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Evaluation of the compressive strength and antibacterial Properties of Glass Ionomer Cement Modified with Different Concentrations of Nanochitosan

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ABSTRACT:
Statement of problem:GICs have certain drawbacks or restrictions, such as early moisture sensitivity, brittleness, and lower mechanical strength.Conventional GICs' antibacterial activity is ineffective in influencing the formation of biofilms and the viability of cells.[1]
KEYWORDS: glass ionomer cement, chitosan, antibacterial effect, compressive strength.

I.INTRODUCTION

Glass ionomer cement (GIC) is categorized as an acid-base cement made up of a basic calcium aluminosilicate powder and an aqueous solution of polyalkenoic acid.Polyacrylic acid (PAA) or an acrylic/maleic/itaconic acid copolymer is typically included in the polyalkenoic acid family of complex acids.Cement hardening results from the crosslinking of polyalkenoic acid chains caused by multivalent counterions (such as Ca^{+2} and Al^{+3}) leaking out of glass particles during the acid-base setting reaction.[2]

Glass ionomer cements are extensively used as bone cements, luting cements, liners, fissure sealants, and tooth fillings.[3] Glass ionomers exhibit remarkable characteristics and benefits, such as chemical attachment to tooth structure, fluoride release-induced anticariogenicactivity, biocompatibility, and a low coefficient of thermal expansion that resembles that of tooth structure.[4]But when compared to resin-based restorative materials, GICs have certain drawbacks or restrictions, such as early moisture sensitivity, brittleness, and lower mechanical strength.[1]

The addition of reinforcement phases (such as metal oxides like ZrO_2, minerals like hydroxyapatite, polymeric materials like N-vinyl pyrrolidone, fibers, or ceramic additives),were explored in order to improve the mechanical properties of GICs.[5-7]Although the mechanical properties were somewhat improved by each strategy, these findings have not yet been used in clinical settings.[8]

Because of the diversity of bacterial species present in the oral cavity, the sophisticated surface chemistry of GIC, as well as its rough surface, all contribute to the growth of biofilms on GIC surfaces.[9]Conventional GICs' antibacterial activity is ineffective in influencing the formation of biofilms and the viability of cells.Chlorhexidine was added to conventional GIC to increase its antibacterial action, but this had a negative impact on its mechanical and physical properties. The need for a different biocompatible additive that could strengthen GIC's antibacterial action without sacrificing its mechanical and physical qualities.[10]

Chitosan (CH) has been deemed safe by the US Food and Drug Administration, naturally occurring polysaccharide that is both biocompatible and biodegradable. Natural polymer chitosan is produced by alkaline N-deacetylation of chitin.The most prevalent organic substance in nature, following cellulose.[11]

Chitosan has antibacterial qualities, but it's crucial that the structure's fundamental physical characteristics aren't compromised when adding it to glass ionomer cement.[12]It has been reported that GIC modified with chitosan has improved antibacterial properties against Streptococcus mutans. Chitosan prevents the release of phosphorus and the loss of minerals, which impedes the demineralization of teeth.Furthermore, flexural strength and the quantity of fluoride ions released are both increased by the addition of chitosan to GIC.[13]Clinical significance may arise from GIC modification with CH. Thus, the purpose of this study is to assess how adding various concentrations of nanochitosan to glass ionomer cement affects the material's mechanical and physical characteristics.

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II. MATERIALS AND METHOD

1.Materials

Glass ionomer restorative material (promedica, Germany),Medium molecular weight chitosan (Oxford Lab Fine ChemicalsIndia), Acetic acid (El Nasr Pharmacological chemicalsEgypt) and Sodium tripolyphosphate (Hubi xingfa chemicals groupChina).

2.Preparation of nanochitosan solution

The approach used was described by Tang et al. A solution of 20 mg chitosan in 40 ml of 2.0% (v/v) acetic acid was created. 20 ml of sodium tripolyphosphate aqueous solution containing 0.75 mg/ml were added gradually while being stirred. A suspension of chitosan nanoparticles was formed. [14]

3.Characterization methods

3.1. Fourier transform infrared spectroscopy (FT-IR) analysis.

The functional groups were assessed by FTIR spectroscopy (Thermo Fisher Scientific, Nicolet iS10, USA)The scale range of frequency in the range of ν = 4000 to 500 cm^{-1} was plotted against the transmittance percentages. The analysis of FT-IR was utilized to identify the characteristic functional groups of both samples.

3.2. X-ray diffraction analysis (XRD)

The crystalline phases were identified using XRD analysis (XRD, PANalytical X'Pert PRO, Netherlands)

3.3. Scanning electron microscopy (SEM)

Chitosan nanoparticles size and morphology were examined using the scan electron microscopy (SEM, Quanta 250 FEG, Netherlands).

4. Preparation of Nanochitosan Modified Glass Ionomer Liquid

Nanochitosan solution was added to the GIC liquids in varying volume percents (v/v%): 0% CH (control; unmodified group), 5% CH, 10% CH, and 15% CH. The CH- modified GIC liquids were then, air-sealed, and gently mixed for 5 minutes at room temperature. Before combining with the GIC powder, the CH-modified GIC liquids were kept at room temperature for 24 hours.

5. Specimens grouping

Specimens were grouped according to the volume percent of added nanochitosan solution to GIC liquid as follow:

- Group 1: Conventional glass ionomer cement as a control group.
- Group 2**:** 5%CH - modified glass ionomer cement.
- Group 3**:** 10%CH - modified glass ionomer cement.
- Group 4**:** 15%CH - modified glass ionomer cement.

For each group, half of the specimens were tested right away, and the other half were tested after 10,000 thermal cycles in distilled water at 5 and 55 ^{0}C.

6. In vitro antibacterial activity

A total of 10 discs specimens for each group were created using a split plastic moldswith a 10 mm internal diameter and 2 mm height.The cement was mixedaccording to the manufacturer's recommended ratio of powder to liquid and packed into plastic molds. The Molds will be slightly overfilled, tucked between two sheets of glass, and the extra material will be cut away. After 10 minutes, the specimens will be taken out of the Molds and allowed to set in air for 24 hours at room temperature. The samples will be kept until use in a 37°C, 100% humidity environment.[15]

6.1. Microbial strain and growth media

Streptococcus mutans (EMCC No. 1815) served as the test organism for the antibacterial activity. The Brain Heart Infusion (BHI) broth was used to transfer S. mutans strains from stock culture, and the mixture was then incubated at 37° C for 24 hours. The bacterial growth was measured by the presence of turbidity in the broth after incubation.

6.2. Agar diffusion test

Fresh S. mutans culture was inoculated onto BHI agar plates and evenly distributed with sterile cotton swabs using turbid brain heart infusion broth. To ensure proper sterilization, the prepared GIC specimens were heated for one hour in a hot air oven. The samples were then placed on BHI agar plates with a uniformly-contact bacterial strain and incubated at 37° C for two intervals of 24 and 48 hours. The distance in millimetres (mm) between each specimen and the bacterial growth inhibition zone (I.Z.) was calculated by deducting the specimen's diameter (10 mm) from the average diameter of the zones.

7. Compressive strength (CS) testing

7.1. Specimens preparation

A total of 10 discs specimens for each group were created using a split plastic moldswith a 4 mm internal diameter and 6 mm height.The

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cement was mixed and prepared as mentioned in antibacterial test.

7.2 Compressive strength measurement

The Universal Testing Machine (UTM) (Model LRXplus. Lloyd Instruments Ltd. Fareham, UK)conducted the compressive strength testing at a cross-head speed of 2mm/minute. Before testing, each specimen's diameter and height were measured with a digital calliper. The specimens' flat ends were positioned between the UTM machine plates to apply a gradually increasing compressive load throughout the specimen's long axis. Using software (Nexygen 4.2), the force-displacement curve was drowned. The highest force at fracture that could be recorded was obtained, and compressive strength was measured in MPa. [16]

III. RESULTS

1.Characterization of the nano-solutions

1.1. The FT-IR spectroscopy

The FT-IR analysis revealed the disappearance of certain functional groups and the shift in frequencies of other groups, indicating the formation of chitosan nanoparticles.The characteristic vibrations of sharp O-H stretching groups in both samples indicated the presence of alcoholic groups in the fundamental framework of both structures, revealing the presence of absorption bands at ν = 3373 and 3396 cm-1.a weak absorption band for the amino group was observed at ν = 3255 cm 1; this band disappeared when the chitosan nanoparticles were analysed. This suggested that chitosan's amino group played a role in the nanoparticles' formation.

1.2. X-ray diffraction analysis (XRD)

An XRD pattern was considered to confirm the crystalline nature of the chitosan nanoparticles that were purely synthesizedThe Bragg's reflections plane of face-centered cubic chitosan showed different noticeable diffraction peaks at 8.6579°, 18.4569°, 22.5126°, 23.3745°, 31.6806°, 33.7907°.indicating the high degree of crystallinity of the material. Comparable studies for XRD orientation of chitosan nanoparticles were previously reported by Divyaet al.[17]

1.3. Scan electron microscope (SEM)

The aggregated chitosan nanoparticles with the nano-sized particles were confirmed, 0.022 to 0.036 µm. The chitosan nanoparticles' available SEM micrograph showed that the sample had a smoother, more uniform size and sheet-like structure.

2. Antibacterial activity

Intragroup comparison (comparison between before and after thermocycling):

There was a significant decrease in antibacterial activity in all groups after thermocycling as (P<0.05).

Intergroup comparison (comparison between different groups):

Before thermocycling: There was a significant difference between all groups as group 1 (3.56 ± 2.55) showed the lowest antibacterial activity, while group 3 (10.74 ± 0.46) and group 4 (11.84 ± 0.34) showed the highest antibacterial activity with insignificant difference between them as (P<0.05).

After thermocycling: There was a significant difference between all groups as group 1 (0.9 ± 0.38) showed the lowest antibacterial activity, while group 4 (6.18 ± 0.53) showed the highest antibacterial activity as (P<0.05).

3. Compressive strength:

Intragroupcomparison (comparison between before and after thermocycling):

There was a significant increase in compressive strength in all groups after thermocycling as (P<0.05).

Intergroup comparison (comparison between different groups):

Before thermocycling: There was a significant difference betweendifferent groups as (P <0.05). group 1 (177.66 ± 2.55) showed the lowest compressive strength, while group 3 (224.11 ± 4.56) and group 4 (219.04 ± 4.89) showed the highest compressive strength as (P<0.05).

After thermocycling: There was a significant difference between different groups as (P <0.05). group 1 (191.57 ± 5.27) showed the lowest compressive strength, while group 3 (235.12 ± 4.08) and group 4 (229.2 ± 3.87) showed the highest compressive strength with as (P<0.05).

IV. DISCUSSION

The antibacterial activity testing proved the modified cements' antibacterial efficacy against Streptococcus mutans. Because S. mutans produce an acid tolerance response that enables them to live and grow in low-pH environments, they are the most cariogenic bacteria. The most frequent cause of dental caries is the S. mutans bacteria, which is also responsible for producing acids and fermenting carbohydrates. [18]

The study's findings demonstrated that, depending on the CH volume content, altering the GIC commercial PAA liquid with CH solutions improved its antibacterial capabilities against S. mutans. Although the exact mechanisms of

chitosan's antimicrobial action are still unknown, it is thought to work by altering the cellular membrane's electric potential to affect the microorganism's cellular wall.[19, 20] It was suggested that the microbial cells could become agglutinated due to the protonated amino groups of chitosan binding to the anionic groups of the microorganisms.[21, 22]

Other proposed mechanisms that could account for the noteworthy antibacterial effect of CH-modified GIC as reported in this study include chitosan's promotion of Ca^{++} displacement from the anionic sites of the membrane, which damages cells[20] and the potential for rupture and loss of significant intracellular components due to the interaction between the positive load of the chitosan and the negative load of the microbial cell wallLow molecular weight and viscosity chitosan may be able to enter bacterial cells and attach itself to the DNA of the microorganism, blocking transcription and translation.[19, 23]The bactericidal effects of CH-modified GICs against S. mutans are strongly supported by statistical significance found with an increase in CH volume content.Additionally, prior research revealed a decrease in the proportion of S. mutans and a higher fluoride concentration in dental plaque on or near GIC restorations.[24] Therefore, it is important to consider that the current study should take into account the potential antibacterial effect of the fluoride ions that were released from the CH-modified and unmodified GIC. Fluoride release was previously reported to be catalysed by chitosan modification of GIC.[25]

The antimicrobial activity of both conventional and modified glass ionomer significantly decreased with aging. This could be attributed to the decrease in fluoride release with thermocycling as well as the initial decrease in high acidity of freshly mixed glass ionomer.[26, 27]As GIC matures over time, CH may also be affected because of the ongoing ionic crosslinking of the gel matrix, which causes the modified cement's antibacterial activity to diminish. [28, 29]

In nature, chitosan (CS) is the only polysaccharide that has a positive charge.[30]Three functional groups are present in chitosan: a primary hydroxyl group, a secondary hydroxyl group, and an amino/acetamido group.[31]The primary reactive group is the amino group, which is connected to their biological activities and chelation.[31]The amino group (NH_2) of the chitosan can react with the carboxyl groups (COOH) of the polyacrylic acid in the GIC fluid when the chitosan is added, creating polymer networks[32, 33]modifying the GIC's initial mechanical and biological characteristics.

In the study adding chitosan to glass ionomer cement lead to significant increase in compressive strength. Adding CS to GIC can greatly increase the compressive strengthbecause of chitosan and polyacrylic acid reactions which can produce polymer networks that lower the interfacial tension between the GIC components, hence enhancing the mechanical characteristics.[10]Furthermore, some researchers have suggested that chitosan functions as a binder to strengthen the binding of GIC components, enhancing the GIC's resistance to outside forces.[1, 32, 34]The mechanical performance deteriorated and eventually reached a level comparable to commercial GIR when the CH content was increased. This effect could be explained that some of chitosan chains segregate, interacting with each other, but not with Polyacrylic acid. [25]

It has been demonstrated that varying the duration of storage of glass ionomers in saline, saliva, water, or fruit juice can significantly alter the material's physical characteristics, frequently leading to improvements in those properties.[35, 36]These findings demonstrated that glass ionomers continue to set up to three months after the restoration is placed, increasing the material's compressive strength.[36]Many distinct mechanisms, including the formation of a phosphate network or the reduction of porosity in glass ionomer over time, have been proposed as explanations for this increase in compressive strength with age. It is known that glass ionomers get stronger and more brittle with age, regardless of the mechanism.[37]

V. CONCLUSION

Addingchitosan to glass ionomer cement leads to significant increase in antibacterial effect without negative impact on compressive strength of glass ionomer cement.

Limitations

1- Effect of nanochitosan on different types of bacteria.
2- Using different types of storage medias
3- Further studies for relation between GIC gel crosslinking and antibacterial efficiency

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