

Research Article

Antimicrobial leakage assessment of glass ionomer restoration enhanced by indium oxide nanoparticles suspension compared to conventional glass ionomer restoration *in vitro* study

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Abstract: Background: Nanoparticles in restorative materials have enhanced oral hygiene and cleanliness by increasing the quality and usefulness of dental restorations, due to small sizes, high surface-area-to-mass ratios, antimicrobial activity and strong chemical reactivity. This study aimed to evaluate the microbial leakage of laser prepared indium oxide nanoparticles added to glass ionomer filling compared to conventional glass ionomer fillings in permanent teeth. Materials and methods: Indium oxide nanoparticles were prepared using laser ablation in liquid, their properties were examined by field emission scanning electron microscopy, Ultraviolet Visible spectrophotometer and mass concentration. A predetermined amount of glass ionomer has been added to attain a homogenous content. Leakage evaluation was performed using a microbiological infiltration technique with *Streptococcus mutans* as an indication. Results: revealed that spherical as well as uniform nanoparticles, with particle size less than 10nm. The absorption spectra have peaks between (260-300) nm for differently prepared samples. It was found that prolonged time for microleakage with increased nanoparticles concentration with statistically highly significant difference ($P < 0.001$), using the Mantel-Cox log-rank test and the Kaplan-Meier plot. Conclusion: it can be concluded that adding Indium oxide nanoparticles to glass ionomer restoration makes it more effective in preventing microbiological leakage. It was considered an alternative to conventional glass ionomer restoration.

Keywords: Dental leakage, *Streptococcus mutans*, glass ionomer restoration, indium oxide nanoparticles suspension solutions

Introduction

Treatment of cervical lesions resulting from early childhood decay, adult dental decay, or erosion, has encountered a special challenge for pediatrics or restorative dentistry, this is because dental fillings need to adhere to the dentin or cementum in the cervical margin of a class V cavity preparation ^(1,2). In the mouth, secondary caries is a condition that develops during restoration and causes the failure of restorative materials. Oral *Streptococcus mutans*, contribute to the formation of dental plaque and is one of the causes of secondary caries. It must be stopped with the use of appropriate restorative material to prevent dental caries and boost tooth resistance to secondary caries ^(3,4). Due to the chemical adherence of glass ionomer restorations to the tooth structure, fluoride releases, almost acceptable esthetics, and virtually thermal expansion to tooth coefficient, glass ionomers restorations are appropriate for cervical lesions. Glass ionomer restoration is produced when an acidic polymer reacts with a basic glass in the presence of water ⁽⁵⁾. Due to their chemical binding to dental tissue, adhesion to base metals, anticariogenic properties

brought on by fluoride

release, thermal compatibility with tooth structure, biocompatibility, and minimal side effects, glass ionomer restorations make great adhesive as well as restorative material ⁽⁶⁾. A key therapeutic advantage of glass-ionomers is their capacity to attach to the tooth's structure. Glass-ionomers are formed from poly (acrylic acid) or related polymers, and this substance has been found to increase adhesion, because of the adherence of the zinc polycarboxylate cement ⁽⁷⁾. One of the most important aspects of the adhesion process is the interaction of acids or acidic monomers with hydroxyapatite. Adhesion is crucial because it helps to keep glass ionomer cement inside the tooth and reduces or eliminates marginal leakage, which makes it impossible for harmful microorganisms to access the area under the restoration and cause decay. Despite their positive characteristics, they have considerable porosity and internal fissures, which result in poor mechanical qualities and the development of secondary caries ^(7,8). Since the beginning of dentistry, numerous studies have been done to address the problem of microleakage with dental restorations, especially for class V lesions; yet, no restorative materials or techniques can eliminate microleakage ⁽⁹⁾. Because of the small particle size of nanomaterials, high surface area, and antimicrobial activities, which can interact harmoniously with the micro-cracks on the GI surface during glass ionomer repair ^(10, 11, 12), which aims to enhance the problems of brittle restoration, protection against tooth decay, prevention of acidification, reducing the sensitivity level, as well as other characteristics. Against both Gram-positive and Gram-negative bacteria, nanoparticles have demonstrated a broad range of antimicrobial properties. They can more easily interact with bacterial membranes, because of their small size ^(13,14). Among its many uses, indium oxide nanoparticles (In₂O₃) are essential antibacterial properties ⁽¹⁴⁾.

Due to the low cost, great efficiency, and accuracy, pulsed laser ablation in liquid is one of the most often used dominant technologies to create homogeneous and stable NPs solutions. Recent research have demonstrated the effectiveness of metal nanoparticles as antibacterial agents. They enable the neutralization of the bacterial membrane's surface and reactive oxygen species (ROS) inhibits the antioxidant defense system ^(15,16). The coronal microbial penetration method for class V restoration leakage was chosen over other methods for assessing anti-microbial leakage of glass ionomer restoration, such as the electrochemical method, fluid filtration, and colour dye penetration method. So even though brain heart infusion broth (BHI-B) doesn't precisely replicate environments in the oral cavity. This procedure was quantitative (amount of days until turbidity may be noticed) despite its extended duration ⁽¹⁷⁾. This technique was introduced by Krausin ⁽¹⁸⁾ and Seltzer ⁽¹⁹⁾ and recently was used by Nematollahi et al ⁽²⁰⁾.

So far, no previous foreign study has been reported to evaluate the antimicrobial microleakage of indium oxide nanoparticles enhanced glass ionomer filling in comparison to conventional glass ionomer filling, for this reason, this study was conducted.

Materials and Methods

Preparation of indium oxide nanoparticles

The indium bulk (99.9%) high-purity substrate with a thickness 2 mm was used to fabricate indium oxide-colloidal. A convex lens approximately 5 cm diameter was employed to concentrate the beam of a Nd:YAG laser of 1064 nm (type HUAFEI) at a 6 cm distance from the top of such an indium target. 5 ml of pure water was contained in the glass chamber where the target was placed. The maximum energy of the laser pulse reached 600 mJ, with a pulse repetition rate of 1 Hz and period of 10 ns. Using a set laser energy and varying the number of pulses (125, 250, 500 pulses) used for every sample, the nanoparticles have been produced. According to the laser-plume shooting technique, during the laser pulse attached target surface, an amount of ablation can be seen and a sound can be heard ⁽¹⁵⁾. Nanoparticle characterizations are accomplished by mass concentration, field emission scanning electron microscope (FESEM) and Ultraviolet Visible spectrophotometer (UV).

Preparation of Indium oxide nanoparticles– Glass Ionomer Composite

The powder/liquid (P/L) proportion was 1:0.5:0.5 (one scooped powder and 0.5 drop of liquid + 0.5 drops of InONPs) while mixing the glass ionomer (Riva, Australia) ⁽²²⁾.

A constant quantity of glass ionomer was added after varying the laser pulses (125, 250, and 500) to form 3 groups with variable concentrations of indium nanomaterials.

Preparation of teeth

The Teeth sample in this study consisted of fifty maxillary first premolars, randomly distributed into five groups, first was the control positive: glass ionomer without NPs and the second control negative: teeth without cavity preparation, the rests were glass ionomer with NPs. Those teeth have been taken from individuals from numerous Hilla city center private orthodontics clinics. The teeth were cleaned with deionized water, and then each tooth was cleaned with acetone to remove any derbies. After that, 20 ml of deionized water with 0.1% thymol was applied to it for storage. Until used, the sample was then maintained in a refrigerator at 4 °C. Crowns were cleaned and polished with non-fluoridated pumice and deionized water using a typical handpiece with a gently rotating rubber cap. To get rid of any greasy pumice on the tooth enamel; teeth were cleaned using cotton dipped in acetone ⁽²⁵⁾. A class V cavity (4*4mm) was created in the buccal surface of every tooth along the cervical border using a diamond bur with a 1mm diameter and periodontal probes to gauge the depth. A 0.6 mm thin fissure bur was used to create a channel leading toward the pulp cavity. This channel's entry was then sealed with a thin layer of blue inlay wax, preventing the entry of any restorative materials. The same operator carried out all of the cleaning and restoration tasks. The inlay wax washes out through the pulp chamber using boiling water. The root portion of each specimen including the capillary tube embed in one end of a plastic microtube in which the microtube reaches 2 mm under the gingival border of class V restoration, the capillary tube stabilizes with the epoxy resin glue. On the other side, glass tubes, as the container of brain–heart infusion (BHI) sterile BHI were poured into a glass tube and the crown occlusal portion of the specimen was inserted through a foramen into the head of the glass tube containing sterile BHI broth until the broth will be at least 2.0 mm above the gingival border of the class V cavity then incubated at 37°C for 40 days, and 0.5 ml of BHI broth containing approximately 1.5×10^8 *Streptococcus mutans* was introduced with a sterile syringe to reach through the capillary tube under the restoration at daily intervals. Bacteria migrate through the capillary tube and the channel established between the pulp chamber and the tooth-restoration interface. The daily examination was performed to the different groups for bacterial leakage, as evidenced by turbidity in BHI broth surrounding the tooth-restoration interface. The verification that the cause of turbidity was *S. mutans*, all turbid samples of *S. mutans* was identified and confirmed by Mitis-salivarius bacitracin agar and Gram staining under a microscope, figure (1).

Media preparation for culturing

Mitis-Salivarius Bacitracin Agar (MSB Agar)

This selective media for cultivation of *Streptococci mutans* was prepared by the addition of selective agents: bacitracin and sucrose, at the optimal levels determined to the Mitis-Salivarius Agar (MSA) composition to be effective in inhibiting bacteria other than *mutans streptococci*, since the relative resistance of *mutans streptococci* to a high concentration of both sucrose and bacitracin had been reported. MSA was prepared according to the manufacturer's direction by dissolving 90 gm of MSA in one liter of distilled water, sucrose was added to obtain a concentration of 150 gm/L before sterilization of the agar medium. The pH was adjusted to 7.2 using HCL or NaOH, sterilized by autoclave, and left to cool till 45 C°, thereafter bacitracin antibiotic solution was added under a septic condition. Bacitracin stock solution was prepared by dissolving 0.364 gm of powder in 100 ml of sterile de-ionized water, this will provide a concentration of 200 IU /L (1 unit of bacitracin=0.0182 mg). The solution was sterilized by a millipore filter (0.22 µm); a new fresh solution was prepared every 2-3 weeks, then the media was poured into plates, left to cool for 24 hours at room temperature then stored in a refrigerator until use ⁽²³⁾.

Brain Heart Infusion Broth (BHI-B)

To prepare this media, its manufacturers recommended dissolving 37gm in one liter of deionized water. The mixture was then allowed to cool to room temperature before being placed in the refrigerator till usage. The pH was adjusted to 7.2 and then it was autoclaved to 121 C° at 15 pounds per square inch for about fifteen min.

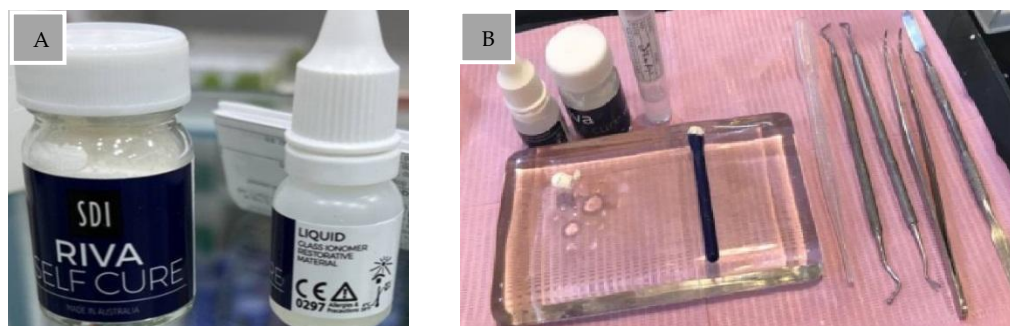


Figure 1: A-Riva self-cure restoration and B-The glass ionomer-nanoparticles restorative enhancement preparation.

Statistical analysis

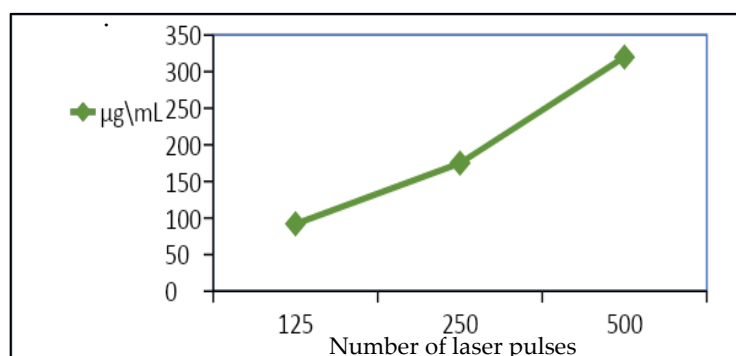
Statistical Package for Social Research was used for descriptive analysis, and presentation (SPSS ver-sion -22, Chicago, Illinois, USA). A Kaplan-Meier curve for such a quantitative variable includes the minimum, maximum, mean, standard deviation (SD), and standard error. Repeated Action To use the Bonferroni posthoc test, an ANOVA evaluates the mean differences in K-related means between groups. Kaplan-Meier curve examines the variable's survival distribution that uses the Mantel-Cox log-rank test and several pairwise comparisons with the Bonferroni post hoc test. All tests were done at $P < 0.05$.

Results:

The Characteristics of nanoparticles

Figure 2 demonstrates the impact of laser pulses upon this quantity of indium oxide nanoparticles produced using laser ablation on indium bulk with deionized water at (125, 250, 500) pulses at constant laser energies of 3%, 5%, and 9%, respectively. Figure (3) shows field emission scanning electron micrographs (FESEM) of indium oxide NPs produced by laser at various laser pulse lengths. The produced nanoparticles were nearly spherical in appearance as well as uniform in size. With more laser pulses, there are more nanoparticle concentrations. InONPs produced by pulsed laser ablation in liquid was calculated to have ultraviolet-visible absorption spectra spanning the spectral range of (200-1100 nm). Figure (4) shows the optical absorption of colloidal for InONPs samples prepared with various laser pulses and fixed energy to 600 mJ. For various produced samples, the absorption spectra contain peaks between (260-300) nm. Increased pulses were observed to promote absorption.

Figure 2: Mass concentration of indium oxide nanoparticles prepared by PLAL with laser pulses.



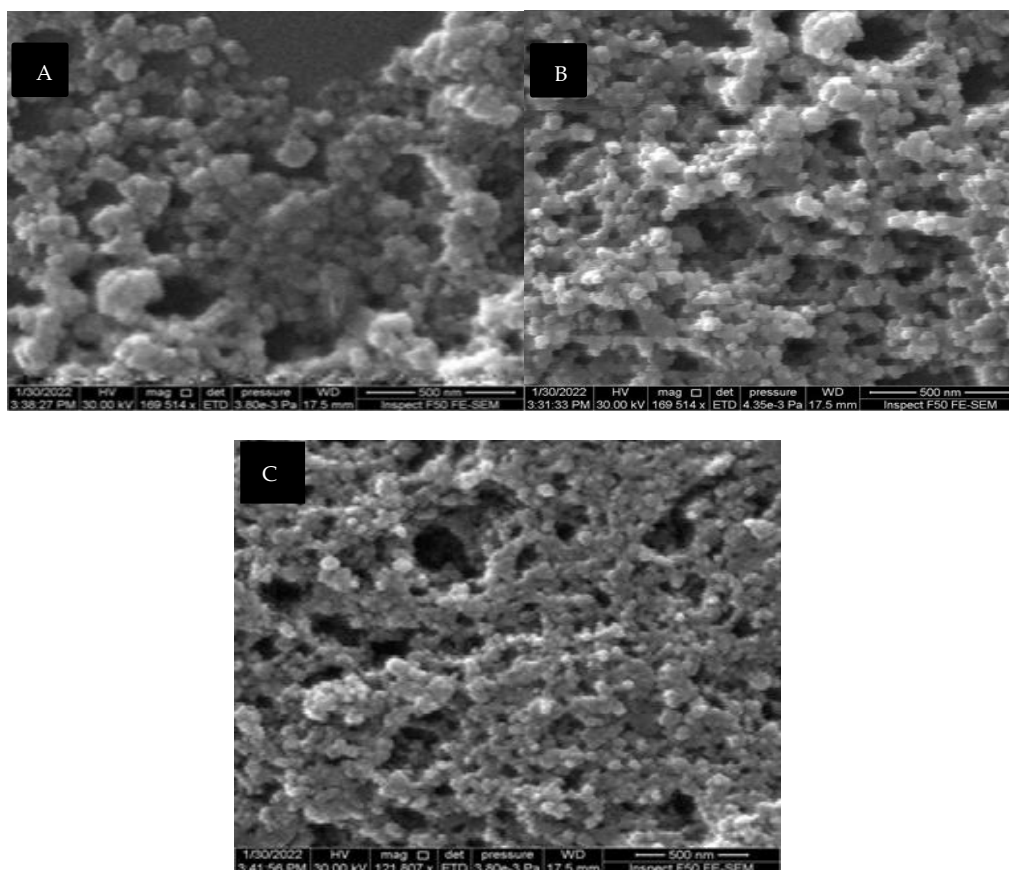


Figure 3: FESEM of indium oxide NPs prepared by laser ablation at different laser pulses. A:125 pulses, B: 250pulses, C: 500 pulses.

Table 1: The crystal size of InONPs prepared with different pulses.

Number of pulses	Grain size (nm)
125	9
250	7
500	6

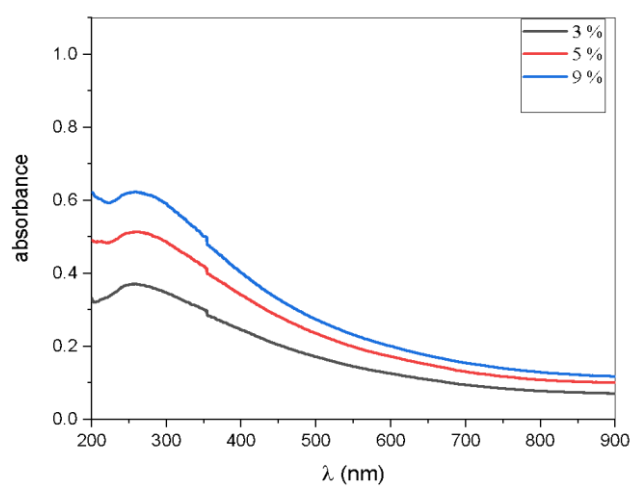


Figure 4: UV- visible absorption spectra of InONPs that prepared by pulsed laser ablation in liquid for different pulses.

Microbial microleakage test of conventional glass ionomer restoration in comparison with glass ionomer restoration enhanced by InONPs: Preparation of the two tubes and tooth with BHI-B in addition to different turbidity after adding *Streptococci mutans* to BHI-B, at different times shown in Figure (5).

Turbidity in figure (6) is used to identify *S. mutans*. On Mitis Salivarius Bacitracin agar media, *Streptococcus mutans* colonies were examined and determined based on their morphological features. *Mutans streptococci* colonies had a spherical or ovoid shape with and an elevated or measured 1-2 mm in diameter. They also adhered well to the agar surface. *Streptococci mutans* cells were gram-positive, spherical or ovoid, and grouped in short or medium-length non-spore producing chains, according to microscopic analysis using Gram stain. Table 2 illustrates the mean day, minimum and maximum days for five groups: negative control, positive control (conventional glass ionomer), glass ionomer restoration with 3% InONPs, glass ionomer restoration with 5% InONPs and glass ionomer restoration with 9% InONPs. During the first day of the study, the positive control exhibits tiny leakage, whereas the negative control shows none (40 days). All studied glass ionomer repair specimens containing nanomaterials showed microleakage after forty days. The result showed the increased time for microleakage occurrence with increased nanoparticle concentration.

The Kaplan-Meier plot of the minimum microleakage occurrence rates across the forty days of the study is shown in Figure 7. According to this figure, the group of conventional glass ionomer restoration had the greatest minimal days of microleakage rate, whereas the group of glass ionomer containing 9% InONPs had the least.

The Mantel-Cox log-rank test of minimum time for microleakage multiple pairwise comparisons between the four groups: conventional glass ionomer restoration, GI-3%InONPs, GI-5%InONPs and GI-9%InONPs, in table 3 It was found statistically significant differences between conventional GI and GI-3%NPs, between conventional GI and GI-5%NPs, between conventional and GI-9%NPs, between GI-3%NPs and GI-9%NPs, and between GI- 5%NPs and GI-9%NPs. However the microleakage rate was lower in GI-5%NPs than GI-3%NPs, but with no significant differences between them.

The Kaplan-Meier plot of the maximum time for the rates of microbiological microleakage occurring in each of the five groups throughout the investigation's 40 days is shown in Figure 8. According to this figure, the group of GI-9%NPs seemed to have the highest recorded days for microleakage rate, whereas standard GI had the least.

The Mantel-Cox log-rank test of maximum time for microbial microleakage, multiple pairwise comparisons between the four groups: conventional glass ionomer restoration, GI-3%InONPs, GI-5%InONPs and GI-9%InONPs in table 4. It was found statistically significant differences between all groups: conventional GI and GI-3%NPs, between conventional GI and GI-5%NPs, between conventional and GI-9%NPs, between GI-3%NPs and GI-5%NPs, between GI-3%NPs and GI-9%NPs, and between GI- 5%NPs and GI-9%NPs.

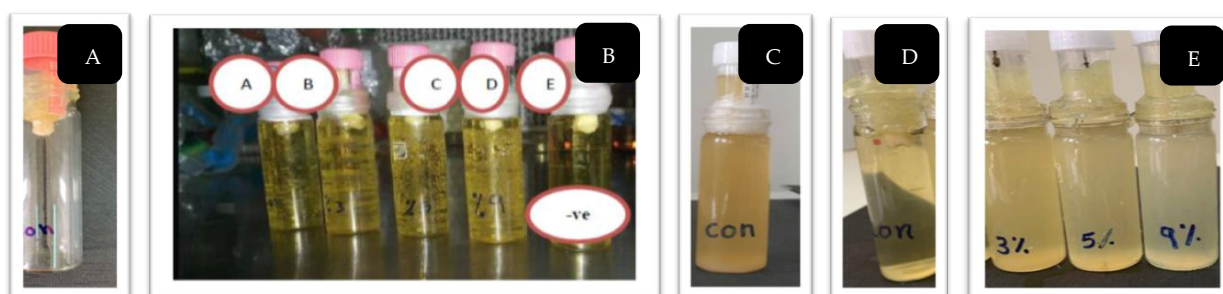


Figure 5: A: illustrates the preparation of the tooth with the two tubes B: Preparation of the two tubes and tooth after putting brain heart infusion, a. Conventional GI, b. sample 1 (3%) NPs+GI, c. Sample 2 (5%)NPs+GI, d. Sample 3 (9%)NPs+GI e. Negative control. C,D,E: after putting *S mutans* in brain heart infusion, C: -ve control, D: + control. E: it showed different turbidity at different times for GI+NPs samples.

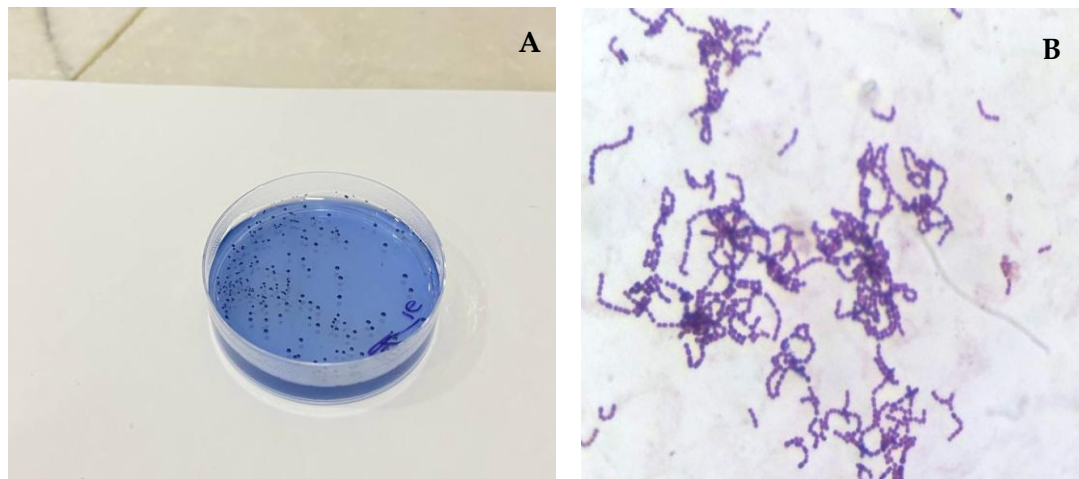


Figure 6: Identification of *S. mutans* from turbidity. A: Mitis-Salivarius Bacitracin agar plate, B: Gram stain.

Table 2: Descriptive statistics of minimum and maximum time (days) microleakage among groups.

Groups		Mean	±SD	±SE	Minimum	Maximum
Minimum	negative control	0.000	0.000	0.000	0.000	0.000
	positive control	1.200	0.422	0.133	1.000	2.000
	GIC + 3%NPs	2.900	0.876	0.277	2.000	4.000
	GIC + 5%NPs	3.200	0.632	0.200	2.000	4.000
	GIC + 9%NPs	7.300	0.823	0.260	6.000	9.000
Maximum	negative control	0.000	0.000	0.000	0.000	0.000
	positive control	12.600	0.843	0.267	12.000	14.000
	GIC + 3%NPs	21.500	1.269	0.401	19.000	23.000
	GIC + 5%NPs	33.800	2.300	0.727	29.000	36.000
	GIC + 9%NPs	39.400	0.699	0.221	38.000	40.000

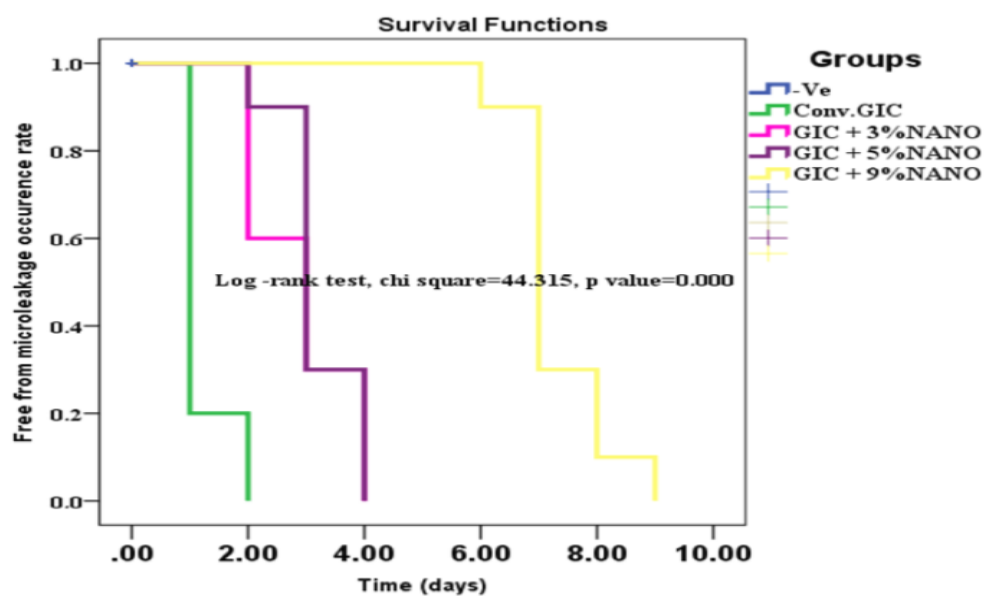
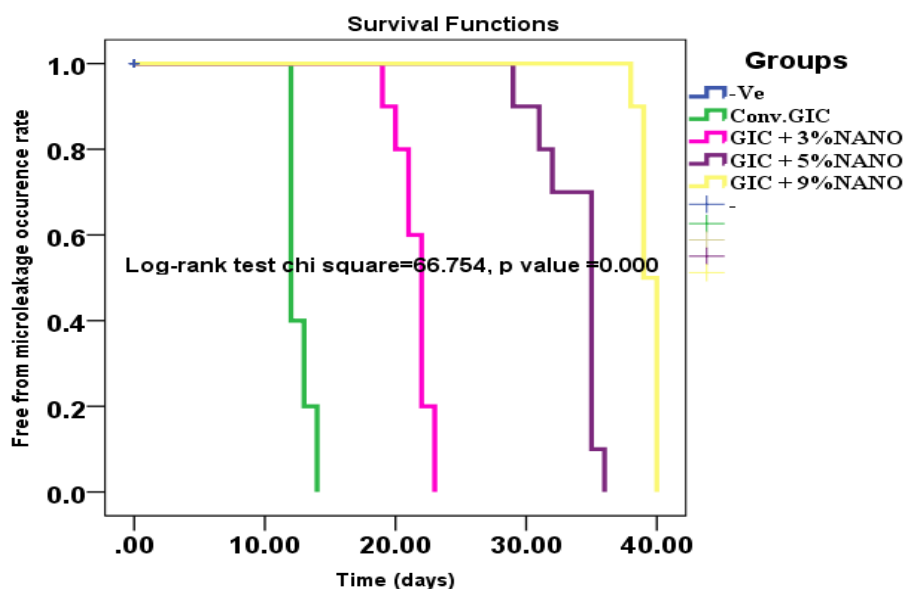


Figure 7: Figure Survival function of minimum time microbial microleakage

Table 3: Multiple pairwise comparisons of minimum time (days) among groups by Bonferonni

Log Rank (Mantel-Cox)	Groups	GI + 3%NPs P value	GI + 5%NPs P value	GI + 9%NPs P value
	Conventional GI	0.00014	0.0001	0.0001
	GI + 3%NPs	-	0.51519 NS	0.0001
	GI + 5%NPs	-	-	0.0001

**Figure 8:** Survival function of maximum time microbial microleakage.**Table 4:** Multiple pairwise comparisons of maximum time (days) among groups by Bonferonni.

Log Rank (Mantel-Cox)	Groups	GIC + 3%NPs P value	GIC + 5%NPs P value	GIC + 9%NPs P value
	Conv.GIC	0.0001	0.0001	0.0001
	GIC + 3%NPs	-	0.0001	0.0001
	GIC + 5%NPs	-	-	0.0001

Discussion

The preparation of indium oxide nanoparticles suspension method by laser ablation was done in this study, with different pulses, which was used by a previous study ⁽¹⁵⁾. Pulsed laser ablation in liquid is presently recognized as an attractive technique for creating nanomaterials because it produces a high-purity final result through a clean and straightforward chemical Process ⁽¹⁹⁾. By adjusting the pulse duration or the laser's energy, colloidal NPs allow for fine-tuning of such distribution. Additionally, the technique works at standard temperature and pressure settings ⁽²⁸⁾.

For every sample, a different number of pulses with fixed laser energy were used to create the indium oxide nanoparticles. Owing to the laser-plume shoot technique, an amount of ablation can be seen during the laser pulse attached target surface and a sound can be heard ⁽¹⁵⁾. By estimating the change in mass concentration between before and after laser ablation, the amount of NPs in the suspension solution that has been ablated

increases with increasing laser pulses ⁽²⁹⁾. This outcome is explained by laser-material interaction with bulk by a series of laser pulses, which allows for greater heat transfer toward the target, raising its surface temperature and causing a sizable quantity of surface targets to evaporate ⁽³⁰⁾. This was supported by another Iraqi study ⁽³¹⁾.

FESEM micrograph displayed the resulting nanoparticles, they were almost spherical in shape and homogeneous, this was confirmed by previous studies ^(32,33), despite small aggregation in high pulses because of high laser energy due to the electrostatic attractive force between NPs. The concentration of NPs increase with increasing laser pulse. The absorption spectra have peaks between (260-300) nm of InONPS for different pulses prepared with UV absorption spectroscopy. The absorption spectra peaks of this study were nearly the same as the previous study ⁽¹¹⁾. Amount of NPs in the colloidal increases with increasing laser pulses ⁽²⁷⁾. The deposition of nanoparticles that stayed in the liquid surrounding the target, created what is known as a colloidal solution, which has been identified to cause a rise in absorbance.

This interaction of laser energy is lengthened by the free electrons absorbing its energy, which lowers the intensity of the incident laser light ⁽³⁴⁾. The coronal microbiological infiltration approach for class V restoration leakage was found to be superior to the current investigation methods such as the electrochemical method, fluid filtration and color dye penetration method for assessing anti-microbial leakage of glass ionomer because this is method is nondestructive, reassessment of leakage is possible, quantitative (number of days before turbidity can be observed), despite long duration and BHI-B doesn't wholly mimic oral cavity condition⁽¹⁷⁾. Because Riva Self Cure vitreous ionomer is still a biologically active compound that aims to protect teeth against caries, it is the perfect restorative material for use in minimally invasive dentistry, because the preservation of the natural tooth structure, extensive cavities and lower incisions were not required⁽⁷⁾. It showed the increased time of microleakage occurrence with increased nanoparticle concentration with statistically highly significant differences in comparison to conventional GI. This is due to elevated concentrations of therapy ions published at low filler content improved or at least did not compromise the mechanical characteristics of the used adhesive, its ability to transfer with the bonding material among the collagen fibrils and small dentinal tubules, which improved adhesion. The small particle size of indium oxide nanoparticles and the high surface area of the nanoparticles incorporated in the adhesive resin offered main advantages ⁽³⁵⁾.

There was no previous study to compare with, but the present result confirmed the effects of other types of nanoparticles in a previous study ⁽²⁰⁾. In addition to the antibacterial actions of InONPs, this metal oxide damages the structure of bacterial cell membranes and controls the activity of some membranous enzymes of gram-positive and negative bacteria and releases reactive oxygen species (ROS) that lead to bacterial cell death. This result was supported by previous studies ^(11,16) and other metal nanoparticles explanation studies ^(36,37), which revealed that antibacterial activity increased by increasing the concentration of nanoparticles. All of the restorations in this investigation demonstrated a bacterial leakage by 40 days. This finding is corroborated by other investigations and can be related to the restoration being exposed daily to high concentrations of new germs that eventually passed through the tooth-restoration interface ^(17,20).

Conclusion

The results of the current study revealed that a positive control (ordinary glass ionomer) exhibited microleakage on the first day of the examination, while the negative control exhibited none all through the duration of the examination (40 days). All of the glass ionomer repair specimens with nanoparticles showed microleakage after forty days. The outcome demonstrated a statistically highly significant difference between a rise in the concentration of nanoparticles and an increase in the duration for microleakage to occur. Indium oxide nanoparticles incorporated into a new composite of glass ionomer restoration can therefore be seen as a better antibacterial leakage as well as a potential replacement for current glass ionomer restoration.

Conflicts of interest:

The authors have no conflicts of interest to declare.

Author contributions

DMH; study conception and design. DMH; data collection. DMH and MJA.; Methodology. MJA, and BHH; statistical analysis and interpretation of results. BHA, MJA and BB; original draft manuscript preparation. All authors reviewed the results and approved the final version of the manuscript to be published.

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Informed consent

The study was approved by the College of Dentistry/University of Baghdad's local ethics commission (reference number 561 in April 17-2022).

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تقييم التسرب الميكروبي لحشوه glass ionomer المعززة بتعليق جزيئات أكسيد الإندسيوم النانوية مقارنة بالحشوه glass ionomer التقليدية (في المختبر)

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الخلفية: عززت الجسيمات النانوية في المواد التصالحية صحة الفم ونظافته من خلال زيادة جودة وفائدة حشوات الأسنان ، بسبب الأحجام الصغيرة ، وارتفاع نسبة مساحة السطح إلى الكتلة ، والنشاط المضاد للميكروبات ، والتفاعل الكيميائي القوي. الهدف من هذه الدراسة هو تقييم التسرب الميكروبي لجزيئات أكسيد الإندسيوم النانوية المحضرة بالليزر المضاف إلى حشوة الـ glass ionomer مقارنة بحشوات glass ionomer التقليدية في الأسنان الدائمة.

المواد وطرق العمل: تم تحضير الجسيمات النانوية من أكسيد الإندسيوم باستخدام الاجتثاث بالليزر في السائل ، وفحصت خصائصها بالمجهر الإلكتروني الماسح للانبعثات الميدانية ، ومقياس الطيف الضوئي المرئي فوق البنفسجي والتركيز الكتلي. تمت إضافة حشوة الـ glass ionomer بالكمية المحسوبة للحصول على تركيبة متجانسة. تم اختبار طريقة الاختراق الميكروبي باستخدام Streptococcus mutans كمؤشر لتقييم التسرب.

النتائج: أظهرت النتائج وجود جسيمات نانوية كروية ومتجانسة ، بحجم جسيم أقل من 10 نانومتر ، كان لأطياف الامتصاص ذروتها بين (260-300) نانومتر للعينات المحضرة المختلفة. تم العثور على إطالة زمن التسرب المجهر مع زيادة تركيز الجسيمات النانوية مع وجود فروق ذات دلالة إحصائية عالية ($P > 0.001$) ، باستخدام اختبار رتبة Mantel Cox و مخطط Kaplan-Meier

الاستنتاج: يمكن اعتبار الجسيمات النانوية المضافة من أكسيد الإندسيوم إلى حشوة الـ glass ionomer بمثابة مضادات للميكروبات بتسريب أقل ، وقد تم اعتباره بديلاً لحشوة الـ glass ionomer التقليدية.