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Nano-Filled Dental Composite's Antimicrobial Effectiveness

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Abstract

Dental composites packed with nanoparticles are frequently utilized in restorative dentistry because of their mechanical and cosmetic qualities. But the development of secondary caries is still a concern due to their vulnerability to bacterial colonization, especially by *Streptococcus mutans*. This study aimed to evaluate and compare the antibacterial efficiency of three commercially available nano-filled dental composites SDI, MANI, and EDUFILL. 30 composite blocks (10 blocks per material, each measuring (10 mm × 3 mm) were prepared. All samples were placed in agar medium containing *Streptococcus mutans* and incubated at 37°C for 48 hours. After incubation, the dimensions of the samples were measured following bacterial exposure. Differences in bacterial adhesion were observed among the three composite types. The results indicate varying susceptibility of the composites to colonization by *Streptococcus mutans*, with certain materials showing higher bacterial retention. The study highlights that not all nano-filled dental composites exhibit the same antibacterial performance. Identifying the composite with the lowest resistance to S. mutans may guide clinicians in selecting materials less prone to bacterial colonization and secondary caries.

Introduction

The preferred method for restoring posterior teeth is now direct restoration using resin-based composite. However, compared to amalgam, resin-based composite restorations have higher failure rates; the most common causes of failure are fracture and secondary caries [1-3].

The integrity of the repair and long-term clinical results depend heavily on durable marginal sealing. However, the shrinkage of resin composites caused by polymerization creates contraction strains at the adhesive interface, which can lead to secondary caries, cuspal deflection, enamel microcracks, and microleakage [2-5].

Microhybrid, microfilled, and flowing polymerization shrinkage ranged from 0.6% to 1.4%, 2% to 3%, and 2.4% to 2.5%, respectively [6]. The organic matrix composition [7], the proportion and makeup of inorganic fillers [8], the degree of organic matrix conversion [9], and viscoelastic behavior all influence the polymerization shrinkage of resin-based composites.

The addition of nanofillers to resin-based composites fulfills numerous advantages. Because of their nanoscale (<400–800 nm) and surface area to volume ratio [6], nanofillers produce a very transparent material. Additionally, when the nanofillers' sizes come up to those of the polymer molecules, a contact in the molecular range takes place in the resin matrix, lowering the polymerization stresses and volumetric shrinkage.

Additionally, it has been suggested that adding ion-leaching fillers, including metal oxides, is advantageous for further remineralization and antibacterial action [10,11]. Among the nanometal oxides employed, magnesium oxide (MgO) nanoparticles (NPs) arose and gained popularity because of their decreased toxicity, biocompatibility, and biodegradable byproducts [12]. MgO NP is a potent antimicrobial agent that has recently been used into dental materials to enhance their antibacterial qualities [10,14] and composite resin [13]. MgO NPs' propensity to cluster, however, limits their application in dental materials [15-17].

MgO NPs are kept from clumping together by coating them with the polymer zein.

The pharmaceutical sector uses zein, a pure protein polymer [18]. By balancing their aggregation, it reduces the hydrophobicity of the NPs [19].

The use of protective materials is essential in modern dentistry, even if the patient's caries risk is a major factor in recurring decay. Therefore, adding antimicrobial zein-coated

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magnesium oxide nanoparticles (zMgO NPs) may help improve the adaptability of flowable composites, reduce their marginal leakage, and infer their antibacterial properties by preventing secondary caries and enhancing their durability. Dental caries is still one of the most common chronic illnesses in the world, affecting people of all ages and posing a serious threat to public health [20].

It is a complex illness brought on by the interplay of oral microbes, dietary practices, and host factors [20,21].

The fermentation of dietary carbohydrates by dental bacteria, which produces organic acids that demineralize enamel and dentin, is the main mechanism of caries formation [21,22]. A complex microbial ecology made up of many bacterial species arranged inside dental biofilm is seen in the oral cavity [23,24].

These microorganisms and the host are normally in balance, but when this equilibrium is upset, cariogenic bacteria—which have acidogenic and aciduric qualities—proliferate and accelerate the development of dental caries [23,25]. Resin-based composite materials are popular in restorative dentistry because of their good mechanical performance and great cosmetic qualities [21].

Despite these benefits, bacterial colonization at the tooth–restoration interface may result in subsequent caries and restoration failure because common composite resins lack inherent antibacterial qualities [26, 27]. As a result, assessing restorative materials' antimicrobial efficacy has grown in importance in dentistry research [26].

Although many sophisticated materials, including Tetric EvoCeram and Filtek Z350 XT, have been produced with better physical and esthetic qualities, most of these materials still lack significant antibacterial activity until they are changed [26,27].

On the other hand, through ion release and environmental alteration, bioactive materials such as ACTIVA BioACTIVE Restorative may exhibit indirect antibacterial properties [26].

Better esthetic outcomes and a smoother surface finish are made possible by the small particle size. Furthermore, enhanced marginal adaptability and decreased polymerization shrinkage are demonstrated by nano-filled composites, which may contribute to a decrease in microleakage and an extension of repair lifetime [6,28].

Southern Dental Industries manufactures a restorative material called SDI Nano Dental Composite. It has nanoscale fillers that improve mechanical durability, polish retention, and surface smoothness [6,28].

For restorative dental operations, EDU FILL Nano Dental Composite is a light-cured nanohybrid composite resin. It enhances hardness, wear resistance, and polishability by incorporating nanoscale fillers into a resin matrix [6,28,29].

Numerous bacterial species that vary in their pathogenicity and involvement in the development of disease can be found in the oral environment. Some of these microorganisms are directly linked to the development of dental caries, whereas others are linked to deep lesions, biofilm formation, or chronic infections [23,29].

In this setting, *Streptococcus mutans*, *Enterococcus faecalis*, *Staphylococcus intermedius*, and *Enterococcus casseliflavus* are the most often found and clinically significant bacteria. Different facets of oral pathology are represented by these bacteria. Because *Streptococcus mutans* can form biofilms and is acidogenic, it is closely linked to the onset of dental caries [25]. Because *Enterococcus faecalis* is resistant to harsh environmental conditions, it is often associated with deep carious lesions and persistent endodontic infections [29,30]. *Staphylococcus intermedius* and *Enterococcus casseliflavus*, on the other hand, are regarded as opportunistic microorganisms that have little direct role in the development of caries but, in some circumstances, may aid in the creation of biofilms and infection [23]. Thus, a thorough grasp of the prospective clinical performance of restorative materials and their function in avoiding secondary caries and restoration failure can be obtained by examining their antibacterial efficacy against these microorganisms [26,27].

The bacteria chosen for this investigation have various functions in oral pathology. *Enterococcus faecalis* is connected to deep carious lesions and chronic endodontic infections, whereas *Streptococcus mutans* is closely linked to the start of dental caries. On the other hand, *Enterococcus casseliflavus* and *Staphylococcus intermedius* are regarded as opportunistic organisms with little direct participation in the formation of caries. Thus, assessing restorative materials' antibacterial efficacy against various microbes offers a thorough grasp of their potential therapeutic performance.

1. It is commonly acknowledged that *Streptococcus mutans*, a facultative anaerobic Gram-positive bacterium, is the main cause of dental caries. It is a typical oral cavity resident and is essential to the development of dental plaque, or biofilm. This microbe can convert dietary carbohydrates into organic acids and thrive in low pH conditions because it is very acidogenic and aciduric. Furthermore, *Streptococcus mutans* uses glucosyltransferase enzymes to produce extracellular polysaccharides, which improve its adherence to enamel surfaces and encourage demineralization [21,23].

2. Rather than being a main oral pathogen, *Staphylococcus intermedius* is a Gram-positive coccus that is regarded as an opportunistic microorganism. Although it is not frequently found in the typical human oral microbiota, it can occasionally be found, especially in immunocompromised people or by zoonotic transmission. Although its involvement in dental caries is minimal, it may aid in the production of biofilms and generate enzymes that promote inflammation and tissue invasion. Therefore, rather than direct cariogenic activity, its presence is more closely linked to opportunistic infections [23,30].

The Gram-positive, facultative anaerobic bacteria *Enterococcus casseliflavus* is not usually linked to the ordinary oral flora. Sometimes, especially in contaminated surroundings or root canal infections, it is isolated from oral infections.

Although this organism is not directly responsible for the development of dental caries, it is well-known for its capacity to endure challenging environmental circumstances. Rather than cariogenicity, its clinical significance is seen in its resilience to unfavorable settings and its role in persistent infections [23,30].

A Gram-positive, facultative anaerobic bacterium called *Enterococcus faecalis* is commonly isolated from deep carious lesions and chronic endodontic infections. It contributes significantly to the advanced stages of dental caries even if it is not the main cause of the condition. It may enter dentinal tubules, endure harsh environments (such as high pH and nutrition deprivation), and withstand a variety of antimicrobial treatments. Because of these traits, it is a major pathogen in root canal therapy failures and subsequent infections [21,22]. This study aimed to compare the antibacterial efficiency of three commercially available nano-filled dental composites (SDI, MANI, and EDUFILL).

Material and Methods

Thirty composite specimens were fabricated using flexible plastic molds with standardized dimensions (10 mm diameter × 3 mm thickness), following previously described in-vitro protocols [31]. Composite materials (Mani, Edu-Fill, and SDI nano-composite) were packed into the molds, covered with glass slabs, and gently pressed to obtain flat and uniform surfaces. Each specimen was light-cured from both sides using overlapping irradiation cycles according to the manufacturers' instructions to ensure optimal polymerization [32]. The curing unit tip was placed in direct contact with the slab surface.

After polymerization, specimens were removed from the molds, and excess material

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was trimmed. Surface finishing was performed using 1200-grit silicon carbide paper
to standardize surface roughness prior to bacterial testing [33,34].

This in vitro investigation employed a laboratory strain of *Streptococcus mutans*, a Gram-positive cariogenic bacterium that is closely linked to dental caries [25,35]. To assess bacterial adherence and biofilm formation on dental restorative materials, the bacterial isolate was cultivated under conventional microbiological conditions that are frequently described in earlier in-vitro research [36,37]. The bacteria were first cultivated on selective Mitis Salivarius Bacitracin (MSB) agar, which is frequently used for the selective isolation and growth of Streptococcus mutans because it inhibits oral streptococci that are not mutans [38,39]. To ensure sufficient bacterial growth, the cultures were incubated aerobically at 37 °C for 18–24 hours.

Colonies with distinctive *Streptococcus mutans* morphology were chosen after incubation and sub-cultured to produce a pure bacterial solution.

Standard microbiological criteria, such as colony shape, Gram staining (Gram-positive cocci organized in chains), and recognized phenotypic traits reported in the literature, were used to confirm identification [25,35,40].

Following confirmation, the *Streptococcus mutans* culture was suspended in artificial saliva to mimic oral environmental conditions. During the incubation phase, the bacterial adhesion and biofilm formation studies were conducted on composite resin specimens.

An agar diffusion assay was carried out in accordance with earlier research [41-43] to assess the antibacterial activity of composite resin materials. Using sterile swabs, a standardized suspension of *Streptococcus mutans* was evenly applied to the surface of agar plates. After that, the inoculated agar surface was directly covered with composite resin disks.

For a full day, the plates were incubated aerobically at 37 °C. Following incubation, the diameter and existence of inhibitory zones surrounding the composite disks were assessed. The development of inhibitory zones was thought to be a sign of antibacterial activity, which might be connected to the release of fluoride or other antibacterial elements included in the composite material [27,44,45].

This technique has been widely used to evaluate how well bioactive and fluoride-releasing restorative materials can stop the growth of cariogenic bacteria, especially Streptococcus mutans [27,41,42].

Bacterial cell counting was carried out using a hemocytometer in accordance with conventional microbiological protocols to objectively evaluate the antibacterial efficacy of the studied composite material [46–48]. Artificial saliva was combined with a suspension of *Streptococcus mutans*. Two experimental setups were assessed. The control group consisted solely of artificial saliva combined with bacterial solution. The test group had artificial saliva combined with bacterial suspension while the composite resin specimen was present.

A drop of the bacterial suspension was placed onto a hemocytometer counting chamber for each condition, and it was then inspected under a light microscope. Five sizable grid squares were used to count the bacterial cells, and the total number of cells was noted [49-51].

Control group (Bacteria + Artificial Saliva)

The total number of bacterial cells counted in five squares was 500,000 cells.

The average number of cells per square was calculated as follows:

Average cell count per square = 500,000 ÷ 5 = 100,000 cells

The bacterial concentration was calculated using the standard hemocytometer
formula:

Cells/mL = Average cell count × 10^4 × dilution factor

Since no dilution was applied, the dilution factor was equal to 1:

Cells/mL = 100,000 × 10^4 = 1.0 × 10^9 cells/mL

Test group (Bacteria + Artificial Saliva + Composite Resin)

The total number of bacterial cells counted in five squares was 300,000 cells.

The average number of cells per square was calculated as follows:

Average cell count per square = 300,000 ÷ 5 = 60,000 cells

Cells/mL = 60,000 × 10^4 = 6.0 × 10^8 cells/mL

Inhibition Percentage Calculation

The antibacterial effect of the composite resin was expressed as a percentage of
bacterial growth inhibition using the following equation [45]:

Inhibition (%) = (Control − Test) / Control × 100

Inhibition (%) = (500,000 − 300,000) / 500,000 × 100

Inhibition (%) = 40%

The reduction in bacterial count from 1.0 × 10^9 cells/mL in the control group to
6.0 × 10^8 cells/mL in the test group indicates that the composite material exhibited a
measurable antibacterial effect, resulting in a 40% reduction in bacterial growth.

Results

In this study, there is difference anti-bacterial activity of three types of composite restorative materials were tested: SDI, Mani Nano, and Edu Fill. From each material, eight samples (blocks) were prepared, with each sample measuring 10 × 3 mm. The total number of samples was 30.

All samples were placed in agar medium containing *Streptococcus mutans* and incubated at 37°C for 48 hours. After incubation, the dimensions of the samples were measured following bacterial exposure.

Among the three nano restorative materials tested, Mani Nano demonstrated the greatest antibacterial effect, showing clear inhibition of bacterial growth. In contrast, SDI showed no antibacterial effect, as it did not produce any inhibition zone against bacterial growth. Edu Fill exhibited a moderate antibacterial effect, demonstrating bacterial inhibition, but to a lesser extent than Mani Nano.

A one-way analysis of variance (ANOVA) demonstrated a statistically significant difference in the mean dead *Streptococcus mutans* values among the three composite types ($F = 28.67$, $p < 0.001$). The Mani composite showed the highest mean value, followed by Edu, while the SDI composite exhibited the lowest mean value, indicating a significantly reduced antibacterial effect compared with the other materials (Table 1).

One-way analysis of variance (ANOVA) revealed a statistically significant difference in the mean live *Streptococcus mutans* counts among the three composite types ($F = 6.23$, $p = 0.008$). The Mani composite demonstrated the lowest mean bacterial count, indicating a superior antibacterial effect, whereas the SDI composite showed the highest mean value, suggesting reduced antibacterial activity. The Edu composite exhibited intermediate results between the two groups (Table 2).

Discussion

Obtaining the necessary bacterial strain (*Streptococcus mutans*) was one of the study's primary constraints and difficulties. Several bacterial species were found when a patient's carious lesion sample was taken and cultivated on agar. *Staphylococcus intermedius*, *Enterococcus casseliflavus*, and *Enterococcus faecalis* were among them; Streptococcus mutans, the target bacterium, was found in trace amounts. This result is in line with other research showing that rather than a single bacterial species, dental caries lesions typically comprise a heterogeneous microbial population [23,52].

The purpose of this study was to analyze the antibacterial qualities of several restorative dental materials and identify the material with the most inhibitory effect against Streptococcus mutans. The study also sought to assess these materials' capacity to lessen biofilm formation and bacterial colonization, two important elements in the development of secondary caries and restoration failure [3,21].

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The MANI composite showed the strongest antibacterial activity among the materials examined, according to the current study's findings. This result could be explained by variations in surface properties, filler distribution, and material composition, all of which can affect bacterial adherence and growth. Different physicochemical characteristics of traditional resin-based composites can lead to different antibacterial activities even though they typically lack intrinsic antibacterial agents [3,27].

These results are consistent with earlier research that found differences in the antibacterial efficacy of various composite resins, even when no antimicrobial agents were used. It has been demonstrated that bacterial colonization and the formation of biofilms are influenced by surface smoothness, polymerization degree, and resin matrix composition. Materials with less surface roughness typically show less biofilm formation and bacterial adhesion [5,27].

Additionally, some research has shown that adding antimicrobial agents such chlorhexidine, quaternary ammonium compounds, or bioactive fillers can improve antibacterial activity. Bioactive restorative materials, like ACTIVA BioACTIVE Restorative, have demonstrated the capacity to release calcium, phosphate, or fluoride ions, which may support remineralization and have antimicrobial properties [53]. The absence of antibacterial additions in the materials examined in this investigation, however, raises the possibility that the observed antibacterial variations are primarily due to intrinsic material characteristics rather than actual antimicrobial mechanisms [27,53].

However, several studies have highlighted that the antibacterial activity of restorative materials seen in vitro does not always correspond with their clinical efficacy. The composition of saliva, dietary carbohydrates, oral hygiene habits, and microbial interactions can all have a substantial impact on bacterial growth and biofilm formation in the complex oral environment [24].

Furthermore, rather than just the presence of bacteria, tooth caries is mainly linked to acid generation brought on by bacterial metabolism of fermentable carbohydrates. As a result, antibacterial qualities might not be enough to totally stop the advancement of caries [20,24].

Therefore, even though the MANI composite showed the best antibacterial performance in this study's experimental setup, its clinical efficacy should be evaluated cautiously. These results imply that rather than actual antimicrobial activity, the antibacterial efficacy of restorative materials is mostly material-dependent and controlled by 22 physicochemical parameters, such as surface features and filler makeup [5,27].

According to several earlier research, the antibacterial activity of restorative materials can vary based on their composition.

Zhang et al. [26] showed that due to variations in their physicochemical characteristics, some dental materials can prevent bacterial development. These results are in line with the current study's findings, which indicated that the MANI composite had the strongest antibacterial activity of all the materials examined. According to Imazato [27], certain resin-based restorative materials may have antibacterial qualities based on their surface features and chemical makeup. These outcomes are consistent with the current study's findings, which also showed differences in the tested materials' antibacterial activity. However, some experts contend that the antibacterial qualities of restorative materials are not the only factor contributing to the formation of dental caries.

According to Nedeljkovic et al. [3], oral hygiene, dietary practices, and the composition of biofilms all have an impact on secondary caries surrounding restorations, rather than just the restorative material. The current findings are somewhat at odds with this perspective. Additionally, Marsh [54] put out the ecological plaque theory, which contends that rather than the existence of a particular bacterial species, dental caries is caused by ecological shifts within the oral microbiota. Therefore, preventing bacterial colonization in the oral cavity may require more than just restorative materials' antibacterial activity.

Among the studied restorative materials, the MANI composite showed the strongest antibacterial activity, according to the study's findings. This discrepancy could be explained by differences in the materials' 23 surface characteristics, filler content, and chemical makeup. According to Ferracane [55], bacterial adherence and biofilm formation on restorative surfaces can be greatly impacted by the physical and chemical properties of composite resins.

The higher antibacterial activity of the MANI composite in this investigation could be attributed to variations in the surface properties, filler particle size, and resin matrix composition. Smoother surfaces and stable polymer networks may lessen bacterial adhesion and biofilm development, according to earlier research [56].

The improved antibacterial effectiveness of the MANI composite in comparison to other investigated materials could be explained by these characteristics.

Several limitations should be noted, even though this in vitro model offers valuable insights into bacterial adhesion and biofilm inhibition on composite materials [49,52]. First, the complex oral environment—which includes saliva flow, mechanical shear forces, dietary carbohydrates, and host immunological responses—is not entirely replicated by the experimental circumstances [52]. Second, while dental biofilms in vivo are multispecies ecosystems with both antagonistic and synergistic microbial interactions, the current work used a single bacterial species (*Streptococcus mutans*) [52]. Lastly, spectrophotometric quantification techniques and multispecies biofilm models should be used in future studies to improve accuracy and clinical relevance because visual or semi-quantitative evaluation may include observer-dependent variability [49,50].

Notwithstanding the useful results of this investigation, several restrictions should be considered. The complex environment of the mouth cavity cannot be entirely replicated in the in-vitro circumstances used for this investigation. Saliva, temperature fluctuations, masticatory forces, and the existence of multispecies biofilms are some of the factors that can affect bacterial behavior and the antibacterial efficacy of restorative materials [56]. Therefore, more in-vivo research is advised to assess these materials' therapeutic efficacy in actual oral settings.

Conclusion

The MANI Nano restorative composite demonstrated the strongest antibacterial effectiveness against Streptococcus mutans among the studied materials, according to the study's limitations. The examined composites varied antibacterial performance might be explained by variations in their surface properties, filler distribution, and material composition, all of which can affect bacterial adherence and biofilm formation.

However, because this study was carried out in vitro, the findings might not accurately represent the intricate biological milieu of the oral cavity.

Therefore, more in-vivo and clinical research is needed to validate and corroborate the results of this study.

The selection of restorative dental materials with greater resistance to bacterial colonization may be improved as a result of these findings, thereby lowering the incidence of secondary caries and extending the life of dental restorations.

It is advised that future research examine the antibacterial qualities of restorative composite materials against additional oral microbes linked to oral infections and tooth caries. Future studies should assess other physical and chemical characteristics of composite resins, such as water sorption and solubility, since these characteristics may have a major impact on the long-term clinical performance,

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stability, and durability of restorative materials in the oral environment.

Conflict of Interest

The authors declare no conflict of interests.

Ethical Approval

The Ethical approval is not applicable as the tested bacterial samples were donated by Microbiology Lab of the Faculty of Dentistry at the University of Kufa.

Data Availability Statement

The corresponding author can provide the data upon reasonable request.

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Table 1. Comparison of mean dead *Streptococcus mutans* values among different composite types.

Composite type	**Mean value**	**SD**	**F test**	**P-value**
Mani	13.67	0.23	28.67	< 0.001
Edu	12.84	0.38		
SDI	12.03	0.63		

Table 2. Comparison of mean live *Streptococcus mutans* values among different composite types.

Composite type	**Mean value**	**SD**	**F test**	**P-value**
Mani	1.6	0.41	6.23	0.008
Edu	1.97	0.32		
SDI	2.23	0.32		