“COMPARATIVE EVALUATION OF LASER SURFACE PATTERNING, PHOTO-FUNCTIONALIZATION AND ARGON PLASMA SURFACE TREATMENT OF TITANIUM ON ITS OSTEOGENIC POTENTIAL – AN INVITRO STUDY”

BY

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LIST OF ABBREVIATIONS USED IN THE STUDY

ABBREVATIONS	FULL FORMS
ANOVA	Analysis of variance
BIC	Bone Implant Contact
CV	Coefficient of Variation
CO_2	Carbon dioxide
°C	Degree Celsius
DMEM	Dulbecco's Modified Eagle Medium
DMSO	Dimethylsulfoxide
Hrs	Hours
IL	Interleukin
L	Litre
M	Milli
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5- diphenyltetrazolium bromide
OD	Optical density
PBS	Phosphate Buffer Saline

RANKL	Receptor activator of nuclear factor kappa B ligand
S.D.	Standard Deviation
S.E.	Standard of error
SEM	Scanning Electron Microscope
Ti6Al4V	Titanium-6 aluminum-4 vanadium
TNF	Tumor Necrosis Factor

ABSTRACT

STATEMENT OF PROBLEM

The success of dental implants relies on osseointegration. Over time, various techniques and materials have been developed to enhance bone-implant contact and expedite the osseointegration process. Ensuring a rapid and predictable outcome for implant prosthetics underscores the importance of achieving successful osseointegration. Conversely, unsuccessful osseointegration can lead to premature implant failure, resulting in an inability to withstand chewing forces, ultimately leading to implant mobility or discomfort. The long-term clinical success of dental implants is closely tied to the surface quality of the implant. Modifying the surface quality of the implant can improve factors such as roughness, free energy, and chemical composition, which in turn promotes faster cell migration and attachment to the implant, thereby enhancing secondary stability. Recognizing the potential benefits of UVC surface treatment, Argon plasma surface treatment, and laser surface patterning, this study aims to evaluate their impact on the osteogenic potential of titanium implants.

AIM

To assess the osteogenic potential of titanium treated with photo-functionalization, Argon plasma surface treatment and laser surface patterning.

MATERIALS AND METHODS

A total of 168 identical titanium disc-shaped specimens were divided into 4 Groups, namely- Group A -sandblasted titanium discs(n=42), Group B -

Photofunctionalization (n=42), Group C- Laser Surface Patterning (n=42), Group D- Argon Plasma Surface Treatment(n=42)

To study the osteogenic potential, the specimens in the study and control groups were further sub divided in to 2 groups based on cell attachment and cell proliferation assays (n=21). These groups were further subdivided into 3 groups (n=7) based on different time interval tested i.e., 24,48,72 hrs.

All the specimens from each group were subjected to Surface roughness evaluation quantitatively using Profilometer and Scanning Electron microscope to assess the surface roughness qualitatively. A mean roughness profile was evaluated for each specimen to describe overall roughness of the surface. SEM evaluation was done at 100x, 500x and 1000x magnification to visualize and compare the surface profile of all the groups qualitatively so as to provide a constant surface roughness. After surface roughness evaluation, Osteogenic Potential was evaluated by assessing the Cell Attachment and Cell Proliferation of MG-63 cells on test groups (Group B,C,D) and control group (Group A) at 24 , 48, 72 hour time interval. The cell Attachment was evaluated using hemocytometer while Cell Proliferation was assessed using MTT Assay. The resultant data were tabulated and then subjected to statistical analysis to draw conclusion from experimental data. ($p<0.05$)

<u>RESULTS</u>

The cell attachment was higher for the titanium treated with Laser Surface Patterning (Group C) at all three time intervals as compared to other three groups. There was a statistically significant difference in the cell attachment between the time intervals in Group C. The cell proliferation was higher in the titanium treated with

Argon Plasma Surface Treatment (Group D) at 24-48 hour time interval when compared with Group A, B, C.

CONCLUSION

Within the constraints of this study, it was observed that Argon plasma surface treatment and Laser Surface Patterning yielded favorable outcomes on the cellular activities of MG-63 cells which can be thought of as an added advantage along with the decontamination procedure for titanium to help in the process of osseointegration.

KEYWORDS

Photofunctionalization, osseointegration, Laser Patterning, Argon Plasma, surface modification

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INTRODUCTION

Implants which are being used in dentistry demonstrated a dependable and reliable method of replacing lost teeth. In order to support and hold in place a fixed or removable dental prosthesis, a dental implant, is placed into the oral tissue beneath the mucosa, inside the bone and periosteum.[1] Compared to traditional fixed partial dentures, dental implants offer benefits, including a high success rate (over 97%), improved bone maintenance in the edentulous location, a decreased incidence of caries and endodontic problems in neighboring teeth, and the avoidance of discomfort in neighboring teeth.[1]

Now coming to the implant materials, titanium is most extensively used, as it is stable in body and doesn't spark a foreign body reaction when fitted into body. In implant dentistry and orthopedics, commercially available pure titanium (cp Ti) and other alloy of titanium (Ti6Al4V) are utilized due to their superior osseointegration, biocompatibility, erosion resistance, and strength.[2]

Asa Leonhardt's study assessed titanium implant survival rates in edentulous areas ten years after surgery. According to the study's findings, implants had a 94.7% survival rate and an average 1.7 mm loss of crestal bone. Because titanium is a bioinert substance, it has little to no negative effects on the tissue around it and its high degree of bio-conduction, strong corrosion resistance, and biocompatibility; titanium is considered best material for dental implants.[4] Even though the material has a lot of inherent benefits, if the surface is inadequately treated, it may not integrate well with the bone and gingival tissue, which could present problems for dental implants. Implant failure can result from poor osseointegration, which weakens the implant's stability in the bone and can further lead to infections and inflammatory reactions developing in the peri-implant.[5]

Osteointegration is the direct bonding of the implant with the living bone in the absence of fibrous connective tissue.[1] Successful osseointegration is essential for an implant prosthesis to function predictably. Implants that fail early due to osseointegration failure are unable to tolerate masticatory forces, which might result in implant movement or pain.[2]

The surface quality of dental implants determines their long-term clinical success.[6] Enhancing the roughness, free energy, and chemical composition of an implant's surface can hasten cell migration and adhesion to the implant, leading to improved secondary stability.[1]

A variety of surface modifications have been applied to implants using additive treatment (plasma-spraying, sol-gel), subtractive treatment (sand blasting), chemical treatment (acid etching), and electrochemical treatment (electropolishing, anodizing) techniques.[7]

Moreover, modifications designed to improve corrosion resistance (anodization) after SLA have been suggested. It has been demonstrated that they enhance osseointegration and surface roughness. But the surface roughness achieved was not uniform and had porosities, having greater bacterial adhesion along with having better osseointegration.[1,2] Several recent modifications for titanium implants for better osseointegration have come up in recent times, which includes Photo-Functionalization, Plasma Sterilization and Laser Surface Texturing.[4]

UV light photofunctionalization of implants has been highlighted as an easy-to-use and efficient way to promote osseointegration. By modifying its physicochemical characteristics, UV photofunctionalization turns naturally hydrophobic titanium into superhydrophilic titanium. It also turns titanium from a negatively charged metal to a positively charged metal by removing hydrocarbons that eventually builds up on surfaces that have aged.[5] By enhancing its adhesive qualities, obtaining nearly 100% bone implant contact, and boosting osseointegration strength in just 15 minutes by eliminating the hydrocarbon accumulated on titanium surfaces, UV-mediated photo functionalization has been proposed as a means of delaying the biological aging of titanium.[5]

Before gamma irradiation sterilization argon plasma is used as an additional surface sterilization for dental implants. A vacuum chamber emptied using a vacuum pump can produce low-pressure plasma if it has a low breakdown voltage. In order to fabricate semiconductor components, low-pressure plasma systems are crucial. The decontamination and sterilization of medical equipment as well as the creation of free radicals under vacuum have been the main topics of research into low-pressure plasma systems.[8] Surface layer is stripped by active species such as polar groups. By varying the kind of gas, applied gas pressure, supplied gas purity, and sample position, one can alter the oxide layer and surface attributes.[6] Principal microscopic impact of such activation is the removal of microbial contamination. The physicochemical characteristics of implant surfaces, and consequently their biological characteristics and interactions with the environment, can also be changed by this technique.[9,10] Improved osteoblast cell adhesion results from the plasma's modification of the oxide layer and its interaction with surrounding tissue cells.[11]

Laser patterning is a versatile and controlled surface alteration technique. The technique increases the surface area, biocompatibility, and resistance to corrosion by forming oxide layers which additionally improves the way the implant and bone interact.[12] Surface melting is impacted by the laser irradiation parameters, hence by simply altering those parameters, multiple surfaces can be produced. This improved cell attachment was linked to decreased epithelial apical migration to the bone-implant interface and crestal bone preservation.[12]

This investigation was carried out with the intent of evaluating the osteogenic potential by means of cell attachment and cell proliferation of titanium when surface treated with Photofunctionalization, Argon Plasma Treatment, And Surface Laser Patterning, taking into account the benefits and characteristics of each method.

NEED FOR STUDY

The uniqueness of implant dentistry lies in the fact that it is able to achieve the objectives of normal functionality, shape, form, appearance, language and health, regardless of the extent of atrophy or disease or injury of the stomatogenic system. [13]

Because of its mechanical and biological compatibility, titanium is the most widely utilized alloy in implant dentistry. Nevertheless, due to its inability to chemically connect with surrounding soft or hard tissues, titanium as an alloy has some limits. Implant failure is increased because, although it can form a physical bond with bone tissue, this bond is not as strong as an osseous chemical attachment.[14]

With current materials, osseointegration of the titanium implant with neighboring bone takes 4-6 months. Through methods that alter the implant's surface quality, surface energy and chemical composition are improved, hastening the process of cell migration and adhesion to the implant. Surface modification is intended to maximize the implant's osteogenic potential while concurrently maintaining outstanding mechanical and biocompatibility.[14]

Various surface modifications have been tried on implants by additive, which includes plasma spraying, anodic oxidization, hydroxyapatite and calcium oxide coatings, and subtractive methods which includes sand blasting, acid etching, electropolishing, laser micro-grooving. More recent modification for titanium implants for better osseointegration includes Photo functionalization, Argon plasma treatment and surface laser texturing.[7]

Photo functionalization is basically UV treatment of the titanium which alters the physiochemical properties remove the hydrocarbons and enhances the hydrophilicity of the implant surface there by increasing the osteogenic potential [5].

Argon plasma treatment modifies the oxide layer of the titanium which interacts with cells of surrounding tissue leading to improved osteoblast cell adhesion[6]. Laser surface texturing on implants increases the surface area and arranges the osteoblast in an organised manner thereby improving the cell migration and increasing the osteogenic potential of implant[12].

Popularly used surface treatments like sand blasting and acid etching causes irregular roughening of implant surface which causes improved osteogenic potential but also increased microbial adhesion. Photofunctionalization and Argon plasma treatment cause an increase in surface energy thereby improving osteogenic potential.[9,10] Laser patterning causes the osteoblastic cells to organize itself in an orderly manner thereby improving the cell attachment and thus the osteogenic potential[12].

Therefore, taking into account these characteristics and benefits, this Invitro study is conducted with the purpose to evaluate the osteogenic activity of titanium when treated by Photo- Functionalization Laser Patterning and Plasma Sterilization and to determine which surface treatment is more effective in improving osseointegrating activity of titanium surface.

HYPOTHESIS:

1. Null Hypothesis:

There is no effect on the osteogenic potential of titanium treated with Photofunctionalization, Argon Plasma Treatment and Laser Patterning.

2. Alternative Hypothesis:

There is an effect on the osteogenic potential of titanium treated with Photofunctionalization, Argon Plasma Treatment and Laser Patterning.

AIM OF THE STUDY:

To assess the osteogenic potential of titanium treated with Photofunctionalization, Argon Plasma Treatment and Laser Patterning.

OBJECTIVES:

1. To evaluate and compare the effect on cell attachment of titanium disc treated with Photofunctionalization, Argon Plasma Treatment and Laser Patterning.
2. To evaluate and compare the effect on cell proliferation of titanium disc treated with Photofunctionalization, Argon Plasma Treatment and Laser Patterning.

REVIEW OF LITERATURE

1. Francisco et al in 2007 conducted an in-vitro study in which Nd-Yag laser was used to create laser patterning on titanium surface. It was then checked under scanning electron microscope and X-ray powder diffraction. The study concluded that the resulting phases on irradiated surface had controlled the formation of oxide layer which in turn helps in better implant osseointegration.[12]

2. Study conducted by Mi Hee Lee et al in 2009 examined the feasibility of using microwave induced argon plasma under atmospheric pressure to eliminate biofilms and inhibit their re-growth. The authors concluded that inhibition of bacterial growth (which may be required for biofilms to form) and suppression of Sessile bacterial colonisation on surfaces may be responsible for the inhibition of growth in plasma and the formation of biofilm under atmospheric pressure.[15]

3. In 2010, Takeshi et al. carried out an in-vivo investigation using a gap healing model for the osteogenic potential of titanium with and without photo-functionalization. After acid etching of titanium disks and rods, titanium was kept in a dark environment for four weeks. Invitro osteogenic potential was evaluated by cell attachment and cell proliferation. For in-vivo study in a rat femur, titanium rods with and without UV irradiation were inserted in contact (gap healing) with the cortical intrinsic bone. After two weeks of recovery, the titanium implants underwent surface elemental analysis, micro-CT bone morphometry, and a biomechanical push-in test. The study concluded that the bone that developed around UV-treated implant surfaces had a much higher Ca/P ratio than the bone that formed around untreated surfaces, especially in the cortical bone area. This suggests that the UV treatment of the surface increased the quality of the bone. [16]

4. An in-vitro investigation carried out by Fuminori et al in 2010 evaluated the osteoblast adhesion on titanium treated with photo-functionalization. Rat bone marrow derived osteoblasts were cultured on treated and untreated discs and then evaluated for its osteogenic potential. The study concluded that osteoblast adhesion is accelerated and augmented remarkably on UV-treated titanium surfaces which was associated with upregulated expression of vinculin. [17]

5. Duske carried out an in vitro investigation in 2012 to assess how osteoblasts spreadibility and contact angle changed following treatment with plasma argon surface. Titanium discs were treated with an argon plasma jet, and the results were assessed using a goniometer. According to the study's findings, plasma therapy facilitated osteoblastic cell spreading and decreased contact angle. [18]

6. See-wook Pyo et al in 2013 conducted a study on dog jaw bone to evaluate if photo-functionalization enhances BIC, osteogenesis, marginal bone seal, and removal torque value of implants. On dental implants photo-functionalization was performed by treating implants with UV light for 15 minutes. Four weeks after placement, bone-implant integration was evaluated using a removable torque test and static and dynamic histology. Study concluded that photo-functionalization enhanced the morphology, quality, and behavior of peri-implant osteogenesis, including the increased BIC, expedited robust interfacial bone deposition, and improved marginal bone seal and support.[5]

7. Ketabi et al in 2013 conducted a literature revie to evaluate the effect of micro grooving in the implant collar on soft and hard tissue attachment. The study concluded that laser abladed implant collar had lesser peri-implantitis and marginal bone loss when contrasted with regular machined implants. [19]

8. Takahiro et al in 2014 a literature review summarized the findings of invitro and invivo studies related to photo-functionalization of titanium implants. The review concluded that photo-functionalization can be used to improve the properties of implants which thereby help in better osseointegration of implant. [20]

9. Renzo et al 2014 executed a retrospective investigation to compare crestal bone heights and clinical parameters between implants with laser-microtextured and machined collars placed and loaded with different protocols. The study concluded that a laser-microtextured surface on the implant collar may mitigate the negative sequelae associated with peri-implant bone loss, regardless of the placement and loading protocols used .[21]

10. In order to assess the effect of the Laser-Lok micro-structured surface on soft tissue peri-implant parameters and esthetics around immediate, functionally loaded implants for single-tooth replacement in the esthetic zone, Renzo et al. (2015) conducted a prospective randomized trial. The study came to the conclusion that Laser-Lok implants outperformed Non Laser-Lok implants in terms of the clinical and aesthetic results of immediate functional loading. [22]

11. Gyeung mi seon et al 2015 conducted a study where different phases of emission spectra from excited species-argon, nitrogen atoms and oxygen atoms were observed. Microwave- induced argon plasma treatment was done on titanium surface which changed it to more hydrophilic on the 5 s short treatment and 30 s, 90 s long treatment. Study concluded MC3T3-E1 attachment and proliferation assay significantly increased in 5 s at short treatment, 30 s, and 90 s at long treatment after 5 days incubation and that microwave-induce argon plasma treatment would be an effective method to modify titanium surface for enhancing cell-material interactions. [23]

12. Manabu et al, 2016 carried out a study which evaluated effect of photo-functionalization on osseointegration under the biologically adverse conditions of aging. The cells from aged rats showed significantly increased cell attachment and the expression of osteoblastic function on photo-functionalized titanium than on untreated titanium. The strength of osseointegration was increased by 40% in aged rats carrying the photo-functionalized implants. The study concluded that photo-functionalization was effective for enhancing osseointegration in aged rats. [24]

13. Makato et al in 2016 carried out an investigation to evaluate implant stability in photo-functionalized implants in regular and complex cases. 49 implants of which 24 were untrated and 25 photo-functionalized were placed and ISQ was evaluated after placement and at stage two surgery. The study concluded that photo-functionalization improved the final level of implant stability development and sped up the rate of development, especially for implants implanted in difficult cases and poor-quality bone. The results of the test indicated that photo-functionalization was a effective for implant stability than other host and implant-related factors. [25]

14. David Charles et al in 2016 carried out an investigation to study the effect of neodymium-doped yttrium aluminum garnet (Nd-YAG) laser surface treatment of TiZr implant alloy on their osteogenic potential. The study concluded that roughness of surface, wettability and cellular activity of cells, was improved with surface modification using Nd-YAG laser thereby improving the osteogenic potential. [26]

15. Tatiana et al in 2017 carried out an investigation to evaluate the osteo-regenerative effect of a laser-modified nano-to-micro-scale hybrid titanium surface on human umbilical cord mesenchymal stem cells. The study concluded that laser treated

titanium surface modulated cellular behaviour depending on cell type, and stimulated osteogenic differentiation. This evidence supports the potential use of laser-processed titanium surfaces as bone implant materials, and their use in regenerative medicine could promote better outcomes.[27]

16. An animal study in 2018 was conducted by Hendrick et al in which 16 implants with a sand-blasted and acid-etched surface were treated with atmospheric plasma after which it was inserted in the frontal bone of four immature pigs. The study conclude that there was no change in the morphology of implant post treatment with plasma. But it showed improved bone implant contact in implants treated with atmospheric plasma.[28]

17. In 2018 Henningsen et al carried out an investigation to evaluate the effect of ultraviolet and non-thermal plasma on surface characteristics of titanium and zirconia. The study concluded that ultraviolet light and non-thermal plasma were found to be able to improve the chemical surface conditions of titanium and zirconia following a short exposure time as x-ray spectrometer showed decrease in carbon content and an increase in oxygen content of either material. [29]

18. Study conducted by Gonzalez et al in 2019 evaluated the cell viability on titanium disc of Grade IV and V treated with SLA and Argon plasma treatment. Study concluded that cells cultivated on those Grade V titanium discs that were decontaminated with argon plasma presented higher levels of cell adhesion and proliferation compared to those which were not decontaminated. [30]

19. Canullo et al performed a study in 2019 to investigate effect of argon plasma and UV irradiation on osteo conductivity of bone grafting materials. After surface treatment with argon plasma and UV irradiation of titanium discs, 4 bone grafts

were evaluated for osteogenic potential. The study concluded that argon plasma increased the osteoconductivity of the bone grafts to the titanium surface.[31]

20. Jun Beom Lee et al in 2019 conducted a study in which machined surface treated with UV light (M + UV) was compared to sandblasted, large-grit, acid-etched (SLA) surface through in vitro and in vivo studies. This study concluded that UV photo-functionalization of a Ti dental implant with machined surface attained an earlier osseointegration compared to an implant with an SLA surface. The enhancement was considered to result from the super-hydrophilicity, the elimination of hydrocarbon on the surface, and the improvement of osteoblastic activities. 3

21. In 2020 Filiberto et al carried out an in vitro investigation to examine the effects of sandblasted and acid-etched surfaces and novel laser titanium surfaces on human osteoblast activity during osteogenesis. Of 60 titanium discs, 40 were subjected to laser surface treatment. The study concluded that laser-induced surface showed high osteogenic process, confirming the critical role of titanium surface characteristics in the cell adhesion and bone deposition during the early phases of osseointegration.[32]

22. Roodabeh et al in 2020 conducted systematic review to investigate the peri-implant marginal bone loss and pocket depths and failure rates of dental implants with laser-microtextured collars. The study concluded that laser micro texturing of implant collar significantly affected the peri-implant marginal bone loss and probing depths. Although no significant differences were noted in the implant failure rates between implants with laser micro textured and machined collar

surfaces, the peri-implant marginal bone loss and probing depths with laser-microtextured collars were significantly lower than the machined collars. [33]

23. Makato et al in 2020 carried out a prospective study which evaluated the seven-year success rate of photo-functionalized implant placement in patients with regular, complex, and cancer-related cases. Seventy implants in 16 patients were included. The study subjects with photo-functionalized implants showed a reliable seven-year success rate in regular and complex cases. However, the success rate in cancer cases was significantly and remarkably lower, suggesting remaining challenges of patho-physiologically compromised conditions, such as bone resection, segmental defect, and radiation. [6]

24. Arturo et al in 2020 conducted an animal study in which one hundred and forty-four implants were placed in the tibiae of 36 Sprague Dawley rats, distributed in four experimental groups: I: mechanized surface; II: mechanized surface treated with non-thermal low pressure argon plasma, III: resorbable blast media (RBM) surface; and IV: RBM surface treated with non-thermal low pressure argon plasma. Bone-to-implant contact (BIC) percentages were calculated. The study concluded that groups treated with NTLP-ArP obtained higher BIC% than those not treated at two and four weeks. [34]

25. Paolo Pesce et al in 2020 in their systematic review on four studies evaluating UV treatment in rabbits showed that bone to implant contact values were significantly increased in the bio-activated groups when follow-up times were relatively homogeneous. The systematic review and meta-analysis concluded that chair-side treatment of implants with UV or non-thermal plasma appear to be effective for improving osseointegration. [35]

26. An invitro study in 2020 was conducted by Lei Wang to assess the bioactive effect of low temperature argon oxygen plasma on titanium surface. In this study, the study group titanium discs were subjected to low temperature argon oxygen plasma and was compared with sandblasted acid etched titanium for surface characteristics and osteogenic potential. The study concluded that titanium treated with low temperature argon oxygen plasma showed better osteogenic potential than the sand blasted acid etched titanium disc. [36]

27. In 2021, Satoshi et al. carried out an in vitro investigation to assess the impact of argon-based atmospheric pressure plasma and its influence on the creation of hard tissue on titanium. The study concluded that the atmospheric pressure plasma treatment with argon gas imparts superhydrophilicity, without changing the properties of the pure titanium plate surface. It was also clarified that it affects the initial adhesion of bone marrow cells and the induction of hard tissue differentiation. [37]

28. Arturo et al in 2021 conducted an invitro study to evaluate the effect of UVC on wettability of titanium in relation of irradiation time. The titanium discs were subjected to UVC irradiation for 15, 30, 60, 120 mins and then was checked for contact angle to check for the wettability. The study concluded that irradiation by UVC after 60 mins on titanium surface increased the surface wettability of titanium thereby improving the biological process of osseointegration in implant therapy. [38]

29. Nagore et al conducted an in vitro study in 2021 to check effect of LED-based UVC photo-functionalization technology to decontaminate the surface chemistry of three different commercially available titanium dental implants. 3 dental

implants of different manufacturers were evaluated before and after the UVC surface treatment for the carbon content present on the surface. The study concluded that decontamination of titanium surfaces occurs after UVC photo-functionalization decreased the carbon compounds therefore improving the chemical characteristics of titanium dental implants. [39]

30. Shivakoti et al,2021, in their systematic review to investigate the biomedical applications of laser patterning of implants. The review concluded that micro texture on titanium surface enhance the cell adhesion and performs an essential function in contact guidance. [11]

31. Long et al in 2022 conducted an animal study in which 6 beagle dogs; Straumann SLActive implants, SLA implants, and SLA implants treated with NTAP were implanted in the mandibular premolar area. After 2 weeks, 4 weeks, and 8 weeks, the animals were sacrificed and specimens were collected. Radiographic and histological analyses were used to measure osseointegration. The study concluded that implants treated with non-thermal argon plasma showed better bone implant contact and bone volume around implants. [40]

32. In a study published in 2022, Marco Roy et al. evaluated the effects of UF- photo-functionalization on TiO2 topographic topography, gene expression patterns, and bioactivity of osteogenic cells (i.e., in vitro osteogenic precursor cells) cultured in the presence of various titanium specimens. The results of the study indicated that UF-photo-functionalized TiO2 implants can be restored in terms of biocompatibility, osteointegration, and bioavailability, suggesting that this is at least partially due to stimulation of osteogenic differentiation of precursor cells. [10]

33. In 2023, Sophie Jones et al assessed the osteogenic potential and differentiation of pre-osteoblasts on titanium, including machined (M), resorbable blast textured (RBT), and Laser-Lok© (LL), in comparison to tissue culture plastic (TCP) control. The surface roughness of specimen was determined, and the hydrophilicity was assessed by water contact angle. This study found that the textured surface alterations on dental implants, which were created by laser microgrooves and resorbable blasts, improved aligned cell proliferation. This could eventually contribute to implant osseointegration and long-term dental implant viability. [41]

34. In 2023, Poonam et al. carried out an in vitro investigation to assess the osteogenic capacity of titanium discs following surface modification with argon plasma after which its potential for osteogenicity was assessed by measuring cell attachment and proliferation. According to the study's findings, argon plasma treatment affected the early cellular processes that increases the adhesion and multiplication of osteoblast-like cells. [42]

MATERIAL AND METHODOLOGY

DATA SOURCE: This invitro study was performed in Department of Prosthodontics and Crown & Bridge, KAHER KLE VKIDS, Belgaum, Karnataka; KAHER Dr. Prabhakar Kore’s BSRC, KAHER KLE VKIDS, Belgaum, Karnataka; Shivaji University, Kolhapur, Maharashtra; Gogte Institute of Technology, Belgaum, Karnataka; KLES Dr. Prabhakar Kore Hospital, Belgaum, Karnataka and KLE Engineering college Belgaum, Karnataka.

The aim was to evaluate titanium's osteogenic potential following Photo-functionalization treatment, Laser Patterning and Argon Plasma Treatment and to compare which among these surface treatment are better to enhance osseointegration of titanium surface.

METHOD OF COLLECTION OF DATA:

Criteria for inclusion:

- The investigation included identical disc-shaped specimens made of titanium, with dimensions of 10 mm in diameter and 2 mm in thickness. (B348, ASTM)

Criteria for exclusion:

- Samples having internal and external porosity.
- Samples with surface irregularities.

Table No 1: Materials used for assessment osteogenic potential

Material	Description	Manufacturer
Ti-6Al-4V	Type V	Special Metals, Mumbai, India
Phosphate buffer solvent (6.8 ph)	Sodium chloride, sodium dihydrogen orthophosphate dihydrate, potassium chloride, distilled water	Himedia, Mumbai, India
Distilled Water	Batch;- 007M15	Ranken Chemicals, Avantor, India
70% Ethanol	20151011	Changshu Hongsheng Fine Chemicals Co, Ltd
Phosphate Buffer Saline	0000237353	Hi Media, Mumbai
Dulbecco's Eagle Medium	0000284902	Hi Media, Mumbai
Trypsin EDTA	0000297540	Hi Media, Mumbai
MTT Reagent	000017715	Hi Media, Mumbai
Tryphan Blue	2024334	Hi Media, Mumbai
MG 63 cell line	-	NCCS, Pune

Table No 2. Equipment used to analyze the surface properties and determine the test specimen's osteogenic potential

Material	Description	Manufacturer
Profilometer	Contact profilometer Model- Surfcomflex S-128	Taylor Hobson, Brazil
SEM	FE-SEM SIGMA HD	Carl- Zeiss, Germany
UVC Chamber	E2/40	Lab solutions India
Argon plasma sterilizer	Sterad 100S	Advanced Sterilization Products, Texas
Laser machine	GS8466	GG Enterprise Pvt Ltd, Haryana
Micropipette to handle media	Model no: 299932	Riviera Glass Pvt, Ltd, Mumbai
Culture Plate	24 well plate	Tarsons, Korea
Haemocytometer	PPHB 205	Rohem, India
Microscope	TCM400	Labomed, USA
CO_2 Incubator	Galaxy 170R	Eppendorf, India
Microtitre plate reader	Epoch	BioTek, USA

Methodology:

1. Preparation of test specimen:

For the purpose of the investigation, 168 identical Grade V Titanium (10 mm x 2 mm) disc-shaped specimens from the commercial market (ASTM B348) were employed (Fig 1).

The specimens were separated into 4 groups: GroupA (n = 42) was the control group, which only received sandblasted titanium discs; GroupB (n = 42) was the test group; GroupC (n = 42) was the surface laser texturing group; and GroupD (n = 42) was the plasma argon surface treatment group.

Cell attachment and proliferation tests were used to further separate the specimens in the study and control groups (n= 21). These groups (n=7) were divided according to the various time intervals that were tested, namely 24, 48, and 72 hours.

For one minute, 50 µm alumina was used to sandblast all of these identical titanium discs at a constant pressure of 4 kg/cm2. To eliminate any last traces of impurities, discs were ultrasonically cleaned for 180 seconds with acetone.

2. Surface treatment of Titanium Discs

i) Photofunctionalization of the test specimen was performed in UV chamber. The discs were placed in the chamber and a wavelength of 278 nm was selected for irradiation with UVC. The specimens were then irradiated for 15 minutes and later sealed and subjected to high pressure steam sterilization and drying within 24 hours (Fig 2).

ii) Plasma treatment of the test specimen was done by subjecting the discs to low temperature gas plasma. The titanium discs were then packed into gas permeable packing and were kept in sterilization vessel. Gas molecules were excited by radiofrequency under deep vacuum in an enclosed vessel so that gas plasma was formed. Then it was ionized when it was subjected to an electric field. The total cycle was of 51 minutes. After sterilization, the packet mark turns to yellow from red (Fig 3).

iii) Lasers surface patterning of the test specimen was carried out using laser power which was controlled through software interface. The software was programmed to generate a linear pattern with dimensions measuring 500 µm. Selective laser melting was done thereby creating linear, equally spaced micro textured grooves on it (Fig 4).

3. Surface Profile Analysis:

The Contact Stylus Profilometer (Surfcomflex S-128 - Taylor Hobson) was used to quantitatively analyze the specimens' surface roughness (Fig 5). To check the overall roughness of the surface, an average roughness profile (Ra) was evaluated for each specimen. Every disc was positioned on a level surface, with the test surface facing up. The Profilometer was able to ascertain the profile of surface along three lines on the surface. A diamond point-stylus with a cut-off length of 0.8 millimeter and a transverse length of 4 millimeter was made after mounting the disc specimens. All absolute roughness profile distances from the center line of the measuring length were calculated to find their arithmetic average value. Using the profilometer, the Ra of the 42 test specimens in each group was ascertained. A field emission SEM (Carl Zeiss) was used to examine specimens from each group in order to qualitatively evaluate the surface roughness. For improved visibility, SEM images were captured at magnifications of 250X, 500X, and 1000X (Fig. 6a, 6b, 6c, 6d). The specimens were dried and attached to an aluminum cylinder measuring 13 millimeter in diam and 10 millimeter in ht after being cleaned with distilled water. Apart from the numerical results derived from the evaluation of surface roughness, the topographical observations of the polished surface were juxtaposed.

4. Assessment of Osteogenic Potential:

Surface characterisation was performed on 168 titanium discs in total. Which were divided into Study GroupB (n = 42), Study GroupC (n = 42), Study GroupD (n = 42), and control GroupA (n = 42). Specimens in each group were split into 2 groups for the attachment of cells experiment (n = 21) and proliferation of cells(n = 21). The specimens underwent additional division into 3 subgroups, one for each of the time intervals investigated (24, 48, and 72 hours). The osteogenic potential was evaluated using MG-63 cell lines (Fig 7).

a. Cell attachment-

The quantity of live cells was determined using the tryphan blue. Whereas nonviable cells showed blue cytoplasm, viable cells with an intact cell membrane did not absorb the dye and had clear cytoplasm. Both the control and study groups' specimens were tested using MG 63 cells. After 24, 48, and 72 hours, the culture media was taken out. To get rid of unattached cells, the wells were 3 times washed with phosphate-buffered solution (PBS) at 37°C. After adding tryphan blue to the cell solution, the adhering cells were quantified enzymatically using a hemocytometer (Fig. 8a and 8b). After counting the unstained cells in the first 16 squares, the next set was counted. The number of cells was used to represent cell attachment.

b. Cell Proliferation-

Each well microtiter plate was seeded with 50µl of a 1 x 10 cells/ml cell suspension, and DMEM medium was added to bring the total volume up to 1500 µl. 100µl of the previously indicated dilutions were added to the wells, and they were then incubated for 72 hours at 37°C in a CO_2 incubator with 5% CO2. Following a 72-hour interval, 200µl of the 5 milligram/millilitre Dimethylthiazole reagent was added. Incubation was done for four hours at room temperature in a dark area. After the supernatant was removed, 1000 µl of Dimethyl sulfoxide was added to dissolve the precipitated Formazan crystals, without disturbing them. Using a micro titre plate reader, the optical density (OD) was determined at 24, 48, and 72 hours at a wavelength of 570 nm. (Fig 9)

Formula:

$$\text{Survived cells (\%)} = \frac{\text{Mean Optical Density of test compound}}{\text{Mean Optical Density at control}} \times 100$$

The study and control groups' cell attachment and proliferation were assessed as a percentage (%), tabulated, and statistically analyzed to derive conclusions from the resulting data.

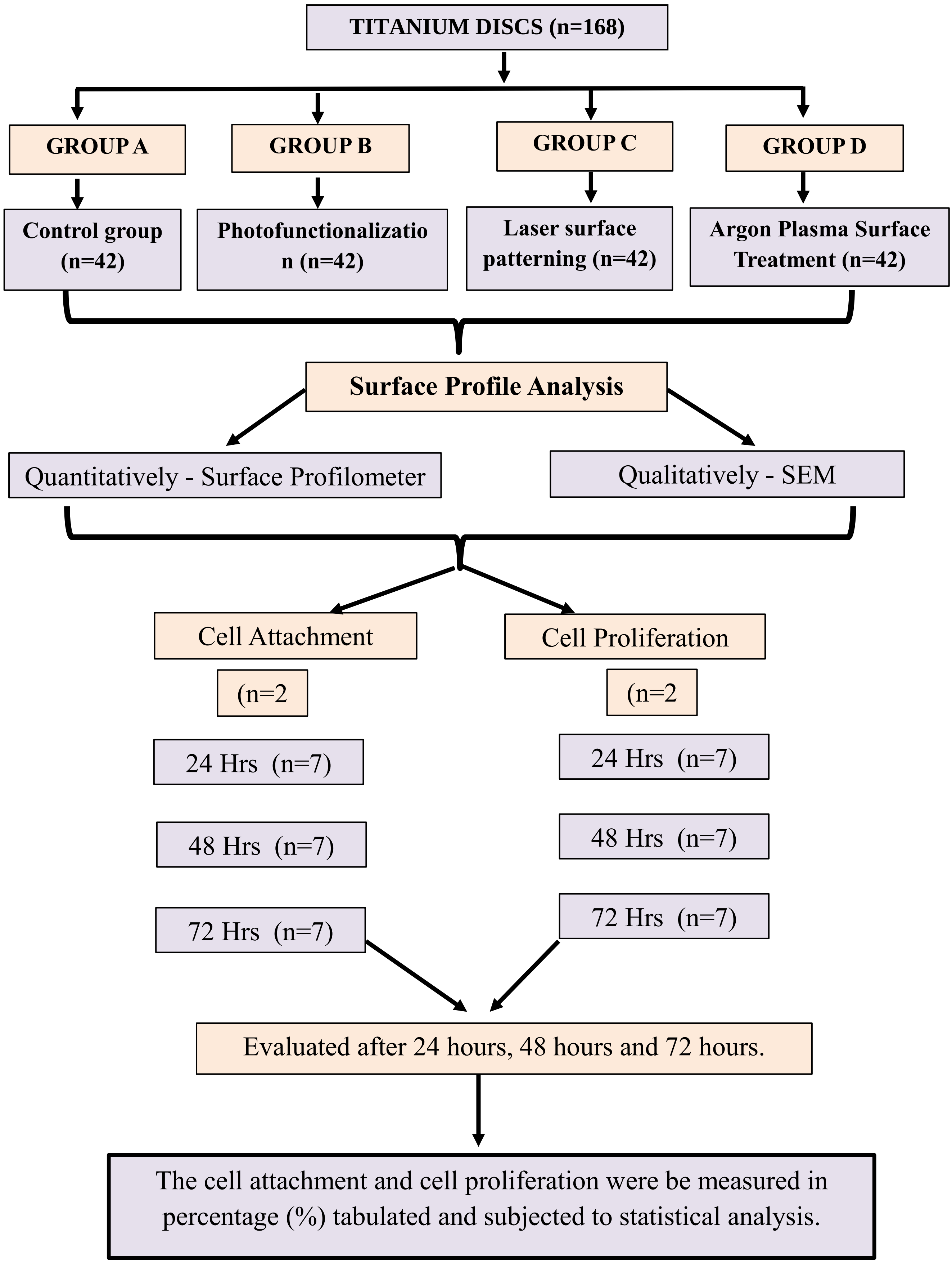

TITANIUM DISCS (n=168)
GROUP A
GROUP B
GROUP C
GROUP D
Control group (n=42)
Photofunctionalization (n=42)
Laser surface patterning (n=42)
Argon Plasma Surface Treatment (n=42)
Surface Profile Analysis
Quantitatively - Surface Profilometer
Qualitatively - SEM
Cell Attachment
Cell Proliferation
(n=2
(n=2
24 Hrs (n=7)
48 Hrs (n=7)
72 Hrs (n=7)
24 Hrs (n=7)
48 Hrs (n=7)
72 Hrs (n=7)
Evaluated after 24 hours, 48 hours and 72 hours.
The cell attachment and cell proliferation were be measured in percentage (%) tabulated and subjected to statistical analysis.

Fig 1: Disc shaped titanium specimen

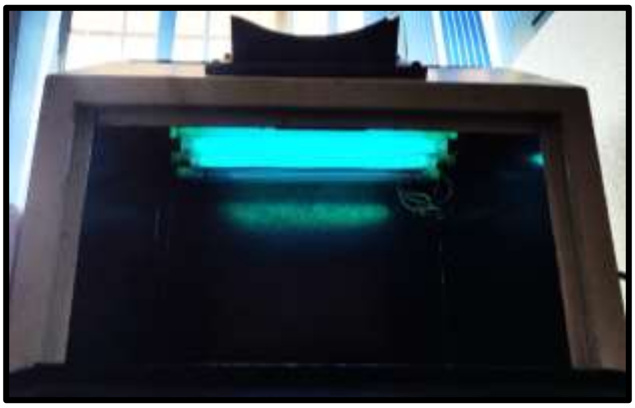


Fig 2: Photofunctionalization (UVC) Chamber and Inside of Photofunctionalization Chamber

Fig 3: Argon Plasma Sterilizer and Pouch used for Argon Plasma Surface Treatment and change in color of pouch post treatment.

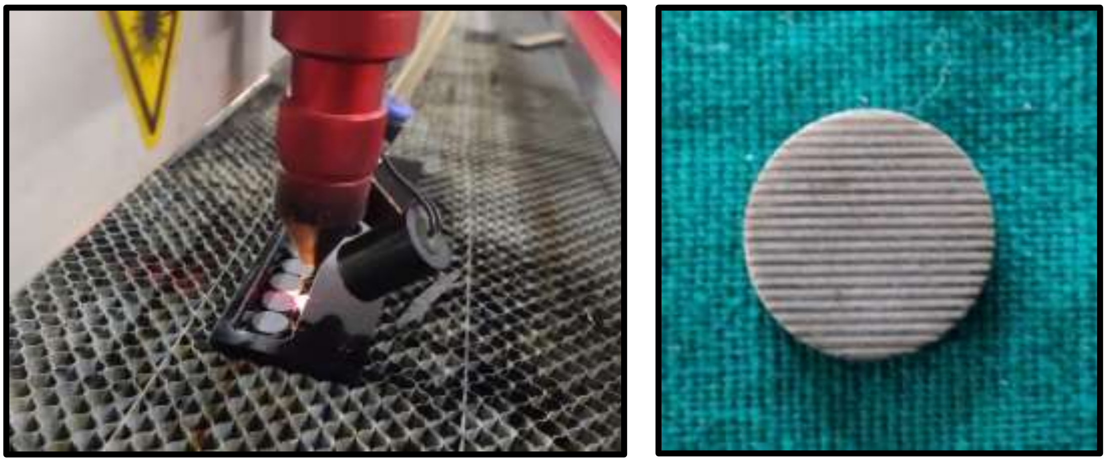


Fig 4: Laser Surface Patterning of Titanium Disc and Laser Surface Patterned Titanium Disc

Figure 5: Surface Profilometer for quantative analysis of surface roughness

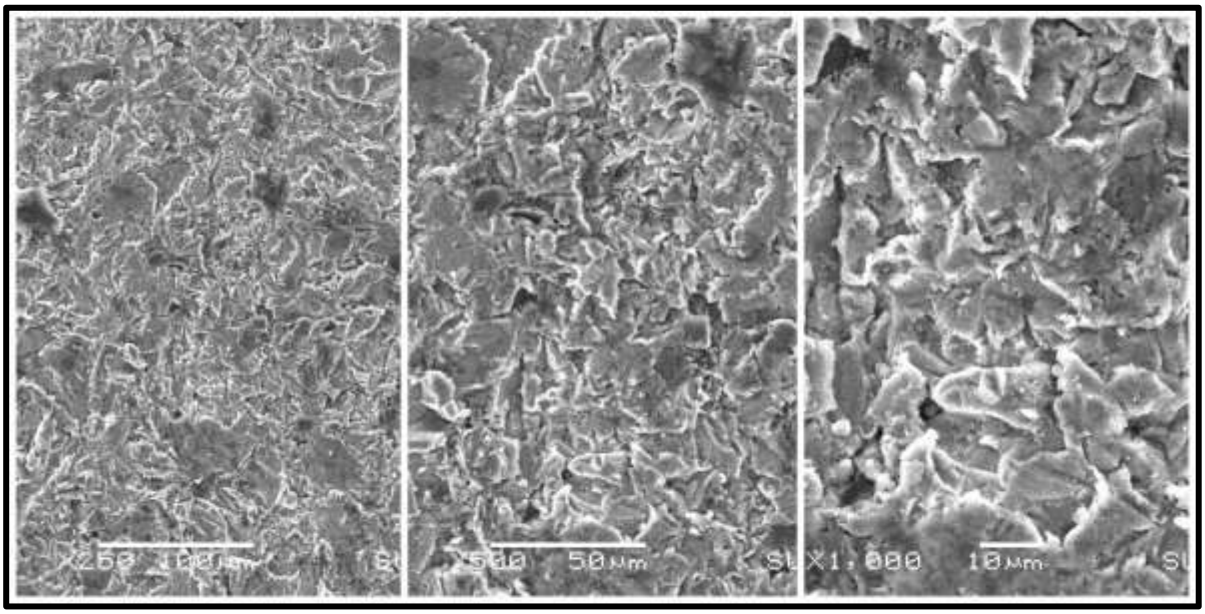


Figure 6 a: SEM image of Control group for qualitative analysis of surface roughness

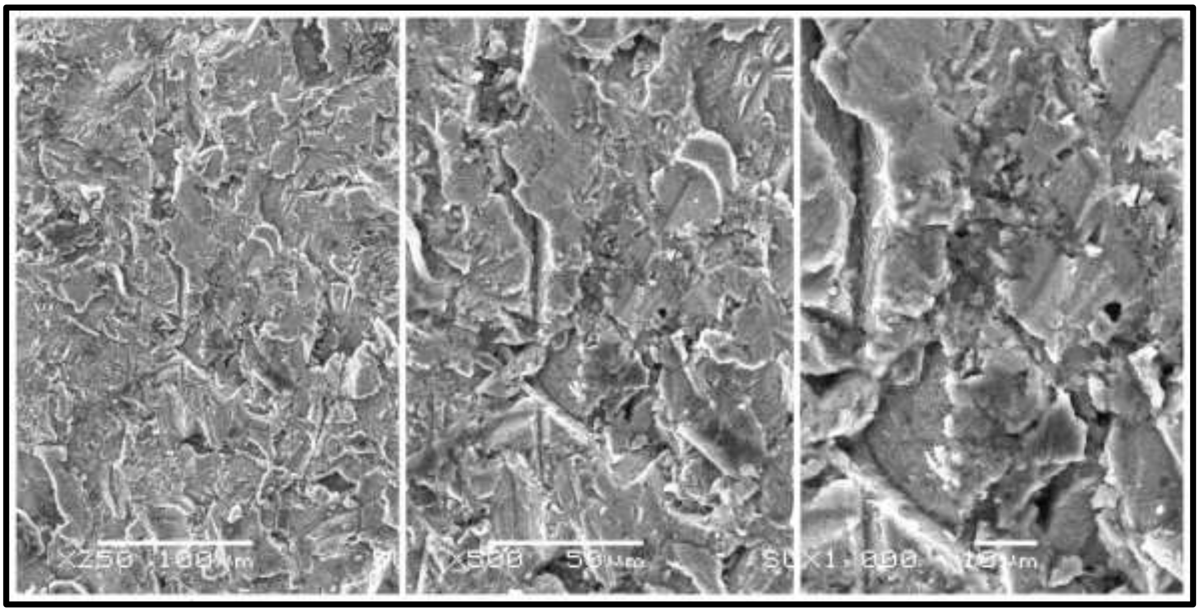


Figure 6 b: SEM image of Photofunctionalization group for qualitative analysis of surface roughness

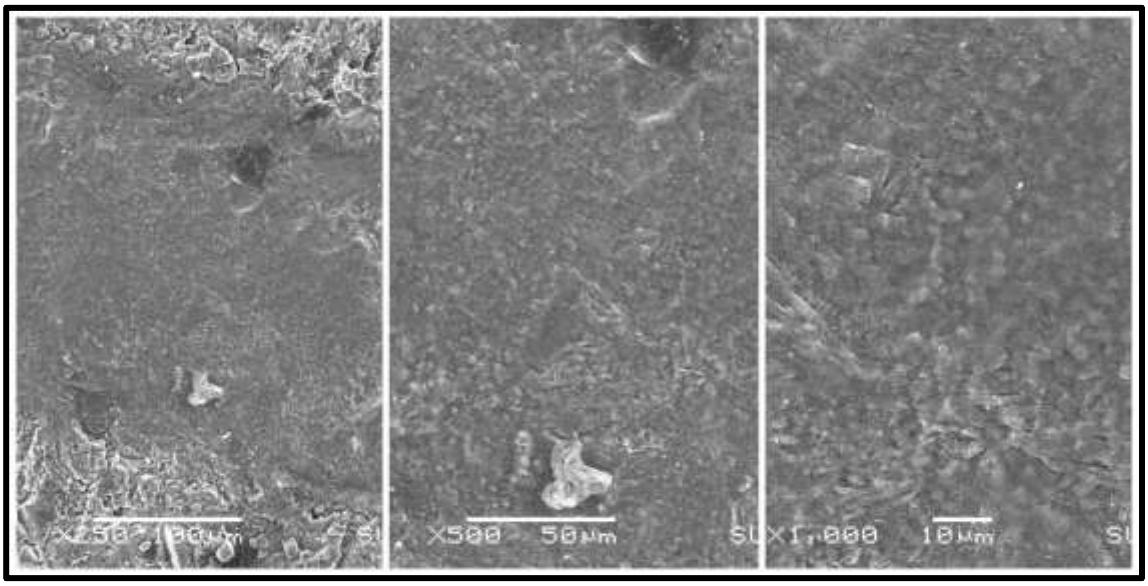


Figure 6 c: SEM image of Laser surface patterened group for qualitative analysis of surface roughness

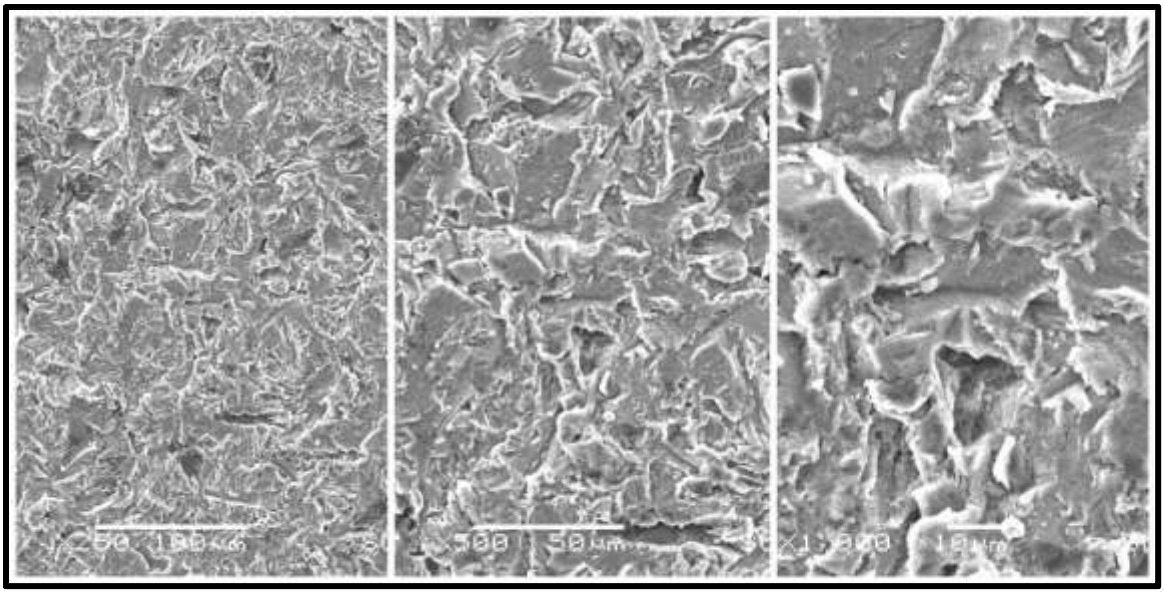


Figure 6 d: SEM image of Argon Plasma Surfaec Treated group for qualitative analysis of surface roughness

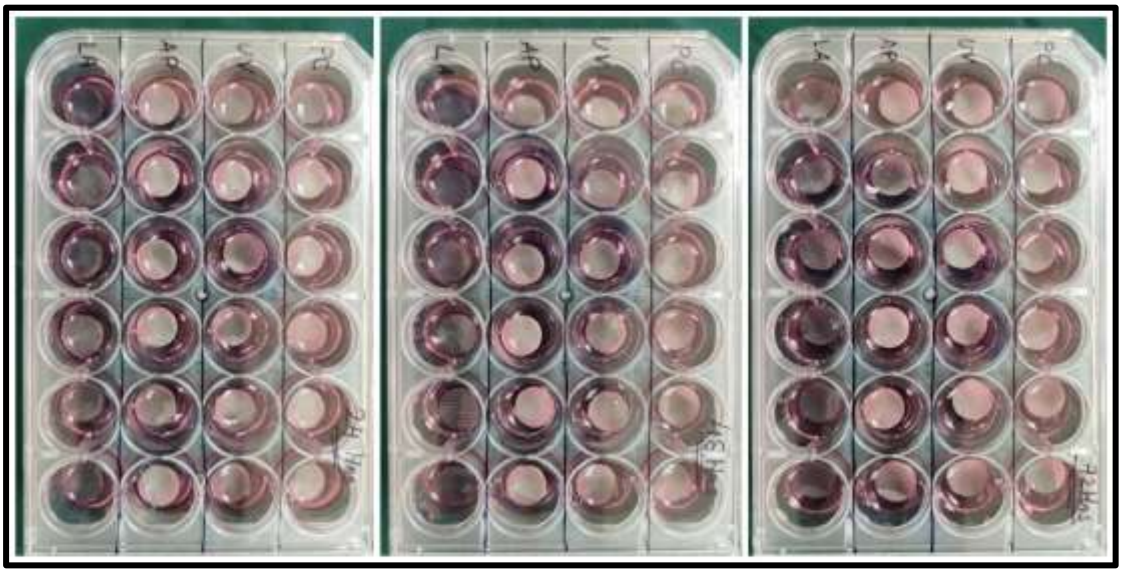


Figure 7: MG-63 cell seeding

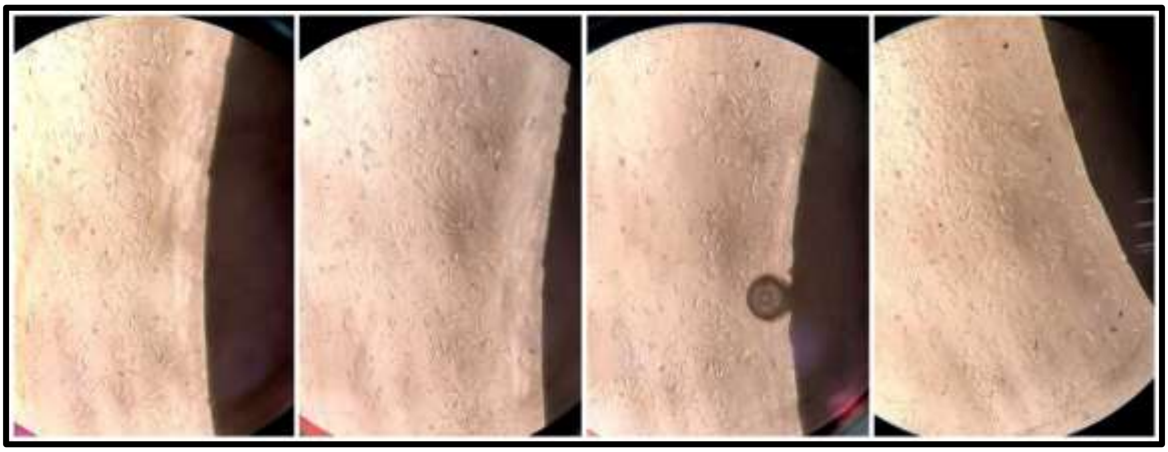


Figure 8a: Cell attachment as seen on seeding plates for Group A, Group B, Group C and Group D

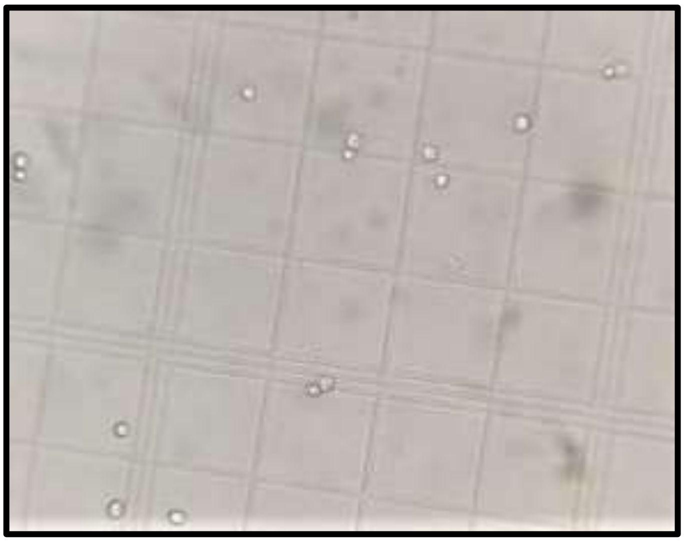


Figure 8b: Hemocytometer used to count cell attachment

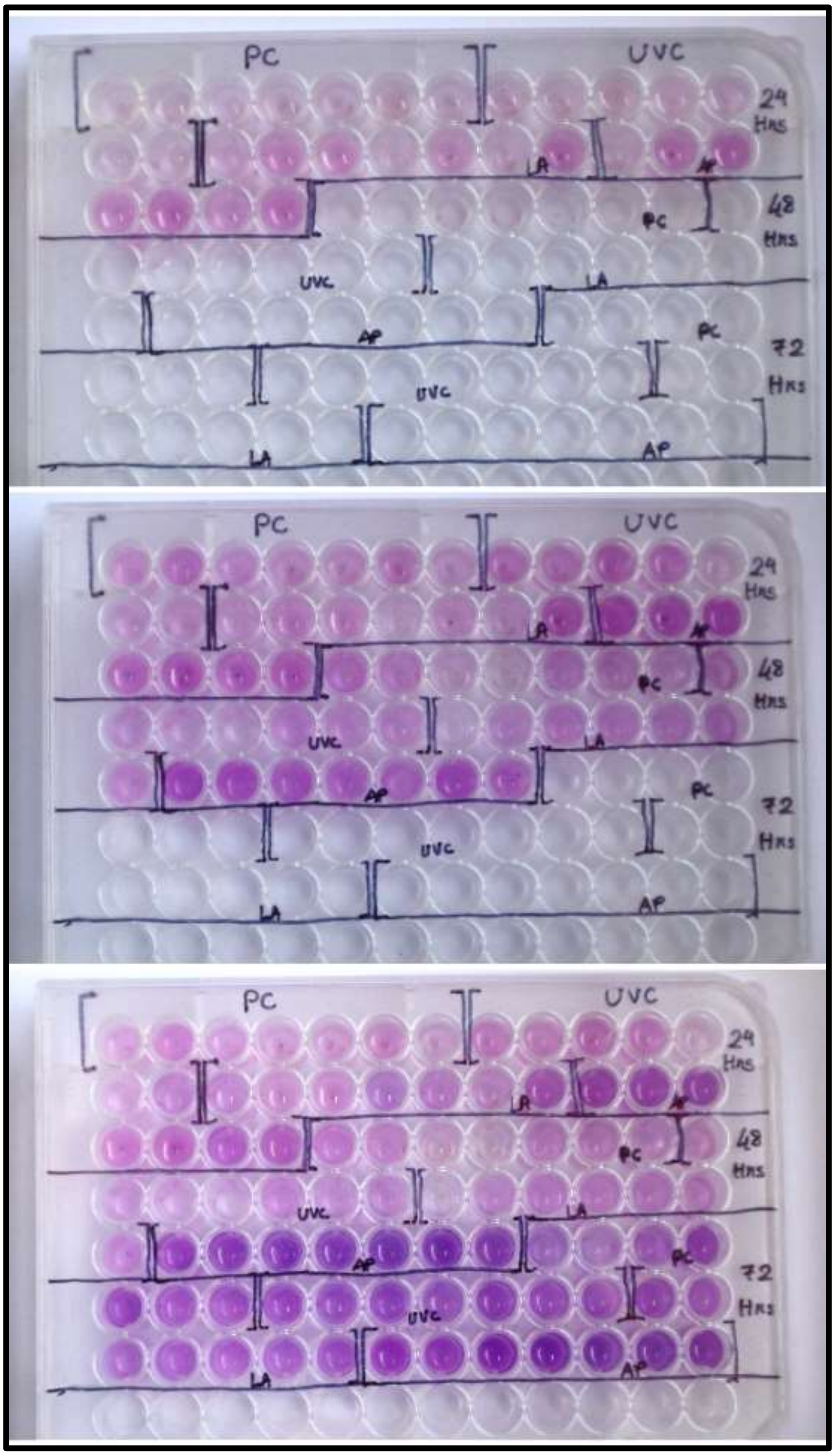


Figure 9: MTT Assay showing the cell proliferation at 24, 48 and 72 hours for all 4 groups.

RESULTS

In the current investigation, the osteogenic potential of titanium was assessed at 24, 48, and 72-hour intervals after surface treatment with Photofunctionalization, Laser patterning, and Argon Plasma. Hemocytometer was used to measure cell attachment, and MTT assay was used to measure cell proliferation at 24, 48, and 72 hours. Data obtained was entered in MicrosoftExcel 2020 and analysis was done using the IBMCorp. Released 2012. IBMSPSS®

To derive conclusions from the experimental results, statistical analysis was performed on the resultant values of osteogenic potential by means of cell attachment (number of cells) and cell proliferation (%) of specimens in GroupsA,GroupB,GroupC,andGroupD.

For the titanium disc surface treated with sandblasting (ControlGroup,GroupA) and the study group (GroupB: photo-functionalization, GroupC: laser patterning, GroupD: Argon plasma treatment), mean, standard deviation were computed.

The mean and standard deviation of descriptive statistical metrics were computed for every group. Two way ANOVA was used to compare osteogenic potential pairwise, and Tukey's Multiple Post Hoc was used to compare data over various time intervals. Result was considered to be statistically significant when p-value was<0.05.

Table No.3 and Graph No. 1: Tukeys multiple posthoc procedures used for pair wise comparison of four groups for surface roughness scores

On comparison of surface roughness between all 4 groups, GroupC (1.45±0.11) showed statistically significant difference in surface roughness when compared with other 3 groups on comparison of surface roughness between groups, GroupB

(0.99±0.29 μm) showed the lowest mean surface roughness and GroupC has maximum mean surface roughness (1.45±0.11μm)

Table No.4: Comparison of four groups at three time intervals (24hrs, 48hrs and 72hrs) with cell attachment scores by Two way ANOVA.

After putting the sum of squares of the cell attachment values through a Two Way ANOVA, the cell attachment across all four groups and at various time intervals was ($p < 0.0001^*$).

Table No.5 and Graph No.2: Pair wise comparison of four groups with cell attachment scores by Tukeys multiple posthoc showed the total mean values and standard deviations of cell attachment were of GroupA (196904±45556.4), GroupsB (246666 ± 61185), C (403952 ± 83552), and D (410142 ± 67548). On subjecting these values to Tukeys Post Hoc, GroupA, GroupB, GroupC were found to be p = 0.0001* compared to the control group (GroupA). However, GroupD did not exhibit a statistically significant difference when compared with GroupC (0.7887).

Table No.6 and Graph No.3: Pair wise comparison of interactions between four groups at three time intervals (24hrs, 48hrs and 72hrs) with cell attachment scores by Tukeys multiple posthoc procedures.

On comparing mean and standard deviation at different time intervals within groups and after subjecting them to Tukeys Post Hoc test, a statistically significant difference in cell attachment was observed for GroupC (48 hours: 330000 ± 20800.64; 72 hours: 615000 ± 10214.37) and GroupD (48 hours: 360428.6 ± 21180.6; 72 hours: 500000 ± 14832.4) when compared with the control group (GroupA) and GroupB at 48 and 72 hours. When compared between GroupC and D, GroupC showed highly statistically significance difference in cell attachment at 72 hours ($p < 0.0001^*$).

Table No.7: Comparison of four groups at three time intervals (24hrs, 48hrs and 72hrs) with cell prolifertaion scores by Two way ANOVA.

After running the sum of squares of the cell proliferation data through a Two Way ANOVA, it was 0.0002* in the cell proliferation between all four groups. But when compared at different time intervals = 0.1180.

Table No.8 and Graph No.4: Pair wise comparison of four groups with proliferation of cells scores by Tukeys multiple posthoc.

The total mean values and standard deviations of cell proliferation were of GroupA (100), Groups B (106.98 ± 18.08), C (112.14 ± 21.67), and D (127.62 ± 29.98). On subjecting these values to Tukeys Post Hoc, GroupD, was found to be 0.0003* and 0.0063^* compared to the GroupA and GroupB. However, GroupD did not exhibit a difference when compared with GroupC.

Table No.9 and Graph No.5: Pair wise comparison of interactions between four groups(ABCD) and three time intervals (24hrs, 48hrs and 72hrs) with cell proliferation scores by Tukeys multiple posthoc procedures.

On comparing mean and standard deviation at different time intervals within groups and after subjecting them to Tukeys Post Hoc test, a statistically significant difference in cell proliferation was observed for GroupD when compared with the GroupA (0.001^*), GroupB (0.02^*)and GroupC (0.01^*)at 48 hours.

Table No.3: Tukeys multiple posthoc procedures used for pair wise comparison of four groups for surface roughness scores (p values)

Groups	GroupA	GroupB	GroupC	GroupD
Mean	1.17	0.99	1.45	1.08
SD	0.14	0.29	0.11	0.25
GroupA	-			
GroupB	0.0001*	-		
GroupC	0.0003*	0.0001*	-	
GroupD	0.7420	0.0024*	0.0001*	-

*p<0.05

The above table shows that GroupB (0.99±0.29 μm) has the lowest mean surface roughness and GroupC has maximum mean surface roughness (1.45±0.11μm)

Graph No. 1: Comparison of four groups with surface roughness score.

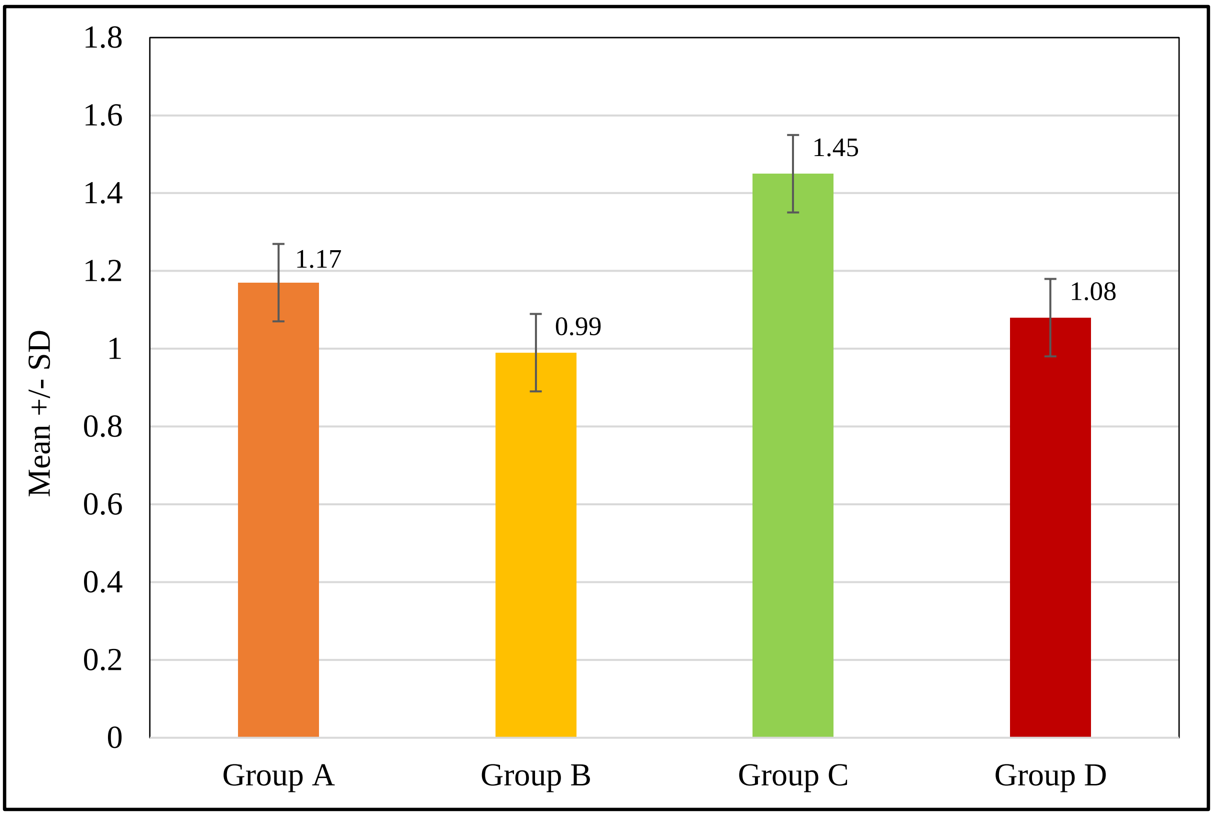


Table No 4: Comparison of four groups at three time intervals (24hrs, 48hrs and 72hrs) with cell attachment scores by Two way ANOVA.

Sources of variation	Sum of squares	Degrees of freedom	Mean sum of squares	F-value	p-value
Main effects					
Groups	747164416667.00	3	249054805556.00	535.9356	0.0001*
Times	259085809524.00	2	129542904762.00	278.7606	0.0001*
2-way interaction effects					
Groups*Times	54713047619.00	6	9118841270.00	19.6226	0.0001*
Error	33459142857.00	72	464710317.00		
Total	1094422416667.00	83			

*p<0.05

This table concluded that there was a significant difference in osteogenic potential in terms of cell attachment between control GroupAnd all study groups at different time intervals.

Table No.5: Pair wise comparison of four groups with cell attachment scores by Tukeys multiple posthoc procedures (p values)

Groups	GroupA	GroupB	GroupC	GroupD
Mean	196904.76	246666.67	437285.38	410142.86
SD	45556.45	61185.24	83552.66	67548.71
GroupA	-			
GroupB	0.0001*	-		
GroupC	0.0001*	0.0001*	-	
GroupD	0.0001*	0.0001*	0.7887	-

*p <0.05

On pair wise comparison of groups, it showed statistically significant difference in cell attachment in all except between GroupC and GroupD which did not show a statistically significant difference

Graph No. 2: Pair wise comparison of four groups with cell attachment scores

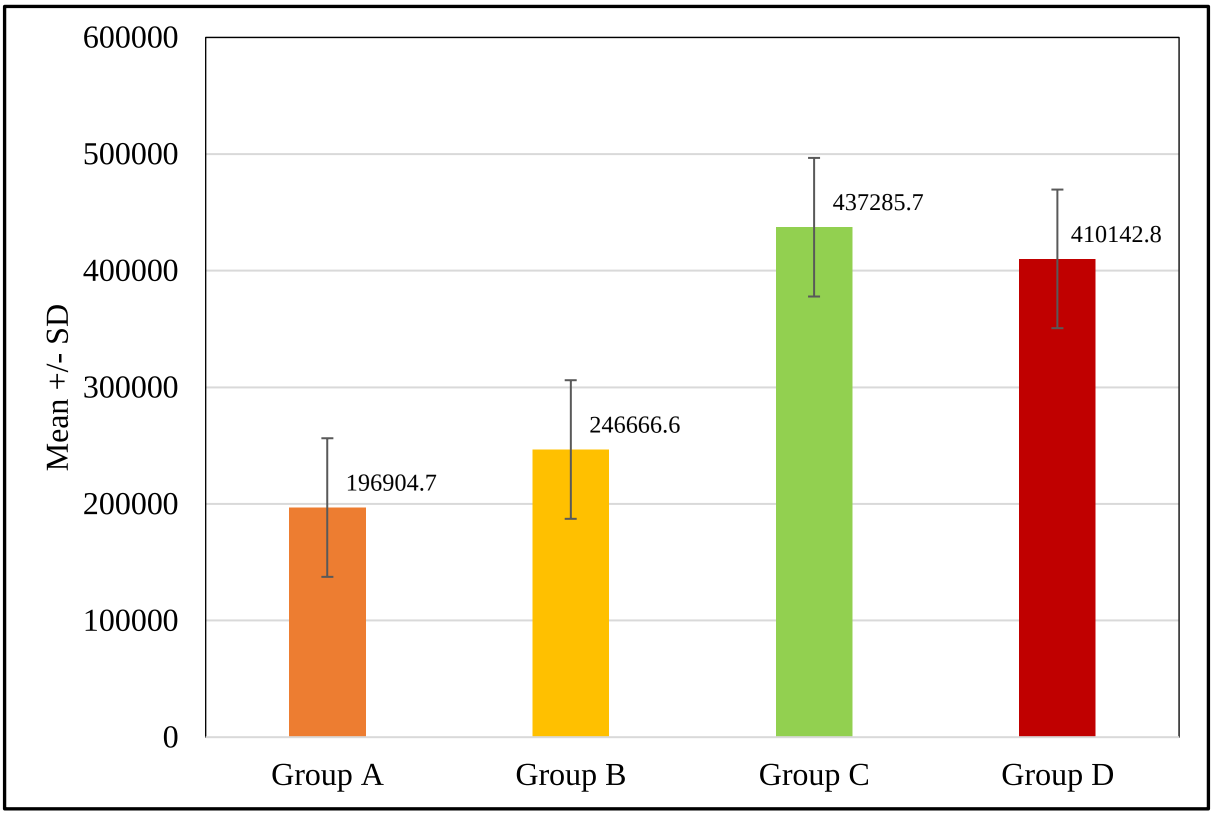


Table No.6: Pair wise comparison of interactions between four groups and three time intervals (24hrs, 48hrs and 72hrs) with cell attachment scores by Tukeys multiple posthoc procedures (p value)

Interactions	GroupA 24hrs	GroupA 48hrs	GroupA 72hrs	GroupB 24hrs	GroupB 48hrs	GroupB 72hrs	GroupC 24hrs	GroupC 48hrs	GroupC 72hrs	GroupD 24hrs	GroupD 48hrs	GroupD 72hrs
Mean	158571.4	187142.9	245000.0	265000.0	170000.0	305000.0	366857.1	330000.0	615000.0	370000.0	360428.6	500000.0
SD	23208.17	34328.94	26012.82	20132.89	23416.52	17483.33	18986.21	20800.64	10214.37	18903.26	21180.63	14832.40
GroupA with 24hrs	-											
GroupA with 48hrs	0.3693	-										
GroupA with 72hrs	0.0001*	0.0003*	-									
GroupB with 24hrs	0.0001*	0.0001*	0.8455	-								
GroupB with 48hrs	0.9975	0.9393	0.0001*	0.0001*	-							
GroupB with 72hrs	0.0001*	0.0001*	0.0002*	0.0387*	0.0001*	-						
GroupC with 24hrs	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*	0.0002*	-					
GroupC with 48hrs	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*	0.5763	0.0802	-				
GroupC with 72hrs	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*	-			
GroupD with 24hrs	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*	1.0000	0.0387*	0.0001*	-		
GroupD with 48hrs	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*	0.0006	1.0000	0.2776	0.0001*	0.9995	-	
GroupD with 72hrs	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*	0.0197*	0.0001*	0.0001*	-

*p<0.05 This table demonstrates statistically significant difference in cell attachment between Control and Test groups at 24,48,72 hours

Graph No. 3: Comparison of interactions between four groups and three time intervals (24yrs, 48hrs and 72hrs) with cell attachment scores

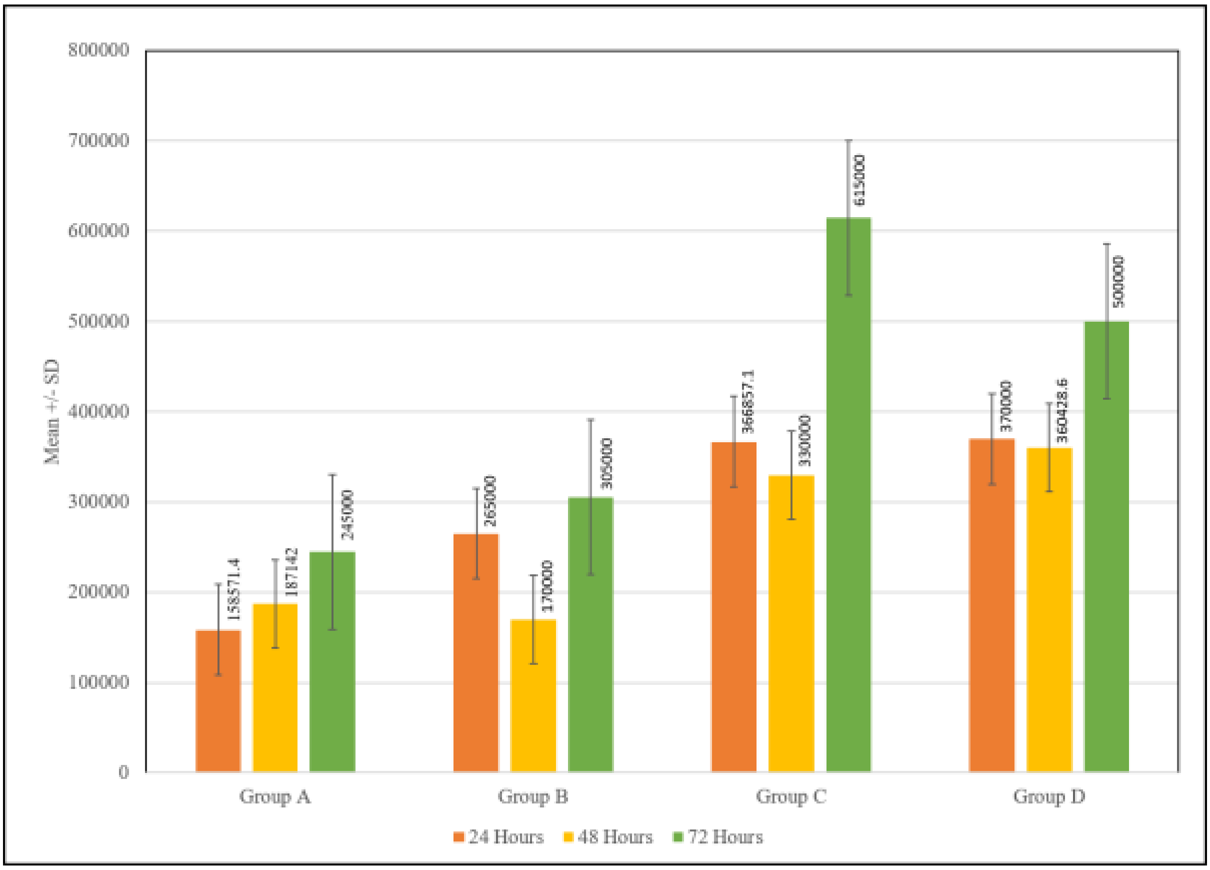


Table No.7: Comparison of four groups at three time intervals (24hrs, 48hrs and 72hrs) with cell proliferation scores by Two way ANOVA.

Sources of variation	Sum of squares	Degrees of freedom	Mean sum of squares	F-value	p-value
Main effects					
Groups	8669.13	3	2889.71	7.4078	0.0002*
Times	1717.79	2	858.90	2.2018	0.1180
2-way interaction effects					
Groups*Times	3972.33	6	662.05	1.6972	0.1341
Error	28086.64	72	390.09		
Total	42445.89	83			

*$p<0.05$ This table concluded that there was a significant difference in osteogenic potential in terms of cell proliferation between control GroupAnd all test groups at different time intervals.

Table No.8: Pair wise comparison of four groups with cell proliferation scores by Tukeys multiple posthoc procedures(p values)

Groups	GroupA	GroupB	GroupC	GroupD
Mean	100.00	106.98	112.14	127.62
SD	0.00	18.08	21.67	29.87
GroupA	-			
GroupB	0.6634	-		
GroupC	0.2006	0.8315	-	
GroupD	0.0003*	0.0063*	0.0624	-

*$p<0.05$

On pair wise comparison of groups, it showed statistically significant difference in cell proliferation in GroupD when compared with GroupA and GroupB

Graph No. 4: Comparison of four groups (A, B, C, D) with cell proliferation scores

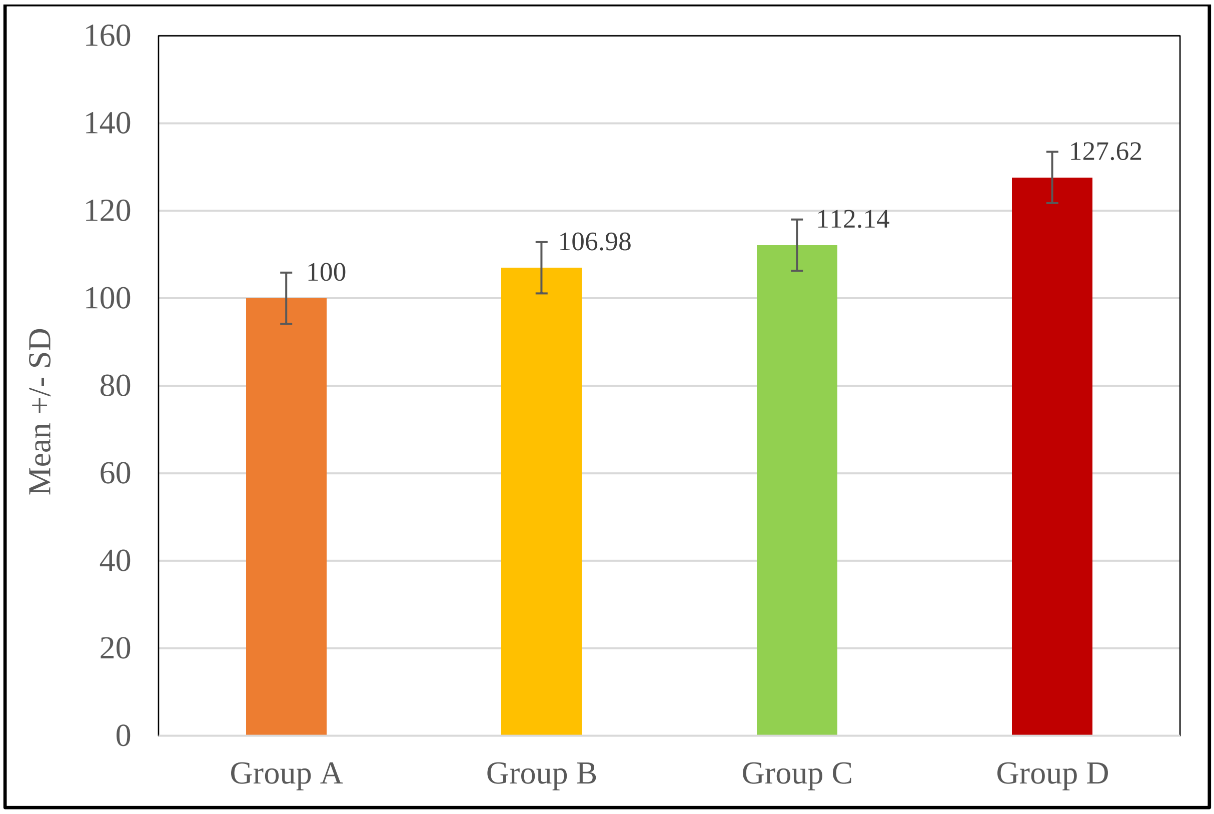
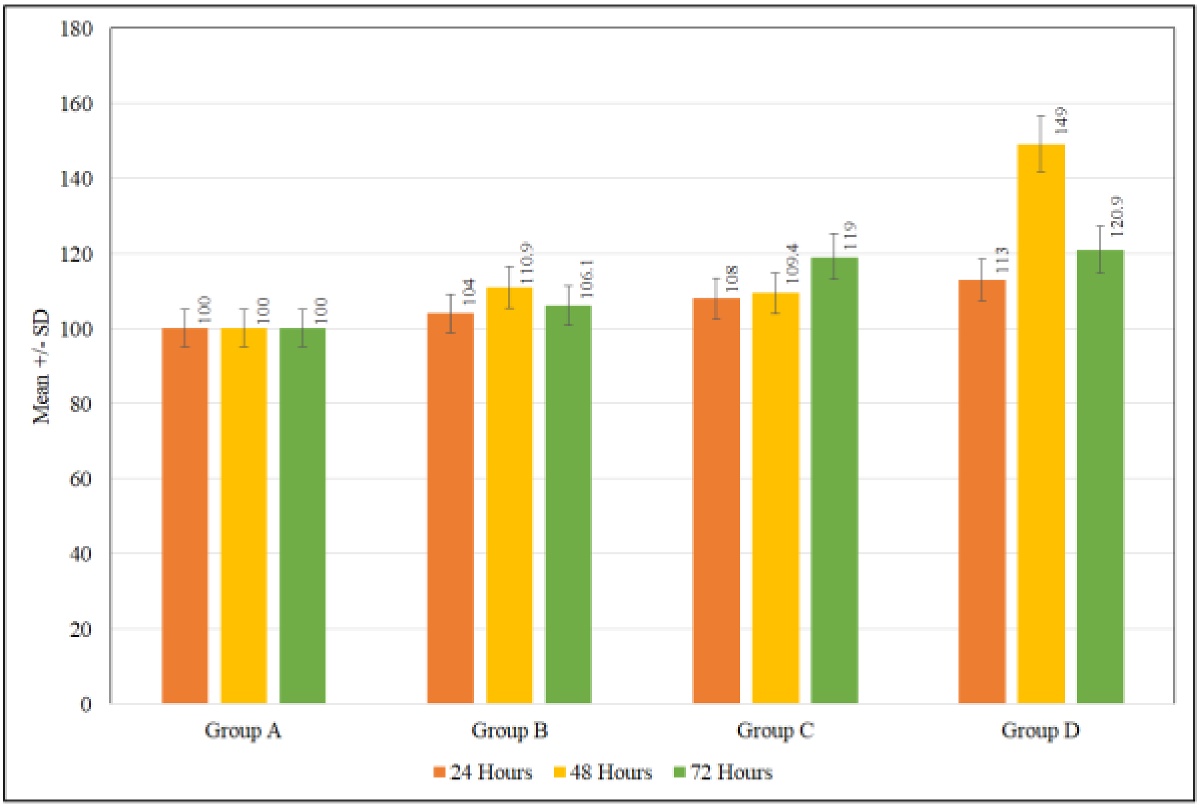


Table No.9: Pair wise comparison of interactions between four groups and three time intervals (24hrs, 48hrs and 72hrs) with cell proliferation scores by Tukeys multiple posthoc procedures (p values)

Interactions	GroupA with 24hrs	GroupA with 48hrs	GroupA with 72hrs	GroupB with 24hrs	GroupB with 48hrs	GroupB with 72hrs	GroupC with 24hrs	GroupC with 48hrs	GroupC with 72hrs	GroupD with 24hrs	GroupD with 48hrs	GroupD with 72hrs
Mean	100.0	100.0	100.0	104.0	110.9	106.1	108.0	109.4	119.0	113.0	149.0	120.9
SD	0.00	0.00	0.00	12.91	27.48	11.78	16.67	26.99	21.81	29.93	20.50	28.67
GroupA with 24hrs	-											
GroupA with 48hrs	1.0000	-										
GroupA with 72hrs	1.0000	1.0000	-									
GroupB with 24hrs	1.0000	1.0000	1.0000	-								
GroupB with 48hrs	0.9965	0.9965	0.9965	1.0000	-							
GroupB with 72hrs	1.0000	1.0000	1.0000	1.0000	1.0000	-						
GroupC with 24hrs	0.9998	0.9998	0.9998	1.0000	1.0000	1.0000	-					
GroupC with 48hrs	0.9990	0.9990	0.9990	1.0000	1.0000	1.0000	1.0000	-				
GroupC with 72hrs	0.8128	0.8128	0.8128	0.9555	0.9998	0.9852	0.9961	0.9989	-			
GroupD with 24hrs	0.9845	0.9845	0.9845	0.9994	1.0000	1.0000	1.0000	1.0000	1.0000	-		
GroupD with 48hrs	0.0010*	0.0010*	0.0010*	0.0034*	0.0258*	0.0064*	0.0114*	0.0173*	0.1852	0.0459*	-	
GroupD with 72hrs	0.7078	0.7078	0.7078	0.9047	0.9983	0.9598	0.9858	0.9946	1.0000	0.9998	0.2647	-

*p<0.05 This table demonstrates statistically significant difference in cell proliferation between Control and Test groups at 24,48,72 hours.

Graph No 5: Comparison of interactions between four groups and three time intervals (24hrs, 48hrs and 72hrs) with cell proliferation scores



DISCUSSION

Presently, dental implants have emerged as a fundamental component within the realm of dentistry. Improving the process of dental implant osseointegration has been a focal point of investigation for over four decades. Researchers have explored various avenues, ranging from macro-geometry to micro-geometry adjustments, alterations in materials, drill sequences, and more; all aimed at enhancing osseointegration. It is widely recognized that the ultimate triumph and longevity of implants hinge upon the caliber and quantity of host bone, the attainment of adequate primary stability during implant insertion, and the establishment of a direct bone-to-implant contact[43]

Osseointegration encompasses a sequence of bone modelling and remodelling activities. Its definition is direct, structural, and functional fusion of biological bone tissue with a load-bearing artificial implant surface.. The amount, distribution, and quality of bone present at the dental implant site are among the variables that must be met for osseointegration to be successful. [44]

However, the majority of what improves osseointegration and BIC is due the implant surface, including its topography, chemistry, roughness, and energy.[44] Titanium stands as the predominant material of choice for dental implants owing to its low toxicity, corrosion resistance, high mechanical strength, and biocompatibility. Throughout various studies, titanium implants consistently exhibit superior biocompatibility outcomes and a more favorable long-term prognosis.[44]

Albrektsson and Wennerberg categorized implant surfaces into four main types: “Smooth surfaces with a surface roughness (Sa < 0.5 μm), Minimally rough surfaces (Sa = 0.5-1 μm), Moderately rough surfaces (Sa = 1-2 μm), and rough surfaces (Sa > 2 μm)". Their findings suggest that moderately roughened surfaces may offer certain clinical advantages compared to smoother or rougher surfaces.[45]

In the current investigation, a Contact stylus profilometer (Surcomflex S-128 - Taylor Hobson) was used to quantitatively evaluate each specimen for surface characterisation. A contact stylus tool called a profilometer is used to gauge roughness and profile of specimen' s surface. This is the mean of all absolute roughness profile distances from the center line of the measurement length. Using a tracking device, Profilometer was able to calculate the surface profile along three lines. To describe the overall surface roughness, (Ra) was evaluated for each specimen. According to the surface profilometry results, the mean average surface roughness (Ra) of all tested specimens fell between 0.99 and 1.45 μm. The specimens were scanned at 500X and 1000X magnifications to assess surface roughness in addition to the quantitative evaluation's results. Apart from the numerical analysis of surface roughness values, the specimens were assessed using SEM at 250X, 500X, and 1000X magnifications to provide further qualitative assessment of the surface topography.

The ideal range for average roughness as reported in the literature is between one and two micrometers, [45] which matches the average roughness values that were derived from the profilometric measurements in this investigation. Osteoblastic shape is influenced by surface topography, according to Lukaszewska et al. Cells are less distributed and have a lower coverage area on rough surfaces than on smooth surfaces. On the other hand, they show a lot of phylodapy, linkages, and cytoplasmic extensions, all of which point to increased adhesion.[46]

According to Lima et al., there is an assertion that implants featuring a textured surface promote osseointegration more effectively compared to those with a machined surface. It is imperative to handle them with precision since this surface modification could potentially exert adverse effects or alter the titanium oxide surface. Therefore, it is crucial to establish a standardized level of surface roughness for titanium to ensure consistent surface characteristics for different surface treatments. [47]

While Titanium implants boast high clinical success rates, there has been advocacy for coatings made of various materials. An ideal surface should fulfil several key functions: enhancing cell attachment, promoting cell differentiation, and facilitating bone apposition; enabling bone fixation; controlling the rate of dissolution in bodily fluids; and potentially offering therapeutic benefits. [48]

The efficacy of rehabilitation with dental implants hinges on multiple factors. The implantation procedure necessitates favorable interactions between the adjacent bone tissue and titanium surface.[49]

The sustained clinical viability of dental implants relies heavily on the intricacies of their surface properties. Improving surface quality through modifications positively impacts parameters such as roughness, free energy, and chemical composition, thereby facilitating swift cell migration and attachment onto the implant surface. This, in turn, promotes enhanced secondary stability, thus underscoring the importance of surface modifications in optimizing the longevity of dental implant treatments.[1]

Implant surfaces have been altered using a variety of techniques, including blasting and turning, chemical processes like acid etching and alkali treatments, deposition techniques like sol-gel coatings and plasma-spraying, electrochemical processes like electropolishing and anodizing, and biochemical approaches involving proteins.

Improvements in dental implants have been made possible by surface roughening methods like acid etching and sandblasting. Additionally, methods aiming to enhance corrosion resistance, like anodization, have been proposed, proving beneficial for surface roughness improvement and osseointegration promotion. However, the achieved surface roughness may not be uniform and may contain porosities, leading to increased bacterial adhesion despite facilitating better osseointegration.[1,2]

Recent modifications for titanium implants to enhance osseointegration include photo-functionalization, plasma sterilization, and laser surface texturing. Photo-functionalization involves the application of UVC radiation to sterilize the implant surface, resulting in an enhancement of the implant's surface energy. This process contributes to improving the osteogenic potential of the implant[3]. Argon plasma treatment is utilized for decontaminating the titanium surface. Through this treatment, the plasma alters the oxide layer, facilitating interactions with surrounding tissue cells and ultimately leading to improved osteoblast cell adhesion[4]. Laser patterning on titanium implants serves to increase surface wettability, organize cell arrangement, enhance cell adhesion, and consequently elevate the osteogenic potential of the implant.[5]

Osteogenic Potential for the specimens in the control group- GroupA (titanium disc surface treated with only sandblasting) and study groups GroupB- Photo-functionalization, GroupC- Laser patterning, GroupD- Argon plasma treatment, were evaluated by assessing their cell adhesion and cell proliferation with MG-63 cell line (osteoblast like cells).

MG-63 cell lines were selected due to their ability to maintain a differentiated phenotype across successive subcultures and demonstrate faster growth rates when compared to 1º bone-forming cell lines, rendering them suitable in vitro model. These cells are particularly relevant for initial cell material characterization owing to their high proliferation potential, enabling continuous division and growth. Consequently, they are widely utilized in in vitro studies and investigations pertaining to biocompatibility. [31]

Cell adhesion was assessed using the trypan blue exclusion test in this investigation. This method involves the evaluation of viable cells with intact cell membranes that take up the dye, resulting in clear cytoplasm, whereas nonviable cells exhibit a blue cytoplasm. This test provides a straightforward and swift means of measuring cell viability and adhesion. Additionally, cell proliferation was determined using the MTT Assay, which gauges cellular metabolic activity and serves as an indicator of viability, proliferation, and cytotoxicity. The MTT Assay entails a colorimetric reaction easily quantifiable from cell monolayers plated on multiwell plates. It relies on mitochondrial respiration and offers an indirect assessment of a cell's energy capacity[50].

To compare the osteogenic potential among different groups and time intervals, there was a definite increase in the cell attachment in GroupC (Laser patterning) between 48-72 and 24-72 hours, showing a greater effect size in terms of cell attachment, as compared to the GroupA.

A notable enhancement in cell adhesion observed on laser-surface patterned titanium surfaces (GroupC) at both 24 and 72 hours. A significant finding was the increased initial adhesion of MG-63 cells to the titanium alloy surface facilitated by the microgrooves generated by the laser. This phenomenon can be attributed to the phenomenon of contact guidance, wherein cells align themselves along the direction of the grooves. Such alignment offers potential advantages for cell integration and adhesion, and these findings are in accordance with an investigation by Soboyaejo et al. [51]

Several in vitro studies investigating the influence of surface microgeometry on fibroblast attachment, spreading, orientation, and growth have indicated that fibroblasts cultured on microgrooved surfaces tend to align and migrate parallel to the grooves, while those on non-grooved surfaces exhibit random growth patterns. Moreover, it has been observed that laser-ablated microgrooves enhance directed cell filipodial contact and the alignment of fibrin fibrils.[9]

The increased cell attachment observed could also be attributed to an increase in surface area and roughness. Surface topography is recognized in playing a significant role in cell attachment and proliferation on implants, as extensively studied by Lukaszewska et al.[46]

Evaluation of proliferation of was done 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 for the specimens in Argon plasma treatment (GroupD) at 48 hours, which was statistically significant at 24-48 hours compared to GroupA, B, and C. This significant disparity at the 24-48 hour time interval, suggests functional maturation and proliferation by the 48-hour mark. The absence of statistical change between 48 and 72 hours might be attributed to lesser cell confluence within the study group.

Significant changes in cellular activity were seen when titanium surfaces treated with argon plasma were compared to other groups; thus, necessitating the rejection of the null hypothesis of equivalent cellular activity. Cell confluence caused an increase in cell viability in cells cultivated on treated surfaces at 24-48 hours.

These results of the current study, in which a statistically significant alteration in cell proliferation was observed after 24 hours, mirror the findings of González-Blanco et al. Their research indicated that Argon plasma treatment reduced surface contamination particles, thereby enhancing cell viability, spreading, and mitochondrial activity. Additionally, González Blanco et al. discovered that argon plasma-treated titanium surfaces showed bigger average cell areas and larger cells in comparison to other groups, indicating superior cellular behavior on such surfaces.[31]

Additionally, Han et al. confirmed the effect of the plasma system on cellular responses under a range of treatment scenarios. For the implant to be accepted early on, cells and biomaterial interaction is essential. Research confirmed that applying atmospheric pressure glow discharge (APGD) to the titanium surface led to a significantly increased uptake of fibronectin, a protein essential for the interaction between cells and biomaterials. As such, the increased cellular response depends on the cell attachment process mediated by fibronectin and integrin. [52]

The research used photofunctionalization, argon plasma treatment, and surface laser patterning to investigate the reaction of osteoblast-like MG-63 cells grown on titanium disc surfaces. Comparing the specimens to the Control Group, the results showed that those that had laser patterning and Argon plasma treatment showed better cell adhesion and proliferation.

This study showed that the Argon Plasma Treatment and Laser Patterning may represent an alternative method to promote early osseointegration.

SCOPE OF THE STUDY

The current investigation assessed the osteogenic potential of titanium discs that underwent Argon plasma treatment, photo-functionalization, and laser patterning. This study can also include the other implant biomaterials like zirconia and polyetheretherketone (PEEK). Future study is advised to evaluate criteria such as ALP, RANKL, alizarin red staining, and calcium deposit deposition using Von Kossa staining in order to differentiate the cells.

LIMITATIONS OF STUDY

- This is an invitro study, some of the clinical parameters like host response and type of bone might have variable findings.
- The study used an osteosarcoma cell line, MG-63 cells, that lacks coherence in the capacity of cell differentiation.
- The morphological differentiation of cells cannot be performed using the present methods used in the study.

CLINICAL IMPLICATIONS

In this study, the findings revealed that Argon Plasma Treatment and Laser Patterning resulted in superior cell proliferation and attachment of osteoblast-like cells, showing a significantly larger effect size compared to Photofunctionalization and machined titanium. The objective of enhancing success rates in cases of bone deficit and systemic conditions like osteoporosis prompted the investigation into these surface treatments, aiming to expedite bone healing. This, in turn, facilitates immediate or early loading protocols and stimulates bone growth, allowing for implant placement in sites with insufficient residual alveolar ridge.

Given these advantages, Argon Plasma Treatment and Laser Patterning emerge as promising and beneficial alternatives to promote early osseointegration, offering potential solutions for enhancing treatment outcomes in implant dentistry.

CONCLUSION

The following conclusions were obtained within the constraints of this in vitro study

When evaluating osteogenic potential, titanium surfaces treated with Photofunctionalization, Argon Plasma Treatment and Laser patterning consistently demonstrated higher cellular attachment across all tested time intervals. Particularly, the effect size of cell attachment was significantly greater on surfaces treated with Laser Patterning compared to Photofunctionalization, Argon Plasma Treatment and machined titanium surfaces.

These findings highlight the enhanced osteogenic capabilities of surface treatments such as Argon plasma treatment, and laser patterning, which can be used as an added advantage for improving osseointegration in dental implantology.

SUMMARY

The present in vitro investigation was carried out to assess and evaluate titanium's osteogenic potential after it underwent surface laser patterning, Argon plasma treatment, and photofunctionalization.

Four groups of 168 identical titanium disc-shaped specimens were created: GroupA consisted of 42 photofunctionalized titanium discs, GroupB of 42 sandblasted titanium discs, GroupC of 42 laser patterning specimens, and GroupD of 42 Argon Plasma Surface Treated specimens. The specimens in the study and control groups were subsequently separated into two groups (n=21) based on assays for cell attachment and proliferation in order to examine the osteogenic potential. Thes groups were further split into three groups (n=7) according to various time periods.

In order to evaluate the surface roughness qualitatively and quantitatively, every specimen from each group was examined under a SEM and profilometer. To characterize the surface's overall roughness, a mean roughness profile was assessed for every specimen. The surface profiles of each group were visually and qualitatively compared by SEM evaluation at magnification of 250X, 500X and 1000X in order to maintain a consistent surface roughness. Following the assessment of surface roughness, the osteogenic potential of MG-63 cells was examined by measuring their cell attachment and proliferation at 24,48, and 72 hours intervals in test goups and control group.

A hemocytometer was used to determine the proliferation of cells, and the MTT assay was used to measure their proliferation. After results were collated, statistical analysis was performed to extract conclusions from experimental data.

The outcomes demonstrated that, in comparison to other three groups, the titanium treated with Laser patterning (GroupC) had a higher cell attachment at each of three time intervals. In Laser patterning, there was a statistically significant variation with cell attachment over the various time intervals. When compared to other groups, the titanium treated with Argon plasma treatment (GroupD) showed higher cell proliferation at 24-48-hour intervals.

These results demonstrate the improved osteogenic properties of surface treatments, such as Laser patterning and Argon plasma treatment, which can be applied as a supplement to promote osseointegration.

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ANNEXURES

ETHICAL CLEARANCE CERTIFICATE

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
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Sl. No. : 1579

CERTIFICATE

This is to Certify that the synopsis titled Comparative Evaluation of Photo-functionalization, Laser Surface Patterning and Argon Plasma Surface Treatment of Titanium on its Osteogenic Potential - An Invitro Study *Submitted by Dr.* IM0221003 ____________ *P. G. Student / Staff, Guided by* ____________ *from Department of* Prosthodontics Crown and Bridge *has been critically evaluated by committee members and granted ethical clearance to conduct the above mentioned study*

Date : 30/3/24

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

Chairman
Research and Ethical Committee
KLEVK Institute of Dental Sciences
Belagavi

CERTIFICATION OF GRADE V TITANIUM DISCS

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.
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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