“EVALUATION OF ANTIMICROBIAL EFFICACY AND OSTEOGENIC POTENTIAL OF HYDROGEL MADE WITH PALLADIUM NANOPARTICLES AND CHITOSAN COATED ON TITANIUM DISCS: AN IN VITRO STUDY”

BY

REG. NO- IM0222005

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Head of Department

Dr. RAMESH NAYAKAR MDS
Professor & Head,
Department of Prosthodontics
and Crown & Bridge,
KAHER'S Vishwanath Katti Institute
of Dental Sciences, Belagavi.

Date: 16/04/25

Place: Belagavi

Professor and Head
Department of Prosthodontics
KLE V. K. Institute of Dental Sciences,
Belagavi

Principal

Dr. ALKA D. KALE MDS, PhD
Principal,
KAHER's Vishwanath Katti Institute of
Dental Sciences,
Belagavi.

PRINCIPAL
KLE V.K. Institute of Dental Sciences
Nehru Nagar, BELAGAVI-590010.

Date: 17/4/25

Place: Belagavi

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Nehru Nagar, Belagavi - 590 010, Karnataka State

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☎: 0831-2470362 **Web: http://www.kledental-bgm.edu.in**

FAX: 0831-2470640 **E-mail:principal@kledental-bgm.edu.in**

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Date: 17/03/2025

Dr. Mridula Tak
Statistics Expert
Ph: 9928840140
E mail: statisticsolutiononline@gmail.com

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LIST OF ABBREVIATIONS USED IN THE STUDY

Ti-6Al-4V	Titanium 6%Aluminum 4%Vanadium
PdNP	Palladium nanoparticles
S. mutans	Streptococcus mutans
CP	commercially pure
ALP	alkaline phosphatase
ROS	reactive oxygen compounds
DNA	Deoxyribonucleic acid
R_a	Average roughness
MIC	Minimum Inhibitory Concentration
S. aureus	Staphylococcus aureus
E. coli	Escherichia coli
AgNPs	Silver nanoparticles
ECM	Extracellular matrix
GO	Graphene oxide
MBC	Minimum Bactericidal Concentration
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
BHI	Brain Heart Infusion
CTAB	cetyltrimethylammonium bromide
$PdCl_2$	palladium(II) chloride

$NaBH_4$	sodium borohydride
CLSI	Clinical and Laboratory Standards Institute
CFU	colony-forming units
P.gingivalis	Porphyromonas *gingivalis*
DMSO	Dimethyl sulfoxide
SEM	Scanning Electron Microscopy
PBS	phosphate-buffered saline
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme-linked immunosorbent assay
DMEM	Dulbecco's Modified Eagle Medium
CO_2	Carbon dioxide
OD	optical density
ZnO	Zinc oxide
TiO_2	Titanium dioxide
CHX	chlorhexidine

ABSTRACT

STATEMENT OF PROBLEM

The loss of natural teeth due to caries, trauma, or periodontal disease often necessitates restorative interventions such as removable partial dentures, fixed prostheses, or dental implants. Among these, osseointegrated dental implants have demonstrated high success rates, ranging from 83.9% to 98%. However, despite successful osseointegration, implant failure may still occur due to peri-implantitis—a pathological condition marked by inflammation and progressive bone loss around the implant site. Management strategies for peri-implantitis vary depending on severity and are broadly classified into non-surgical and surgical approaches. Non-surgical treatment commonly involves mechanical debridement and adjunctive use of antimicrobial agents such as chlorhexidine gluconate (CHX). Although effective, long-term use of 1% CHX gel is associated with adverse effects, prompting interest in safer alternatives. Herbal and nanomaterial-based formulations have gained attention due to their antimicrobial efficacy and biocompatibility. Palladium nanoparticles and chitosan, in particular, have shown promising antibacterial activity against pathogens implicated in early peri-implant infections. Their incorporation into hydrogels offers a potential site-specific drug delivery system.

The present study aims to develop and evaluate a palladium nanoparticle–chitosan hydrogel for its antimicrobial efficacy against early peri-implantitis pathogens, exploring its potential as a novel therapeutic alternative for localized, controlled drug delivery in implant dentistry.

PURPOSE

To evaluate the antimicrobial efficacy and the osteogenic potential of palladium nanoparticles and chitosan hydrogel coated on titanium discs.

METHODOLOGY

A total of 120 titanium discs (10 mm × 2 mm, ASTM B348 Grade 4) were fabricated and divided into control and experimental groups. Surface modification was done via sandblasting with 50 μm alumina and roughness evaluated using a profilometer. Discs were sterilized prior to use. Palladium nanoparticles (PdNPs) were synthesized using $PdCl_2$, $NaBH_4$, and CTAB, followed by centrifugation and purification. Antimicrobial activity against *Staphylococcus aureus* and *Porphyromonas gingivalis* was assessed using Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) protocols as per CLSI guidelines. A hydrogel was formulated using Carbopol 930, glycerin, preservatives, and incorporation of PdNPs and chitosan. Titanium discs were coated with the hydrogel and characterized using SEM. Osteogenic potential was assessed using MG-63 osteoblast-like cells. Cell attachment was evaluated by incubating cells on coated and uncoated discs for 24 hours, followed by detachment and hemocytometer counting. Cytotoxicity was determined using an MTT assay, where cell viability was measured via absorbance after exposure to the test gel. Antibacterial efficacy was evaluated using the disc-diffusion test. Discs were placed on agar inoculated with test bacteria, and inhibition zones were measured after incubation (24 hours for *S. aureus*, 48 hours for *P. gingivalis*). Discs were compared across three groups: hydrogel-coated, uncoated, and 1% chlorhexidine-coated controls.

RESULTS

This in vitro study investigated the antimicrobial efficacy and osteogenic potential of titanium discs coated with a hydrogel containing 0.125% palladium nanoparticles (PdNPs) and chitosan. Antibacterial activity against *S. aureus* and *P. gingivalis* was assessed using the disc diffusion method and compared with 1% chlorhexidine (CHX) gel. The PdNP–chitosan hydrogel showed a comparable zone of inhibition to CHX, with statistically significant differences ($p < 0.05$) indicating effective antimicrobial activity.

Osteogenic potential was evaluated by assessing MG-63 cell attachment and proliferation at 24, 48, and 72 hours. Cell attachment in the PdNP–chitosan group increased from 1,200,000 ± 55,025 (24h) to 1,440,000 ± 30,276 (72h), compared to the blank gel group, which ranged from 1,100,000 ± 57,445 (24h) to 1,249,700 ± 100,903 (72h). Similarly, MTT values for the PdNP–chitosan group rose from 93.00 ± 4.59 (24h) to 104.00 ± 4.47 (72h), while the blank gel group ranged from 81.60 ± 6.02 to 95.00 ± 4.08. All results showed statistically significant differences ($p = 0.000$) between groups and across time intervals.

These findings suggest that the PdNP–chitosan hydrogel not only demonstrates antimicrobial effects comparable to CHX but also significantly enhances cell attachment and proliferation, indicating strong osteogenic potential for improving dental implant surface integration.

CONCLUSION

This study suggests that palladium nanoparticles with chitosan-based hydrogel offer effective antibacterial action against early peri-implant pathogens, comparable to 1% CHX gel, with potentially less cytotoxicity. Though promising, further clinical studies are needed to confirm its long-term safety and effectiveness.

KEYWORDS

Palladium nanoparticles; chitosan; hydrogel coating; antimicrobial; osteogenic potential; periimplantitis; titanium discs; in-vitro study.

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INTRODUCTION

Tooth decay, periodontal disease and trauma are among the commonest causes of tooth loss. These conditions can impair both, look and functionality, making it harder to chew and communicate. Nowadays, dental implants are a common choice for replacing lost teeth since they improve appearance and help patients regain their ability to chew, both of which improve the patient's general quality of life.[1]

Biocompatible prostheses that are surgically inserted and secured into the alveolar bone are known as dental implants. They support dental restorations and encourage osseointegration by acting as a substitute to roots of teeth.[2]

Biocompatible materials, mostly titanium and its alloys, are used to make dental implants. Ti-6Al-4V, the most often used alloy, has 4% vanadium and 6% aluminum. This alloy is favored due to its exceptional biocompatibility, corrosion resistance and mechanical strength, all of which support efficient osseointegration.[3]

The process of osseointegration, which occurs when the implants are first placed into the bone, creates an environment for healing that promotes regeneration of bone as well as integration with the implant surface. The durability and long-term success of the implant depend heavily on this biological relationship. Osteointegration can be influenced by a number of parameters, including the geometry of the design, surface texture and the physicochemical properties of the implant material.[4]

When bone volume and density are reduced, usually as a result of systemic conditions like osteoporosis or even as a side effect of radiation therapy, osseointegration may be impeded. These conditions may hinder the healing of bone, which could lower the dental implant survival rate to about 55%. In these situations, surface changes that encourage bone formation and expedite the integration process are crucial for improving osseointegration.[5]

For implants to be successfully integrated, the interaction involving biological tissues and a material's surface is essential. Surface improvements can increase biological responses and encourage osseointegration while preserving the material's general characteristics.[4]

Therefore, improving dental implants' osteoconductive properties is essential to getting good clinical outcomes.

In order to enhance the biological properties of dental implants and ultimately facilitate osseointegration, a variety of surface modification techniques have been extensively studied and used. These techniques fall into two categories: additive and subtractive. In order to promote cellular adherence, subtractive procedures such as sandblasting, acid etching or their combination, concentrate on making the surface rougher. Conversely, additive techniques including hydroxyapatite coating, plasma spraying and biomimetic agent deposit are meant to increase bioactivity and improve the possibility of osseointegration.[6]

Most implant surface alterations are intended to support cells that are osteogenic in nature and the signaling chemicals they produce, both of which in turn stimulate the formation of new bone. This procedure increases secondary stability and facilitates the implant's quick osseointegration.[6]

Using biological coatings to chemically modify implant surfaces is a crucial tactic for enhancing osseointegration. A variety of surface coatings including peptides, growth hormones, calcium phosphates and lipids have been investigated by researchers. Additionally, the potential of organic protective coatings, bio polymers and biomimetic agents to affect cellular reactions during implant recovery has been investigated. These alterations facilitate direct contact between the implant and bone, ultimately improving osseointegration.[7]

The utilization of hydrogels, nanoporous structures and polymeric scaffolds as prospective biomaterial surface modification methods to enhance implant integration has been investigated.[8]

Hydrogels are polymeric frameworks that can contain bioactive materials or tissue-engineering matrices and are used to change implant surfaces. They stimulate bone regeneration, offer antibacterial qualities or enable the localized and regulated release of medicinal drugs at the site of implant placement.[9]

Infections associated with biomaterials and those with effective tissue integration are two major issues in dental implant treatment. Infections after implant placement remain a significant risk that can compromise the implant's success, despite rigorous adherence to sterile surgical protocols and cutting-edge antibiotic treatments.[10]

The chances of successful tissue integration are greatly enhanced when cells attach and osseointegration occurs before bacteria can colonize the surface of the implant.[11]

Significant advancements have been made in modifying implant surfaces to tackle key issues, such as reducing biofilm formation to avoid peri-implantitis, promoting osseointegration for faster loading and enhancing implant stability when there is limited bone at the implantation site.[12]

An optimal implant or implant coating should exhibit both antibacterial properties and the ability to promote osseointegration.[12]

As antibiotic resistance rises and some antimicrobials cause negative side effects that hinder osseointegration and may be toxic to cells, there is a heightened interest in natural alternatives. Chitosan, a polysaccharide sourced from the exoskeleton of crustaceans, has emerged as a promising option for hydrogel coatings thanks to its natural antimicrobial properties, compatibility with biological systems, and ability to promote cell adhesion and growth.[13,14]

Chitosan-based hydrogels show significant promise in biomedical uses, especially in enhancing the surface characteristics of implants by displaying antimicrobial properties and supporting osteoblast activity.[14]

Nanoparticles, due to their small size and large surface area, possess distinct physicochemical characteristics that can be utilized to enhance the antimicrobial effectiveness and bone-forming ability of implant surfaces. Among the various types of nanoparticles, palladium nanoparticles (PdNP) have garnered considerable attention because of their potent antimicrobial effects and their capacity to enhance osteogenic differentiation.

By rupturing bacterial membranes and causing oxidative stress, palladium nanoparticles exhibit potent antibacterial activity against a variety of bacterial species.[3*]

Furthermore, PdNP have been shown to promote osteoblast development, which makes them a viable option for implant coatings that combine osteogenic and antibacterial qualities.[15]

PdNP potential for usage in implant coatings is further highlighted by their capacity to inhibit biofilm development, which is a major contributing factor to persistent implant-related illnesses.[17]

The combination of PdNP bone-forming properties and chitosan's antibacterial properties may produce a coating that not only successfully inhibits colonization by bacteria, but also promotes osteoblast growth and development, thus improving titanium implant performance.

This study is sought to evaluate the antibacterial efficacy and osteogenic potential of titanium surfaces augmented through the use of PdNP-chitosan hydrogel coating, taking into account the beneficial properties of both chitosan and PdNP.

NEED FOR THE STUDY

Dental implants have become a cornerstone in contemporary restorative dentistry, providing a reliable and functional alternative to replace missing teeth. Compared to traditional fixed and removable prostheses, implants offer superior aesthetics, stability, and preservation of oral function. These biocompatible devices integrate with the jawbone to serve as artificial tooth roots, forming a stable foundation for prosthetic restorations that closely mimic the natural dentition.

Despite adherence to established surgical and prosthetic protocols, implant failures can still occur, primarily due to peri-implantitis. Peri-implantitis is a progressive inflammatory condition characterized by the destruction of peri-implant soft tissues and supporting alveolar bone, leading to the formation of peri-implant pockets and potential implant loss.[5,18]

Chlorhexidine gluconate (CHX) is commonly used for the antimicrobial management of early peri-implantitis due to its broad-spectrum activity against gram-positive and gram-negative bacteria, fungi, yeasts, and some viruses.[12*] Typically administered as a 0.2% solution or 1% gel, CHX remains a gold standard in local antimicrobial therapy. However, its long-term use is associated with several adverse effects, including staining of teeth and restorations, altered taste sensation, mucosal irritation, and rare allergic reactions.[15]

Given these limitations and the multifactorial etiology of peri-implantitis, there is a compelling need for safer and more effective therapeutic alternatives that not only combat microbial biofilms but also support peri-implant tissue regeneration.

To improve the osseointegration of metallic implants with surrounding bone tissue, various surface modification techniques—both mechanical and chemical—have been employed. An ideal implant or implant coating should therefore possess dual functionality: promoting osseointegration while exhibiting antimicrobial activity.

Hydrogels, which are three-dimensional crosslinked polymeric networks capable of absorbing large volumes of water, have gained significant attention as carriers for cells, drug delivery systems, self-healing materials, and scaffolds in tissue engineering applications.[19]

Chitosan, a naturally derived, biodegradable, and biocompatible polysaccharide, has shown broad applicability in dentistry owing to its functional versatility, accessibility, and inherent antibacterial properties.

Noble metals have long been recognized for their antimicrobial effects, historically used for disinfecting water and promoting wound healing. Among these, nanoscale silver has garnered interest; however, recent reports of microbial resistance to silver nanoparticles highlight the need for alternative materials.

Given these considerations, the present in vitro study aims to evaluate the antimicrobial efficacy of a hydrogel incorporating palladium nanoparticles and chitosan against *P. gingivalis* and *S. aureus*, as well as to assess its osteogenic potential when coated on titanium discs.

HYPOTHESIS

NULL HYPOTHESIS

- There is no difference in the antimicrobial efficacy and osteogenic potential of titanium discs coated with palladium nanoparticles and chitosan gel.

RESEARCH HYPOTHESIS

- There is difference in the antimicrobial efficacy and osteogenic potential of titanium discs coated with palladium nanoparticles and chitosan gel.

AIMS AND OBJECTIVES

- To evaluate the antimicrobial efficacy and the osteogenic potential of palladium nanoparticles and chitosan hydrogel coated on titanium discs.

REVIEW OF LITERATURE

1. K. J. Bundy and associates investigated the antibacterial effects of pure metallic substances on *Streptococcus mutans* development in-vitro in anaerobic as well as aerobic environments in 1980. Their study, which examined 16 distinct pure metals, revealed that the impact of nickel, titanium, iron and vanadium on bacterial proliferation varied. They identified specific threshold concentrations that halted bacterial proliferation, highlighting significant variability in antimicrobial effectiveness among the metals. Cobalt proved particularly effective at lower concentrations. The results indicate that *S. mutans* has varying sensitivities to different metals, with threshold concentrations differing between aerobic and anaerobic conditions; *S. mutans* showed greater resistance in anaerobic conditions, even though corrosion levels were similar.[20]

2. In 1985, Gregory R. Parr and colleagues highlighted the essential properties of titanium and its alloys for dental uses. For successful long-term implants, it is crucial that these materials possess biocompatibility, mechanical strength, toughness, corrosion and wear resistance, along with fracture toughness. Titanium & alloys are preferred in dental implants for their outstanding biocompatibility, high strength and excellent corrosion resistance. The most frequently used alloys for dental implants fall within the alpha-beta phase category, with Ti-6Al-4V (6% aluminum & 4% vanadium) being the most commonly selected. After undergoing heat treatment, these alloys demonstrate improved physical and mechanical characteristics, making them particularly advantageous for implant applications.[21]

3. In 1987, Mombelli A. et al. performed a comparative study on microflora linked with both accomplished and detoriated osseointegrated implants. The research focused on the microbial makeup of endosteal titanium hollow cylindrical implants by using various methods, including microscopic, immunochemical and cultural techniques. Samples were collected by five edentulous patients whose implants had functioned successfully as overdenture abutments for over a year and compared to samples from seven patients with clinically failing implants. The results showed that the peri-implant sites had a diverse microbiota mainly composed of gram-negative anaerobic rods, while control sites in the same patients had significantly fewer bacteria. Interestingly, the microbiota in the control sites of patients with failing implants closely matched that of the successfully integrated implants. The study concluded that peri-implantitis should be viewed as a site-specific infection, sharing many similarities with chronic adult periodontitis.[22]

4. In 1991, Sennerby L. et al. conducted research to explore implant-related factors that relate to the reactions at the bone-metal interface around titanium screws. The research centered on the ultrastructural interfacial reactions to titanium implants, comparing the tissue responses to commercially pure (CP) titanium and Ti-6Al-4V alloys. Additionally, the study investigated essential surface characteristics and structural properties needed for effective osseointegration, as well as possible bonding mechanisms at the bone-titanium interface. The results indicated that the responses at the bone-metal interface are not determined solely by implant-specific factors; surgical techniques and loading conditions also play significant roles in achieving successful and predictable osseointegration.[23]

5. In 1992, Ong and colleagues carried out a study to examine the peri-implant area in 19 patients aged between 22 and 77, each having at least two osseointegrated Brånemark titanium implants. They conducted microbiological evaluations to identify the presence of *A. actinomycetemcomitans, P. gingivalis,* and *P. intermedia* at 37 implant locations. Patients instructed to incorporate a daily regimen of 0.2% chlorhexidine gluconate mouthwash as part of their oral care practice. The findings revealed that 22 out of the 37 sites had a greater number of anaerobic bacteria than aerobic ones.[24]

6. In 1999, Gerald McDonnell and colleagues explored the antibacterial characteristics of chlorhexidine. According to their findings, chlorhexidine has fungicidal and bactericidal effects, however it is useless against mycobacteria and bacterial spores. It has demonstrated the ability to eradicate Acanthamoeba cysts at high doses, despite its limited antiviral properties [27]

7. In 2003, Lauk et al, focused on the potential of chitosan-based hydrogels that are for regenerative medicine to stimulate cell proliferation and differentiation. Their analysis demonstrated how chitosan is a perfect option for biomedical applications, particularly in tissue engineering, due to its biocompatibility and biodegradability. Additionally, the study looked at how chitosan hydrogels foster a healing environment that promotes wound healing, tissue regeneration and cell proliferation. To expand their uses in regenerative medicine, the researchers also noted the potential for chitosan hydrogels to be used as delivery vehicles for bioactive compounds. [28]

8. In 2004, K.M. Woo and his team carried out research on a hydrogel system aimed at promoting bone regeneration. Their results revealed that chitosan-

based hydrogels encourage the adhesion and growth of osteoblasts, underscoring their potential as biomaterials for bone tissue engineering. The research also highlighted the hydrogels' capability to create a supportive microenvironment, crucial for cellular differentiation and extracellular matrix formation, both vital for successful bone regeneration. Furthermore, the researchers examined the mechanical and biochemical properties of the hydrogel system, underscoring its appropriateness for clinical use in regenerative medicine and implantology.[29]

9. In 2006, study by J. Rinaudo looked at use of chitosan in tissue engineering of bone, with an emphasis on how it can encourage bone regeneration. The study emphasized how chitosan is an ideal material for hydrogel coverings on implants because it is biocompatible, biodegradable and can increase osteoblast activity. The results also demonstrated the function of chitosan in promoting cell adhesion, growth and extracellular matrix formation—all of which are essential for successful osseointegration. The study also looked at how chitosan-based hydrogels might be used to distribute growth factors and bioactive compounds, which would increase their usefulness in altering implant surfaces to enhance clinical results. [30]

10. In 2007, C. Gerente et al. conducted a comprehensive evaluation of the biological applications of chitosan and its byproducts, with an emphasis on their antibacterial and osteoconductive qualities. The study highlighted the unique biological characteristics of chitosan, including its capacity to promote cell adhesion and development as well as its biocompatibility and biodegradability. The article also discussed chitosan-based materials' intrinsic antibacterial properties, which mostly originate from their contact with bacterial membranes

and subsequent cell lysis. The study also looked at the osteoconductive capability of chitosan, which demonstrated how well it works as an implant coating to promote bone regeneration and ease osseointegration. These findings demonstrate how versatile chitosan is as a biological material for a variety of biomedical uses, particularly in tissue engineering and implantology.[31]

11. In 2008, Lindhe J. & Meyle et al. conducted a study to identify the causes and treatment approaches for mucositis and peri-implantitis, Lindhe J. & Meyle et al. conducted a study in 2008. They determined that smoking, a history of periodontal disease, poor oral hygiene, and residual dental cements were important risk factors. Mechanical debridement is used to remove plaque in order to treat peri-implant mucositis, and non-surgical mechanical therapy has shown promise in this regard. On the other hand, attempts to mechanically clean the implant surface—whether or not they were paired with antibiotic therapy—were mainly unsuccessful in treating peri-implantitis. [32]

12. in 2009, Antonio Fernández-Barbero and associates investigated the mechanical properties, refractive index, and internal structure of polymer networks on hydrogels. These materials, which are categorized as superabsorbents, can hold several hundred times their weight in solvent and respond quickly to changes in their local environment. Microgels, due to their ability to change size and structure internally, have been investigated for use as carriers for therapeutic agents and diagnostic tools. [33]

13. In 2010, Wennerberg A. and his team emphasized the importance of implant surface roughness for successful osseointegration and long-term stability of implants. Their study found that a surface roughness of 1–2 μm is optimal for

encouraging osseointegration, with moderately rough surfaces (Sa between 1.0–2.0 μm) showing the best clinical results compared to smoother or overly rough surfaces. [34]

14. In 2010, Krisztina Ungvari and her collaborators assessed the effectiveness of three cleaning solutions—3% hydrogen peroxide (H_2O_2) for 5 minutes, saturated citric acid (pH 1) for 1 minute, and chlorhexidine gel for 5 minutes—on commercially pure grade 4 machined titanium discs. They evaluated how these treatments affected the attachment and proliferation of human epithelial cells (at 24 and 72 hours, respectively) using the MTT and BCA protein content assays. The research found that none of the cleaning solutions harmed the titanium surface, and treatment with H_2O_2 resulted in a slight increase in epithelial cell proliferation when compared to chlorhexidine gel. [35]

15. In 2008, Stefan Renvert et al. conducted a literature review focusing on non-surgical treatment methods for peri-implant mucositis and peri-implantitis. Their findings showed that mechanical non-surgical therapy effectively addressed lesions of peri-implant mucositis, with enhanced results when antibacterial mouth rinses were added to the treatment plan. On the other hand, non-surgical methods for managing peri-implantitis showed limited success, with chlorhexidine having little effect on clinical and microbiological outcomes. However, the administration of local or systemic antibiotics was effective in reducing bleeding on probing and lowering probing depths. [36]

16. In 2009, D.R. Monteiro et al. conducted research on the Antimicrobial Effect of Medical Devices Containing Silver, revealing that nanoparticle coatings, including those with palladium nanoparticles (PdNP), are essential for

decreasing microbial attachment and biofilm development on medical devices. The study indicated that nanoparticles provide antimicrobial activity through various mechanisms, such as damaging bacterial membranes, blocking enzymatic functions, and producing reactive oxygen species (ROS), which ultimately leads to the death of bacterial cells. Additionally, PdNP were found to disrupt quorum sensing processes, preventing the maturation and persistence of biofilms on implanted surfaces. The research highlighted the importance of nanoparticle-enhanced medical devices in lowering infection risks in biomedical implants and prosthetics, where bacterial colonization presents a significant issue. These results emphasize the potential of PdNP coatings to improve the long-term effectiveness and biocompatibility of dental and orthopedic implants by reducing infection-related problems. [37]

17. In 2009, M. Shirosaki and colleagues explored the biocompatibility of chitosan-based hydrogels, underscoring their potential for implantable medical devices. Their findings indicated that chitosan hydrogels possess remarkable biocompatibility, fostering an environment suitable for cell adhesion, growth, and differentiation. According to the researchers, chitosan is a great option for biomedical applications because of its inherent qualities, which include biodegradability, low toxicity, while encouraging for tissue regeneration. Additionally, the study suggested that hydrogels made from chitosan might efficiently transport bioactive compounds, increasing their usefulness in implant coating to encourage osseointegration and guard against infections. [38]

18. In 2010, P. Jayakumar and colleagues performed a thorough investigation of chitosan-based nanocomposites in regenerative medicine, emphasizing their capacity to inhibit infections and enhance tissue regeneration. According to the

study, chitosan's inherent biocompatibility and biodegradability make it a great scaffold material. When chitosan-based nanocomposites are mixed with nanoparticles, such as palladium nanoparticles (PdNP), they exhibit improved antibacterial and osteogenic potential. Because of their many uses, they are very promising choices for tissue engineering, particularly for implantable medical devices where it is essential to promote bone mending and infection control at the same time. [39]

19. In 2011, F.J. O'Brien conducted a comprehensive study on the importance of scaffolds based on biomaterials in regenerative medicine is highlighted in Biomaterials & Scaffolding for Tissue Engineering. Palladium nanoparticles' (PdNP) capacity to stimulate osteoblast development was highlighted in this work, which is essential for bone applications in tissue engineering to be effective. By promoting cellular signaling pathways that control osteoblast development, differentiation, and extracellular matrix mineralization, PdNP have been shown to increase osteogenic activity. Their significance in bone regeneration is highlighted by their function in regulating important osteogenic indicators, including alkaline phosphatase (ALP) action and the expression of genes linked to bone. Furthermore, when added to scaffolds, PdNP strong surface reactivity promotes improved cell adhesion and bioactivity. According to these results, PdNP may be useful nanomaterials for creating cutting-edge biomaterials that will speed up bone growth and enhance the functionality of implant coatings and bone transplant alternatives. [40]

20. In 2011, R. Ghosh and colleagues explored the application of nanoparticles of (PdNP) in medical implants, with a focus on the influence of nanoparticles on antimicrobial coatings. Their study highlighted PdNP remarkable

physicochemical qualities, which strengthen their potent antibacterial effects by breaking down bacterial membranes and generating reactive oxygen species. Furthermore, it was discovered that adding PdNP to implant coatings increased surface bioactivity, reduced bacterial adhesion, and prevented the formation of biofilms, all of which increased implant longevity and reduced the risk of implant-related illnesses. These findings demonstrate how PdNP-based nanocoatings, which promote osseointegration and inhibit microbiological development, have promise for improving biomedical implants. [42]

21. In 2011, Y. Xie and colleagues conducted research on the processes and antibacterial qualities of zinc oxide nanoparticles, examining how well they work against different bacterial pathogens in conjunction with palladium nanoparticles (PdNP). According to the research, the primary mechanism by which these nanoparticles have antibacterial effects is through the generation of reactive oxygen compounds (ROS), which cause oxidative stress and cause peroxidation of lipids, protein degradation, and DNA damage in cells of bacteria, all of which kill the cells. Additionally, it was discovered that PdNP weaken the integrity of microorganism membranes, making them more permeable and causing the intracellular contents to be lost. The study also demonstrated that these nanoparticles' antibacterial activity is particularly potent against Gram-positive and Gram-negative bacteria, establishing PdNP as a promising antibacterial substance for biomedical applications where infection prevention is crucial, such as in implant the coatings and wound dressings. [43]

22. In 2013, B.D. Ratner and colleagues published a detailed study titled "Biomaterials Science: An Introduction to Materials in Medicine," which analyzed the properties and medical applications of titanium and other

biomaterials. The investigation validated the extensive employment of titanium in implants, owing to its great biocompatibility, superior mechanical qualities, and high durability against corrosion. However, the investigation identified a major disadvantage: titanium lacks intrinsic antibacterial qualities. This flaw increases the possibility of pathogenic bacteria attachment and biofilm formation on implant surfaces, which could result in peri-implant infections and endanger the implants' long-term viability. The results highlighted the need for antimicrobial coatings and surface modifications to enhance titanium's biological properties and lower the risk of implant-related infections. [44]

23. In 2013, H. Liu and colleagues examined Hydrogels based on biodegradable chitosan for biomedical applications, with a focus on their dual use in implanted devices. According to the study, these hydrogels are essential for promoting bone development and preventing bacterial infections because of their remarkable biodegradability. Because of these properties, chitosan-based hydrogels are perfect for implant coverings, where fostering bone regeneration and having antibacterial properties are essential for implants' long-term viability. [45]

24. In 2014, D. Patra and colleagues explored the Palladium nanoparticles (PdNP) have antibacterial and bone-promoting properties for use in biomedicine. The study highlighted PdNP dual functions, emphasizing both their potential to inhibit microbial development and promote bone regeneration. By rupturing bacterial membranes and producing oxidative stress, the scientists discovered that PdNP effectively prevent biofilm development, reducing the likelihood of implant-related illnesses. The study also looked at PdNP osteogenic qualities, highlighting how they can promote osteoblast development and mineralization.

These findings imply that PdNP can be used as a multipurpose coating for implants, providing improved osseointegration in addition to antimicrobial protection.[46]

25. In 2015, V. Singh et al. investigated applications of palladium-based nanoparticles in the biomedical field, emphasizing their versatility and encouraging prospects for use in medical implants. The study demonstrated the remarkable antibacterial properties of nanoparticles of (PdNP) and elucidated how they work by causing bacterial membranes to rupture and generating reactive oxygen compounds (ROS). It also took into account the potential uses of PdNP in fields like tissue engineering, medication delivery, and biosensing. The findings demonstrated the adaptability of palladium-based nanoparticles, particularly in the development of sophisticated implant coatings that enhance the longevity and efficacy of implants by combining osteogenic and antibacterial qualities.[47]

26. In 2015, J. Venkatesan and colleagues examined chitosan-alginate biocomposites infused with fucoidan for use in bone tissue engineering. Their research demonstrated how chitosan-based hydrogels can improve implant surface characteristics by increasing osteoblast activity and antibacterial efficiency. A naturally produced polysaccharide called chitosan has built-in antibacterial properties that stop germs from growing on implants, reducing the risk of peri-implant infections. Better osseointegration is also made possible by its biocompatibility and bioactivity, which encourage osteoblast adhesion, proliferation, and differentiation. The biofunctionality of this composite is further enhanced by the inclusion of fucoidan as a sulfated polysaccharide known for its bone-forming and anti-inflammatory properties. This study

demonstrated the value of chitosan-based hydrogels that are as adaptable biomaterials that promote bone regeneration and provide antimicrobial protection, which makes them perfect for implant coatings and bone tissue engineering. [48]

27. In 2015, Xureb M. et al. performed a systematic review examining materials and cutting-edge coating methods for dental implants. They discovered a number of substances that could enhance implant performance, including calcium, a mineral hydroxyapatite, and bone-stimulating factors. The most widely utilized methods, which involve directly applying a coating material from a solution onto the implant's surface, were specifically mentioned in the review as hydrocoating and plasma spraying. [49]

28. In 2016, Z. Huang and colleagues investigated the potential of Multifunctional Nanocomposite Hydrogels for Biomedical Applications, specifically boosting osteogenic activity. PdNP displayed excellent antibacterial activity by creating reactive oxygen species (ROS) and damaging bacterial membranes, hence preventing biofilm formation on implants. Simultaneously, these nanoparticles increase osteoblast adhesion, development, and differentiation, which improves bone repair and osseous integration. Additionally, hydrogels function as effective delivery vehicles, enabling for controlled and sustained release of PdNP at the implant site, resulting in longer-lasting therapeutic effects. These findings suggest that hydrogels combined with PdNP could be a promising method for next-generation implant coverings, enhancing biocompatibility, infection resistance, and bone healing abilities. [50]

29. In 2016, Amparo Mendoza-Arnau et al. explored the surface morphology of implants, focusing on how surface irregularities influences cellular attachment and osseointegration. They found that while moderately rough surfaces foster cell attachment, excessive roughness can hinder osseointegration and biological reactions. The study recommended an optimal Ra roughness of 0.775µm ± 0.058µm and an Rt range of 5.258µm ± 0.554µm, although the implant systems examined generally had lower measurements. The results reinforced that a moderate Ra value is conducive to optimal cell adhesion and emphasized that excessive roughness isn't necessary for achieving a favorable cellular response. [51]

30. To investigate the antibacterial efficacy of chitosan and its quaternized form against both Escherichia coli and Staphylococcus Embrapa et al. carried out an in vitro investigation in 2016. The Disc Diffusion Method was used to test the antibacterial activity, and the Microdilution technique was used to determine the Minimum Inhibitory Concentration (MIC). The findings showed that chitosan particles effectively prevented the growth of both bacterial species, with the gram-positive S. aureus being more effectively suppressed than the gram-negative E. coli. The unique makeup of bacterial cell walls is responsible for this variation in sensitivity; gram-positive bacteria's thick peptidoglycan coating improves their interaction with chitosan, increasing their antibacterial efficacy. These findings demonstrate how chitosan-based materials can be used to make antimicrobial coatings for implant surfaces and other biomedical environments to help stop bacterial colonization and illness. [52]

31. In 2017, Darshan et al. carried out a study to evaluate the antimicrobial effectiveness of a natural bioactive substance, chitosan, in combination with

silver nanoparticles (AgNP), against common pathogens associated with dental implants. The results showed that using Ag-chitosan nanoparticles as a coating for titanium dental implants not only increased antimicrobial activity but also improved resistance to corrosion. Additionally, the study indicated that this nanocomposite coating could enhance the passivation of titanium implants, potentially boosting their durability and success in clinical settings. The research highlights the importance of nanotechnology-based surface modifications in creating multifunctional coatings for implants that provide both antimicrobial protection and improved material stability. [53]

32. In 2017, A. Kumar and colleagues conducted research on how nanoparticle coatings can improve the performance of biomedical implants. Their review discussed the effectiveness of various nanoparticles, particularly palladium nanoparticles (PdNP), in enhancing the antimicrobial and osteogenic characteristics of implant surfaces. The study pointed out that PdNP possess significant antibacterial properties by disrupting bacterial membranes and inducing oxidative stress, which helps to lower the likelihood of infections associated with implants. Furthermore, the inclusion of PdNP in implant coatings was shown to promote osteoblast differentiation and mineralization, leading to quicker and more effective osseointegration. The results highlighted the promise of nanoparticle coatings in addressing current challenges in implantology by providing a versatile surface that both prevents bacterial colonization and facilitates bone integration. [54]

33. In 2018, Jyoti and colleagues conducted an in vivo study to investigate the effectiveness of a chitosan-gold nanoparticle gene delivery system for c-myb in osteoporotic rats. The research aimed to determine if delivering c-myb through

this nanocomposite could improve implant integration in osteoporotic conditions. Results showed that c-myb, delivered via chitosan-gold nanoparticles, significantly enhanced the osseointegration of dental implants, even in weaker bone environments. This indicates that c-myb may be useful in promoting the stability and integration of dental implants, particularly in cases of age-related bone loss like osteoporosis. The study underscores the potential of nanomaterial-based gene delivery methods to boost bone regeneration and enhance clinical results for patients with compromised bone metabolism. [55]

34. In 2018, Sara and colleagues performed an animal study to evaluate the osseointegration capabilities of titanium implants that were coated with an antimicrobial nanostructured noble metal. The results indicated that the bone response to these coated implants, which demonstrated anti-adhesive effects against *Staphylococcus aureus* in vitro, was comparable both qualitatively and quantitatively to that of standard machined titanium screws used clinically. These results imply that the nanostructured noble metal coating did not hinder the osseointegration process, presenting a promising method for improving implant surfaces by introducing antimicrobial features without negatively impacting bone integration. [56]

35. In 2021, A. Sathiyaseelan et al. conducted a comprehensive study on the creation and analysis of palladium nanoparticles (PdNP) embedded in a graphene oxide (GO) nanocomposite. Their findings revealed that PdNP exhibit exceptional antibacterial properties, mainly due to their capacity to disrupt bacterial cell membranes and trigger oxidative stress. The research emphasized that PdNP produce reactive oxygen species (ROS), which result in cellular damage, protein denaturation, and ultimately bacterial cell death. Additionally,

the interaction of PdNP with bacterial membranes modifies membrane permeability, jeopardizing cellular integrity and hindering microbial growth. The inclusion of graphene oxide further amplifies these antimicrobial effects by offering a large surface area for nanoparticle distribution, enhancing stability, and supporting prolonged antibacterial activity. These results highlight the potential applications of PdNP-based nanocomposites in the biomedical field, especially in implant coatings and wound healing, where effective infection management is essential. [57]

MATERIALS AND METHODOLOGY

SOURCE OF DATA:

- KLE Academy of Higher Education (KAHER's)

1. The Department of Prosthodontics, Crown and Bridge, KLE Vishwanath Katti Institute of Dental Sciences, Belagavi
2. KAHER Dr. Prabhakar Kore's BSRC Belagavi (Evaluation of adhesion and proliferation of osteoblast-like cells)

- Department of Mechanical Engineering, KLS Gogte Institute of Technology, Belagavi (For analysis of surface roughness using surface profilometer)

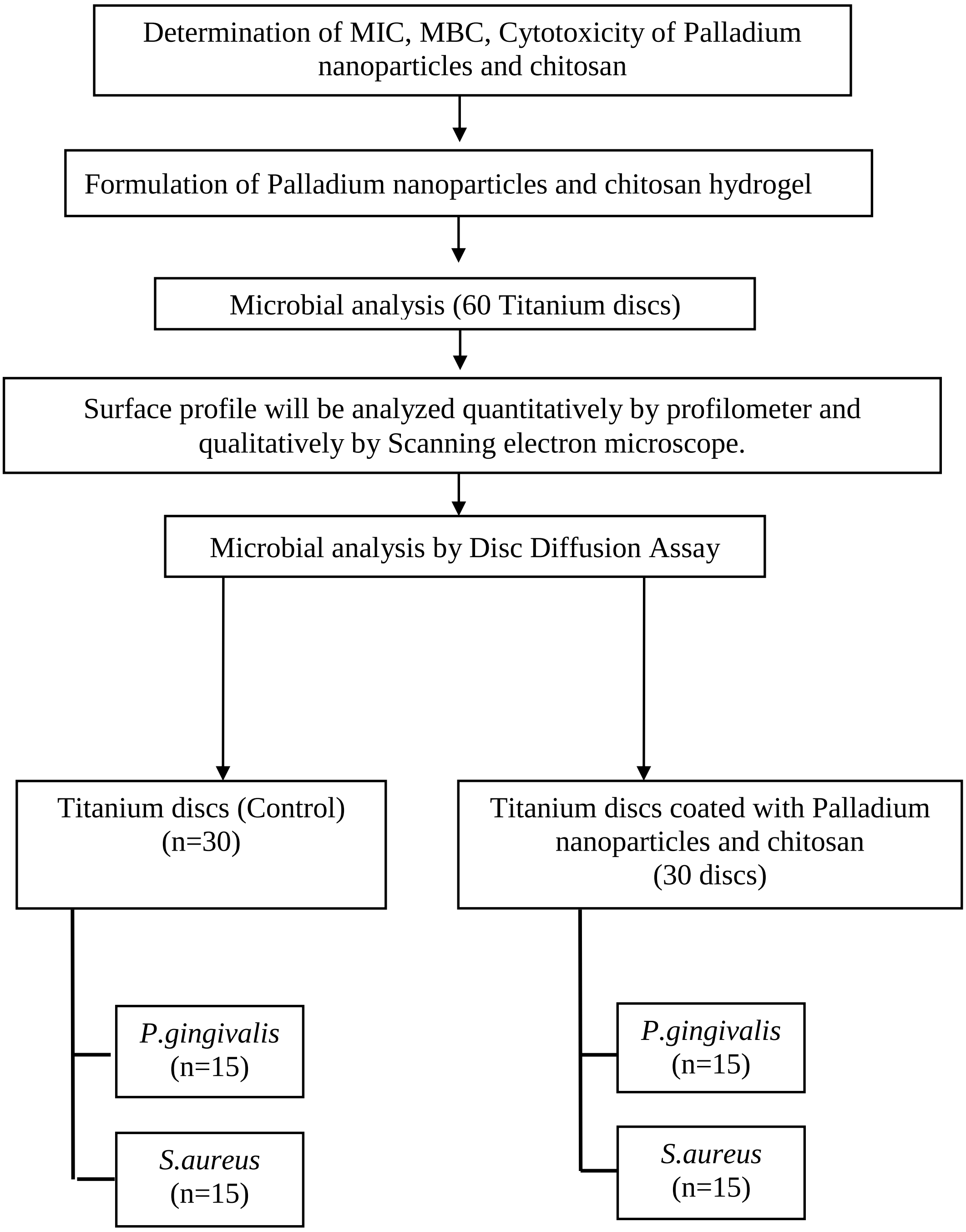
METHODS OF COLLECTION OF DATA:

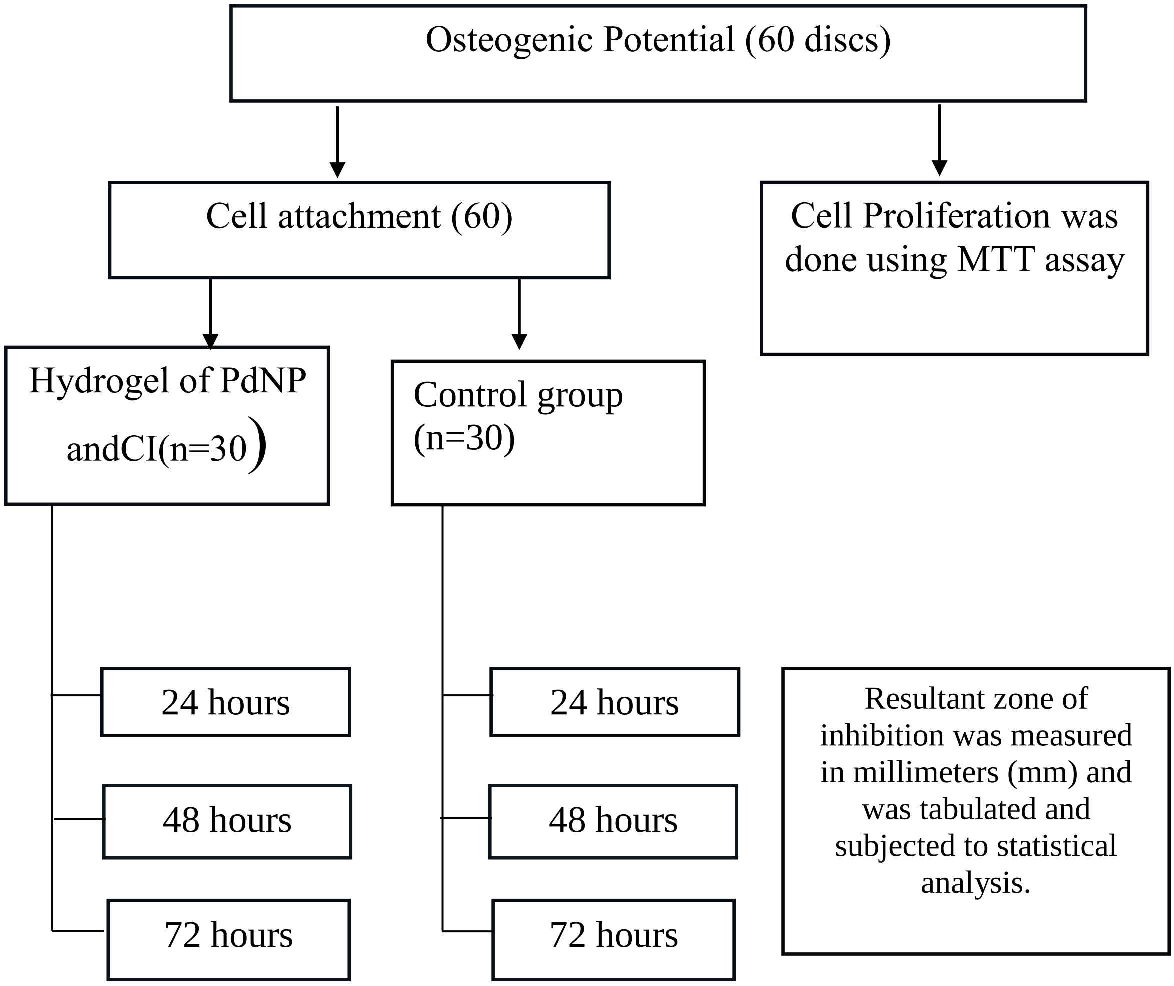
INCLUSION CRITERIA

- Identical Titanium disc shaped specimens measuring 10 mm in diameter and 2 mm in thickness was included in the study. (ASTM B348)

EXCLUSION CRITERIA

- Specimens with internal and external porosities.
- Specimens with surface irregularities (R_a> 5µm)


Determination of MIC, MBC, Cytotoxicity of Palladium nanoparticles and chitosan
Formulation of Palladium nanoparticles and chitosan hydrogel
Microbial analysis (60 Titanium discs)
Surface profile will be analyzed quantitatively by profilometer and qualitatively by Scanning electron microscope.
Microbial analysis by Disc Diffusion Assay
Titanium discs (Control) (n=30)
Titanium discs coated with Palladium nanoparticles and chitosan (30 discs)
P.gingivalis (n=15)
S.aureus (n=15)
P.gingivalis (n=15)
S.aureus (n=15)



Osteogenic Potential (60 discs)
Cell attachment (60)
Cell Proliferation was done using MTT assay
Hydrogel of PdNP andCI(n=30)
Control group (n=30)
24 hours
48 hours
72 hours
24 hours
48 hours
72 hours
Resultant zone of inhibition was measured in millimeters (mm) and was tabulated and subjected to statistical analysis.

MATERIALS

Table 1: Materials used in the study

Materials	Description	Manufacturer
Titanium alloy	TYPE V (Ti-6Al-4V alloy)	Special Metals, Mumbai, India
Hydro-alcoholic solvent	Absolute Ethanol	Analytical reagent
Mueller hinton agar	Culture media	Hi-media
BHI blood agar	Culture media	Hi-media
Carbopol gel base	Carbopol 940	Hi- media
Distilled water	-	-
Palladium nanoparticles and chitosan incorporated Bio Gel	Palladium nanoparticles	Novel

Table 2: Armamentarium used in the study

ARMAMENTARIUM	DESCRIPTION	MANUFACTURER
Incubator	Sr.no: ZBCI-08444	kemielektrotechnik ltd India
Weighing machine	Unibloc weighing balance, ELB series	Shimadzu Balances
Profilometer	Contact prophylometer	Taylor Hobson, Brazil
Water bath	Rectangular water bath	Labline
Anaerobic Jar	Mcintosh Fildes’ Jar	Techno source
Laminar Air Flow	Model ANC 4D	Esco Air stream
Microtitre Plate Reader	Epoch	BioTek, USA

METHODOLOGY

A. SPECIMEN PREPARATION

B. SURFACE CHARACTERIZATION

C. FORMULATION OF EXTRACT

D. DETERMINATION OF MIC

E. DETERMINATION OF MBC

F. FORMULATION OF HYDROGEL

G. OSTEOGENIC POTENTIAL:

H. CELL ATTACHMENT

I. DISC-DIFFUSION TEST

SPECIMEN PREPARATION -

A total of 120 discs of commercially available pure Titanium grade 4 were fabricated of diameter 10 mm and a width of 2 mm (ASTM B348). The discs were subdivided into four groups as control and experimental. Minimum sample size per sub group was estimated to be 30.(Fig 1)

SURFACE CHARACTERIZATION

Identical titanium discs measuring 10 mm × 2 mm were sandblasted for 1 minute at a constant pressure of 4 kg/cm² using 50 µm alumina. The discs were then ultrasonically cleaned with acetone for 180 seconds to eliminate any residual contaminants. Surface roughness was assessed using a contact stylus profilometer, (Fig 2)and an average roughness value was calculated for each specimen. Each disc was positioned on a level surface with the testing side facing upward. The profilometer measured surface roughness along three lines on the disc surface. After mounting the disc specimen, the mathematical average of all absolute surface roughness distances from the center-line within the measuring length was determined using a diamond-point stylus with a lateral length of 4 mm and a cut-off length of 0.8 mm. In this study, discs with an ideal surface roughness ranging from 0 to 5 μm were used. Finally, the discs were sterilized in an autoclave at 121°C for 15 minutes.(Fig 3)

FORMULATION OF EXTRACT[22]

Palladium nanoparticles (PdNP) were synthesized using sodium borohydride ($NaBH_4$) as a reducing agent and cetyltrimethylammonium bromide (CTAB) as a surfactant. First, a 0.5 mM palladium(II) chloride ($PdCl_2$) solution was prepared by dissolving 0.5 mg of $PdCl_2$ in 10 mL of deionized water. A 0.1 M CTAB solution was then prepared by dissolving 3.4 g of CTAB in 50 mL of deionized water. Similarly, a 0.1 M $NaBH_4$ solution was prepared by dissolving 0.38 g of $NaBH_4$ in 50 mL of deionized water, ensuring it was freshly prepared before use.

In a clean beaker, 10 mL of the 0.5 mM $PdCl_2$ solution was combined with 10 mL of the 0.1 M CTAB solution and stirred at room temperature for 15 minutes to allow CTAB to adsorb onto the $PdCl_2$. Subsequently, 10 mL of the 0.1 M $NaBH_4$ solution was added dropwise while stirring continuously. A color change from pale yellow to black was observed, indicating the formation of Pd nanoparticles. The reaction mixture was stirred for an additional 30 minutes to ensure complete reduction.

Following the completion of the reaction, the mixture was centrifuged at 10,000 rpm for 10 minutes to isolate the Pd nanoparticles. The supernatant was discarded, and the pellet was resuspended in 20 mL of deionized water. The washing and centrifugation process was repeated three more times to remove any residual

CTAB and $NaBH_4$. The purified Pd nanoparticles were then stored for future use. (Fig 5)

DETERMINATION OF MIC [41]

To evaluate the antimicrobial properties of palladium nanoparticles and chitosan, an in vitro study was conducted using standard strains of *S. aureus* (ATCC 25923) and *P. gingivalis* (ATCC 33277), following the Clinical and Laboratory Standards Institute (CLSI) guidelines.

The bacterial inoculum was standardized by adjusting the opacity to 0.5 McFarland standard, corresponding to a concentration of 1×10^6 to 5×10^6 colony-forming units (CFU/mL). The antimicrobial activity was assessed using the sequential dilution method.

A standardized protocol was followed to perform the Minimum Inhibitory Concentration (MIC) test. *P. gingivalis* was revived by culturing on Blood Agar and incubating in an anaerobic chamber, while *S. aureus* was revived on Brain Heart Infusion (BHI) Agar and incubated under aerobic conditions. Isolated bacterial colonies were transferred to sterile BHI broth and incubated at 37°C—*P. gingivalis* for 48 hours under anaerobic conditions and *S. aureus* for 24 hours under aerobic conditions. The bacterial concentration was adjusted to 10^5 organisms/mL using a 0.5 McFarland turbidity standard.

A stock solution was prepared by dissolving 0.1 gram of palladium nanoparticles and chitosan extract in 100 mL of solvent consisting of 1% DMSO and 99% distilled water. For the minimum inhibitory concentration (MIC) test, six test tubes containing 1 mL of BHI broth each were set up. In the first tube, 1 mL of the palladium nanoparticle and chitosan stock solution was added and mixed thoroughly. Then, 1 mL of this mixture was transferred to the second tube, and this serial dilution process (Fig 7) was continued up to the sixth tube, from which 1 mL was removed to achieve the desired gradient of palladium nanoparticles and chitosan concentrations.

Following the dilution, 50 µL of the prepared strains of *P. gingivalis* and *S. aureus* were introduced into each tube. The tubes were incubated under anaerobic conditions at 37°C for 48 hours for *P. gingivalis* and under aerobic conditions at 37°C for 24 hours for *S. aureus*. After incubation, turbidity was evaluated in comparison to the control. The presence of turbidity in the MIC tubes indicated bacterial growth, suggesting resistance of *P. gingivalis* and *S. aureus* to the palladium nanoparticles and chitosan.

DETERMINATION OF MBC [41]

The solution from the MIC tubes was sub-cultured onto BHI agar and incubated anaerobically for 48 hours in an anaerobic jar for *P. gingivalis*. Likewise, *S. aureus* was sub-cultured onto BHI agar and incubated aerobically for 24 hours. The absence of bacterial growth was indicative of a bactericidal effect.

Based on the MIC and MBC values, extract was incorporated into the hydrogel.

To determine the bactericidal concentration, MIC dilution tubes showing no visible growth (absence of turbidity), along with the control tube, were subcultured onto blood agar and incubated anaerobically at 37°C for 48 hours. For *S. aureus*, subculturing will be performed on BHI agar, followed by incubation at 37°C for 24 hours under aerobic conditions. The resultant colony counts in the test group were compared with those in the control group.(Fig 9)

The interpretation of the test results was as follows:

- A similar number of colonies in both groups indicates bacteriostatic activity.
- A reduced colony count suggests partial or slow bactericidal activity.
- The absence of bacterial growth signifies a complete bactericidal effect.

The formulation of the hydrogel was guided by the MIC and MBC findings.

FORMULATION OF HYDROGEL

Carbopol 930 was allowed to hydrate in 50 mL of distilled water for 24 hours. After that, glycerin was added to the Carbopol 930 solution while continuously stirring with a propeller mixer at 100 rpm for 10 minutes. A preservative solution was made by dissolving methyl paraben, propyl paraben, and sodium benzoate(Fig 10) in 25 mL of water, which was then mixed into the polymeric solution using the propeller mixer at the same speed for 5 minutes.(Fig 14)

Next, palladium nanoparticles and chitosan were slowly incorporated into the polymeric solution while stirring constantly at 200 rpm for 30 minutes.(Fig 11) The gel's weight was adjusted by adding distilled water, and the pH was set to 7 by mixing in triethanolamine. The resulting hydrogel was stored in airtight containers at room temperature.

Titanium discs were immersed in the newly formulated hydrogel for 45 to 60 seconds and then characterized using Scanning Electron Microscopy (SEM). Afterwards, the gel was kept at room temperature in an airtight container.(Fig 13)

OSTEOGENIC POTENTIAL:

The osteogenic potential were evaluated by cell attachment and cell proliferation using MG-63 (human osteoblast like) cell lines.The cell attachment and cell proliferation were evaluated after 24 hours, 48 hours and 72 hours.

CELL ATTACHMENT

To evaluate cell attachment, MG-63 cells were incubated for 24 hours on both uncoated titanium discs and titanium discs coated with hydrogel. After the incubation period, the culture medium was discarded, and the wells were washed three times with 37ºC phosphate-buffered saline (PBS) to remove any cells that did not adhere. The attached cells were then detached using an enzymatic solution of 1 mM EDTA and 0.25% trypsin, and cell counts were performed using a hemocytometer. The level of cell attachment will be expressed as a percentage of the initial number of cells seeded. (Fig 18)

DETERMINATION OF CYTOTOXICITY OF PALLADIUM NANOPARTICLES AND CHITOSAN INCORPORATED BIOGEL[11]

The MTT solution was made by dissolving 5 mg of MTT reagent in 1 mL of phosphate-buffered saline (PBS, pH 7.4),(Fig 17) and a cytotoxicity assay was performed on MG-63 cells. The effect of palladium nanoparticles and chitosan on in vitro growth inhibition was assessed using an ELISA reader to measure the conversion of MTT to formazan blue by metabolically active cells.

A 50 µL suspension of 4000 cells/mL was placed into each well of a 96-well microtiter plate, and the total volume was brought to 150 µL by adding Dulbecco's Modified Eagle Medium (DMEM). Palladium nanoparticles and chitosan were diluted to a final concentration of 10% in DMEM, and 100 µL of this solution was added to each well. The plate was incubated for 24 hours at 37°C in a CO_2 incubator with 5% CO_2.

After incubation, 20 µL of a 5 mg/mL MTT reagent was introduced to each well, and the plate was allowed to incubate for an additional four hours in the dark at room temperature, covered with aluminium foil to safeguard the photosensitive MTT reagent. Following this incubation, the supernatant was carefully discarded without disturbing the formazan crystals, and 100 µL of dimethyl sulfoxide (DMSO) was added to dissolve the crystals. The optical density (OD) was then measured at 570 nm using an ELISA reader.

The experiment was performed in triplicate, and the results are reported as the average of three independent measurements.

Formula:

$$\text{Surviving cells \%} = \frac{\text{Mean optical density of test component}}{\text{Mean optical density of control (untreated cell)}} \times 100$$

DISC-DIFFUSION TEST

The disk-diffusion agar method was utilized to assess the antibacterial properties of a hydrogel containing palladium nanoparticles and chitosan against *Staphylococcus aureu*s and *Porphyromonas gingivalis*. Bacterial suspensions were plated using the lawn culture technique, employing Mueller-Hinton agar for *S. aureus* and blood agar for *P. gingivalis*.

Sterile titanium discs (ASTM B348 Grade 5) were coated and divided into three categories:

- Experimental group: Discs coated with the palladium nanoparticle- and chitosan-infused hydrogel.
- Negative control: Uncoated discs.
- Positive control: Discs coated with 1% chlorhexidine gel.

Each agar plate contained three discs. They were incubated at 37°C in aerobic conditions for 24 hours for *S. aureus*,(Fig 19) while *P. gingivalis*.(Fig 20) was incubated in an anaerobic jar for 48 hours. Antibacterial activity was indicated by the absence of bacterial growth around the discs, resulting in a clear region referred to as the zone of inhibition. The effectiveness of the antibacterial action was determined by measuring the diameter of this zone.

IMAGES

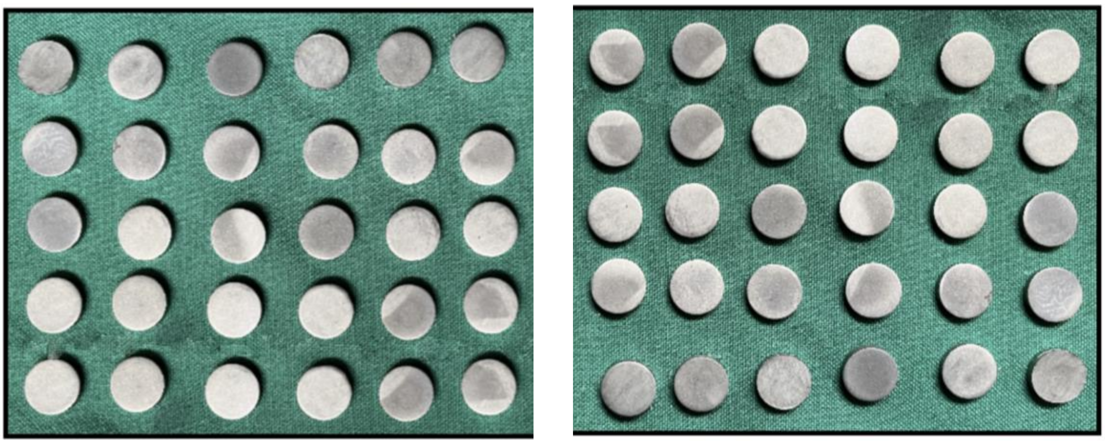


Fig 1: Titanium Discs

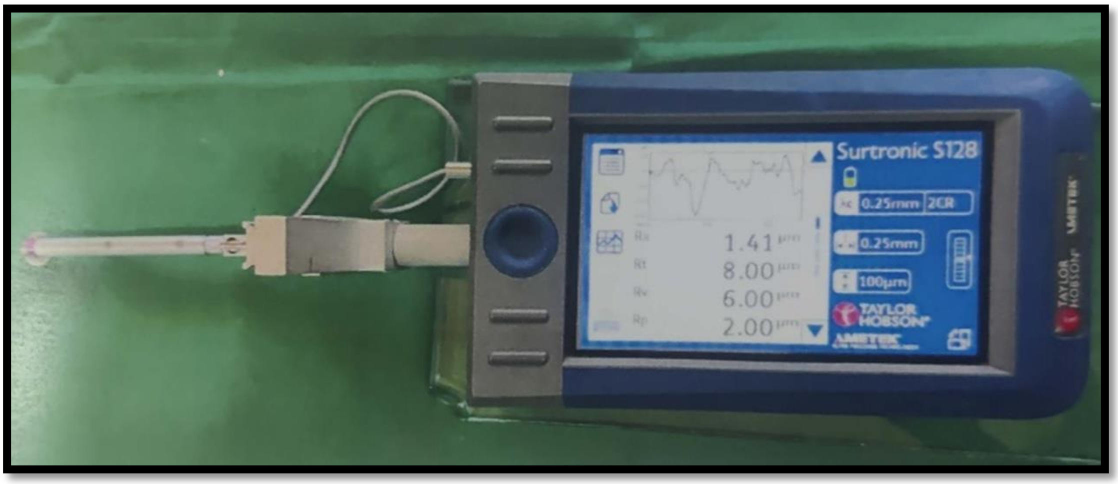


Fig 2: Profilometer.

A: 100X B:500X C:1000X

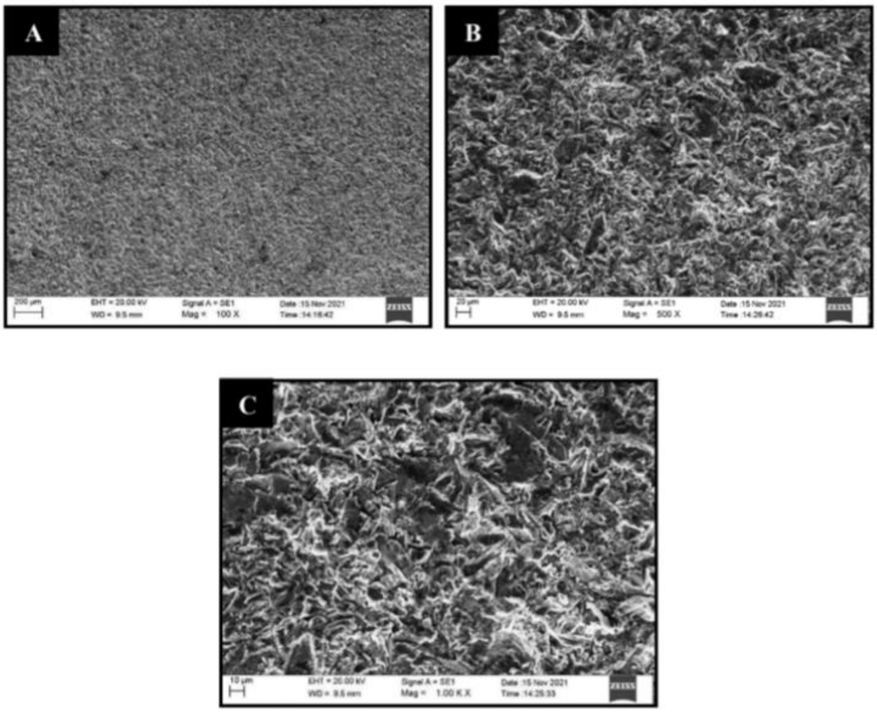


Fig 3: Qualitative evaluation of surface roughness Of specimen using scanning electron microscopy.

Fig 4: Chlorhexidine gel

Fig 5: Palladium nanoparticles

Fig 6: Chitosan powder

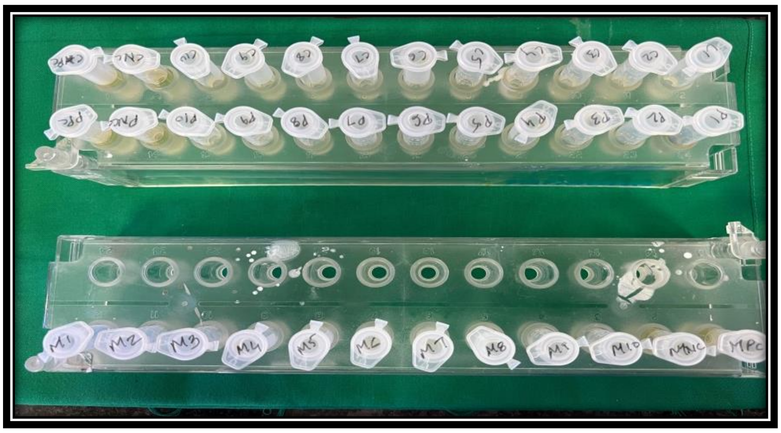


Fig 7: Preparation of Serial dilution done for 96 well plate for MIC

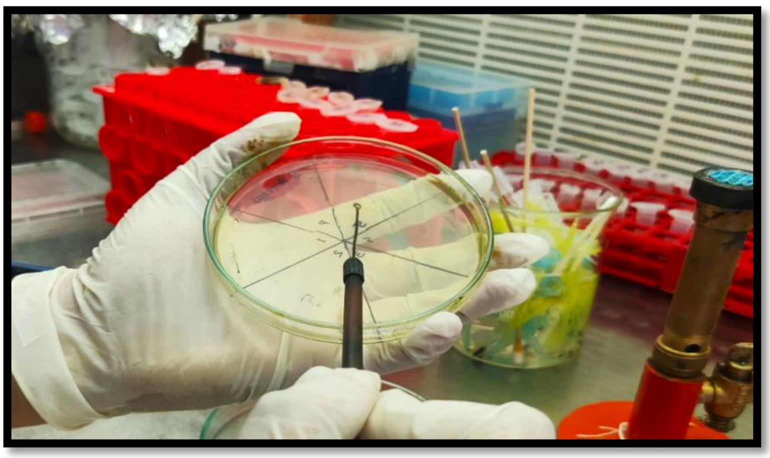


Fig 8: Streaking for MBC evaluation

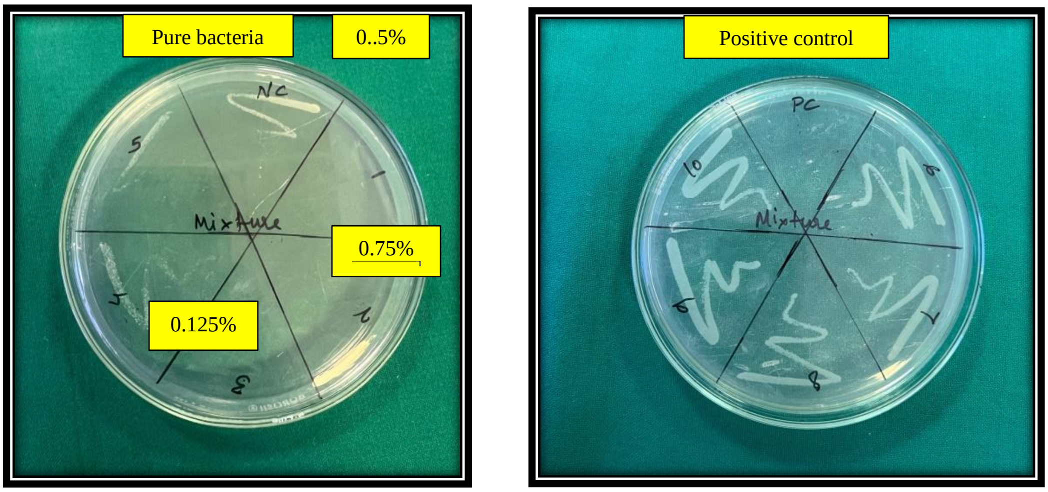


Fig 9 : MBC results of *S. aureus* and *P.gingivalis* for both bacteria growth is absent at a conc. of 0.125% hence it is selected as MBC value for the hydrogel preparation.

Fig 10: Materials used for hydrogel preparation: Carbopol, Glycerine, Sodium benzoate, Propyl Paraben, Methyl paraben

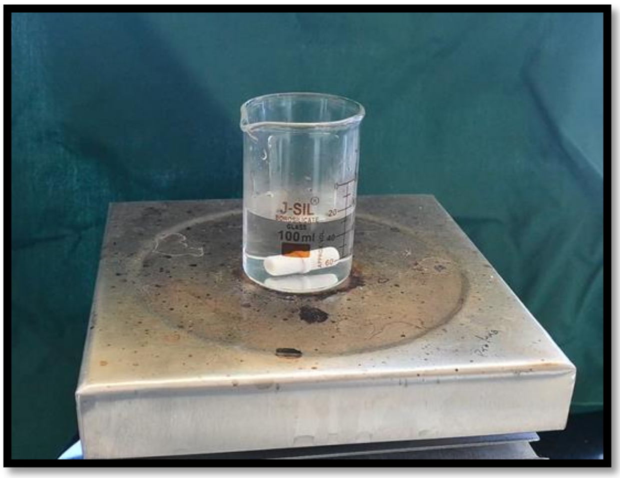


Fig 11: Magnetic stirrer   Carbopol with Distilled water

Fig 12: Adjusted pH is verified by digital meter

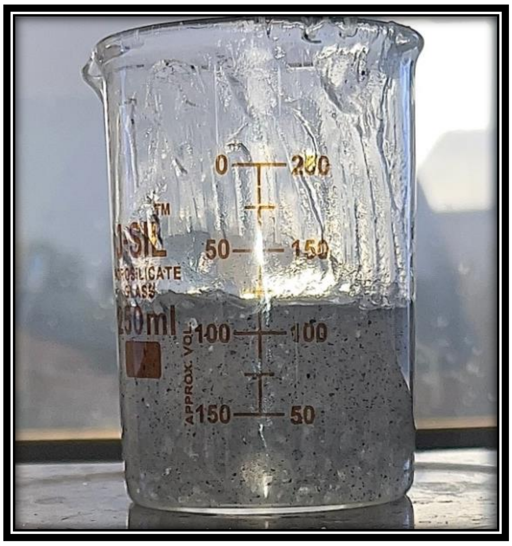


Fig 13: 0.125% Palladium nanoparticles and chitosan

Fig 14: Blank hydrogel

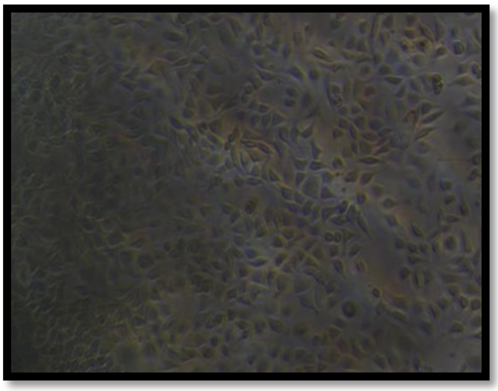


Fig 15: Viable L929 cell line

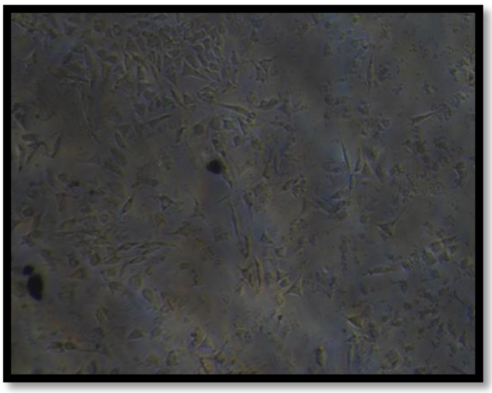


Fig 16: Results of MTT assay for CHX

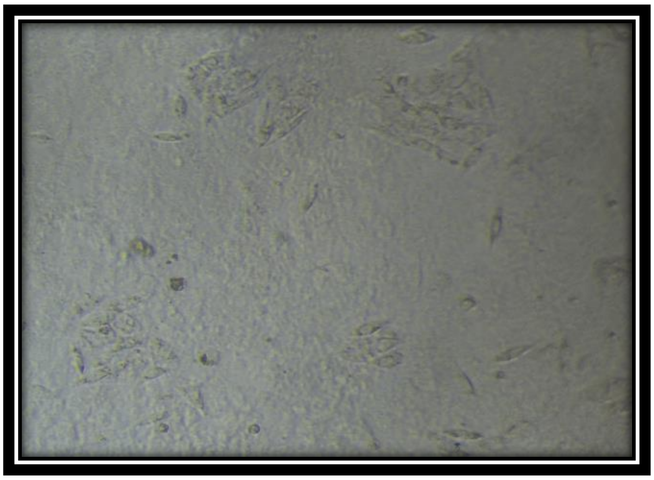


Fig 17: Results of MTT assay for Palladium nanoparticles and chitosan hydrogel

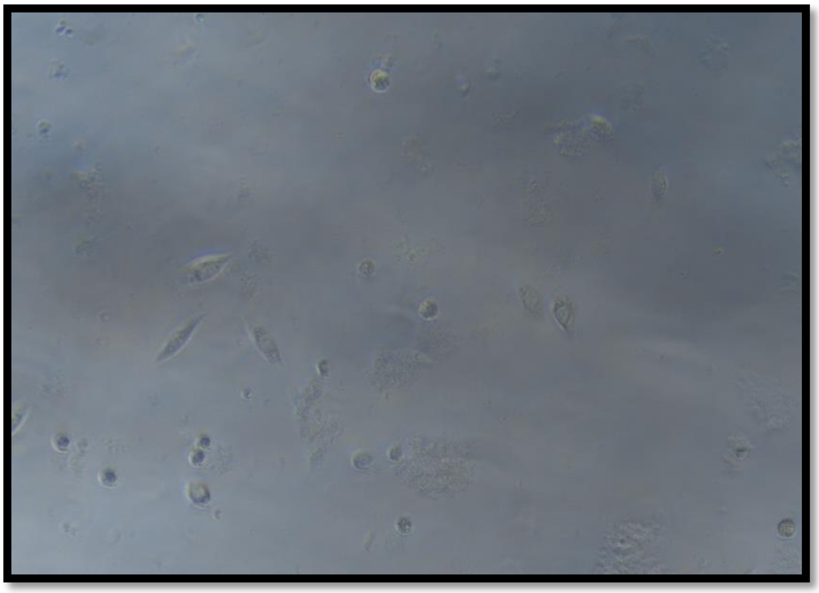


Fig 18: Evaluation of attached cells on hemocytometer

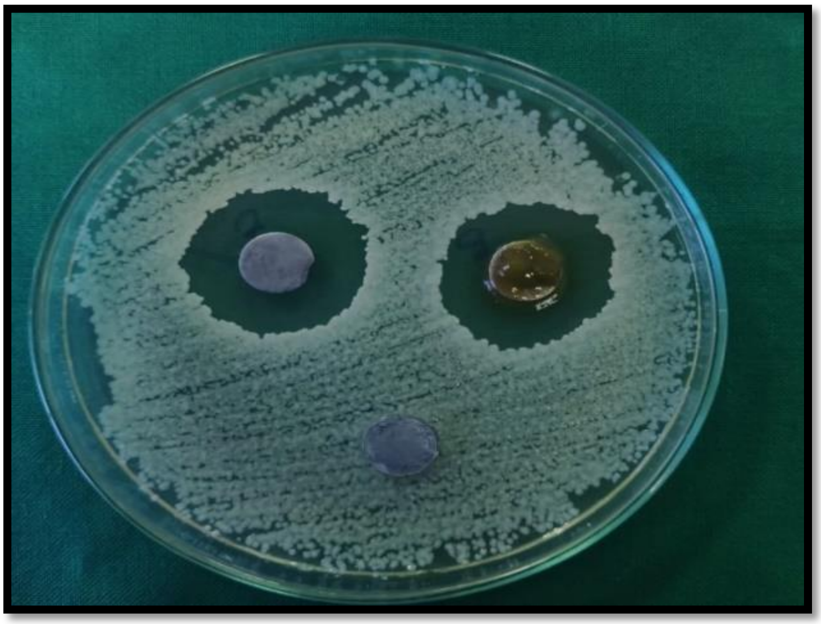


Fig 19: Disc diffusion test for titanium discs *S. aureus*

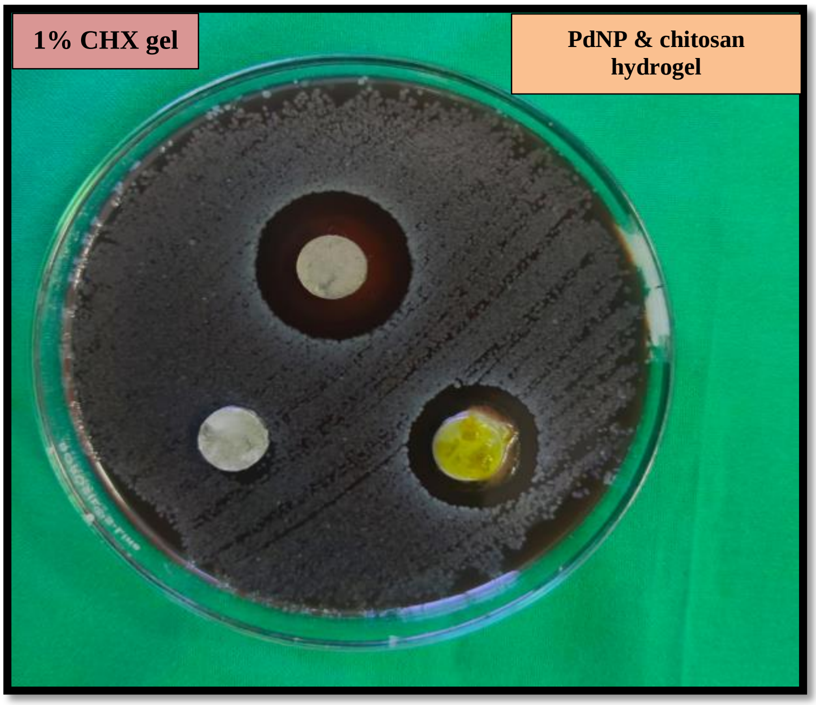


Fig 20: Disc diffusion test for titanium discs *P. gingivalis*

RESULTS

The current study examined the osteogenic potential and antibacterial effectiveness of palladium nanoparticles and chitosan hydrogel coated on titanium discs with palladium nanoparticles.

The disc diffusion method was used to assess antimicrobial activity against Staphylococcus aureus and Porphyromonas gingivalis. By monitoring MG-63 cell adhesion and propagation on hydrogel-coated surface at 24, 48, and 72-hour intervals, osteogenic potential was evaluated. At the appropriate time points, the MTT test was used to measure cell proliferation, and a hemocytometer was used to quantify cell attachment.

The antibacterial efficacy of both the widely accessible 1% chlorhexidine (CHX) gel and the hydrogel containing 0.125% Palladium nanoparticles and chitosan was assessed by measuring the zone of inhibition (in millimeters). To draw inferences based on experimental results, statistical analysis was done on the recorded data.

The antimicrobial efficacy was measured by the zone of inhibition (in mm), and osteogenic potential, assessed through cell attachment (number of cells) and cell proliferation percentage, of specimens from the Study Group, Control Group were analysed statistically to draw conclusions from the experimental findings. Descriptive statistics, including mean and standard deviation, were calculated for each group. The antimicrobial efficacy of the Study Group was compared using two-way ANOVA, while the osteogenic potential was evaluated through dependent t tests and repeated measures ANOVA, with comparisons across different time intervals made using independent t tests. A p-value of less than 0.05 was deemed statistically significant.

The average and standard deviation were determined for the study group, which consisted of titanium discs coated with Palladium nanoparticles and chitosan hydrogel, as well as the control group comprising titanium discs treated with 1% Chlorhexidine gel.

Table 3 summarizes the antimicrobial effectiveness in terms of the zone of inhibition for the study group, which used discs coated with Palladium nanoparticles and chitosan hydrogel, compared to the control group that utilized 1% Chlorhexidine gel against Porphyromonas gingivalis and Staphylococcus aureus.

As shown in Tables 3 and 4, descriptive statistical characteristics, such as the mean and the standard deviation, were computed to compare the group performing the experiment (hydrogel combined with 0.125% nanoparticles of palladium and chitosan) with the control group(1% chlorhexidine gel).

Using titanium discs, the antibacterial effectiveness of chlorhexidine gel and palladium nanoparticles and chitosan hydrogel was tested against S. aureus and P. gingivalis was compared using an unpaired t-test.

When examining cell proliferation using a dependent t-test and repeated measures ANOVA across different time intervals, no statistically significant differences were found in the study group from 24-48 hours and 48-72 hours. However, there was a significant difference in the 24–72hour time interval.

Table 3: Comparative assessment of mean cell attachment at various intervals according to study groups

Study groups	Cell attachment (Mean ± SD)			p value
	24 hours	48 hours	72 hours	
Blank Gel	1100000.00 ± 57445.62	1020000.00 ± 67494.85	1249700.00 ± 100903.75	0.000*
PD NP & chitosan hydrogel	1200000.00 ± 55025.24	1400000.00 ± 40824.82	1440000.00 ± 30276.50	
p value	0.000*			

Test applied: Repeated measures two way ANOVA, *indicates statistically significant difference, SD= Standard deviation.

Graph 1: Comparative assessment of mean cell attachment at various intervals according to study groups

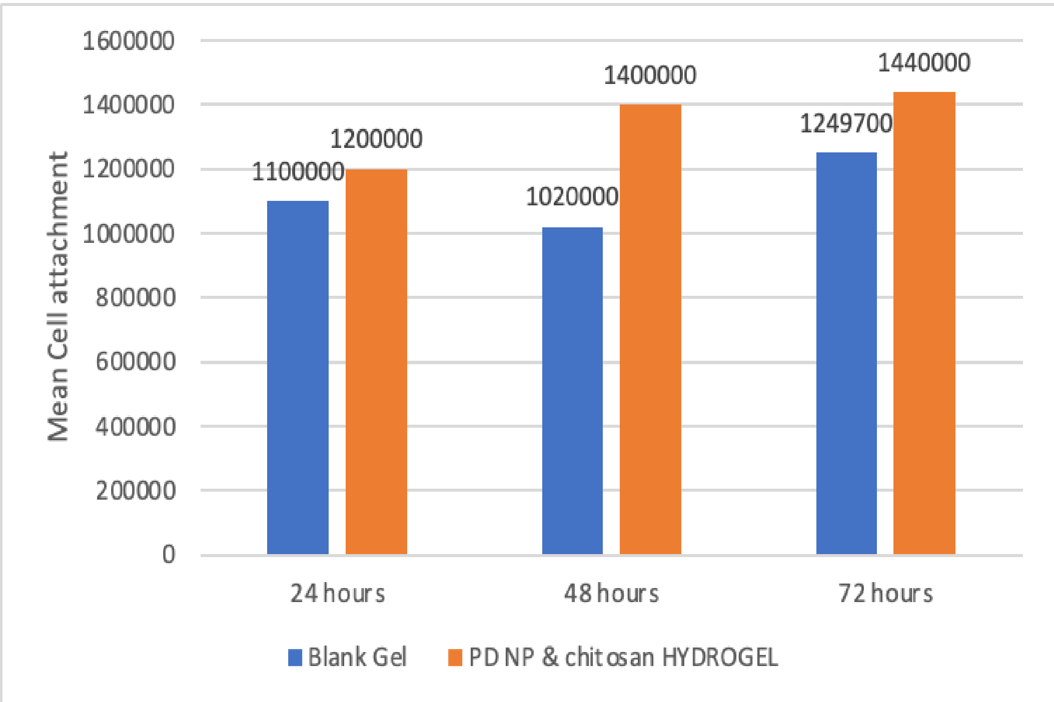


Table 3 and Graph 1 reveals a significantly (p=0.000) higher mean cell attachment level at 24 hours, 48 hours and 72 hours interval among PdNP & chitosan HYDROGEL group as compared to Blank gel group. Also among both study groups, there was a statistically significant (p=0.000) increase in mean cell attachment with increase in duration from 24 to 72 hours. The mean cell attachment at 24 hours, 48 hours and 72 hours were 1100000.00 $\pm$ 57445.62, 1020000.00 $\pm$ 67494.85 and 1249700.00 $\pm$ 100903.75 among blank gel group, respectively. Also, The mean cell attachment at 24 hours, 48 hours and 72 hours were 1200000.00 $\pm$ 55025.24, 1400000.00 $\pm$ 40824.82 and 1440000.00 $\pm$ 30276.50 among PD NP & chitosan hydrogel group, respectively.

The PdNP & chitosan hydrogel group showed a significantly higher mean cell attachment at all three time points: 24, 48, and 72 hours. The p-value = 0.000 indicates that the difference is highly statistically significant (commonly interpreted as $p < 0.001$**).** In both groups, the cell attachment increased over time from 24 to 72 hours

Table 4: Comparative assessment of mean MTT at various intervals according to study groups

Study groups	MTT (Mean ± SD)			p value
	24 hours	48 hours	72 hours	
Blank Gel	81.60 ± 6.02	87.70 ± 6.00	95.00 ± 4.08	0.000*
PD NP & chitosan hydrogel	93.00 ± 4.59	95.00 ± 3.49	104.00 ± 4.47	
p value	0.000*			

Test applied: Repeated measures two way ANOVA, *indicates statistically significant difference, SD= Standard deviation

Graph 2: Comparative assessment of mean MTT at various intervals according to study groups

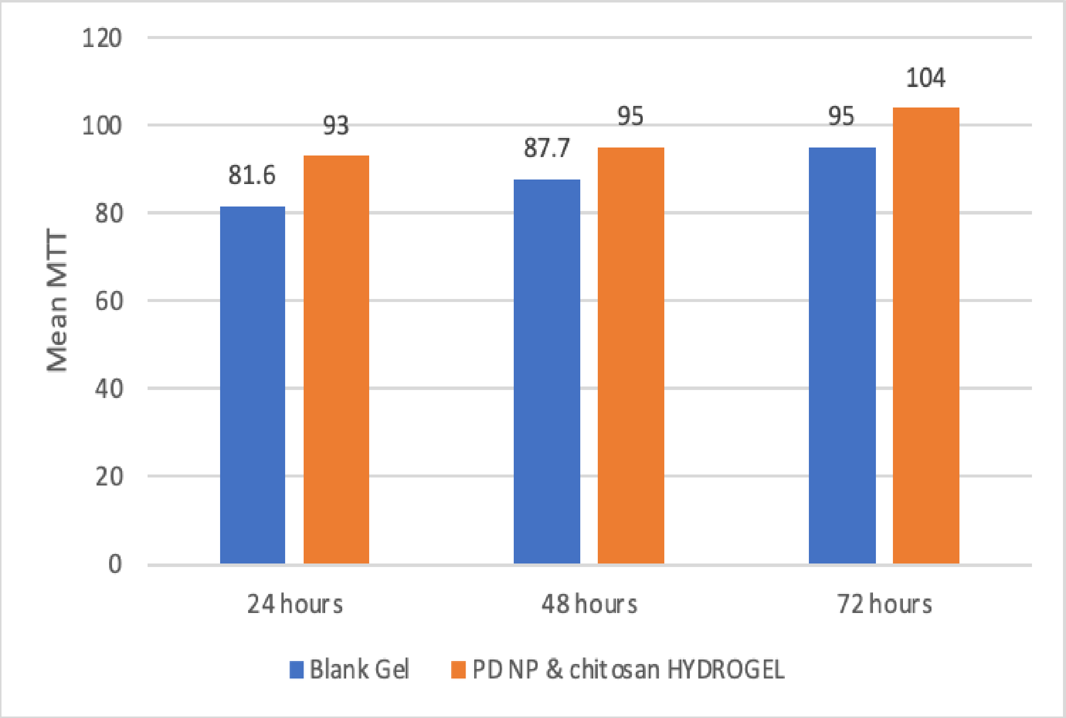


Table 4 and Graph 2 shows a significant (p=0.000) rise in mean MTT at various time intervals from 24 to 72 hours among the study groups. Also, the mean MTT level was significantly (p=0.000) higher among PdNP & chitosan hydrogel group as compared to Blank gel group at all three time intervals. The mean MTT level among blank gel group were 81.60 $\pm$ 6.02, 87.70 $\pm$ 6.00 and 95.00 $\pm$ 4.08 at 24 hours, 48 hours and 72 hours, respectively. The mean MTT level at 24 hours, 48 hours and 72 hours among PD NP & chitosan hydrogel group were 93.00 $\pm$ 4.59, 95.00 $\pm$ 3.49 and 104.00 $\pm$ 4.47, respectively. There is a significant increase in MTT values from 24 to 72 hours in both groups (p = 0.000), indicating that cell viability/metabolic activity increases over time. At each time interval (24, 48, and 72 hours), the PdNP & chitosan hydrogel group showed significantly higher MTT levels compared to the blank gel group (p = 0.000), demonstrating that the PdNP & chitosan hydrogel supports better cell viability/metabolic activity.

Table 5: Pairwise comparison of the two groups—hydrogel containing 3% palladium nanoparticles and chitosan, and 1% chlorhexidine (CHX) gel—was conducted on titanium surfaces against *P. gingivalis*.

Study groups	Zone of inhibition (Mean ± SD)	p value
Chlorhexidine 1% gel	19.13 ± 0.41	0.000*
PdNP and Chitosan 0.125% gel	16.32 ± 0.68	

Test applied: Independent t test, *indicates statistically significant difference, SD= Standard deviation

Graph 3: Comparative assessment of mean zone of inhibition against Porphyromonas gingivalis level according to study groups

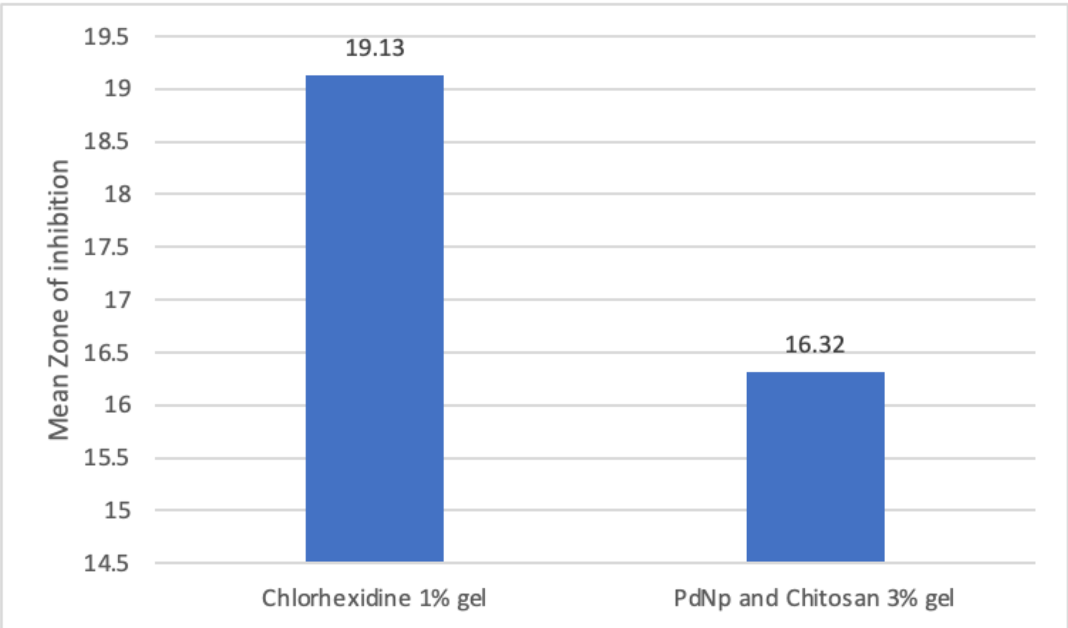


Table 5 and Graph 3 shows a significantly (p= 0.000) higher mean zone of inhibition against Porphyromonas gingivalis pathogen among Chlorhexidine 1% gel group (19.13 ± 0.41) as compared to PdNp and Chitosan 0.125% gel group (16.32 ± 0.68). Chlorhexidine 1% gel demonstrates superior antimicrobial activity against *P. gingivalis* compared to the PdNP and Chitosan hydrogel.

Table 6: Pairwise comparison of the two groups—hydrogel containing 0.125% palladium nanoparticles and chitosan, and 1% chlorhexidine (CHX) gel—was conducted on titanium surfaces against *Staphylococcus aureus*.

Study groups	Zone of inhibition (Mean ± SD)	p value
Chlorhexidine 1% gel	25.06 ± 0.51	0.123
PdNP and Chitosan 0.125% hydrogel	24.73 ± 0.39	

Test applied: Independent t test, *indicates statistically significant difference, SD= Standard deviation.

Graph 4: Comparative assessment of mean zone of inhibition against Staphylococcus aureus level according to study groups

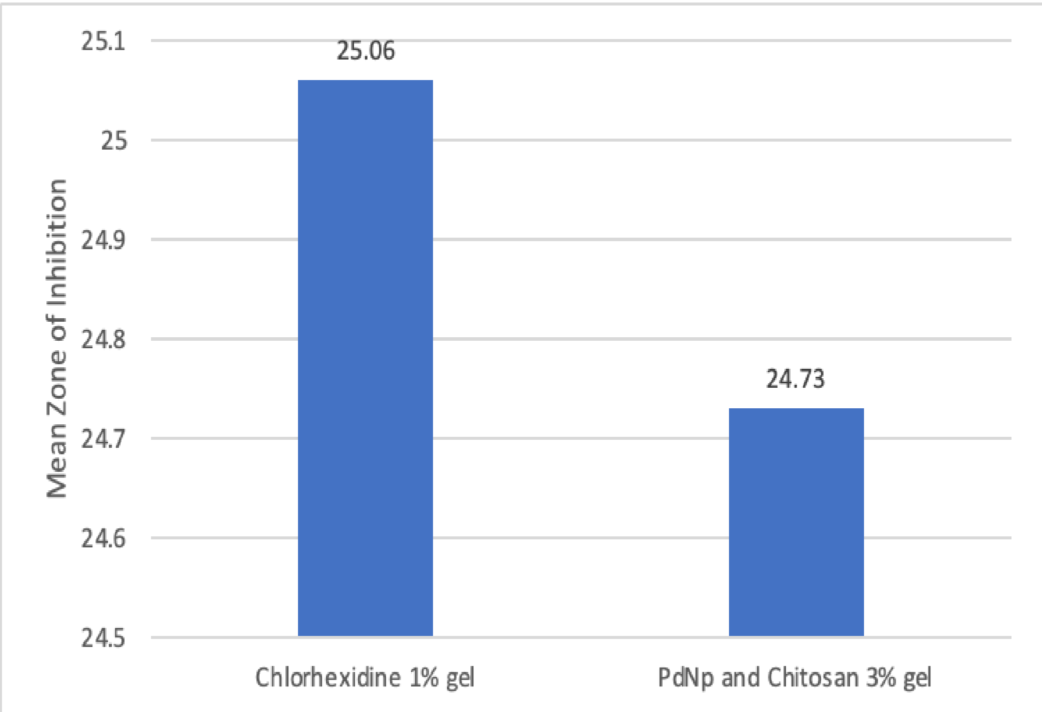


Table 6 and Graph 4 shows higher mean zone of inhibition against *Staphylococcus aureus* pathogen among Chlorhexidine 1% gel group (25.06 ± 0.51) as compared to PdNP and Chitosan 0.125% gel group (24.73 ± 0.39), however, the difference was statistically non-significant (p=0.123). While Chlorhexidine still had a marginally larger zone, the lack of statistical significance suggests that PdNP and Chitosan gel at 0.125% concentration exhibits a similar level of antimicrobial activity against *S. aureus*. This is especially relevant because PdNP and Chitosan-based gels offer additional benefits like osteoconductivity and biocompatibility, potentially making them a valuable alternative to traditional antimicrobials like chlorhexidine in certain clinical applications.

DISCUSSION

The present study was designed with three core objectives: (1) to evaluate the antimicrobial efficacy of palladium nanoparticles (PdNP) and chitosan hydrogel when applied to titanium discs, (2) to assess the osteogenic potential of these materials on titanium surfaces, and (3) to comparatively analyze their dual functionality in terms of both antimicrobial activity and osseoinductive capabilities. To achieve these objectives, a comprehensive experimental approach was adopted, including inhibition zone assays to quantify antimicrobial effects, cytotoxicity testing to ensure biocompatibility, and osteoblastic cell studies to evaluate cell attachment and proliferation. The outcomes of these experiments were critically interpreted and discussed within the context of existing implantology literature, allowing for a comparative understanding of the performance of PdNP–chitosan hydrogel coatings. Through this integrative analysis, the study highlights the potential of these nano biomaterials as promising candidates for improving both infection control and osseointegration in dental implant applications.

4.1 Antimicrobial Efficacy

The antimicrobial properties of implant surfaces play a pivotal role in preventing early bacterial colonization and biofilm formation, which are leading contributors to peri-implant infections and early implant failure. In this study, titanium discs coated with a palladium nanoparticle–chitosan (PdNP–chitosan) hydrogel demonstrated robust antimicrobial activity against both Gram-negative and Gram-positive bacteria—*Porphyromonas gingivalis* and *Staphylococcus aureus,* respectively. The inhibition zones recorded were 16.32 ± 0.68 mm for *P. gingivalis* and 24.73 ± 0.39 mm for *S. aureus*. These results are highly promising when compared to the 1% chlorhexidine (CHX) gel controls, which exhibited zones of 19.13 ± 0.41 mm and 25.06 ± 0.51 mm, respectively.

Although chlorhexidine remains the gold standard antiseptic in dental practice, its long-term use is often limited by cytotoxicity, tooth staining, and development of microbial resistance. The nearly equivalent efficacy of the PdNP–chitosan system to CHX, especially against *P. gingivalis* a keystone pathogen in periodontal disease demonstrates the formulation’s broad-spectrum potential and provides a promising alternative with dual benefits in terms of both antimicrobial function and biocompatibility.

The disc diffusion assay employed, while inherently semi-quantitative, is well-established for comparative antimicrobial screening. The observed inhibition zones place PdNP–chitosan hydrogel coatings within the performance range of previously studied metal-based antibacterial agents. Silver–chitosan composites, for example, typically show inhibition zones in the 23–26 mm range against *S. aureus*, while our PdNP–chitosan system closely matches this efficacy without the associated risks of silver toxicity or rising microbial resistance. [63]

Furthermore, metal oxide nanoparticles such as zinc oxide (ZnO) and titanium dioxide (TiO_2) have demonstrated antibacterial activity, but often at the expense of osseo-compatibility or requiring UV activation [8]. In contrast, PdNP demonstrate stability, redox activity, and biocompatibility under physiological conditions.

Mechanistically, the antimicrobial activity of PdNP and chitosan is attributed to distinct yet complementary processes. PdNP are known to generate reactive oxygen species (ROS) under aqueous and physiological conditions, which disrupt bacterial membranes, denature enzymes, and damage intracellular nucleic acids. [63,66] This oxidative stress overwhelms the bacterial antioxidant defense systems, ultimately leading to cell death. Meanwhile, chitosan, a cationic polysaccharide derived from chitin, interacts electrostatically with negatively charged bacterial membranes. This polycationic effect disrupts the cell wall structure, increases membrane permeability, and causes leakage of intracellular contents. [61] Furthermore, chitosan chelates essential divalent metal ions (e.g., Mg^{2+} and Ca^{2+}), which are critical for bacterial enzyme systems and membrane integrity.

The synergy between PdNP and chitosan in the hydrogel matrix is crucial. The hydrogel serves as a sustained release platform, ensuring prolonged exposure of bacteria to active agents, while also providing a moist and conformal barrier that can physically impede microbial adhesion. Monteiro et al. highlighted the efficacy of PdNP embedded in polymeric matrices, showing enhanced and sustained antimicrobial effects compared to nanoparticles alone. [61] Our findings support this, showing inhibition zones that match or exceed many previously studied nanomaterials.

Several comparative studies help contextualize the antimicrobial strength of this novel coating. Bundy et al. examined the antibacterial properties of various metals and found that palladium had a lower inhibitory threshold against oral streptococci compared to many base metals, such as titanium or stainless steel. [58] In another benchmark study, Xie et al. reported that ZnO nanoparticles produced an inhibition zone of ~15 mm against *Escherichia coli* [8]; in contrast, the PdNP–chitosan hydrogel in our study demonstrated superior efficacy against *P. gingivalis* (16.32 mm), a more clinically relevant periodontal pathogen.

Moreover, growing evidence suggests that overuse of silver-based antimicrobial materials may contribute to selective pressure and bacterial resistance. Palladium, which shares a similar ionic radius and group chemistry with silver, offers a stable and less explored alternative with a lower risk of inducing resistance mechanisms.[66] Its resistance to oxidation and ability to retain its nanoparticulate form under physiological conditions makes PdNPs uniquely suited for long-term implant coatings.

Additionally, our results suggest that PdNP–chitosan may be particularly effective against biofilm formation. The initial inhibition zones may not fully reflect the long-term antimicrobial effect; however, given the sustained release profile of the hydrogel and the known antibiofilm properties of both chitosan and PdNP, it is likely that this coating could reduce early biofilm establishment—a critical window during the first 24–72 hours post-implantation.

Taken together, the antimicrobial data clearly establish PdNP–chitosan hydrogel as a strong dual-function coating that matches current antiseptic standards while offering enhanced biocompatibility and a lower risk of adverse effects. These results form a strong rationale for future in vivo studies that assess long-term biofilm resistance, peri-implant health, and overall implant success rates in dynamic biological environments.

4.2 Osteogenic Potential

Successful osseointegration remains the cornerstone of long-term implant stability and functionality. In this study, the osteogenic performance of the PdNP–chitosan hydrogel coating was evaluated through MG-63 osteoblast-like cell attachment and proliferation assays, revealing significant enhancements when compared to unmodified chitosan controls. The data demonstrate that after 72 hours, cell attachment on PdNP–chitosan-coated titanium discs increased from 1.1×10^6 cells in the blank group to 1.44×10^6 cells in the experimental group. Similarly, the MTT assay revealed proliferation indices of 93–104% for the PdNP–chitosan group, in contrast to 81.6–95% in the control ($p < 0.001$). These findings substantiate the material's biocompatibility and capacity to support early-stage osteoblast activity—two critical factors in early-stage osseointegration.

The observed osteogenic effects can be attributed to both the physical and chemical properties of the PdNP–chitosan composite. Chitosan has long been recognized for its osteoconductive and biocompatible characteristics. As Khor and Lim demonstrated, chitosan mimics the natural glycosaminoglycan (GAG) structure of extracellular matrix (ECM) components, promoting cell adhesion, nutrient diffusion, and favorable ionic interactions with osteoblast membranes.[59] Furthermore, its porous structure facilitates cellular infiltration, matrix deposition, and mineralization—all essential for the early phases of bone regeneration.

The addition of PdNPs significantly enhances the bioactivity of the chitosan scaffold. Palladium nanoparticles have been shown to participate in redox-sensitive cellular signaling pathways. As O'Brien highlighted, nanoparticles can regulate cellular behaviors such as proliferation and differentiation through moderate induction of reactive oxygen species (ROS), which serve as intracellular signaling molecules rather than merely cytotoxic agents. [64] Patra et al. further demonstrated that PdNPs upregulate osteogenic markers such as alkaline phosphatase (ALP) and osteocalcin (OCN) in preosteoblast cultures, suggesting that these particles actively promote osteogenic lineage commitment. [66]

In our study, the PdNP–chitosan hydrogel enhanced not only initial adhesion but also sustained osteoblast viability. This dual benefit may be attributed to the hydrogel's ability to maintain a hydrated surface environment conducive to protein adsorption (e.g., fibronectin, vitronectin), which supports integrin binding and focal adhesion formation. The presence of PdNPs likely enhances these effects by modifying the surface energy and charge distribution, thereby improving cell-material interactions at the nanoscale.

Surface topography also plays a vital role in osteoblastic responses. In this study, surface profilometry of the coated titanium revealed an Ra value of approximately 1.2 µm—well within the optimal range of 1–2 µm as established by Wennerberg et al. for maximizing osseointegration and bone-implant contact.[62] This moderate roughness mimics the trabecular morphology of cancellous bone, supporting osteoblast migration and matrix synthesis. Additionally, scanning electron microscopy (SEM) confirmed uniform hydrogel coverage with micro/nano-scale features favorable for cytoskeletal anchorage and mechano-transduction.

Chitosan's ability to chelate calcium ions further contributes to biomineralization and bone matrix formation. When combined with PdNPs, the hydrogel system likely amplifies local ionic signaling—particularly calcium and phosphate flux—which further supports osteoblast activity. These interactions may stimulate mitogen-activated protein kinase (MAPK) and bone morphogenetic protein (BMP) signaling pathways, enhancing osteogenic gene expression.

Comparative literature supports our findings. Rinaudo emphasized that chitosan-based scaffolds, when structurally optimized, can outperform several synthetic polymer matrices in supporting cell proliferation and ALP expression. [60]

Additionally, Jayakumar et al. promoted the development of nanocomposite hydrogels that merge mechanical integrity, osteoconductivity, and controlled drug/nanoparticle delivery. [69] Our study builds on this foundation, illustrating the translational value of PdNP-conjugated chitosan coatings in clinical implant settings.

In light of these results, the PdNP–chitosan hydrogel not only supports early cellular events but may also influence the longer-term phases of osteogenesis, including ECM maturation and mineral deposition. Future investigations incorporating mineralization assays (e.g., Alizarin Red S staining), osteocalcin quantification, and in vivo histomorphometry would help further validate its osteoinductive capacity.

Taken together, the results affirm that PdNP–chitosan coatings significantly enhance the osteogenic profile of titanium implant surfaces by improving cell adhesion, metabolic activity, and surface integration parameters—all while maintaining antimicrobial efficacy. These dual functionalities are crucial for developing next-generation implant materials that accelerate healing while minimizing the risk of infection.

4.3 Comparative Evaluation

In the realm of implant surface engineering, achieving a balance between antimicrobial defense and osteointegration enhancement is both scientifically complex and clinically vital. The dual functionality of a coating material—capable of simultaneously preventing bacterial colonization and promoting osseointegration—offers a significant advantage in reducing early implant failures, especially in immunocompromised or periodontally susceptible individuals. However, this balance often represents a trade-off: materials with strong antibacterial properties, such as chlorhexidine and silver nanoparticles, frequently exhibit cytotoxic effects on host tissues and bone-forming cells; conversely, bioactive polymers like chitosan are biocompatible and osteoconductive but possess limited innate antimicrobial efficacy .[61,69]

In this study, we addressed this dual-performance dilemma by developing and testing a palladium nanoparticle–chitosan (PdNP–chitosan) hydrogel coating. The integrated performance was quantitatively assessed using a composite performance index (CPI), calculated by multiplying the average inhibition zone (mm) by the osteoblastic proliferation percentage. For PdNP–chitosan, this yielded a value of ~1996—significantly higher than that of either PdNP or chitosan alone, or 1% chlorhexidine, thereby underscoring the synergistic advantage of the composite formulation.

This index offers a novel and simplified metric for quantifying multifunctionality, integrating biological and antimicrobial performance into a single comparative framework. While other metrics exist for evaluating implant coatings—such as ALP activity, bacterial colony forming units (CFUs), or bone-to-implant contact ratios (BIC%)—the CPI provides a quick yet meaningful snapshot of surface functionality during early-stage in vitro evaluation.

The rationale for integrating PdNPs into a biopolymeric chitosan matrix draws support from earlier research. Jayakumar et al. emphasized the importance of combining structural and bioactive elements within a hydrogel scaffold to create a “smart” material that responds to both microbial and cellular stimuli.[68] Similarly, Darshan et al. reported that nanoparticle conjugation improves corrosion resistance and bioactivity in metallic biomaterials, further supporting our decision to incorporate PdNPs as both biofunctional and antimicrobial agents.

Moreover, while silver and copper nanoparticles have dominated prior antimicrobial strategies in implantology, their widespread use has raised concerns about cytotoxic thresholds and environmental accumulation. The choice of palladium nanoparticles in this study offers a new direction, exploiting palladium's similar electron structure to silver and gold while benefiting from its lower redox reactivity in physiological environments. This ensures more predictable long-term performance and reduced oxidative damage to host tissues. [63,66]

From the osteogenic perspective, pure chitosan provides a favorable matrix for osteoblast activity due to its cationic nature and similarity to extracellular matrix glycosaminoglycans. However, its bacteriostatic performance is often inadequate, particularly against Gram-negative anaerobes like *P. gingivalis*. In contrast, the PdNP–chitosan hydrogel demonstrates a stronger and more balanced performance profile. The chitosan serves as both a structural scaffold and a controlled-release matrix for PdNPs, which ensures that neither osteoblastic activity nor bacterial suppression is compromised during the early healing phase.

The clinical implications of such a coating are considerable. Infections within the first 72 hours of implantation are largely due to microbial colonization on the implant surface before host bone integration has stabilized. Thus, a material that can prevent bacterial adhesion without impairing osteoblastic cell adhesion is invaluable in reducing early implant failures and promoting rapid healing.

Additionally, Embrapa's studies on quaternized chitosan derivatives highlight the role of polymer functionalization in enhancing antimicrobial properties without diminishing cytocompatibility. [64] Our PdNP–chitosan system aligns with this approach by integrating a noble metal nanoparticle into a non-toxic polymeric matrix, offering comparable performance with added osteogenic support.

Overall, this comparative evaluation demonstrates that the PdNP–chitosan hydrogel coating successfully addresses one of the most persistent challenges in dental implantology: the simultaneous enhancement of antimicrobial protection and osteointegration potential. This multifunctional synergy not only improves early-stage clinical outcomes but also reduces the reliance on systemic antibiotics or antiseptic rinses, thereby contributing to antimicrobial stewardship. Future clinical and in vivo investigations are warranted to validate the long-term impact of such coatings on bone remodeling, implant stability, and peri-implant tissue health.

SCOPE OF THE STUDY

- The study aimed to evaluate the antimicrobial efficacy and the osteogenic potential of palladium nanoparticles and chitosan hydrogel coated on titanium discs.
- Further research can be conducted where, the osteogenic potential and antimicrobial efficacy of palladium nanoparticles and chitosan hydrogel on different implant materials such as Polyetheretherketone (PEEK) and Zirconia.
- Given that periimplantitis involves a polymicrobial biofilm, the hydrogel's effectiveness can be tested against various bacteria associated with periimplantitis.
- The hydrogel can undergo additional testing to assess the duration of drug release, disintegration rate, and other factors.
- Additional studies are recommended to evaluate cell differentiation using factors such as alkaline phosphatase activity (ALP), receptor activator of nuclear factor kappa B ligand (RANKL), Alizarin Red staining, and calcium deposition assessed through Von Kossa staining.
- Since this is an in-vitro study, in-vivo studies can be carried out since intraoral parameters such as pH, presence of saliva, different factors play a major role.
- Furthermore, long term studies can be conducted for validation of results.

LIMITATION OF THE STUDY

- This is an in vitro study, not all clinical conditions have been examined, and the sample size is relatively small.
- The research utilized MG-63 cells, an osteoblast-like osteosarcoma cell line, which demonstrates inconsistencies in their ability to differentiate.
- Since peri-implantitis arises from various groups of microorganisms, the study focused solely on the main microbial organisms associated with peri-implantitis and did not investigate the effect of PDNP chitosan hydrogel on different microbial strains.
- Although the dipping method is widely recognized as a popular technique for hydrogel coating, the uniformity of the coating's thickness across the substrate may vary. The optimal hydrogel coating technique should ensure a strong bond with the substrate and adapt well to substrates of various shapes.

CLINICAL IMPLICATIONS

The current study indicates that palladium nanoparticles combined with chitosan hydrogel exhibit lower cytotoxicity, implying a greater safety profile for clinical applications than CHX gel. This is especially beneficial in cases that require frequent or long-term use of the gel, such as in the treatment of early peri-implantitis or other oral infections.

Additionally, although the palladium nanoparticles and chitosan extract-based hydrogel exhibits lower cytotoxicity, it shows similar bactericidal efficacy to CHX gel. This suggests that it can efficiently target oral pathogens and help manage infections like peri-implantitis while maintaining safety.

Additionally, adding palladium nanoparticles and chitosan to a hydrogel offers a natural substitute for CHX, reflecting the increasing interest in herbal and holistic methods in healthcare. This option could attract patients who favor natural treatments or are worried about the possible adverse effects of synthetic substances.

In summary, palladium nanoparticles combined with chitosan extract-based hydrogel show great potential in clinical settings, providing a safer and equally effective substitute for CHX gel in treating oral infections like peri-implantitis. Additional studies and clinical trials are needed to confirm these results and fully investigate the capabilities of palladium nanoparticles and chitosan extract in dental applications.

CONCLUSION

This study leads to several conclusions based on the discussions and findings presented. Firstly, it shows that the combination of palladium nanoparticles with chitosan extract-based hydrogel demonstrates significant antibacterial effectiveness against early peri-implant pathogens, similar to that of traditional 1% CHX gel. This indicates that this combination could be a viable alternative to CHX for preventing early peri-implant infections, potentially offering similar antibacterial effects while reducing cytotoxicity.

Additionally, the use of disk diffusion tests highlights an affordable and accessible method for assessing antibacterial activity, making it a practical tool for research and development in this area. However, the study identifies limitations, such as the necessity for further clinical validation, long-term effect assessments, and a more comprehensive evaluation of peri-implant pathogens. Future studies should aim to address these gaps to strengthen the findings' reliability and application.

In summary, the research suggests that palladium nanoparticles coupled with chitosan extract-based hydrogel shows promise as an alternative antibacterial agent for treating early peri-implantitis, with comparable effectiveness to CHX and potentially reduced cytotoxicity. Further research and clinical validation are essential to confirm these results and to establish this combination as a safe and effective therapeutic option in dental practice.

SUMMARY

This in vitro study investigated a novel palladium nanoparticle–chitosan (PdNP–chitosan) hydrogel coating applied to titanium discs, aiming to enhance both antimicrobial efficacy and osteogenic potential for dental implants. The study had three main objectives: evaluating the antimicrobial effects, assessing osteoblastic cell responses, and comparing the combined performance of PdNP and chitosan.

Antimicrobial testing using P. gingivalis and S. aureus showed that the PdNP–chitosan hydrogel produced significant inhibition zones, comparable to 1% chlorhexidine. Osteogenic potential was assessed using MG-63 osteoblast-like cells, revealing increased cell attachment and proliferation with PdNP–chitosan coatings over 24 hours, 48 hours and 72 hours. These results confirmed the coating's biocompatibility and bone regenerative ability.

PdNP contributed antibacterial activity via ROS generation and membrane disruption, while chitosan enhanced cellular adhesion and acted as a supportive matrix. The PdNP–chitosan composite exhibited synergistic properties, combining the benefits of both components without compromising biocompatibility.

The study concludes that PdNP–chitosan hydrogel offers a promising dual-functional surface modification for titanium implants, capable of preventing peri-implantitis while promoting osseointegration. This material may serve as a valuable advancement in implant surface coating technology, with potential for improved clinical outcomes and reduced reliance on systemic antimicrobials.

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ANNEXURE – I

ETHICAL CLEARANCE

Research and Ethics Committee
KLE VK INSTITUTE OF DENTAL SCIENCES
A Constituent Unit of KLE Academy of Higher Education & Research
Accredited 'A' Grade by NAAC — Placed in Category 'A' by MHRD (GoI)
Nehru Nagar, Belagavi - 590 010, Karnataka State
☎: 0831-2470362 — Web: http://www.kledental-bgm.edu.in
FAX: 0831-2470640 — E-mail:principal@kledental-bgm.edu.in

Sl. No. : 1662

CERTIFICATE

This is to Certify that the synopsis titled Evaluation of Antimicrobial Efficacy and Osteogenic Potential of Hydrogel made with Palladium Nanoparticles and Chitosan coated on titanium Discs - An In vitro study *Submitted by Dr.* **REG. NO- IM0222005.** *P. G. Student / Staff, Guided by* DR. SOUMYA .RAYANNAVAR *from Department of* Prosthodontics Crown and Bridge *has been critically evaluated by committee members and granted ethical clearance to conduct the above mentioned study*

Date : 16/4/18

Member Secretary	**Chairman**
Research and Ethical Committee	Research and Ethical Committee
KLEVK Institute of Dental Sciences	KLEVK Institute of Dental Sciences
Belagavi	Belagavi

MEMBER SECRETARY
Research & Ethical Committee
KLEVK Institute of Dental Sciences
BELAGAVI

Chairman
Research and Ethical Committee
KLE VK Institute of Dental Sciences
Belgaum

ANNEXURE – II

METAL TEST LAB

(Recognised By Government Deptts & Undertakings)

UQSR. ISO 9001

ISO 9001:2015 Certified
- Optical Emission Spectrometry
- PMI
- Hardness Testing
- Ultrasonic Flaw Detection
- Ultrasonic Thickness Gauging
- Dye Penetrant Testing

Office : Gr. Flr. Bhavnagari Bldg., 72, Nanubhai Desai Rd., Khetwadi Main Road, Mumbai - 400 004.
Phone : 6743 7546 • Mobile : 9224778882 / 9223371637 • E-mail : metaltestlab2016@gmail.com

TEST REPORT

T/C No : 1820 DATE 04/03/2022

PARTY NAME :

125, C.P. TANK ROAD,
MUMBAI - 400 004.

REFERENCE : -
MATERIAL DESCRIPTION: TITANIUM DISC
GRADE : TI GR 5

%	C %	Si %	Mn %	P %	S %	Cr %	Mo %	Ni %	Al %
COMP	0.0600								6.10
REQD	-- 0.0800	--	--	--	--				5.5000 6.7500

%	Co %	Cu %	Nb %	Ti %	V %	W %	Pb %	Fe %	N %
COMP				87.88	4.40		--	0.069	--
REQD	-- --	-- --	-- --	-- --	3.5000 4.5000	-- --	-- --	-- 0.4000	-- --

REMARK: THE ABOVE MATERIAL CONFIRMS TO TITANIUM GR. 5 W.R.T. ELEMENTS SPECIFIED.

For METAL TEST LAB

METAL TEST LAB MUMBAI 400004

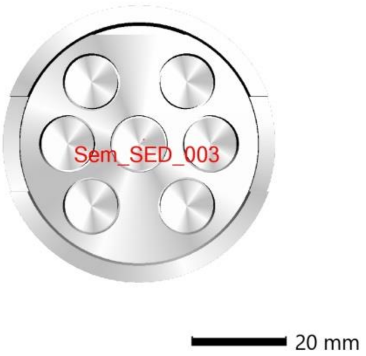
AUTHORISED SIGNATORY

1. The above Test Reports relate only to the sample submitted.
2. The above samples are not drawn by the laboratory.
3. The company or its partners shall in no way responsible for any financial liability due to any act of omission or error made.
4. No part of this Test Report shall be reproduced without the written permission of this laboratory.

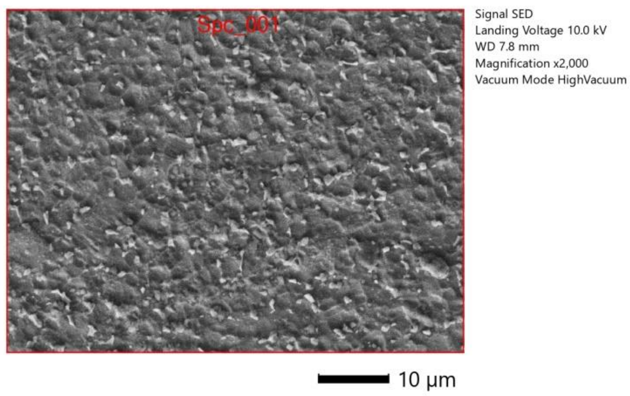
QUALITY IS OUR MOTTO

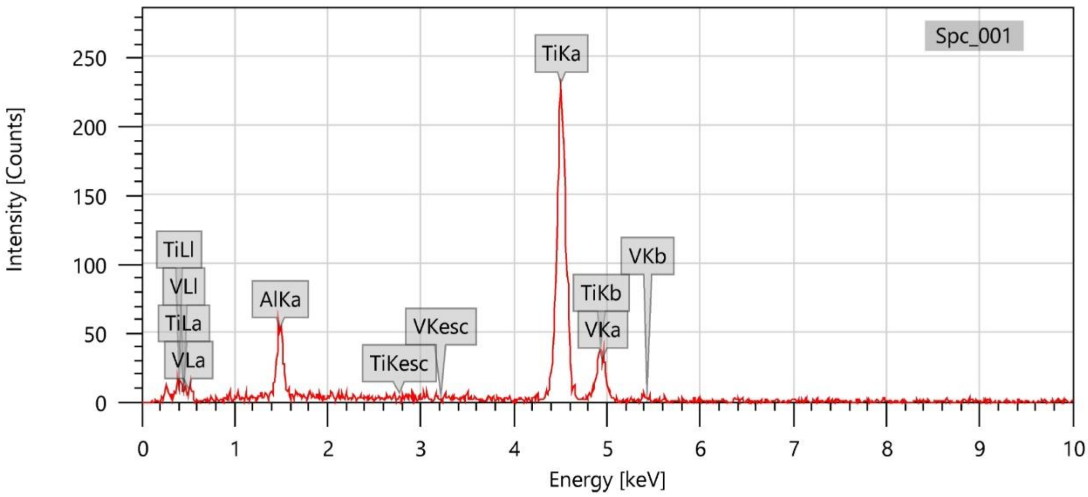
ANNEXURE – III

04-04-22



Sem_SED_003





Items	Value
measurement conditions	
Acceleration voltage	20.00 kV
Probe current	0.00 nA
Magnification	x 2000
Process time	T3
Measurement detector	First
Live time	30.00 seconds
Real time	30.26 seconds
Dead time	1.00
Count rate	212.00 CPS

Display name	Standard data	Quantification method	Result Type
Spc_001	Standardless	ZAF	Metal

Element	Line	Mass%	Atom%
Al	K	9.05±0.56	15.06±0.92
Ti	K	85.96±1.74	80.55±1.63
V	K	4.98±0.60	4.39±0.53
Total		100.00	100.00
Spc_001			Fitting ratio 0.1390