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Evaluation of Antimicrobial Efficacy Cyanoacrylate Infused with Chitosan Nanoparticles: An *in Vitro* Study

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ABSTRACT

Aim: To evaluate the antimicrobial activity and efficiency of the cyanoacrylate adhesive incorporated with chitosan nanoparticles

Objective: to formulate an adhesive for membrane stabilisation in grafting and optical determine its anti-microbial efficacy

Materials and methods: Chitosan nanoparticles were made by dissolving chitosan in 1percent acetic acid and stirred at 1000 rpm for 24 hrs. Presence of nanoparticles was confirmed by the use of a spectrophotometer. Medical grade Isoamyl 2 cyanoacrylate was used to mix with the chitosan nano particle. Setting time, efficacy and anti-microbial effects were evaluated

Results: Anti-microbial activity test showed significant results.

Key words: Chitosan, Cyano acrylate, GBR, Antibactial activity, Membrane stabilisation

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INTRODUCTION

Nanoparticles of chemical substances are attracting a lot of scientific attention these days because they can effectively bind bulk materials to atomic or molecular structures. A bulk material's physical properties should remain constant regardless of its size, but size-dependent properties are often observed to change at the nanoscale [1].

The tooth is a vital element of the body that helps with personality development, digestion, infection, and a variety of other physiological functions [2]. Poor oral hygiene is greatly exacerbated by an infected tooth [3]. Many major problems, such as cardiovascular disease, diabetes, neurological illnesses, and others, are linked to oral health [4]. Various materials or treatments have been produced in the field of dentistry to maintain clean teeth. Conventional dental materials can harm tooth qualities including whiteness and integrity, as well as increase biofilm formation on the teeth [5]. Various materials have been developed that address the drawbacks of traditional materials [1]. Chitosan is a marine derived polysaccharide and its a relatively newer biomaterial that have numerous applications in medicine and has been in the field of nanomedicine, and newer drug delivery methods with better results, advanced bioavailability ,reduced toxicity [6]. Chitosan is a polycationic linear polysaccharide generated from chitin that is naturally polycationic [7]. Chitosan's limited solubility in neutral and alkaline solutions limits its use. Chemical alteration of composites or hydrogels, on the other hand, confers new functional qualities for a variety of applications. Because of their non-toxicity, low allergenicity, biocompatibility, and biodegradability, chitosans are widely used as biomaterials [6]. The amount of protonated amino groups in the polymeric chain, and thus the proportion of acetylated and non-acetylated D-glucosamine units, affect chitosan solubility, biodegradability, reactivity, and adsorption of several substrates [8].

Cyanoacrylate adhesives have been used in medical and dentistry for a long time, however there are some mixed feelings about them [9]. The goal of this research was to compile all available information on the use of cyanoacrylate adhesives for oral wounds during dental and surgical operations with an emphasis on uses, indications, benefits, and drawbacks. In conclusion, in vivo and clinical research have proved in the last few years that all types of cyanoacrylate adhesives used in intra- and extraoral operations are safe, effective, easy to apply, and feasible [10].

Cyanoacrylate adhesive is a substance made by combining cyanoacetate with formaldehyde in the presence of a

catalyst [11]. Rapid polymerization results in the formation of a cyano acrylate sticky layer [12]. Ice.

This anionic polymerisation can be activated with the help of water as a catalyst , induced by the presence of hydroxyl groups on the surface.

CyanoAcrylates are particularly adherent to biological tissue because of their proteins. It might even come in handy for grafting CyanoAcrylates to keep their sticky properties, even in the presence of wetness, and they also have the ability to adhere [13].

Bacteriostatic and haemostatic properties of CyanoAcrylate have further advantages [14]. The aim of the study conducted was to evaluate the antimicrobial activity of chitosan nanoparticles infused in cyanoacrylate.

The rationale for the study was to create an adhesive that have the desirable adhesive properties of the cyanoacrylate and anti microbial activity of chitosan for membrane fixation for grafting.

MATERIALS AND METHODS

Preparation of chitosan nanoparticles

Ionic gelation of chitosan (MW 190–370 kDa, deacetylation degree 75%) with sodium tripolyphosphate (TPP) anions was used to make Chitosan Nanoparticles .

Chitosan was dissolved at a concentration of 0.1 percent (w/v) in 1 percent (v/v) acetic acid, then stirred overnight at 200 rpm on a magnetic stirrer.a PVDF syringe filter with a 0.22 m pore size TPP was dissolved in sterile distilled water at a concentration of 0.25 percent (w/v).

A PVDF membrane syringe filter (pore size 0.22 m) was used to filter the water. Cross-linking is a process in which two or more molecules are linked together.

Drop by drop, under a magnetic stirrer at 700 rpm, chitosan was mixed with TPP in an equal volume. The particle was resuspended in water after being centrifuged for 10 minutes at 10,000 rpm in sterile distilled water, then ultrasonication at 28 percent pulse ratio for 100 seconds at 4 degrees Celsius.

The precipitated nanoformulation was then subjected to three rounds of ultrasonication. For further investigation, the samples were freeze-dried and kept in a desiccator (Figure 1).

Figure 1: IsoAmyl cyanoacrylate was was procured from medical sources.

RESULTS AND DISCUSSION

Characterization of nano- particles

Characterization of nano- particles was performed using TEM analysis and UV-Vis spectroscopy.

TEM Analysis

The TEM analysis was done to understand the crystalline characteristics and size of the Chitosan nanoparticles. TEM analysis revealed that the Chitosan Nanoparticles were spherical in shape with slight variation in thickness. The average particle size of the Chitosan Nanoparticles by histogram was around 100-200nm. The image revealed that the nanoparticles were almost spherical in shape and the average particle size was around 150 nm (Figure 2).

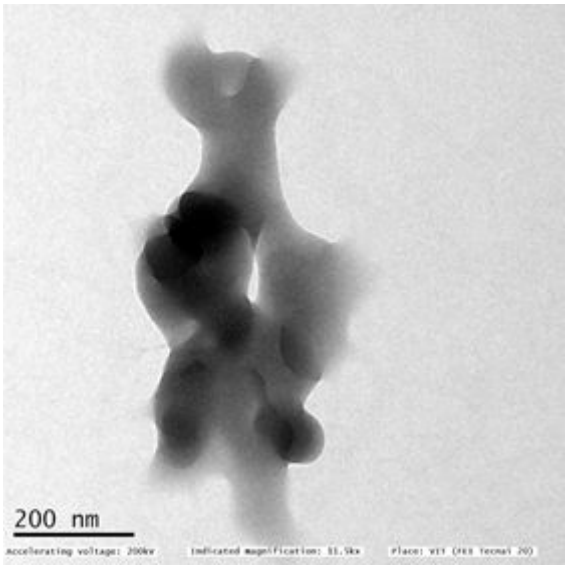

Figure 2: Transmission electron microscopy of chitosan nanoparticles.

UV-Visual spectroscopy

UV-Visual spectroscopy device is used to find out the optical properties.A nanoparticles size is considered as a significant factor in changing the entire properties for materials .

The Chitosan nanoparticles were dissolved with the ethanol, then solution was used in Uv-visible test. Absorption spectra of pure Chitosan NPs are shown in fig.This spectrum indicate that absorption peak is strong in the wavelength (345) nm, due to the band-gap absorption for zinc oxide result from removed electrons from the valence band to the conduction band.
Furt2`her, because ZnO particles are in nano-size and the particle size distribution is narrow, this 11lead0 s to a sharp absorption peak . UV-Visual spectroscopy was done at 24,hrs and it wa1s found that zinc oxide nanoparticles have a good absorption in UV region (300-400) nm, which makes convenient to medical applications like antiseptic ointments and antibiotic properties (Figure 3).

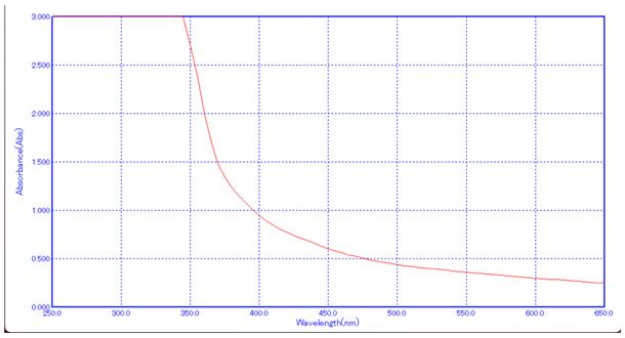

Figure 3: UV spectroscopy.

Infusion of chitosan in cyanoacrylate

Chitosan nanoparticles were incorporated into medical grade isoamyl cyanoacrylate and stirred using a tabletop vibrator.

Setting time was calculated, consistency was evaluated . The resultant compound was tested for antibacterial activity against S. Mutans and E. Fecalis.

The infusion of cyanoacrylate with chitosan resulted in a colourless, odorless gelatinous mixture with a viscous consistency .

Setting time was clocked around 8.4 minutes which was very high when compared to the conventional setting time of the material which was 5- 10 seconds (Figure 4).

When antimicrobial activity was tested for the prepared compound Zone of inhibition was seen in both the cultures suggesting good antibacterial activity.

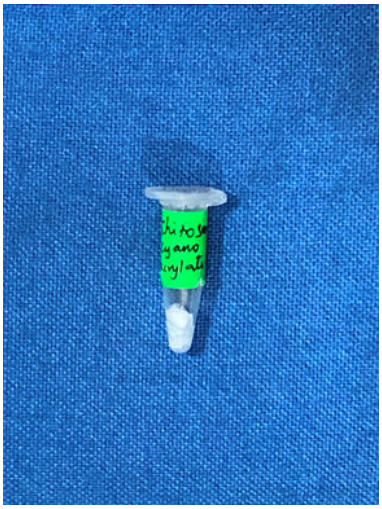

Figure 4: Cyanoacrylate infused with chitosan.

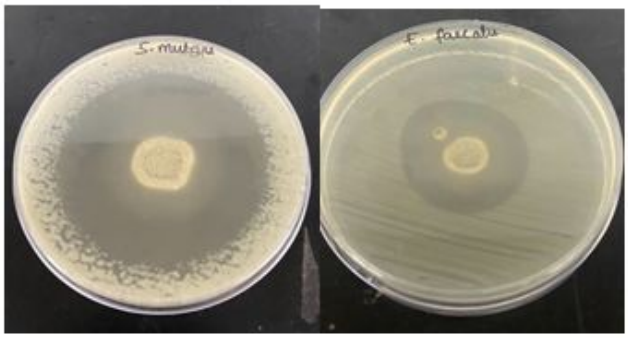

Figure 5: Antimicrobial activity.

DISCUSSION

Chitosan (-(1,4)-2amino-2-deoxy-D-glucose) is a nontoxic, biodegradable amino polysaccharide made from the deacetylation of chitin, one of the most prevalent biopolymers on the planet. This biopolymer has a lot of potential.When cast from organic acidic water solutions, it has film- and coating-forming capabilities [15]. Due to the presence of the amino group in conjunction with the primary alcohol activity, chitosan can be converted into a variety of derivatives, including N-modified Chitosan, O-modified Chitosan, and N,O-modified Chitosan [16].

Chitosan's antibacterial action is one of its most studied features, with applications ranging from biomedicine to cosmetics and food to agriculture. Many studies have been undertaken to date in order to make use of chitosan's antibacterial activity as well as its unique properties in order to create self-preserving materials. This has resulted in the development of a wide range of chitosan-based products such as beads, films, fibres, membranes, and hydrogels for a variety of applications [17]. In investigations including in vivo and in vitro interactions with chitosan in various forms, chitosan has been examined as an antibacterial substance against a wide range of target species such as algae, bacteria, yeasts, and fungi (solutions, films and composites) [18]. The precise mechanism of antibacterial action is still

unknown. The antibacterial activity of chitosan is known to be influenced by a variety of variables that work in an ordered and independent manner. The most widely hypothesised antibacterial effect of chitosan is that it binds to the negatively charged bacterial cell wall, disrupting the cell and altering membrane permeability, then attaches to DNA, inhibiting DNA replication and causing cell death [19]. Another suggested mechanism is that chitosan serves as a chelating agent, producing toxin production and limiting microbial growth by binding to trace metal elements on an opt-in basis [20]. Chitosan's polycationic structure is required for antibacterial action. Electrostatic interaction between the polycationic structure and the predominantly anionic components of the microorganisms' surface (such as Gram-negative lipopolysaccharide and cell surface proteins) plays a primary role in antibacterial activity because environmental pH is below the pKa of chitosan and its derivatives [18].

The first use of cyanoacrylate glue in open Lichtenstein repair was reported in 1993 , while the first use of cyanoacrylate glue in laparoscopic hernia repair was documented in 1998 . N-butyl cyanoacrylate is a cyanoacrylate butyl polymer.In the presence of heat, acrylic resin conducts an exothermic reaction.In around 5 seconds, water forms a connection that nalises in about a minute.As a result, the mesh must be secured against the underlying tissue.Among the several types of polymers,The stronger binding is formed by cyanoacrylate and butyl polymer [21].

Studies have proved cyanoacrylate provide a low form of anti microbial activity .Gram-positive bacteria are more resistant to cyanoacrylate than gram-negative bacteria, probably because the latter are protected by an outer carbohydrate capsule [22]. For Streptococcus pneumoniae, Staphylococcus aureus, and Escherichia coli, the polymerization reaction in EC improved antibacterial activity. Without exposure to the active polymerization activity, inhibitory halos were completely absent in Escherichia coli. Only gram-positive bacteria were found to have inhibitory halos in BC. With or without exposure to the active polymerization reaction, no inhibitory halo was seen for Pseudomonas aeruginosa in either EC or BC [23].

The drawbacks of this study were that limited varieties of microbes were used to check antibacterial activity . More extensive study is required in this area on a larger scale . Potential uses of this material include to create an adhesive with qualities of both chitosan and cyanoacrylate . To formulate a material with the antimicrobial effectiveness of chitosan and cyanoacrylate and the adhesive properties which will help in membrane stabilization during grafting . It can also be used as a tissue adhesive to approximate flaps .

CONCLUSION

The study was conducted keeping in mind the formulation of an adhesive that can be used for membrane stabilization during gbr. The resulting compound did show a significant antimicrobial activity as well as retained the properties of the cyanoacrylate adhesive . However, the prolonged setting time rendered it not helpful for the surgical procedures as of now. Further studies are required to find a specific combination of the two materials to formulate an ideal compound that can be used immediately as well as possess the characteristics of chitosan.

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