' DEAE-Dextran coated AgNPs for antibacterial composite based dental fillings '

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Abbreviations:

BISGMA - bisglycidil methacrylate

DEAE – Diethylaminoethyl

E. faecalis - *Enterococcus faecalis*

HEMA - hydroxyethyl methacrylate

Hrs. – Hours

NPs – Nanoparticles

S. mutans - *Streptococcus mutans*

TEGDMA - triethyleneglycol dimethacrylate

UDMA - urethane dimethacrylate

Abstract

The major dental problem now a days is occurrence of dental caries. To cope with them, the best treatment option considered today is restorative material fillings. There is observed few problems with utilization of composite material. The accumulation of biofilm over these dental composite resins is a major cause of secondary caries. Antibacterial restorative resins are an effective option to avoid biofilm accumulation. The aim of this research was to synthesize novel Diethylaminoethyl (DEAE)-dextran silver nanoparticles (AgNPs) and to modify dental composite resins utilizing these NPs. The antibacterial and antibiofilm properties of these composites were evaluated and compared with unmodified composites. Furthermore, these modified composite resins were assessed for their mechanical and cytotoxic activity. DEAE-Dextran AgNPs of mean size i.e., 170nm were successfully synthesized with chemical reduction method which was confirmed by Ultraviolet–visible (UV-Vis) spectroscopy, Scanning electron microscopy (SEM), Fourier-transform infrared spectroscopy (FTIR), Zeta potential and Energy-dispersive X-ray *spectroscopy* (EDS). Antibacterial activity of modified composite disc specimens was tested against Streptococcus mutans (*S. Mutans), Enterococcus faecalis* (*E. Faecalis)* and microcosm biofilms. The composite discs prepared with DEAE-Dextran AgNPs exhibited excellent antibacterial activity when compared with composite resin reinforced with simple AgNPs. Also, composite resins with DEAE-Dextran AgNPs exhibited enhanced antibiofilm properties when compared with the silver modified resin composites ($P<0.05$). The mechanical properties were significantly enhanced by the addition of DEAE-Dextran into composite resin($P<0.05$). Moreover, unlike AgNPs, DEAE-Dextran AgNPs were less haemolytic. In short, these results established strong ground applications for DEAE-Dextran modified dental composite resins.

Chapter 1

1. Introduction

1.1. Dental caries

Dental caries is a multidimensional, chronic bacterial illness that causes death of the tissues, most commonly by the creation of acid from microbial fermentation on the tooth surface. It is a common infectious disorder which is caused mainly due to *Streptococcus mutans*, a gram-positive bacterium which is mostly found in the oral cavity. This bacterium metabolizes sugars and produces acid that demineralizes the structure of teeth very slowly (Rathee & Sapra, 2021). It's a complex disease that is caused by biofilms which causes the periodic demineralization of enamel as well as dentine. Dental caries also affect the tooth crown and, then later in the life, these exposed surfaces develops permanent dentitions within them (Pitts et al., 2017). Caries is the one of most commonly affecting illness bothering the people all over the world. Individuals are mostly prone to this illness through the rest of their lives. According to dentists, dental caries affects around 36% of the world's population. It affects roughly 9% of the population when it comes to infant teeth. Physical, biochemical, environmental, psychological, and lifestyle-related variables all contribute to the risk of caries (Al-Shahrani, 2019).

1.2. Global Prevalence of dental caries

Dental caries mostly affects 60 to 90 percent of the children worldwide, primarily in the underdeveloped nations, as per the reports of the World Health Organization (WHO). While dental caries mostly prevail under control in developed nations, but it is more prevalent in underdeveloped countries (James et al., 2018). Children with caries experience discomfort, which can compromise their ability to eat, sleep, and communicate, as well as their focus, affecting their academic development. This infection causes significant pain and inflammation if not treated early, may necessitate expensive surgical procedure. The global prevalence rate of caries among adults and children was expected to reach 46.2 percent and 53.8 percent, respectively, in 2020, which was considered significantly high (Teshome et al., 2021).

1.3. Dental caries prevalence in Pakistan

Dental caries must be managed in order to attain optimal oral health. To control them in a community, the very first step is to understand its prevalence and trends. Unfortunately, many poor nations have a relatively high incidence of dental caries and Pakistan is no different. According to available data, over 60percent of the total of Pakistani population suffers from dental caries. In all provinces, the share is about the same. The majority of all the studies involved were deemed to be at high-risk (Siddiqui et al., 2021). According to a study conducted by Islamia medical and dental college in 2016, Patients with dental caries were found in 52 percent of men and 48 percent of females. According to the statistics, dental caries is a widespread condition that affects both men and women, with males being more affected than women. Communities must be educated about the risk factors for dental caries, as well as given adequate hygiene recommendations, by healthcare workers and dentists (S Rashid et al., 2016).

1.4. Main cause of dental caries

1.4.1. Etiology

Dental caries is considered a complex illness that is involving the host, an agent, and its environment. The etiological agent of dental caries is Streptococcus mutans (*S. mutans*). *S. mutans* adheres to the dental pellicle and then uses carbohydrates as its energy to make lactic acid, which results in an acidic condition all around the tooth. As a consequence, enamel and, then eventually, the whole dentin become demineralized. The tooth structure, microorganisms present in form of a dental biofilm, and also the sugary diet are all variables in this process of dental decay. Sugar consumption, has a significant impact on caries the frequency and severity (Lee, 2013).

1.4.2. Biofilm formation

The dental biofilm is a very complex network with the well-classified set of the operational microorganisms and their symbiotic interactions. Hydrolytic bacteria present in the oral biofilm matrix which have the potential of delivering acids, that causes the demineralization of the teeth structure. The biofilm, which is linked to the dental cavities, has numerous roles. Microbial propagation and development, acid-base modulation at tooth surface, and the calcium ion interaction among the tooth and the saliva all contribute to this plaque formation (Stefanovska & Bilbilova, 2021). Caries-associated colonists include *S. mutans* and *E. faecalis*. Previously conducted researches have revealed that the biofilm resistance to the antibiotics, the preservatives, and the anti-adhesion chemicals is all linked with the microbial diversity. This microbial abundance basically falls with development of the cariogenic biofilms due to great diligence of these cariogenic bacteria. This dominance of cariogenic microbes between the microbial species that are associated with the health is considered the main cause of the caries. Therefore, there is intricacy of this biofilm matrix and the number of bacteria that provide hurdles in prevention of the caries.

1.5. Treatment for caries

Dental Fillings, also known as restorations, are the most common method of treating dental caries. These fillings are composed of a various type of materials such as gold, silver, or it includes the dental composite resins, also porcelain fillings, or sometimes a mixture of elements that is called dental amalgam. Today, composite resin restorations are most commonly used because they are most effective and are inexpensive than silver or gold fillings. These composite resins are tooth-colored polymers that were proposed as mercury-free alternatives to silver amalgam fillings. with the increased physiochemical characteristics of dental resin composites.(Gupta et al., 2012). Although they are the most often utilized materials for repairing tooth enamel because of their high aesthetic characteristics and strength. Unfortunately, investigations have revealed failures, with secondary caries being the primary cause. It was discovered that resin composites accumulated more dental biofilm. The absence of inhibitory activity against the cariogenic bacteria like *S. mutans* adds to major biochemical breakdown in resin composites, i.e., biofilm development. In addition, microorganisms that have clung to nearby tissues infect it. As a result, persistent caries develops around these restorations, which are managed by restorative replacement, resulting in further tooth loss. As a result, antimicrobial prophylaxis is one of the ways for extending the life of dental resin composites (Beyth et al., 2014).

1.5.1. Antimicrobial agent modification

The incorporation of an antimicrobial component to composite resin materials could be accomplished with modifying the matrix of resin material. Some antibacterial agents just as the antibiotics, phosphate, hypochlorite, Ag ions, selenium, and the ammonium compounds have been introduced. Among all of these, silver possesses most effective antibacterial activity. Endodontics, root canal therapy, restorative dentistry, orthodontics, and oral malignancies have all benefited from the use of silver nanoparticles (AgNPs). Due to their antibacterial properties, they also have been used in the medicine and dentistry field. Also biomaterials that contain AgNPs have been employed in preventing and minimizing production of the biofilms (Bapat et al., 2018a). However, AgNPs have been found to be harmful in several laboratory tests. Silver ions build up in the organs. Some scientists are concerned regarding their toxicity. The toxicity of AgNPs is almost proportional with amount of the free Ag ions present. They may easily disrupt biomolecules, tissues, and organs due to their tiny size. According to certain laboratory research, AgNPs can generate free radicals in living cells and impede mitochondrial function. Scientists are also anxious about AgNPs' potential to breach blood-brain barrier to accumulate in the brain via cross transport (Yin et al., 2020b). The toxicity of AgNPs was known for sure, therefore limited their usage in oral diseases. AgNPs Silver nanoparticles are frequently coated with a capping substance to reduce their toxicity. The antimicrobial, antibiofilm, and toxicity of AgNPs were all impacted by the capping agent (Niska et al., 2016). For this research, we have used DEAE-Dextran as capping agent for AgNPs. DEAE-dextran is a strong cationic polymer that electrostatically stabilizes metallic nanoparticles in extremely concentrated suspensions. Furthermore, it stabilizes particles at an advanced production stage, allowing for superior nanoparticle dispersity and resilience in aqueous solution (Mikac et al., 2017). Also, dextran helps in reducing the toxicity of AgNPs. According to the findings, DEAE-Dextran AgNPs modified restorative materials may reduce the expansion of periodic dental caries while increasing their durability of the tooth restorations, and have been shown to be helpful in reducing emergence of the microbial dental biofilms over tooth surfaces and the restorations that is without jeopardizing mechanical properties as well as toxicity of composite resins (Corrêa et al., 2015).

1.6. Objective

The objectives of this research included:

- Synthesize and characterizations of DEAE-dextran coated AgNPs.
- Preparation of composite discs with incorporation of NPs.
- Testing of antimicrobial activity against *S. Mutans*, *E. faecalis* and microcosm.
- Evaluation of hemolytic activity
- Testing of mechanical properties.

The successful therapy for secondary caries is DEAE-Dextran integrated silver nanoparticles, which have less adverse effects and a faster commencement of action.

Chapter 2

2. Literature Review

2.1. DEAE-Diethyl Amino Ethyl Dextran HCl

DEAE-Dextran hydrochloride is a cationic dextran derivative that can be utilized for vaccine manufacture, gene therapy, protein stability, dyslipidemia control, flocculation, and a variety of other uses. It is also utilized to inject foreign DNA into animal cells. The DEAE dextran molecule forms a strong bond with the nucleic acids highly reactive core, and this complex's positive net polarity enables it to cling to the cellular membranes and enter the plasma through endocytosis.

The structure of DEAE-Dextran HCl is:

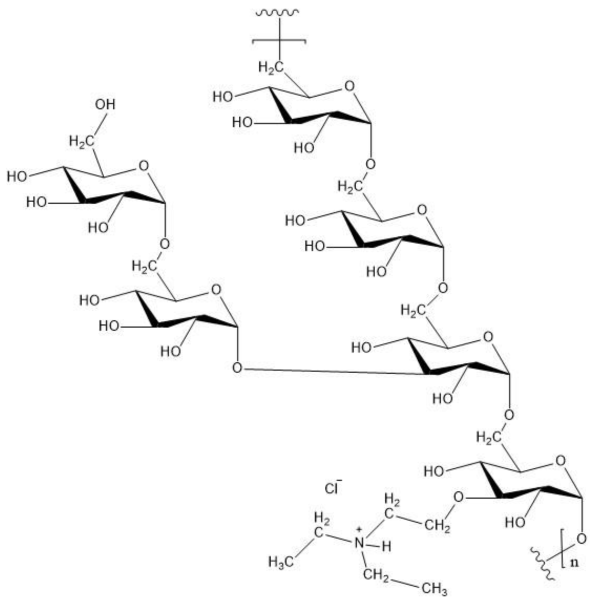

Figure:1 *Structure of DEAE-Dextran HCl (adapted from information)*

2.2. Dental Caries

The damage on a tooth's surface, or to the enamel, is known to be the tooth decay. It occurs usually when oral bacteria mostly produce lactic acid that destroys enamel. Dental caries, the holes in teeth that are caused by the tooth decay. If its left untreated, it can also lead to the discomfort, then infection, and can even cause tooth loss.

People in India, Egypt, Japan, and China believed that dental caries was caused by a "tooth worm" as long back as 5,000 BC. The phrase "dental caries" first emerged in the print in the 1634, and it is derived from a Latin word Caris, that means rotting. With limited information of the disease's etiology and pathophysiology, the phrase was originally used to merely describe holes in the teeth (Conrads & About, 2018).

2.2.1. Definition

Asa it can be defined, the dental caries is among one of most common disorders that affects people all over the world, and it can affect anybody at any moment. Teeth caries is the acidic metabolic end of microbial fermentation of dietary components that cause localized damage of sensitive dental tissues(Selwitz et al., 2007). They develop with time due to the result of a very complicated interplay among the acid-producing microorganisms, simple sugars, as well as the variety of the host components just like the tooth and the saliva. This illness affects the crowns and the roots of teeth, and also it may start as a severe issue of tooth decay in newborns and in the toddlers' primary teeth in early infancy.

It is a common infectious illness that is traditionally linked to the interaction of biological, psychological, and socioeconomic factors. In developed nations, it affects roughly 40–50 percent of individuals(Vergnes et al., 2012).

2.2.2. Pathophysiology

Dental caries is caused through activities and interactions involving acid-producing bacteria, a precursor that the pathogens can utilize, and a variety of host components such as teeth and saliva. Dental caries occurs when the physiological balance between dental nutrients and oral bacteria biofilms is disrupted. Bacteria exist in biofilm communities on teeth that are incapsulated in the matrix material of the polysaccharides, enzymes, and the DNA released by the cells. This organic matrix protects the bacteria from evaporation, host defenses, and pathogens, as well as providing greater antibiotic resistance(Selwitz et al., 2007).

The presence of the fermentable carbohydrates, some other environmental circumstances, microorganisms, and the host factors all play a role in development of the caries lesions. Streptococcus mutans and Enterococcus faecalis both are most significant acidogenic-aciduric bacterium which are discovered to date. While the mutans operate as sort of initiators, faecalis take part as boosters. Some specific environmental circumstances, like the availability of the fermentable dietary carbohydrates and lack of the oxygen, operate as the stimulants for the microbial activity. Exposure to reduced carbohydrates, particularly sucrose, and a weak redox potential are two environmental factors that facilitate the growth and metabolism of such organisms. When sugar levels are high and oxygen levels are low, fermentation and acid generation occur quickly(Conrads & About, 2018). Acid products of plaque biofilm metabolism are the most major factor mediating Caries. Plaque acid degrades the calcium hydroxyapatite particles at carbonate-rich prism and crystalline surfaces at first. While the lesion front moves swiftly, the lesions interface seems to stay intact mainly to dissolved crystal reprecipitation aided by increased fluoride surface concentrations(Robinson, 2014).

2.2.3. Risk Factors

Although most risk factors seem to be dynamic, but a person's risk for caries might shift over the period of time. Insufficient salivary stream and composition, large numbers of microbial pathogens, insufficient quantity of fluoride exposure, the gingival recession, also the immunological aspects, requirements for the specific health care, and the hereditary variables are all physiological and chemical major risk factor for enamel decay (Featherstone et al., 2003).

2.2.4. Epidemiology

The diagnostic criteria may vary from research to the study confounding comparisons of worldwide occurrence and the spread of these dental caries. However, in many affluent nations, the frequency and intensity of caries in adults has decreased in recent decades. In addition, as people get older, the pace of illness progression decreases. Caries decrease has been larger on interproximal as well as smooth areas in permanent teeth than on decayed or occlusal regions. Caries frequency and intensity in teeth may have maintained a grown modestly in various demographic groups (Ismail, 2004; Pitts, 2004; Selwitz et al., 2007).

Caries is one of the most prevalent chronic pediatric illnesses in the United States, five times more frequent than asthma. Dental caries has become increasingly common among the elderly in the United States and internationally, since more individuals are keeping their teeth longer(Guiguimde et al., 2014). New caries formation may be comparable or greater in older people than in youngsters. This is the most prevalent dental disorder in US, and it is one of leading reasons of the tooth loss in the young adults: by the age of 35, average American has already lost five teeth as well as has 11 more caries-affected teeth (M, 2002); (Ismail, 2004).

2.2.5. Levels of caries infection

Dental plaque plays a vital role in tooth decay. Plaque is a whitish, sticky coating that forms on the teeth's surfaces. Microbes, food debris, and saliva make up this substance.

Plaque may build up on the teeth if don't brush them regularly. It can also harden with time, resulting in tartar formation. Tartar may assist to preserve germs even more, rendering them quite difficult to eliminate.

There are five phases of tooth decay, in general. Let's take a closer look at them now:

Stage 1: Initial demineralization

Enamel is kind of the tissue that usually makes up outer covering for the teeth. Toughest tissue in our body is this enamel, that is mainly composed of another mineral. When tooth is subjected to plaque bacteria's acids, unfortunately, the enamel tends to lose those minerals.

One may notice a white spot on one of the teeth if it happens. Then loss of the minerals in this region is first sign of the decay of tooth.

Stage 2: Dental Enamel deterioration

The enamel would continue deteriorating if this dental caries left to fester. A white spot may deepen toward a brownish tone over time. Tiny holes in teeth called the dental caries, may occur as the enamel weakens. Then dentist will fix such cavities.

Stage 3: Dentin degeneration

Dentin is indeed layer that is underneath the enamel. It is lighter than the enamel, making that more vulnerable to acid erosion. As a result, tooth decay progresses more quickly once it penetrates the dentin.

Dentin also includes tubes that connect to the tooth's fibers. As a result, when the dentin is impacted by dental caries, patients may notice sensitivity. This is most noticeable while eating or drinking hot or cold meals or soft drinks.

Stage 4: Pulp damage

Pulp is just tooth's deepest surface. It houses blood vessels and the nerves which sustain these teeth in good shape. The pulp contains nerves that provide feeling to tooth.

When this pulp is damaged, then it becomes irritable and therefore begins to swell. Due to the supporting tissues that are unable to grow to compensate the swelling, results in stress on the nerves. This might cause discomfort.

Stage 5: Abscessed tooth

Bacteria can penetrate the pulp as tooth decay progresses and create an infection. An abscess is a pocket of pus that forms at the bottom of the tooth as a result of increased dental inflammation.

Abscessed teeth can cause excruciating discomfort that can spread to jaw. The swelling of gums, cheeks, or the jaw, fever, and enlarged lymph nodes in neck are all the possible signs. The tooth abscess demands immediate attention because the infection can spread to the jaw bones and then to other parts of the head and neck. Also, in some circumstances, the damaged tooth can be removed (*Tooth Decay Stages*, 2020).

Figure 2: stages of tooth decay(Bekisheva & Barbasheva, n.d.)

2.2.6. Treatment options

Regular dental checkups help to detect the cavities and some other oral issues before they can produce bothersome symptoms or may worsen into any more serious issues. The sooner the people seek the treatment, the higher is the chance of reversing as well as avoiding dental decay in its early stages. If a cavity is cured before it becomes more painful, then it won't require any significant therapy.

Cavity treatment is usually determined by severity of cavity and the specific circumstances. Most commonly used treatment option is **dental fillings**. When a decay has been advanced beyond its first stage, dental fillings, also known as the restorations, becomes primary therapeutic option. These fillings are composed of various type of materials, including some tooth-colored resin composites, few porcelain fillings, and also mixture of the elements called as the dental amalgam(Zero et al., 2009).

2.2.7. Dental Fillings

Fillings are often used to restore teeth that have already been worn due to usage, such as those that are chipped or shattered. The dentist would remove the decaying piece of tooth and afterwards "fill" the region in which the decaying material was extracted to cure a cavity.

There are a variety of dental filling materials available today. Gold, porcelain, silver amalgamation (mercury combined with metal, lead, zinc, and copper), and tooth-colored, plastics, and resin composites fillings are all options for filling teeth. Glass ionomer is another substance that comprises glass particles. This substance is employed in the same manner as composite resin fillings are.

The sort of filling that is appropriate is determined by the location and degree of the degradation, as well as the expense of the filling product (Bhaduri & Bhaduri, 2009).

Advantages and Disadvantages:

- **Gold fillings**

Gold fillings last for more than 10 years and does not corrode but they are way more expensive. They are almost 10 times more costly than other dental fillings. They can cause electric shock in mouth, if came in contact with silver. This is because of the presence of the saliva in the oral cavity, that conducts current among the two metals producing a sharp shock (“Gold And Amalgam Fillings,” 2021).

- **Amalgams**

Amalgam fillings are strong and more durable than gold fillings and have been used for more than a century. But there lie few problems with them such as they do no stick properly to tooth and so dentist has to cut a whole cavity to make them stay. They are indeed less costly than gold fillings but amalgam fillings discolor the tooth structure, giving it a grey appearance. Also, it makes teeth more sensitive towards hot and cold food. It contains mercury, and many patients get allergic towards that mercury within amalgam fillings (Rangreez & Mobin, 2019).

- **Porcelain fillings**

Porcelain fillings, which are more stain resistant than resin composites fillings, are the most common. This substance may endure more than ten years and be as expensive as gold. Porcelain or ceramic filling can resist more abrasion than any other dental filling. The drawback of these fillings is they are brittle and mostly used in large cavities to avoid breakage. (Kelly & Benetti, 2011).

- **Glass Ionomer**

It's constructed of acrylics and a particular sort of glass. Fillings underneath the gum line are the most typical uses for this material. Fluoride is released by glass ionomers, which might help prevent teeth from future decay. This substance, however, is weaker to resin composites and is more prone to stress and breakage(Sidhu & Nicholson, 2016).

Figure 3: (*Tooth Cavity Filling by Silver and Composite Material | Preferred Dental*, 2020)

2.3. Resin Based-Dental Composite materials

The dental composites, also known as the resin-based composite materials, are the synthetic polymers which used coupling agents to join the polymeric substrate with the mixture of the minerals, or the resin filler particles and the short fiber. This basically are intended to replace the tooth structure that has been lost because of some trauma, or any caries, or some other disorders, just as dental amalgam. Composites can also be utilized to cement crowns and dentures, among other things. Composite materials have become one of the most extensively utilized cosmetic restoration solutions as amalgam is phased out in dentistry. It is a blend of plastic and granulated glass that closely resembles the natural look of teeth. Resin composites fillings are used to improve the appearance of the teeth by altering their color, recovering decaying teeth, mending broken teeth, filling gaps among teeth, and making them more even. The resin is prepared by the dentist and applied to the tooth in layers. Each layer is hardened using a specific curing light. After the composite has hardened, the dentist will mold it to fit in the tooth. To avoid stains and early wear, the composite material is then smoothed and highly polished (Xu et al., 2013).

2.3.1. Types

There are two types of dental composites:

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

1. **Self-cure resins:**

The self-cure resins are delivered as two pastes that must be combined together 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.

2. **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., 2013).

2.3.2. Compositions

Dental composites are made up of inorganic filler particles covered in silane and dimethacrylate resin, usually bisglycidil methacrylate (BISGMA) or urethane dimethacrylate (UDMA). To reduce viscosity, a percentage of a lower-molecular-weight monomer, such as triethyleneglycol dimethacrylate (TEGDMA), may be added. Barium silicate glass, quartz, or zirconium silicate are utilized as filler particles, which are commonly mixed using 5% to 10% content of tiny (0.04-m) colloidal silica particles. Quartz or porcelain particles are distributed in a photopolymerizable synthesized resin matrix in new dental composite materials. The polymer ingredients are mixed with a sharply split inorganic substance, such as barium aluminosilicate glass or another crystal composition that has an appropriate quantity of radiopaque oxide, making the resulting glass radiopaque to x-rays (Hervás García et al., 2006).

The handling and filler content of resin-based dental composites are divided into two categories. Lightweight composites are viscous and include a variety of filler sizes, making them strong. Some flowable composites including the monomers HEMA, that lowers the viscosity without losing the filler loading (Pratap et al., 2019).

2.3.3. Limitation

Dental composite resins are more cosmetically attractive because they match the color and appearance of the natural teeth. Also, they need less drilling, so less tooth structure must be removed. Unlike other materials, they harden in moments rather than day and form a strong link with the tooth, preventing it from breaking. lastly, if they are damaged, they can be repaired.

But the formation of **biofilm** over restorative materials that causes **secondary caries** is an issue that is considered as the restoration failure. Due to this problem, composite resins are preferred to be modified using nanoparticles(Erickson, 2013).

2.3.4. Biofilm formation and Secondary Caries

Secondary caries are caused by creation of the minute fracture points between the fillings and tooth tissue, allowing saliva to enter. When environment of the micro fissures is more favorable, then cariogenic bacteria in saliva aims its acid attack at the tooth tissues and surface, resulting in secondary caries (Feng, 2014). Biofilms of microbes stick to all tissue surfaces with in oral cavity. The deterioration of individual dental structures is caused by these bacterial biofilms. They also have an impact on the longevity of dental restorations(Engel et al., 2020). Due to formation of biofilm, secondary caries developed on the tooth just after filling has been in place for a while. This is also the leading cause of dental restorative material failure. They cannot be totally prevented, regardless of the filling material employed. After filling in the teeth, the proportion of secondary caries is quite high. The main challenge here is that of the secondary caries, which is resultant of a very complex interaction between injured soft tissue, overlying biofilms which often preserve the microbial circumstances that ultimately led to the primitive lesion, and orthodontic biomaterials which may assist, or even exacerbate the situation (Brambilla & Ionescu, 2021).

2.4. Nanotechnology

Nanotechnology is being used to generate new dental restorative materials with enhanced characteristics and anticaries potential. Nanoparticles are used in dental resin composites as effective caries control techniques. They have wide range of applications in the research and also innovation, especially in development of the novel materials. They are usually created with the unique features that mostly make them more appealing towards material scientists and the biologists. Nanomaterials offer a lot of potential for reducing biofilm formation, inhibiting demineralization, remineralizing the tooth structure, and combating caries-causing bacteria. (Melo et al., 2013). The identification and treatment of illnesses affecting the tooth and its associated structures is the focus of restorative dentistry. Advanced procedures are required for repairing and replacing damaged teeth structures, as well as restoring dental function and improving aesthetics. Nanotechnology has aided in the development of biodegradable and normally nontoxic resin composites greatly. In recent years, resin-based dental composite materials have made significant progress. Nanoparticles added to resin composites medium can improve mechanical qualities, such as minimal polymerization shrinkage and good abrasion resistance as well as surface hardness (Barot et al., 2021).

2.4.1. Silver nanoparticles

Silver nanoparticles had become a well-known research topic in the recent years among the different nanoparticles. These silver nanoparticles are usually composed of 20 to 15,000 of the silver atoms and they have a size normally less than 100 nanometers. They display exceptional antibacterial action, even at low concentrations, because of their large surface to the volume ratio. Furthermore, they are inexpensive and have demonstrated little cytotoxicity and immunological reactivity. As a result, silver nanoparticles have a wide range of biological uses (Yin et al., 2020b). They're employed in drug delivery activities, medical image processing, and molecular testing, as well as therapies including surgical mesh, artificial joint substitution fabrication, wound dressing, and wound healing medication (Shanmuganathan et al., 2019).

Action mechanism of Ag nanoparticles:

Silver nanoparticles (AgNPs) have shown good antibacterial properties through the following mechanism:

- Cell wall and cellular membrane disruption: silver nanoparticles discharge silver ions (Ag+), which attach to or penetrate through the cell wall and cytoskeleton.
- Ribosome denaturation: silver ions successfully denature ribosomes, inhibiting proteosynthesis.
- Adenosine triphosphate (ATP) generation is disrupted: silver ions disable a respiratory enzyme on the cytoplasmic membrane, halting ATP production.
- Membrane disruption caused by reactive oxygen radicals: reactive oxygen generated by a disrupted electron transport system can disrupt the membrane.
- Silver and reactive oxygen species attach to deoxyribonucleic acid (DNA) and hinder its replication and cell proliferation.
- Membrane denaturation: silver nanoparticles gather in the gaps of the cell wall, causing membrane denaturation.
- Membrane perforation: silver nanoparticles go directly over the cytoplasmic membrane, allowing organelles to be released from the cell (Yin et al., 2020a).

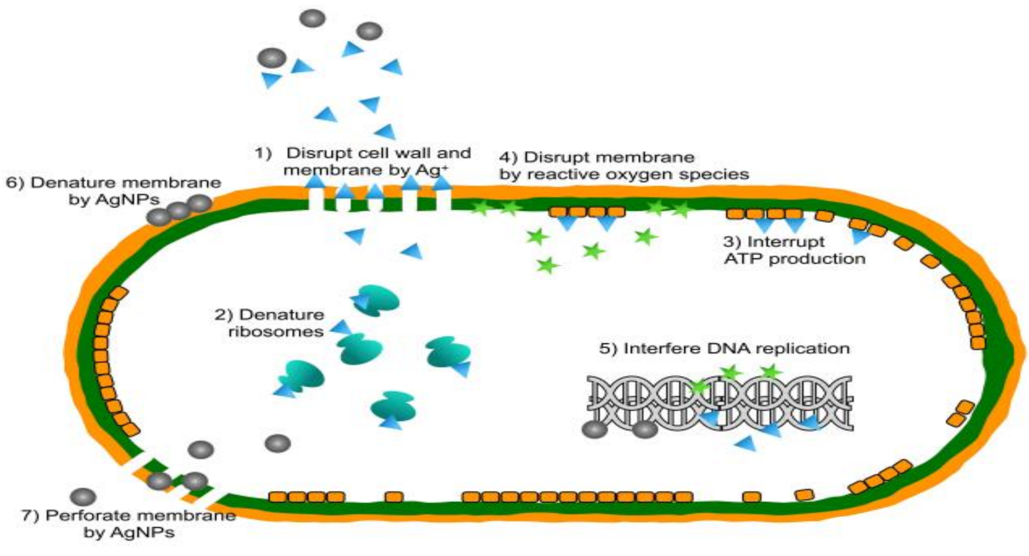

Figure 4: Mechanism of action of silver ions(Yin et al., 2020b)

Role of AgNPs In dentistry:

Silver nanoparticles are utilized in dentistry to create antimicrobial materials that enhance the reliability of dental products and improve treatment outcomes. Antimicrobial characteristics and bioavailability against the bacteria, and fungi, and also enveloped viruses have made AgNPs stand out in scientific studies. The release of cationic silver and its oxidative potential are key to mechanism of the action of the Ag nanoparticles. This mechanism of action of Ag nanoparticles can also be influenced by their size and morphology (Fernandez et al., 2021).

When AgNPs were included with the dental composite resin, it demonstrated significant antimicrobial properties specifically against the *S mutans* and *E faecalis* bacteria, as well as enhanced mechanical qualities. The generation of silver ions following immersion in the composite was linked to the antibacterial actions (Kassaee et al., 2008).

Drawback of using AgNPs:

The knowledge that silvers ions are hazardous to organisms has been effectively utilized in research. Due to the massive higher surface area of nanoparticle formation, silver nanoparticle formulation will boost broad spectrum antibacterial capabilities. Furthermore, these antimicrobial characteristics have been shown to be effective against antibiotic-resistant bacteria and to have a synergistic impact with standard antimicrobials. AgNPs have a broad range of applications in medicine, including inhibition of the bacterial adhesion, their growth, and the biofilm development in a variety of dental treatments. As a result, AgNPs in oral treatments come into direct touch with teeth as well as other cellular components in the oral cavity. To meet its safety criteria, major adverse occurrences must be handled in addition to AgNPs' indisputable support to dental health against infectious bacteria and causing disorders. Because of the presence of argyria (skin coloring) and argyosis (eye discoloration) as well as some other connected adverse effects on kidneys, hepatic, gastrointestinal, and respiratory adverse effects, free Ag+ adverse events in industrial wastes are common. Furthermore, some studies on AgNPs toxicity can relate to the co-exposure with the fluorides or the cytotoxicity to gingival fibroblasts caused by their capping agent. The major drawback concerning the use of AgNPs is their **cytotoxicity** (Bapat et al., 2018b).

According to recent studies, composites containing 1% silver nanoparticles (w/v) have better antibacterial activity than the control group. This is the optimal AgNPs concentration. Any more than this would result in cytotoxicity(Kasraei et al., 2014a).

2.4.2. DEAE-Dextran coated AgNPs

Coating of nanoparticles is a remarkable way to enhance the benefits of any nanoparticle substance. This can enhance the physiochemical properties of the material. This coating approach can improve AgNP stability by reducing agglomeration and increasing electro steric stabilization among particles. One of the most essential functions of coating is to protect live cells from the cytotoxic effects of AgNPs. Many coating compounds have also been shown more useful in stabilizing of AgNPs, keeping the unique form, and then reducing silver ions, all of these are the key considerations in AgNPs toxicity (Mohamed Fahmy et al., 2019). Silver nanoparticles agglomerate quickly and are not particularly stable, hence a capping agent that improves their stability is preferred (Shanmuganathan et al., 2019). The metal nanoparticles have been stabilized by the combined effect of DEAE-Dextran, a cationic polymer of dextran, and silver nanoparticles (Mikac et al., 2017). Individual silver nanoparticles are less efficient against bacteria than DEAE-Dextran AgNPs. The addition of silver nanoparticles to DEAE-Dextran decreases toxicity and improves antibacterial activity. Incorporating DEAE-Dextran silver nanoparticles into composite materials would minimize biofilm from forming on the composite resins and avoid secondary caries from forming (Corrêa et al., 2015).

Chapter 3

3. Materials and Method

3.1. Preparation of AgNPs and DEAE-AgNPs

3.1.1. Materials

All Chemicals DEAE-Dextran hydrochloride, silver nitrate ($AgNO_3$), Sodium Borohydride ($NaBH_4$), Ammonia, and Distilled water were all purchased from the Sigma-Aldrich for use in the experiments.

3.1.2. Methodology

The chemical reduction approach was used to make AgNPs (Mulfinger et al., 2007). $NaBH_4$ (2.0 mM) and $AgNO_3$ (1.0 mM) were utilized. As a reducing agent, $NaBH_4$ was utilized. In a biuret, 10 ml $AgNO_3$ was added dropwise (one drop per second) into 30 ml $NaBH_4$ solution while constantly stirring. After adding 2ml $AgNO_3$, the solution begins to become yellow and eventually turns into a brilliant yellow color. As soon as the addition is finished, the stirring stops.

Figure 5: Bright Yellow AgNPs

DEAE-AgNPs were made using the technique described before (Mikac et al., 2017). In 10 ml distilled water, $AgNO_3$ (0.51g) and DEAE-dextran HCl powder (0.17g) solutions were produced, yielding a final concentration of 20ml. DEAE was mixed with $AgNO_3$ at a 3:1 mass ratio. The two solutions were combined together with steady stirring, yielding a milky white solution. Because of the production of AgCl in solution, this happened. Then, until the solution was colorless, 23% NH_3 solution was added. Then 5-6 drops of 2.0mM $NaBH_4$ were added. The solution turns a dark brown color almost quickly.

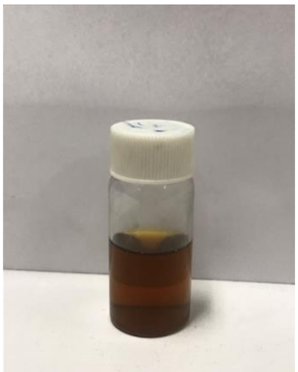

Figure 6: Brownish DEAE-Dextran AgNPs

3.2. Characterizations

The size, net charge on their surface, and aggregation of DEAE-Dextran coated nanoparticles were determined and evaluated to ensure they were of the proper size and type to be employed in the dental composite resins for antibacterial models testing.

3.2.1. UV-Vis absorption Spectroscopy

UV-Vis absorption spectroscopy is the most often utilized technology in labs. A light beam is directed through a sample within a cuvette. The individual molecules in the sample absorb a certain wavelength of light. Because certain wavelengths pass through the samples, the amount of absorption is determined. To begin, a cuvette containing just solvent (Reference) is put inside the hood and absorption is measured. The absorption spectrum is then obtained by placing a second cuvette filled with sample material in the hood. The laser beam separates into two pieces, one of which is focused at the Reference. The second one was pointed at the second cuvette carrying the sample. An absorbance spectrum is created for the whole wavelength range. At any given wavelength, lambda max is the maximum absorption value. The Beer Lambert Law, according to which the absorbance of a sample mixture is proportional to concentration (molar) in the sample cuvette; this value of absorption is known as molar absorptivity and is used to compare the spectra of various chemicals.

3.2.2. Particle size and surface distribution determination

The morphology of DEAE-Dextran coated AgNPs were studied by using a Scanning Electron Microscope (SEM). For instance, glass slides containing DEAE-Dextran AgNPs were coated with gold (30nm) to make them functional and conductive for SEM investigation. SEM study, in particular SEM, analyses the physical dispersion of nanoparticles and confirms their spherical form.

3.2.3. FTIR analysis

Fourier Transform Infrared (FTIR) is the most efficient infrared spectroscopy method. In this technique, infrared (IR) radiation is sent through a specimen, where it absorbs IR radiations while transmitting some of it. As a consequence, the resultant spectrum displays molecule absorption and the transmission, forming molecular fingerprint of that sample that may be used to identify unknown material and evaluate the quality and quantity of the substance present.

3.2.4. Zeta sizer and surface Potential

In terms of the level of force of attraction between nanoparticles and their stability, the zeta potential value often defines their net charge upon them.

3.3. Isolation of Bacterial strains

Saliva was obtained from ten individuals. Only those individuals who had never undergone any previous dental operations were chosen. For the last two hours, volunteers did not brush or eat anything in order to get saliva. These samples were well mixed, and half of them were serially diluted with autoclaved distilled water before being distributed on Tryptic soy agar plates (1% sucrose) as shown in figure 4. This half diluted saliva was used in isolation of strains while other half was used in preparation of inoculum for microcosm (Li et al., 2014). Later, colonies of bright and dark yellow hue were streaked on separate TSA plates and further used blood agar plates to isolate *S. Mutans* and *E. faecalis*.

Figure 7: Diluted Saliva spreading of TSA (1% sucrose) plates

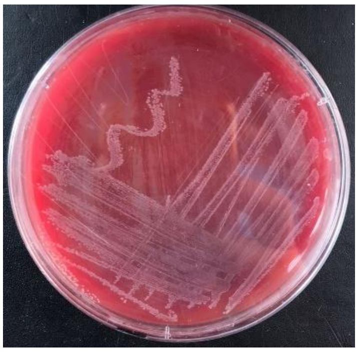

Figure 8: *S. mutans* on blood agar plate

Figure 9: *E. faecalis* on blood agar plate

3.4. Modification of composite resin discs

In order to prepare discs for antibacterial testing, Nexcomp-META BIOMED dental composite resin was employed. Organic polymers Bis-GMA, UDMA, and Bis-EMA make up this resin. A consistent methodology for weight-percentage incorporation of AgNPs and DEAE-Dextran coated AgNPs into composite resins was used. Different concentrations of DEAE-dextran coated AgNPs, such as 1%, 2.5%, and 5%, were manually added to composite resins. They were mixed for 1 minute to ensure homogeneity. The plastic mould was used to make discs out of this resin. The composite was cured for 2 hours with blue UV radiation at 400 mV/cm^2 intensity and a wavelength of 430-480 nm. All of the discs were 2×4 mm in diameter and 0.1g in weight. NPs were manually inserted and blended in discs using a glass rod.

Figure 10: 1% AgNPs incorporated composite resin disc

Figure 11: DEAE-Dextran AgNPs incorporated disc

3.5. Antibacterial *in vitro* models

Saliva was taken from 10 healthy adult volunteers, falling within the criteria of natural teeth with no caries history and no antibiotic usage in the previous three months. Prior to giving saliva, individuals did not clean their teeth for 24 hours and did not consume any food or drink. Each donors' saliva was combined in an equal amount. Half of this saliva mixture was diluted to 30% proportion in sterile glycerol and kept at -80°C for use as microcosm inoculums (Li et al., 2014). *S. mutans* and *E. faecalis* bacterial strains were isolated and differentiated on blood agar plates. Using autoclaved distilled water, serial dilutions were performed.

3.5.1. Culture conditions

The bacterial growth medium was Tryptic Soy Broth with 1% Sucrose (TSBS). Sucrose was added for enhancing growth scope of tryptic soy broth. This media was used for preparing pre culture of both bacterial models. All incubations were performed at 37ºC. A 10 ml tryptic soy broth with 1% Sucrose was prepared for every bacterial culture.

3.5.2. Single Specie Model

Bacterial strains were isolated as described earlier. Blood agar plates were used to isolate *S. mutans* and *E. faecalis* bacteria. Both strains were placed in separate 5ml TSBS tubes and incubated overnight. 200 µl of this preculture were placed in 5 ml of TSBS medium and incubated for 3 hours in shaking water bath. After 3 hours, absorbance was then measured at the 600 nm, and then culture was serially diluted. 500 µl of this diluted culture was coupled with a composite disc sample in an Eppendorf tube and placed in an incubator. 8 hours later 50 µl from each Eppendorf was collected and spread out on TSA plates and incubated overnight.

Figure 12: Eppendorf's with 500 µl diluted culture and immersed composite discs immersed

3.5.3. Microcosm Model

The inoculum was mixed with 10ml TSBS and incubated overnight. After obtaining the culture's OD at 600nm, it was serially diluted and 500µl was put to Eppendorf's with composite samples and incubated for 8 hours. Then, from each Eppendorf, 50µl was collected and put on TSA plates, and was incubated overnight.

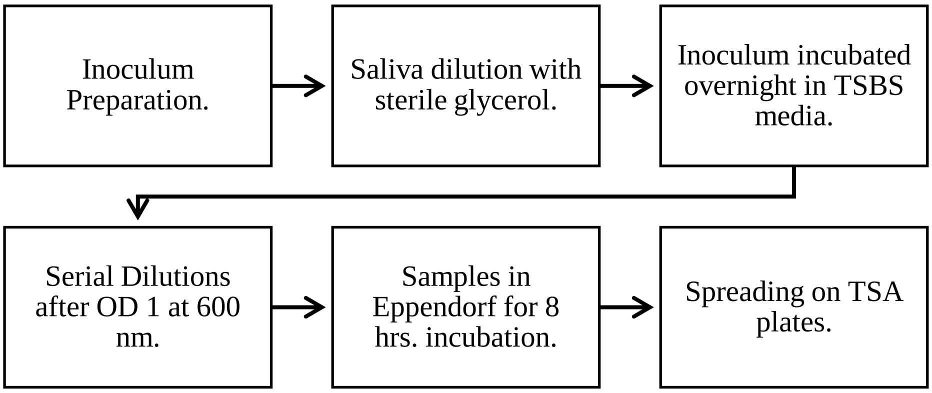

Figure 13: Scheme of Antibacterial models

3.6. Colony Forming Unit (CFU)

Colonies on the plates for each model were manually counted, and log CFU/ml was calculated. To eliminate any uncertainty, all models' trials were repeated three times. The 5th dilution was used in all of the trials.

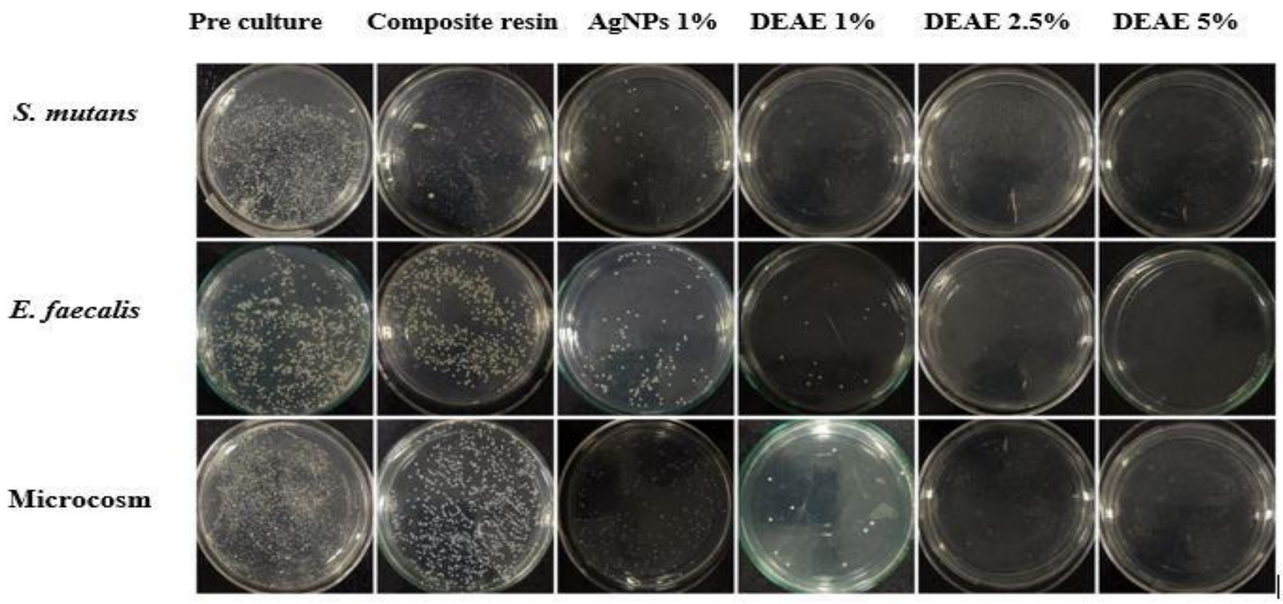

Figure 14: Bacterial colonies on TSA plates

3.7. Hemolytic Testing of AgNPs and DEAE-Dextran AgNPs

Hemolytic activity is an analysis based on erythrocyte lysis caused by metal ion interaction and the influence of metal ions on RBCs lysis.

Composite discs were put in a test tube with 10 ml PBS solution. Each tube was filled with 0.2 ml of the diluted blood solution, gently inverted, and kept incubated in the water bath for another 2 hours. A favorable positive control that indicates 100% hemolysis i.e., Triton X-100 (1%) was prepared by gently mixing 0.2 ml of the diluted blood solution with 10 ml of the 0.1 percent sodium carbonate solution. The negative control was made by mixing 0.2 ml of diluted blood with 10 ml of PBS solution. Positive and negative controls tubes were made and incubated for almost the same way as sample containing test tubes. After the incubation, all of these tubes were then centrifuged for another 10 minutes to pellet the erythrocytes and the supernatant was passed to spectrometric cuvettes (Thom et al., 2003).

Readings of optical density (OD) at 350 nm were obtained and documented to compute the % hemolysis using the formula below:

$$\%Hemolysis = \frac{Sample\ OD - Negative\ Control\ OD}{Positive\ Control\ \ OD - Negative\ Control\ OD} \times 100$$

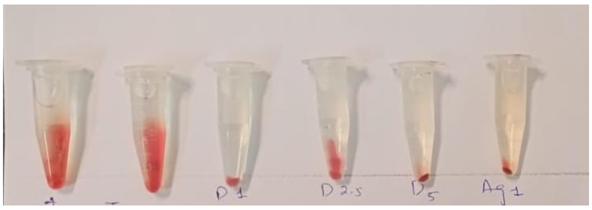

Figure 15: Hemolytic testing

3.8. Mechanical testing of composite resin discs

Stainless metal split molds were used to make composite resin samples during compressive strength testing (4mm in diameter and 2mm in height). After being removed from the mold, the samples were light cured. Prior to testing, the specimens were kept in saliva and then incubated at 37°C for 24 hours. A maximum load cell of the 5 KN was used to test compressive strength at quite a cross-speed of 0.5mmmin1. The composite disc samples were put with their flat sides between both the plates of the testing equipment for compressive testing, and the compressive force was administered along the specimens' entire length (Dias et al., 2019).

3.9. Statistical Analysis

Graph Pad Prism 9.2 was used to do statistical study of the multiple group comparison analysis using one-way ANOVA at a significance level of 5%. On untreated and treated composite resin samples by NPs, mean values of log CFU/ml of *S. mutans*, *E. faecalis*, and microcosm colonies were obtained. In contrast to unmodified samples and samples with 1% AgNPs, specimens containing different concentrations of DEAE-Dextran Ag nanoparticles were tested for the development of bacterial biofilms, with statistically significant differences ($P<0.05$). The Shapiro–Wilk test was used for determining data's normal distribution. For multiple comparisons, one-way ANOVA and Tukey's test were used for antibacterial activity across the biofilm, hemolytic activity, and mechanical testing. The level of significance for all of the tests was set at 5%.

Chapter 4

4. Results and Discussion

4.1. Characterizations of AgNPs and DEAE-Dextran AgNPs

The DEAE-dextran AgNPs were characterized by UV spectrophotometry by UV-2800 BMS Biotechnology Medical Services, Madrid, Spain spectrophotometer. FTIR was done by Bruker FTIR Spectrometer ALPHA ll (Westborough, MA, USA). SEM and EDX were performed with SEM VEG 3 LMU (Tescan, Czech Republic), and zeta potential was analysed by Malvern Zeta sizer (Malvern).

4.1.1. UV-Vis Spectroscopy

UV analysis was performed for AgNPs and DEAE Dextran AgNPs. Within the region of 400-420 nm, AgNPs showed a very strong and wide peak. The decrease of Ag^+ to Ag^0 is confirmed by a distinct band (Mohanta et al., 2020). AgNPs had a peak absorption of 0.5 at 412 nm. The peak spectrum of DEAE-AgNPs was 420 nm, with 1.2 units absorption. Because of the dextran coating on AgNPs, the peak migrated forward. The synthesis of DEAE-AgNPs was confirmed with these observable peaks. This absorption maxima have migrated within the range of 420 - 450 nm with the addition of DEAE-Dextran, which is near to the original peak and indicates the stability of Dextran coated nanoparticles. Silver nanoparticles were effectively encapsulated with DEAE-Dextran macromolecules. (Figure 11)

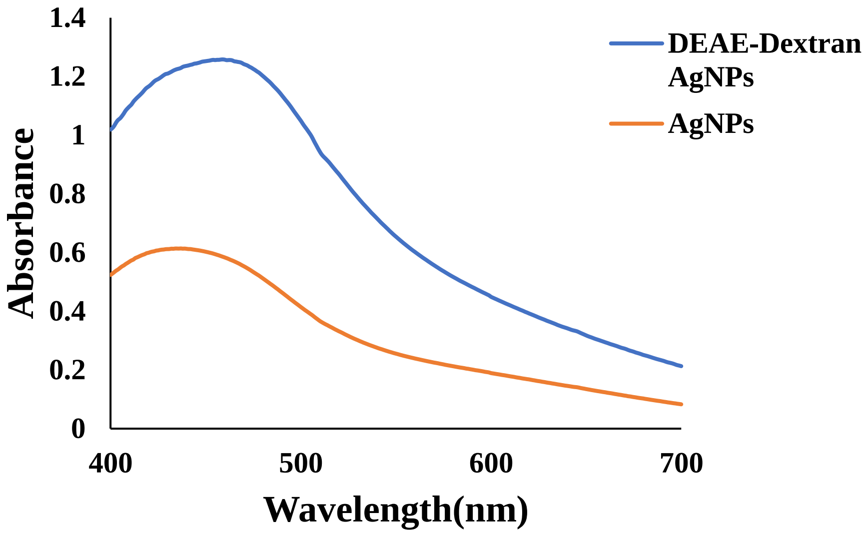

Figure 16: UV- absorbance

4.1.2. Zeta size and charge

Zeta analysis was performed to examine the average size and charge potential of the synthesized NPs. The average particle size according to zeta sizer, for AgNPs was found to be 72.70 nm. Whereas, the particle size of DEAE-Dextran coated AgNPs, increases to 170 nm.

	Z-Average (d. nm)	St. Dev (d. nm)	Zeta Potential (mV)
AgNPs	72.70	30.7	-15.1
DEAE-Dex AgNPs	170	41.66	16.2

Table 1: zeta size and charge of DEAE-Dextran AgNPs

The values of zeta size and charge of DEAE-Dextran AgNPs are presented in table 1. The zeta potential of AgNPs was -15.1 mV, which changed to +16.2 mV for DEAE-Dextran AgNPs, indicating DEAE-Dextran coating on AgNPs. The electrostatic interaction between the negative charge of AgNPs and the positive charge of Dextran resulted in improved antibacterial activity (Davidović et al., 2017). DEAE-Dextran AgNPs were also shown to be more stable in suspension as the value was closer to +20 mV (Mikac et al., 2017).

4.1.3. SEM analysis

The SEM analysis at 20kV was used to examine the morphology of AgNPs and DEAE-Dextran AgNPs. This analysis was carried out by dropping the NP suspension directly onto glass slides and drying them at room temperature. The AgNPs were around 23-30 nm in size, and there were irregular hexagonal shaped DEAE-Dextran AgNPs that were nearly 5 times larger due to the DEAE-Dextran coating. The SEM images revealed AgNPs with a spherical form, but DEAE-Dextran AgNPs were mostly hexagonal with few exhibiting irregular shapes. (Figure 14 and 15)

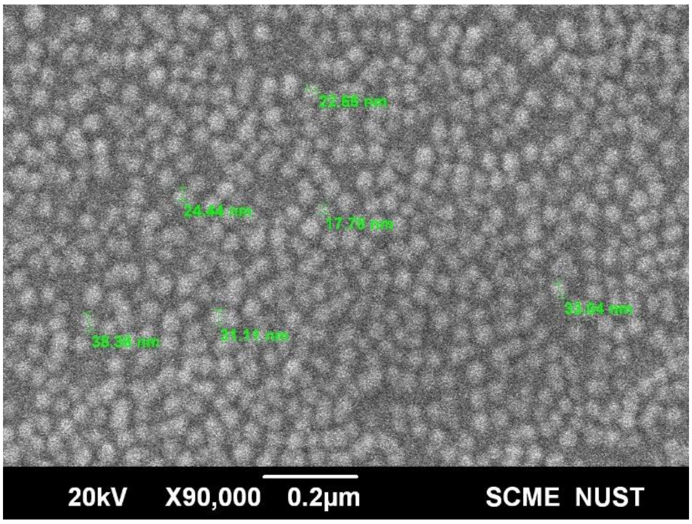

Figure 17: SEM image of AgNPs

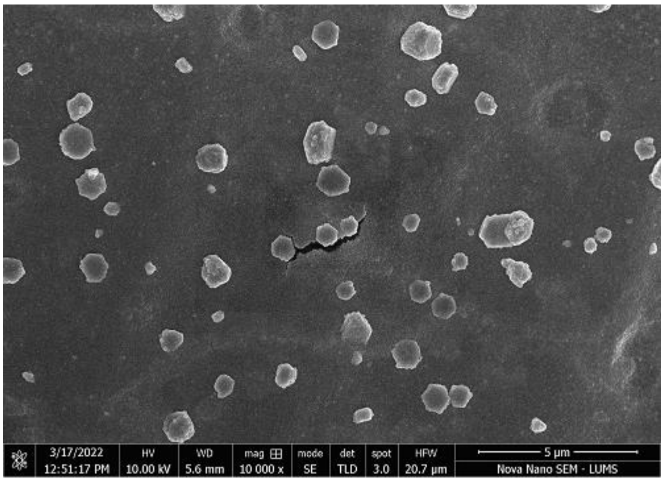

Figure 18: SEM image of DEAE-Dextran AgNPs

4.1.4. FTIR analysis

The DEAE Dextran coating on AgNPs was validated by FTIR analysis. FTIR analysis was used to better understand how Dextran stabilizes the AgNPs. The figure 17 shows the FTIR of

AgNPs, DEAE-Dextran AgNPs, and DEAE Dextran HCl powder. It represented the spectra of simple AgNPs and DEAE-Dextran AgNPs that was recorded at 400 cm^{-1} - 4000 cm^{-1}. For DEAE-Dextran AgNPs, the stretching peaks for amide bond were found at 1517 cm^{-1}. Some peaks in these spectra were almost identical, however there were a few peak changes in the area above 1000 cm^{-1} due to DEAE-Dextran covering on AgNPs. Dextran coating was found at 1091 cm^{-1}, 1972 cm^{-1}, and 2008 cm-1. These peak changes implied dextran stretching (İspirli et al., 2021). OH, band stretching is allocated to a large span surrounding 3600 cm^{-1}. This is due to water adsorption in the samples, which decreases the signals on the 2860 cm^{-1} band. The C-O-H deformation at 1517 cm^{-1} contributes to the symmetric stretching of the carboxylate group's O-C-O. The absorption of the stronger band's spans from 1188 cm^{-1} to 826 cm^{-1}. C-O-C vibrations are visible. The peak in the spectra at 966 cm^{-1} is typical dextran adsorption, which displays α1, 3 glycosidic linkages. Some particular shifts were noticed in the range above 1000 cm^{-1} after coating. The stretching of DEAE-dextran can be detected on 1091 cm^{-1}, 1972 cm^{-1}, 2008 cm^{-1} and 2161 cm^{-1} peaks, which reflect dextran adsorption on AgNPs (Can et al., 2018).

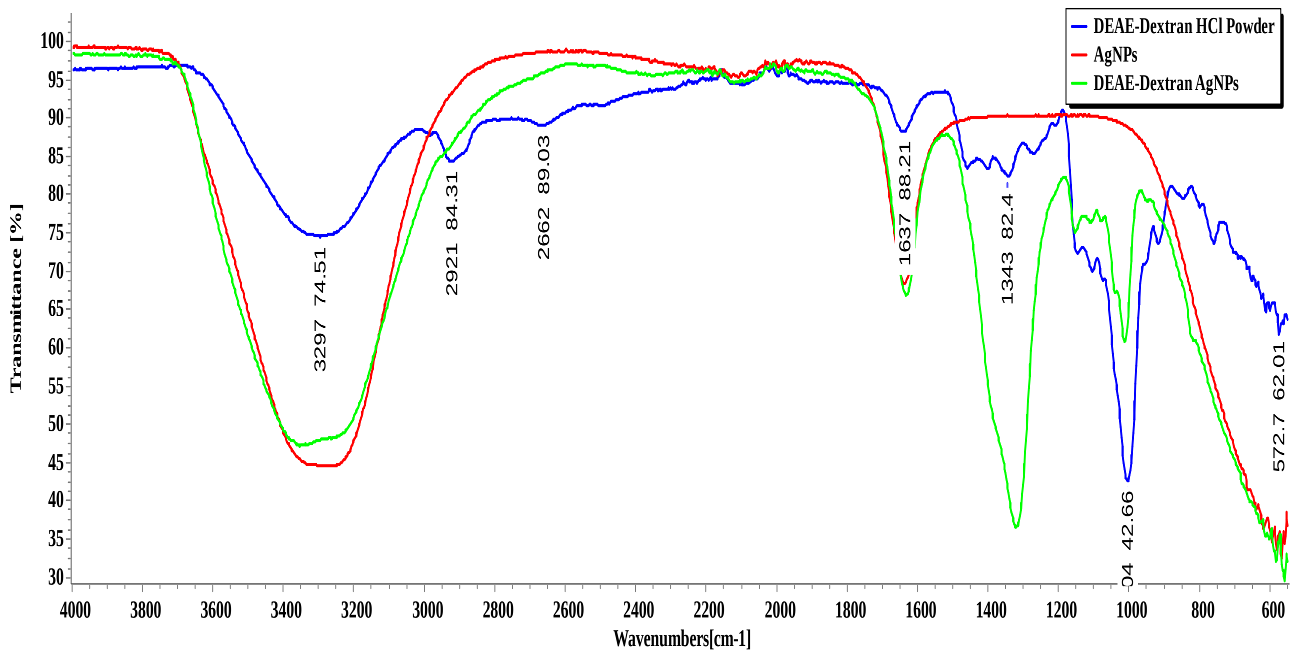

Figure 19: FTIR analysis

4.1.5. EDS Analysis

EDS analysis is performed for calculating the elemental composition of NPs. Figure 20 (a) showed the EDS analysis of AgNPs. The results of this analysis showed a significant peak of the silver. That confirmed the synthesis of AgNPs. Figure (b) represents that EDS analysis of DEAE-Dextran AgNPs exhibited a strong peak in the carbon zone as well as reduction in silver, indicating that DEAE-Dextran was successfully coated on AgNPs.

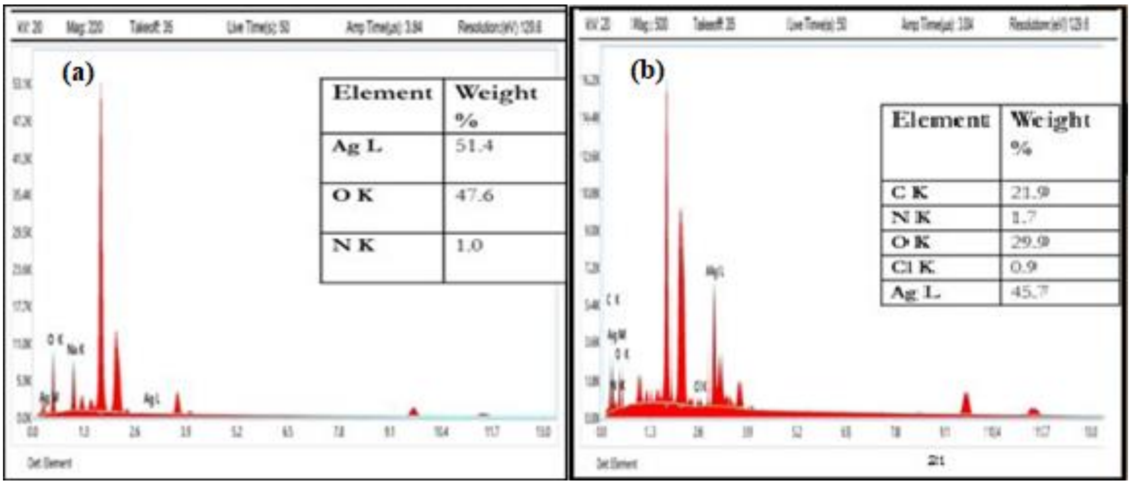

Figure 20: EDS analysis of (a) AgNPs, (b) DEAE-Dextran AgNPs

4.1.6. Antibacterial activity of AgNPs and DEAE-Dextran AgNPs

The antibacterial activity of AgNPs and DEAE-Dextran AgNPs was performed and compared at different concentrations i.e., 1% , 2.5% and 5%. For the comparison study, different DEAE-NPs concentrations were utilized. Figure **22** indicated that DEAE-Dextran AgNPs modified composite resin discs reduced the bacterial colony count significantly when compared to AgNPs and non-modified composite discs. The graphical representation of in vitro antibacterial activity was shown in Figure 15. Addition of 1% AgNPs into composite resin had a higher antibacterial impact than unmodified resin. Lesser concentrations of AgNPs had lower toxicity and better mechanical characteristics(Kassaee et al., 2008). *S. mutans* and *E. faecalis* growths were reduced to nearly half with 1% AgNPs incorporation in the composite material. Increasing the concentration would increase cell toxicity and compromise the mechanical characteristics of the composite resin (Kasraei et al., 2014b). *S. mutans* growth was fully suppressed by composite resins containing 1% DEAE-Dextran AgNPs as compared to 1 % AgNPs and unmodified composite resin. *E. faecalis* and microcosm model demonstrated that concentration of 2.5 % DEAE-Dextran AgNPs was more effective. DEAE-Dextran added to composite resins suppressed the growth of *S. mutans* and *E. faecalis* substantially.

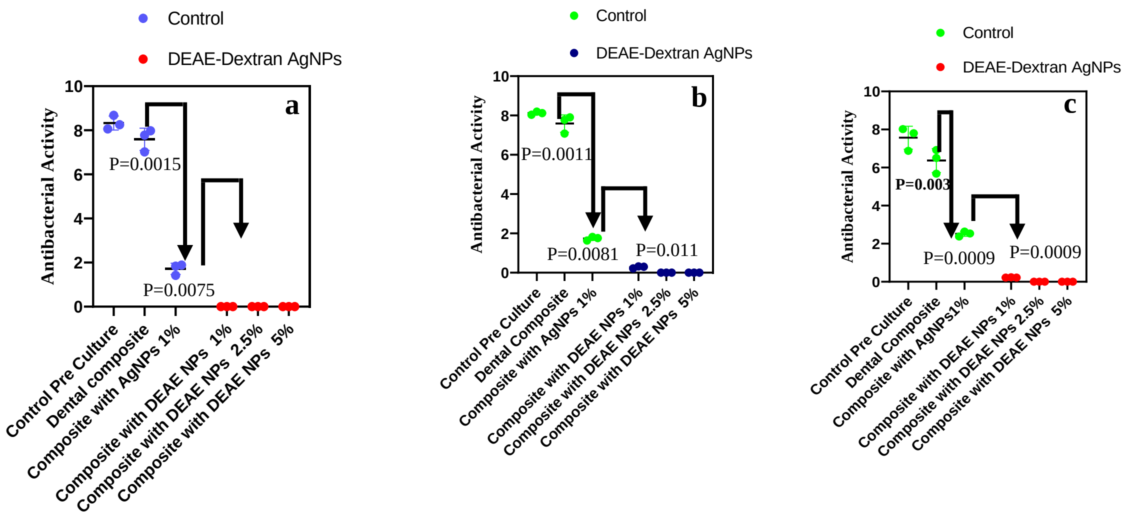

Figure 21: Antibacterial activity of (a) *S. mutans*, (b) *E. faecalis* and (c) Microcosm models: *Antibacterial effect of modified composite resin disks**Indicate statistically significant changes from nonmodified composite resin ($P<0.05$).

4.1.7. Hemolytic assay

Hemolytic assay was performed to examine the cytotoxicity of different doses of DEAE-Dextran AgNPs and AgNPs. Healthy volunteers provided blood samples. Triton X-100 (1%) was used as positive control, whereas PBS was employed as the negative control. At the wavelength of 500 nm, the absorbance of samples was measured. Figure 22 shows the hemolytic activity of various concentrations of DEAE-Dextran AgNPs, AgNPs, and non-modified composite resin. So according to ISO/TR 7406, the safe limit of hemolytic proportion is 5%. The results of this current study showed that 1% AgNPs had higher hemolytic activity i.e., 4.8 % than non-modified composite resin and DEAE-Dextran AgNPs.

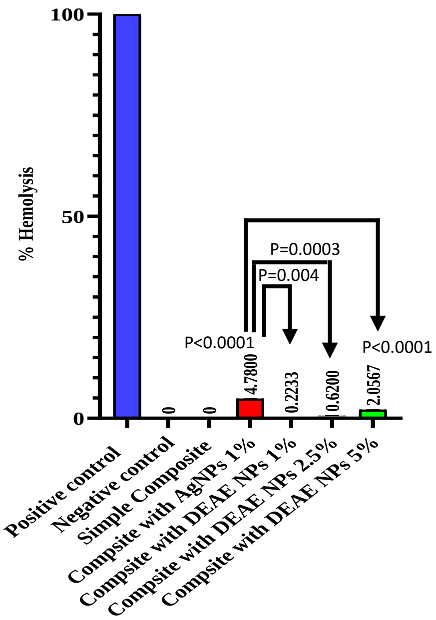

Figure 22: Hemolytic Activity of Composite resin, AgNPs and DEAE-Dextran AgNPs****statistically significant changes from untreated composite resin (P< 0.05).

Although AgNPs demonstrated hemolytic activity and it was in the safe range. This 1% of concentration is the optimum concentration for AgNPs. Whereas, when DEAE-Dextran was added to AgNPs, cytotoxicity was significantly reduced. There was no prominent hemolysis activity seen with the addition of 1% DEAE Dextran (i.e., 0.2%) and 2.5 % DEAE-Dextran AgNPs (i.e., 0.64%) while raising the concentration to 5% indicates some activity but is still less than AgNPs i.e., 2.08%.

4.1.8. Mechanical Testing

The mean compressive strength (MPa) for unmodified and NPs modified resin composite discs (wt.%) is represented in figure 22. As DEAE-Dextran AgNPs were added to the resin composites at 1%, 2.5%, and 5%, the compressive strength of composites resin enhanced considerably (P<0.05) when compared to the unmodified group.

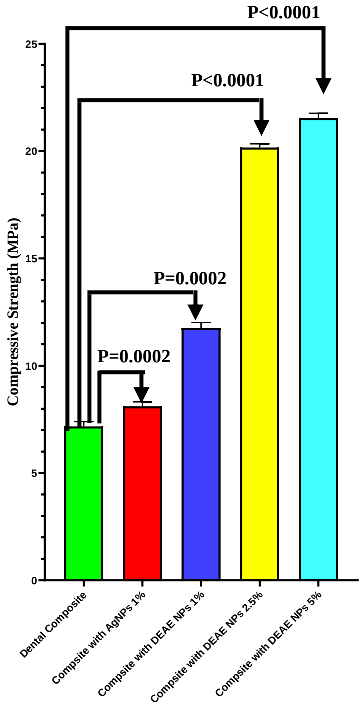

Figure 23: Compressive strength of Modified and non-modified composite resins****statistically significant changes from untreated composite resin ($P < 0.0001$)

The incorporation of DEAE-Dextran AgNPs showed improvement in the compressive strength of the composite material.

Conclusion

As the dental caries is one of most serious health issue that almost everyone had to face once in a lifetime. The major aim of this study was synthesizing of DEAE-Dextran AgNPs that could be effectively used as a treatment for caries. Different sort of NPs is utilized across the world in this regard, but the development of biofilm has created a barrier in treatment of the dental caries, limiting advantages for composite materials. Nanotechnology has revolutionized the dental science. It entails designing and constructing a high-quality nano-sized framework that can stick to resin materials and improve their efficiency and antibacterial characteristics. DEAE-Dextran is a biocompatible stabilizing and capping agent that is a cationic derivative of dextran. In order to make DEAE-Dextran coated AgNPs, Mulfinger's chemical reduction approach and Mika's synthesis methodology was used. The molar ratio of Ag: Dextran (3:1) synthesized hexagonal and few irregular shaped DEAE-Dextran AgNPs in this study. DEAE-Dextran AgNPs have demonstrated highly favorable results in the prevention of polymicrobial biofilms and specific *S. mutans* and *E. faecalis* strains in dental composites in tests. Also, as the results of analysis have revealed raising concentration of the DEAE-Dextran AgNPs increased the antibacterial action without causing cellular toxicity or changing the mechanical qualities of the composite resin. Because of the consistency and modest number of NPs included into composite resin, they hindered *S. mutans* and *E. faecalis* composed biofilm formations on surface. Also, the usage of AgNPs in conjunction with an optimal concentration of DEAE-Dextran improved the distinctive behavior. This research might aid future clinical protocols for employing DEAE-Dextran AgNPs as dental adhesives.

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