Antibacterial Activity of Dental Composite integrated with Ciprofloxacin loaded Silver Nanoparticles

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Acknowledgments

In the name of Allah, the Beneficent, the Merciful

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Abbreviations

BISGMA - bisglycidil methacrylate

CIP-AgNPs - Ciprofloxacin loaded Silver Nanoparticle

E. faecalis - Enterococcus faecalis

HEMA - hydroxyethyl methacrylate

Hrs. – Hours

NPs – Nanoparticles

S. mutans - Streptococcus mutans

TEGDMA - triethyleneglycol dimethacrylate

UDMA - urethane dimethacrylate

Abstract

Resin composites have been widely used in dental restoration. However, polymerization shrinkage and resultant bacterial microleakage is a major limitation that may leads to secondary caries. To overcome this, a new type of antibacterial resin composites containing ciprofloxacin loaded silver nanoparticles were synthesized. Ciprofloxacin loaded silver nanoparticles 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) and Zeta potential. Ciprofloxacin loaded silver nanoparticles were added into resin composites. The antibacterial properties of these ciprofloxacin loaded silver nanoparticles modified resin composites against *Enterococcus faecalis, Streptococcus mutans* and Saliva Microcosm model were evaluated. Cytotoxicity of these modified resin composites was determined by performing hemolytic assay. Compressive strength (CS) of these modified resin composites was assessed by a universal testing machine. The results indicated that the antibacterial activity and compressive strength of resin composites containing Ciprofloxacin loaded silver nanoparticles were superior to the control group and also exhibited less cytotoxicity as compared to the resin composites containing silver nanoparticles. In short, these results established strong ground applications for CIP-AgNPs modified dental composite resins.

Chapter 1

1. Introduction

1.1. Dental Caries

Cavity, tooth decay, and dental caries all refer to the same phenomenon and are interchangeable. In our mouths, there are hundreds of different kinds of microorganisms. While some bacteria are helpful, others might be hazardous. Some bacteria produce acids from the sugar in meals, which causes illness. These infections gradually demineralize enamel, leading to cavities. Every time we consume food that contains sugar or starch, oral bacteria interact with it to create acid that attacks the enamel. Enamel demineralizes when acid attacks occur repeatedly. It is a typical infectious disease that is primarily brought on by the gram-positive bacterium Streptococcus mutans, which is primarily found in the oral cavity. This bacterium breaks down sugars to create acid, which slowly demineralizes the structure of teeth (Rathee & Sapra, 2021). The disease that affects people the most frequently over the world is caries. The majority of people are susceptible to this condition throughout the remainder of their life. Dental caries affects about 36% of the world's population, according to dentists. When it comes to baby teeth, around 9% of the population is affected. Caries risk is affected by a number of factors, including biological, physiological, environmental, psychological, and lifestyle-related factors (Al-Shahrani, 2019).

According to data from the World Health Organization, dental caries largely affects 60 to 90 percent of children worldwide, primarily in the underdeveloped countries (WHO). Dental caries is more common in underdeveloped countries than it is in industrialised ones, where it is mostly under control (James et al., 2018). Children who have caries feel discomfort, which can interfere with their ability to focus, eat, sleep, and communicate, which can impair how effectively they learn in school. If not treated promptly, this infection causes severe discomfort and inflammation and may require costly surgical intervention.

Data on hand indicate that dental caries affects more than 60% of Pakistan's population as a whole. Dental caries is seen as a complicated disease that affects the host, an agent, and its surroundings. Streptococcus mutans is the cause of dental caries (S. mutans). In order to produce lactic acid, S. mutans attaches to the dental pellicle and uses carbohydrates as fuel. This creates an acidic environment around the tooth. Enamel demineralizes as a result, followed by the entire dentin ultimately. Variables in the process of dental decay include tooth structure, bacteria present in the form of a dental biofilm, and a diet high in sugar. Sugar consumption significantly affects the frequency and severity of caries (Lee, 2013).

1.1.1. Treatment

The most popular way to treat dental cavities is with dental fillings, sometimes referred to as restorations. These fillings are made of a variety of materials, including porcelain, dental composite resins, gold, silver, and occasionally a compound of elements known as dental amalgam. Due to their superior effectiveness and lower cost than silver or gold fillings, composite resin restorations are currently the most often utilised type of restoration. With the improved physiochemical properties of dental resin composites, these composite resins—tooth-colored polymers—were offered as mercury-free substitutes for silver amalgam fillings (Gupta et al., 2012).

Despite the fact that they are the materials that are most frequently used for rebuilding tooth enamel due to their great aesthetic qualities and strength. Investigations unfortunately turned up failures, with secondary caries as the main culprit. It was found that dental biofilm accumulated more on resin composites. The development of biofilm in resin composites, which is caused by the absence of inhibitory activity against cariogenic bacteria like S. mutans, is a significant biochemical breakdown. Additionally, it becomes infected by microbes that have stuck to adjacent tissues. Because of the continuous caries that grows around these restorations and is treated with restorative replacement, more tooth loss results. As a result, one method for increasing the lifespan of dental resin composites is antimicrobial prophylaxis (Beyth et al., 2014).

1.2. Antimicrobial Dental Composite

By altering the matrix of the resin material, composite resin materials can incorporate an antibacterial component. Phosphate, Ag ions, and ammonium compounds have all been developed as antibacterial agents, similar to how antibiotics were first used. Silver has the strongest antibacterial activity of any of these. Silver nanoparticles have been helpful in treating oral cancer, endodontics, root canal therapy, restorative dentistry, orthodontics, and other dental conditions. They have also been employed in dentistry and medicine due to their antimicrobial characteristics. Additionally, biomaterials containing AgNPs have been used to prevent and minimise the development of the biofilms (Bapat et al., 2018a). However, numerous laboratory investigations have revealed that AgNPs are toxic. In the organs, silver ions accumulate. Their toxicity is a source of concern for several scientists. The number of free Ag ions present has a nearly linear relationship with the toxicity of AgNPs. Due to their small size, they might easily disrupt biomolecules, tissues, and organs. AgNPs have been shown to impair mitochondrial function and produce free radicals in living cells in several laboratory studies. AgNPs To lessen their toxicity, silver nanoparticles are usually covered with a capping material.

1.3. Objectives

The objectives of this research included:

• To synthesize and characterize the Ciprofloxacin loaded AgNPs

•To prepare dental composite discs with incorporation of synthesized nanoparticles

• To evaluate the antimicrobial activity against bacterial models of *S. mutans, E. faecalis* and Microcosm

• To evaluate the hemolytic activity of modified composite discs

• To test the mechanical strength of modified composite discs

Chapter 2

2. Literature Review

2.1. Tooth Anatomy

Human tooth is formed of two main parts – crown and root. The top portion is crown and the bottom portion is root, as shown in (Figure 1).Crown is the exposed portion and root is covered in gum. Enamel covers the outer portion of crown. It is the hardest substance in human body. Enamel is made up of keratin and mineral salts (of calcium and magnesium). It is brittle and its brittle property is due to its high elastic modulus and low tensile strength. Enamel has no nerves therefore it does not sense any pain, hot or cold. Dentine is present under enamel and is yellow in color. Dentine is biological composite material and is comprised of 18 % organic matrix, 70 % inorganic material and 12% water (wt. %). Dentine is present along the length of tooth and its structural components and properties vary with the location.

Cementum is present in the root portion of tooth. Cementum is made up of mineral water and salt and is as hard as bone. Pulp is present in the inner portion of tooth and it consists of blood vessels and nerves.

Figure 1: Structure of tooth

2.2. Classes of Human Teeth

Human teeth are classified on the basis of form and function. The classes are: incisor, canine, pre molar and molar. Incisors have a function of cutting and shearing of food and located near the entrance of oral cavity. The main function of incisor is cutting of food but contributes to esthetics and phonetics as well. Incisors are total eight in number. Canines are located near the dental arch. They are pointed and have the longest roots. Function of canines is piercing and tearing of food. Canines are total four in number. Premolars are located between canines and molars therefore it possess properties of both. They act like canines in piercing and tearing of food and helps in grinding of food like molars. There are eight premolars in human mouth. Molars are large in size and have multiple cusps. The major role of molar teeth is chewing, crushing and grinding of food. Premolars and molars both maintain the vertical dimension of the face. Molars are twelve in number. Half of the maxillary bone is shown in the figure below. Other half portion of the jaw is just its mirror.

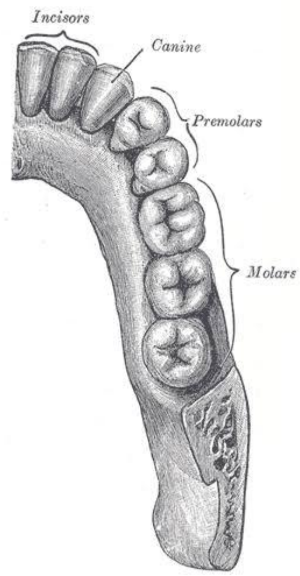

Figure 2: Classes of teeth

2.3. Dental Problems

In the field of oral health, dental disorders predominantly tooth decay and dental caries have been found to be the most prevalent issues. WHO has revealed that almost 65-90% of the children and approximately 99% of the adults have had the problem of dental caries. This problem is triggered due to an imbalance between demineralization and remineralization. (Abou Neel et al., 2016). Due to poor hygienic practices, dental plaque is formed over the tooth which results in the biofilm growth over a period of time. The pH at the infection site declines and the carcinogenic bacteria start acidic attack by fermenting the carbohydrates ultimately resulting in demineralization of tooth leading to cavities and dental caries (Gabrilska and Rumbaugh, 2015).

Calcium phosphate (hydroxyapatite) is the essential mineral component of both dentin and enamel and also serves as a main target for bacterial acidic attacks. In normal physiological conditions, Ca2+ and PO42- are constantly deposited on enamel surface because these are present in supersaturated concentration in the oral fluids. But this mineral ion balance gets disturbed at the site of infection where carcinogenic bacteria start producing acids thereby decreasing the pH up to 5.5. When pH declines the demineralization process is initiated through the chemical dissolution of Ca2+ and PO42- resulting in tooth decay (Leitão et al., 2018). Different restorative materials or dental fillings are used to treat such dental problems. According to a study, nearly 200 million teeth cavity restoration problems occur in USA per year and more than half of the restorations or fillings used for the treatment fail because of their poor performance. In the recent years, dentistry problems have been found to place a major economic burden because teeth cavity restorations cost nearly US46 billion dollars per year (Cheng et al., 2015).

Recently, dental composites and dental acrylic resins are gaining more attention in the restorative dentistry due to their excellent performance and properties. Different resin composites have been investigated and emerged as a very good option because of their ability to be directly applied in dental caries and teeth cavities. But these resin composites are highly porous so they overcome the problem of tooth decay but the plaque biofilms can easily accumulate on them, causing infection again. Therefore, the resin composites should have the capability to inhibit the bacterial plaques and biofilms growth as well as should possess the remineralization power. This can be achieved through nanotechnology which can provide novel approaches for the treatment and prevention of dental caries (Angel Villegas et al., 2019).

2.4. Oral Microbes

Oral cavity of humans provides habitat for the growth of many pathogenic microorganisms because of its high nutrient content (lipids, proteins, carbohydrates), ambient temperature, pH and moisture. A wide variety of microorganisms including bacteria, fungi, yeast, viruses can therefore inhabit and cause oral infections. But the principal bacteria isolated from the site of oral infections include *Staphylococcus aureus, Streptococcus mutans, Escherichia coli* and several *Lactobacillus* species. Among these, *Staphylococcus aureus* has been recognized to be involved in many oral diseases like periodontitis, endodontic, peri implantitis, dental caries and many other infections because it has been found to be a consistent microbe of oral microflora (Smith et al., 2001). This oral pathogen has been isolated repeatedly from numerous oral infections with 25-85% carriage rates in young adults and nearly 48% among the patients. *S. aureus* is also found to be involved in several other dental infections such as staphylococcal-mucositis, parotitis, angular-cheilitis etc. Recently, the role of this oral pathogen for the failure of dental implants is also recognized (McCormack et al., 2015). *S. aureus* is significantly resistant to many drugs, therefore its prevalence is increasing in the dental caries (Vellappally et al., 2017). In a recent study, 64 samples were collected from the patients having dental caries, and among them 62.4% (40 samples) were found to contain *S. aureus* based on its morphological and cultural properties (Das et al., 2019). Due to the favorable environment of the oral cavity a large variety of pathogens are found to be accumulated at different sites in the oral cavity but a few species are recognized as pathogenic because they are responsible for causing tooth decay, dental caries and demineralization. These distinct microbes belong to Streptococcus and Lactobacillus genera. *Streptococcus mutans* is a putative etiological agent and has been the most frequently isolated microbe from the dental caries in both animals and humans because it is a carcinogenic bacterium and is involved in acidic attacks causing tooth decay and creating acidic environment for the growth of other pathogens (Ahmadian et al., 2018). The other pathogens isolated from the oral diseases and implant infections are *Porphromonas gingivalis, Actinomycete coomitansad, Aggregtibacter, Candida albicans*. These act as primary stimulants for the development of implant and denture infections (Divakar et al., 2018). Among the pathogenic fungi isolated from the oral infections, Candida albicans has been suggested as the most opportunistic pathogen involved in the denture failures by colonizing dental acrylic materials (Acosta-Torres et al., 2012).

2.5. Need for Dental Restoration

There are various reasons in which teeth require restoration which are explained below.

i. Dental carries- to repair a tooth after carious lesion is the foremost need for dental restoration.
ii. Replacement of the restoration- sometime tooth need restoration for replacing previous restoration with some serious defects, such as defective open margin, improper proximal contact or poor aesthetics.

iii. Fractured teeth- restoration is needed when tooth is being fractured. In this case restoration is done to restore the proper form and function of tooth.
iv. Form or function- tooth may require restoration to restore the form or function which is absent due to congenital malformation.
v. Aesthetics- Aesthetic desire of patient is another reason for dental restoration.
vi. As a part of fulfilling other restorative needs- some restorations, for example fixed or removable partial dentures, require some other types of restorative procedures for proper placement of the dentures.

2.6. Dental Carries

Dental carries, tooth decay and cavity all refer to same thing and can be used interchangeably. There are hundreds of different types of bacteria in our mouth. Some of bacteria are harmful while others are useful. Some bacteria make acids from sugar in the food and results in infection. These infection over time demineralize enamel and results in cavity. Whenever we eat food containing sugar or starch, bacteria in our mouth combines with it forming acid which attack the enamel. Repeated cycles of acid attacks make the enamel demineralize. The first sign of mineral lose is the appearance of white spot. There is immunity to this demineralization. Minerals in our saliva (phosphate and calcium) and fluoride from toothpaste or other sources like milk, provide minerals to the enamel. This process of mineralization and demineralization continues all day long. If the demineralization process continues then it damages the hardest substance i.e., enamel and form a cavity. This cavity cannot be recovered naturally. In this case a dentist must be consulted. There are different types of cavities developed depending on their location. Greene Vardiman Black classified the cavities depending on their size and location.

Classes of caries

- Class I

Class I cavities affect pits and fissures. These are located in the occlusal surface of molars and premolars and in the lingual surfaces of upper incisors, and often found in the lingual surfaces of upper molars.

- Class II

These cavities are located in the proximal surfaces of molars and premolars.

- Class III

Cavities are present in the proximal surfaces of incisor and canines.

- Class IV

Cavities affect the proximal surfaces of incisors or canines and also involving one or both of the incisal edges of anterior teeth.

- Class V

Cavities located in the buccal or lingual surfaces of any tooth.

- Class VI

Class VI cavities are located in the cusp tips of molars, premolars, and canines.

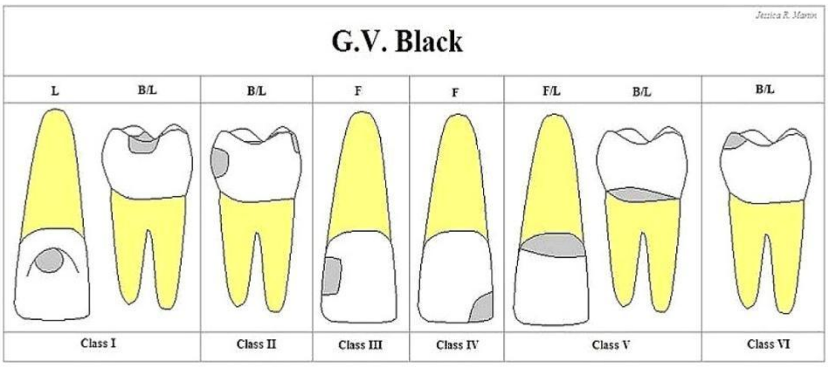

Figure 3: Classes of Caries

Different Types of Dental Restoration

There are different types of dental restorations designed to do a specific job. Following is the breakdown of the most common.

i. Fillings: fillings are dental restorative material directly places into prepared tooth to restore its structure. Fillings are placed where the decay is not very safer. Material used for fillings are amalgam, porcelain, composite resin and sometimes gold.
ii. Inlays- inlay is a restoration technique use to repair posterior teeth where the decay is moderate and the cusp of the tooth is not affected. Inlay is an indirect

restoration technique where the solid material is prepared outside of the mouth and then fitted in the cavity of prepared tooth.

iii. Onlays- onlay is an indirect restoration, just like inlay, where the material is prepared outside of mouth. It is just like inlays except the damage is more sever and it covers the cusp of tooth.

iv. Crowns- it is also an indirect restoration technique. Crowns are extended form of onlays which completely covers all surfaces of tooth. Crowns are used when the damage is very large and where filling, inlay and onlay can’t be done. In crown the whole cap of the tooth is replaced. Crowns are also performed when there is very limited tooth structure lift or when the tooth be crowned is anchoring tooth for bridge.

v. Veneer – Veneers are used for esthetic purpose. They are thin porcelain facings used to change the shape, length and color of teeth.

vi. Bridge- Bridge covers the gap created by missing teeth. Bridge is made up of two or more crown for the teeth on each side of the gap. Teeth prepared for crown placement is called abutment and the false teeth in between are called pontics. Pontics are made from porcelain, gold or alloys.

vii. Partial dentures- partial dentures are removable false teeth and is a replacement to bridge when there are no sufficient teeth left to support bridge. Another case in which partial dentures are preferred over bridge is when patient does not test well for bridge. Metallic framework is support to dentures and natural teeth is support to the metallic framework.

2.7. 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)

2.7.1. Composition

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)

2.7.2. 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).

2.8. Biofilm formation and Secondary Caries

Secondary caries is 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).

2.9. Nanotechnology

Nanotechnology is emerging as a most promising technology in the 21st century. This theory involves manufacturing of the matter within nanometer range (1-100nm) through observing, manipulating, measuring, assembling and controlling of the materials. The prefix —nano‖ is derived from a Greek work which means —dwarf‖ or something exceptionally small and is equal to 1,000 millionth of meter (100nm). Nanotechnology and Nanoscience should not be intermixed. Nanoscience is the field of science which deals with the study of matter and structures on the scale of nm i.e., somewhere within 1-100nm and the technology which makes use of the practical applications of these structures is referred to as Nanotechnology (Bayda et al., 2020). The concept of nanotechnology was firstly introduced by a noble prize laureate and American physicist Richard Feynman in 1959. During his lecture —There is a plenty of room at bottom‖, he gave the concept of utilizing machines for constructing smaller structures down to the molecular size (Leon et al., 2020). Recently, the research is more extensively focused in nanotechnology in order to identify the physical, optical, magnetic and electrical properties od different nanomaterials (Butt et al., 2015). The prime objective of nanotechnology is to synthesize diverse range of monodispersed and small sized nanomaterials having different chemical composition and morphologies with probable applications in different sectors of daily life. The interest in the study and synthesis of nanoparticles is constantly increasing due to their unique physico-chemical properties such as photocatalytic, optical, antibacterial, mechanical, magnetic and electrical which enables them to be utilized in diverse fields (Osuntokun et al., 2018). Due to their profound applications in medical sectors and other industries, nanomaterials can have a direct impact on the global economy because there are thousands of commercially available products produced from nanotechnology and this global business is expected to be exponentially increased in the next few years (Roco, 2011)

Nanotechnology is the study and manipulation of particles smaller than 100 nanometers (Goddard 2007). Because of their small size and huge surface area, nanoscale particles have unique features those larger ones lack (Adams and Barbante, 2013). Nanotechnology allows for the creation of smaller, lighter, cheaper, stronger, smarter, cleaner, and more precise products (Merkle, 2000). Nano-sized materials also have substantially better magnetic, optical, photocatalytic, thermal, and electrical characteristics than their bulk counterparts (Basavaraj, 2012). Medicine, optics, textiles, farming, cosmetics, aircraft, construction, semiconductor devices, and catalysis are some of the fields that have profited from nanotechnology (Kango et al., 2013).

Carbon black and fumed silica, as well as microgram amounts of luminous quantum dots, are examples of nanomaterials (Hoet et al., 2004). Nanomaterials come in a variety of forms, including nanotubes, nanoclusters (Terrones et al., 2002), liposomes, nanoparticles, and dendrimers (Terrones et al., 2002). (Gupta et al., 2012). Nanoshells, nanoeggs, and nanocups are three different types of core shell nanostructures (Knight and Halas, 2008). Nanomaterials offer unique features that make them more valuable in commercial applications such as cosmetics, skincare, and sanitary products (Wu et al., 2013). Nanoparticles have enabled targeted drug delivery and personalized drug formulations in medicine. Hughes (Hughes, 2005). Nanoparticles can be utilized in imaging techniques to get high-resolution images for cancer and other disorders diagnosis. (Torchilin 2007).

2.9.1. Classification of the Nanoparticles

Nanoparticles are referred to the products of nanotechnology having dimensions between 1-100nm and lying in the range of 10-9 m. These materials are transients between molecular structures and bulk materials with smaller size and higher surface area (Sangeetha et al., 2012). Nanoparticles are generally classified in two categories: organic and inorganic. Organic nanoparticles are based on carbon such as dendrimers, liposomes, ferritin, fullerenes, carbon nanotubes etc. Such nanoparticles are non-toxic, biodegradable as well as extremely sensitive to heat and light. These have enormously applied in different biomedical applications because of their efficiency and biocompatibility i.e., in targeted drug delivery. Other class of nanoparticles which does not contain carbon is classified under inorganic nanoparticles and mostly include particles that are made up of metals and metal oxides. Almost every metal can be used to synthesize its nanoparticle depending upon its properties.

The properties of metallic based nanoparticles are further modified by synthesizing their respective metallic oxides nanoparticles e.g., the reactivity of iron (Fe) nanoparticles can be increased by changing the oxidation state of iron from Fe to Fe2O3. Thus, iron oxide nanoparticles have exceptionally increased reactivity and efficiency as compared to the metal nanoparticles (Ealia and Saravanakumar, 2017).

2.9.2. Different Approaches for the Synthesis of Nanoparticles

After Feynman presented the idea of nanotechnology, this field gained attention of many researchers to develop different approaches for the synthesis of nanostructures. Two manufacturing approaches were developed named as top down and bottom up. Both approaches differ with respect to speed, quality and cost (Iqbal et al., 2012). In top-down approach, large macroscopic particles are synthesized initially and then their size is reduced to nano ranges through milling or plastic deformation. However, this approach is costly and takes a lot of time therefore it is not feasible for the production of nanoparticles at large scale (Agarwal et al., 2017). In bottom-up approach, nanomaterials are built by different physical and chemical synthesis methods from bottom through the controlled self-assembly of atoms and the molecules (Bayda et al., 2020).

2.9.3.Different Methods for the Synthesis of Nanoparticles:

Different methods have been developed for the synthesis of nanoparticles having desirable characteristics and among these physical and chemical methods are categorized as the most conventional and common methods (Fig 2.1). The physical synthesis methods mostly employ top-down approach and involve grinding and milling of the bulk materials. Some of the examples for physical methods include Sol-gel, Chemical vapor deposition, Spinning, Pyrolysis and Electrochemical methods. In all these methods physical forces such as high voltage, temperature and pressure are involved for the production of stable and well-defined nanoparticles (Rudramurthy et al., 2016). However, in chemical synthesis methods, several reducing and stabilizing agents such as sodium dihydrogen phosphate, potassium bitartrate, sodium borohydride, polyvinyl pyrrolidone etc., are added in a liquid medium to reduce the metallic salts into small sized and monodispersed nanoparticles (Agarwal et al., 2019). Both of these methods are conventional and have been used for the production of nanoparticles since long. These methods have some drawbacks such as physical methods require more energy and are unaffordable while chemical methods have negative environmental impacts as they produce large number of toxic by-products. Therefore, such synthesis methods should be developed which are affordable and involve eco-friendly approaches for the production of nanoparticles (Agarwal et al., 2019).

2.9.4. Dental Applications

The oral cavity inhabits a natural microflora and when the oral habitat is disturbed, numerous pathogenic microorganisms accumulate and cause infections by resisting against the defense system of host. Initially, a complex community of bacteria and fungi is accumulated on the teeth forming a plaque biofilm, which stimulates different dental diseases such as periodontitis, caries and cavities (Fernandes et al., 2018). Dental hard tissues are comprised of dentin and enamel, both of these are highly susceptible to acid attacks by carcinogenic bacteria through fermenting carbohydrates (Ahmadian et al., 2018). There are numerous methods and techniques available which overcome this problem by inhibiting the growth and proliferation of oral pathogenic microorganisms through different antimicrobial agents or drugs. However, they fail to achieve the desired objectives due to their lower efficacy, toxicity, rapid release and fast degradation (Saafan et al., 2018). Nanotechnology can provide new strategies in this field by developing novel approaches for the treatment and prevention of oral infections. Nanoparticles have been proved to exhibit superior antibacterial activity because of their small size, high charge density and high surface area. These properties enable them to strongly interact with the bacterial cell surface with an enhanced antibacterial action. The nanoparticles can also be conjugated with different polymeric materials or coated on the surface of different biomaterials to increase their antimicrobial and mechanical properties (Cao et al., 2018). Denture based applications mostly employ different kinds of acrylic resins because they exhibit relatively simple fabrication. They are commonly used for the replacement of hard tissues, filling the dental cavities and for denture bases due to their excellent mechanical properties and substantial ability to wear load. However, they don‘t exhibit antibacterial activity due to which there are chances of recurrent infections are emerging as powerful tools in the field of dentistry for the prevention and treatment of dental infections. Their distinguished and unique characteristics like very small size, high surface area, enhanced chemical reactivity and increased charge density make them an ideal material to be used in antimicrobial therapy. Nanoparticles alone or in combination with different polymers and acrylic resins have been repeatedly used recently in order to achieve the desired objectives (Ana-Paula-Rodrigues Magalhães et al., 2016). Both inorganic and organic nanoparticles have been tested for their potential in different areas of dentistry due to their wide spectrum physical, biological and mechanical properties. More importantly, nanoparticles have gained attention to be utilized in dental diseases due to their antimicrobial potential because dental infections mostly arise due to the oral microorganisms. Therefore, nanoparticles can be proved promisingly advantageous in dentistry (Fernandes et al., 2018). Different studies have incorporated different nanoparticles depending on the desired objective and application. Among them, silver nanoparticles have been most commonly employed because of its ability to exhibit strong antibacterial activity in dental materials. Ag nanoparticles can be used alone in the acrylic resins (quaternary ammonium methacrylate) or in combination with different biomaterials (chitosan) to achieve desired properties. However, addition of silver nanoparticles in acrylic composites is desirable only in small amounts as higher concentrations will affect the texture, surface properties, color and mechanical properties of the composites (Cheng et al., 2015). Organic or mineral based nanoparticles have been proved to be more advantageous because they reduce demineralization and help in the remineralization of the hard dental tissues. For example, calcium phosphate nanoparticles are incorporated into different acrylic resins such as dimethyl aminohexadecyl methacrylate, quaternary ammonium methacrylate, quaternary ammonium polyethleneimine to develop modified composites with increased durability and strong antibacterial properties (Pietrokovski et al., 2016).

Some nanoparticles are additionally added into orthodontic biomaterials as nanofillers for enhancing their antibacterial and mechanical properties. Different nanoparticles have been studied and tested for their potential to be used in dental biomaterials such as titanium dioxide, silicon dioxide, zinc oxide, celenium oxide, iron oxide, silver, calcium phosphate, hallosite nanostructures (Yang et al., 2017). In a study, titanium dioxide nanoparticles were incorporated in two different acrylic resins and their effect on the biological and physical properties of the resins were observed. The results revealed that adding a very small amount of titanium dioxide nanoparticles can improve the mechanical strength and the microhardness of the dentures but negatively affect the flexural strength (Alrahlah et al., 2018). Furthermore, specific mineral-based nanoparticles have also shown a great potential to inhibit demineralization and initiate remineralization of the tooth structure with the controlled released of the minerals required to form the dental structure. Different antimicrobial agents can be incorporated in these formulations in specific concentrations to kill the pathogens of the dental caries. Recently, nanoparticles of zinc oxide have been suggested as novel nanoantibacterial agents for dental composites because they are more biocompatible, nontoxic and do not cause discoloration when compared with other metallic nanoparticles (Ag and TiO2) (Angel Villegas et al., 2019).

2.9.6. Silver Nanoparticles

Silver nanoparticles are one of the most popular and simple to make metallic nanoparticles, and they've seen a lot of use in recent years because they're more effective than silver ions (Lara et al., 2010). By rupturing the cell wall, attaching to the thiol group of proteins, denaturing DNA, and triggering cell death, silver ions have the ability to impede bacterial replication (Russel and Hugo, 1994). Because of its antibacterial characteristics, silver has been used as an antiseptic for ages (Moyer et al., 1965). Silver has also been used to cure burns and skin conditions (Parikh et al., 2005). Due to their efficiency at small doses and low adverse effects, silver nanoparticles are interesting candidates for use as microbicides (Sondi and Sondi, 2004).

Many key aspects of nanoparticles are determined by their size and shape, including in vivo circulation, cytotoxicity, biological providence, genotoxicity, and targeting ability (Panyam and Labhasetwar, 2003). With the rapid advancement of nanotechnology, applications have been expanded even further, and silver is now the most often used designed nanomaterial in consumer items (Rejeski, 2009). Silver nitrate, silver sulfadiazine, silver powder, and silver chloride are examples of silver compounds that have been utilized as antimicrobials (Rai et al., 2012).

2.9.7. Synthesis of Silver Nanoparticles

Chemical, physical, and biological processes have all been used to create silver nanoparticles. Vapor condensation and arc discharge are two physical ways for producing silver nanoparticles (Sharma et al., 2009). Chemical reduction, photochemical reduction, and electrochemical synthesis are all used to make silver nanoparticles (Khan et al., 2011). Silver nanoparticles have also been created from bacteria, algae, fungi, insects, plants, and other biological sources (Mukherjee et al., 2009). The ultimate size, shape, morphology, and stability of silver nanoparticles are affected by a variety of experimental parameters such as pH, temperature, reaction time, and so on (Li et al., 2011).

Silver nanoparticles are made chemically by reducing silver ions with reducing agents such as hydrogen, hydrazine, Dextrose, ethylene glycol, citrate, and ascorbate (Hiramatsu et al., 2004). Various compounds are reduced with silver Ag^+ ions in this process, resulting in the formation of silver atoms, which then aggregate to form oligomeric clusters. Colloidal silver nanoparticles are formed from these clusters (Evanoff and Chumanov, 2004; Sondi et al., 2003). Sodium Borohydrate with a size distribution of 3-28nm was produced in 5 minutes by changing the experimental conditions, resulting in nanoparticles with varied size and form (Ghorbani et al., 2011).

Condensation during evaporation in a tube furnace is one of the physical ways used to make silver nanoparticles. This procedure necessitates a significant amount of time and energy (Simchi et al., 2007). Another way is to use a ceramic heater to gradually and slowly heat the solution to evaporate the liquid, resulting in small sized nanoparticles at high concentrations (Jung et al., 2006). Silver nanoparticles have also been synthesized using lasers. Silver nanoparticles of various shapes, sizes, and structures have been created using laser ablation procedures (Barcikowski et al., 2009). The features of the silver nanoparticles generated and the ablation effectiveness can be influenced by varying elements such as laser pulse time, pulse frequency, and the effective liquid medium (Mafune et al., 2003).

A solvent medium, a non-toxic reducing agent, and a stabilising agent are the three essential components of the biological technique for the synthesis of silver nanoparticles (Prabhu and Poulose, 2012). Silver nanoparticles are commonly synthesized using microorganisms such as prokaryotic bacteria (Kaushik et al., 2010). Biocompatible silver nanoparticles have also been created using yeast and other fungi (Kowshik et al., 2003). Biocompatible silver nanoparticles have also been synthesized using various plant extracts. Because there is no need to maintain a microbial culture, using plant extract provides a quick and simple way to make nanoparticles (Sastry et al., 2004).

2.9.8. Mechanism of Silver Nanoparticle

Many hypothesized mechanisms of silver nanoparticle activity have been presented, with size, mobility, and composition of silver nanoparticles all playing a role in antimicrobial activity (Quang et al., 2013). Silver nanoparticles may also act as a specie-independent anti-biofilm, allowing them to be employed to restrict the proliferation of resistant microorganisms (Kalishwaralal et al., 2010). Changes in bacterial cell wall permeability, antimicrobial drug excretion through membrane efflux pumps, and antimicrobial agent inactivation are some of the reasons that make microbes resistant to antibiotics (Giraud et al., 2006). Because of active mechanisms unique to silver nanoparticles, silver nanoparticles can reduce resistance (Kim et al., 2007).

Many theories have been offered to explain how silver nanoparticles affect cells. The silver nanoparticle may cling to the bacterial cell wall, causing conformational changes in its structure and, as a result, bacterial cell destruction (Klasen, 2000). Silver nanoparticles are also hypothesized to change cell membrane permeability, either directly or indirectly, by interacting with the phospholipid bilayer or by releasing reactive oxygen species, which alters membrane permeability and has bactericidal effects (Fayaz et al., 2010). Silver ions released in the presence of silver nanoparticles may create Ag-S bonds with certain thiol groups in enzymes, causing the activity of bacterial enzymes involved in transmembrane energy production and ion transport to be altered (Matsumura et al., 2003). Another reason could be that silver nanoparticles internalize and aggregate within bacterial cells, causing "pits" to form on bacterial membranes, leading to bacterial cell lysis (Nair et al., 2009). Silver nanoparticles may also block several enzymes via Ag-S and interfere with transmembrane energy generation within bacterial cells, resulting in the development of reactive oxygen species (ROS) (Yamanaka et al., 2005). Both silver nanoparticles and oxidative stress cause the creation of reactive oxygen species, which affects cell membrane permeability (Raghupathi et al., 2011). By interacting with the 30S ribosomal subunit, Ag ions can cause bacterial death by deactivating the complex and halting protein translation (Yamanaka et al., 2005).

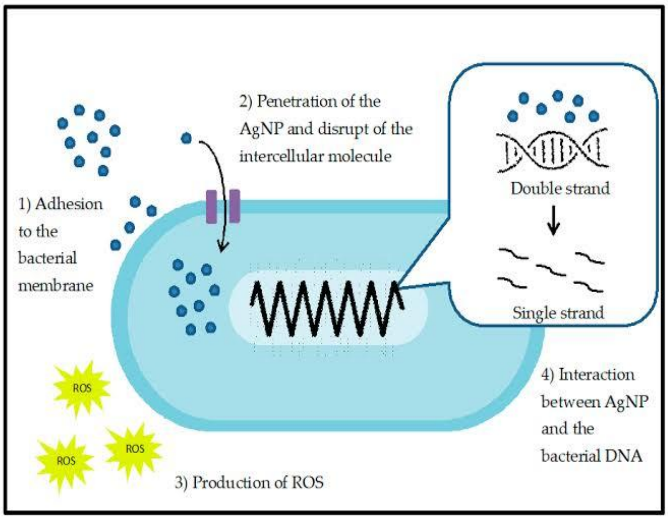

Figure 4: Mechanism of Action of AgNPs

2.9.9. Application of Silver Nanoparticles

In recent decades, silver nanoparticles have seen widespread use. Their most well-known application is in the medical field. The majority of multicellular creatures' cells are about 10m in size (Feynman 1991). Cellular organelles are significantly smaller, measuring in the sub-micron range. Proteins, carbohydrates, nucleic acids, and other biomolecules have a normal size of 5 nm, which is comparable to the average size of man-made nanoparticles (Murray et al., 2000). The benefit of utilizing nanoparticles as probes to infiltrate the cellular machinery without causing too much damage may be seen in this size comparison (Taton et al., 2002). Our understanding of biological processes at the nanoscale is critical to the advancement and development of nanobiotechnology (Whitesides 2003).

Because of rising worldwide demand and low food supplies, food preservation has become a serious issue. Maintaining the quality of food products is difficult due to increased respiration rates and ethylene generation, both of which lead to quality degradation (Aguilar et al., 2010). Food packaging with antimicrobial active ingredients has the potential to extend the shelf life of fresh foods. Natural compounds, such as plant essential oils, inhibit the growth of microorganisms (Zivanovic et al., 2005). Silver nanoparticles' exceptional antibacterial qualities have made them useful in the food business (Bosetti et al., 2002). The use of silver nanoparticles to preserve vegetables and vegetables-derived products is one example of application of silver nanoparticles to food (An et al., 2008).

This problem of pathogen contamination can be solved with antimicrobial packaging of fresh cut vegetables. Solvent casting was used to coat lettuce and paprika samples with silver nanoparticles and polylactide (PLA) sheets. In vitro, the PLA silver nanoparticles films demonstrated substantial antibacterial, antifungal, and antiviral activity, with increasing effects as silver concentrations increased (Abad et al., 2013). To regulate the quality deterioration of Fior di Latte and mozzarella cheeses, different amounts of silver montmorillonite embedded in agar were utilized as an antimicrobial packaging solution (Incoronato et al., 2011). Because acidophilic microorganisms like lactic acid bacteria and yeast can grow across a wide pH range, they are important pollutants in citrus juices (Alwazeer et al., 2003).

2.10. Ciprofloxacin

Ciprofloxacin is a well-known antibiotic that is used against a huge number of infections of bacteria. The infections could be off joints or bones, abdomen or respiratory tract, skin infection or urinary tract infection. This antibiotic is approved by the World Health Organization to be the safest and most effective antibiotic for mankind against microbial diseases. These drugs are fluorescent and can be probed using different techniques even with the low concentration of the drug. Fluoroquinolones such as ciprofloxacin, norfloxacin, clinafloxacin, sparfloxacin, and levofloxacin are antibacterial which are being used against several microbial infections. As far as the mode of action is concerned, the fluoroquinolones are well known anti-bacterial agents that target two important enzymes of bacteria. One is the DNA topoisomerase IV and the other is the DNA Gyrase. The DNA gyrase introduces supercoiling in the DNA of bacteria. On the other hand, DNA topoisomerase IV causes the recognition of the crossovers in DNA and therefore is a decatenating enzyme. It restricts the repair, as well as the reproduction of the genetic material of bacteria, thus stopping the multiplication of the bacteria.

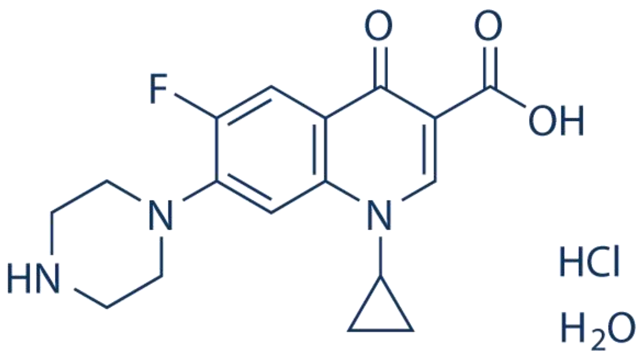

Figure 5: Chemical Structure of Ciprofloxacin-Hcl

Chapter 3

3. Materials and methods

3.1. Materials

All Chemicals ciprofloxacin hydrochloride, silver nitrate ($AgNO3$), Sodium Borohydride ($NaBH4$), were all purchased from the Sigma-Aldrich for use in the experiments.

3.2. Methodology

To prepare AgNPs, a chemical reduction technique was applied (Mulfinger et al., 2007). AgNO3 (1.0 mM) and NaBH4 (2.0 mM) were used. NaBH4 was used as an agent to reduce silver nitrate. In a biuret, 30 ml of NaBH4 solution was mixed continuously while 10 ml of AgNO3 was added dropwise (one drop per second). The solution turns yellow after the addition of 2ml of AgNO3, and eventually attains a bright yellow golden colour. The stirring comes to an end as soon as the addition is complete.

Figure 6: AgNPs Solution

Ciprofloxacin loaded AgNPs were made by adding 0.001M Ciprofloxacin aqueous solution to 100 mL synthesized AgNPs with continuous stirring. To enhance the interaction between ciprofloxacin and silver nanoparticles, ultrasonication was performed to enhance the interaction of drug with the nanoparticles.

3.3. Characterizations

The size, net charge on their surface, and aggregation of CIP-AgNPs 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.

3.3.1. UV-Vis absorption Spectroscopy

Spectroscopy is very powerful technique which is used to analyze the size, concentration and stability of silver nanoparticles quantitatively. Silver nanoparticles were characterized initially by observing the specific peak of silver nanoparticles via UV–visible spectra on Spectrophotometer. Quartz cuvette was filled with sample solutions and loaded in spectrophotometer chamber where silver nanoparticles absorbed the photons of particular wavelength, depending upon the particle size distribution and the absorption spectra, was recorded between wavelengths ranging from 380-410 nm.

3.3.2. Particle size and surface distribution determination

The morphology of CIP-AgNPs were studied by using a Scanning Electron Microscope (SEM). SEM is a useful technique used for analyzing surface topography, morphology, size and composition in which a beam of electrons is used to excite the target sample which forces the conducting sample to lose electrons from their own shell. For instance, glass slides containing CIP-AgNPs were coated with gold (30nm) to make the sample conductive for SEM investigation. SEM study, in particular SEM, analyses the physical dispersion of nanoparticles and confirms their spherical form.

3.3.3. FTIR analysis

The most effective infrared spectroscopy technique is called Fourier Transform Infrared (FTIR). In this method, IR radiation is passed through a sample, which partially transmits and partially absorbs the IR radiation. As a result, the resulting spectrum shows how molecules absorb and transmit light, creating a molecular fingerprint of that sample that may be used to distinguish between known and unidentified materials and gauge their quality and amount.

3.3.4. Zeta sizer and surface Potential

The zeta potential value frequently specifies their net charge onto them in terms of the strength of the force of attraction between nanoparticles and their stability.

3.4. Isolation of Bacterial Strains

There were 3 antibacterial activity models for *Enterococcus faecalis, Streptococcus mutans* and saliva microcosm. For isolation of bacterial strains, 10 saliva samples were collected from people that never had any orthodontic treatment, those samples were mixed and serially diluted and spread on tryptic soy agar (TSA) plates. These plates were then incubated for 24 hours. *Enterococcus faecalis, Streptococcus mutans* colonies were differentiated morphologically. For confirmation, these colonies were streaked on Blood Agar plates and were isolated after incubation. For microcosm model, an inoculum was prepared by the saliva sample dilution with sterile glycerol of 30% in concentration.

Figure 7: Isolation of E. faecalis on Blood Agar Plates

Figure 8: Isolation of S. mutans on Blood Agar Plate

3.5. 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 CIP-AgNPs into composite resins was used. Different concentrations of ciprofloxacin coated AgNPs, such as 1% 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/cm2 intensity and a wavelength of 430-480 nm. All of the discs were 2×4 mm in diameter and 0.1g in weight. The nanoparticles were manually added in discs using a glass rod.

Figure 9: Modified Composite discs

3.6. Antibacterial Activity

The bacterial growth medium was Tryptic Soy Broth with 1 percent Sucrose (TSBS). Overnight, the pre-culture was incubated. A culture derived from this preculture, on the other hand, was incubated for 3 hours. The culture was serially diluted once the optical density (OD) reached 1 at 600 nm. To prepare antibacterial activity model, 500µL of bacterial suspension was added in Eppendorf along with experimental composite discs that were incubated for 7 hours. After 7 hours, 50µL of incubated samples was spread on TSA plates that were incubated for 18 hours. After 18 hours of incubation, Colony Forming Units were counted using the formula CFU/ml = (no. of colonies x dilution factor) / volume of culture plate. log10 CFU/ml was determined. Antibacterial activity of pure composite discs and 1% AgNPs containing composite discs were used as control groups. Experiment was performed in triplicates.

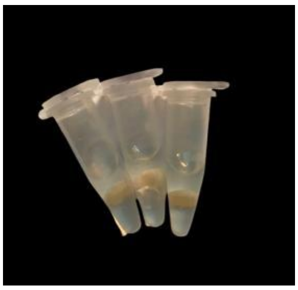

Figure 10: Eppendorf with 500uL bacterial suspension & composite discs

3.6.1. 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

TSB 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 and placed in an incubator. 8 hours later 50µl from each Eppendorf was collected and spread out on TSA plates and incubated overnight.

3.6.2. Microcosm Model

10ml of TSB were added to the inoculum, which was then incubated overnight. The culture was serially diluted after reaching its OD at 600 nm, and 500µl of it was added to Eppendorf's with composite samples before being incubated for 8 hours. Then, 50µl was taken from each Eppendorf and placed on TSA plates before being incubated overnight.

3.7. Hemolytic testing of experimental composite resin discs

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 545 nm were obtained and documented to compute the % hemolysis.

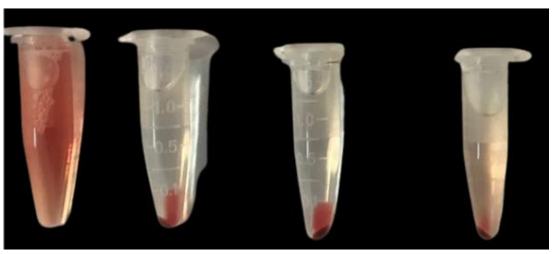

Figure 11: Hemolytic Analysis

3.8. Mechanical testing of composite resin discs

During compressive strength testing, composite resin samples were created using stainless steel split moulds (4mm in diameter and 2mm in height). The samples underwent light curing after being taken out of the mould. The samples were stored in saliva before being tested, and they were then incubated at 37°C for 24 hours. Compressive strength was tested using a load cell with a maximum capacity of 5 KN at a cross-speed of 0.5 mm/min. In order to conduct compressive testing, the composite disc samples were placed with their flat sides between the two plates of the testing apparatus. A compressive force was then applied along the specimens' entire length (Dias et al., 2019).

3.9. Statistical analysis:

Graphpad prism 9.2 was used for statistical analysis. T-test was done for analyzing all the results with significance level of 5% to determine the p-value of experimental dental composite's antibacterial activity, hemolytic activity and compressive strength.

Chapter 4

4. Results

4.1. Characterizations

CIP-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 analyzed by Malvern Zetasizer (Malvern).

4.1.1. Uv Analysis

UV analysis was performed for AgNPs and CIP-Dextran AgNPs. Within the region of 400-420 nm, AgNPs showed a very strong and wide peak. The decrease of Ag+ to Ag0 is confirmed by a distinct band (Mohanta et al., 2020). AgNPs had a peak absorption of 1 at 404 nm. The peak spectrum of CIP-AgNPs was 414 nm, with 2.3 units absorption. Because of the coating of Ciprofloxacin on AgNPs, the peak migrated forward. The synthesis of CIP-AgNPs was confirmed with these observable peaks. This absorption maxima have migrated within the range of 410-420 nm with the addition of ciprofloxacin which is near to the original peak and indicates the stability of ciprofloxacin coated nanoparticles.

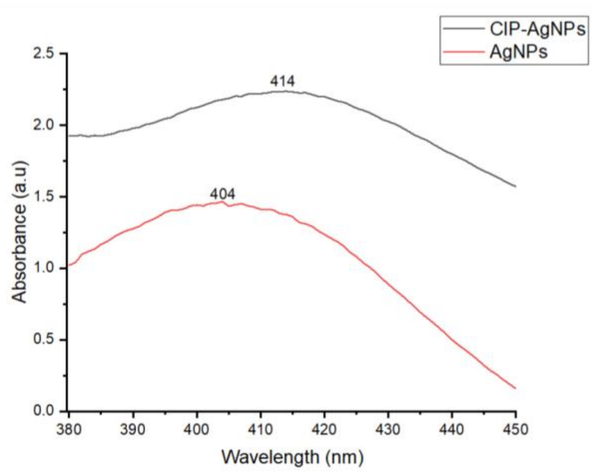

Figure 12: UV-Spectrograph

4.1.2. FTIR Analysis

The results demonstrated that there was interaction between Cipro and capped AgNPs when the major peaks of the AgNPs were matched with pure Cipro and Cipro-AgNPs nanocomposites. The stretching of hydroxide and hydrogen bonding caused a peak at 3340 cm^{-1}. The peak at 1380 cm^{-1} shifted to 1405 cm^{-1}. This was due to electrostatic interaction between - NH group of ciprofloxacin and borohydride of silver nanoparticles. So, these interactions confirmed the loading of ciprofloxacin on silver nanoparticles. CF stretching was observed at 1085 cm^{-1}.

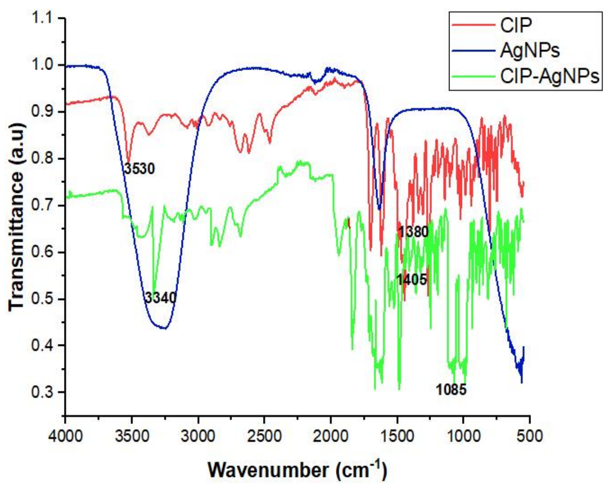

Figure 13: FTIR Spectra

4.1.3. SEM Analysis

AgNPs and CIP-AgNPs morphology was examined using the SEM analysis at 20kV. The NP suspension was dropped directly onto glass slides for this study, which was then dried at room temperature. The CIP-AgNPs were almost three times larger than the AgNPs, which were roughly 30–40 nm in size. The spherical shape of AgNPs and CIP-AgNPs was visible in the SEM images.

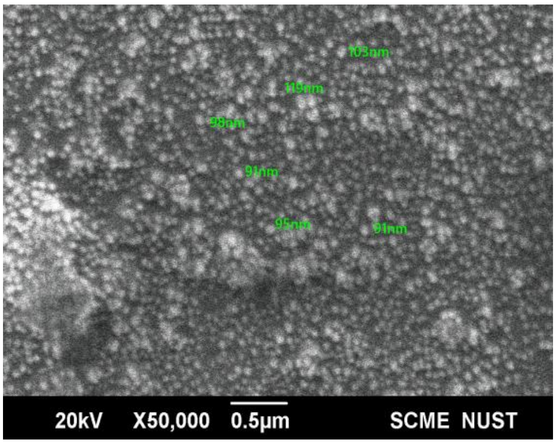

Figure 14: SEM Image of CIP-AgNPs

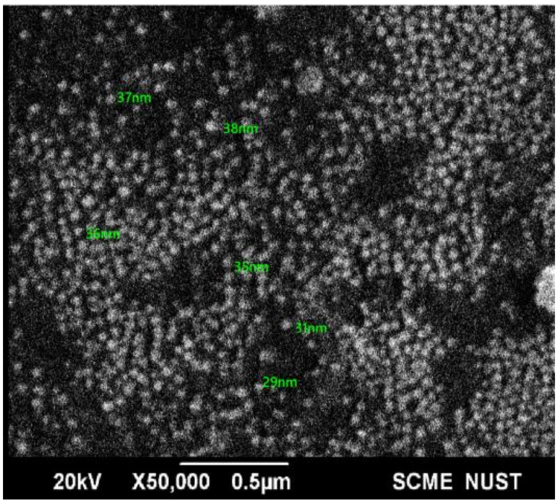

Figure 15: SEM Image of AgNPs

4.1.4. Zeta analysis

AgNPs exhibited zeta potential of - 14.5 mV while CIP-AgNPs exhibited zeta potential of +15.8 mV. The opposite charges supported the electrostatic interaction between silver nanoparticles and ciprofloxacin loaded silver nanoparticles, that leaded to the successful loading of ciprofloxacin on silver nanoparticles.

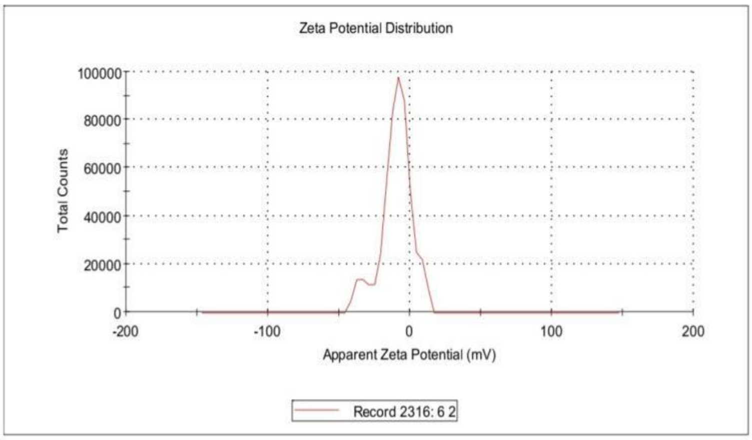

Figure 16: Zeta Potential of AgNPs

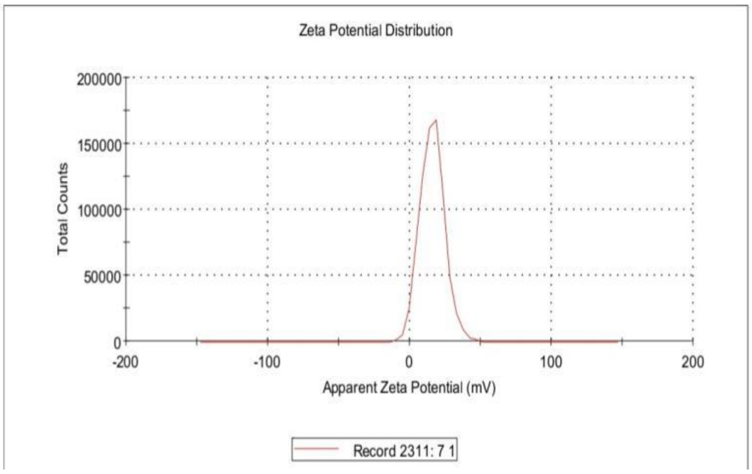

Figure 17: Zeta Potential of CIP-AgNPs

4.1.5. Antibacterial activity of CIP-AgNPs and AgNPs composite discs

Ciprofloxacin loaded silver nanoparticles exhibited a high antibacterial activity than pure resin composite and pure AgNPs for all three models. The resin composite containing 1% Ciprofloxacin loaded silver nanoparticles exhibited clear plates and very significantly high antibacterial activity than the control groups that were pure resin composite and composite containing 1% AgNPs. Since, 1% CIP-AgNPs exhibited significant anti-bacterial activity, it shows that even 1% CIP-AgNPs were very strong antibacterial agents. CIP-AgNPs incorporated dental composite exhibited really good antibacterial activity against the experimental models ($P<0.05$).

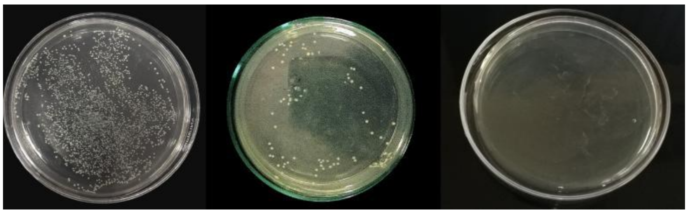

Figure 18: *E. faecalis* growth of unmodified composite resins, composites resin having 1% AgNPs & composite resin having 1% CIP-AgNPs

Figure 19: *S. mutans* growth of unmodified composite resins, composites resin having 1% AgNPs & composite resin having 1% CIP-AgNPs

Figure 20: Microcosm bacterial growth of unmodified composite resins, composites resin having 1% AgNPs & composite resin having 1% CIP-AgNPs

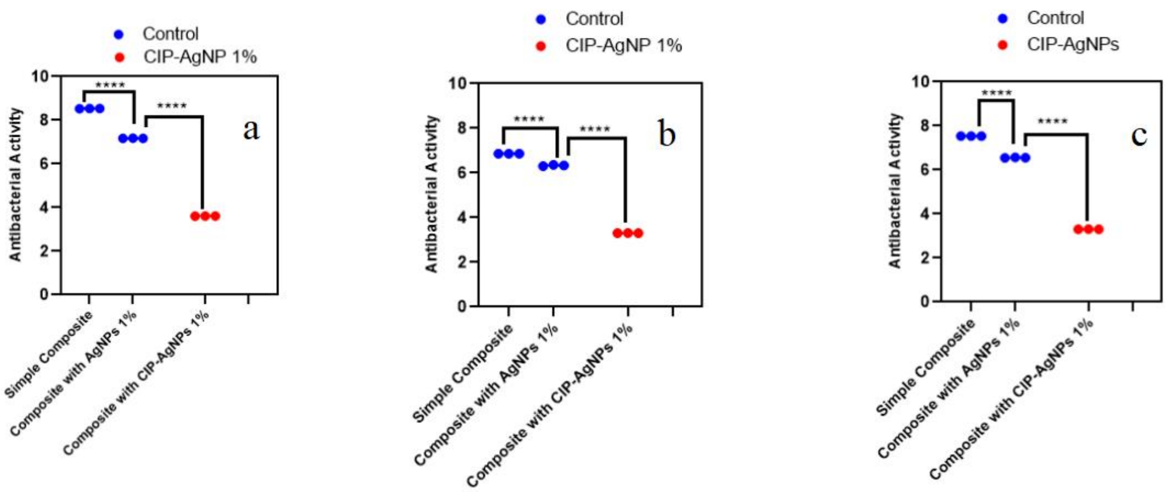

Figure 21: CFU/ml (Antibacterial activity) of a) *E. faecalis*, b) *S. mutans*, c) Saliva Microcosm Model

4.1.6. Hemolytic Assay

AgNPs and CIP-AgNPs were tested for cytotoxicity using a hemolytic assay. Blood samples were obtained by healthy participants. PBS served as the negative control, and Triton X-100 (1% concentration) served as the positive control. The absorbance of the samples was calculated at 545 nm. The hemolytic activity of different doses of CIP-AgNPs, AgNPs, and unaltered composite resin is depicted in Figure 22. So the safe limit of hemolytic percentage is 5%, under ISO/TR 7406. According to the findings of the current investigation, CIP-AgNPs and non-modified composite resin both had hemolytic activity levels that were lower than 1% AgNPs.

AgNPs showed hemolytic activity, it was more toxic as compared to CIP-AgNPs. The ideal concentration for AgNPs is at this 1% concentration. On the other hand, cytotoxicity was dramatically decreased (P<0.05) when ciprofloxacin was added to AgNPs. With the addition of 1% CIP-AgNPs (i.e., 1.1%), there was little to very less noticeable hemolytic activity.

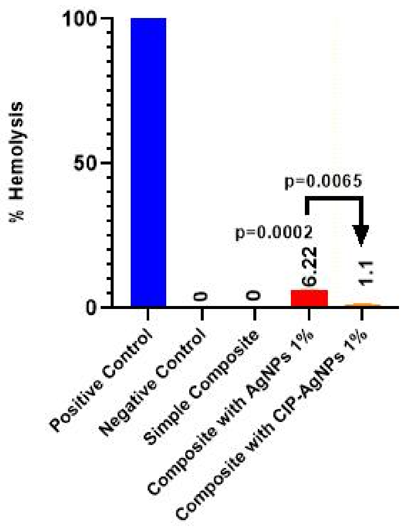

Figure 22: Hemolytic Activity of Composites

4.1.7. Mechanical testing

The mean compressive strength (MPa) for unmodified and NPs modified resin composite discs (wt.%) is represented in (Figure 22). As CIP-AgNPs were added to the resin composites at 1%, the compressive strength of composites resin enhanced considerably (P<0.05) when compared to the unmodified. The addition of CIP-AgNPs showed improvement in the compressive strength of the composite discs. This proved that adding CIP-AgNPs did not lower the mechanical properties of dental composites. In fact, it enhanced the compressive strength of dental composites

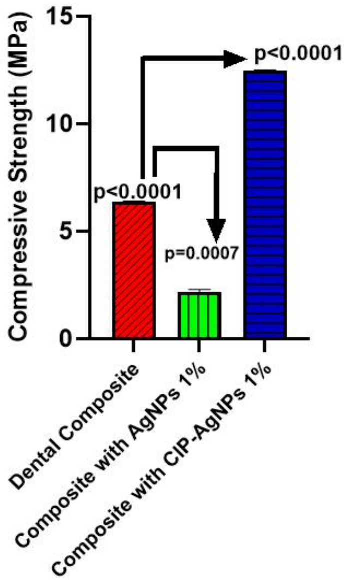

Figure 23: Compressive strength of composites

5) Discussion

Ciprofloxacin is a broad spectrum antibiotic that is highly effective for Streptococcus species and enterococus species. Ciprofloxacin was loaded on AgNPs to enhance the antibacterial activity and mechanical properties.

The loading of Ciprofloxacin on AgNPs was confirmed by performing UV-Visable Spectroscopy and FTIR. The absorbance maxima of AgNPs showed red-shift upon the loading of ciprofloxacin and shifted from 404 nm to 414 nm (Figure 12)

The positive charge of Ciprofloxacin and negative charge of AgNPs resulted in electrostatic interaction that favoured the loading of ciprofloxacin on AgNPs successfully. The charge of AgNPs was changed from -14.5 mV to +15.8 mV, that showed the loading of positive charged ciprofloxacin on AgNPs (Figure 16 & 17). CIP-AgNPs were also shown to be more stable in suspension as the zeta potential was closer to +20 mV (Mikac et al., 2017). FTIR analysis showed the interaction of ciprofloxacin with AgNPs. There was hydrogen bonding between - OH of ciprofloxacin and $-BH_4$ of AgNPs. This interaction exhibited a characteristic peak shift from 3530cm^{-1} to 3340cm^{-1}. Due to electrostatic interaction between amine group of ciprofloxacin and borohydride of AgNPs, another characteristic peak shift of 1380cm^{-1} to 1405cm^{-1} was observed (Figure 13)

Dental composites containing CIP-AgNPs showed enhanced antibacterial activity and lower hemolytic activity as compared with dental composites that contained AgNPs. AgNPs were 3 times more toxic than CIP-AgNPs when hemolytic assay was performed. Upon the addition of ciprofloxacin on silver nanoparticles, the cytotoxicity was decreased and anti-bacterial activity was increased (Figure 22).

Mechanical strength is really important for dental fillings as teeth exert forces on each other. A good dental composite should not only have a better antibacterial activity but also improved mechanical properties (Figure 23). Addition of ciprofloxacin loaded AgNPs in dental composites, resulted in improved compressive strength along with enhanced anti-bacterial activity ($P<0.05$).

6) Conclusion

The results indicated that the antibacterial activity and compressive strength of resin composites containing Ciprofloxacin loaded silver nanoparticles were superior to the control group and also exhibited less cytotoxicity as compared to the resin composites containing silver nanoparticles. In short, these results established strong ground applications for CIP-AgNPs modified dental composite resins.

Nanotechnology is a promising field to combat secondary caries. It has proved to be of great assistance not only for the treatment of dental caries but also for the prevention of restoration failure due to secondary caries. Ciprofloxacin loaded silver nanoparticles can be used to enhance the antibacterial properties of resin composites and improve the longevity of tooth restoration procedure. CIP-AgNPs have exhibited highly favorable results in the prevention of polymicrobial biofilms, especially *S. mutans* and *E. faecalis* strains in dental composites. The long-term antibacterial effect of Ciprofloxacin loaded silver nanoparticles in resin composites remains to be further studied. In addition, in vivo and in situ studies on application of ciprofloxacin loaded AgNPs in resin composites are also of great importance.

References

Al-Shahrani, M. A. (2019). Microbiology of Dental Caries: A Literature Review. AnGorain, Medical and Health Sciences Research. https://www.amhsr.org/abstract/microbiologyof-dental-caries-a-literature-review-5239.html

Bapat, R. A., Chaubal, T. V., Joshi, C. P., Bapat, P. R., Choudhury, H., Pandey, M., Gorain, B., & Kesharwani, P. (2018b). An overview of application of silver nanoparticles for biomaterials in dentistry. Materials Science and Engineering: C, 91, 881–898. https://doi.org/10.1016/j.msec.2018.05.069

Barot, T., Rawtani, D., & Kulkarni, P. (2021). Nanotechnology-based materials as emerging trends for dental applications. REVIEWS ON ADVANCED MATERIALS SCIENCE, 60(1), 173–189. https://doi.org/10.1515/rams-2020-0052

Bekisheva, E. V., & Barbasheva, S. S. (n.d.). 5.2 The Stages of Tooth Decay. Retrieved September 20, 2021, from https://www.elibrary.ru/item.asp?id=23890122 Beyth, N., Farah, S., Domb, A. J., & Weiss, E. I. (2014). Antibacterial dental resin composites. Reactive and Functional Polymers, 75, 81–88. https://doi.org/10.1016/j.reactfunctpolym.2013.11.011

Bhaduri, S. B., & Bhaduri, S. (2009). Biomaterials for Dental Applications. In R. Narayan (Ed.), Biomedical Materials (pp. 295–326). Springer US. https://doi.org/10.1007/978-0-387-84872-3_11

Brambilla, E., & Ionescu, A. C. (2021). Oral Biofilms and Secondary Caries Formation. In A.C. Ionescu & S. Hahnel (Eds.), Oral Biofilms and Modern Dental Materials: Advances Toward Bioactivity (pp. 19–35). Springer International Publishing. https://doi.org/10.1007/978-3-030-67388-8_3

Conrads, G., & About, I. (2018). Pathophysiology of Dental Caries. Caries Excavation: Evolution of Treating Cavitated Carious Lesions, 27, 1–10. https://doi.org/10.1159/000487826

Corrêa, J. M., Mori, M., Sanches, H. L., Cruz, A. D. da, Poiate, E., & Poiate, I. A. V. P. (2015). Silver Nanoparticles in Dental Biomaterials. International Journal of Biomaterials, 2015, e485275. https://doi.org/10.1155/2015/485275

Davidović, S., Lazić, V., Vukoje, I., Papan, J., Anhrenkiel, S. P., Dimitrijević, S., & Nedeljković, J. M. (2017). Dextran coated silver nanoparticles—Chemical sensor for selective cysteine detection. Colloids and Surfaces B: Biointerfaces, 160, 184–191. https://doi.org/10.1016/j.colsurfb.2017.09.031

Dias, H. B., Bernardi, M. I. B., Bauab, T. M., Hernandes, A. C., & de Souza Rastelli, A. N. (2019). Titanium dioxide and modified titanium dioxide by silver nanoparticles as an anti biofilm filler content for composite resins. Dental Materials: Official Publication of the Academy of Dental Materials, 35(2), e36–e46. https://doi.org/10.1016/j.dental.2018.11.002

Engel, A.-S., Kranz, H. T., Schneider, M., Tietze, J. P., Piwowarcyk, A., Kuzius, T., Arnold, W., & Naumova, E. A. (2020). Biofilm formation on different dental restorative materials in the oral cavity. BMC Oral Health, 20(1), 162. https://doi.org/10.1186/s12903-020-01147-x

Erickson, D. J. (2013, June 20). Composite Resin Fillings: What are the Advantages and Disadvantages? Danville Family Dentistry. https://www.danvilledentalcare.com/composite-resin-fillings-advantages-anddisadvantages/

Featherstone, J. D. B., Adair, S. M., Anderson, M. H., Berkowitz, R. J., Bird, W. F., Crall, J. J., Den Besten, P. K., Donly, K. J., Glassman, P., Milgrom, P., Roth, J. R., Snow, R., & Stewart, R. E. (2003). Caries management by risk assessment: Consensus statement, April 2002. Journal of the California Dental Association, 31(3), 257–269.

Feng, X. (2014). [Cause of secondary caries and prevention]. Hua Xi Kou Qiang Yi Xue Za Zhi = Huaxi Kouqiang Yixue Zazhi = West China Journal of Stomatology, 32(2), 107–110.

Fernandez, C. C., Sokolonski, A. R., Fonseca, M. S., Stanisic, D., Araújo, D. B., Azevedo, V., Portela, R. D., & Tasic, L. (2021). Applications of Silver Nanoparticles in Dentistry: Advances and Technological Innovation. International Journal of Molecular Sciences, 22(5), 2485. https://doi.org/10.3390/ijms22052485

Gold And Amalgam Fillings. (2021, March 17). Westerville Dental Associates.

https://www.westervilledental.com/why-dont-dentists-use-gold-and-amalgam-fillingsanymore/

Guiguimde, W. P. L., Bakiono, F., Ouedraogo, Y., Millogo, M., Gare, J. V., Konsem, T., Ouedraogo, A., & Ouedraogo, D. (2014). [Epidemiology and clinic of dental extractions in University Teaching Hospital Yalgado Ouedraogo, (Burkina Faso)]. Odonto-stomatologie tropicale = Tropical dental journal, 37(148), 32–38.

Gupta, S. K., Saxena, P., Pant, V. A., & Pant, A. B. (2012). Release and toxicity of dental resin composite. Toxicology International, 19(3), 225–234. https://doi.org/10.4103/0971-6580.103652

Hervás García, A., Martínez Lozano, M. A., Cabanes Vila, J., Barjau Escribano, A., & Fos Galve, P. (2006). Composite resins: A review of the materials and clinical indications. Hervás García, A. ; Martínez Lozano, M.A. ; Cabanes Vila, Jose ; Barjau Escribano, Amaya ; Fos Galve, P.. Composite Resins : A Review of the Materials and Clinical Indications. En: Medicina Oral, Patología Oral y Cirugía Bucal. Ed. Inglesa, 11 2 2006: 23-. https://roderic.uv.es/handle/10550/63556

Ismail, A. I. (2004). Visual and Visuo-tactile Detection of Dental Caries. Journal of Dental Research, 83(1_suppl), 56–66. https://doi.org/10.1177/154405910408301s12 İspirli, H., Sagdic, O., & Dertli, E. (2021). Synthesis of silver nanoparticles prepared with a dextran-type exopolysaccharide from Weissella cibaria MED17 with antimicrobial functions. Preparative Biochemistry & Biotechnology, 51(2), 112–119. https://doi.org/10.1080/10826068.2020.1795673

James, S. L., Abate, D., Abate, K. H., Abay, S. M., Abbafati, C., Abbasi, N., Abbastabar, H., Abd-Allah, F., Abdela, J., Abdelalim, A., Abdollahpour, I., Abdulkader, R. S., Abebe, Z., Abera, S. F., Abil, O. Z., Abraha, H. N., Abu-Raddad, L. J., Abu-Rmeileh, N. M. E., Accrombessi, M. M. K., … Murray, C. J. L. (2018). Global, regional, and national incidence, prevalence, and years lived with disability for 354 diseases and injuries for 195 countries and territories, 1990–2017: A systematic analysis for the Global Burden of Disease Study 2017. The Lancet, 392(10159), 1789–1858. https://doi.org/10.1016/S0140-6736(18)32279-7

Kasraei, S., Sami, L., Hendi, S., AliKhani, M.-Y., Rezaei-Soufi, L., & Khamverdi, Z. (2014a). Antibacterial properties of composite resins incorporating silver and zinc oxide nanoparticles on Streptococcus mutans and Lactobacillus. Restorative Dentistry & Endodontics, 39(2), 109–114. https://doi.org/10.5395/rde.2014.39.2.109

Kassaee, M., Akhavan, A., Sheikh, N., & Sodagar, A. (2008). Antibacterial Effects of a New Dental Acrylic Resin Containing Silver Nanoparticles. Journal of Applied Polymer Science, 110, 1699–1703. https://doi.org/10.1002/app.28762

Lee, Y. (2013). Diagnosis and Prevention Strategies for Dental Caries. Journal of Lifestyle Medicine, 3(2), 107–109.

Li, F., Weir, M. D., Fouad, A. F., & Xu, H. H. K. (2014). Effect of salivary pellicle on antibacterial activity of novel antibacterial dental adhesives using a dental plaque microcosm biofilm model. Dental Materials: Official Publication of the Academy of Dental Materials, 30(2), 182–191. https://doi.org/10.1016/j.dental.2013.11.004

M, A. (2002). Risk assessment and epidemiology of dental caries: Review of the literature. Pediatric Dentistry, 24(5), 377–385. Melo, M. A. S., Guedes, S. F. F., Xu, H. H. K., & Rodrigues, L. K. A. (2013). Nanotechnologybased restorative materials for dental caries management. Trends in Biotechnology, 31(8), 459–467. https://doi.org/10.1016/j.tibtech.2013.05.010

Mikac, L., Jurkin, T., Štefanić, G., Ivanda, M., & Gotić, M. (2017). Synthesis of silver nanoparticles in the presence of diethylaminoethyl-dextran hydrochloride polymer and their SERS activity. Journal of Nanoparticle Research, 19(9), 299. https://doi.org/10.1007/s11051-017-3989-1

Mohamed Fahmy, H., Mahmoud Mosleh, A., Abd Elghany, A., Shams-Eldin, E., Serea, E. S. A., Ashour Ali, S., & Esmail Shalan, A. (2019). Coated silver nanoparticles: Synthesis, cytotoxicity, and optical properties. RSC Advances, 9(35), 20118–20136. https://doi.org/10.1039/C9RA02907A

Mohanta, Y. K., Biswas, K., Jena, S. K., Hashem, A., Abd_Allah, E. F., & Mohanta, T. K.(2020). Anti-biofilm and Antibacterial Activities of Silver Nanoparticles Synthesized by the Reducing Activity of Phytoconstituents Present in the Indian Medicinal Plants. Frontiers in Microbiology, 11. https://www.frontiersin.org/article/10.3389/fmicb.2020.01143

Mohsen E, El-Borady OM, Mohamed MB, Fahim IS. Synthesis and characterization of ciprofloxacin loaded silver nanoparticles and investigation of their antibacterial effect. J Radiat Res Appl Sci. 2020;13(1):416–25.

Mulfinger, L., Solomon, S. D., Bahadory, M., Jeyarajasingam, A. V., Rutkowsky, S. A., & Boritz, C. (2007). Synthesis and Study of Silver Nanoparticles. Journal of Chemical Education, 84(2), 322. https://doi.org/10.1021/ed084p322

Niska, K., Knap, N., Kędzia, A., Jaskiewicz, M., Kamysz, W., & Inkielewicz-Stepniak, I. (2016). Capping Agent-Dependent Toxicity and Antimicrobial Activity of Silver Nanoparticles: An In Vitro Study. Concerns about Potential Application in Dental Practice. International Journal of Medical Sciences, 13(10), 772–782. https://doi.org/10.7150/ijms.16011

Pitts, N. B. (2004). "ICDAS"—An international system for caries detection and assessment being developed to facilitate caries epidemiology, research and appropriate clinical management. Community Dental Health, 193–198.

Pitts, N. B., Zero, D. T., Marsh, P. D., Ekstrand, K., Weintraub, J. A., Ramos-Gomez, F., Tagami, J., Twetman, S., Tsakos, G., & Ismail, A. (2017). Dental caries. Nature Reviews Disease Primers, 3(1), 1–16. https://doi.org/10.1038/nrdp.2017.30

Pratap, B., Gupta, R. K., Bhardwaj, B., & Nag, M. (2019). Resin based restorative dental materials: Characteristics and future perspectives. The Japanese Dental Science Review, 55(1), 126–138. https://doi.org/10.1016/j.jdsr.2019.09.004

Prevalence of dental caries among the patients visiting islam dental college hospital sialkot | Virtual Health Sciences Library. (n.d.). Retrieved April 26, 2022, from https://vlibrary.emro.who.int/imemr/prevalence-of-dental-caries-among-the-patientsvisiting-islam-dental-college-hospital-sialkot-2/

Rangreez, T. A., & Mobin, R. (2019). Polymer composites for dental fillings. In Applications of Nanocomposite Materials in Dentistry (pp. 205–224). Elsevier. https://doi.org/10.1016/B978-0-12-813742-0.00013-4

Rathee, M., & Sapra, A. (2021). Dental Caries. In StatPearls. StatPearls Publishing. http://www.ncbi.nlm.nih.gov/books/NBK551699/

Robinson, C. (2014). Dental Caries. In E. Lammert & M. Zeeb (Eds.), Metabolism of Human Diseases (pp. 87–92). Springer Vienna. https://doi.org/10.1007/978-3-7091-0715-7_1545

Roger Warwick & Peter L. Williams, ed. (1973), Gray's Anatomy (35th ed.), London: Longman, pp. 1218-1220

Selwitz, R. H., Ismail, A. I., & Pitts, N. B. (2007). Dental caries. Lancet (London, England), 369(9555), 51–59. https://doi.org/10.1016/S0140-6736(07)60031-2

Shanmuganathan, R., Karuppusamy, I., Saravanan, M., Muthukumar, H., Ponnuchamy, K., Ramkumar, V. S., & Pugazhendhi, A. (2019). Synthesis of Silver Nanoparticles and their Biomedical Applications—A Comprehensive Review. Current Pharmaceutical Design, 25(24), 2650–2660. https://doi.org/10.2174/1381612825666190708185506

Siddiqui, A. A., Alshammary, F., Mulla, M., Al-Zubaidi, S. M., Afroze, E., Amin, J., Amin, S., Shaikh, S., Madfa, A. A., & Alam, M. K. (2021). Prevalence of dental caries in Pakistan: A systematic review and meta-analysis. BMC Oral Health, 21(1), 450. https://doi.org/10.1186/s12903-021-01802-x

Sidhu, S., & Nicholson, J. (2016). A Review of Glass-Ionomer Cements for Clinical Dentistry. Journal of Functional Biomaterials, 7(3), 16. https://doi.org/10.3390/jfb7030016

Stefanovska, E., & Bilbilova, E. Z. (2021). Introductory Chapter: Dental Biofilms Associated with Caries. In Dental Caries. IntechOpen. https://doi.org/10.5772/intechopen.96384

Teshome, A., Muche, A., & Girma, B. (2021). Prevalence of Dental Caries and Associated Factors in East Africa, 2000–2020: Systematic Review and Meta-Analysis. Frontiers in Public Health, 9, 645091. https://doi.org/10.3389/fpubh.2021.645091

Thom, D. C., Davies, J. E., Santerre, J. P., & Friedman, S. (2003). The hemolytic and cytotoxic properties of a zeolite-containing root filling material in vitro. Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontics, 95(1), 101–108. https://doi.org/10.1067/moe.2003.90

Vergnes, J.-N., Kaminski, M., Lelong, N., Musset, A.-M., Sixou, M., Nabet, C., & Group, for the E. (2012). Frequency and Risk Indicators of Tooth Decay among Pregnant Women in France: A Cross-Sectional Analysis. PLOS ONE, 7(5), e33296. https://doi.org/10.1371/journal.pone.0033296

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Xu, H. H. K., Cheng, L., Zhang, K., Melo, M. A. S., Weir, M. D., Antonucci, J. M., Lin, N. J., Lin-Gibson, S., Chow, L. C., & Zhou, X. (2013). Nanostructured Dental Composites and Adhesives with Antibacterial and Remineralizing Capabilities for Caries Inhibition. In Nanobiomaterials in Clinical Dentistry (pp. 109–129). Elsevier. https://doi.org/10.1016/B978-1-4557-3127-5.00006-4

Yin, I. X., Zhang, J., Zhao, I. S., Mei, M. L., Li, Q., & Chu, C. H. (2020b). The Antibacterial Mechanism of Silver Nanoparticles and Its Application in Dentistry. International Journal of Nanomedicine, 15, 2555–2562. https://doi.org/10.2147/IJN.S246764

Zero, D. T., Fontana, M., Martínez-Mier, E. A., Ferreira-Zandoná, A., Ando, M., González Cabezas, C., & Bayne, S. (2009). The Biology, Prevention, Diagnosis and Treatment of Dental Caries: Scientific Advances in the United States. The Journal of the American Dental Association, 140, 25S-34S. https://doi.org/10.14219/jada.archive.2009.0355

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