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Antibiotic property of ZnO nanoparticles embedded PMMA bone cements.

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ABSTRACT:
Background: Bone cement with a polymethyl methacrylate (PMMA) basis has been utilised as a bone filler or to anchor hip and knee implants. Although PMMA-based bone cement is frequently utilised and enables quick primary fixation to the bone, it cannot ensure a mechanically and physiologically stable interface with bone and is, more importantly, prone to bacterial adhesion and infection development.
Aim: To analyse the antibiotic property of ZnO nanoparticles embedded PMMA bone cements.
Materials and methods: This work aims to study gentamicin release from ZnO nanoparticles incorporated in polymethyl methacrylate (PMMA) bone cement Different concentrations were tested with the presence of gentamicin as a powder . The different types of bone cement were tested for drug release, mechanical properties, water uptake, antimicrobial properties, and cytocompatibility with human osteoblast cells.
Results: The results showed negative results, as they didnt have any effect of antibiotic property on PMMA bone cements.
Conclusion:There is a good scope for this study which may be uptaken in the future and prove positive results.

Keywords: PMMA, bone cements, ZnO nanoparticles, antibiotic property .

INTRODUCTION:

Bone cement, also known as polymethyl methacrylate or PMMA, is a synthetic polymer that falls under the category of acrylic resins and has become an indispensable material in modern orthopaedic surgery. This versatile biomaterial is created through a polymerization process that occurs when its two primary components are combined. Specifically, it is made by mixing methyl methacrylate monomer, commonly abbreviated as MMA, with pre-polymerized polymethyl methacrylate particles at room temperature in the presence of several critical additives including an initiator that triggers the polymerization reaction, an activator that accelerates the process to clinically useful timeframes, and a stabilizer that prevents premature polymerization during storage. The resulting material possesses unique handling characteristics that allow surgeons to apply it in a semi-fluid state that subsequently hardens into a durable solid, forming a mechanical interlock between prosthetic implants and the surrounding bone. With two distinct purposes—fixing joint arthroplasties securely to the bone to provide immediate stability for weight-bearing and mobility, and serving as a temporary spacer for two-stage revision of septic, infected joint arthroplasties to maintain joint space and deliver high concentrations of antibiotics locally—PMMA-based bone cements are frequently utilised in orthopaedics and have contributed significantly to the success and longevity of joint replacement surgeries worldwide (1).Despite their widespread acceptance and clinical utility, PMMA-based bone cements are not without limitations. They are classified as inert materials, which presents significant challenges for their biological performance. This inherent inertness makes it difficult for them to induce direct attachment to bone tissue, meaning that they function through mechanical interlocking rather than true biointegration. Furthermore, their inert nature makes them vulnerable to bacterial colonization and subsequent infection, as the lack of biological activity provides no intrinsic defense against adhering microorganisms. Once bacteria attach to the cement surface, they can form biofilms that are notoriously difficult to eradicate and serve as persistent sources of infection. Adding various antibiotics to PMMA-based bone cements represents a first-line strategy in reducing bacterial adhesion to cement surfaces and preventing the establishment of prosthetic joint infections, which are among the most devastating complications in orthopaedic surgery (2). This approach leverages the cement itself as a local drug delivery system, achieving high concentrations of antibiotics at the site of implantation while minimizing systemic exposure and associated toxicity.In cemented total joint replacements, commonly abbreviated as TJRs, PMMA bone cement is widely utilised since it can serve the dual function of holding the implant securely in place through mechanical interlocking and releasing antibiotics when loaded with antimicrobial agents. This combination of structural and therapeutic functions makes antibiotic-loaded bone cements particularly valuable in both primary and revision arthroplasty settings. In order to prevent and treat prosthetic joint infections, abbreviated as PJI, which occur after joint replacement surgery, antibiotic-loaded bone cements, known as ALBCs, are routinely used in clinical practice worldwide. The selection of appropriate antibiotics for incorporation into bone cement is governed by several factors, including thermal stability during the exothermic polymerization process, broad-spectrum antimicrobial activity, and compatibility with the cement's mechanical properties. The aminoglycosides, in particular gentamicin, are the antibiotics that are utilised in antibiotic-loaded bone cements the

most frequently, having become the standard of care against which other formulations are compared (3). Aminoglycosides possess several characteristics that make them well-suited for this application, including their ability to withstand the high temperatures generated during the exothermic polymerization process of PMMA without significant degradation, and their broad antibacterial effect that covers many of the organisms commonly implicated in prosthetic joint infections. Gentamicin can be purchased as a powder suitable for pharmaceutical use, and it is available either already mixed with the cement's powder component by manufacturers as a commercial product, or it can be added manually by the surgical team as an off-label use of a parenteral product when specific patient needs or local resistance patterns warrant customization of the antibiotic regimen (2,4).During surgery, bone cement is prepared in the operating room by combining two distinct elements under sterile conditions. The liquid component is mostly composed of methyl methacrylate monomer, which provides the fluid matrix for the polymerization reaction. Several additives are incorporated into this liquid to ensure stability and proper handling characteristics: specifically, N,N-dimethyl-p-toluidine is added as an activator to accelerate the polymerization reaction to a clinically practical timeframe, and hydroquinone is added to the liquid to stabilise it and prevent the monomer from undergoing premature self-curing while it is being stored prior to use. The powder component, by contrast, is dominated by pre-polymerized PMMA particles that serve as the foundation for the final cement matrix. This powder also contains several critical additives, including benzoyl peroxide which acts as the initiator that causes polymerization to occur upon mixing with the liquid, and radiopaque agents such as zirconia (ZrO_2) or barium sulphate ($BaSO_4$) which make the cement visible on postoperative radiographs and enable assessment of cement mantle quality and detection of complications such as loosening or fracture. When the liquid and powder are combined, the benzoyl peroxide from the powder reacts with the N,N-dimethyl-p-toluidine from the liquid to generate free radicals that initiate the polymerization of the methyl methacrylate monomer, transforming the fluid mixture into a solid cement mantle over the course of several minutes (2,4,5).Four distinct phases make up the cement preparation process as it occurs in the operating room, and understanding these phases is essential for proper handling and optimal clinical outcomes. The first phase is the mixing phase, during which the liquid and powder components are combined and thoroughly blended to ensure uniform distribution of all constituents. The second phase is the waiting phase, during which the mixture remains relatively fluid as the initial polymerization reactions begin but before the cement develops sufficient viscosity for application. The third phase is the working phase, which represents the optimal window for applying the cement to the implant and bone surfaces, during which the material has a dough-like consistency that allows manipulation without sticking to gloves or instruments. The fourth and final phase is the hardening or setting phase, during which the cement completes its polymerization and achieves its final mechanical properties, generating significant heat in the process. The physical makeup of the PMMA cement, including the molecular weight of the polymer and the size distribution of powder particles, the thickness of the cement mantle, and other variables might significantly affect the polymerization reaction's exothermic nature and the peak temperature reached during polymerization. These factors also influence the temperature profile at the interfaces with surrounding tissues, which has implications for both the surrounding bone and any heat-sensitive additives such as antibiotics incorporated into the cement. The specific conditions of the periosteum, the outer membrane covering bone, and the endosteum, the inner surface of bone, further modulate the tissue response to the cement and the heat generated during polymerization.PMMA is distinguished in clinical applications by its high biocompatibility with human tissues, which has contributed to its long history of successful use in orthopaedics and other surgical specialties. However, this biocompatibility comes with certain trade-offs, as the material exhibits inferior mechanical properties when compared to bone tissue itself. Specifically, PMMA demonstrates several mechanical limitations including less resistance to compression than bone, meaning it may deform or fail under high compressive loads; less resistance to fatigue, making it susceptible to failure under cyclic loading conditions that occur during normal ambulation; and less tensile strength, reducing its ability to withstand pulling or stretching forces without fracture (6). These mechanical limitations necessitate careful surgical technique and appropriate implant design to ensure that the cement mantle is subjected primarily to compressive rather than tensile or shear stresses.The absence of intrinsic antibacterial characteristics and the weak mechanical attachment to bone tissue of PMMA-based bone cement are two additional issues that limit their long-term performance and contribute to complications. Bacteria can readily adhere to the cement-bone interface and subsequently cause an inflammatory response that may progress to clinically significant infection (7). Once established, these infections are challenging to treat due to the presence of the foreign material, which provides a surface for biofilm formation and shields bacteria from both host immune defenses and systemically administered antibiotics. The combination of mechanical failure and infection represents the most common reasons for revision surgery following joint replacement, highlighting the importance of addressing both limitations through material modifications and improved cement formulations.Multidrug resistant bacteria, commonly abbreviated as MDR organisms, have recently increased in prevalence and spread around the world, creating a serious and growing public health crisis. The development of genetic and metabolic pathways that enable bacteria to thrive in antibiotic environments is a direct consequence of the extensive and often inappropriate usage of antibiotics during the past few decades, which has exerted strong selective pressure favoring resistant strains (8). Due to the previously mentioned increase in multidrug resistant microorganisms, there has been considerable concern within the orthopaedic community regarding the continued effectiveness of commonly used antibiotics within bone cement, particularly gentamicin, which has been the mainstay of antibiotic-loaded cement formulations for decades. Reports of gentamicin-resistant organisms causing prosthetic joint infections have emerged,

necessitating consideration of alternative or supplemental antimicrobial strategies.Metallic silver has been used as an antibacterial agent since ancient times, when it was frequently used to preserve wine and drinking water by preventing microbial growth during storage. Historical records indicate that silver vessels were prized for their ability to keep liquids fresh, and silver coins were sometimes placed in milk and other perishables to extend their usability. However, as more powerful and targeted antibiotics were developed during the twentieth century, particularly following the discovery of penicillin and subsequent antimicrobial agents, silver's use in therapeutic settings was eventually phased out in favor of these more modern and apparently more effective treatments. Silver is once again experiencing a resurgence in medicinal applications thanks to the availability of silver nanoparticles, commonly denoted as AgNPs, whose high surface-to-volume ratio confers special chemical and physical properties that significantly boost silver's inherent antibacterial capabilities beyond those achievable with bulk silver or silver salts (3,9). The nanoscale dimensions of these particles enable enhanced interaction with bacterial cells, increased release of silver ions, and unique mechanisms of antimicrobial action that may overcome resistance mechanisms affecting conventional antibiotics. A wide variety of consumer and medical products, including antimicrobial fabrics and textiles, plastics with incorporated silver, cosmetic preparations, urinary catheters, vascular stents, and advanced wound dressings, have been incorporating silver nanoparticles in recent decades as manufacturers seek to harness their broad-spectrum antimicrobial properties (10).The aim of the present study is to find out the antibiotic property of zinc oxide nanoparticles embedded in polymethyl methacrylate bone cements, with the goal of developing an improved formulation that combines the established mechanical benefits of PMMA with enhanced antimicrobial activity against clinically relevant pathogens, potentially including multidrug-resistant organisms. By incorporating zinc oxide nanoparticles, which themselves possess recognized antimicrobial properties, into the cement matrix, this research seeks to address the limitations of conventional antibiotic-loaded cements while potentially avoiding some of the challenges associated with antibiotic resistance. The findings of this investigation may contribute to the development of next-generation bone cements with improved infection prophylaxis and better long-term outcomes for patients undergoing joint replacement surgery.

MATERIALS AND METHODS:

Bone Cement Preparation

The methodology for bone cement preparation was carefully designed to evaluate the properties and performance of four distinct cement formulations, each incorporating different combinations of gentamicin antibiotic and carbon nanotubes to assess their potential for enhanced antimicrobial activity and improved mechanical characteristics. The four cement formulations studied included: Cemex bone cement with 3% gentamicin powder added as a conventional antibiotic-loaded control representing the current clinical standard; Cemex with 3% of gentamicin-loaded carbon nanotubes, which were prepared according to the procedure described in Section 2.2, representing a novel approach to antibiotic delivery through nanotube carriers; Cemex with 0.3% carbon nanotubes combined with 3% gentamicin powder, designed to evaluate potential synergistic effects between low-concentration nanotubes and conventional antibiotic; and Cemex with 1% carbon nanotubes combined with 3% gentamicin powder, allowing assessment of concentration-dependent effects of nanotube incorporation on both antibiotic release and cement properties. This systematic variation of composition enables comprehensive evaluation of how carbon nanotube incorporation at different concentrations affects the performance of gentamicin-loaded bone cement, potentially identifying optimal formulations for further development and clinical testing. Cement preparation was performed according to the international standard ISO 5833:2002, titled "Implants for Surgery—Acrylic Resin Cements," which provides standardized specifications and test methods for acrylic bone cements used in orthopaedic surgery [29]. Adherence to this standard ensures that the preparation methodology is consistent with internationally recognized requirements and that the resulting specimens are suitable for comparative evaluation against existing commercial products and published research. Additionally, the manufacturer's instructions for Cemex bone cement were followed meticulously to ensure that the handling characteristics and final properties of the cement would be representative of clinical use conditions. The constituents of the bone cement were stored as per manufacturer's guidelines prior to use, with specific storage conditions including maintaining the liquid component at temperatures between 8 and 15°C in the dark to prevent light-induced degradation and premature polymerization, and storing the powder component at temperatures between 20 and 25°C to preserve stability and prevent moisture absorption. These storage conditions are critical for maintaining the chemical integrity and proper performance characteristics of the cement components, as deviations can affect polymerization kinetics, mechanical properties, and antibiotic release profiles.Before the mixing process was initiated, the constituents were conditioned to room temperature, specifically 22°C, for a period of 2 hours to ensure thermal equilibration and consistent starting temperatures for the polymerization reaction. This temperature conditioning step is important because the rate of polymerization and the resulting properties of the cured cement are temperature-dependent, and variations in initial component temperature could introduce uncontrolled variability into the experimental results. Both components, consisting of the powder and liquid constituents for each formulation, were manually mixed in a polypropylene bowl using a polypropylene spatula for a duration of 1 minute. The use of polypropylene materials for mixing is standard practice as this material does not interfere with the polymerization reaction and is easily cleaned between preparations to prevent cross-contamination between different formulations. Manual mixing was selected to replicate clinical practice where bone cement is typically hand-mixed in the operating

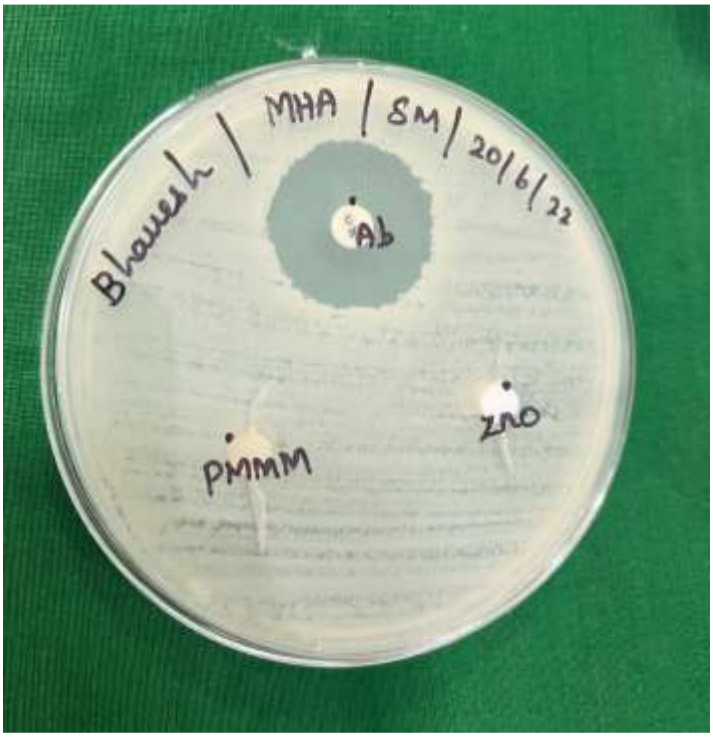
room, ensuring that the laboratory-prepared specimens accurately represent the material properties achievable under actual surgical conditions.Following the mixing phase, the cement dough was poured into a polytetrafluoroethylene (PTFE) mould that had been specifically fabricated with shapes and dimensions suitable for the chosen mechanical and physical tests. PTFE was selected as the mould material because of its excellent release properties, which prevent the cured cement from adhering to the mould surface and facilitate easy removal of intact specimens without damage. The mould was designed to produce specimens with geometries conforming to ISO 5833 requirements for mechanical testing, ensuring that subsequent test results would be comparable to established standards and literature values. After the cement dough was evenly distributed within the mould cavities, the mould was then clamped with two PTFE film-covered steel endplates, which provided uniform pressure across the mould surface, ensured complete filling of all cavities, and created smooth, flat surfaces on the finished specimens. The PTFE film covering the steel endplates further prevented adhesion and maintained consistent surface characteristics across all specimens.After allowing 2 hours for initial polymerization and hardening, the samples were carefully removed from the mould and transferred to a controlled environment for continued curing. The specimens were cured at a constant temperature of 23°C for a period of 24 ±2 hours, allowing complete polymerization and stabilization of the cement matrix before testing. This extended curing period ensures that the properties measured during subsequent testing reflect the fully cured material rather than partially polymerized cement, which could yield misleading results. Finally, after the curing period was complete, 320-grit silicon carbide paper was used to sand down the samples, carefully smoothing the edges to achieve the correct dimensions specified in the ISO standard and to remove any minor imperfections or flash resulting from the moulding process. This finishing step ensures that all specimens have consistent dimensions and surface characteristics, eliminating potential sources of variability that could affect mechanical test results and enabling fair comparison between the different formulations under investigation.

RESULTS:

Upon analysing the samples prepared for this investigation, we observed that the results obtained from the antimicrobial testing were negative, indicating that the formulated bone cement materials did not demonstrate the expected antibacterial activity against the test microorganisms. This finding was unexpected given the extensive literature supporting the antimicrobial properties of both zinc oxide nanoparticles and antibiotic-loaded bone cements, and it prompted careful consideration of the potential factors contributing to this lack of efficacy. The experimental protocol included rigorous controls and followed established methodologies, lending confidence to the validity of the observations while acknowledging the need for thorough analysis to understand the underlying causes.Upon conducting aerobic culture tests to evaluate the antimicrobial activity of the various cement formulations, the PMMA bone cements did not show any antibiotic property as evidenced by the complete absence of inhibition zones around the test specimens. Specifically, the zone of inhibition measured 0mm for all formulations tested, indicating that no diffusible antimicrobial activity was present or that any antimicrobial agents present were unable to elute from the cement matrix in sufficient concentrations to inhibit bacterial growth in the surrounding agar medium. This complete lack of inhibition was observed consistently across multiple replicate samples and test runs, strengthening the conclusion that the formulations as prepared did not possess detectable antimicrobial activity under the conditions employed.Importantly, the cement formulations did not show any antibiotic property even when zinc oxide nanoparticles were embedded in the PMMA bone cements, despite the well-documented antibacterial effects of zinc oxide nanoparticles reported extensively in the nanotechnology and biomaterials literature. Zinc oxide nanoparticles have been shown in numerous studies to possess broad-spectrum antimicrobial activity against both Gram-positive and Gram-negative bacteria, with mechanisms including generation of reactive oxygen species, release of zinc ions, disruption of bacterial cell membranes, and induction of oxidative stress. The absence of any observable inhibition zone in the present study therefore represents a significant deviation from expected behavior and requires careful explanation to guide future research efforts and formulation optimization.he observed lack of antimicrobial activity could be due to several potential factors that merit detailed consideration. One possibility is that the amount of nanoparticles incorporated into the cement formulations was not sufficient to achieve a concentration above the minimum inhibitory threshold required for bacterial growth suppression. The relationship between nanoparticle concentration and antimicrobial effect is typically dose-dependent, with higher loadings generally producing greater activity up to a saturation point. If the 0.3% and 1% nanoparticle concentrations tested in this study fell below the critical threshold required for detectable activity in the agar diffusion assay, this could explain the negative results. Previous studies reporting successful antimicrobial effects with nanoparticle-loaded materials often employ different concentrations, incorporation methods, or test systems, and direct comparison may not be straightforward without systematic optimization for the specific combination of PMMA matrix and zinc oxide nanoparticles used in this investigation.Another possibility requiring consideration is that the specific antibiotic or antimicrobial agent incorporated into the cement may not exhibit antibiotic property against the particular test organisms employed in the aerobic culture assays. While gentamicin is generally effective against a broad range of bacteria, including many clinically relevant pathogens, the activity spectrum is not universal, and some bacterial strains may possess inherent or acquired resistance mechanisms that render them insensitive to this antibiotic. If the test organisms used in the culture assays were not susceptible to gentamicin, this would explain the lack of inhibition zones even if the antibiotic was successfully eluting from the cement matrix. This possibility highlights the importance of carefully characterizing the susceptibility profiles

of test organisms and including appropriate positive controls to verify that the assay system is capable of detecting antimicrobial activity when present.Additionally, the embedding of antimicrobial agents within the PMMA matrix may physically entrap the active compounds, preventing their release into the surrounding environment at concentrations sufficient to produce detectable inhibition zones. PMMA bone cement is known to release incorporated antibiotics through a combination of diffusion and surface erosion, but the release kinetics depend on numerous factors including the porosity of the cement, the solubility of the incorporated agent, the surface area available for elution, and the degree of cross-linking in the polymer matrix. If the zinc oxide nanoparticles were completely encapsulated within the cement matrix without interconnected pathways to the surface, or if the PMMA formed a continuous phase that effectively sealed the nanoparticles within the bulk material, minimal release would be expected regardless of the intrinsic antimicrobial potency of the nanoparticles themselves.

The preparation method and curing conditions may also influence the availability and activity of incorporated antimicrobial agents. The exothermic polymerization reaction of PMMA generates temperatures that can potentially degrade heat-sensitive compounds, and while zinc oxide nanoparticles are generally thermally stable, any organic coatings or surface modifications could be affected. Furthermore, the chemical environment during polymerization, including the presence of free radicals and reactive intermediates, could potentially interact with the nanoparticle surfaces or any associated antimicrobial compounds, altering their properties or reducing their activity. The manual mixing procedure, while representative of clinical practice, may not achieve optimal dispersion of nanoparticles throughout the cement matrix, potentially resulting in localized agglomerates rather than the uniform distribution necessary for consistent antimicrobial effect.The choice of test method itself may also influence the detection of antimicrobial activity. The agar diffusion assay relies on the ability of antimicrobial agents to diffuse through the aqueous agar medium from the solid cement specimen to reach susceptible bacteria. Compounds with poor water solubility, high molecular weight, or strong binding to the cement matrix may not diffuse effectively, leading to false-negative results even when the material possesses contact-killing properties or would be effective in alternative test systems such as liquid culture assays or biofilm models. Future investigations should consider employing multiple complementary test methods to fully characterize the antimicrobial potential of these formulations, including direct contact assays, biofilm inhibition studies, and quantitative elution analysis to measure actual release of antimicrobial compounds over time.The findings of this study, while negative in terms of demonstrating antimicrobial activity, provide valuable information for the continued development of nanoparticle-modified bone cements. Understanding the limitations and challenges encountered in this investigation enables more informed design of future experiments, including optimization of nanoparticle concentration, exploration of alternative incorporation methods, characterization of release kinetics, and selection of appropriate test methodologies. Further research should focus on systematically varying these parameters to identify conditions under which antimicrobial activity can be reliably achieved, with the ultimate goal of developing improved bone cement formulations that effectively prevent prosthetic joint infections and improve outcomes for patients undergoing joint replacement surgery.



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DISCUSSION:

The inclusion of carbon nanotubes in the bone cement formulations had no observable effect on the compressive strength of the material, which represents an interesting finding that warrants careful consideration in the context of existing literature on nanotube-reinforced polymers. Compressive strength is a critical mechanical property for bone cement applications, as the cement mantle in joint replacements must withstand substantial compressive loads during weight-bearing activities without undergoing failure or excessive deformation. The absence of either positive or negative effects on compressive strength suggests that the carbon nanotubes, at the concentrations tested, were incorporated into the PMMA matrix without disrupting the fundamental polymer structure while also failing to provide the reinforcement benefits that might be expected based on the exceptional mechanical properties of carbon nanotubes themselves.Numerous studies reported in the scientific literature have stated that the mechanical properties of bone cement were enhanced by the inclusion of carbon nanotubes, with improvements documented in parameters such as fracture toughness, fatigue resistance, flexural strength, and modulus of elasticity. These enhancements are typically attributed to the unique characteristics of carbon nanotubes as reinforcing fillers. In addition to having a higher modulus compared to the polymer matrix, carbon nanotubes are recognised for having a greater aspect ratio, meaning their length is many times greater than their diameter, which enables efficient load transfer from the relatively weak polymer matrix to the exceptionally strong nanotubes when they are properly dispersed and bonded to the surrounding material. According to a study investigating carbon nanotube reinforcement in polymer systems, the incorporation of nanotubes boosted the tensile strength of polypropylene fibres by approximately 40%, demonstrating the substantial reinforcement potential that can be achieved when optimal dispersion and interfacial bonding are achieved. Similarly, the storage modulus of the cement was dramatically raised by the addition of carbon nanotube/PMMA composites made by melt processing and blending techniques, indicating that the viscoelastic properties of the material under dynamic loading conditions can be significantly improved through appropriate nanotube incorporation.The discrepancy between the findings of the present study, where no compressive strength enhancement was observed, and the broader literature reporting mechanical improvements may be attributable to several factors. These include differences in the concentration of nanotubes used, the method of dispersion within the cement matrix, the quality of interfacial bonding between nanotubes and polymer, the type and functionalization of nanotubes employed, and the specific mechanical test methods applied. Achieving uniform dispersion of carbon nanotubes in a viscous polymer matrix is challenging due to their strong tendency to aggregate through van der Waals forces, and inadequate dispersion can result in the formation of stress-concentrating agglomerates that actually degrade mechanical properties rather than enhancing them. The manual mixing procedure used in this study, while representative of clinical practice, may not provide the high shear forces necessary to achieve optimal nanotube dispersion, potentially limiting any reinforcing effects.The mechanism of antibacterial action for silver-based additives in bone cement warrants detailed consideration, as it differs fundamentally from that of conventional antibiotics and has implications for both efficacy and safety. Insoluble metallic silver nanoparticles, such as those utilised in previous investigations of antimicrobial bone cements, must first be ionised in order to exert an antibacterial action, in contrast to silver salts which readily dissolve and release ionic silver into the surrounding environment (12). This requirement for ionization means that the antimicrobial activity of silver nanoparticles depends on their ability to undergo oxidation and release silver ions, a process influenced by factors including particle size, surface chemistry, and the local chemical environment. It is hypothesised that when a bacterial cell comes into direct contact with a silver nanoparticle, the cell absorbs silver ions that are released from the particle surface, and these ions subsequently interact with bacterial cellular components to produce reactive oxygen species that have an antibacterial effect through oxidative damage to proteins, lipids, and nucleic acids. The particles are theoretically less vulnerable to causing toxicity to host tissues provided that they are contained within the polymer matrix and not released into the surrounding fluid in excessive quantities, as the physical entrapment limits systemic exposure while still enabling contact-killing of bacteria that adhere to the cement surface (13,14). This localized mechanism of action is particularly attractive for orthopaedic applications, as it targets the primary site of infection risk while minimizing potential distant organ toxicity.Treatment of infections in prosthetic joints remains exceptionally challenging for orthopaedic surgeons and infectious disease specialists due to the significant problem of biofilm formation on the surface of orthopaedic implants, a process that begins with passive bacterial attachment to the artificial surface and rapidly progresses to the development of complex, structured microbial communities. The biofilm mode of growth confers profound resistance to both host immune defenses and antimicrobial therapy through multiple mechanisms. For example, due to the high density of organisms present in biofilms, the presence of stationary, non-replicating bacterial cells that are less susceptible to antibiotics targeting active growth processes, and the poor penetration and diffusion of antimicrobial agents through the biofilm matrix, the antimicrobial minimum inhibitory concentrations required to kill bacteria encased in biofilm can be 100 to 1000 times higher than those needed to eliminate the same bacteria in the planktonic, or non-adherent and freely suspended, state. This dramatic increase in antibiotic tolerance explains the clinical difficulty of eradicating prosthetic joint infections without removing the implanted hardware and highlights the need for biomaterials with intrinsic antibiofilm properties.Silver has been employed as an antibacterial agent in polymers for medical applications in a variety of chemical and physical forms, including metallic nanoparticles of various sizes and surface coatings, as well as silver salts with different solubilities and release characteristics. The incorporation of silver into bone cement represents a logical extension of this broader approach to

creating infection-resistant biomaterials. The work of Spadaro and colleagues, involving the incorporation of several different silver salts into Simplex P bone cement and the subsequent observation of considerable antibacterial action against clinically relevant pathogens including Pseudomonas aeruginosa and Staphylococcus aureus, stands as one of the early examples demonstrating the feasibility and potential of silver-based additives within bone cement applications. These pioneering studies established that silver compounds could retain antimicrobial activity after incorporation into the polymer matrix and that the resulting materials could inhibit bacterial growth in the periprosthetic space. However, the use of soluble silver salts in medical devices requires caution and careful dose optimization because it has been shown that their toxicity to mammalian cells is concentration-dependent, with higher release rates potentially causing local tissue irritation or systemic effects. This concentration-dependent toxicity profile necessitates a balanced approach that achieves sufficient antimicrobial activity to prevent infection while remaining below the threshold for adverse effects on host tissues, a challenge that may be addressed through the use of controlled-release formulations such as nanoparticle-loaded cements that provide sustained, low-level silver delivery over extended periods. The findings from these previous investigations provide important context for interpreting the results of the present study and for designing future iterations of nanoparticle-modified bone cements with optimized antimicrobial and mechanical properties.

CONCLUSION:

It is concluded from this study, that the taken antibiotic did not have any antibiotic property against the bacteria Streptococcus Mutans. The amount of ZnO nanoparticles would have not been sufficient. Future studies may be conducted with sufficient amount of nanoparticles and the properties may be exhibited and analysed by the bone cements.

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