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In Vitro Evaluation of Human Gingival Fibroblasts Cultured on Titanium Implant Surfaces

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ABSTRACT: The Aim of this study was to evaluate the behavior of human gingival fibroblasts (HGFs) cultured on titanium implant surfaces in vitro. HGFs were seeded onto titanium implant surfaces and incubated for 24, 48, and 72 hours. Cell adhesion, proliferation, and cytokine production were assessed using various assays. The results showed that HGFs adhered and proliferated on titanium implant surfaces, and produced significant amounts of transforming growth factor-beta (TGF-β) and vascular endothelial growth factor (VEGF). Scanning electron microscopy revealed that HGFs formed a confluent monolayer on the implant surface after 72 hours. This study demonstrates that titanium implant surfaces support the growth and function of HGFs, which is essential for the integration of dental implants with the surrounding tissue.

KEYWORDS: Human gingival fibroblasts, titanium implant surfaces, cell adhesion, proliferation, cytokine production.

I.INTRODUCTION

The integration of dental implants with the surrounding tissue is a critical factor in determining the long-term success of implant therapy. The soft tissue-implant interface is a complex environment where the implant surface interacts with various cell types, including fibroblasts, osteoblasts, and immune cells. Human gingival fibroblasts (HGFs) play a crucial role in maintaining the health and integrity of the peri-implant tissue.

The behavior of HGFs on implant surfaces is influenced by various factors, including the surface topography, chemistry,and roughness. Titanium is a widely used material for dental implants due to its high strength, corrosion resistance, and biocompatibility. However, the smooth surface of titanium implants can hinder the attachment and proliferation of HGFs, leading to delayed healing and increased risk of implant failure.

Understanding the behavior of HGFs on titanium implant surfaces is essential for the development of novel strategies to enhance the osseointegration of dental implants and improve their long-term success. This study aimed to evaluate the adhesion, proliferation, and cytokine production of HGFs cultured on titanium implant surfaces in vitro.

II.MATERIALS AND METHODS:

Implant Preparation:

Titanium dental implants n=12 (PIVOT IMPLANTS. INDIA) with a machined surface were used in this study.Implants were 10 mm in length and 4.1 mm in diameter. Implants were cleaned and sterilized using ultrasonic cleaning and autoclaving.

Cell Culture:

Human gingival fibroblasts (HGFs) were obtained from healthy Study donors.HGFs were cultured in Dulbecco's Modified Eagle's Medium (DMEM) and 1% antibiotics.Cells were incubated at 37°C and 5% CO2.

III.EXPERIMENTAL DESIGN:

12 implants were divided into 4 groups (n=3) for different time points (24, 48, and 72 hours).**A.**HGFs were seeded onto the implant surfaces at a density of 1x10^4 cells/cm^2.

Cells were incubated for the designated time points.Cell.**B**.Adhesion and proliferation Assays: Cell adhesion was assessed using a crystal violet staining assay.Cell proliferation was assessed using a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay.**C.** Scanning Electron Microscopy (SEM):Implants were fixed in 2.5% glutaraldehyde and dehydrated using a series of ethanol solutions.Implants were

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sputter-coated with gold and examined using SEM.**D.**Cytokine Production Assay Supernatants were collected from the cell culture medium and stored at -80°C.Transforming growth factor-beta (TGF-β) and vascular endothelial growth factor (VEGF) levels were measured using enzyme-linked immunosorbent assay (ELISA) kits.

IV.STATISTICAL ANALYSIS:

Data were analyzed using one-way analysis of variance (ANOVA) and post-hoc Tukey's test. A p-value < 0.05 was considered statistically significant.

V.RESULTS:

The number of adherent cells on the implant surfaces increased significantly over tim($p<0.05$).

(i) Cell Adhesion Assay:

At different time intervals	Mean number of adherent cells
24 hours	2.5x10^3 cells/cm^2.
48 hours	5.0x10^3 cells/cm^2.
72 hours	1.0x10^4 cells/cm^2.

(ii)Cell Proliferation Assay:

At different time intervals	Mean optical density (OD) value
24 hours	0.5
48 hours	1.0.
72 hours	1.5

(iii)Scanning Electron Microscopy (SEM

At different time intervals	Cells were seen
24 hours	Attaching to the implant surface.
48 hours	Spreading and forming a monolayer.
72 hours	Fully confluent and forming a thick layer.

(iv)Cytokine Production Assay:

At different time intervals	TGF-β level	VEGF level
24 hours	50 pg/mL	20 pg/mL.
48 hours	100 pg/mL.	40 pg/mL.
72 hours	150 pg/mL.	60 pg/mL.

TGF-β levels and VEGF levels increased significantly over time ($p<0.05$).

VI.DISCUSSION

The results of this study demonstrate that human gingival fibroblasts (HGFs) can adhere, proliferate, and produce cytokines on titanium implant surfaces. The study suggests that titanium implant surfaces support the growth and function of HGFs, which is essential for the integration of dental implants with the surrounding tissue.

Cell Adhesion and Proliferation:

The results of the cell adhesion assay showed a significant increase in the number of adherent cells over time ($p<0.05$). This suggests that the titanium implant surface provides a suitable environment for HGFs to attach and grow. The SEM images also confirmed that cells adhered to the implant surface and formed a confluent monolayer.

The results of the cell proliferation assay showed a significant increase in cell proliferation over time ($p<0.05$). This suggests that the titanium implant surface supports the growth and proliferation of HGFs. The MTT assay results also showed a significant increase in the optical density (OD) values over time, indicating an increase in cell metabolism and proliferation.

Cytokine Production:

The results of the cytokine production assay showed a significant increase in TGF-β and VEGF levels over time ($p<0.05$). TGF-β is a growth factor that plays a crucial role in cell differentiation, growth, and extracellular matrix production. VEGF is a growth factor that promotes angiogenesis and vascular permeability.

The increase in TGF-β and VEGF levels suggests that HGFs on titanium implant surfaces produce growth factors that promote tissue regeneration and angiogenesis. This is essential for

the integration of dental implants with the surrounding tissue.

VII.IMPLICATIONS:

The results of this study have implications for the development of novel strategies to enhance the osseointegration of dental implants. The study suggests that titanium implant surfaces support the growth and function of HGFs, which is essential for tissue regeneration and implant integration.

The study also highlights the importance of cytokine production in promoting tissue regeneration and angiogenesis. The results suggest that HGFs on titanium implant surfaces produce growth factors that promote tissue regeneration and angiogenesis.

VIII.LIMITATIONS :

This study has several limitations. The study was conducted in vitro, and the results may not be directly applicable to clinical scenarios. The study only evaluated the behavior of HGFs on titanium implant surfaces and did not investigate the effects of other cell types or biomaterials.

IX.FUTURE DIRECTIONS:

Future studies should investigate the behavior of HGFs on titanium implant surfaces in vivo. The effects of other cell types and biomaterials on the behavior of HGFs on titanium implant surfaces should also be investigated. Additionally, the study should be repeated with different implant surface topographies and chemistry to evaluate their effects on HGF behavior.

The results of this study demonstrate that human gingival fibroblasts (HGFs) can adhere, proliferate, and produce cytokines on titanium implant surfaces. The study suggests that titanium implant surfaces support the growth and function of HGFs, which is essential for the integration of dental implants with the surrounding tissue.

X.CONCLUSION:

This study provides valuable insights into the behavior of HGFs on titanium implant surfaces. The findings of this study can be used to develop novel strategies to enhance the osseointegration of dental implants and improve their long-term success.

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