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ASSESSMENT OF HUMAN GINGIVAL FIBROBLAST ATTACHMENTS TO DIFFERENT TYPES OF ABUTMENT MATERIAL

Ahmad Alamoudi[1*], Nouf Aldahri[2], Yara Alsanea[2]

[1]Department of Oral Biology, Faculty of Dentistry, King Abdulaziz University, Jeddah 21589, Saudi Arabia
[2]General Dentistry, Faculty of Dentistry, King Abdulaziz University, Jeddah 21589, Saudi Arabia

Correspondence author: Ahmad Alamoudi; **Email**: ahmalamoudi@kau.edu.sa

Abstract:

Background: The success of dental implants is heavily dependent on the material properties of the abutments used. Understanding how gingival fibroblasts interact with different abutment materials is crucial for optimizing peri-implant health and ensuring the long-term success of the implants.

Objective: This study aimed to evaluate the adhesion and proliferation of human gingival fibroblasts on four commonly used abutment materials: titanium, zirconium, polyether ether ketone (PEEK), and stainless steel, to determine which materials promote the best cellular response and peri-implant seal.

Methods: Disk-shaped specimens of each material were prepared and subjected to fibroblast adhesion assay. The surfaces were analyzed for roughness, while fibroblast response was quantified using optical density measurements of cell viability assays.

Results: The results indicated that titanium and zirconia abutments exhibited significantly higher levels of fibroblast adhesion compared to PEEK and stainless steel. Both materials demonstrated favorable surface properties that enhance biocompatibility and support peri-implant tissue integration. In contrast, PEEK and stainless steel showed lower biocompatibility, which could potentially limit their use in clinical scenarios requiring optimal biological responses.

Conclusion: Titanium and zirconia are superior to PEEK and stainless steel in terms of supporting gingival fibroblast activities crucial for successful dental implant. These findings suggest that choosing the right abutment material is fundamental for improving implant success rates and long-term patient outcomes. Further in vivo studies and clinical trials are necessary to validate these findings and fully understand the implications of abutment material selection in dental implantology.

Keywords: Titanium, zirconia, dental implant, adhesion.

Introduction:

The prosthetic dentistry paradigm has undergone a significant shift. One of the most popular options for replacing missing teeth is dental implants (1). During the past 20 years, implantology has advanced more quickly than any other branch of modern dentistry in conjunction with the creative use of computer-aided design/computer-aided manufacturing (CAD/CAM) technology (2). Following tooth

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extraction, a thrombus fills the socket, which gradually increases in density over the next two to seven days to replace the entire tissue. Furthermore, following periodontal surgical procedures, the first epithelium healing takes roughly 7–14 weeks, and it takes 6-8 weeks to establish the biological width and barrier function around transmucosal implants or abutments. For these reasons, an effective peri-implant seal depends on the interaction of the connective tissue and implant material (3) . The principal cellular constituent of connective tissues is fibroblasts, crucial for preserving structural integrity in these tissues. They generate and release diverse extracellular proteins, such as proteinases, which govern the biochemical makeup and tissue remodeling (4). For long lasting implant supported prostheses, it is essential to produce not just sound implant to hard tissue interfaces, but also a firm implant to soft tissue junction (4).

Implant abutments play a critical role in the field of dental implantology, acting as the crucial interface between the dental implant and the prosthetic restoration (5). These components are vital for the structural and aesthetic integration of dental prostheses supported by dental implants. Implant abutments can be fabricated from materials like titanium, zirconia, gold, polyether ether ketone (PEEK) and stainless steel (5). The abutment material is chosen based on their compatibility, mechanical properties, and esthetic quality to match the individual clinical needs for the patient (5).

The design and fabrication of implant abutments have evolved significantly, driven by the advancements in dental technology and materials science. Modern abutments are precisely engineered to ensure optimal load distribution, enhance soft tissue contours, and promote long-term peri-implant health (5).

The interaction between gingival fibroblasts and implant abutments is a crucial aspect of long-tern success of dental implants (6). Gingival fibroblasts, the predominant cell type within the gingival connective tissue, play a significant role in the maintenance and repair of the periodontal ligament and surrounding soft tissues (6). Their behavior in the presence of implant abutments is key to understanding the peri-implant health, particularly in how these cells adhere to, proliferate, and integrate with different abutment materials (6).

The material composition and surface characteristics of implant abutments can significantly influence fibroblasts behavior, impacting everything from cellular attachment and proliferation to the synthesis of extracellular components (7). These interactions are pivotal as they dictate the soft tissue's barrier integrity around the implant, a critical factor in preventing implant peri-implant diseases and ensuring the success of implant-supported prostheses (7).

This study aimed to explore how different abutment materials influence gingival fibroblasts attachment and subsequent tissue integration. By examining the cellular mechanisms and interactions at biomaterial interface, this study aimed to shed light on the fundamental process that govern soft tissue interaction and stability, providing insights that could guide the design and selection of implant abutments for enhanced clinical outcomes.

Research question: How does titanium differ from other abutment materials in terms of gingival fibroblast adhesion?

Research Hypothesis:

- Null Hypothesis: No difference in adherence of gingival fibroblasts between implant abutment materials.

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- Alternative Hypothesis: Titanium abutment has better gingival fibroblast adhesion than other abutment materials (Peek, Zirconia, Stainless steel).

Objectives:

- Evaluate the attachment of human gingival fibroblasts to different implant abutment materials aiming to elucidate the interactions between these cells and various abutment materials in the context of dental implantology.
- Determine which material is more biocompatible and promotes a better peri-implant seal.

Significance Of The Study:
Enhancing our understanding of how abutment materials interact with peri-implant soft tissues. This study holds the potential to significantly impact both the practice and outcomes of dental implant therapies by providing deeper scientific understanding of the interactions at the biomaterial-tissue interface.
Key aspects of the study's significance include:
1- Improving implant success rates.
2- Enhancing soft tissue integration.
3- Material innovation.
4- Reducing complications and costs.

Literature review:

The field of prosthetic dentistry has witnessed a significant transformation, marked by the emergence of dental implants as a leading solution for replacing missing teeth over the past two decades (1). This rapid advancement in implantology is tightly linked with the innovative application of computer-aided design and manufacturing (CAD/CAM) technologies, which have uplifted this specialty to new heights (2). After a tooth is extracted, the socket is initially filled by a blood clot, which gradually increases in density from two to seven days to completely replace the extracted tissue. Additionally, the initial healing of the epithelium following periodontal surgery takes approximately 7–14 weeks, and establishing the biological width and barrier function around transmucosal implants or abutments typically requires 6-8 weeks (2). Consequently, the effectiveness of the peri-implant seal is highly dependent on the interaction between the connective tissue and the implant material (3). Fibroblasts, the primary cellular component of connective tissues, are vital for maintaining structural integrity. They synthesize and release a variety of extracellular proteins, such as proteinases, which regulate the biochemical composition and remodeling of tissues (4). A dental implant is technically defined as a medical device that interacts with the jawbone (5). Customized implant abutments are fundamental to modern prosthetic implant dentistry, uniquely designed to align with the soft tissue contour of the implant site, thus offering dual benefits: they support the soft tissues and strategically place the cementation margin for easier removal of excess cement (6). Nowadays, a diverse collection of materials including base metals, zirconia or alumina ceramics, and high noble alloys are utilized to manufacture these uniquely designed prosthetic abutments (7).

Historically, cast gold abutments were viewed as the cutting edge of customized prosthetic solutions; however, their use has declined due to high costs and questions regarding their biocompatibility. Animal

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studies have indicated that peri-implant soft tissues fail to establish a strong seal with gold abutments, leading to tissue recession and crestal bone loss (8). Similarly, dental porcelain has not proven effective in forming a reliable soft tissue attachment (8). Outcomes with feldspathic ceramics were particularly unfavorable, exhibiting the most significant soft tissue recession and bone loss (8). Composite resin abutments have been proposed as alternatives to zirconium in various in vitro experiments (8). However, the reaction of soft peri-implant tissues to composite is concerning, with significant plaque accumulation and subsequent mucosal inflammation reported in randomized clinical trials, limiting the use of resin composite abutments (7). Titanium and zirconium are recommended for the construction of patient-specific abutments, with titanium long favored for its strength, resistance to distortion, and the possibility of being manufactured as a single piece (8). Systematic reviews have attested to the exceptional reliability of titanium abutments. However, a significant drawback of these abutments is their dark color, which can penetrate thin peri-implant tissues and give the peri-implant mucosa a displeasing gray appearance (6). In response to heightened aesthetic demands and concerns regarding the biocompatibility of dental alloys, ceramics, particularly zirconia, have increased in popularity. Known for its strength, zirconia meets the demand for aesthetically pleasing, durable, and metal-free prostheses, though its brittleness remains a concern (2).

The establishment of durable connections between implants and soft tissues, as well as stable connections between implants and hard tissues, is essential for the longevity of implant-supported prostheses (9). Prior research examining soft tissue responses to different abutment materials has shown no difference in peri-implant tissue conditions between submerged and non-submerged healing, and peri-implant hard and soft tissue configurations were consistent across various implant systems (9). The success or failure of implants is significantly influenced by the oral flora surrounding the implants (1). Preventing the adhesion of bacteria or microorganisms to implant abutment surfaces is crucial because biofilm formation can lead to periodontitis and peri-implant mucositis. This adverse reaction not only increases the risk of early implant failure but also imposes a psychological burden on patients (10). Moreover, it has been noted that peri-implant mucositis can alter various bodily parameters, including serum biochemical markers, cytokine levels, and blood cell counts, potentially affecting other diseases and systemic conditions (10). After implant-abutment placement, surrounding fibroblasts and keratinocytes initiate regenerative processes that form the collagen matrix beneath and the epithelial keratinocyte layer above the implant-abutments through cell migration from a soft tissue barrier (10). This barrier enables the surrounding soft tissue to act as a protective seal with the adjacent bones, thus extending the longevity of dental implant abutments by preventing early implant failure due to bone loss and protecting the implant-abutment connection from peri-implant mucositis caused by the invasion of harmful bacteria (10). In conclusion, the primary considerations for the use of dental materials in dental implant abutments are their ability to promote cell adhesion and their resistance to biofilm formation (11). Maintaining crestal bone levels and establishing a soft tissue barrier are vital for the long-term success of dental implants. To prevent the downward migration of epithelial tissue, a robust connective tissue graft can be attached to the transmucosal section of the implant, acting as a biological barrier that blocks bacterial toxins and resists bone resorption (12). This feature is crucial for aesthetic considerations, as the stability of the papilla and gingival margin depends on the health of the crestal bone (12). The surface characteristics of dental implants and their abutments affect osseointegration and the behavior of cells exposed to implanted surfaces, such as titanium, which promotes cell attachment (12). Research suggests that the properties of the abutment surface, including topography, roughness, chemistry, surface energy (charge), surface treatment, and hydrophilicity, influence the migration,

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adhesion, and dispersion of cells at the interface between soft tissue and implants (13). To promote rapid adhesion and proliferation of fibroblasts and epithelial cells to the abutments, as well as to facilitate faster osseointegration and implant stability, researchers have explored strategies to modify surface chemistry (13).

Studies on both humans and animals have shown that an Ra value of 0.2 μm is necessary to create an optimal abutment/soft tissue seal (12). Smooth surfaces can lead to unwanted adhesion and apical migration of epithelial cells (12). Additionally, creating micrometer-scale grooves on the implant surface perpendicular to the direction of migration of epithelial cells has proven to improve HGF attachment and inhibit apical proliferation of epithelial cells (12). The results of a study investigating the surface characteristics correlated with human gingival fibroblast attachment to various implant abutment materials indicate that wettability, particularly on surfaces with roughness less than 0.2 mm, affects the attachment of human gingival fibroblasts to implant abutment surfaces (12). Surface roughness within the range of 0.2–0.5 μm can potentially affect the implant. Therefore, it is recommended that any implant abutment should have a smooth and hydrophilic surface with a minimal water contact angle and a surface roughness value of approximately 0.2 mm or less (9). In a study conducted to compare the attachment and growth patterns of epithelial and fibroblast cells on variously shaped abutment materials, both materials produced comparable surface topographies (13). Zirconia surface topographies showed noticeably greater fibroblast proliferation rates than those of the titanium alloy (13). On various substrate/topography combinations, fibroblast and epithelial cell growth was most optimal. The study found that cell spreading was generally higher on polished and machined surfaces compared to sandblasted surfaces. While rough surfaces positively affected the adherence of fibroblast cells, they did not have a similar impact on epithelial cell adhesion. The study confirmed that complex interactions between soft tissue cells and substrate occur and that both the surface topography and the material can affect the response of fibroblast and epithelial cells (7). Three implant abutments were compared for biocompatibility using human gingival keratinocytes: titanium uncoated, titanium nitride coated, and modified polyetheretherketone (PEEK). They were made and contrasted with controls, which were polyester cell culture discs without coating. After 7 days of growth, it was found that PEEK materials significantly increased HGEK cell proliferation in all three groups when compared to the control. In a similar vein, the effects were observed by both TiN-coated and uncoated Ti. In comparison to PEEK material, it was also noted that after 7 days, the HGEK cells migrated and covered over 50% of the wound area for all abutment discs, whereas the TiN and uncoated Ti showed less migration and less migration, respectively.

Regarding the rate at which fibroblasts proliferate, it was possible for the HGEK to adhere to the disks and multiply. Over the period of up to 14 days in culture, the rate of cell proliferation increased. Cell proliferation was observed to be almost at the same level after 21 days as it was at 14 days. There were no noticeable differences between the disk surface types. It's clear that the samples have cell colonization. HGFs were seen to have evenly distributed and adhered to the sample surfaces after 14 days, forming a continuous monolayer. After 21 days in culture, the cells reached confluence and ceased to proliferate.

In comparison to uncoated and coated titanium abutments, the PEEK surface was found to be more biocompatible and to have a favorable effect on the viability, migration, and proliferation of HGEK. Moreover, TiN coating shows increased antibacterial activity (1). The viability, proliferation, and adhesion abilities of HGFs at the cellular level on polymer -infiltrated ceramic networks (PICN), polyetheretherketone (PEEK), hydroxyapatite- reinforced polyetheretherketone (HA-PEEK), and polyetherketoneketone (PEKK) were evaluated in vitro in 2019 for the first time and compared to the

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traditional abutment materials Ti and Zr. HGF proliferation and adhesion characteristics are significantly influenced by the wettability of dental implant abutment materials. The finest wettability characteristics were displayed by PICN, although with severe shaking, Zr had the strongest adhesive strength. Zr is still the most biocompatible abutment material as a result (14).
To assess the potential effects of argon and UV light on chair side surface treatments on fibroblast adherence on various titanium surfaces intended for soft tissue repair. Surface observation was carried out in line with the expected features. The range of roughness ratings from very rough to smooth. After 20 minutes, the cells on the treated samples were more widely distributed with broad lamellipodia, whereas all the untreated surfaces displayed hemispherical cells with reduced filopodia. At 24 and 72 hours, however, these variations in spreading behavior disappeared. After 20 minutes, argon plasma was found to significantly increase the number of fibroblasts, regardless of the surface type. However, there was no statistically significant increase in cellular adhesion observed with any surface plus treatment combination. Early-stage cell adhesion research indicates that treating implant abutment surfaces with argon plasma may have biological benefits. It should be noted that UV light did not have the same effect on fibroblast growth as argon plasma (13).
In summary, dental implants are an effective solution for individuals with good oral health who have lost teeth due to a variety of reasons including periodontal disease and trauma. The amount of bone that is present at the implant site is crucial to its capacity to support a dental restoration. The materials' physical and chemical properties, as well as specific surface qualities of the implant, determine the implants' biocompatibility. The dental implant materials' surface should promote a strong bond between the implant and the bone, soft connective tissue, and junction epithelium. Several indicators generated by osteoblasts or periodontal ligament cells have been suggested for the assessment of dental implant biocompatibility. Alkaline phosphatase and collagen I are the most common indicators for osteoblasts and fibroblasts, respectively. Tumor necrosis factor α and pro-inflammatory interleukins are used to measure the contribution of both cell types to the inflammatory response (15). The findings demonstrated complex relationships between soft tissue cell substrate, with the fibroblasts and epithelial cells responding differently depending on the surface topography and material used (15).

Materials & Methods:

Implant abutment materials preparation:

Four common implant abutment materials Grade 1 titanium (CpTi), zirconia (Y-TZP), PEEK and Stainless steel were used in the study (Figure 1). The specimens were designed as disk-shaped specimens with a diameter of 10.0 mm and a thickness of 2 mm and fabricated using a dental CAD/CAM milling machine (Zirkonzahn M1; Zirkonzahn GmbH, Gais, Italy) (Figure 2). All the specimens were washed with de-ionized water in an ultrasonic cleaner and air dried. Zirconia samples were finished and highly polished using DIACERA DIASYNT® PLUS Zirconia Polishers, PEEK samples were polished using polishing paste and rubber cup, and Stainless steel and titanium samples were polished using RABBIT ® Rubber Bonded Abrasive Wheel, Silicone Rubber Polishing Wheel (Figure 3).

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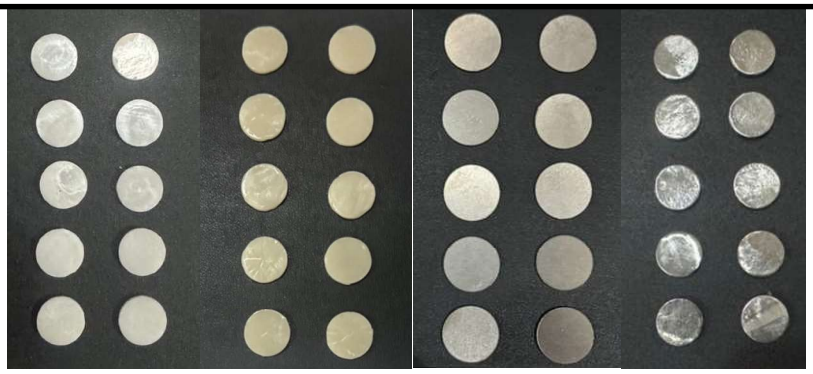

Figure 1. (A) Zr (B) PEEK (C) Ti (D) St

Figure 2. Milling machine.

Figure 3. A) PEEK Polishers, B) Zr Polishers, C) Ti & St Polishers.

Human gingival fibroblasts isolation:

1. Primary human gingival fibroblasts (HGFs) were isolated from fragments of healthy gingival tissues (1-2mm). Gingival tissues were obtained from patients underwent crown lengthening procedures after obtaining consent (Figure 4).

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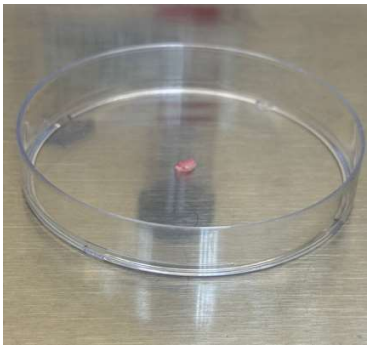

Figure 4. Gingival tissue (1-2 mm).

2. The tissues were cut, minced, and distributed on petri dishes immediately in cell culture medium (DMEM, Sigma-Aldrich, St. Louis, MO, USA) supplemented with 10% fetal bovine serum (FBS, Sigma-Aldrich, St. Louis, MO, USA) and penicillin/streptomycin (Sigma-Aldrich, St. Louis, MO, USA) (Figure 5).

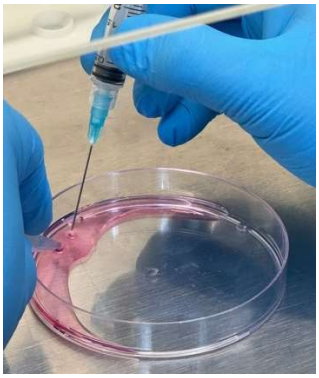

Figure 5. Tissue was cut and minced using blade and needle.

3. After cleaning, the samples were transferred into another 60 mm petri dish containing 5 ml HBSS and 5 ml CVS complete media and incubate for 24 hours at 37 C° in CO2 incubator (Figure 6).

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Figure 6. Incubator.

4. Following incubation, the samples were transferred into new another 60 mm petri dish containing 5ml of 0.25% trypsin EDTA and ineubate for one hour at 37 C° in CO2 incubator.
5. Under inverted microscope, the out layer of cells was examined and should be coming off and single cell was observed floating in trypsin.
6. If floating cell in trypsin was not obtained, 1-2 ml of fresh 0.25% trypsin EDAT was added and ineubated for 30 minutes at 37 C° in CO2 incubator.
7. Following incubation, samples were transferred into 60 mm petri dish containing 5 ml collagenase solution and incubated for 2 hours then transfered the collagenase cells suspension into 15 ml centrifuge tube with using sterile pasture pipette. Then centrifuged at 1000 rpm for 10 min and supernatant was removed (Figure 7 and 8).

Figure 7. Collagenase preparation.

Figure 8. Centrifuge machine.

8. Cell pellets were resuspended in 8 ml of CVS complete media then centrifuge at 1000 rpm for 10 minutes and supernatant was removed.
9. Cell pellets were resuspended into 3 ml CVS complete media (Figure 9).

Figure 9. Cell pellet in CVS media.

10. Then cell pellet suspension was divided into two different T.25 culture flask and incubated at 37C in CO2 incubator for 2-3 days.

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11. Cells were checked using inverted microscope and the cells were fed with 2 ml of CVS complete media then iincubated for 24 hours (Figure 10 and 11).

Figure 10. Microscope.

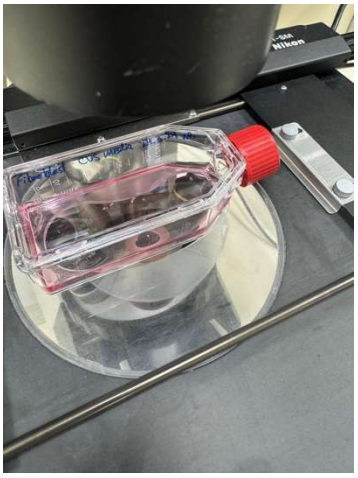

Figure 11. Cells in flask under microscope**.**

12. Using inverted microscope cells were fed with 2 ml fresh media. Media were changed when growth observes or every 2-3 days.

Surface roughness:

Each material's surface was polished to the highest possible degree. For this phase, Mitutoyo roughness tester was used to assess roughness of each material (Figure 12).

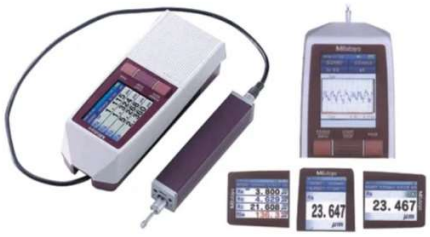

Figure 12. Mitutoyo roughness tester.

To study the surface of each material, digital camera stereomicroscope (RaySmart Technology Co., Ltd., Shenzhen, China) was used (Figure 13,14, 15, 16 and 17).

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Figure 13. Digital camera stereomicroscope (RaySmart Technology Co., Ltd., Shenzhen, China).

(A)

(B)

Figure 14. Representative pictures of surface topography of Zirconia by stereomicroscope:

(A) x200
(B) x4

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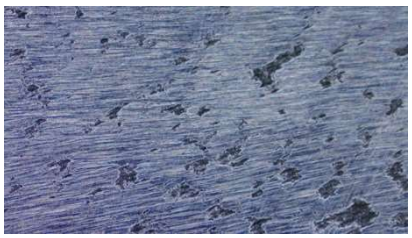


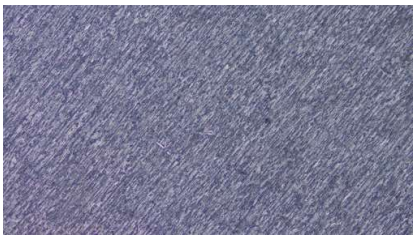
(A)

(B)

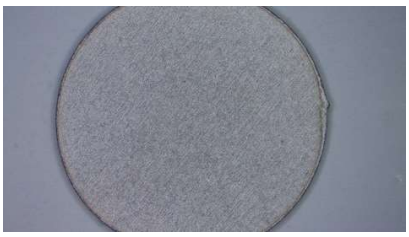
Figure 15. Representative pictures of surface topography of Stainless steel by stereomicroscope:

(A) x200

(B) x4



(A)



(B)

Figure 16. Representative pictures of surface topography of Titanium by stereomicroscope:

(A) x200

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(B) x4
(C)

(A)

(B)

Figure 17. Representative pictures of surface topography of PEEK by stereomicroscope:

(A) x200
(B) x4

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Titanium abutment (CpTi)

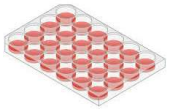

PEEK abutment


Zirconia abutment (Y-TZP)


Stainless steel

The testing specimens were designed as disc-shaped specimens with a diameter of 10 mm and thickness of 2 mm and fabricated using a dental CAD/CAM milling machine.
All specimens were washed with de-ionized water in an ultrasonic cleaner and air dried.

Ten implant abutment discs from each material were placed in a 24-well cell culture plate (total samples = 120).



Human gingival fibroblast were isolated then seeded on different implant surface in 1 mL of fully supplemented DMEM at a density of 2x10^4 cells/well of viability assays.

Cell viability was determined by cell counting Kit 8 (CCK-8)

100 ul of CCK-8 reagent solution were pipetted into each well, and cells were intubated at 37 C for 2 hours.

100 ul of the solution was transferred into 96-well plate.

100 ul solution was absorbed at 450 nm and was measured using the Synergy HTX multi-detection reader.

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Sample size calculation rational based on the POWER selected:

- A power calculation was calculated using nQuery Advisor. Based on the results of SILVA et al assuming no difference between the implant abutment materials, a sample size of 84 is adequate to obtain type I error rate of 5% and a power of 88%. Therefore, the decision was made to include 40 samples in triplicate which will be a total of 120 samples. Cell viability assay was applied in triplicate.

Statistical Analysis:

The analysis began with the Independent-Samples Kruskal-Wallis Test, a non-parametric alternative to one-way ANOVA, to compare the optical density (OD) values across the four abutment material groups (zirconium, PEEK, titanium, and stainless steel).
To identify which specific pairs of materials exhibited statistically significant differences in OD values, pairwise comparisons were performed as a post-hoc analysis. The Bonferroni correction was applied to adjust the significance values (p-values) for multiple comparisons. The p-value of <0.001 indicates statistically significant between groups.The results were presented in the form of tables and graphically using boxplots and a node-link diagram.

Results:

Surface roughness of implant abutment samples:
To minimize differences caused by the physical characteristics of surfaces, it was crucial to polish each material to achieve consistent surface roughness. After polishing all samples, the surface roughness was measured for all samples of each implant abutment material.
Tables 1,2,3 and 4. shows the surface roughness values for zirconia, titanium, PEEK and stainless steel samples from three readings.
The mean value of surface roughness (Ra) of zirconia samples was 0.338 ± 0.01, titanium was 0.354 ± 0.02, PEEK was 0.353 ± 0.031 and for stainless steel was 0.401 ± 0.03.
All groups showed no statistical differences between them analyzed by Kruskal-Wallis test as shown in **figure 18.** $P<0.001$ to consider the difference statistically significant.

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Table 1. Surface roughness for zirconia specimens.

Zirconia	1st reading	2nd reading	3rd reading	Mean	SD
1	0.271	0.361	0.353	0.328	0.04
2	0.279	0.344	0.347	0.323	0.031
3	0.27	0.312	0.367	0.316	0.039
4	0.334	0.308	0.322	0.321	0.01
5	0.332	0.332	0.334	0.332	0.0009
6	0.354	0.284	0.314	0.317	0.028
7	0.287	0.303	0.296	0.295	0.006
8	0.383	0.391	0.38	0.384	0.004
9	0.319	0.352	0.371	0.347	0.021
10	0.431	0.411	0.398	0.413	0.013
Mean of total samples				0.338	0.019

Table 2. Surface roughness for titanium specimens.

Titanium	1st reading	2nd reading	3rd reading	Mean	SD
1	0.384	0.309	0.408	0.367	0.051
2	0.35	0.393	0.358	0.367	0.022
3	0.396	0.364	0.351	0.37	0.023
4	0.329	0.351	0.381	0.353	0.026
5	0.374	0.382	0.352	0.369	0.015
6	0.412	0.398	0.424	0.411	0.013
7	0.337	0.332	0.335	0.334	0.002
8	0.311	0.346	0.332	0.329	0.017
9	0.314	0.365	0.358	0.345	0.027
10	0.288	0.306	0.296	0.296	0.009
Mean of total samples				0.354	0.02

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Table 3. Surface roughness for PEEK specimens.

PEEK	1st reading	2nd reading	3rd reading	Mean	SD
1	0.461	0.377	0.443	0.427	0.044
2	0.437	0.352	0.453	0.414	0.054
3	0.39	0.324	0.332	0.348	0.036
4	0.29	0.305	0.288	0.294	0.009
5	0.372	0.33	0.391	0.364	0.031
6	0.335	0.357	0.388	0.36	0.026
7	0.322	0.318	0.319	0.319	0.002
8	0.375	0.337	0.328	0.346	0.024
9	0.373	0.325	0.343	0.347	0.024
10	0.322	0.372	0.256	0.316	0.058
Mean of total samples				0.353	0.031

Table 4. Surface roughness for stainless steel specimens.

Stainless steel	1st reading	2nd reading	3rd reading	Mean	SD
1	0.386	0.454	0.414	0.418	0.034
2	0.433	0.376	0.465	0.424	0.045
3	0.45	0.458	0.485	0.464	0.018
4	0.37	0.36	0.419	0.383	0.031
5	0.359	0.497	0.361	0.405	0.079
6	0.365	0.345	0.421	0.377	0.039
7	0.332	0.363	0.381	0.358	0.024
8	0.412	0.424	0.396	0.41	0.014
9	0.312	0.358	0.403	0.357	0.045
10	0.394	0.429	0.412	0.411	0.017
Mean of total samples				0.401	0.034

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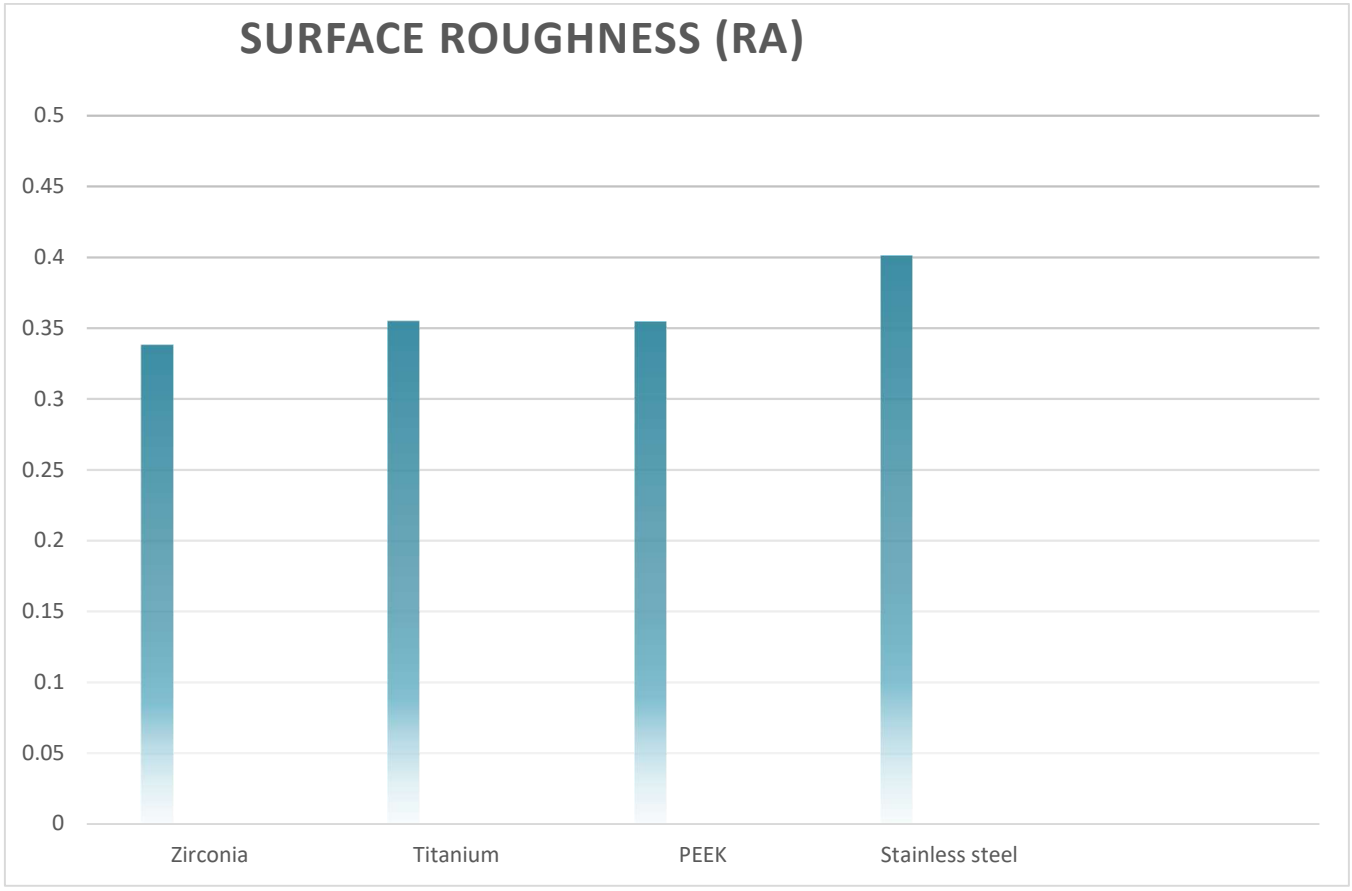

Figure 18. Means of surface roughness of implant abutment samples (n=10)
There were no significant difference between the groups.

Assessment of human gingival fibroblasts adhesion on different implant abutment materials:
Cells attachment on zirconia, titanium, PEEK and stainless steel samples were evaluated by viability assay.
Optical density mean was the highest for zirconia samples (OD= 1.088 ± 0.023), followed by titanium (OD = 1.046 ± 0.018), PEEK (OD = 0.734 ± 0.122) and the least material was stainless steel (OD = 0.049 ± 0.002) **(Tables 5,6,7,8)**

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Table 5. Optical density (OD) of fibroblasts adhesion on zirconia abutments samples.

Zirconia samples	OD - well1	OD- well2	OD - well3	Mean	SD
Zr-1	1.027	1.026	1.023	1.025	0.002
Zr-2	1.046	1.048	1.044	1.046	0.002
Zr-3	1.144	1.142	1.251	1.179	0.062
Zr-4	1.016	1.019	1.021	1.018	0.002
Zr-5	1.082	0.968	1.076	1.042	0.064
Zr-6	1.031	1.037	1.039	1.035	0.004
Zr-7	1.104	1.125	1.113	1.114	0.010
Zr-8	1.233	1.202	1.198	1.211	0.019
Zr-9	1.195	1.176	1.124	1.165	0.036
Zr-10	1.073	1.056	1.021	1.05	0.026
Mean of total samples				1.088	0.023

Table 6. Optical density (OD) of fibroblasts adhesion on titanium abutments samples.

Titanium samples	OD-well1	OD- well2	OD - well3	Mean	SD
Ti-1	1.035	1.032	1.039	1.035333333	0.00351188
Ti-2	1.195	1.176	1.124	1.165	0.03675595
Ti-3	1.062	1.041	1.052	1.051666667	0.01050397
Ti-4	1.038	1.033	1.014	1.028333333	0.01266228
Ti-5	1.051	1.129	1.11	1.096666667	0.0406735
Ti-6	1.011	1.04	1.038	1.029666667	0.01619671
Ti-7	1.031	1.019	1.081	1.043666667	0.03288363
Ti-8	0.974	0.944	0.956	0.958	0.01509967
Ti-9	1.046	1.032	1.045	1.041	0.00781025
Ti-10	1.015	1.012	1.029	1.018666667	0.00907377
Mean of total samples				1.0468	0.01851716

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PEEK samples	OD-well1	OD- well2	OD - well3	Mean	SD
P-1	0.751	0.754	0.746	0.750333333	0.00404145
P-2	0.755	0.703	0.758	0.738666667	0.03092464
P-3	0.771	0.796	0.776	0.781	0.01322876
P-4	0.685	0.694	0.693	0.690666667	0.00493288
P-5	0.799	0.781	0.761	0.780333333	0.01900877
P-6	0.779	0.822	0.796	0.799	0.02165641
P-7	0.674	0.691	0.688	0.684333333	0.00907377
P-8	0.792	0.799	0.784	0.791666667	0.00750555
P-9	0.622	0.612	0.623	0.619	0.00608276
P-10	0.701	0.713	0.711	0.708333333	0.0064291
Mean of total samples				0.734333333	0.01228841

Table 7. Optical density (OD) of fibroblasts adhesion on PEEK abutments samples.

Stainless steel samples	OD-well1	OD- well2	OD - well3	Mean	SD
St-1	0.044	0.048	0.049	0.047	0.00264575
St-2	0.046	0.041	0.042	0.043	0.00264575
St-3	0.041	0.055	0.058	0.051333333	0.00907377
St-4	0.059	0.056	0.051	0.055333333	0.00404145
St-5	0.049	0.047	0.048	0.048	0.001
St-6	0.057	0.056	0.056	0.056333333	0.00057735
St-7	0.048	0.047	0.042	0.045666667	0.00321455
St-8	0.047	0.044	0.047	0.046	0.00173205
St-9	0.052	0.056	0.054	0.054	0.002
St-10	0.055	0.051	0.053	0.053	0.002
Mean of total samples				0.049966667	0.00289307

Table 8. Optical density (OD) of fibroblasts adhesion on stainless steel abutments samples.

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Table 9a and 9b present the results of a statistical analysis comparing the optical density (OD) values, which represent greater cell adhesion and greater biocompatibility, across four different abutment materials: zirconium, PEEK (polyetheretherketone), titanium, and stainless steel. Zirconium had the highest mean rank (95.22), followed by titanium (85.78), PEEK (45.50), and stainless steel (15.50). The p-value of <0.001 indicates that the differences in OD values among the material groups are statistically significant.

Table 9a. Comparison of Optical Density (Cell Viability) Across Different Abutment Materials

	Material	Mean Rank	Sig
Optical Density	Zirconium	95.22	<.001
	PEEK	45.50	
	Titanium	85.78	
	Stainless Steel	15.50	

Table 9b. Comparison of Optical Density (Cell Viability) Across Different Abutment Materials

Null Hypothesis	Test	Sig.[a]	Decision
The distribution of Optical Density is the same across categories of Material.	Independent-Samples Kruskal-Wallis Test	<.001	Reject the null hypothesis.

a. The significance level is .050.

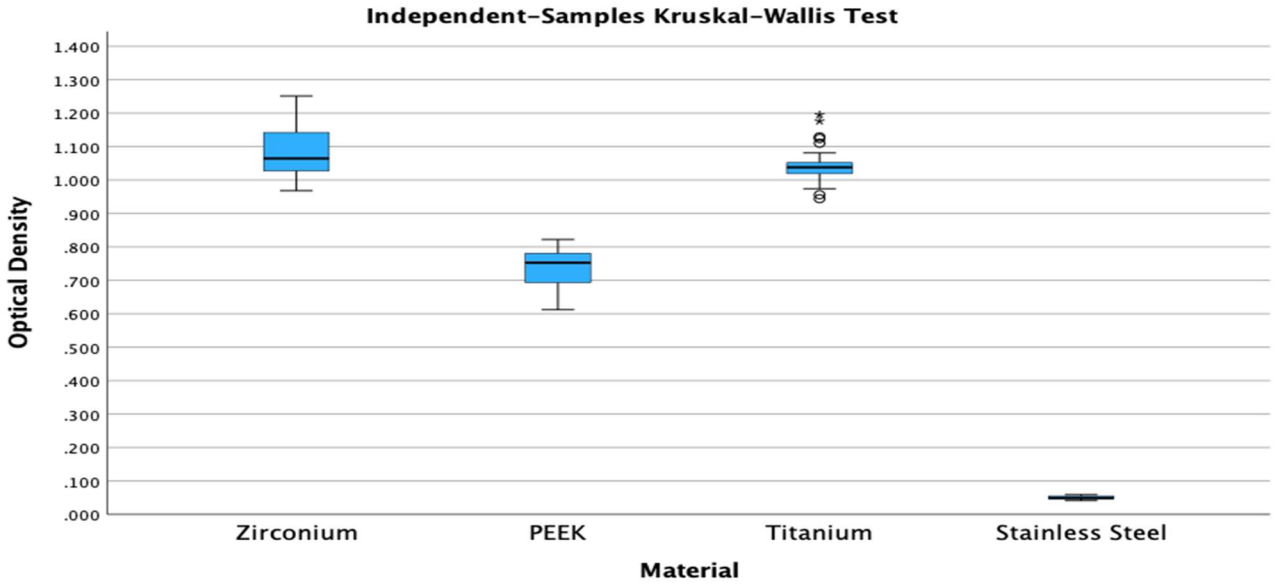

Figure 19. Boxplot for Optical Density Values according to material type.

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Table 10. presents the results of pairwise comparisons between the different abutment material groups, performed as a post-hoc analysis after the initial Kruskal-Wallis test. These comparisons aim to identify which specific pairs of materials exhibit statistically significant differences in optical density (OD) values, representing differences in biocompatibility.
Stainless steel exhibited significantly lower OD values compared to PEEK ($p = 0.005$), titanium ($p < 0.001$), and zirconium ($p < 0.001$), indicating poorer biocompatibility than these three materials
PEEK, while performing better than stainless steel, still had significantly lower OD values compared to titanium ($p < 0.001$) and zirconium ($p < 0.001$). This suggests that PEEK may not be as biocompatible as titanium and zirconium in terms of promoting gingival fibroblast adhesion and viability.
Notably, there was no statistically significant difference in OD values between titanium and zirconium ($p = 1.000$) after adjusting for multiple comparisons. Both materials exhibited comparable and the highest levels of cell viability among the tested materials, indicating similar biocompatibility and potential for providing an optimal peri-implant seal.

Table 10. Pairwise Comparisons of Optical Density (Cell Viability) Across Abutment Materials

Sample 1-Sample 2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj. Sig.[a]
Stainless Steel-PEEK	30.000	8.981	3.340	<.001	.005
Stainless Steel-Titanium	70.283	8.981	7.826	<.001	.000
Stainless Steel-Zirconium	79.717	8.981	8.876	<.001	.000
PEEK-Titanium	-40.283	8.981	-4.485	<.001	.000
PEEK-Zirconium	49.717	8.981	5.536	<.001	.000
Titanium-Zirconium	9.433	8.981	1.050	.294	1.000

Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same.
Asymptotic significances (2-sided tests) are displayed. The significance level is .050.
a. Significance values have been adjusted by the Bonferroni correction for multiple tests.

Figure 20. presents a graphical representation of the pairwise comparisons between the average ranks of four different materials: PEEK, zirconium, titanium, and stainless steel. The average ranks are displayed as numerical values next to each material name, with PEEK having the highest rank of 45.50, followed by zirconium (95.22), titanium (85.78), and stainless steel (15.50).The lines connecting the materials indicate the pairwise comparisons, with the line colors (blue or red) representing the statistical significance of the differences between the materials. Blue lines signify an adjusted significance value (Adj. Sig.) less than 0.05, indicating a statistically significant difference, while red lines represent an adjusted significance value greater than or equal to 0.05, suggesting no significant difference between the connected materials.

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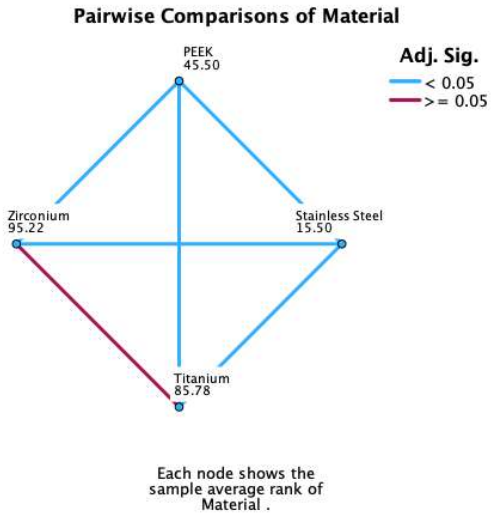

Figure 20. Pairwise Comparisons of Material Average Rank.

Discussion:

Methodology and Techniques Justification:

The findings of this study provide crucial insights into the interactions between various abutment materials and gingival fibroblasts, with significant implications for dental implantology.
This research aimed to determine how different abutment materials influence the attachment and subsequent tissue integration of human gingival fibroblasts, focusing on the comparison between titanium and alternative materials such as PEEK, zirconia, and stainless steel.
Surface Preparation Rationale: To reduce variability resulting from physical surface characteristics, the process of polishing for each material to produce uniform surface roughness was essential. Standardizing this feature focused on separating the impact of material composition on fibroblast attachment, shedding light on how surface chemistry and material characteristics affect cell behavior. Surface roughness is a critical factor in the cellular response to biomaterials.
Optical Density Measurement Explanation: In cellular biology, the use of optical density (OD) measurements to evaluate fibroblast attachment is a commonly utilized method. In order to measure the amount of associated cell growth on different surfaces, this method includes labeling the cells and measuring absorbance at particular wavelengths. It provides a trustworthy, quantitative assessment of the important markers of material biocompatibility, cell attachment and proliferation. The sensitivity and speed of optical density's results are especially beneficial for comparative studies such as ours.
In conclusion, the methods and approaches used in this investigation were created with the specific goal of thoroughly assessing and contrasting the biocompatibility of various implant materials. The objective of this research was to improve patient outcomes in implant procedures by directing material selection in clinical applications through the use of strong statistical analysis and control of critical experimental factors.

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Justification of each results:
The experiment's outcome, which measures optical density (OD) values across various wells for four implant abutment materials—zirconia (Zr), (PEEK), titanium (Ti), and stainless steel (St)—provides a quantitative basis for comparing fibroblast attachment and proliferation on these surfacres.

Analysis of Zirconia (Zr) Results: The results for zirconia display a range of OD values, with certain wells (Zr-3, Zr-7, Zr-8, Zr-9) exhibiting higher values (average OD over 1.1), indicating robust fibroblast attachment and proliferation. This variability across samples might be attributable to minor differences in surface treatment or intrinsic material properties. Zirconia is known for its good biocompatibility, and these results support its efficacy in supporting cellular activities necessary for successful implant integration.

Analysis of PEEK Results: PEEK shows generally lower OD values compared to zirconia, with averages mostly below 0.8, suggesting a less favorable environment for fibroblast attachment in this experiment. Despite this, PEEK still shows consistent results across the wells, supporting its known properties of biocompatibility and stability. The lower attachment levels could be due to surface characteristics not captured solely by polishing or inherent material properties influencing cell-material interactions.

Analysis of Titanium (Ti) Results: Titanium, consistent with its reputation as the material of choice for many implant applications, shows generally high OD values, with many results clustering around or above 1.0. Samples like Ti-2, Ti-5, and Ti-7 exhibit particularly high attachment, aligning with previous findings in literature that laud titanium's ability to enhance fibroblast proliferation due to its excellent surface characteristics and chemical stability.

Analysis of Stainless Steel (St) Results: Stainless steel shows the lowest OD values across all groups, with all results significantly below 0.1. These findings underscore the limitations of stainless steel in biological applications, likely due to ion release and potential cytotoxic effects, which inhibit fibroblast proliferation. This aligns with the shift away from stainless steel in clinical settings favoring materials with better biological integration.

Comparison and Implications: Titanium's superior performance in fibroblast attachment is well-documented in the literature and can be attributed to its excellent biocompatibility, corrosion resistance, and the unique ability to support osseointegration. Numerous studies have highlighted titanium's rough surface texture at the microscopic level, which may enhance protein adsorption and cell attachment (Buser et al., 1991) (16). The favorable results for titanium in this study confirm these characteristics, underscoring its suitability for dental and orthopedic implants where direct bone contact is crucial (16). Zirconia and PEEK also showed considerable fibroblast attachment, which supports recent trends in dental implantology that favor these materials for their aesthetic qualities and mechanical properties. Zirconia, in particular, has been praised for its high tensile strength and low thermal conductivity, making it an excellent material for implants (Özkurt & Kazazoğlu, 2011) (17). Although slightly behind titanium, the performance of zirconia in our study suggests that with further surface modifications, it could match or even exceed the biocompatibility of titanium. PEEK's results were comparable to zirconia, which is noteworthy given its increasing use in the medical field due to its excellent mechanical properties, chemical stability, and radiolucency (Toth et al., 2006) (18). However, the surface treatment

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of PEEK is critical for its performance, as its naturally hydrophobic surface can inhibit cell attachment (18). Future investigations could explore various surface modifications to enhance PEEK's bioactivity and fibroblast compatibility. Conversely, stainless steel demonstrated the lowest levels of fibroblast attachment among the materials tested. This result is consistent with earlier studies suggesting that stainless steel may release ions that could be cytotoxic or interfere with cell adhesion processes (Rahman et al., 2012) (19). The poor performance of stainless steel highlights its limitations for use in scenarios where long-term biocompatibility is critical, despite its widespread use due to mechanical properties and cost-effectiveness (19).

Future Directions in Material Innovation

The ongoing development of abutment materials should focus on optimizing the biocompatibility, surface roughness, and chemical properties to enhance soft tissue integration.

Further investigations could explore the biochemical surface modifications of PEEK and zirconia to enhance their biocompatibility further. Additionally, in vivo studies would be instrumental in validating these in vitro findings, providing a more comprehensive understanding of each material's performance in a dynamic biological environment. Understanding the specific interactions between cell types and material surfaces at the molecular level could also lead to innovations in implant surface treatments that enhance biocompatibility across all used materials.

Innovations in surface treatment technologies, such as argon plasma and UV light treatments, show promise in improving the attachment and proliferation of soft tissue cells around abutments. The exploration of these techniques could lead to the development of next-generation abutment surfaces that better support the biological requirements of peri-implant tissues.

This study's findings suggest that while all materials tested have potential applications in medical implants, their surface properties must be carefully considered and optimized for specific clinical applications. The significant variation in fibroblast attachment across materials underlines the importance of selecting the appropriate material based on the intended use and required biological interactions. Overall, the preferential attachment of fibroblasts to titanium confirms its status as the material of choice for many biomedical applications, while also highlighting the potential of zirconia and PEEK, provided that their surfaces are appropriately modified. Further comparative studies and developments in surface engineering will be crucial to optimize these materials for broader clinical applications.

Future Directions and Longitudinal Studies

Given the mixed outcomes observed with different materials and surface treatments, there is a clear need for longitudinal studies that can assess the long-term clinical outcomes associated with these abutments. It would be particularly useful to evaluate the performance of these materials in a clinical setting, where factors such as patient-specific responses and oral hygiene can significantly influence the success of dental implants.

Moreover, ongoing advances in materials science might lead to the development of new abutment materials that combine the best properties of both traditional and novel materials. For example, composite materials that integrate the mechanical strength and biocompatibility of titanium with the aesthetic qualities of ceramics could potentially overcome the limitations noted with single-material abutments.

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Limitation of the study:

- In Vitro Model Limitations: One primary limitation is the use of in vitro models to study cellular interactions with abutment materials. While in vitro experiments provide controlled conditions to understand basic cellular behaviors and material properties, they cannot fully replicate the complex biological and mechanical environments present in the human mouth. Factors such as saliva, blood, immune responses, and the dynamic loading of dental implants during chewing are not accounted for, which could influence the actual biological response in vivo.

- Lack of Long-term Data: The study likely focuses on short-term interactions between fibroblasts and abutment materials, typically over days. However, the long-term behavior of these interactions, crucial for understanding the success of dental implants over years, is not captured. Longitudinal studies in a clinical setting would be necessary to provide insights into the durability and stability of these materials over time.

- Limited Range of Materials and Surface Treatments: Although the study might include several commonly used materials like titanium, zirconia, PEEK, and stainless steel, the field of dental materials is vast, with continuous innovations. The study might not cover newer or less conventional materials that could offer enhanced biocompatibility or mechanical properties. Additionally, variations in surface treatments that could affect the results are often not comprehensively examined.

- Cell Type Specificity: The study focuses on human gingival fibroblasts, which are crucial for peri-implant health but do not represent all cell types involved in peri-implant biology. Other significant cell types, such as osteoblasts (bone-forming cells) and epithelial cells, play critical roles in implant integration and the formation of a biological seal. The exclusion of these cells from the study limits understanding of the complete peri-implant environment.

- Homogeneity of Samples**: The variability in the processing and preparation of abutment materials could introduce inconsistencies in the samples used for testing. Differences in manufacturing, polishing, and cleaning procedures can affect surface properties and thus the cellular response, potentially leading to variability in the results that isn't accounted for by the study design.

- Generalization to Clinical Settings**: The conditions under which the experiments are conducted might not adequately mimic the clinical conditions under which abutments are used. Factors such as patient-specific variations in oral hygiene, health status, and individual biological responses to implant materials can significantly influence outcomes. This makes it difficult to directly translate in vitro findings to clinical scenarios without further validation.

Addressing these limitations in future research could involve the development of more complex in vitro models, inclusion of additional relevant cell types, longer study durations, and eventually, clinical trials that can validate in vitro findings and provide comprehensive insights into the performance of different

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abutment materials in real-world settings.

Conclusion:

This study has provided valuable insights into the interaction of gingival fibroblasts with various dental abutment materials, highlighting critical aspects that influence the success of dental implants. Through rigorous in vitro experimentation, we observed that titanium and zirconia exhibit superior biocompatibility, supporting enhanced cell adhesion and proliferation, which are pivotal for establishing a robust peri-implant seal. These findings affirm the suitability of these materials for clinical use, providing a foundation for achieving optimal aesthetic and functional outcomes in dental implantology. Conversely, PEEK and stainless steel demonstrated lower levels of fibroblast viability and adhesion, indicating that while they may hold certain mechanical advantages, their biological integration is comparatively inferior. This discrepancy underscores the necessity for ongoing material innovations and surface treatments that could potentially elevate their biocompatibility to levels seen with more traditionally favored materials.
In conclusion, our research contributes to a deeper understanding of how different abutment materials interact with gingival tissues at the cellular level, offering a scientific basis for material selection in dental implantology. By continuing to bridge the gap between in vitro studies and clinical reality, we can better tailor dental implant treatments to meet the specific needs of patients, ultimately enhancing the longevity and success of dental implants in clinical practice.

Impact of the study:

- Aid healthcare professionals in choosing the superior implant abutment and determining its ability to adhere to gingival fibroblast for long-term success of implants and healthier peri-implant tissues.

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