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DIFFERENTIAL PROTEIN EXPRESSIONS ASSOCIATED WITH DENTAL IMPLANT HEALING

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ABSTRACT

Purpose of the research- Dental implants' topographical features are critical in regulating the healing process and, consequently, the production of proteins involved in multiple stages, from the initial inflammatory response to the following remodelling of the bone. Improved bone-to-implant contact and favourable cellular responses have been shown when a rough implant surface is present. But to get the appropriate level of roughness, additional procedures are usually required, which raises the total cost of dental implants. Using metal injection moulding technology, a porous dental implant composed of nickel titanium (NiTi) has been produced successfully. This novel method makes it unnecessary to make additional surface alterations, making it a more economical alternative. However, further research needs to be done to determine which protein expressions are associated with the faster healing of porous implants, as shown by an earlier study in an animal model. The objective of this investigation was to identify and compare the proteins that are produced during the healing process in dental implants, both with and without porous properties. **Methods-** The study comprised a total of fourteen participants, with seven participants receiving the NiTi dental implant and seven participants receiving the Mega Gen dental implant (control group). Saliva samples were obtained at three distinct time intervals: prior to implantation (T0), one-week post-implantation (T1), and eighteen weeks' post-implantation (T2). The proteins involved in bone regeneration were analyzed in saliva using liquid chromatography-mass spectrometry (LC-MS, Model 6520-Accurate Mass Q-TOF). **Results-** Gene ontology analysis revealed 15 proteins associated with osseointegration in the MegaGen group, detected at various time points (T0, T1, and T2). These proteins included Pancreatic alpha-amylase (T0, T1, T2), Fibronectin (T0), Putative POU domain, class 5, transcription factor 1B (T0), Titin (T0, T2), Cellular tumor antigen p53 (T1, T2), Neuroserpin (T1), Collagen alpha-3(VI) chain (T1), Dystonin (T1), Ubiquitin carboxyl-terminal hydrolase 19 (T1), basement membrane-specific heparan sulfate proteoglycan core protein (T2), EH domain-containing protein 1 (T2), myosin-11 (T2), TLR4 interactor with leucine-rich repeats (T2), Talin-1 (T2), and Thrombospondin-1 (T2). In the NiTi group, 7 proteins were identified, including Fibronectin at T0 and T2, Pancreatic alpha-amylase at T0, T1, and T2, Titin and Hepatocyte growth factor at T0, T1, and T2, Zinc finger and BTB domain-containing protein 40 at T1, Endothelin-1 at T2, and Fibrillin-2 at T2. **Conclusion-** The proteins associated with the MegaGen group offer a detailed elucidation of the osseointegration process, particularly during the phases of cell proliferation and bone remodelling. The proteins associated with the NiTi group may have a link not only to osseointegration but also to inflammation.

Keywords: Dental implants, bone healing, proteins, saliva, biomarkers.

INTRODUCTION

Dental implants have been used to replace missing teeth since the 1960s. However, due to their unparalleled level of effectiveness and convenience, their use has become much more widespread in recent years, and it is estimated that one million dental implants are placed worldwide each year (1). This is because dental implants are now regarded as the gold standard in tooth replacement. The process of osseointegration, also known as bone to implant contact (BIC), is critical to the success of dental implants. This procedure starts with the surgical placement of the implant and continues through the healing process as well as the continuous loading of the implants while they are in use. Osseointegration is only considered successful if new bone forms on the implant's surface. During the contact osteogenesis process, osteogenic cells in various stages of differentiation are continuously recruited and migrating to the implant site.

The purpose of this study, which was part of a larger clinical trial on a novel porous Nickel Titanium dental implant, was to look into the impact of the implant's porous surface on the osseointegration process. It would be beneficial to identify the biomarkers involved in the osseointegration of this novel porous implant because research has shown that different surface characteristics can elicit different cellular responses (2,3,4). We anticipate seeing some differences in the biomarkers involved, particularly in the early stages of the healing process, because the animal study showed that this porous implant enhances osseointegration (5).

Homeostasis, granulation tissue production, and bone remodelling have all been shown to be critical components of the osseointegration process. These processes have the potential to initiate the production of cytokines that promote inflammation, inflammatory cell infiltration, osteogenic cell activation, and immune cell stimulation. As a result, each stage of the osseointegration process stimulates the release of healing-related factors that have yet to be fully identified and understood. These factors linked to the healing process have the potential to be used as clinical biomarkers. As a result, more molecular studies are needed to provide a clear explanation of the molecular mechanism underlying osseointegration.

MATERIAL AND METHOD

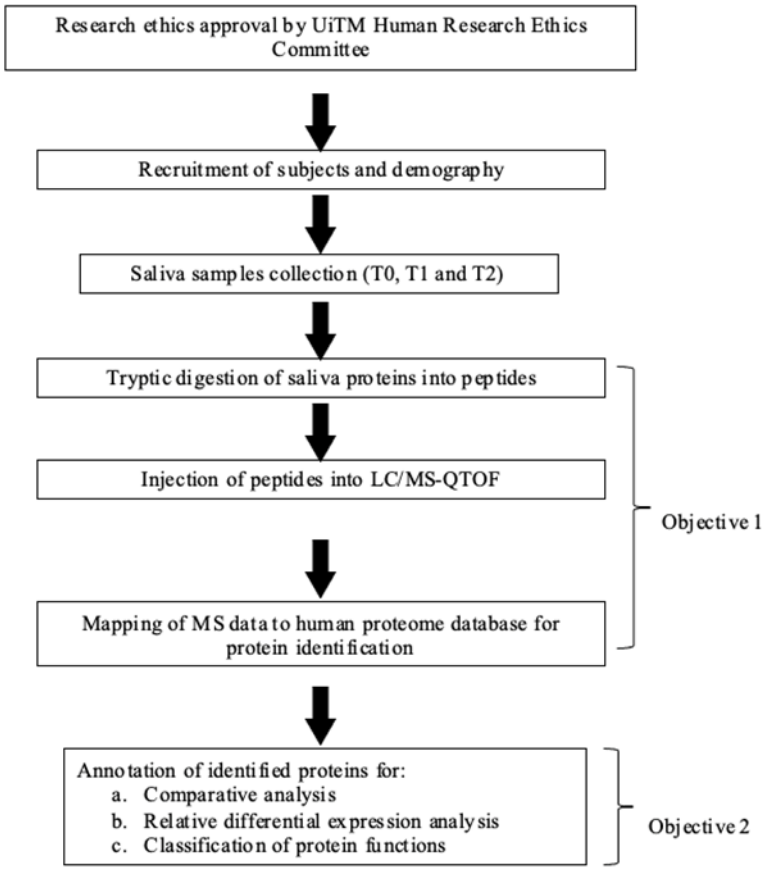


Figure 3.1: A flowchart summarizes the methodology used in this study.

Saliva samples were taken from participants in a parallel, single-blinded, superiority clinical trial (registered under ClinicalTrial.Gov (U.S. National Library of Medicine, ID:NCT04618055)) following ICH GCP, ISO 14155:2011 guidelines.

This study was conducted at the Faculty of Dentistry, Sungai Buloh and Integrative Pharmacogenomics Institute (iPromise), Puncak Alam Campus. 14 individuals received a single implant in the mandibular posterior region: 7 received porous NiTi and 7 received MegaGen Anyridge implants from February 2022 to April 2023.

Three saliva samples were taken before, one week after, and 18 weeks after implant installation. MegaGen was selected as the control group due to their recent entry into the implant market and the absence of comprehensive performance research. Moreover, MegaGen implants were notably affordable compared to other dental implants in Malaysia, and the NiTi implant manufacturers intended to provide their product at a comparable price to MegaGen implants.

Subject recruitment was open to individuals of both genders who are 18 years or older and have good dental hygiene. This study excludes individuals who are medically compromised, smokers, individuals with a history of drug abuse or alcoholism, individuals with severe periodontal disease, individuals who have had unsuccessful implant treatment, individuals undergoing chemotherapy or radiotherapy, and pregnant women.

RESULTS AND DISCUSSION

RESULT

As for the result, 42 saliva samples were collected for testing. The majority of the participants were Malay (85%), female (93%), and aged 44 to 64 years old, with a mean age of 52 years old. The demographic data for both groups were very similar.

Comparative Analysis of Peptide Profiles

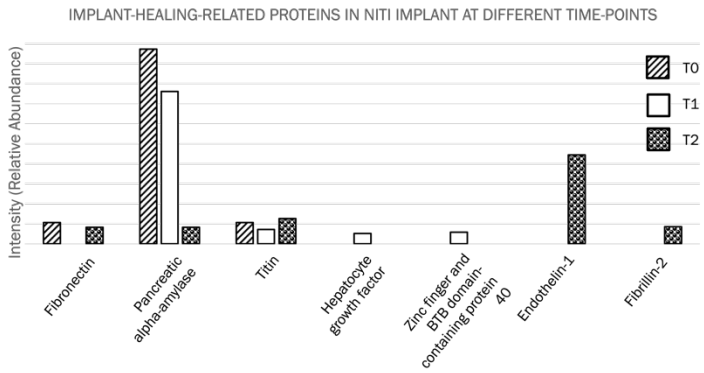
Chromatograms show the relative concentration and intensity of peptides in porous and non-porous dental implants throughout time. These various chromatographic patterns may indicate different protein expression profiles or protein copy numbers across timepoints. T0 and T1 show that non-porous implant peptides are more abundant than porous implant peptides. This suggests different protein expression kinetics at these stages. The chromatograms show a significant increase in peptide abundance in the porous implant group compared to the non-porous implant group in T2. This change in peptide intensity may indicate a major change in protein expression or copy numbers during healing.

Protein Identification and Functional Annotation

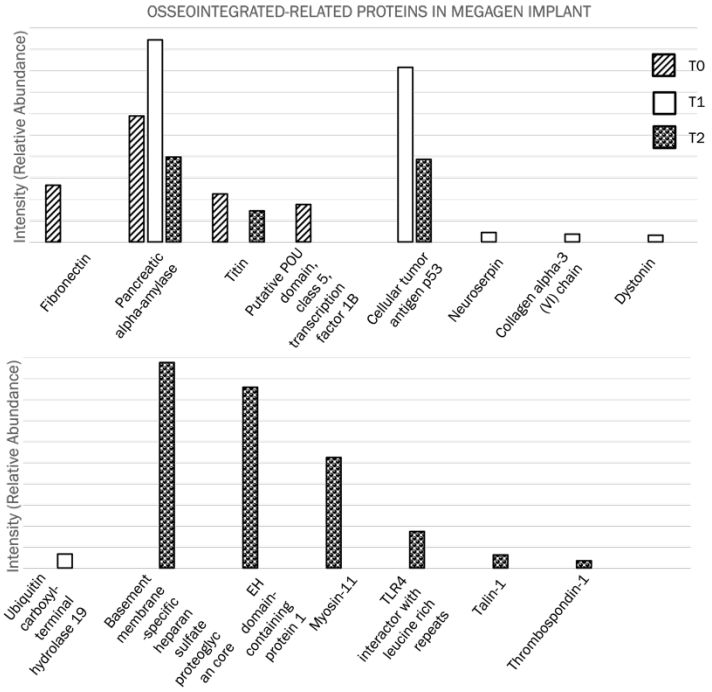
This research discovered proteins, each with unique proteome profiles, highlighting the dynamic nature of protein expression during dental implant healing. In particular, 17 proteins were consistently expressed at all timepoints, but different protein combinations were coexpressed at different times. Using PANTHER webtools for protein classification revealed a key difference: MegaGen implant patients have a wider range of proteins involved in many biological processes than NiTi implant patients. This protein involvement discrepancy suggests different biological pathways. The SalivaDB database was used to compare the proteins. Of the proteins discovered, 13.21% proteins matched SalivaDB entries.

Protein Annotation: Comparative Analysis

MegaGen implants detected 8.14% salivary proteins, while NiTi implants found 6.30%. NiTi discovered 7 implant healing-related proteins after functional annotation, with the first 3 proteins also found in MegaGen. Different regulatory patterns defined these proteins. Fibronectin downregulated from T0 to T2, pancreatic alpha-amylase consistently downregulated, and Titin downregulated from T0 to T1 and upregulated from T1 to T2. Hepatocyte growth factor and Zinc finger and BTB domain-containing protein 40 were exclusively expressed at T1 in NiTi implants, while Endothelin-1 and Fibrillin-2 were expressed at T2.



MegaGen discovered 15 osseointegration-associated proteins with diverse regulation patterns. Pancreatic alpha-amylase upregulated during T0 to T1 and downregulated during T1 to T2. Both fibronectin and Putative POU domain, class 5, transcription factor 1B were only expressed at T0. Cellular tumour antigen p53 downregulated from T1 to T2, while Titin consistently downregulated from T0 to T2. Neuroserpin, Collagen alpha-3(VI) chain, Dystonin, Ubiquitin carboxyl-terminal hydrolase 19 were exclusively expressed at T1 while Basement membrane-specific heparan sulfate proteoglycan core protein, EH domain-containing protein 1, Myosin-11, TLR4 interactor with leucine-rich repeats, Talin-1, Thrombospondin-1 were expressed at T2.



DISCUSSION

This dental implantology study uses salivary proteomics to analyse osseointegration, a pioneering effort (6,7,8,9). This work tracks healing during osseointegration, unlike previous research that examined salivary proteome alterations at a single timepoint. T1 proteins reflect the inflammatory to proliferative phase, while T2 proteins show healing maturation. No other study has provided as complete and dynamic salivary proteome data for dental implant operations, which enhances our understanding of implant healing dynamics.

Several proteins showed unexpected patterns with NiTi implants. Pancreatic alpha-amylase was detectable at all three timepoints but decreased. This protein may affect bone repair and calcium binding. Throughout recovery, striated muscle structural protein titin was constantly found. Fibronectin, hepatocyte growth factor, zinc finger and BTB domain-containing protein 40, endothelin-1, and fibrillin-2 were also connected to bone healing but only found once. Cell adhesion, collagen binding, osteoblast development, bone mineralization, cell population proliferation, and bone trabecula creation are among their roles (10).

The salivary proteomes of participants who received MegaGen and NiTi implants differed significantly due to variations in implant surface material and osseointegration outcomes. The paucity of research on salivary proteins and MegaGen osseointegration renders comprehension challenging. Within the MegaGen group, there are variations in the levels of pancreatic alpha-amylase, which can potentially impact the process of bone repair and the binding of calcium (11). The levels of T0 and T2 titin decreased without a definitive correlation to bone regeneration. The levels of cellular tumour antigen p53 decreased from T1 to T2, potentially because it plays a role in inhibiting DNA synthesis following DNA damage. This decrease may not have an impact on bone repair. At T1, active bone healing resulted in the production of neuroserpin and collagen alpha-3(VI) chain. The proteins dystonin, ubiquitin carboxyl-terminal hydrolase 19, myosin-11, TLR4 interactor with leucine-rich

repeats, talin-1, and thrombospondin-1 were found only at T1 or T2, indicating that they play a role in the process of bone healing and osseointegration. Further investigation is required to ascertain their precise functions.

This study provides a database of MegaGen dental implant patients' saliva protein expressions during two critical healing periods. Future research could compare these protein profiles to those of other calcium-coated implants to gain a better understanding of the molecular mechanism that promotes osseointegration in calcium-coated implants. The atypical protein expressions observed in the NiTi group may suggest the presence of metal contamination resulting from surgical drills, which could potentially lead to complications in osseointegration. This study emphasizes the potential of saliva as a diagnostic tool for assessing the healing process of dental implants, which could have a significant impact on the field of dental implantology.

CONCLUSION

We believe this is the first proteomics work to assess saliva proteins at several timepoints after dental implant therapy, especially with varied implant types. Protein profiles differ from pre-implant treatment (T0) to 1 week (T1) and 18 weeks (T2) after implant implantation. Surgical drill metal contamination may affect seven implant-healing proteins in NiTi implants. In MegaGen implants, 15 proteins were linked to osseointegration phases. The unique protein profiles of each implant group reveal their healing processes and lay the groundwork for future research. They enable clinical biomarker research and dental implant surface coating development to expedite implant healing.

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