

RESEARCH PAPER

Evaluation of Antibacterial Activity of Dental Nanocomposite Filling After Medical X-ray Exposure

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ABSTRACT

This study aimed to evaluate the antibacterial activity of dental nanocomposite filling (Filtek™ Z350 XT) after medical radiographic techniques (OPG, CBCT, CT-Scan) exposure. 16 Samples of Filtek™ Z350 XT (3M ESPE, USA) dental filling material were prepared by placing the filling syringes in an electric heater (composite heater) and raising their temperature to (50°C). The material was then placed in various shaped Teflon molds according to (ISO 4049) specifications under specific conditions. These samples were cured (polymerized) using light curing with a specialized light source for (40 sec). After sample preparation, their dimensions were measured, and they were placed in small, transparent bags, immersed in artificial saliva, and exposed to X-rays emitted from various medical imaging techniques (OPG, CBCT, and CT-Scan). A FESEM scan was performed on the prepared samples to study their surface morphology and determine the nanodiameters of the particles. The antibacterial activity of the prepared samples was investigated against gram positive *Streptococcus mutans* using agar well diffusion assay. After culturing the organisms, the samples were placed directly on agar after spreading bacteria. The cultured plates containing the samples and the test organisms were incubated overnight at 37°C before measuring and recording the average the zones of inhibition diameter. The FESEM results confirmed that all prepared samples exposed to the medical imaging techniques exhibited positive results. The samples (OPG, CBCT, CT) and those not exposed to radiation, have a nanostructure and fine particles with an average nanoparticle size ranging from (36.478 - 51.08) nm. The best positive radiation effect was observed in the sample exposed to CT radiation, followed by the sample exposed to OPG radiation. All samples exposed to medical imaging techniques showed significant increase in antibacterial activity and inhibition zones compared to unexposed sample. The diameters of inhibition zone were respectively 18, 20, 24 mm. The sample exposed to CT radiation showed the best antibacterial effect against *Streptococcus mutans*, followed by the sample exposed to CBCT radiation and then OPG. Exposing nanocomposite filtek z350xt to medical imaging techniques radiation improved the antibacterial activity against *Streptococcus mutants* without compromising mechanical performance.

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INTRODUCTION

X-rays, which are electromagnetic radiation of extremely short wavelength and high frequency, are widely used as a diagnostic tool in modern medicine. Their high energy allows X-rays to pass through the body's biological tissues, creating images of internal structures [1,2]. X-rays are used in several different medical fields to diagnose various diseases. One of these fields is dentistry [3]. Dental X-rays are a common procedure in dentistry as part of a clinical examination due to the lower radiation dose compared to most other medical X-ray examinations [4]. There are two main types of dental radiography equipment: intraoral and extraoral. Intraoral radiography involves inserting an X-ray film inside the patient's mouth. This equipment creates images that provide comprehensive information about the condition of the patient's teeth, jawbone, and tooth roots, as well as confirming the presence of caries. Extraoral radiography equipment places the X-ray image receptor outside the mouth, providing images of the teeth and information about the jaw and skull [5,6].

Intraoral and extraoral X-rays can be used to diagnose cavities, periodontal disease, and other pathological problems, or to assess disturbances tooth growth and its developmental [7]. Several types of medical imaging techniques are available for diagnosis, including 2D imaging (intraoral, cephalometric radiography, panoramic x-ray) and 3D imaging (multislice computed tomography [CT] and cone beam computed tomography [CBCT] techniques [8]. Intraoral and panoramic radiography, along with three-dimensional (3D) Cone Beam Computed Tomography CBCT, are among the most common methods for daily clinical imaging in dental medicine [9]. Panoramic dental radiography is a basic imaging technique often used as a primary diagnostic tool to evaluate both the mandible and maxilla using a single image [10]. In panoramic radiography, the X-ray tube and receptor holder rotate in a semicircle around the front of the head, creating a composite image of the entire mouth in a single photograph [11]. This type of imaging offers several advantages to clinicians, including wide coverage of the oral, facial, and jaw structures, accurate imaging of pathological lesions, lower cost, and reduced radiation dose [12]. Cone-beam computed tomography (CBCT) is a specialized imaging technique primarily used in dentistry and maxillofacial surgery. It utilizes a

cone-shaped X-ray beam and a flat-panel detector to provide high-resolution three-dimensional images while reducing radiation exposure compared to standard medical CT scans [13,14]. Conventional computed tomography (CT) uses a fan-shaped X-ray beam to capture a series of axial planes, which requires continuous helical movement over the axial plane, conversely cone beam computed tomography (CBCT) uses a cone-shaped x-ray beam and a solid flat detector, one cycle around the patient, covering the entire anatomical volume. This one cycle significantly reduces the amount of X-rays absorbed, reducing radiation exposure by 6 to 15 times compared with CT scans [15,16]. A computed tomography (CT) scanner works by rotating an X-ray tube around the patient's body inside a circular structure called a portal. With each complete rotation, the scanner collects computer data to produce separate cross-sectional images [17]. Each type of such device can emit a range of doses of radiation, depending on the imaging technique being used [18]. Over the last decade, composite fillings have gained increasing popularity in posterior dental restorations and this shift is largely due to patients' preference for non-metallic fillings for aesthetic reasons and concerns about mercury toxicity in metal alternatives options, although concerns are not yet proven and as a result dentists are increasingly being asked to offer composite fillings to meet these evolving requirements [19]. X-rays remain necessary in human diagnosis, as they provide detailed images to detect restorative materials in the teeth [20]. Although current composite resins exhibit mechanical properties suitable for all fields of dentistry, resin composite are preferred for their multiple properties as they consist of an organic resin matrix, inorganic fillers, silane agents, catalysts and pigments [21]. Their aesthetic appeal, with a wide range of enamel-like shades, making them ideal for anterior restoration, where color matching is critical to achieve seamless dental restorations [22,23]. Restorative materials that contain resin are polymer structures and contain monomers such as BISGMA, TEGDMA, BISEMA, UDMA. Monomers form a major part of the organic matrix of composite resins and significantly influence polymerization, reaction, mechanical properties, water absorption of resin [24]. Resin-containing restoration materials do not polymerize completely. Depending on the material, the polymerization degree of these materials ranges

from 50% to 70% [25]. Many factors, such as the intensity of light used and its wavelength, type and quantity of monomers, catalysts and initiators materials in a material affect the degree to which the material is polymerized [26]. Resin composites have drawbacks such as polymerization shrinkage and susceptibility to recurrent decay [27]. The colonization of bacteria or the formation of biofilm leads to a secondary decay at the margin of the restoration, which affects its durability [28]. In addition, as plaque accumulates on the surfaces of the reconstruction, the surrounding tooth structure becomes more susceptible to mineral loss [29]. The most common reason for replacement of restorations made from resin-composite materials is the development of secondary caries [30]. During a reaction with polymerization of resin compounds, a gap is formed at the separating surface between the compound and patient tissue. This gap provides an ideal environment for the growth of bacterial biofilm, which in the long term can lead to chemical and mechanical degradation of the filling [31]. Smooth composite resin surfaces were found to be less prone to bacterial adhesion and accumulation compared to rough composite resins. They have a significant effect in preventing the adhesion and development of biofilms. Rough surface of composite dental fillings also plays a crucial role in initiating bacterial adhesion to substances present in the oral cavity [32,33]. The best qualities of several different composites have recently been combined to create new types such as nanofilled and suprananocomposite. The size of nanoparticles that make up these modern restorative materials ranges from 1 to 100 nm. In order for these particles to withstand the chewing pressures that occur in the oral cavity, they must possess high physical and mechanical properties. Superior polishing properties and surface smoothness are among the most prominent advantages of these nanoparticles. This phenomenon is attributed to the minuscule gaps that exist between inorganic particles known as nanoparticulates [34,35]. Hybrid nano-resin composites contain nanoscale inorganic particles distributed in the resin matrix, resulting in a smoother surface, less shrinkage, better color stability, and an improved aesthetic appearance [36]. Therefore, the light-cured direct nanohybrid Filtek Z350 XT can be used for anterior and posterior tooth restoration.

Zirconium and silica particles, or nanoclusters,

which range in size from (5-20) nm, exhibit superior wear resistance and retain their shine during polishing [34,37]. Despite the development of composite resin technology over the years, the problem of bacterial adhesion and accumulation on restoration materials surfaces, tooth decay and failure restorations still persist [28,38]. The oral biofilm is formed of diverse microbes found on the tooth surface, and surrounded in a matrix of polymers of bacterial and salivary origin. The initial and critical step in plaque formation includes the adhesion of bacteria known as early colonizers such as oral streptococci [39].

Streptococcus mutans is a Gram-positive, anaerobic, facultative bacterium endogenous to the oral cavity [40]. It's the primary etiological agent in the development of dental caries, due to its high ability to colonize restoration materials surfaces and form microbial biofilms [41]. They survive in low pH, and their pathogenesis is related to the production of organic acids resulting from carbohydrate metabolism [42]. The secretion of these acids from *Streptococcus mutans* bacteria can even affect composite dental fillings, increasing the risk for secondary caries [43]. The longevity of the restoration can also be affected by the shrinkage of the composite during polymerization, which may promote the occurrence of tooth decay [44]. According to microbial bacterial strain the esterase activity of *Streptococcus mutans* may deteriorate, altering the surface morphology of composite resins [45], also it may inhibit the activity of enzymes associated in glycolysis process [46].

Streptococcus mutans bacteria can form biofilms on solid surfaces by producing of glucosyltransferase (GTFs) enzymes to catalyze extracellular polysaccharide synthesis (EPS) [47]. It's biofilm forming capacity superior that of many other *Streptococcus* species that live in oral cavity environment [48]. Some specific adhesion of *Streptococcus mutans*, known as double-antigen I/II, promote biofilm formation, which facilitates the bacterium's adherence to the salivary membrane of the tooth and its interaction with other bacterial species and host proteins [49,50]. Biofilms lead to roughness and degradation of composite resin surfaces, which promotes bacterial colonization at the tooth-composite interface and leads to secondary caries [51]. This study aimed to evaluate the antibacterial activity of nanocomposite Filtek™ Z350XT against *Streptococcus mutans*

after medical radiographic techniques (OPG, CBCT, CT) exposure.

MATERIALS AND METHODS

Materials Used in Sample Preparation

In this study composite restoration material was used 3M Filtek™ Z350XT universal restorative (ENAMEL A2) (3M ESPE, USA) and the Fig. 1 shows the filling syringes used.

This filling is multi-purpose, light-activated nanocomposite material and used in both direct and indirect dental restoration procedures. utilizing advanced nanotechnology, it possesses a truly nanocomposite structure that ensures wear resistance and long-lasting shine, in addition to its high x-ray transparency that makes the material is clearly visible in dental x-ray images. The ease of use of this product makes it highly moldable and resistant to sagging.

Samples Preparation

The samples were prepared according to (ISO 4049) specifications, using dental filling material (3M Filtek™ Z350XT), this material is a paste-like resin packaged in syringes, as shown in Fig. 1. These syringes are placed in an electric heater or composite heater, the electric heater used is from (3M, USA), designed to raise the temperature of

composite resin fillings. It has openings specifically designed for inserting composite syringes or capsules, along with a digital display that allows accurate temperature control. The material syringes are heated to 50°C to reduce viscosity and make the material more flexible and flowable. This facilitates its conformation within the mold and reduces the possibility of voids [52]. A quantity of this resin was injected into a Teflon mold with dimensions of (3 mm) in thickness and (2 mm) in diameter (according to ISO 4049 specifications) [53], at a temperature of 37°C and under yellow illumination to prevent curing. The bottom layer was pressed into the mold against a glass slab covered with celluloid tape. Once the top layer was in place, its surface was covered with celluloid tape and then with a glass slab to provide a flat, smooth surface and to remove excess material. Transparent Mylar strips were used over the molds before curing to prevent the formation of an oxygen-inhibited layer on the sample surface. The glass slabs and strips were then removed, and the excess material was removed using a sharp scalpel.

A (3M™, Elipar Deep Cure-L LED, USA) curing light was used to cure (polymerize) these samples. This process is essential to ensure the polymerization of the filling and to guarantee its



Fig. 1. Dental filling (syringes) of 3M Filtek™ Z350XT universal restorative.

Table 1. Sample codes prepared in this study.

Sample Name	Sample Description
A	Dental filling prepared without exposure to any technique(Control sample)
B	Dental filling prepared and exposed to the technique OPG
C	Dental filling prepared and exposed to the technique CBCT
D	Dental filling prepared and exposed to the technique CT-Scan

strength, durability, and longevity. The samples were exposed to this light for 40 seconds. The dimensions of the samples were then measured, and they were placed in small, transparent bags and labeled. After preparation, a quantity of artificial saliva was added to cover the sample, ensuring an environment close to that of the mouth. The sample was then exposed to radiation emitted from specialized medical radiography techniques used in dental clinics. (Three types of dental radiography techniques were used: OPG, CBCT, and CT-Scan) The type of radiation used in all these techniques is X-rays, but the radiation doses used in each technique vary from one technique to another depending on the function that these techniques perform.

After exposing the samples to the radiation used in these three techniques, the samples are removed from the bags, dried of saliva, and stored for (24) hours after being immersed in distilled water to prevent drying and to simulate the oral environment. After that, they are dried, placed in other bags, and labeled to be ready for examination. (4) samples were selected to be tested for each radiographic technique. The first sample (A) represents the prepared dental filling that was immersed in saliva for the same duration as its counterparts but was not exposed to

radiation. this sample was then compared with the other samples that were exposed to radiation. The second sample (B) represents the prepared dental filling that was immersed in saliva and exposed to Orthopantomogram (OPG) radiation. The third sample (C) represents the prepared dental filling that was immersed in saliva and exposed to cone beam computed tomography (CBCT) radiation. The fourth sample (D) represents the prepared dental filling that was immersed in saliva and exposed to computed tomography (CT-Scan) radiation. Table 1 shows the codes of the samples prepared in the current study.

Techniques used in exposing samples to radiation Orthopantomogram Technology (OPG)

The OPG device (OP 3D, KaVo dental, Biberach, Germany) was used. After operating the device and adjusting the settings (with the same settings as the patient's radiographic imaging to capture an OPG image to provide the same conditions), the sample was placed inside small transparent plastic bags and immersed with artificial saliva. The bags were placed in the device's designated area, and the sample was exposed to the X-rays emitted by this technique with an energy of (66 kV), a radiation dose of (62 mGy cm²), a current of 7.1 mA, and an exposure time of (9.0 sec).

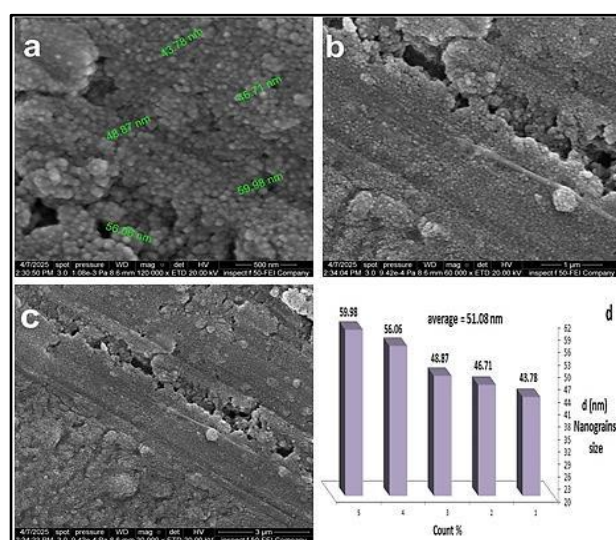


Fig. 2. FESEM images of sample A: (a) at 500 nm scale and 120 KX magnification. (b) at 1 Micro scale and 60 KX magnification. (c) at 3 Micro scale and 30 KX magnification, (d) Represents nanoparticle size distribution of the sample.

Cone beam computed tomography technology (CBCT)

The CBCT device (OP 3D, KaVo dental, Biberach, Germany) was used. After operating the device and adjusting the settings (with the same radiographic imaging settings to capture a CBCT image of the

patient to provide the same conditions), the sample was placed inside transparent plastic bags and immersed in artificial saliva. The bags were placed in the device's designated location, and the sample was exposed to the X-rays emitted by this technology, with an energy of (95 KV), a radiation

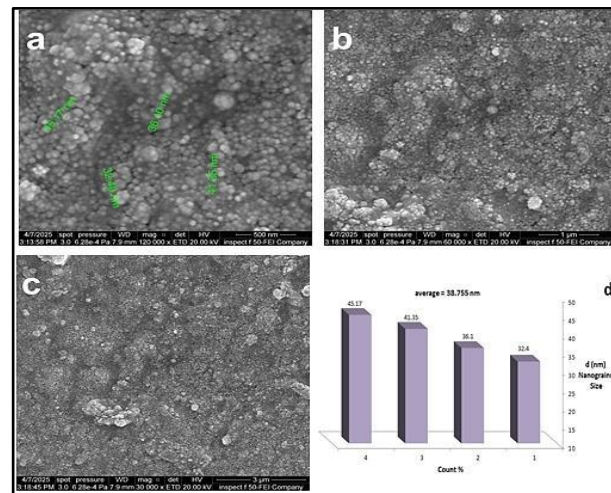


Fig. 3. FESEM images of sample B: (a) at a scale of 500 nm and a magnification of 120 KX. (b) at a scale of 1 Micro and a magnification of 60 KX. (c) at a scale of 3 Micro and a magnification of 30 KX. (d) Represents the nano-particle size distribution of sample B.

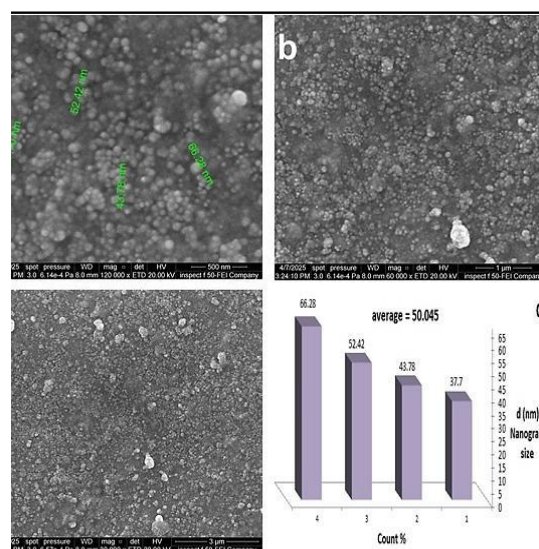


Fig. 4. shows FESEM images of sample C exposed to CBCT irradiation: (a) at a scale of 500 nm and a magnification of 120 KX. (b) at a scale of 1 Micro and a magnification of 60 KX. (c) at a scale of 3 Micro and a magnification of 30 KX. (d) represents the nano-particle size distribution of the sample.

dose of (662 mGy cm²), a current of (8.0 mA), and an exposure time of (3.6 sec).

Computed tomography technology (CT- scan)

A 128-slice CT-Scan device (PHILIPS, Netherlands) was used. After operating the device and adjusting the settings (with the same radiographic settings for taking a CT-Scan image of the patient's head to provide the same conditions), the sample was placed inside transparent plastic bags and immersed with artificial saliva. The bags were placed in the device's designated location, and the sample was exposed to the X-rays emitted by this technique, which had an energy of (120 KV), a radiation dose of (1552.5 mGy cm²), a current of 120 mA, and an exposure time of (12.3 sec).

Field Emission Scanning Electron Microscopy (FESEM)testing

The FESEM test was performed, and the device used was a MODEL INSPECT F50 from THERMO FISHER SCIENTIFIC.

Measurements Of Field Emission Scanning Electron Microscopy (FESEM)

Through this technique, the surface shapes of the granular particles comprising the prepared samples were studied. Fig. 2 shows the FESEM analysis of the control sample A, with measurements of 500nm, 1µm, 3µm. Fig. 2a shows a nanostructure with fine grains ranging in size from 43.78 to 59.98 nm, with an average nano- grain size of (51.08 nm). This nanoscale

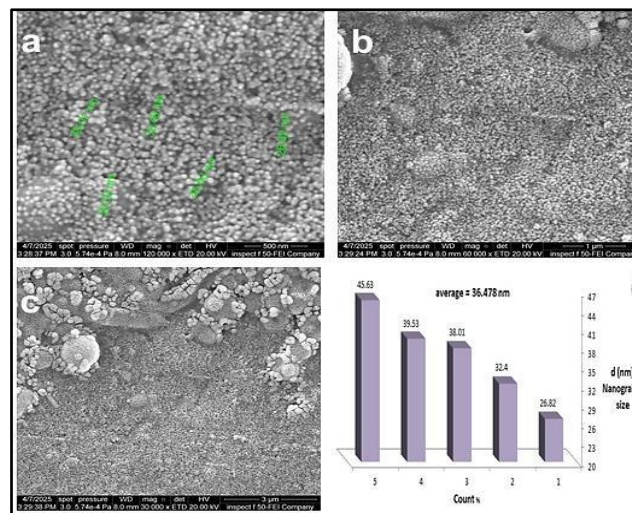


Fig. 5. FESEM images of sample D: (a) at a scale of 500 nm and a magnification of 120 KX. (b) at a scale of 1 µm and a magnification of 60 KX. (c) at a scale of 3 µm and a magnification of 30 KX. (d) represents the nano-particle size distribution of the sample.

Table 2. Materials and devices used in antibacterial activity testing.

No.	Items	Company	Country
1	Mueller Hinton Agar	Accumix	Spin
2	Hot plate Magnetic stirrer	Four E's	China
3	Glass Erlenmeyer Flask	Pyrex	Germany
4	Autoclave	Westtline	Chain
5	Incubator	Ningbo	Chain
6	Ethanol	Duksan	Korea
7	Round Flask	labmax	Germany
8	Balance 4 digits	Hangzhou	China

size increases the total surface area, which can positively influence chemical properties such as adhesion and interaction with the oral medium. Figs. 2b and c show the sample surface covered with tightly packed, finely distributed spherical (nanoparticles). This indicates that the grains are tightly packed and have a relatively homogeneous grain distribution, suggesting good manufacturing quality the absence of large agglomerates, and increased mechanical and abrasion resistance. The presence of some cracks or voids in the structure may be due to the preparation process or the nature of the curing.

Fig. 3 shows the FESEM analysis of sample B exposed to OPG radiation, at 500 nm, 1 μ m, 3 μ m. Image (a) shows the particles with very small and close sizes, with high compactness indicating a relatively regular distribution and fine grains with grain sizes ranging from (32.40-45.17) nm and an average nano-grain size of (38.755) nm, which is smaller than the particle size in sample A and more homogeneous. This indicates that exposure

to the X-rays emitted from the OPG caused partial recrystallization or stimulation of particle condensation and structural rearrangement or slight dissociation of the material, which reduced the particle size as a result of the energy absorbed from the OPG radiation [54]. Images (b) and (c) show particles with higher compactness and a smoother surface, indicating a possible improvement in the filler distribution. We conclude that the radiation from the OPG device affected the filler's nanostructure, causing a reduction in particle size and agglomeration density, and an increase in compactness, surface homogeneity, and more regular distribution.

Fig. 4 shows the FESEM analysis of sample C exposed to CBCT radiation at 500 nm, 1 μ m, and 3 μ m. Image (a) shows a nanostructure with particle sizes ranging approximately from (37.70 to 66.28) nm and an average grain size of 50.045 nm. The particles exhibit an irregular distribution, appearing coarser compared to control sample A, with some clearly visible clumping. This may

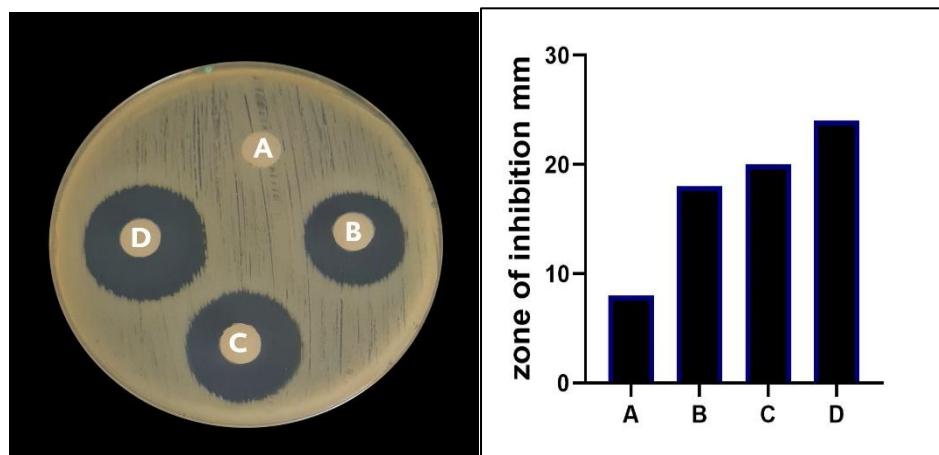


Fig. 6. Antibacterial activity of (Zones of inhibition) for the prepared samples (A, B, C, D) against *S. mutans* . and A diagram showing the distribution of inhibition zone values.

Table 3. Explain the antibacterial activity of nanoparticles prepared samples (A, B, C, D).

Antibacterial analysis (Zone of inhibition (mm))				
Sample	A	B	C	D
<i>S. mutans</i>	8	18	20	24

indicate changes in surface structure and a significant increase in the size of some particles due to CBCT radiation exposure. CBCT radiation uses a higher radiation dose, a broader energy spectrum, and a longer exposure time than OPG, which increases the effect on the particles (due to the stimulated electromagnetic energy). This caused slight electronic and thermal excitation that affected the filler structure, leading to an increased rate of atomic rearrangement on the surface and the potential for nanoparticle growth or adhesion. This may explain the presence of larger particles. Compared to the control sample A [55].

Fig. 5 shows the FESEM analysis of sample D exposed to CT radiation at 500 nm, 1 μm , and 1 μm . Image (a) reveals a relatively homogeneous nanostructure with nearly spherical and closely spaced particles, indicating good distribution without significant agglomeration. The particle sizes range from (26.82 to 45.63) nm, with an average particle size of (36.478) nm, which is smaller than the other samples. Since smaller particle size implies a larger surface area, the mechanical properties or tooth adhesion may be improved. CT radiation exposure appears to have smoothed the surface structure and reduced particle size, resulting in a more homogeneous sample compared to the OPG and CBCT samples (samples B and C). This may be due to changes in the chemical composition or the rearrangement of bonds within the material under the influence of radiation. This sample can be considered the best in terms of nanostructure, which may positively impact its clinical performance. Figs. 5b and c show a smooth surface covered with small, nearly homogeneous nanoparticles, with few visible voids or cracks. The regular distribution of the particles indicates structural homogeneity resulting from the CT radiation effect. The absence of large clumps or cracks indicates high structural stability. The radiation emitted from the CT technique may have helped to rearrange or break surface bonds, resulting in smaller, more stable particles and increased surface density. This reduces the surface area for interaction, which explains the high anti-corrosion performance [56].

Prepare of Mueller Hinton agar

Chemicals and instruments

Muller-Hinton (M-H) prepared by adding (38 gm) of the powder into (1 L) distilled water and

then heated on a burner with shaking. M-H must be autoclaved for 15 minutes at (121°C) to be sterilized. Then it was allowed to cool to (50°C) before pouring into a petri dish and leaving for about (15 min) for solidification before flipping upside down and storing in the refrigerator at (4°C).

Antibacterial activity

The antibacterial potential of the prepared Samples (A, B, C, D) was investigated against Gram's positive bacterial strain using agar well diffusion assay [57, 58]. About (20mL) of on Muller-Hinton (MH) agar was aseptically poured into sterile Petri dishes. The bacterial species were collected from their stock cultures using a sterile wire loop [59]. After culturing the organisms, the samples were placed directly on agar after spreading bacteria. The cultured plates containing the Samples (A, B, C, D) and the test organisms were incubated overnight at (37°C) before measuring and recording the average the zones of inhibition diameter [60,61].

RESULTS AND DISCUSSION

All result of antibacterial activity with different concentration shown by the Fig. 6. all details explained by Table 3.

This study investigates the antibacterial activity of the most common oral caries-causing bacteria (*Streptococcus mutans*) of 3M's FILTEK Z350XT nano-filling before and after exposure to dental X-ray techniques such as panoramic radiography (OPG), cone beam computed tomography (CBCT), and computed tomography (CT). This filling is entirely nano-based, containing silica nanoparticles and zirconia nanoclusters with a high loading ratio [62,63]. Based on the results, the null hypothesis, which stated that there would be no significant increase in the antibacterial activity of the FILTEK Z350XT filling before and after exposure to x-ray techniques, was rejected. The results showed a significant increase in antibacterial activity after x-ray exposure. This is because the zirconia nanoparticles, which are densely present in the FILTEK Z350XT filling material, undergo radiocatalytic reaction when exposed to high-energy x-rays. This reaction generates free radicals and reactive oxygen species (ROS), such as hydroxyl (OH) and hydrogen peroxide (H_2O_2) [64-66]. Since the material utilizes fully nanotechnology, the surface area exposed to the

reaction is enormous compared to conventional fillings, thus significantly increasing the amount of these free radicals produced when exposed to x-rays [67,68].

The active free radicals resulting from the interaction of x-rays with silica and zirconia nanoparticles attack the cell wall of *Streptococcus mutans*, a Gram-positive bacterium commonly found in the mouth. This attack causes lipid peroxidation in the bacteria's biofilm, leading to their death and the appearance of an inhibition zone around the filling [68,69]. This means that the X-rays prevented the bacteria from secreting the complex sugars (glucans) they use to adhere to the filling and this clinically reduces the chances of secondary caries occurring [70]. The results of the current study showed that there was a noticeable increase between the samples exposed to different X-ray techniques and the sample not exposed to radiation (the control group) in the size of the area of attachment of *mutans streptococcus* bacteria. Where computed tomography (CT) shows the largest diameter because it has the highest radiation energy and exposure time, it achieved the highest absorbed dose by the nanoparticles in the filling [71-74]. The high absorption led to the maximum levels of radiation stimulation of the zirconia and silica compounds, and this led to an intense influx of free radicals (ROS) that destroyed the *Streptococcus mutans* bacteria, causing the largest inhibition zone to appear [75,76]. This was followed by cone beam computed tomography (CBCT) because it relies on a cone beam that rotates one revolution and releases a radiation dose much less than CT. This made the production of free radicals and their penetration into the material relatively less, so a moderate inhibition zone appeared [77-79]. While panoramic radiography (OPG) recorded the lowest diameter because panoramic radiography is a two-dimensional scanning technique and its effective radiation dose is the lowest compared to three-dimensional techniques (CBCT, CT) [80,81]. When these results are compared with the sample not exposed to radiation, it will be clear that the antibacterial effect is the result of the filling's exposure to X-rays, as the FILTEK Z350XT nanofilling showed no significant antibacterial activity was observed in its natural state and before it was exposed to radiation. The explanation for this is that the FILTEK Z350XT filling contains in its composition nanoparticles of zirconia and silica.

In the natural state without exposure to radiation, these molecules are inert and chemically stable [82-84]. These results are consistent with what researchers have reached in previous literature.

This behavior is consistent with the scientific review conducted by researcher [85]. In it, they confirmed that many conventional nanofillers and polymers do not show significant intrinsic antimicrobial activity unless they are structurally modified or combined with catalytic active materials such as graphene. In order for these molecules to release reactive oxygen species (ROS) that are lethal to *Streptococcus mutans*, they require high external energy (such as x-rays) to excite the electrons. the same principle applies to the addition of graphene and its derivatives, as discussed in the previous study. one of the antibacterial mechanisms is oxidative stress, defined as increase in the production of reactive oxygen species (ROS) compared to antioxidant activity. Reactive oxygen species are oxygen-based molecules and free radicals with high reactivity. Examples of reactive oxygen species include peroxides (H_2O_2), superoxide (O_2^-), hydroxyl radical (OH), hydroxyl ion (OH^-), and singlet oxygen ($O_2(1)$). Reactive oxygen species can be harmful to cells due to oxidative damage to lipids, proteins, and DNA. Moreover, reactive oxygen species are extremely destructive to living organisms at high concentrations, as they cause lipid peroxidation, protein oxidation, damage to nucleic acids, enzyme inhibition, and activation of the programmed cell death (PCD) pathway, which ultimately leads to cell death [85]. In the sample not exposed to radiation, this reaction does not occur, so it lacks an attack mechanism against bacteria [86]. Because the filling, after being light-cured, is a complex and highly stable polymer network, and it is a hydrophobic and insoluble material, and there are no substances that leach or dissolve and diffuse from the filling into the agar (the bacterial culture), so no inhibition zone appears around it [87-89]. This sample was not exposed to x-rays, which means that it did not undergo an additional radiation-induced polymerization, and its surface free energy remained unchanged. therefore, the preservation of filling's physical and chemical surface in its original state, allowed bacteria to grow normally around it, and this explains that the inhibition zone does not appear. This is consistent with the findings of [51]. This study aimed to evaluate the effect of

additional photo-polymerization against thermal polymerization protocols on the adhesion and film formation of *Streptococcus mutans* bacteria on the surfaces of composite resin fillers. The results demonstrated that thermal polymerization significantly reduces bacterial accumulation compared to photo-polymerization alone, which leaves a percentage of unpolymerized monomers that facilitate bacterial adhesion. The superiority of thermal polymerization in the study confirms the importance of surface modification to reduce bacterial accumulation [51].

The positive effect of X-ray irradiation the FILTEK Z350XT filling against the growth and accumulation of *Streptococcus mutans* bacteria is due to several physical and chemical changes resulting from radiation, as the radiation energy contributed to increasing the efficiency of the transformation of polymers in the nanofiller structure and thus reducing surface voids and porosity [90] and also led to modifying the surface energy properties [91]. This is consistent with the findings of [92], as ionizing radiation stimulates additional polymerization of the remaining monomers in the resin fillings, and this leads to an increase in their hardness and a decrease in surface porosity, which is exploited by *Streptococcus mutans* bacteria for adhesion [92]. Inhibit *Streptococcus mutans* bacteria in our study is attributed to the unique nanostructure of the FILTEK Z350XT filling because it contains zirconia and silica nanocomposites. The zirconia nanoclusters in this filling acted as energy receptors. As the applied radiation energy from (OPG) to (CT) imaging systems increases, the excitation of electrons in the zirconia molecules intensifies, promoting their transition from the valence band to the conduction band, The transfer leaves positive gaps that interact with the humidity of the surrounding medium (bacterial culture) to produce free hydroxyl radicals that are toxic to *streptococcus mutans* bacteria [93]. This is consistent with research indicating that nanomaterials exhibit enhanced catalytic activity when exposed to high-energy radiation such as x-rays. This leads to the production of large amounts of reactive oxygen species (ROS), causing oxidative stress that destroys bacterial biofilms [94-96]. A study by [97] confirms that most current research relies primarily on adding elements and nanoparticles to the resin matrix to enhance the antimicrobial properties [97]. For example, [98] added selenium nanoparticles to the FILTEK 350XT

filling, and [99] added titanium dioxide particles to the same composite [100] added nanoparticles of hydroxyapatite and silver for the same filling. In contrast to these studies, the current study focuses on utilizing x-rays to stimulate the intrinsic molecules originally present within the formulation of filtek z350xt. This approach serves as the primary mechanism, thereby avoiding the introduction of extraneous physical additives that could potentially compromise the mechanical properties of the material [98-100]. The Filtek Z350XT is characterized by its unique nanostructure based on true nanotechnology, it contains nanoparticles and nanoclusters of silica and zirconia [101] which gives it a polished surface that reduces the formation of bacterial biofilm [102] When comparing this to studies investigating the effects of radiotherapy, we find that high and cumulative doses lead to the degradation of the resin matrix and change the mechanical and surface properties of nanocomposite fillings [103,104]. However, in our study, exposure of these fillings to X-ray techniques did not cause surface degradation as occurs in high-energy radiotherapy studies. Instead, it acted as a positive catalyst. This positive enhancement is attributed to the ability of low-dose X-ray techniques to induce surface radioactivation, stimulating nanocomposite particles such as zirconia to generate free oxygen radicals (ROS). Recent research has demonstrated the effectiveness of this mechanism in destroying bacterial cell membranes and preventing biofilm formation [96,105,106]. Based on the results of our study, and given that dental patient undergoes radiographic examinations during their clinic visits, this procedure, from the researcher's perspective, turns into an indirect preventive treatment because it automatically activates the filling (FILTEK 350XT) to disinfect its edges and prevent the return of the bacteria causing tooth decay around it. This reduces the risk of secondary caries that often appears on the edges of restoration materials.

CONCLUSION

This study demonstrated that the Filtek™ Z350XT nanocomposite exhibited no intrinsic antibacterial activity in its untreated state, but its performance changed significantly after exposure to dental X-ray techniques. All irradiated samples showed inhibition against *Streptococcus mutans*, confirming that ionizing radiation can activate

the nanofiller surface and enhance antibacterial behavior. Among the tested modalities, CT-Scan produced the strongest effect, followed by CBCT and OPG, which is consistent with their relative radiation dose and exposure conditions. FESEM analysis supported these findings by showing radiation-induced alterations in particle size, surface compactness, and homogeneity, with CT-exposed samples showing the most favorable nanostructure. These structural changes likely increased the generation of reactive oxygen species from zirconia- and silica-based nanofillers, leading to bacterial membrane damage and reduced biofilm formation. Clinically, these results suggest that routine diagnostic radiography may have a secondary beneficial effect by activating the surface of nanocomposite restorations and reducing microbial adhesion at restoration margins. However, further studies are needed to confirm the long-term biological relevance of this effect, evaluate other composite systems, and determine whether repeated exposure produces stable antibacterial benefits without compromising material integrity.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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