"COMPARATIVE EVALUATION OF ADHESIVE AND PROLIFERATIVE NATURE OF OSTEOBLAST-LIKE CELLS ON ZIRCONIA SURFACE AND ALTERED TITANIUM SURFACE WITH NANOTUBES – AN IN-VITRO STUDY"

BY

REG. NO- IM0221002

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KLE V.K. INSTITUTE OF DENTAL SCIENCES,
BELAGAVI, KARNATAKA

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Professor and Head
Department of Prosthodontics
LE V. K. Institute of Dental Scie
Belagavi

Head of Department

Dr. RAMESH P. NAYAKAR M.D.S.
Professor & Head,
Department of Prosthodontics
and Crown & Bridge,
KAHER Vishwanath Katti Institute
of Dental Sciences, Belagavi.
Date: 23/04/24
Place: Belagavi

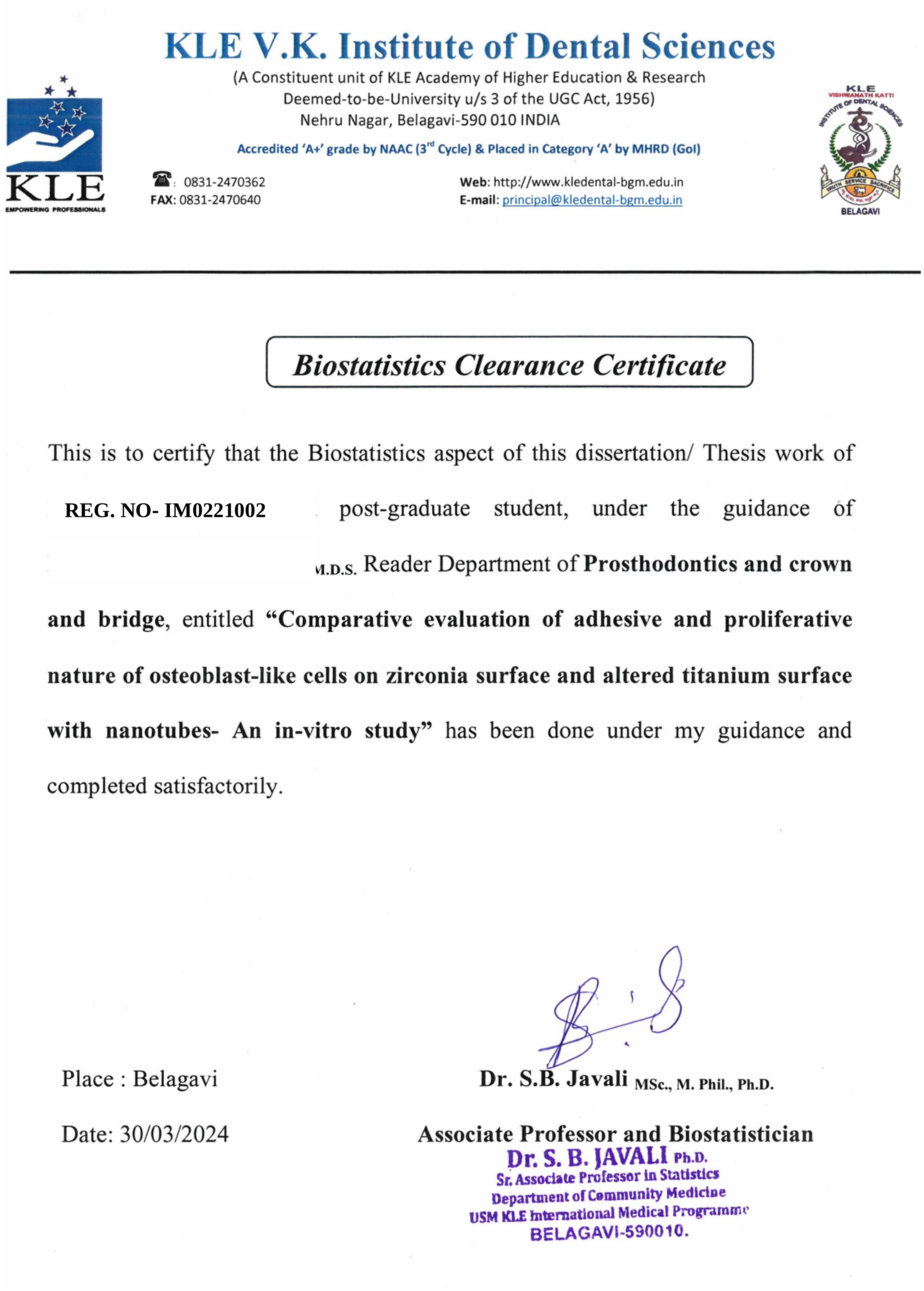
Principal

Dr. ALKA D. KALE M.D.S.
Principal,
KAHER Vishwanath Katti Institute of
Dental Sciences,
Belagavi.

Date: 23/04/24
Place: Belagavi

PRINCIPAL
LE V.K. Institute of Dental Sciences
Nehru Nagar BELAGAVI-5900

BIOSTATISTICS CLEARANCE CERTIFICATE



KLE V.K. Institute of Dental Sciences

(A Constituent unit of KLE Academy of Higher Education & Research
Deemed-to-be-University u/s 3 of the UGC Act, 1956)
Nehru Nagar, Belagavi-590 010 INDIA

Accredited 'A+' grade by NAAC (3rd Cycle) & Placed in Category 'A' by MHRD (GoI)

☎ : 0831-2470362
FAX: 0831-2470640
Web: http://www.kledental-bgm.edu.in
E-mail: principal@kledental-bgm.edu.in

Biostatistics Clearance Certificate

This is to certify that the Biostatistics aspect of this dissertation/ Thesis work of **REG. NO- IM0221002** post-graduate student, under the guidance of [redacted] M.D.S. Reader Department of **Prosthodontics and crown and bridge**, entitled **"Comparative evaluation of adhesive and proliferative nature of osteoblast-like cells on zirconia surface and altered titanium surface with nanotubes- An in-vitro study"** has been done under my guidance and completed satisfactorily.

Place : Belagavi

Date: 30/03/2024

Dr. S.B. Javali MSc., M. Phil., Ph.D.

Associate Professor and Biostatistician

Dr. S. B. JAVALI Ph.D.
Sr. Associate Professor in Statistics
Department of Community Medicine
USM KLE International Medical Programme
BELAGAVI-590010.

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I, **REG. NO- IM0221002** Post-Graduate student in the subject of **Prosthodontics and Crown and Bridge** have completed research work on the topic **“COMPARATIVE EVALUATION OF ADHESIVE AND PROLIFERATIVE NATURE OF OSTEOBLAST-LIKE CELLS ON ZIRCONIA SURFACE AND ALTERED TITANIUM SURFACE WITH NANOTUBES – AN IN-VITRO STUDY”,** in the year **2021-2024**.

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LIST OF ABBREVIATIONS USED IN THE STUDY

TiO_2	TITANIUM DIOXIDE
FESEM	FIELD EMISSION SCANNING ELECTRON MICROSCOPY
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide
ANOVA	ANALYSIS OF VARIANCE
Ti	TITANIUM
Zr	ZIRCONIA
Y-TZP	YTTRIA-STABILIZED TETRAGONAL ZIRCONIA
Ra	AVERAGE ROUGHNESS
TNT	TITANIUM NANOTUBES
DMEM	DULBECCO’S MODIFIED EAGLE MEDIUM
Ti	TITANIUM

ABSTRACT

STATEMENT OF PROBLEM

There are several types of dental implants in use today, with titanium being a popular choice due to its strong bone contact, compatibility with the body, low weight, and mechanical strength. Researchers have explored various methods to modify titanium surfaces, aiming to enhance their properties. These altered surfaces, which mimic the roughness of genuine bone, promote direct interaction with osseous cells and the production of hydroxyapatite, a substance that resembles bone. The creation of bioactive surfaces and biomimetic nano-texturized surfaces, like titanium covered with arrays of TiO2 nanotubes, is the result of recent advances in implant surface technology. These surfaces, produced through controlled electrochemical anodization, feature nanotubular, nanoporous, or nanosponge-like coatings.

Zirconia implants, another biomaterial, are an alternative for patients seeking metal-free treatment. Zirconia has demonstrated good antibacterial properties and the ability to promote bone growth, comparable to machined titanium implants.

PURPOSE

To assess and compare how well osteoblast-like cells adhere to as well as proliferate on the surface of zirconia implants versus titanium surfaces modified with nanotubes.

METHODOLOGY

By using HF solution as an electrolyte, grade 5 titanium alloy, and an improved anodic oxidation procedure with a potential of U = 15V, titanium nanotubes were created. A profilometer was used to measure the samples' surface qualities quantitatively, and field emission scanning electron microscopy (FESEM) to look at samples' surface qualities qualitatively. MTT test used to evaluate the cell attachment and proliferative potential of “MG-63 (human osteoblast-like) cell lines” at 24, 48, and 72 hours in order to assess the osteogenic potential.

RESULTS

These results were analysed and subjected to statistical analysis. Comparison of cell proliferation, cell attachment and surface roughness of the machined titanium surface, titanium nanotubes and zirconia surface at 24, 48, 72 hours was done using two-way ANOVA and Tukeys Multiple Posthoc procedures. A significant difference statistically was observed ($p=0.0001$) when the mean cell proliferation and cell attachment scores at each of the three-time intervals, three groups were compared and when comparison was done for each specimen across all the three time points.

CONCLUSION

The study concluded that the surface analysis of titanium nanotubes revealed greater roughness compared to the zirconia surface. Anodization procedures successfully produced well-aligned arrays resembling titanium dioxide nanotubes. Zirconia demonstrated superior osteogenic potential compared to both titanium nanotubes and machined titanium. Therefore, the in vitro method employed in this study is highly valuable for implant advancement, as it enables the prediction of the in vivo osseointegration potential of surface-modified titanium with nanotubes.

KEYWORDS

Titanium nanotubes, osseointegration, zirconia, surface modification

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INTRODUCTION:

The finding that implants made of commercially pure titanium could achieve direct bone-to-implant contact, anchoring securely in the bone, marked a major advancement in oral rehabilitation. [1,2]. The exceptional mechanical properties, anti-corrosion, and biological compatibility of Ti and its alloys have led to their extensive use in treating clinical bone abnormalities, especially in load-bearing areas of the body[3-5]. Although titanium-based materials have been shown to have favorable mechanical and chemical properties for orthopedic and dental applications, there are still important issues that need to be resolved, including low implant osseointegration, bacterial attachment, and a strong host inflammatory response[6-10].

Furthermore, under prolonged stimulation from stress and bodily fluid erosion, the film on Ti can readily disintegrate, leading to implant loosening and failure. Many modifications been developed to address these issues and have improved the biological characteristics of Ti implants with encouraging results[5].

Numerous studies have effectively demonstrated that topography, chemical makeup, hydrophilicity in implant's surface influence the cellular fate decisions made by osteoprogenitors and their progeny, which in turn affects the implant's potential for osseointegration and overall clinical success[11]. A improved biological response following implantation has been studied through the development of several surface modification approaches for metal biomaterials[12-14].

Implant surface technology underwent a paradigm change in the 1990s. Two surface types in implant market: rough, surface and the relatively smooth machined surface[1]. Later, a variety of surface modification techniques were employed to generate on the necessary substrate surfaces a wide range of functional qualities, including as physical, biological, electric, and anticorrosion characteristics[10]. Zeta potential measurements are an important physicochemical characteristic of interface that connects a coating's functionality to its application environment. Surface engineering is becoming more and more relevant in research and many industrial areas[5,10].

Various methods have been elucidated, such as:

1. Machined Implant surfaces: are created and passified, no polishing is done thereafter, and untreated surface is left[15-16].

2. Utilizing plasma spraying: This is one of the most used methods, wherein high-temperature powders of various materials (such as titanium or calcium phosphates) are projected onto implant surfaces that have been roughened to create coatings that range 30-50 μm[16].

3. Grit-blasting: Hard particles (alumina or TiO2) are propelled at increased speeds to change the roughness[16]. Advantage is it increases bone formation.

4. Etching with Acid: By submerging into acid (HF or HCl), the oxide layer thickness and roughness are increased. This procedure results in the surface eroding and the formation of micropits, which range in size from 0.5-2 μm[16].

5. Laser therapy: The use of dental lasers for implant surface cleaning and sterilizing has grown in popularity. Laser peening is the process of bombarding a metallic surface with tiny spherical particles while a laser beam strikes a protective layer on the surface to create tiny indentations or dimples[16].

6. Coating: Application of $CaPO_4$ to surface to create “bioactive surfaces” that improve BIC is one example[16]. Furthermore, implant surfaces can be coated with immobilized molecules to enhance cell adhesion, protein deposition, and mineralization because osteoblasts are specific molecular recognizers.

7. Anodization: Through the use of an electrolyte and an applied current, the implant undergoes an electrochemical reaction that produces micropores with different dimensions and thickens oxide layer. The anodization process has several pros, including higher biological-compatibility and heightened cell adhesivness and proliferation[16,17].

The type of acid mixed utilized as the electrolytic solution, the formation of titanium anodic oxides might result in diverse structures, according to Gomez Sanchez A et al. The addition of fluorides to the electrolytic media results in the formation of anodic oxide structures characterized by the presence of nanotubes. During the growth-dissolution phase of anodization, fluoride ions cause the titanium oxide to form nanotubular or nanoporous structures[18].

The recent introduction of nanoscale implant surface modification is that by imitating the bone structures, surface charge may be increased. This could improve protein adsorption, cell migration, and ultimately enhance osseointegration[2]. These techniques, which are collectively referred to as "electro-assisted titanium implants," used to create uniform, thin layers. Furthermore, it is possible to engineer many nanostructures, including nanofibers, nanorods, nanowires, nanotubes, nanosheets, nanoparticles, nanosponges, and nanocomposites[12]. Several chemical techniques, such as anodization, sol-gel techniques, and low-temperature solution chemical techniques, can be used to create nanotube oxide layers.

Because it is the easiest to create an ordered geometry of tube oxides on titanium, anodization is most popular technique among them. The surfaces created by this process also have superior optical, electrical, and biocompatibility characteristics[3]. By adjusting the anodization methodology, like time, current, and electrolytic composition, nanotubes with varying dimensions can be created[3,12].

In a study by Meyerink JG and colleagues [19], the TiO_2 nanotubes produced by the anodization process had a tube diameter of 89±24 nm, which demonstrated new bone tissue in an in-vivo rat investigation.

Similar to this, Ying LI., et al. discovered that anodization produces a 70 nm-diameter TiO_2 nanotube structure that has positive effects on osteoblast cell development and proliferation[19].

According to Sailer et al. (2007), titanium's strength and biocompatibility under bite-force loading circumstances are its key advantages. However, it causes obvious gray discoloration in soft tissues, which is unsightly[6]. As a result, ceramics and bioactive substrates been developed (Kaur et al. 2014). Compared to titanium, zirconia (Zr) has a less severe inflammatory reaction and is both osteoconductive and chemically inert (Hisbergues et al. 2009)[6]. Hip replacements frequently employ Y_2O_3-stabilized tetragonal zirconia polycrystals (Y-TZP) due to their pros over conventional ceramics, with high flexural strength and fracture-toughness (Pittayachawan et al. 2009)[6].

According to Cho et al. 2014, the osteogenic potential of TZP discs was shown to be comparable to that of discs produced on anodized titanium, a material commonly used for dental implants[6].

According to a thorough review by Stefan Roehling et al., zirconia can tolerate oral stresses because of its better biomechanical qualities over those of other ceramics. When zirconia is compared, potential biological material-related benefits have been reported, including significantly decreased bacterial adhesion, fewer inflammation in the soft tissues.

Zirconia implants were found to exhibit both qualitative and quantitatively equivalent soft-tissue binding when evaluated against Ti implants in the area of peri-implant soft tissues[20].

To develop implant biomaterials, it is crucial to first investigate the interactions between cells and materials using an appropriate in vitro model system. Cell lines derived from animal or human osteosarcomas are often employed to study the behavior of individual cells. One such well-established model is the ATCC® CRL1427TM human osteoblast-like MG-63 cell line, which has been widely used in bone research. These cells serve as an effective model for studying various aspects of cell-material interactions, such as adhesion, proliferation, and signaling on biomaterial surfaces. Additionally, they exhibit characteristics similar to those of pre-osteoblasts, making them particularly valuable for studying early stages of bone formation.

Utilizing MG63 cell lines, this in-vitro study assessed the surface topography, cell adhesion, & cell proliferation in titanium surface nanotubes and compare it to zirconia surface.

NEED FOR THE STUDY

Dental implants are being used much more frequently to replace missing teeth in patients since the discovery of titanium alloys. Titanium is widely recognized for its excellent mechanical qualities, low density (4.5 g/cm3), and exceptional bone-contact biocompatibility. Different methods for modifying the surfaces of titanium alloys have been investigated, in addition to depending on their natural oxide covering. A number of surface alterations were made in an effort to enhance implant-bone contact. These include roughening, treating with an acid or an alkaline solution, etc[1-5].

One approach to surface modification in contemporary implantology is the creation of TiO_2-based coatings with specific structural, architectural characteristics as nanotubes. This approach aims to improve bioactivity and osteointegration. These surfaces encourage interaction and the development of bone, because of their topology, which is comparable to that of natural bone. By using a regulated electrochemical anodization process, they can be made rapidly[21]. Also, one of the economical techniques that could be used to produce nanotubular structure is anodization.

A definitive regimen must be established in order to create nanotubes with an effective diameter and length that would boost osteogenic potential.

Over the past 20 years, zirconia, another biomaterial, has gained significant use in dentistry as a ceramic material because of its white color and adequate mechanical strength[22]. Tetragonal zirconia polycrystals in particular have exceptional mechanical, biocompatible, and aesthetic qualities[11].

These qualities could compensate for titanium implant disadvantages. Compared to titanium, zirconia is more biocompatible and compatible with esthetic performance. Zirconia inhibits bacterial adherence more than titanium, indicating its suitability for use in abutments.

In order to examine which surfaces have more osteogenic potential and surface roughness, the current study will assess the osteoblastic cell adhesion and proliferation on machined titanium, titanium surface containing nanotubes, and zirconia surface.

HYPOTHESIS:

NULL HYPOTHESIS

There is no difference in adhesive and proliferative nature of osteoblast-like cells between altered titanium surface with nanotubes and zirconia surface.

RESEARCH HYPOTHESIS

There is difference in adhesive and proliferative nature of osteoblast-like cells between altered titanium surface with nanotubes and zirconia surface.

AIM OF THE STUDY:

The evaluation and comparison of the adhesive and proliferative nature of osteoblast-like cells on altered titanium surface with nanotubes and zirconia surface.

REVIEW OF LITERATURE

1. Yasumasa Akagawa et al. (1993) assessed the interface histopathology of partially stabilized zirconia endosseous implants that were loaded both early and unloaded during the early stages of bone healing.

   On partially stabilized zirconia implants in beagle dogs, clinical and histological assessments were performed to evaluate osseointegration under unloaded and early loaded settings. Although there were no notable clinical variations, both groups' direct bone apposition was detected. But there was a discernible reduction of crestal bone height with loaded implants. These results suggested that one-stage zirconia implants may have a better chance of attaining osseointegration under initial unloading settings[54].

2. In 1998, Wennerberg A. et al. assessed "how crucial surface roughness is for implant assimilation".

   The article emphasizes the significance of surface roughness in the integration of implants. It discusses how surface roughness influences the interaction between implants and surrounding tissues, particularly in promoting osseointegration. The study highlights the importance of optimizing implant surface roughness to enhance the biological response and stability of implants. It provides insights into the mechanisms by which surface roughness affects the incorporation of implants, emphasizing the need for careful consideration of surface characteristics in implant design. The findings underscore the critical role of surface roughness in implant success and offer guidance for improving implant performance through surface modifications[35].

3. Using anodic oxidation, Dawei Gong et al. (2001) produced arrays of titanium oxide nanotubes.

   Nanotubular structures were created through anodisation with 0.5 to 3.5 wt% HF. These nanotubes are well-oriented. They feature open tops and closed bottoms. Average diameter, ranged 25-65 nm, increased with higher anodization voltage, while tube length remained unaffected[4].

4. Kohal, Ralph J. et al. (2004) assessed An animal investigation that demonstrates the loading of, custom-made zr and ti implants which exhibit comparable BIC.

   In monkeys, titanium and loaded zirconia implants were evaluated for histological behavior. Crowns were inserted after implantation, and after five months, they were assessed. Throughout the research, no implants were lost. The soft tissue surrounding both kinds of implants had comparable proportions. There was no statistical difference from mineralized BIC between zirconia & titanium, which was 67.4% and 72.9%, respectively. According to the study's findings, titanium and custom-made zirconia implants showed comparable soft tissue dimensions and osseointegrated to the same degree[55].

5. In 2006, Su YT et al. assessed which surface characteristics improve the way the bone responds to implants, additionally implant surfaces made of oxidized magnesium, TiUnite, and Osseotite were compared.

   In rabbit tibiae, this study examined the osseointegration and osteoconductivity of implants made of acid-etched Osseotite, oxidized titanium (TiUnite), and magnesium (Mg). At three and six weeks, magnesium implants significantly outperformed TiUnite and Osseotite in terms of osseointegration and bone development, with more new bone formation and significantly higher removal torque values (RTQ). From three to six weeks, the osseointegration rates of magnesium implants were also faster. Mg implants showed stronger and faster osseointegration despite having less roughness, indicating that they may be able to decrease the rate of early implant failure and speed up bone repair to enable immediate/early loading[39].

6. In pig maxillas, Gehlert M et al. (2007) compared the biological-mechanical and histological properties of zr implants having different surfaces and a ti implant.

   For dental implants, zirconia ceramics are preferred because of its mechanical qualities, biocompatibility, and resemblance to teeth. Surface alterations can improve osseointegration; this is a topic that needs more research. This study compared a titanium surface with a high bone-implant interaction (SLA) to two zirconia surfaces—one machined and the other sandblasted. Implants were positioned in healed maxillary sites and assessed biomechanically and histologically during a 6-month period in a study involving 13 miniature pigs. Findings indicated that, in comparison to the machined zirconia surface, the sandblasted zirconia surface demonstrated better bone apposition and greater stability in bone, indicating the significance of surface roughness for implant success[57].

7. Depprich et al. (2008) used SEM examination of the interface between bone and metal to study osseointegration in zirconia implants.

   Zirconia ceramics are becoming more and more popular in dental implants because of its biomechanical qualities, biocompatibility, and natural appearance. They are well-known for their effectiveness in orthopedic surgery. Osseointegration of Ti implants and zirconia implants having altered ablative surfaces in minipigs was compared. For both implant types, SEM examination demonstrated substantial bone attachment after one-week, intimate bone contact after four weeks, and full osseointegration without interfacial layers after twelve weeks. The study found that, from an ultrastructural perspective, zirconia implants with altered ablative surfaces showed comparable osseointegration to titanium implants[56].

8. A study by Ferguson S.J. and colleagues (2008) compared the biological-mechanical properties in various modifications.

   In order to evaluate osseointegration, this study examined various dental implant surface treatments using a sheep model. A range of surface treatments were tested on implants: zirconia, ti with calcium phosphate, ti altered by anodic mode, containing chondroitin, and sandblasted and acid-etched titanium. Certain modified titanium implants showed high removal torque values after 8 weeks than others, suggesting improved osseointegration. Although the conventional titanium implant, which is sandblasted or acid-etched, is still successful, the study indicates that surface modifications such as collagen coatings or bisphosphonate may be able to improve early bone development surrounding implants[44].

9. The impact of pretreatment and cyclic loading on the fracture strength of zirconium dioxide implants was examined by Ralph J. Kohal et al. (2010).

   The impact of preparation and cyclic loading on fracture strength of zr oral implants has been assessed in this study. Groups consisting of 48 implants were created depending on their preparation and cyclic loading levels. After loading, the fracture strength was evaluated. Cyclic loading and preparation both decreased fracture strength, the implants continued to be able to sustain typical occlusal stresses even after prolonged artificial loading. Significant differences between some groups were found by subgroup analysis, suggesting that preparation and cyclic loading had an effect on the fracture resistance of zirconia implants[53].

10. Kansong Chen and colleagues (2012) assessed a superior hydrogen sensor based on titania nanotube arrays that works at ambient temperature

    Hydrogen sensors were developed using nanotubular anatomy by anodic method in NH4F solution. The response to hydrogen is significant even without oxygen in the atmosphere, suggesting that the variation of the Schottky barrier height at the Pt/TiO_2 interface primarily contributes to the excellent sensing properties in air[46].

11. Current trends in dental implants were examined by Laura Gaviria et al. (2014)

    Dental implants are used widely. Research on implant design, materials, and procedures has increased. In addition to reviewing developed implant kinds and current design specifications, this study examines past and future. It also looks at how implant surfaces are getting better and new developments in analysis and design technology. It is anticipated that further developments in these fields will improve the efficiency and durability of dental implant procedures[16].

12. A study by Y. Cho et al. (2015) focuses on how bones respond to zirconia coated with hydroxyapatite applied via aerosol deposition.

    In this study, the osteogenic capabilities of zirconia coated with hydroxyapatite (HA) were evaluated using an aerosol deposition technique to improve osseointegration. Surface analysis revealed a thin, uniform HA film that enhanced surface wettability. While cell attachment did not significantly differ between titanium and zirconia surfaces, proliferation was lower on HA-coated zirconia. However, osteogenic markers indicated positive responses on HA-coated zirconia. These findings suggest that the HA coating applied through aerosol deposition improves surface quality, thus promoting osteogenesis.[6].

13. Daniel Buser et al. (2016) examined the 50 years of advancement, present trends, and unanswered concerns in modern implant dentistry, which is based on osseointegration.

    In the 1960s and 1970s, implant-supported prostheses faced skepticism due to subpar outcomes and lack of scientific backing. Professors P. I. Brånemark and André Schroeder revolutionized implant dentistry, establishing osseointegration as a cornerstone. This progress enabled advancements like guided bone regeneration and sinus floor elevation, expanding implant therapy possibilities. Moderately rough implant surfaces enhanced bone integration, enabling immediate and early loading protocols. The introduction of cone-beam computed tomography improved preoperative analysis, leading to digital planning and surgical advancements. These innovations have greatly enhanced esthetic and patient-centered outcomes in implant dentistry over the past 50 years, though debates on topics like peri-implantitis and the use of zirconia implants continue[1].

14. Long Bai et al. (2016) investigated at how ex vivo hemocompatibility and the reactivity of macrophages and endothelial cells were affected by TiO_2 nanotube arrays of varying sizes.

Percutaneous coronary intervention with stenting is a common treatment for coronary disease, but in-stent restenosis (ISR) remains a concern. Endothelial cell (EC) functionality is key to addressing ISR, yet understanding of the intervention-material mechanism is lacking. Immune response, particularly by macrophages (MΦs), is also crucial but often overlooked in stent design. TiO_2 nanotube arrays (TNAs) with varied diameters were fabricated and tested for their effects on ECs/MΦs behavior and crosstalk. Nano-15 showed enhanced EC vitality and gene expression, along with favorable MΦ activation and inflammation regulation. Nano-15 also exhibited good hemocompatibility, suggesting potential for preventing ISR when used as a coronary stent coating[8].

15. Review of Bioceramic oxides: Inert Ceramics of Dentistry and Medicine by J. Li and G.W. Hastings (2016)

    The paper emphasizes the importance of controlling the structure of advanced ceramics at different levels. It discusses the manufacturing processes involved in producing fine ceramic components and highlights factors such as chemical composition, impurities, crystal structure, grain size, and defects that affect the performance of ceramic materials. The paper also provides recommendations for biological testing of biomaterials for orthopedic applications. It mentions the biocompatibility of alumina and zirconia ceramics and presents testing results for zirconia. The paper warns against steam resterilization and mentions the detection of radioactive impurities in some zirconia ceramics. It also discusses the clinical performance of zirconia balls and the surface degradation caused by phase transformation under loading[51].

16. A study by Ying LI et al. (2018) investigates the process of osseointegration and the adhesion of osteoblasts to the surface of TiO2 nanotubes that have been coated with hydroxyapatite.

    To improve the adhesion of osteoblasts and the process of osseointegration, a layer of TiO2 nanotubes was created on titanium (Ti) using anodic oxidation (AO), followed by an additional coating of hydroxyapatite (HA) (AO/HA). In vitro experiments revealed that both AO and AO/HA surfaces promoted the adhesion, proliferation, and differentiation of osteoblasts, with the AO/HA surface showing superior performance. These surfaces also increased the expression of genes associated with osteogenesis and adhesion. In vivo studies conducted in rats demonstrated that AO, particularly AO/HA, facilitated earlier osseointegration and better bone bonding compared to unmodified Ti. This research emphasizes the combined effect of nanotopography and HA in enhancing osteoblast functions and osseointegration, suggesting a promising surface modification approach for implants.[9].

17. A study by Erick Barrios Serrano et al. (2018) assessed the use of various stabilizing agents and zeta potential.

    Surface engineering is a critical aspect of various industries, where zeta potential measurements serve as a vital parameter connecting coating functionality to its surroundings. This study aimed to improve the stability of TiO2 particles by using different stabilizing agents. By examining electrophoretic deposition (EPD) parameters and suspension concentrations, the optimal deposition performance on titanium substrates was determined. The composition were analyzed by FT-IR; SEM, revealing that TiO2 particles were dispersed within the chitosan matrix during deposition. Corrosion resistance, assessed through electrochemical polarization curves, showed that TiO_2 and TiO_2-chitosan coatings exhibited greater resistance compared to pure titanium when exposed to a sulfuric acid solution[10].

18. Susanne Staehlke et al. (2018) assessed the human MG-63 osteoblastic cell line's phenotypic stability at various stages.

    The MG-63 human cell line is commonly used in osteogenesis studies due to its stable phenotype over multiple passages. This study aims to characterize MG-63 cell behavior from passages five to thirty. Results show that key physiological processes, such as cell morphology, adhesion receptor availability, and expression of proteins, remain stable throughout these passages. This stability provide consistent insights into cell-material interactions[13].

19. A review was conducted by Isabelle Denry et al. (2018) on the state of zirconia technology for dental applications.

    With CAD/CAM technology, zirconia is being used more and more in prosthetic dentistry, especially for crowns and fixed partial dentures. Dental zirconia types and their characteristics are covered in this review. It assesses the long-term performance and clinical implications of various machining processing methods, both hard and soft, on zirconia. An update on international clinical studies utilizing zirconia in dentistry is also given in the paper[52].

20. Using an advanced in-vitro model, Markus Rottmar et al. (2018) evaluated the osteogenic potential of surfaces made of zr and ti.

    Zirconia is becoming more common, particularly among patients who want metal-free restorations. In order to better understand how microstructured surfaces of titanium and zirconia interact with blood, this study assessed their osseointegration capability with and without extra nanostructures. In comparison to titanium, zirconia surfaces exhibited increased thrombogenicity, platelet adhesion, and fibrinogen adsorption. Human bone cell mineralization, on the other hand, was lower on titanium with nanostructures but greater on zirconia. The work sheds light on the interactions between blood and material and surface characteristics, which is crucial for creating implant surfaces that encourage osseointegration[11].

21. Evaluation of Titania Nanotubes/Hydroxyapatite Nanocomposites Made Using the ALD method: Assessment of Biological-activity and mechanical Properties was conducted by Radtke A et al. (2019).

    ALD used in modifying the implant surface of a titanium alloy (Ti6Al4V) to produce titanium dioxide nanotubes/ hydroxyapatite nanocomposites. The structure and surface verified by SEM and XRD. Good biointegration was demonstrated by fibroblast proliferation on the nanocomposite surface, and antibacterial efficacy was seen in the inhibition of bacterial colonization and biofilm formation. Additionally evaluated were mechanical attributes such as resistance, Young's modulus, and hardness. According to the study, these nanocomposites present a viable combination of biofunctionality and biomechanical qualities for contemporary implants[58].

22. ALGHAMDI, Hamdan S. et al. (2020) examined the advancements and prospects for dental implants.

    Since the 1970s, significant efforts have been made in developing dental implants. Despite a reported survival rate above 90%, compromised state can lead to implant failure, especially concerning due to age. Medical conditions and certain medications hinder bone healing around implants. Research must continue to improve osseointegration methods, particularly for compromised bone conditions[2].

23. The impact in TiO_2-Nb_2O_5-ZrO_2 nanotubes with varying nanoscale structures in Ti35Zr28Nb alloy was assessed by Muhammad Qadir and colleagues (2020).

    Nanotubular oxide layers, like TiO_2-Nb_2O_5-ZrO_2, are of interest for biomedical use due to their potential biocompatibility enhancements. In this study, such a layer was created on a Ti35Zr28Nb alloy surface via anodization. Varying the anodization parameters yielded nanotubes with different diameters, wall thicknesses, and lengths, impacting surface roughness and energy. Cell viability was highest on nanotubes with a larger diameter, attributed to increased surface energy and optimal tube spacing, indicating a favorable environment for “cell attachment and growth” compared to the alloy surface[3].

24. The effects of silicon doping and nanotopography regulation on the angiogenic and osteogenic activities of hydroxyapatite coating on titanium implants were assessed by Xi fu et al. in 2020.

    This study aimed to enhance angiogenesis and osteogeneis activities of biomedical (Ti) implants by electrochemically depositing hydroxyapatite (HA) coatings with varying nanotopographies and silicon (Si) doping. Coatings were characterized and evaluated for bioactivity and protein adsorption. All coatings promoted HUVEC growth, but only HS coatings up-regulated angiogenesis-related genes. Overall, Si doping and nanotopography control in

HA coatings can enhance bone regeneration and vascularization in Ti implants[5].

25. Using an in-vivo model, Anton Desch et al. (2020) assessed biofilm formation on titanium and zirconia over time.

    This study assessed biofilm development on materials over time in an in-vivo model. Volunteers wore splints with test specimens for 6 hours to 5 days, after which biofilms were analyzed using confocal laser scanning microscopy and microbiota sequencing. Biofilm volume changed significantly over time on both materials, but material type did not affect volume or live/dead ratio. Microbiome composition varied with biofilm age but not with material type, with *Streptococcus spp.* being most common. Overall, biofilm quantity and diversity increased over time, with minimal differences between zirconia and titanium[7].

26. Surface Characterization of Electro-Assisted Titanium Implants: A Multi-Technique Approach was reviewed by Stefania Cometa et al in 2020.

    Zirconia dental implants are increasingly popular, especially for patients preferring metal-free restoration or with thin gingival biotypes. However, the complex interplay of biochemical and biophysical cues in blood-material interactions and osseointegration remains poorly understood. This study compared microstructured zirconia and titanium surfaces with or without nanostructures. Zirconia exhibited increased fibrinogen adsorption, platelet adhesion, and activation, indicating higher thrombogenicity than titanium. Despite zirconia's superior mineralization for bone cells compared to microstructured titanium, nanostructured titanium outperformed zirconia.

These findings offer valuable insights into implant surface development and the importance of blood-material interactions in osseointegration[12].

27. The modulation of osteoblast morphology and proliferation through implant biomaterial surface micro-topographies was studied by Kristin Rabel et al (2020).

    In order to enhance bone cell response, surface changes are the main focus of current implant biomaterials research. Further research is necessary to ascertain whether these alterations have a direct impact on cell behavior. This study looked at the morphology and proliferation of primary alveolar bone cells in-vitro in response to several surface changes. Surface characteristics such as micro-roughness, textural aspect, surface enlargement, and wettability were examined in the study. Surface enlargement & texture aspect affected osteoblast morphology and proliferation, but not wettability or roughness. According to the study, implant surface topography affects how cells behave, which could result in the creation of biomaterials that are instructional to cells[15].

28. Michelle O'Doherty et al. (2020) looked into “how nanocomplexation with the RALA peptide could increase calcium phosphate's osteogenic potency and intracellular uptake”.

    The aim to improve osteogenesis and bone tissue development by means of calcium phosphate-based materials, specifically alpha tri-calcium phosphate (α-TCP). The problem is that these materials' varied particle sizes and hydrophilicity contribute to their limited bioavailability. The work presents a method of encapsulating α-TCP nanoparticles utilizing RALA, a cationic peptide. It was discovered that these stable, 43 nm-sized nanoparticles stimulated collagen deposition and mineralization in osteoblast lineage cells. The RALA/α-TCP nanoparticles greatly increased osteogenic responses in both MG-63 cells and porcine mesenchymal stem cells, despite an initial drop in cell viability, indicating their promise in bone regeneration therapy[31].

29. In 2020, Mukesh Tak and colleagues studied the fabrication of TNT and its impact in ti electrochemical micromachining.

    The study focused on fabricating titanium nanotubes (TNT) via anodization in an NH4F and ethylene glycol mixture. The TNTs were evaluated for their inner diameter and surface properties. A novel method using electrochemical micromachining (ECMM) was proposed for surface modification by creating TNTs on titanium. Optimal parameters for uniform TNT arrays were determined as "3 hours of anodization with a mixture of 0.3 M H3PO4 and 0.3 M NH4F at 30 V potential". ECMM demonstrated improved machining on TNT-coated titanium surfaces. This study suggests that TNT-coated surfaces enhance ECMM machining performance on titanium[40].

30. Samira Esteves Afonso Camargo and colleagues (2021) conducted a study on nanostructured surfaces of titanium aimed at promoting the proliferation of osteoblasts and reducing bacterial adhesion.

    The effects of titanium nanotubes on human osteoblast proliferation and the reduction of monomicrobial biofilm adhesion, with a specific focus on the influence of silicon carbide (SiC) on these surfaces was evaluated. Anodized titanium sheets with nanotubes ranging from 100 to 150 nm were utilized, some of which were coated with SiC. Following 24 hours of osteoblast cultivation, SEM revealed the presence of cells on all sheets. Cytotoxicity was assessed over 1, 3, and 7 days, demonstrating no harmful effects. Samples were also exposed to periodontal bacteria for 30 days, with SEM indicating decreased biofilm coverage on nanostructured surfaces. These findings suggest that titanium nanotubes can enhance osteoblast proliferation and reduce biofilm adhesion on implant surfaces.[14].

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, Belgavi
2. KAHER Dr. Prabhakar Kore's Basic Science Research centre, Belagavi (Evaluation of adhesion and proliferation of osteoblast-like cells)

- Department of Chemistry, KLS Gogte Institute of Technology, Belagavi (Fabrication of titanium nanotubes by anodization process)
- Department of Mechanical Engineering, KLS Gogte Institute of Technology, Belagavi (For analysis of surface roughness using surface profilometer)
- USIC Karnatak University, Dharwad (Surface characterization of titanium and zirconia disc through scanning electron microscope [SEM])

METHODS OF COLLECTION OF DATA:

INCLUSION CRITERIA

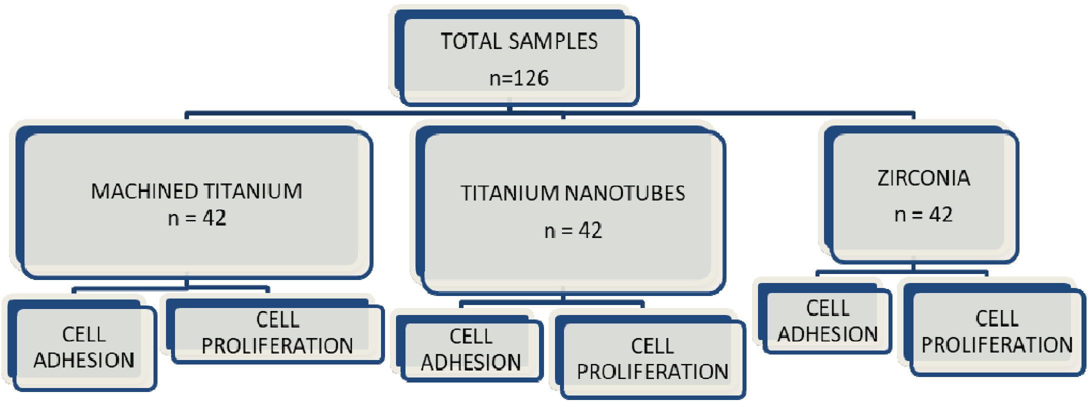
- Identical round shaped specimens of the dimensions - Diameter-10mm, Thickness-2mm

- Specimens without surface deformities & gross irregularities visible to naked eye

- Specimen having uniform surface roughness (Ra values)

EXCLUSION CRITERIA

- Specimens with inaccurate dimensions
- Specimens with porosities
- Specimens with non-uniform surface roughness (Ra values)
- Specimens with visual surface defects, deformity and gross irregularities



MATERIALS

- Titanium alloy disc (grade 5, round, diameter-10mm, thickness-2mm)
- Zirconia discs (round, diameter-10mm, thickness-2mm)
- Hydrofluoric acid
- Copper metal
- Acetone
- Distilled water
- MTT reagent
- Dulbecco’s modified eagle medium (DMEM)
- Tryphan blue
- Phosphate buffered saline (PBS)
- MG-63 cell lines
- Trypsin EDTA

Table 1: Materials used in the study

MATERIALS	DESCRIPTION	MANUFACTURER
Titanium alloy	Type V (ti-al6-4v alloy) ATSM B348	Special metals, Mumbai, india
Zirconia	ISO 13356:2015	Special metals, Mumbai, india
Hydrofluoric acid	product code: 14880	Molychem, Mumbai
Copper	FA Nr.: 433908	RS Components & Controls (I) Ltd, Noida
Acetone	Batch No.:709066	Nice chemicals, Kerala
Distilled water	Batch no.: 007M15	Rankem chemicals, avantor, india
MTT reagent	LOT No.: 0000173715	Hi Media, Mumbai
Dulbecco’s modified eagle medium (DMEM)	LOT No.: 0000284902	Hi Media, Mumbai
Tryphan blue	LOT No.: 2024334	Hi Media, Mumbai
Phosphate buffered saline (PBS)	LOT No.: 0000237353	Hi Media, Mumbai
MG-63 cell lines		NCCS, pune
Trypsin EDTA	LOT No.: 0000297540	Hi Media, Mumbai
Paraformaldehyde	LOT No.:GRM3660-500G	Hi Media, Mumbai

ARMAMENTARIUM

- SEM (JSM-IT500LA JOEL)
- Sonicator (GT SONIC)
- DC rectifier
- Surface profilometer
- Haemocytometer
- Microtitre plate reader
- Laminar air flow
- Microscope
- Micropipette
- Incubator
- Tissue culture plate

Table 2: Armamentarium used in the study

ARMAMENTARIUM	DESCRIPTION	MANUFACTURER
SEM	(JSM-IT500LA JOEL)	Carl-zeiss
Sonicator	(GT SONIC)	Antech
DC rectifier	Model no.:7112	Aplab
Surface profilometer	Contact profilometer Model:- surtronic S-128	Taylor hobson, brazil
Haemocytometer	B.S.748 I.S. 10269	Rohem, india
Microtitre plate reader	epoch	BioTek, usa
Laminar air flow	Model:- vertical	Quest international, bangluru
Microscope	TCM400	LABOMED, USA
Micropipette	Model no.: 299932	Riviera Glass Pvt Ltd., mumbai
Incubator	Galaxy 170R	EPPENDORF, INDIA
Tissue culture plate	96 well plate	Tarsons, korea

METHODOLOGY

A. SPECIMEN PREPARATION

B. TITANIUM NANOTUBULAR COATINGS PREPARATION

C. SURFACE CHARACTERIZATION

D. ASSESSMENT OF OSTEOGENIC POTENTIAL:

1. CELL ATTACHMENT

2. CELL PROLIFERATION

3. CELL MORPHOLOGY

A. SPECIMEN PREPARATION

A total of 84 commercially available identical machined titanium grade V discs, measuring 10mm x 2mm (ATSM B348), were utilized in the study (see Figure 1). For the creation of titanium nanotubes, 42 of these machined titanium discs were employed.

Additionally, a total of 42 commercially available identical zirconia discs were used in the study (see Figure 3). The three groups:

Group A: Machined titanium discs

Group B: Titanium nanotubes (TNT) (see Figure 2)

Group C: Zirconia discs

The groups further sub-divided to smaller group (n=21) for adhesion & proliferation assays. These subgroups (n=7) were according to time periods, namely 24, 48, and 72-hours.

Prior to testing, discs were cleaned with acetone using ultrasonic treatment for 180 seconds.

B. TITANIUM NANOTUBULAR COATINGS PREPARATION

Titania nanotubular coatings were created using an optimized anodic oxidation procedure on machined titanium alloy discs (grade V, 2 mm thick, 10 mm diameter, disc-shaped samples) (see Figure 1).

The titanium discs and copper wire were meticulously cleaned with acetone using ultrasonic treatment for 120 minutes (see Figure 4 and Figure 5).

In the anodic oxidation process, titanium - anode and copper - cathode, with a 0.3% HF solution as electrolyte (see Figure 6a and 6b).

Optimized anodic oxidation procedure was conducted at a potential of U = 15V for 20 minutes. Next, discs were taken out of beaker and kept in a muffle furnace for 3 hours for annealing (see Figure 8). Subsequently, the titanium discs were cleaned with distilled water and acetone using ultrasonic treatment (see Figure 7).

C. SURFACE CHARACTERIZATION

Each group of specimens was numbered from 1 to 42 and subjected to quantitative and qualitative surface roughness evaluation.

Surface roughness analyzed using contact stylus profilometer (Surtronic S-128, Taylor Hobson). The total surface roughness for each specimen was calculated based on the average roughness profile (Ra). Each disc placed on flat surface with the test surface facing upward. Three lines on the surface to capture the surface profile. Diamond-point stylus was used to scan the specimens at cut-off length - 0.8 mm and transverse length - 4 mm after mounting them. All absolute roughness profile distances from the measurement length's centerline were computed as an arithmetical average value (refer to Figure 9). Ra levels were determined for each of the 42 test specimens that made up each group.

Specimens inspected using FESEM, Carl Zeiss to assess surface roughness qualitatively. For improved visualization, FESEM images were captured at magnifications of 25.0kx, 50.0kx, and 100.0kx (see Figure 10). Furthermore, an investigation using energy-dispersive X-rays (EDX) was conducted. Scanners with electron microscopy were used to analyze specimens chosen at random from each category. Figures 12 and 13 show the comparison of topographical observations of the machined ti surface with one zr.

D. EVALUATING OSTEOGENIC POTENTIAL

total sixty-three discs were surface characterized. Group A (n=42) consisted of machined titanium, Group B (n=42) of titanium nanotubes (TNT), and Group C (n=42) consisted of zirconia. The specimens were divided into three groups, and then further divided into two groups—one for cell attachment tests (n = 21) and another for cell proliferation tests (n = 21). Subsequently, each group was subdivided into subgroups (n=7) for cell attachment and cell proliferation assessments at varying durations (24, 48, and 72-hours). The osteogenic potential was assessed using MG-63 cell lines, which are osteoblast-like cells (see Figure 14).

For the revival of cell lines, MG-63 cell line obtained National Center for Cell Sciences (NCCS) in Pune. Data sheet included sixteen short tandem repeat (STR) loci, demonstrating a 100% match with the ATCC STR profile. Upon procurement, the cells were maintained and subcultured by preparing 100ml of complete media containing DMEM (89ml) (Hi-media, LOT No.:0000515574), fetal bovine serum (10ml) (Gibco by Life Technologies LOT No.: 42G5176K), and antibiotics (1ml) (Hi-media, LOT No.:000D482174). Cells cultured in 5% CO_2 incubator. All cell culture procedures were performed in a class II cabinet under strict aseptic conditions. Upon reaching 85% confluency, trypsinization was performed using trypsin, and subculturing was carried out with cell observation under an inverted microscope.

CELL ATTACHMENT

Live cells ascertained by trypan blue exclusion test. While non-viable cells having damaged membranes absorb dye to look blue, viable cells having intact cell membranes don’t take up dye to look clear. On specimens from the test, positive, and negative control groups, MG63 cells were grown (refer to Figure 15).

The growth media was collected at all time points, wells washed three times with PBS at 37°C removed any unadhered cells. After adding trypan blue to cell suspension, adhering cells counted enzymatically with hemacytometer (refer to Figure 16). Counting began with the number of unstained cells in each set of 16 squares and continued until all squares had been counted. The quantity of cells used to quantify cell attachment was used.

CELL PROLIFERATION

The MTT colorimetric test, which assesses mitochondrial dehydrogenase activity, was utilized to measure cell proliferation. Each well of a microtiter plate was seeded with a 50µl cell suspension containing 1 × 105 cells/ml, and the total volume was adjusted to 150 µl with DMEM medium. Subsequently, 100µl of the diluted solution put in wells, and plate kept for 72 hours at 37°C in CO_2 incubator having 5% CO_2. After 72-hour incubation, 20µl of a 5 mg/ml MTT reagent added, followed by a further four-hour incubation at room temperature in a dark environment.

The supernatant was carefully extracted to avoid disrupting the formazan crystals, which had formed. Subsequently, 200 µl of DMSO added for dissolution of crystals. OD measured at 570 nm with microtiter plate reader at 24-hours, 48-hours, and 72-hours. (refer to Figure 17).

CELL MORPHOLOGY

SEM was employed in examining morphology of MG 63 cells cultured on the specimens. Following a 72-hour incubation period, the scaffolds were fixed in 4% paraformaldehyde (see Figure 18).

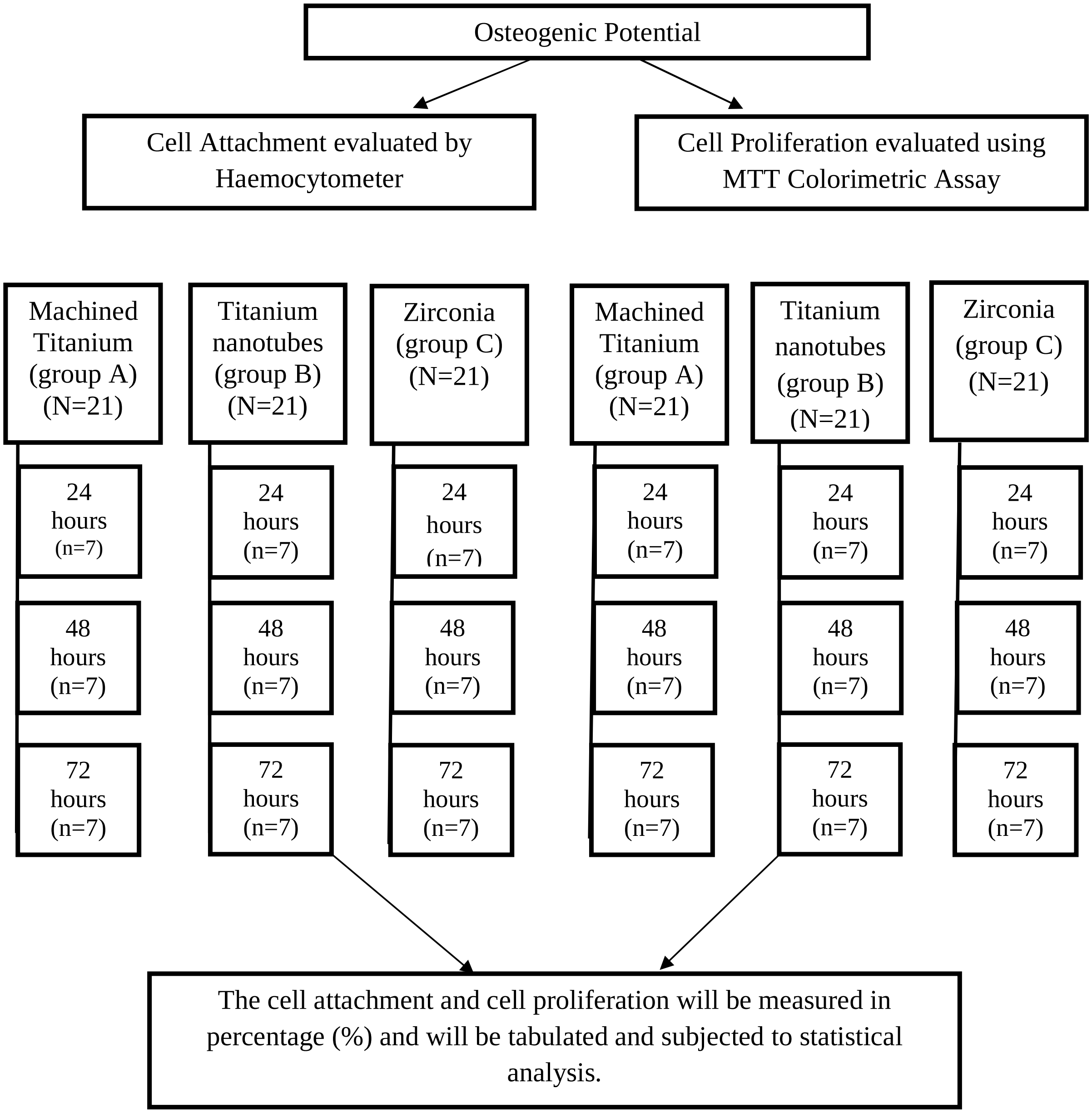


FIGURE 1: GROUP A – MACHINED TITANIUM DISC

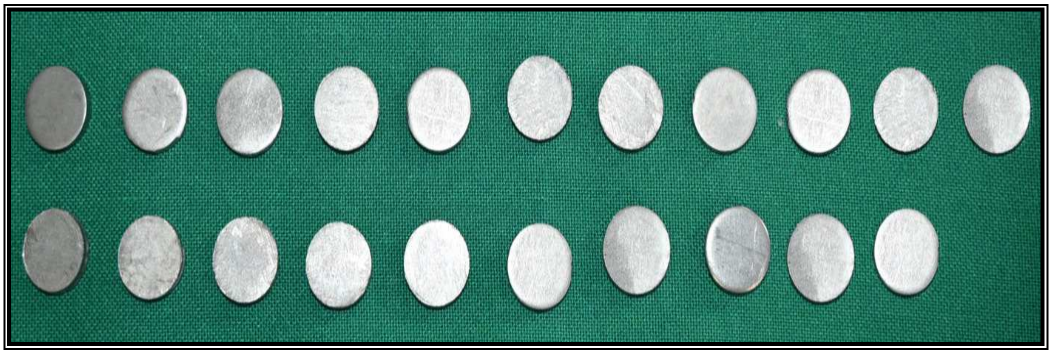


FIGURE 2: GROUP B – ALTERED TITANIUM SURFACE WITH NANOTUBES

FIGURE 3: GROUP C – ZIRCONIA DISC

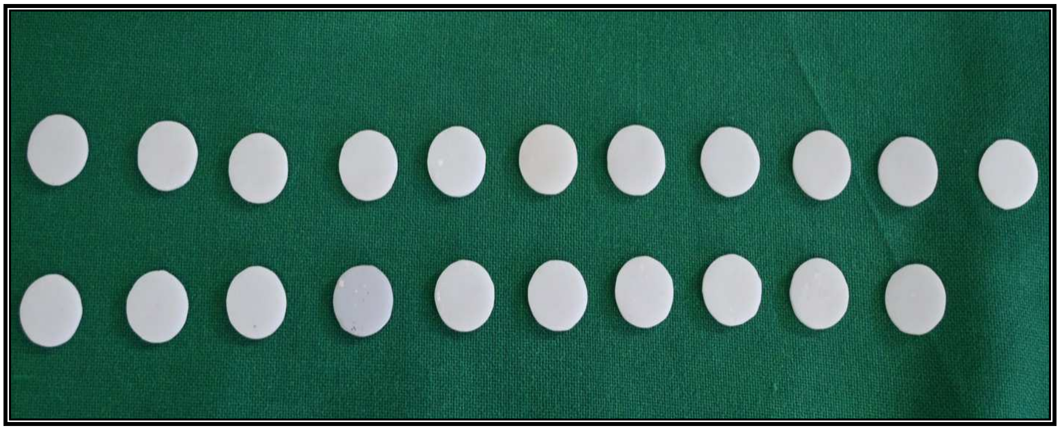


FIGURE 4: ULTRASONIC CLEANER (GT SONIC)

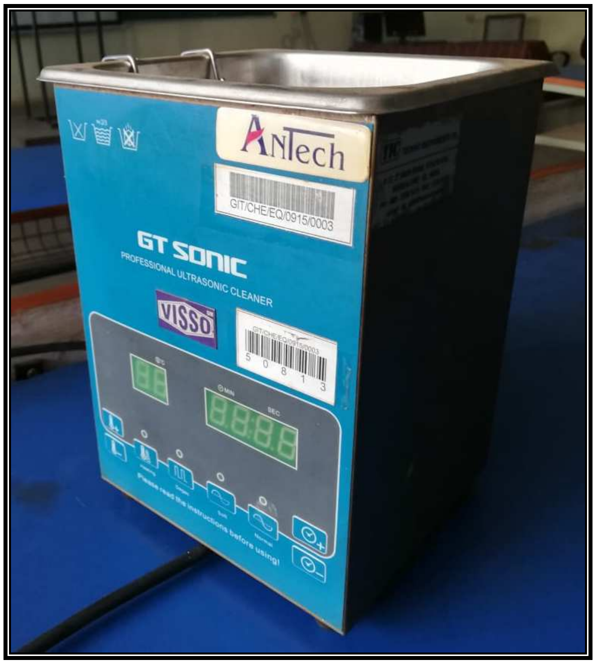


FIGURE 5: ULTRASONIC CLEANING OF COPPER WIRE AND TITANIUM DISC

FIGURE 6: ELECTROCHEMICAL CELL

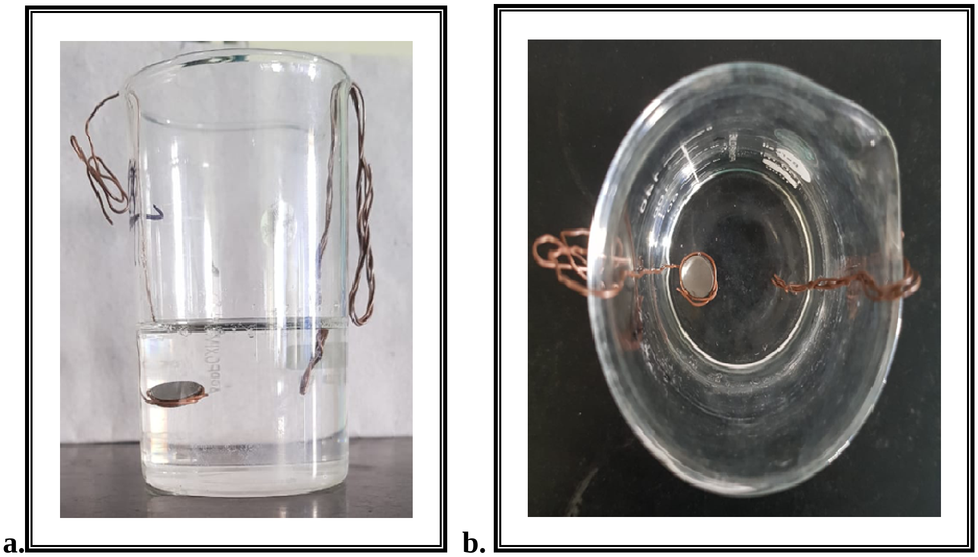


FIGURE 7: ANODIZATION AT U=15V TIME: 20 MINS

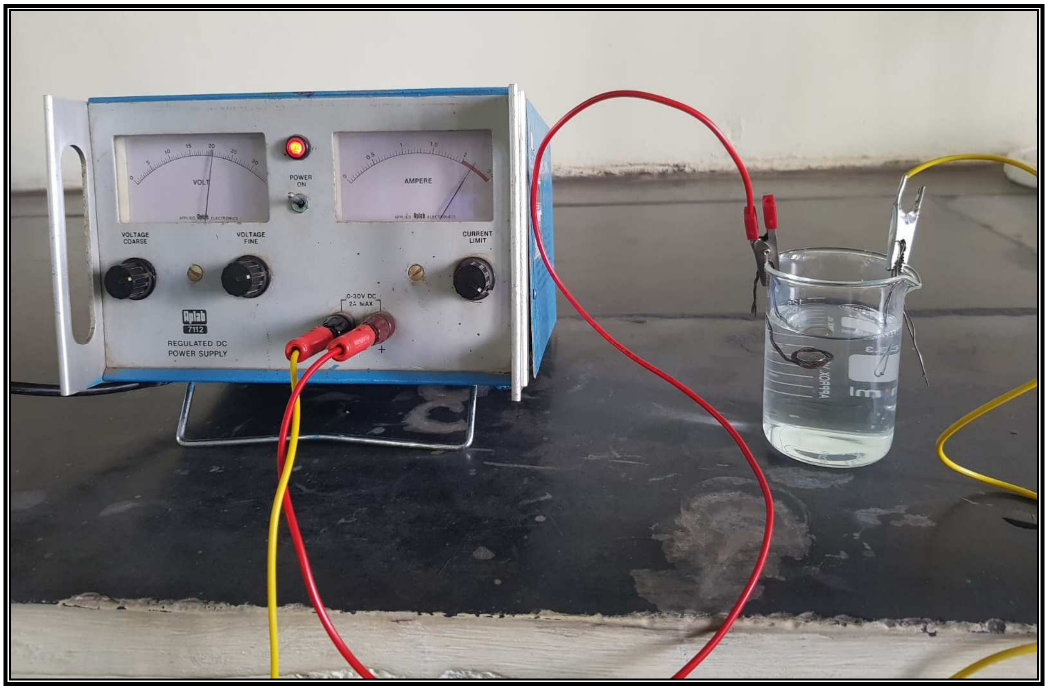
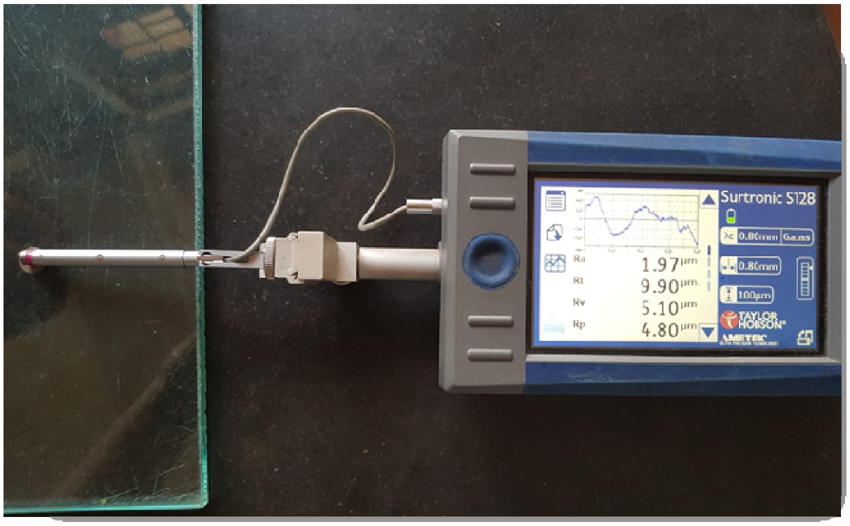


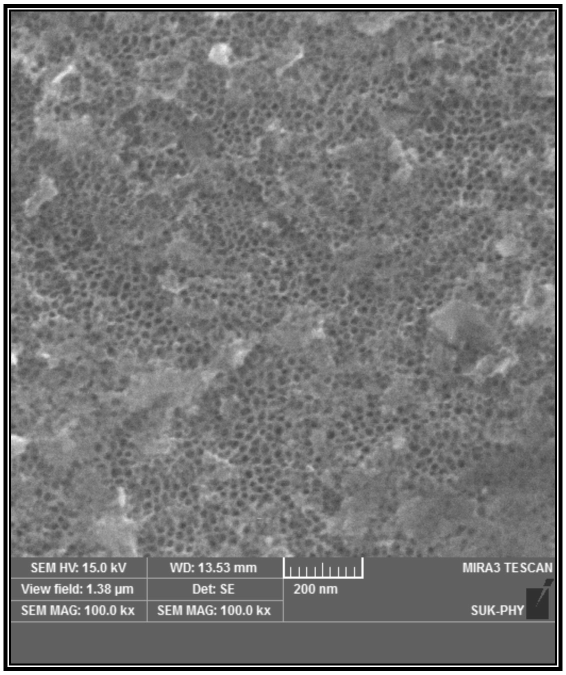
FIGURE 8: ANNEALING IN MUFFLE FURNACE

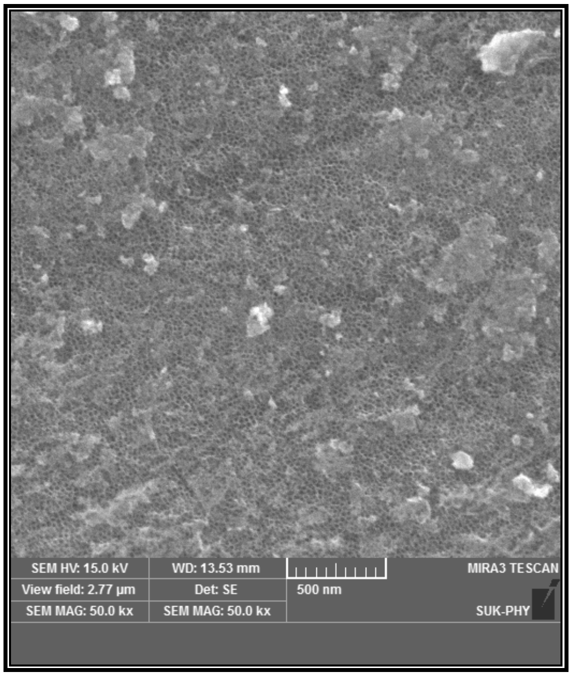
FIGURE 9: SURFACE CHARACTERIZATION – QUANTITATIVE ANALYSIS USING SURFACE PROFILOMETER

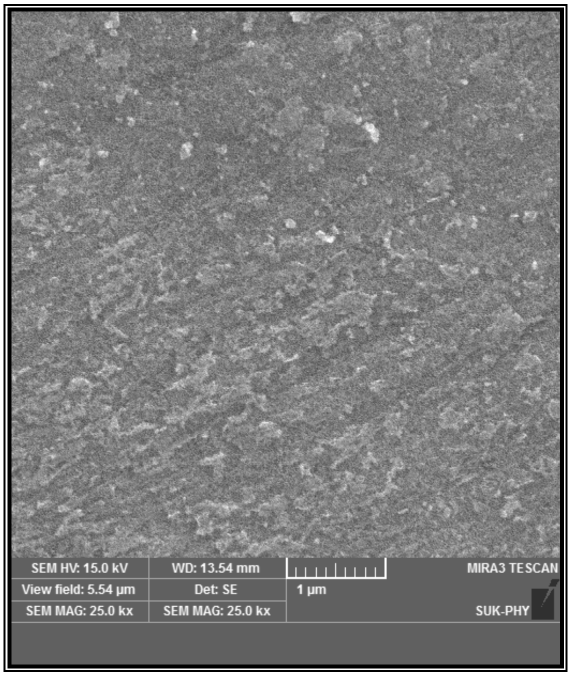


Ra 1.97µm
Rt 9.90µm
Rv 5.10µm
Rp 4.80µm

FIGURE 10: SURFACE CHARACTERIZATION – QUALITATIVE ANALYSIS USING FESEM (25KX, 50KX, 100KX, 226KX(SHOWING DIAMETER OF NANOTUBE)






SEM HV: 15.0 kV
WD: 13.54 mm
MIRA3 TESCAN
View field: 5.54 µm
Det: SE
1 µm
SEM MAG: 25.0 kx
SEM MAG: 25.0 kx
SUK-PHY

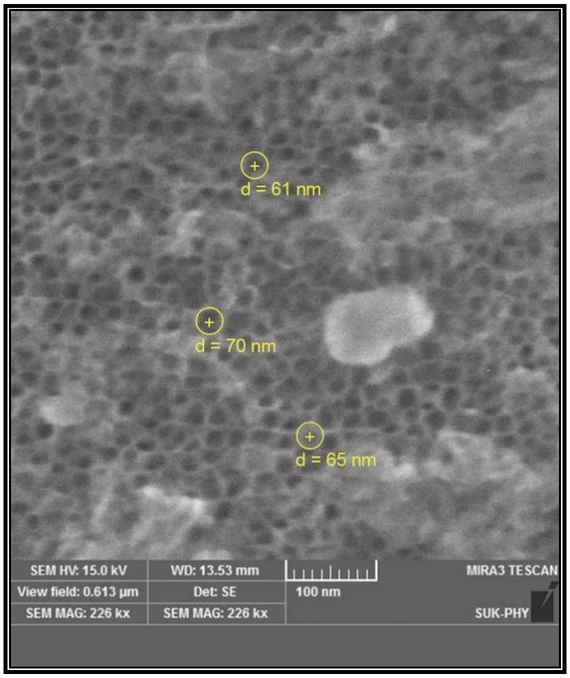

d = 61 nm
d = 70 nm
d = 65 nm
SEM HV: 15.0 kV
WD: 13.53 mm
MIRA3 TESCAN
View field: 0.613 µm
Det: SE
100 nm
SEM MAG: 226 kx
SEM MAG: 226 kx
SUK-PHY

FIGURE 11: EDX ANALYSIS OF TNT SURFACE USING FESEM

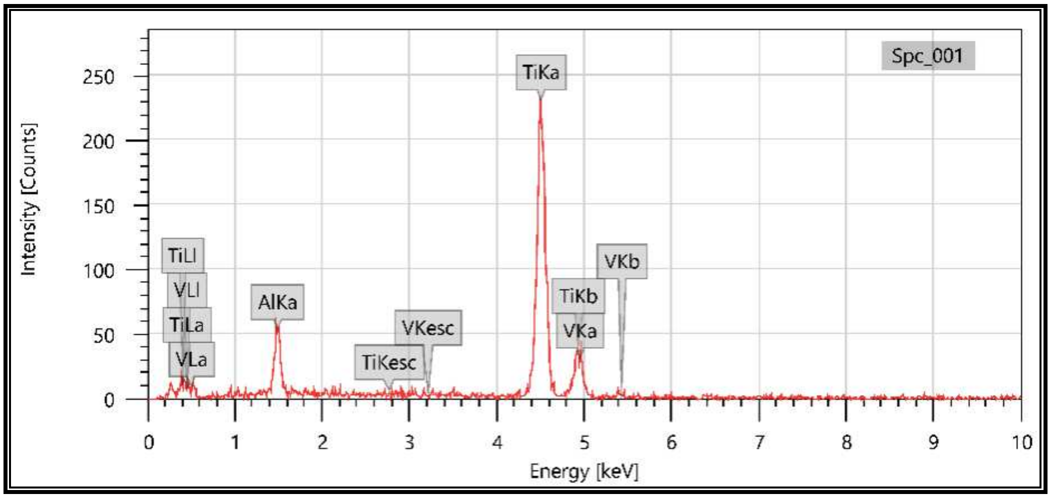


FIGURE 12: QUALITATIVE ANALYSIS OF TITANIUM SURFACE USING FESEM

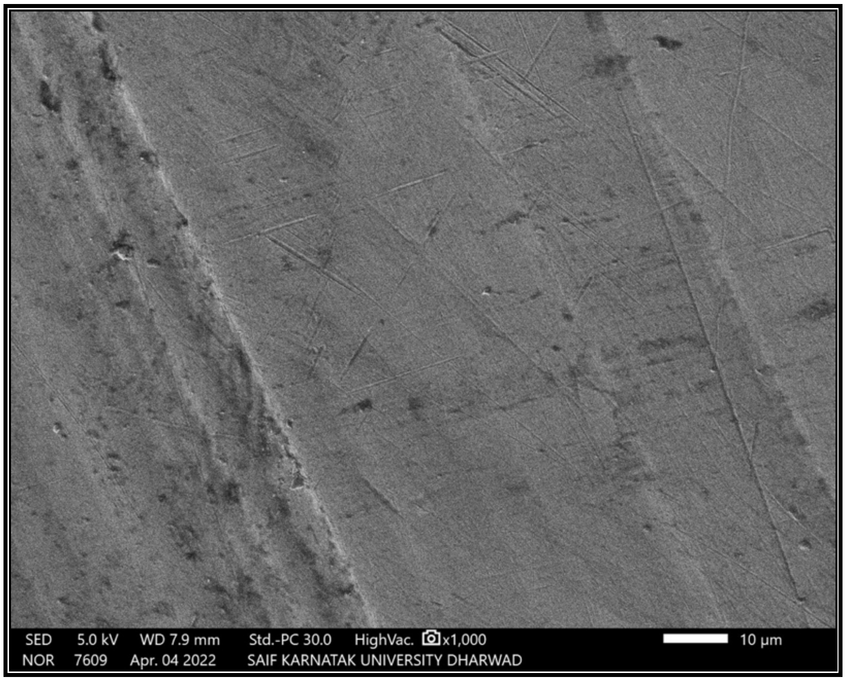


FIGURE 13: QUALITATIVE ANALYSIS OF ZIRCONIA SURFACE USING FESEM

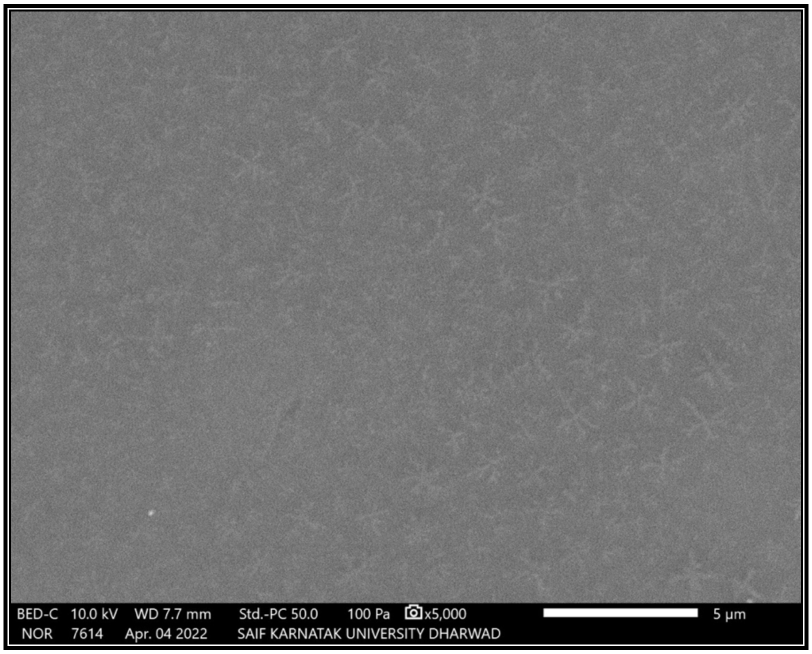


FIGURE 14: MG-63 CELL LINES FOR ASSESSMENT OF OSTEOGENIC POTENTIAL

FIGURE 15: SEEDED SPECIMENS ASSESSING CELL ATTACHMENT

FIGURE 16: HAEMOCYTOMETER

FIGURE 17: MTT ASSAY TO ASSESS PROLIFERATION OF THREE GROUPS

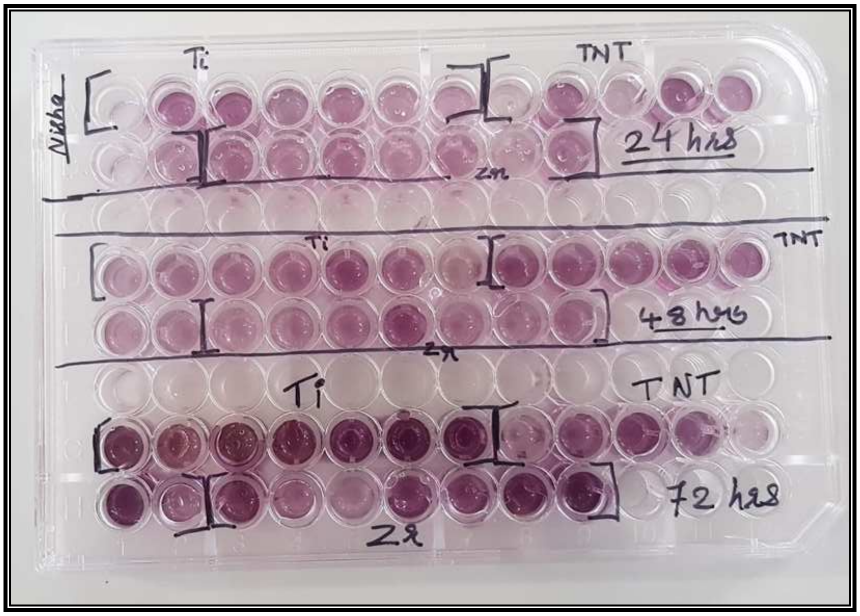


FIGURE 18: PROLIFERATED MG63 CELLS AFTER 72 HOURS OBSERVED UNDER FESEM

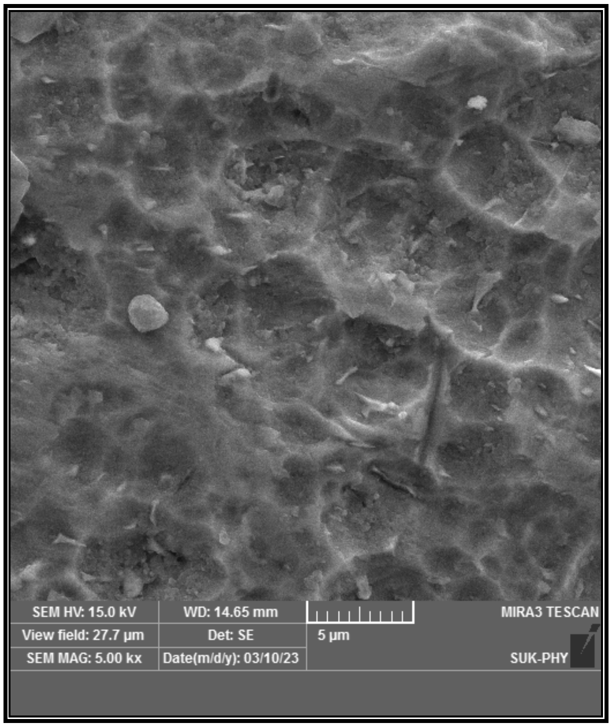


FIGURE 19: MATERIALS USED IN THE STUDY

RESULTS

The results of the cell proliferation, cell attachment and surface roughness values comparing the machined titanium surface, titanium surface with nanotubes and zirconia surface were tabulated.

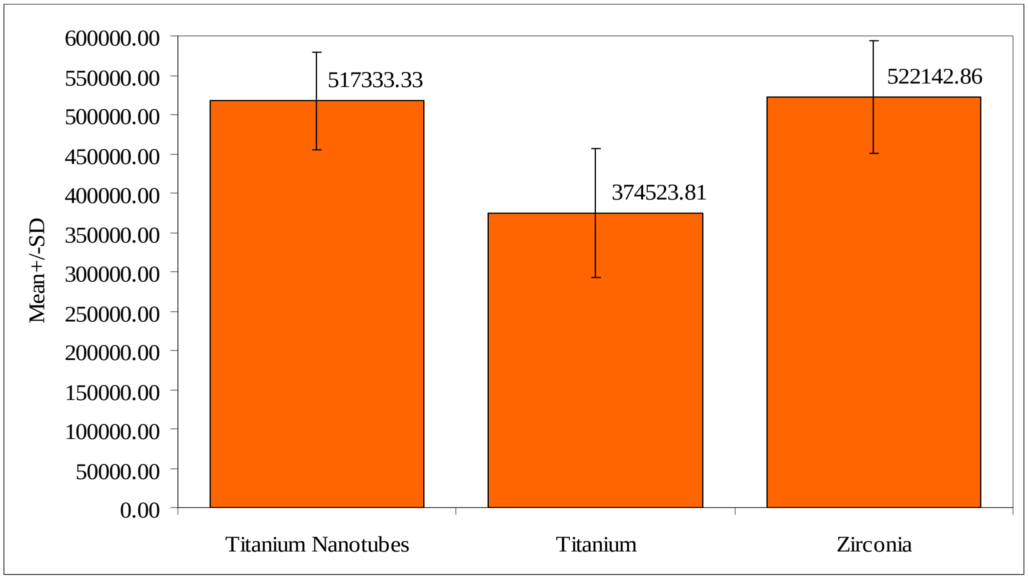
These results were analyzed and subjected for analysis using SPSS software version 21.0. Mean Standard deviation (Descriptive statistical measures) calculated in cell proliferation, cell attachment (Table 3) and surface roughness.

Table 3: Summery of cell attachment scores in three groups and three times (24-hours, 48-hours and 72-hours)

Main	Levels	Mean	SD	SE	95% CI for mean	
					Lower	Upper
Groups	Titanium Nanotubes	517333.33	62240.13	13581.91	489001.97	545664.70
	Machined Titanium	374523.81	82232.97	17944.71	337091.81	411955.81
	Zirconia	522142.86	72138.26	15741.86	489305.92	554979.80
Times	24 hours	385190.48	87193.82	19027.25	345500.33	424880.63
	48 hours	481666.67	53859.38	11753.08	457150.17	506183.17
	72 hours	547142.86	78558.44	17142.86	511383.48	582902.23
Interactions	Titanium Nanotubes with 24 hours	439857.14	6962.48	2631.57	433417.92	446296.37
	Titanium Nanotubes with 48 hours	527857.14	17761.65	6713.27	511430.36	544283.93
	Titanium Nanotubes with 72hrs	584285.71	13671.31	5167.27	571641.86	596929.57
	Machined Titanium with 24 hours	266428.57	22119.80	8360.50	245971.17	286885.98
	Machined Titanium with 48 hours	414285.71	30059.46	11361.41	386485.35	442086.08
	Machined Titanium with 72 hours	442857.14	14960.26	5654.45	429021.21	456693.08
	Zirconia with 24 hours	449285.71	8380.82	3167.65	441534.75	457036.68
	Zirconia with 48hrs	502857.14	11852.27	4479.74	491895.63	513818.66
	Zirconia with 72 hours	614285.71	24567.69	9285.71	591564.39	637007.04

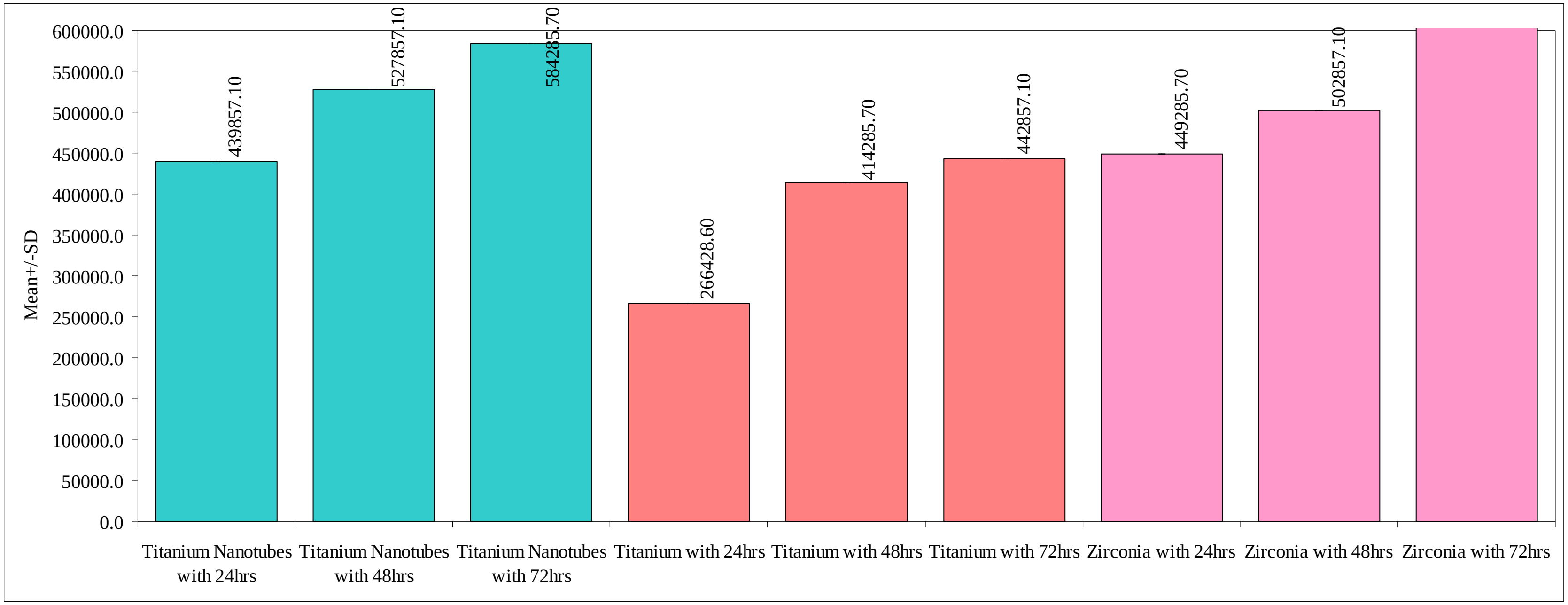
As seen in Table 3 and Graph 1 and 2 across time intervals it was observed that titanium surface with nanotubes had 517333.33 ± 62240.13 cell attachment whereas zirconia surface had 522142.86 ± 72138.26 cell proliferation. The titanium surface with nanotubes has shown increase in number of cells attached per ml cell suspension from 24 hours (439857.14 ± 6962.48) to 72 hrs (584285.71 ± 13671.31). The zirconia surface has shown increase number of cells attached per ml cell suspension from 24 hours (449285.71± 8380.82) to 72 hours (614285.71± 24567.69). The machined titanium surface has shown increase in number of cells attached per ml cell suspension 24 hours (266428.57± 22119.80) to 72 hours (442857.14± 14960.26). The titanium surface with nanotubes and zirconia surface has shown around 37% of increase in attachment of cells from 24 hours to 72 hours whereas machined titanium surface has shown around 66% increase in attachment of cells from 24 hours to 72 hours.

Graph 1: Pair wise comparison of three groups with cell attachment scores



Comparison of mean cell attachment scores at three-time intervals for machined titanium surface, titanium surface with nanotubes and zirconia surface was observed that machined titanium surface had 374523.81 ± 82232.97 cell attachment, titanium surface with nanotubes had 517333.33 ± 62240.13 cell attachment whereas zirconia surface had 522142.86 ± 72138.26 cell attachment as seen in Graph 1.

Graph 2: Comparison of interactions between three groups and three times (24-hours, 48-hours and 72-hours) with cell attachment scores



Comparison of the mean cell attachment scores of machined titanium surface, titanium surface with nanotubes and zirconia surface at 24-hours, 48-hours and 72-hours revealed that there has been difference between groups when compared across time 24-hours, 48-hours, and 72-hours. the zirconia surface showed an increased mean cell attachment when compared to machined titanium surface and titanium surface with nanotubes across all the three times that are compared.

Table 4: Comparing three groups & three times with cell attachment scores using Two-way ANOVA

Sources of variation	Sum of squares	Degrees of freedom	Mean sum of squares	F-value	p-value
Main effects					
Groups	295463523810.00	2	147731761905.00	445.8990	0.0001*
Times	278763523810.00	2	139381761905.00	420.6962	0.0001*
2-way interaction effects					
Groups*Times	20146095238.00	4	5036523810.00	15.2017	0.0001*
Error	17890857143.00	54	331312169.00		
Total	612264000001.00	62			

*p<0.05 indicates statistically significant

Two-way ANOVA was performed to compare the mean cell attachment scores of machined titanium surface, titanium surface with nanotubes and zirconia surface at three points as seen in Table 4 and Graph 2. The test revealed there was significant difference statistically between groups when only the groups were compared, and also when the groups were compared across time (p=0.0001) as seen in Table 4.

Table 5: Pair wise comparison of interactions between three groups and three time intervals with cell attachment scores using Tukeys multiple posthoc procedures

Interactions	Titanium Nanotubes with 24 hours	Titanium Nanotubes with 48 hours	Titanium Nanotubes with 72 hours	Machined Titanium with 24 hours	Machined Titanium with 48 hours	Machined Titanium with 72 hours	Zirconia with 24 hours	Zirconia with 48 hours	Zirconia with 72 hours
Mean	439857.1	527857.1	584285.7	266428.6	414285.7	442857.1	449285.7	502857.1	614285.7
SD	6962.48	17761.65	13671.31	22119.80	30059.46	14960.26	8380.82	11852.27	24567.69
Titanium Nanotubes with 24 hours	-								
Titanium Nanotubes with 48 hours	p=0.0001*	-							
Titanium Nanotubes with 72 hours	p=0.0001*	p=0.0002*	-						
Machined Titanium with 24 hours	p=0.0001*	p=0.0001*	p=0.0001*	-					
Machined Titanium with 48 hours	p=0.1994	p=0.0001*	p=0.0001*	p=0.0001*	-				
Machined Titanium with 72 hours	p=1.0000	p=0.0001*	p=0.0001*	p=0.0001*	p=0.1026	-			
Zirconia with 24 hours	p=0.9872	p=0.0001*	p=0.0001*	p=0.0001*	p=0.0186*	p=0.9991	-		
Zirconia with 48 hours	p=0.0001*	p=0.2237	p=0.0001*	p=0.0001*	p=0.0001*	p=0.0001*	p=0.0002*	-	
Zirconia with 72 hours	p=0.0001*	p=0.0001*	p=0.0724	p=0.0001*	p=0.0001*	p=0.0001*	p=0.0001*	p=0.0001*	-

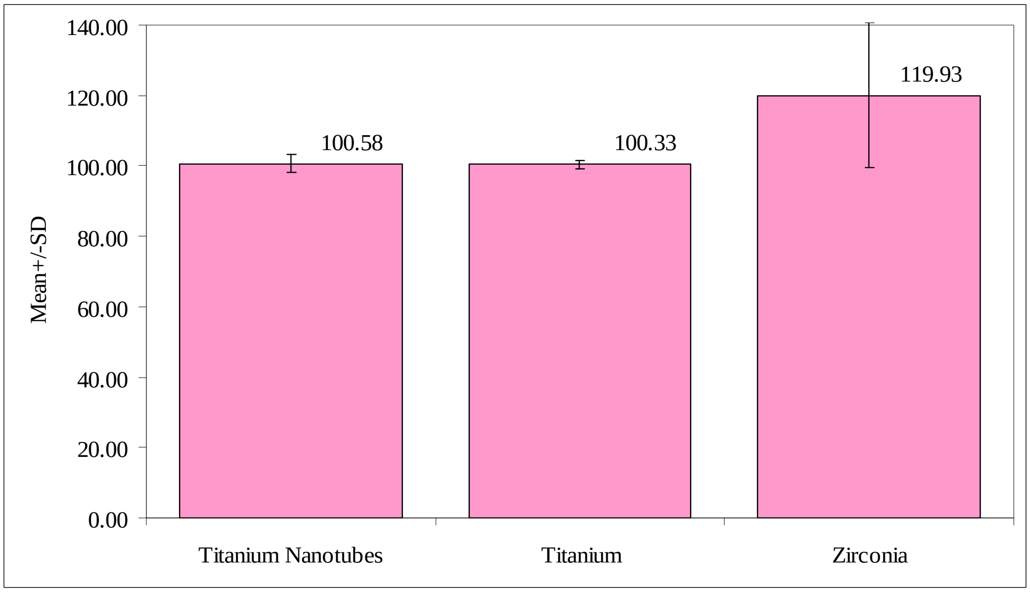
*p<0.05 indicates statistically significant

Pair-wise comparison of machined titanium surface, titanium surface with nanotubes and zirconia surface at three time points to compare mean cell proliferation scores by Tukeys Multiple Posthoc Procedure. Found a significant difference statistically (p=0.0001) when mean cell attachment of machined titanium surface, is compared with titanium surface with nanotubes and zirconia surface at all the three time points and when comparison was done for each surface across all the three time points as seen in Table 5.

Table 6: Summery of cell proliferation scores in three groups and three time intervals

Main	Levels	Mean	SD	SE	95% CI for mean	
					Lower	Upper
Groups	Titanium Nanotubes	100.58	2.56	0.56	99.42	101.74
	Machined Titanium	100.33	1.06	0.23	99.85	100.82
	Zirconia	119.93	20.59	4.49	110.56	129.31
Times	24 hours	97.94	3.40	0.74	96.40	99.49
	48 hours	107.48	10.28	2.24	102.80	112.16
	72 hours	115.43	20.47	4.47	106.11	124.75
Interactions	Titanium Nanotubes with 24 hours	97.60	1.32	0.50	96.38	98.82
	Titanium Nanotubes with 48 hours	101.29	1.25	0.47	100.13	102.45
	Titanium Nanotubes with 72 hours	102.86	1.21	0.46	101.73	103.98
	Machined Titanium with 24 hours	99.86	0.69	0.26	99.22	100.50
	Machined Titanium with 48 hours	100.43	0.98	0.37	99.53	101.33
	Machined Titanium with 72 hours	100.71	1.38	0.52	99.44	101.99
	Zirconia with 24 hours	96.37	5.39	2.04	91.39	101.35
	Zirconia with 48 hours	120.71	6.52	2.47	114.68	126.75
	Zirconia with 72 hours	142.71	9.39	3.55	134.03	151.40

Graph 3: Comparison of three groups with cell proliferation scores



As seen in Table 6 and Graph 3 across the three time periods the mean cell proliferation of machined titanium surface was 100.33 ± 1.06, titanium surface with nanotubes was 100.58 ± 2.56 and zirconia surface was 119.93 ± 20.59.

The titanium surface with nanotubes has shown increase in proliferation from 24 hours (97.60± 1.32) to 72 hours (102.86± 1.21). The zirconia surface has shown increase in attachment from 24 hours (96.37± 5.39) to 72 hours (142.71± 9.39). The machined titanium surface has shown increase in attachment from 24 hours (99.86± 0.69) to 72 hours (100.71± 1.38).

Comparison of machined titanium surface with titanium surface with nanotubes and zirconia surface with mean cell proliferation scores at three time periods revealed that mean cell proliferation for machined titanium surface was 100.33 ± 1.06, titanium surface with nanotubes was 100.58 ± 2.56 and zirconia surface was 119.93 ± 20.59 as shown in Graph 3.

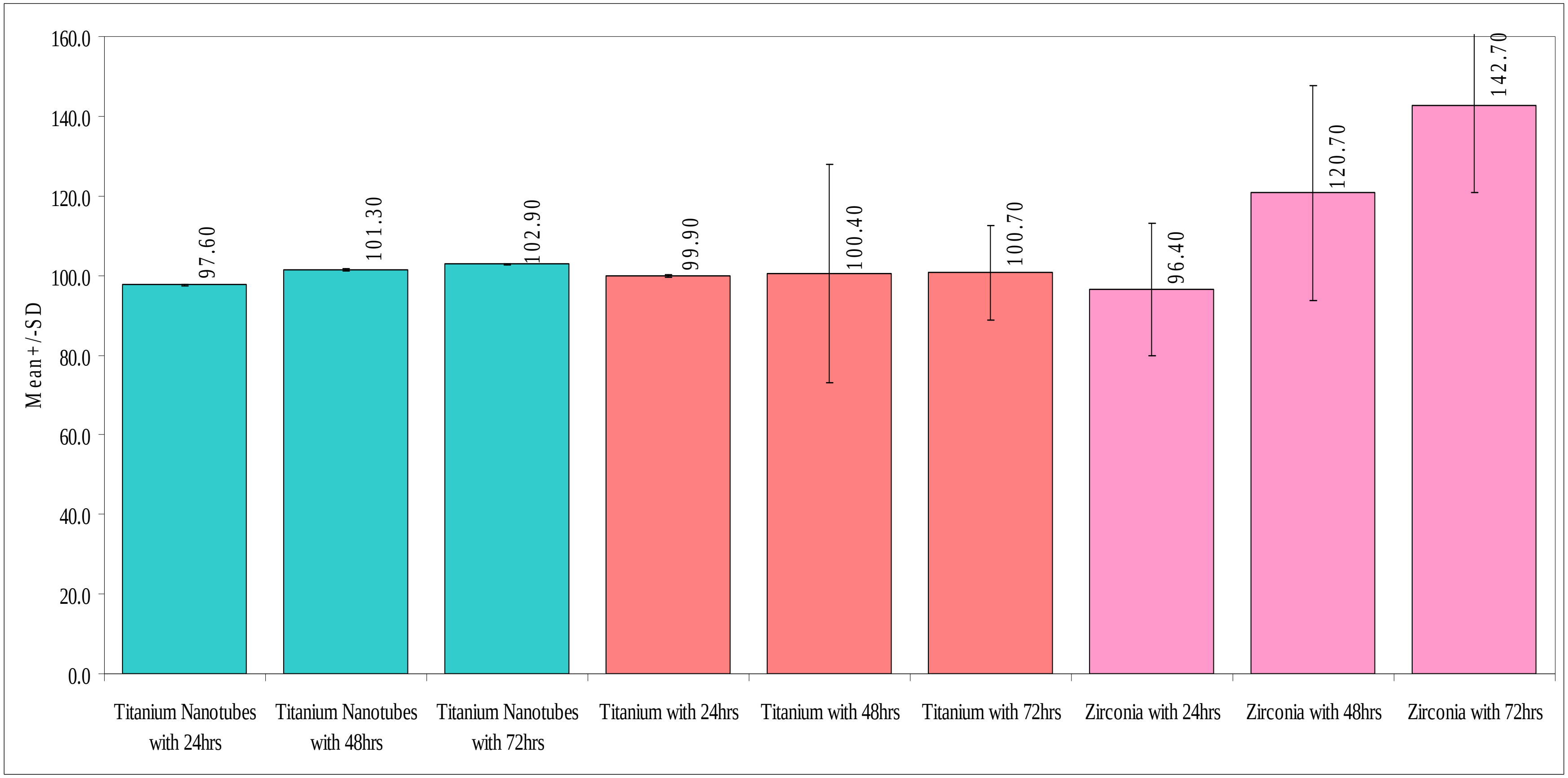
Table 7: Comparing three groups and 3 time points with cell proliferation scores using Two-way ANOVA

Sources of variation	Sum of squares	Degrees of freedom	Mean sum of squares	F-value	p-value
Main effects					
Groups	5311.1518	2	2655.5759	142.3227	0.0001*
Times	3219.1251	2	1609.5625	86.2628	0.0001*
2-way interaction effects					
Groups*Times	4408.7035	4	1102.1759	59.0699	0.0001*
Error	1007.5771	54	18.6588		
Total	13946.5575	62			

*p<0.05 indicates statistically significant

Two-way ANOVA was performed to compare the mean cell proliferation scores of machined titanium surface, titanium surface with nanotubes and zirconia surface at three time points as seen in Table 7 and Graph 4. Test revealed significant difference statistically between the groups when only groups were compared, and also when the groups were compared across time (p=0.0001) as seen in Table 7.

Graph 4: Comparison of interactions between three groups and three time intervals with cell proliferation scores



Comparison of mean cell proliferation scores of machined titanium surface, titanium surface with nanotubes and zirconia surface at different time periods revealed there was difference in between groups when the three groups were compared and also within each group when compared across time. Comparing three groups zirconia surface showed in cell proliferation by 48% from 24 hours to 72 hours whereas titanium surface with nanotubes showed increase of 5.43% from 24 hours to 72 hours and machined titanium surface showed increase by 0.8% from 24 hours to 72 hours in cell proliferation as seen in Graph 4.

Table 8: Pair wise comparison of interactions between three groups and 3 time intervals with cell proliferation scores using Tukeys Multiple Posthoc Procedures

Interactions	Titanium Nanotubes with 24hrs	Titanium Nanotubes with 48hrs	Titanium Nanotubes with 72hrs	Machined Titanium with 24hrs	Machined Titanium with 48hrs	Machined Titanium with 72hrs	Zirconia with 24hrs	Zirconia with 48hrs	Zirconia with 72hrs
Mean	97.6	101.3	102.9	99.9	100.4	100.7	96.4	120.7	142.7
SD	1.32	1.25	1.21	0.69	0.98	1.38	5.39	6.52	9.39
Titanium Nanotubes with 24hrs	-								
Titanium Nanotubes with 48hrs	p=0.8026	-							
Titanium Nanotubes with 72hrs	p=0.3739	p=0.9989	-						
Machined Titanium with 24hrs	p=0.9865	p=0.9995	p=0.9273	-					
Machined Titanium with 48hrs	p=0.9473	p=1.0000	p=0.9786	p=1.0000	-				
Machined Titanium with 72hrs	p=0.9115	p=1.0000	p=0.9903	p=1.0000	p=1.0000	-			
Zirconia with 24hrs	p=0.9998	p=0.4658	p=0.1367	p=0.8462	p=0.7090	p=0.6293	-		
Zirconia with 48hrs	p=0.0001*	p=0.0001*	p=0.0001*	p=0.0001*	p=0.0001*	p=0.0001*	p=0.0001*	-	
Zirconia with 72hrs	p=0.0001*	p=0.0001*	p=0.0001*	p=0.0001*	p=0.0001*	p=0.0001*	p=0.0001*	p=0.0001*	-

*p<0.05 indicates statistically significant

Pair-wise comparison of machined titanium surface, titanium surface with nanotubes and zirconia surface at three time points to compare cell proliferation scoresusing Tukeys Multiple Posthoc Procedure revealed there is a significant difference statistically (p=0.0001) when the mean cell proliferation of machined titanium surface is compared with titanium surface with nanotubes and zirconia surface at all three time points and when comparison was done for each surface across all the three time points as seen in Table 8.

Table 9: Summery of surface roughness scores in three groups

Groups	Mean	SD	SE	95% CI for mean	
				Lower	Upper
Titanium Nanotubes	1.9262	0.0563	0.0123	1.9005	1.9518
Machined Titanium	1.7181	0.0540	0.0118	1.6935	1.7427
Zirconia	0.9952	0.0800	0.0175	0.9588	1.0316

As seen in Table 9 and Graph 5 across groups it was observed that machined titanium surface had 1.7181± 0.0540 value for surface roughness whereas titanium surface with nanotubes had 1.9262± 0.0563 surface roughness and zirconia surface had 0.9952± 0.0800 surface roughness.

Table 10: Comparison of three groups with surface roughness scores using One-Way ANOVA

Sources of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F-value	p-value
Between groups	2	10.0275	5.0137	1204.5376	0.0001*
Within groups	60	0.2497	0.0042		
Total	62	10.2772			

*p<0.05 indicates statistically significant

One-way ANOVA performed comparing mean surface roughness scores in machined titanium, titanium surface with nanotubes and zirconia surface as seen in Table 10 and Graph 5. The test revealed significant difference statistically in groups when groups were compared (p=0.0001) as seen in Table 10.

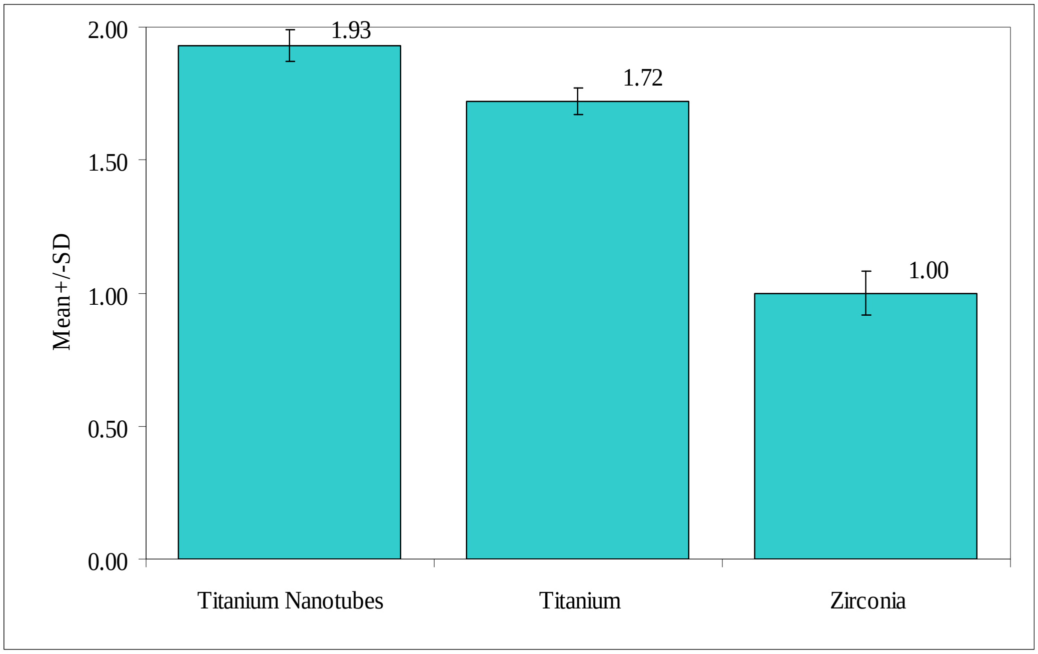
Table 11: Pair wise comparison in 3 groups with surface roughness scores by Tukeys Multiple Posthoc Procedures

Groups	Titanium Nanotubes	Machined Titanium	Zirconia
Mean	1.93	1.72	1.00
SD	0.06	0.05	0.08
Titanium Nanotubes	-		
Titanium	p=0.0001*	-	
Zirconia	p=0.0003*	p=0.0001*	-

*p<0.0 indicates statistically significant

Pair-wise comparison of machined titanium surface, titanium surface with nanotubes and zirconia surface to compare surface roughness scores by Tukeys Multiple Posthoc Procedure revealed significant difference statistically (p=0.0001) when mean surface roughness in machined titanium is compared with titanium surface with nanotubes and zirconia surface as seen in Table 11.

Graph 5: Comparison of three groups with surface roughness scores



Comparison of mean surface roughness scores for machined titanium surface, titanium surface with nanotubes and zirconia surface was observed that machined titanium surface had 1.7181± 0.0540 value of surface roughness whereas titanium surface with nanotubes had 1.9262± 0.0563 surface roughness and zirconia surface had 0.9952± 0.0800 surface roughness as seen in Graph 5.

DISCUSSION

Sixty-nine percent of adults in the 35–44 age range had lost one or more permanent teeth as a result of numerous circumstances, including dental decay, gum disease, unsuccessful root canals, and accidents, according to the American According to the Oral and Maxillofacial Surgeons Association, approximately 26% of individuals have lost all their permanent teeth by the age of 74. The significance of dental implants is underscored by the annual placement of between 100,000 and 300,000 implants, a number comparable to the annual installation of prosthetic hip and knee joints [16].

Dental implant designs, materials, and methods have been the subject of a plethora of recent research, much of which is expected to continue. The expanding demand for cosmetic dentistry and the expanding worldwide market for dental implants are the main drivers of this rise. Establishing sufficient implant anchoring, a critical component in osseointegration, requires a high BIC [16].

According to Brånemark and associates, the osseointegration phenomenon is caused by the growth of new bone that comes into direct touch with metal. Procedures developed because there are a number of characteristics that must be met, from the metal selection to prosthesis positioning. Thus, the implant material, machining methods, surface roughness, bone quality at the implant site, surgical strategy, prosthesis design, and patient postoperative care all have an impact on osseointegration [23].

Dental implants are made from a variety of biomaterials, including metals, ceramics, carbons, polymers, and their mixtures. Titanium, particularly Ti-6Al-4V, stands out as the initial contemporary material utilized for dental implants and remains one of the most favored options in use today. Commercially pure titanium, known for its lightweight properties, remarkable corrosion resistance, moderate stiffness, and excellent biocompatibility, is also highly regarded.[16,24].

Since the early 1970s, titanium has been widely employed in dental implant manufacture. On its surface, titanium naturally forms an extremely thin oxide layer that offers passive defense against corrosion and degradation under a variety of harsh environmental circumstances. These surface oxides, which develop naturally in air and/or physiological fluids, are responsible for titanium's biocompatibility as an implant material (Williams, 1981) [16-17].

In the process of machining titanium, O_2 molecules are taken in. These molecules split up and deposit a monolayer of atomic oxygen in around 10 nanoseconds. After that, the titanium and oxygen combine to form a titanium oxide layer that ranges in thickness from 50 to 100 Å (5 to 10 nm). This film, also known as the "native oxide," develops naturally at normal temperature and pressure. As an alternative, techniques like anodization—can be used to artificially create titanium oxide layers [23].

Smooth surfaces tend to attract fibroblasts and epithelial cells more firmly, whereas moderately rough surfaces encourage higher osteoblast proliferation and collagen formation. There is no set surface roughness criterion for dental implants, despite the fact that surface roughness plays a significant role in osseointegration.

Surface roughness in dental implants describes small flaws that arise from surface treatments or cutting process faults on a micrometer (μm) scale. Roughness in machined implants is mostly defined by the cutting tool and appears as a consistent shallow groove pattern [23].

One can determine the parameters in two dimensions (2D) or three dimensions (3D) to quantify roughness. Roughness average (Ra) drawback is that profiles with identical Ra values might function very differently from one another [23].

According to Menezes et al. (2003), osteoblast attachment is less likely to occur on smooth implant surfaces. The results of this investigation are in line according to Elias and colleagues (2011) that assessed the surface roughness of machined, acid-etched, blasted, and anodized implants. The anodized implants showed higher surface roughness [23].

The contact stylus profilometer was used in the current study to quantitatively evaluate all of the disc-shaped specimens for surface characterization (as seen in Figure 9). Using a tracking device, Profilometer was able to calculate the surface profile along three lines.

According to surface profilometry data, the mean average surface roughness (Ra) of all TNT-tested specimens fell between 1.8 and 1.9 um. The specimens were submitted to FESEM (25kx, 50kx, and 100kx) to evaluate the surface roughness qualitatively, and EDX analysis of the same was done (as seen in Figure 10-13).

Literature's recommended average roughness value is 1-2um [25], which is concurrent with the present study.

In present study, as seen in Table 9 and Graph 5 across groups it was observed that machined titanium surface had 1.7181± 0.0540 value for surface roughness whereas titanium surface with nanotubes had 1.9262± 0.0563 surface roughness and zirconia surface had 0.9952± 0.0800 surface roughness. One-way ANOVA and Tukeys Multiple Posthoc procedure revealed significant difference p=0.0001 of titanium nanotubes with machined titanium and p=0.0003 when compared with zirconia surface. Therefore, from the present study it was evaluated that titanium surface with nanotubes showed more surface roughness when characterized qualitatively as well as quantitatively.

Likászewska et al. claim that surface topography affects osteoblastic shape; on rough surfaces, cells are less distributed and have a smaller coverage area than on smooth surfaces. On the other hand, compared to smooth surfaces, they show more cytoplasmic extentions, phylodapy, and linkages, all of which suggest stronger adhesion qualities. Over time, rough surfaces showed an increase in cellular vitality relative to smooth surfaces [26].

According to Lima et al., implants with a rough surface promote osseointegration more effectively than ones that are machined. However, these implants must be handled cautiously because the surface treatment may alter or adversely affect the titanium oxide surface. Therefore, it's critical to standardize titanium surface roughness in order to employ a consistent surface roughness to assess surface modification features.

The influence of titanium implant surface properties on bone apposition into surface was investigated by a number of researchers. The findings demonstrated that the surface composition, morphology, all affect the biological response. These findings are significant because the dentist determines the implant's roughness based on the insertion site, surgical technique, and amount and quality of accessible bone [25-28].

Present study, anodization method applied for altering surface in titanium discs. A porous surface structure is produced by the electrolyte and current utilized in implant treatment procedure [23,29].

The anodizing parameters determine whether the TiO_2 layer is compact or nanotubular[27].

Cell proliferation is motivated at sizes of 10 nm in height or depth, and becomes undetectable at scales of 100 nm, according to Chen et al.[30]. Human bones with a large macrostructure are made up of ions, proteins, DNA, and viruses as well as other nanoscale organic and mineral phases. Because basement membrane holes, ridges, and fibers include nanoscale features, it has been demonstrated that cells react to nanosurfaces (Sepideh Minagar et al, 2012) [30].

Titanium surfaces with uniform and controllable nanopatterns can be created through electrochemical anodic oxidation, a cost-effective, straightforward, and adaptable technique.

In present study, diameter of nanotubes obtained was 60-70nm (as seen in Figure 10) which was in accordance to the ideal range required to promote bone producing cells [30].

Gurgel and colleagues (2008) examined the effectiveness of anodised implants. Three months after the dog's teeth were extracted, they created flaws that were 4 mm broad and 5 mm high. They then put the implants in place. Three months after the implant was placed, the animals were killed. The percentages of BD and (BIC) for anodized implants was 40.86 ± 22.73% and 57.03 ± 21.86%, respectively, while those for machined implants were 37.39 ± 23.33% and 3.52 ± 4.87%, the researchers discovered [31].

At now, a great deal of research is being done on methods for creating implants based on nanotechnology[16,32,33].

For osseointegration, other metals like zirconium, gold, and titanium-aluminum-vanadium alloys have been used. They have shown comparatively poor bone-to-implant contact, despite the possibility that they will increase implant strength.

In 1992, in order to further improve osseointegration, dental implant manufacturers have since included ceramic surface treatments and ceramic-like components into implants [16].

Among oxide ceramics, zirconia is unique because of its remarkable mechanical qualities. Y-TZP materials outperform other dental ceramics in terms of flexural strength, which ranges from 800 to 1000 MPa, and corrosion and wear resistance [34-37].

Kohal et al. [37] evaluated the soft tissue dimensions and osseointegration of loaded ti and zr implants in a split mouth design in monkey and statistically significant difference not found. Numerous more studies conducted on animals revealed that zirconia implants experience osseointegration that is either better than or comparable to that of titanium implants [38-39,50].

Even though the titanium surface was noticeably rougher than the tested zirconia surfaces, it was discovered that titanium implants were more resistant to removal torque, most likely as a result of the variations in surface roughness [21,40-43].

In the current study, osteogenic potential for the specimens in the experimental groups were evaluated by assessing their cell attachment and cell profileration using MG-63 cells (osteoblast like cells) on the modified surfaces which acted as machined titanium, titanium nanotubes and zirconia discs[28,31].

In this study, cell adhesion was evaluated using the tryphan blue exclusion test. This test is a simple and rapid technique measuring the cell viability and adhesion whereas the cell proliferation was assessed using MTT Assay[34].

Results in study indicate higher cell adhesion in the Zirconia surface in 24-hours at p=0.9872, 48 hours at p=0.0001 and 72 hours at p=0.0001 (as seen in Table 5 and Graph 3).

Titanium surface with nanotubes shower higher cell adhesion and proliferation when compared to machined titanium surface with statistically significant value of p=0.0001 (as seen in Table 5 and 8 and Graph 3 and 4). The higher cell attachment may also because of increase in surface-area and roughness of surface. It is known that surface topography could also play role in attachment and proliferation on implants like has been studied by Lukaszewska et al [26]

When compared between the time intervals within the groups there was a definite increase in the cell adhesion in the test group between 24-48, 48-72 and 24-72 hours showing a greater effect size compared to negative control group (as seen in Graph 2).

Cell proliferation was evaluated by the MTT assay. Cells were seeded onto discs and evaluated at intervals of 24, 48 and 72 hours. There was a higher cell proliferation on the specimens in the zirconia at all the time intervals tested, however these changes were not significant at 24-48 and 24-72 hours between the machined titanium surface and titanium nanotube surface (as seen in Table 6).

When compared between time intervals there were no statistically significant results at 24-48 hours, however, significant difference was seen at 24-72-hour time interval indicating that there was a functional maturation as well as proliferation at 48-hour time interval. There was no statistical change seen between 48-72 hours which could be due to cell confluence in the titanium nanotube group (as seen in Graph 4 and Table 8).

These findings are consistent with study by Michelle Ehrlich and colleagues in which modification increased surface roughness. Additionally, studies involving osteoblasts and fibroblasts corroborate the findings of AFM, which increased cell proliferation and raised the Sa parameter. Furthermore, co-culturing MG-63 osteoblasts with ADSCs provided indirect evidence that TNT-modified scaffolds stimulated ADSCs' osteogenic development [42,44-49].

Hence, based on the above findings the study showed that titanium surface modified with nanotubes had better osteogenic potential than machined titanium surface.

SCOPE OF THE STUDY

The three different implant materials: machined titanium, titanium with nanotubes (TNT), and zirconia were chosen due to their common use in dental and orthopedic implants. The study employed in-vitro techniques to evaluate the osteogenic properties, providing valuable insights into the potential performance of these materials in clinical settings.

Results from this study could be extrapolated and further investigated in in-vivo conditions, offering a more realistic assessment of their osteogenic capabilities. Additionally, future studies could explore the use of other implant materials, such as polyether ether ketone (PEEK), to compare and contrast their osteogenic potential with the materials studied here. To assess the osteogenic nature of these materials, various parameters could be considered, including ALP activity, RANKL expression, Alizarin Red staining, and von Kossa staining. These parameters would provide a comprehensive evaluation of the materials' ability to promote bone formation and mineralization.

Furthermore, expanding the present study to include human bone marrow cells obtained from healthy donors could enhance its clinical relevance. This extension would allow researchers to assess the materials' osteogenic potential in a more biologically relevant context, potentially paving the way for their future clinical applications.

Additionally, assessing the antimicrobial efficacy of TNT and zirconia against various strains of microorganisms that cause peri-implantitis could provide valuable insights into the materials' ability to resist infection.

LIMITATION OF THE STUDY

- In this in-vitro study, the sample groups included were only zirconia and titanium nanotubes, comparison of different sample groups with different surface treatments could yield better understanding of the best surface modification promoting bone contact.
- The study used MG63 cells (osteoblast like cells), an osteosarcoma cell line, from future perspective human osteoblasts could be used which would give more accurate results.
- Other parameters such as antibacterial efficacy can be evaluated in future research.
- Various other parameters like ALP activity, RANKL expression, Alizarin red staining, etc can be employed to evaluate osteogenic potential.

CLINICAL IMPLICATIONS

1. The altered titanium surface with nanotubes has shown increased surface roughness compared to machined titanium surface. The qualitative analysis through FESEM revealed nanotubes of pore diameter ranging from 60-70nm. This has attributed to the increase in cell attachment and proliferation.
2. The clinical use of this surface modification has a great future perspective for having implants with increased bone-to-implant contact.
3. The regimen for obtaining nanotubes is simple, versatile and cost effective, which makes it easier to fabricate nanotubes.

CONCLUSION

The osteogenic potential of titanium with nanotubes (TNT) compared to zirconia and machined titanium surfaces. The TNT surface demonstrated a significant increase in cell attachment scores at 24, 48, and 72 hours, indicating its favorable interaction with osteogenic cells. These findings were comparable to group C, which was the zirconia surface, suggesting that TNT has the potential to promote cell attachment similarly to zirconia.

Moreover, the study observed an increase in cell proliferation from 24 to 72 hours on both the TNT and zirconia surfaces. This increase in proliferation indicates that both surfaces support cell growth, which is a crucial aspect of bone regeneration. Additionally, the surface roughness of TNT was found to be higher compared to the machined titanium surface and zirconia surface. This increased roughness may contribute to the enhanced cell attachment and proliferation observed on the TNT surface, as rough surfaces have been shown to promote osteogenic cell behavior.

Overall, these findings suggest that TNT has osteogenic potential higher to machined titanium, indicating its potential utility as an implant material for promoting bone regeneration. Future studies warranted to fully elucidate the osteogenesis of TNT, to assess its performance in in-vivo models. Additional study could lead in advancement of improved implant materials that enhance bone regeneration and integration, ultimately improving the success rates of dental and orthopedic implants.

SUMMARY

A total of 126 disc, measuring 10 mm-diameter and 2 mm-thickness, used. Specimens divided into three groups: Group A (machined titanium disc) (n=42), Group B (TNT) (n=42), and Group C (zirconia disc) (n=42). Each group was further subdivided in two subgroups for cell attachment assay (n=21) and cell proliferation (n=21), and then into subgroups of seven for different time intervals (24, 48, 72 hours).

Surface roughness evaluated using profilometer, and one specimen from each group was qualitatively evaluated using FESEM at magnifications of 25.0kx, 50.0kx, and 100.0kx to visualize and compare surface profiles. Following surface roughness evaluation, osteogenic potential was assessed for attachment and proliferation of MG63 cells on the disc specimens at 24, 48, and 72-hour intervals. Cell attachment evaluated using hemocytometer, while cell proliferation assessed using MTT assay.

Data obtained tabulated and statistical analysis done. Results indicated that cell attachment was higher on the zirconia surface group at all three-time intervals compared to TNT and machined titanium surfaces. There was a significant difference in cell attachment between time intervals for the study group.

Cell proliferation was higher in Group C (zirconia) with statistically significant results at all three-time intervals. Group B (TNT) showed comparable values to the zirconia disc, suggesting that TNT could be used as a surface modification with osteogenic properties that could promote early osseointegration.

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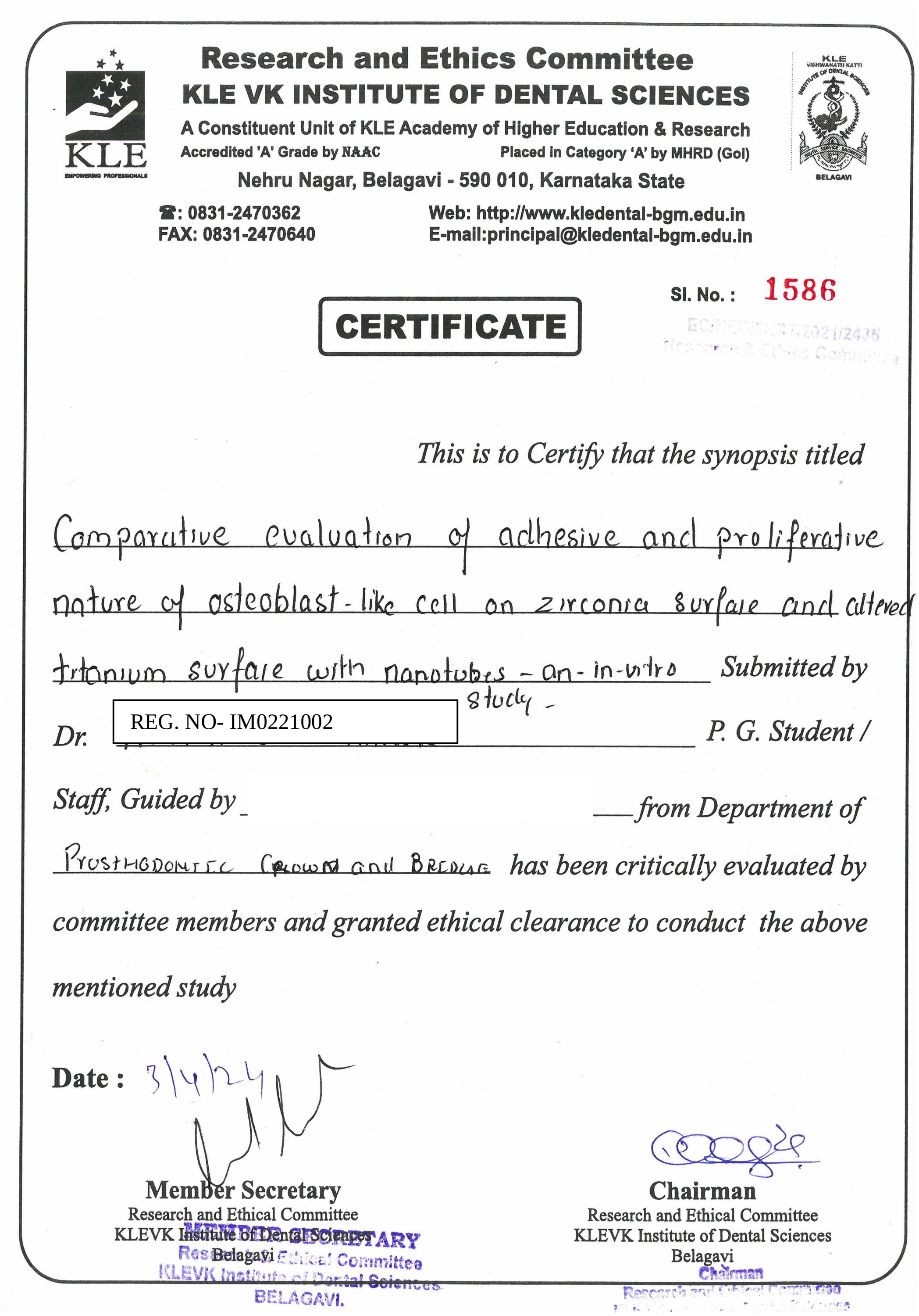
ANNEXURE – I

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. : 1586

EC/NEW/INST/2021/2435
Research & Ethics Committee

CERTIFICATE

This is to Certify that the synopsis titled Comparative evaluation of adhesive and proliferative nature of osteoblast-like cell on zirconia surface and altered titanium surface with nanotubes - an-in-vitro study - *Submitted by Dr.* REG. NO- IM0221002 *P. G. Student / Staff, Guided by* _ ___ *from Department of* PROSTHODONTIC CROWN and BRIDGE *has been critically evaluated by committee members and granted ethical clearance to conduct the above mentioned study*

Date : 3/4/24

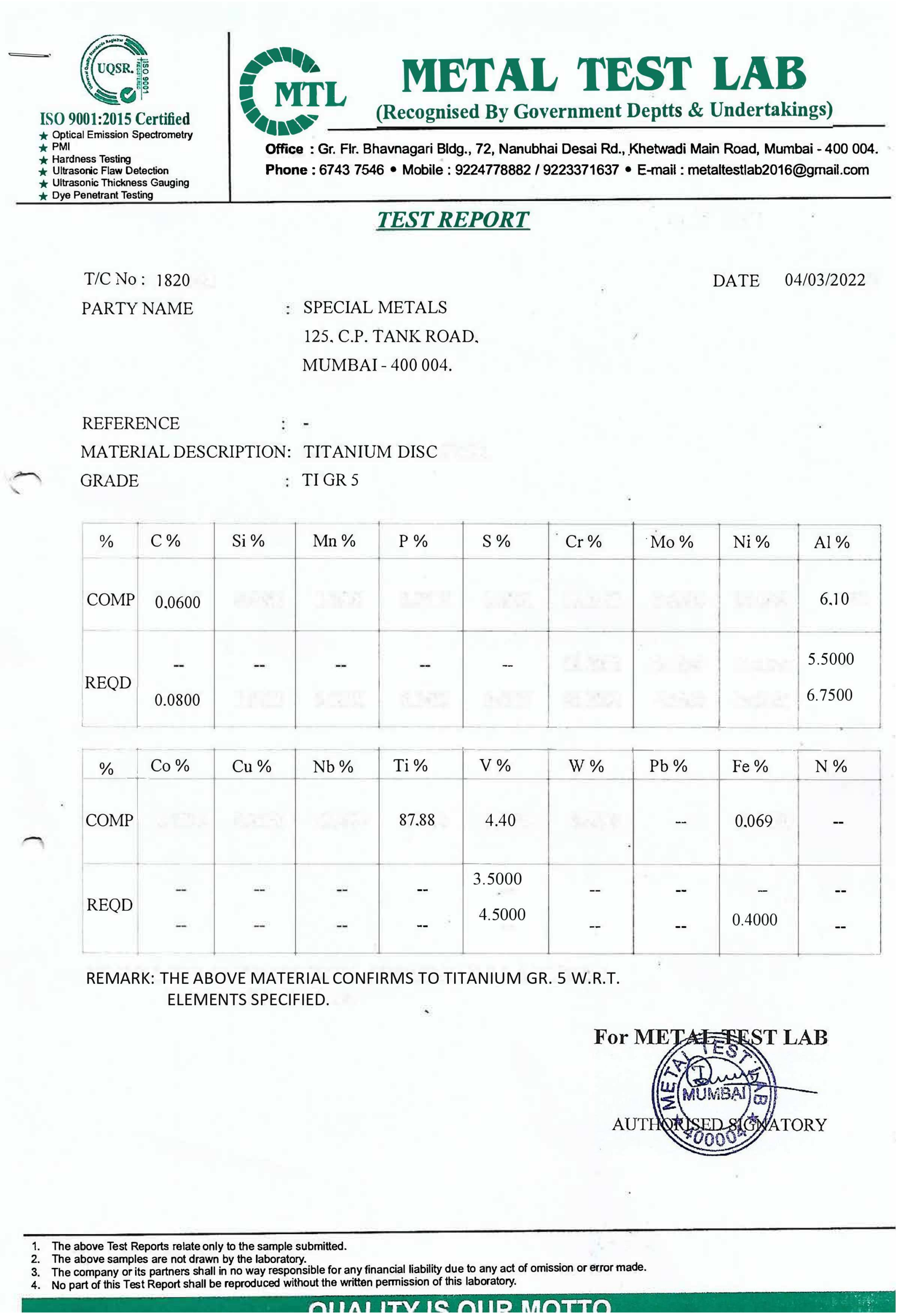
Member Secretary
Research and Ethical Committee
KLEVK Institute of Dental Sciences
Belagavi

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

Chairman
Research and Ethical Committee
KLEVK Institute of Dental Sciences
Belagavi

Chairman
Research and Ethical Committee
KLEVK Institute of Dental Sciences

ANNEXURE – II



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

METAL TEST LAB

(Recognised By Government Deptts & Undertakings)

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 : SPECIAL METALS
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

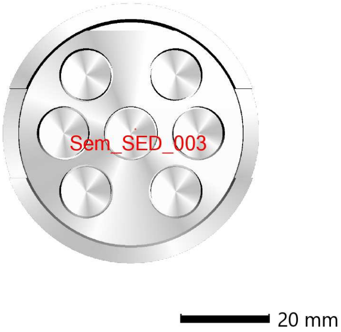
AUTHORISED SIGNATORY

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.

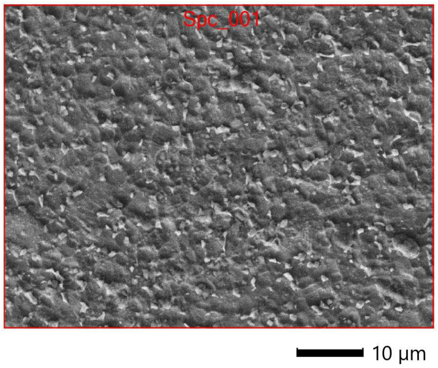
QUALITY IS OUR MOTTO

ANNEXURE – III

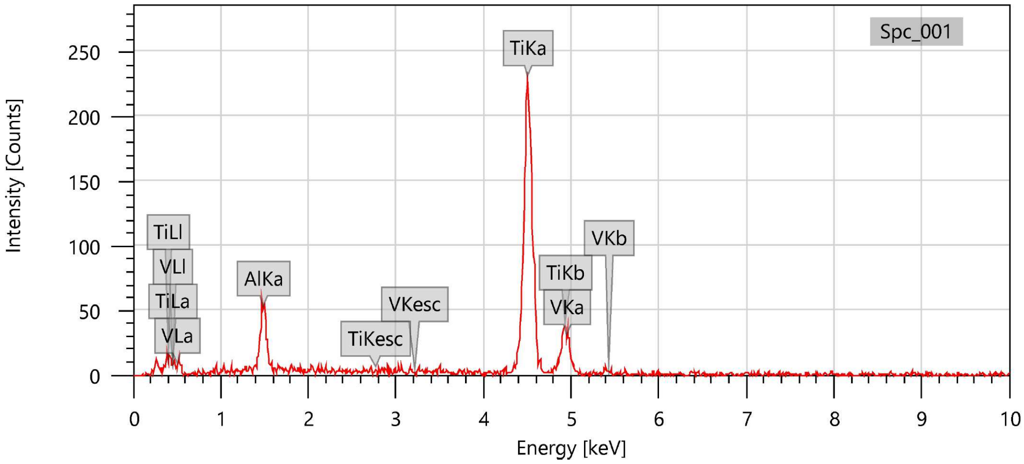
04-04-22



Sem_SED_003



Signal SED
Landing Voltage 10.0 kV
WD 7.8 mm
Magnification x2,000
Vacuum Mode HighVacuum



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

ANNEXURE – IV

OSTEOGENIC POTENTIAL

CELL ATTACHMENT

STUDY GROUP – TITANIUM NANOTUBES n = 21

24 hours	48 hrs	72 hrs
4,40,000	5,20,000	5,85,000
4,35,000	5,10,000	6,10,000
4,45,000	5,40,000	5,90,000
4,50,000	5,60,000	5,85,000
4,30,000	5,25,000	5,70,000
4,44,000	5,10,000	5,70,000
4,35,000	5,30,000	5,80,000

CONTROL GROUP – TITANIUM n = 21

24 hours	48 hrs	72 hrs
2,65,000	4,20,000	4,35,000
2,50,000	4,10,000	4,50,000
2,80,000	4,75,000	4,40,000
3,10,000	3,90,000	4,35,000
2,60,000	3,85,000	4,55,000
2,50,000	4,00,000	4,20,000
2,50,000	4,20,000	4,65,000

POSITIVE CONTROL GROUP – ZIRCONIA n = 21

24 hours	48 hrs	72 hrs
4,45,000	4,95,000	6,10,000
4,50,000	4,95,000	6,10,000
4,40,000	5,10,000	5,90,000
4,55,000	5,15,000	5,95,000
4,65,000	5,20,000	6,10,000
4,45,000	4,90,000	6,20,000
4,45,000	4,95,000	6,65,000

ANNEXURE – V

CELL PROLIFERATION

STUDY GROUP – TITANIUM NANOTUBES n = 21

24 hours	48 hrs	72 hrs
97.6%	102 %	103%
98 %	100 %	101 %
99 %	103%	102 %
97 %	100%	105 %
95 %	102 %	103 %
98 %	102 %	103%
98.6 %	100 %	103%

CONTROL GROUP – TITANIUM n = 21

24 hours	48 hrs	72 hrs
100%	100%	100%
101%	101%	101 %
100 %	100%	102%
99 %	102%	100%
100 %	101%	99%
100 %	99 %	100 %
99 %	100%	103%

POSITIVE CONTROL GROUP – ZIRCONIA n = 21

24 hours	48 hrs	72 hrs
85.8%	121%	145%
93.6%	125%	155 %
100%	130%	145%
95.8 %	122%	130%
98%	115%	149%
100 %	110%	130%
101.4 %	122%	145%

ANNEXURE – VI

SURFACE ROUGHNESS {RA VALUES (µ)} -

	Test: titanium nanotube surface	Control : titanium surface	Positive control: zirconia surface
1.	1.97	1.74	0.97
2.	1.97	1.68	0.90
3.	1.95	1.70	0.98
4.	1.8	1.66	1.22
5.	1.99	1.77	0.94
6.	1.97	1.71	0.96
7.	1.90	1.75	0.94
8.	1.92	1.79	0.98
9.	1.94	1.74	0.99
10.	1.95	1.74	1.11
11.	1.95	1.78	1.15
12.	1.98	1.66	1.10
13.	1.98	1.65	0.97
14.	1.97	1.74	0.97
15.	1.96	1.63	0.97
16.	1.93	1.59	0.97
17.	1.9	1.78	0.95
18.	1.9	1.75	0.98
19.	1.8	1.74	0.97
20	1.87	1.74	0.95
21.	1.85	1.74	0.93