red blood cells of the blood (Buxton, 2005).

Figure 28: Clinical isolates of *S.Mutans*

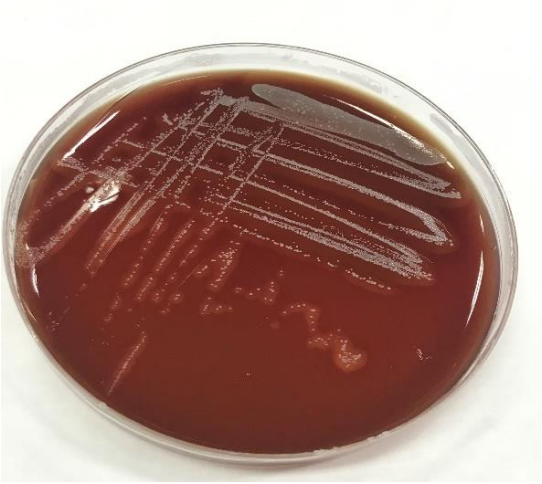

Figure 29: Clinical isolates of *E. faecalis*

4.8. Antibacterial activity of Simple ZnO and Se doped ZnO nanoparticles:

For testing the antibacterial activity of the fabricated nanoparticles two models were tested. In single specie model the nanoparticles were tested on our isolated bacteria. Whereas, in microcosm model nanoparticles were tested against all the oral microorganisms. The CFU/ml was calculated after the experiment and the data was plotted.

2.5.7. 4.8.1. Single specie model:

E. faecalis:

In antibacterial assay of Enterococcus faecalis results showed that the antibacterial activity of fabricated simple ZnO nanoparticles in comparison with simple unmodified composite is higher. 1% ZnO NPs were incorporated and tested for the antibacterial activity against *E. faecalis*. Different concentration of Se/ZnO NPs were also incorporated in the composite resin discs and tested against the strain of *E. faecalis.* Results clearly shown that the antibacterial activity is significantly enhanced by the doping of the Se metal in the incorporated nanoparticles in composite resin. 1% of Se/ZnO NPs showed significantly enhanced antibacterial activity and the higher the concentrations higher the antibacterial activity. Similarly, 2.5% and 5% concentrations also showed that the bacterial growth was significantly reduced in higher concentrations. To avoid any uncertainty CFU/ml were counted in each poured sample of the bacteria.

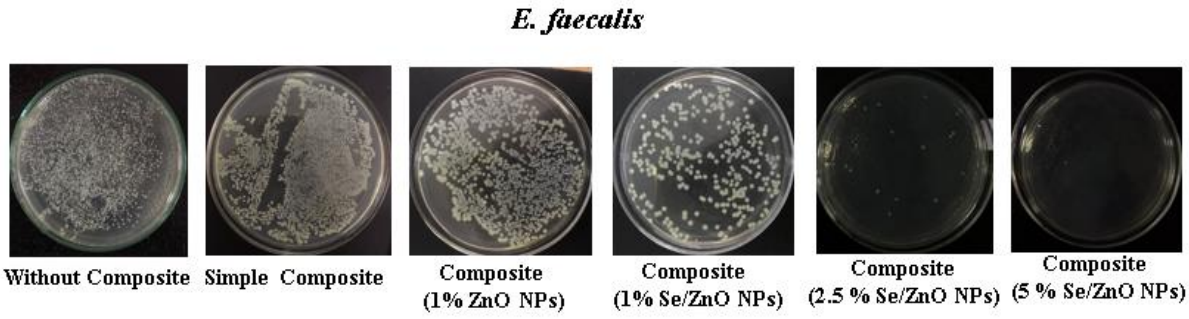

Figure 30: Antibacterial assay against E.faecalis

To avoid any uncertainty CFU/ml were counted in each poured sample of the bacteria. 10^{-5} dilution was used in this regard.

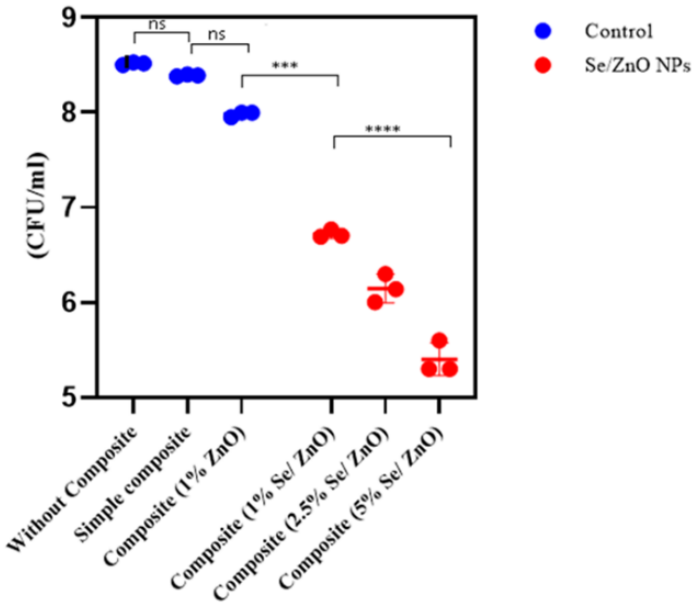

Figure 31:CFU/ml in E. faecalis media

S. mutans:

In antibacterial assay of Streptococcus mutans results showed that the antibacterial activity of fabricated simple ZnO nanoparticles in comparison with simple unmodified composite is higher. 1% ZnO NPs were incorporated and tested for the antibacterial activity against *S. mutans.* Different concentration of Se/ZnO NPs were also incorporated in the composite resin discs and tested against the strain of *S. mutans.* Results clearly showed that the antibacterial activity is

significantly enhanced by the doping of the Se metal in the incorporated nanoparticles in composite resin. 1% of Se/ZnO NPs showed significantly enhanced antibacterial activity and the higher the concentrations higher the antibacterial activity. Similarly, 2.5% and 5% concentrations also showed that the bacteria were almost eliminated from the sample.

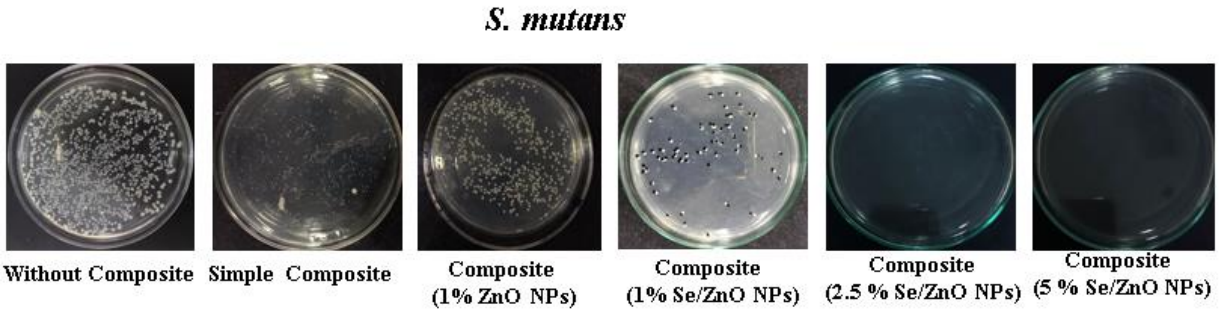

Figure 32: Antibacterial against S. mutans

To avoid any uncertainty CFU/ml were counted in each poured sample of the bacteria. 10^{-5} dilution was used in this regard.

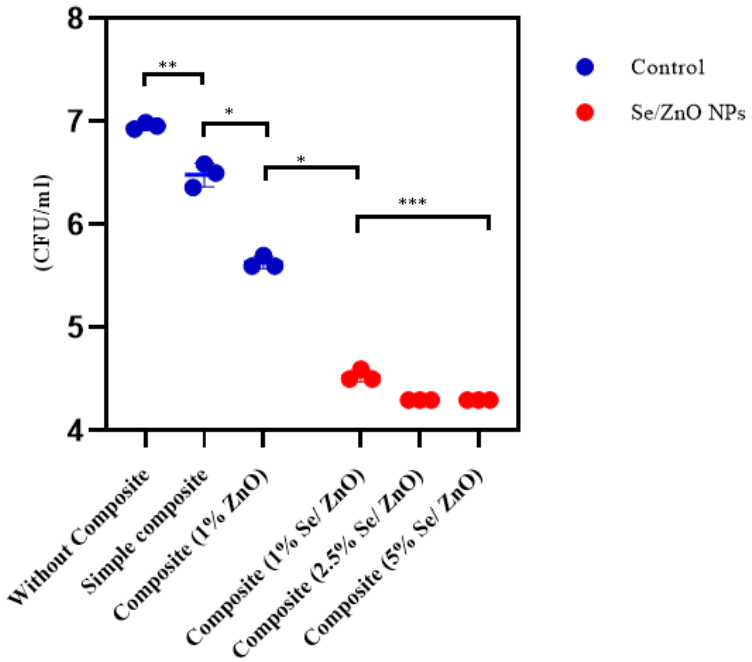

Figure 33: CFU/ml in *S. mutans* media

2.5.8. 4.8.2. Microcosm model:

In antibacterial assay of microcosm model, results showed that the antibacterial activity of fabricated simple ZnO nanoparticles in comparison with simple unmodified composite is higher. 1% ZnO NPs were incorporated and tested for the antibacterial activity against oral bacteria. Different concentration of Se/ZnO NPs were also incorporated in the composite resin discs and tested against the strain of oral bacteria. Results clearly showed that the antibacterial activity is significantly enhanced by the doping of the Se metal in the incorporated nanoparticles in composite resin. 1% of Se/ZnO NPs showed significantly enhanced antibacterial activity and the higher the concentrations higher the antibacterial activity. Similarly, 2.5% and 5% concentrations also showed that the bacteria were almost eliminated from the sample.

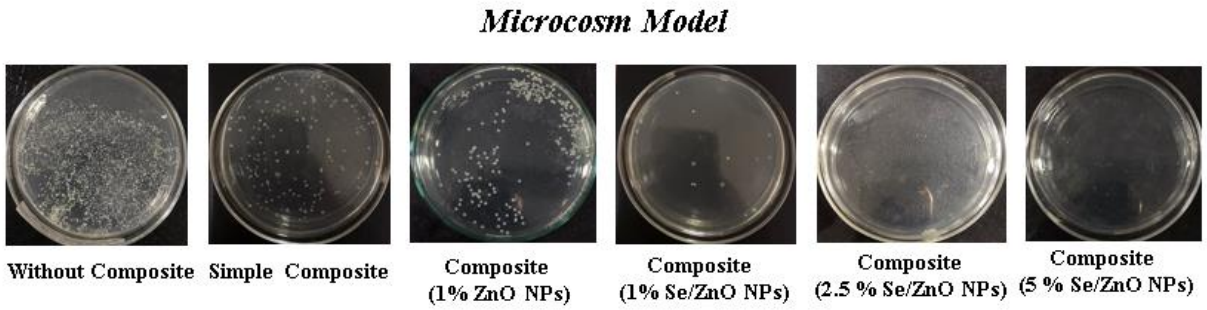

Figure 34: Antibacterial assay against Microcosm

To avoid any uncertainty CFU/ml were counted in each poured sample of the bacteria. 10^{-5} dilution was used in this regard.

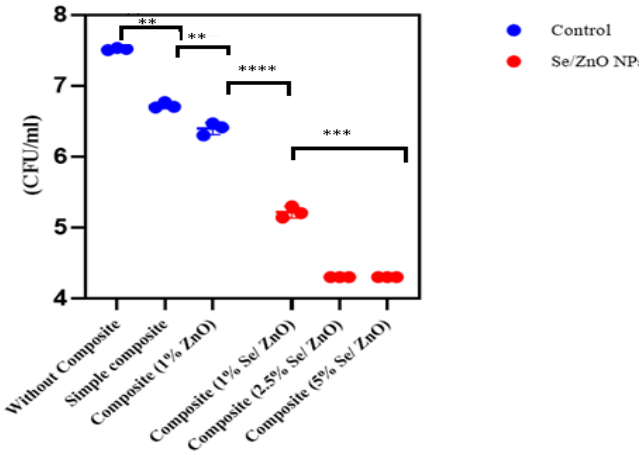

Figure 35:CFU/ml in Microcosm sample

4.9. Hemolytic assay:

In hemolytic analysis the appearance of our supernatant tells us a lot about the hemolytic activity. Our supernatant of positive control showed 100% hemolysis. Whereas our other samples, the supernatant was colorless which means they show non or negligible hemolytic activity.

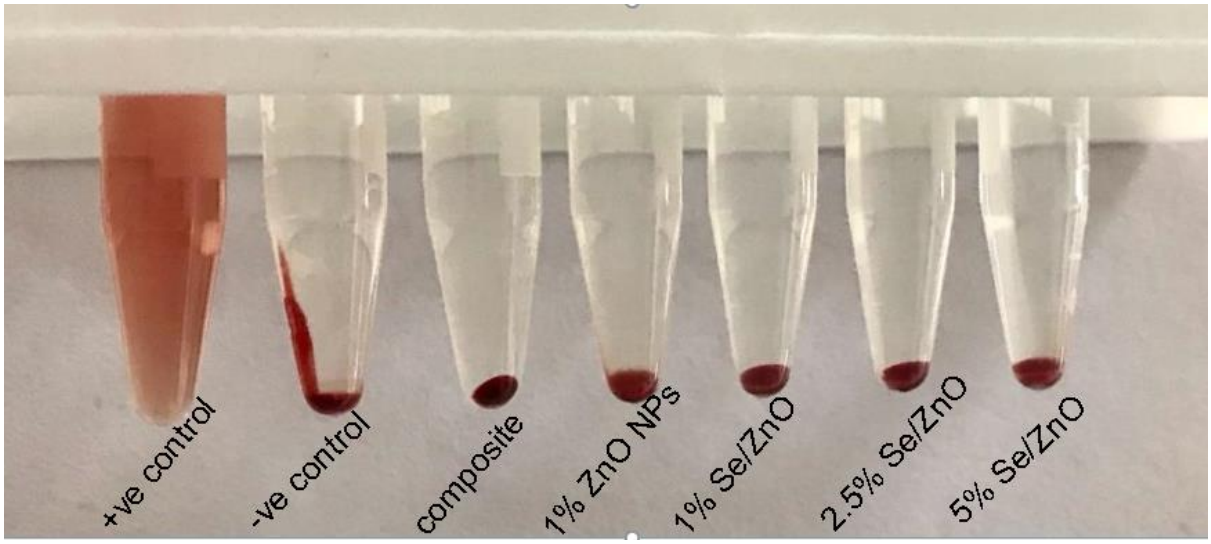

Figure 36: Centrifuge tubes showing the hemolysis of testing samples

In quantitative analysis the optical density (OD) of supernatant was obtained at 545 nm in UV-Vis spectroscopy. By these results the percentage hemolysis was calculated. It was found that our tested samples (Se/ZnO NPs) do not have any hemolytic activity because their percentage hemolysis was obtained in the range of non-hemolytic that is 5% for materials defined by ASTM F756 standard. Our positive control (blood with Triton X-100) showed 100% hemolysis. Negative control (PBS solution with blood) had 0% hemolytic activity. Our first control with simple composite showed 0% hemolytic activity and second control which was composite loaded with bare 1% ZnO NPs showed 5.1% hemolysis that does not fall under the safe range according to ASTM F756. Whereas 0%, 1.65%, 2.32% hemolytic activity was shown by our tested nanoparticles 1% Se/ZnO, 2.5% Se/ZnO, and 5% Se/ZnO NPs respectively. Figure: 31 shows the significant absorbance of hemolyzed samples.

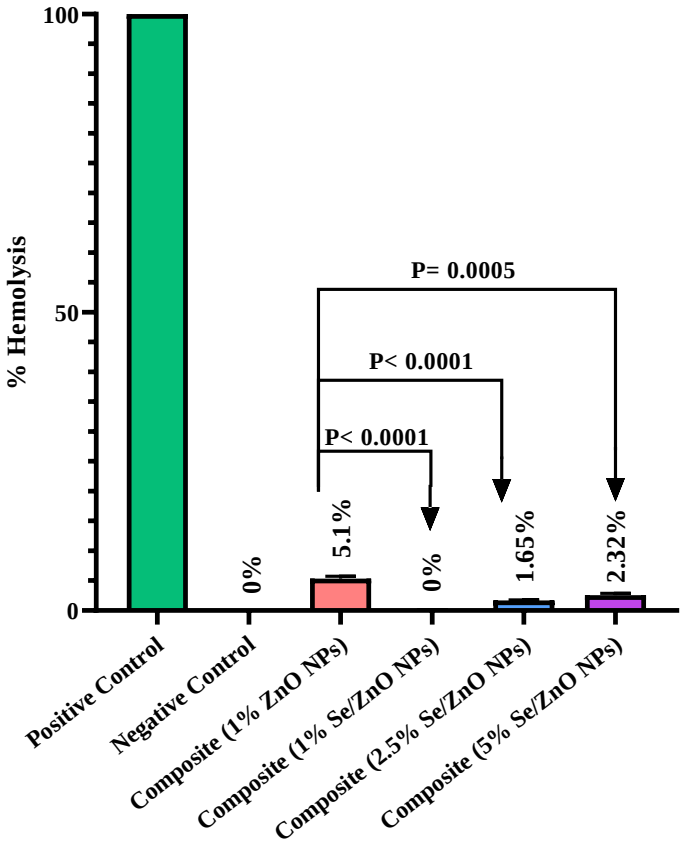

Figure 37: Hemolytic activity of nanoparticles

4.10. Mechanical testing:

Mechanical strength testing reveals that the nanoparticles incorporation decreases its mechanical strength that is why it is recommended to use the lower concentrations of nanoparticles in the composite resin for increased antibacterial activity. Higher antibacterial activity can be achieved at lower concentrations that is 1% and 2.5% of the total weight of the composite resin.

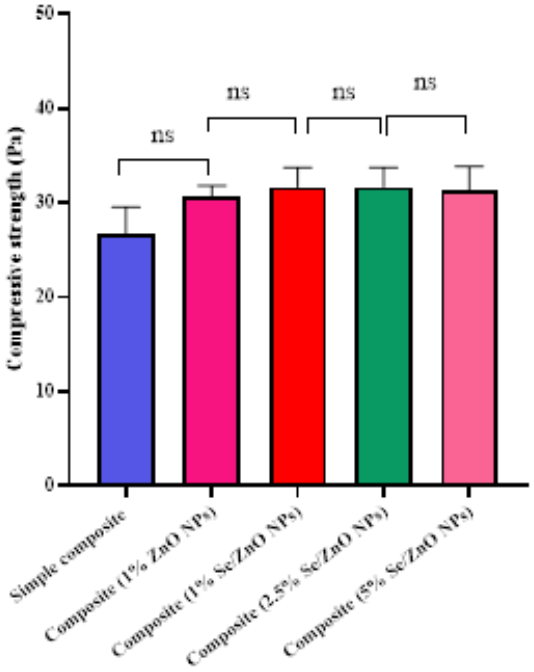

Figure 38: Mechanical strength reduced by enhancing the nanoparticles

CHAPTER 5

5. CONCLUSION

The meritorious achievement of this research was to overcome the problem of secondary caries. Secondary caries is the major cause of composite resin failure. That is usually caused by the biofilm formation and the micro leakage at the interface of the tooth and the composite resin. Much research is done to minimize the occurrence of secondary caries. The most advanced approach is the use of nanotechnology. In which nanosized machinery is used in composite resins to minimize the prevalence of secondary caries. In this regard ZnO NPs are tested by

incorporating them in composite resin but it has some limitations. The major concern was the cytotoxicity and limited antibacterial activity. This study is also nano-based in which the Selenium doped ZnO NPs are synthesized. The purpose was to achieve the prolonged antibacterial activity, reduced cytotoxicity maintaining the aesthetics and the mechanical strength of the commercially practiced dental composite resin, compared to the ZnO NPs incorporated composite resin. The fabricated nanoparticles were characterized by using UV-Vis Spectroscopy, FTIR, ZETA sizer, XRD, SEM, and EDX. All the characterization results support the required synthesis of both the nanoparticles. The smallest sized nanoparticles are more favorable than the larger ones and the doping also undergo the size reduction of the nanoparticles confirmed by the SEM and XRD. The zeta charge of synthesized NPs showed the stability of the nanoparticles, and the elemental analysis confirmed the presence of Se, Zn and O elements. The antibacterial studies showed that the Se/ZnO nano particles have significantly enhanced the antibacterial activity against the polymicrobial biofilm, *E. faecalis* and *S. mutans* microbial strains. The hemolytic studies also revealed that the doped nanoparticles are highly biocompatible even in higher concentrations (5%). According to the ASTM F756 standard the dopped nanoparticles are not cytotoxic even at higher concentration compared to the lowest concentrations of ZnO NPs. The compressive strength test of modified nanoparticles showed that the enhanced concentrations of NPs does not have any negative impact on mechanical strength of composite resin. As the mechanical strength is not affected by increasing the concentrations of doped nanoparticles. The doped nanoparticles are recommended to use for the effective antibacterial properties even with lower concentrations for the treatment of caries or the permanent prevention of secondary caries.

FUTURE ASPECTS

In future the modified composites with Se/ZnO NPs will be tested on animal model and further hemocompatibility, anti-cariogenic properties and gap formation, studies will be tested on the animal model before taking it to humans. The Se/ZnO NPs are also recommended to be used in dental adhesives, tooth paste and mouthwash etc.

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