Original Article

INTERACTIONS OF TITANIUM DIOXIDE WITH THE PERI-IMPLANT MICROBIOME, AN ANALYSIS OF MOLECULAR MECHANISMS - USING IN SILICO VALIDATION TOOLS

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Abstract

Background: Titanium dioxide on the implant surface and/or in the peri-implant tissue might have an influence on the local microflora, the aim of the present study is to understand the molecular mechanisms involved in the interactions between titanium dioxide and peri implant microflora that could lead to the better understanding of peri implantitis.

Objectives: To understand the interactions between titanium oxide and peri-implant microbiome and its influence on peri implantitis.

Methods: the microbes for the present in-vitro observational study were obtained by screening available literature about peri-implant pathogens, the Search Tool for Chemical Interactions (STITCH v5) was used to study titania protein interactions; VirulentPred, VICMpred tools were used to obtain the virulence potential and functional class of the proteins, following which subcellular localization of the virulent protein and epitope prediction done using the tools GNeg-mPLoc and BepiPred v2.0.

Results: Titanium dioxide was found to interact with proteins involved in cellular process, metabolism, and interestingly virulence proteins were also targeted, on further analysis most of these factors were localised to be present in the cytoplasm of the pathogen and finally epitope prediction revealed multiple epitopes in the virulent proteins, these predictions indicate the potential inhibitory effect of titanium dioxide of peri-implant pathogen and specific epitope that can act as a target for future drugs in management of peri-implantitis.

Conclusion: From the entire in silico assessment of the interaction between titanium oxide and virulent proteins revealed that titania has the propensity to modify the peri implant microflora, and this potential of this metallic element should be explored further for clinical management of peri implantitis.

Keywords: Titanium dioxide, periimplantitis, antibacterial, In-Vitro Prediction, titanium dioxide.

Introduction

Dental implant therapy has reported high survival rates, about 96.4% (Moraschini et al 2015) to 99% (Van Velzen et al 2015) over a period of 10 years . This has encouraged clinicians to consider dental implants as a valid option to treat edentulous patients. Implants historically have been made with noble metals like gold and platinum, and more recently ceramic implants are into play but the most reports on success rate of implant therapy is attributed to the titanium which is commonly used to fabricate the dental implant device due to its innate biocompatible property and has been modified to incorporate other elements and surface characterization to improve osseointegration(Gaviria et al 2014). Considering all the benefits, there still happens to be limitations to any treatment modality and one of the common post-implant placement complications is peri implantitis(Alberktsson & Isidor 1994), which is be defined as an inflammatory process around an implant that includes the loss of both soft tissue and supporting bone most commonly peri-implantitis (Schwarz et al 2018), In a retrospective analysis of 588 patients who have received 2277 implant concluded the prevalence rate of periimplantitis was at 45% (Derks et al 2016), the patient is usually unaware of the presence of this disease due to absence of pain in most of the cases and it is noted by the dentist only when the patient reports for recall properly, an increasing probing depth, suppuration, draining sinuses, and peri-implant mucosal swelling and/or recession are considered as common presenting signs of this pathology if present (Mombelli et al 2012).

It has been observed that patients who have multiple implants, when they lost one implant due to peri-implant disease the risk of losing the other implants due to the same cause increases multifold times(Hutton et al 1996). Peri implantitis is known to have a wide array of causative factors and it has been proposed that bacteria that cause periodontal breakdown could migrate and colonise peri-implant sites, resulting in tissue breakdown, to substantiate this sequencing studies (Sanz - Martin et al. 2017) have reported that peri-implantitis sites were heavily colonised by red complex species as well as newly proposed pathogens and observed the peri-implant site which was pathogen enriched and commensal depleted. In addition to the microbial influence, it has been noted that titanium corrosion products, titanium dioxide to be specific are said to have a role in peri-implant disease, greater levels of titanium were detected in submucosal plaque around implants with peri-implantitis when compared to healthy implants (Safioti et al 2017). However, according to recent studies, metal oxides are proposed to have antibacterial effects (Khan ST et al. 2015) (Vargas Reus et al. 2012). In order to add proof to this assumption that titanium dioxide has an antibacterial effect (Daubert D et al. 2018), this in - silico validation study is done and it aims to highlight in brief the interactions between titania and microbes on the peri implant surface which is commonly attributed to the inflammatory disease, carried out using available databases of known and predicted interactions between chemicals and proteins and B- Cell epitope prediction tool.

Materials and Methods

Study Design

This study is set to follow an observational design that aims to screen for proteins or virulence factors of Aggregatibacter actinomycetemcomitans (D7S-1), Bacteroides fragilis (ATCC 25285), Campylobacter gracilis (RM 3268), Fusobacterium nucleatum (ATCC 10953), Porphyromonas gingivalis (ATCC 33277), Treponema denticola (ATCC 35405), Slackia exigua (ATCC700122) and Tannerella forsythia (ATCC 43037) with titania, the interaction of titania with the proteome of bacteria was analysed using STITCH v.5 and the virulence properties of the interacting proteins were deduced using VICMPred and VirulentPred software. The given bacterial strains were included in the STITCH database and analysis was done using a user-defined query.

Prediction of bacterial protein and metal oxide interactions

STITCH database (Version 5) is an open-source platform with an exhaustive collection of data about interactions both physical and functional associations made possible by computational prediction of interactions from primary databases (Szklarczyk D et al. 2016), the repertoire of proteins which interacts with A. actinomycetemcomitans

(D7S-1), B. fragilis (ATCC 25285), C. gracilis (RM 3268), F. nucleatum (ATCC 10953), P. gingivalis (ATCC 33277), T. denticola (ATCC 35405), S. exigua (ATCC700122) and T. forsythia (ATCC 43037).

Virulence prediction:

VICMpred and VirulentPred pipelines were used for the identification of virulence factors targeted by titania among the mentioned peri-implant pathogens, these tools employ Support Vector Machine based five-fold cross-validation process to corroborate the results (Saha S et al 2006). The virulence factors were screened based on amino acid composition using VirulentPred which categorises them into virulent and avirulent. VICMPred tool on the other hand aids in functionally assorts the proteins into the cellular process, metabolism, information storage, and virulence. The amino acid sequence of the interacting protein seen in the STITCH tool is obtained from the NCBI protein database and was used to run the virulence prediction algorithms.

Prediction of subcellular localization of the virulent protein:

Subcellular localization of proteins helps in the identification of drug targets and could serve as a potential target for new medicines (Yu NY et al 2010), cell surface proteins are of great interest as they can be used as vaccine targets.GNeg-mPLoc is an algorithm that assigns a probable localization site to a protein from an amino acid sequence provided (Shen HB et a;. 2010).

Prediction of B – Cell epitopes in the virulent proteins:

The BepiPred-2.0 server predicts B-cell epitopes from a protein sequence, using a Random Forest algorithm based on epitopes and non-epitope amino acids determined from crystal structures (Jespersen MC et al 2017). The residues with scores above the threshold (> 0.5) are predicted to be part of an epitope and colored in yellow on the graph .

Results:

The STITCH v5 tool was utilised to visualise the interaction between the microbe and element of interest, the protein target derivatives of the reactions were further processed with algorithms of VICMpred and Virulentpred to categorise the outcomes as virulent and avirulent.

Titanium dioxide – pathogen interactions

Titanium dioxide as a molecule was found to react with proteins involved with cellular metabolism, and cellular processes. It is interesting to observe that the element of interest also interacted with virulence factors of the peri-implant pathogens which were most commonly enzymes glutaminase, glutamine synthetase, and superoxide group of enzymes.

In addition to these predictions, the subcellular localization of the ten virulent factors and epitope analysis was also carried out and most of the proteins targeted were found to be present at the cytoplasm – periplasm compartment.

Pathogen-specific interactions with titanium dioxide have been listed below,

1. Titania – interactions with A. actinomycetemcomitans

A total of seven interconnected interactions were observed, the interaction with Glutamine synthetase type I, which happened, was predicted as a virulent factor in VirulentPRED.

2. Titania – interactions with B. fragilis

A total of nine interconnected interactions were observed, the interaction was with glutamine synthetase, glutaminase, and superoxide dismutase, which were predicted to be a virulent factor in VirulentPRED.

3. Titania – interactions with C. gracilis

A total of ten interconnected interactions were observed, the interactions with antioxidant aphC family and glutamine synthetase type I, which were predicted to be virulent factors in VirulentPRED.

4. Titania – interactions with F. nucleatum

A total of four interconnected interactions were observed, the oxide seems to have an interaction with glutaminase A, which was detected to be a virulent factor in VirulentPRED.

5. Titania – interactions with P. gingivalis

A total of five interconnected interactions were observed, the interaction with superoxide dismutase which happened to be detected as a virulent factor in VirulentPRED was seen.

6. Titania – interactions with T. denticola

A total of four interconnected interactions were observed, the interaction with peroxiredoxin and alcohol dehydrogenase, which was detected to be a virulent factor in VirulentPRED.

7. Titania – interactions with S. exigua

A total of four interactions were observed, the element's most common reactions were with proteins involved with metabolic and cellular process pathways, the oxide seem to have an interaction with Glutamine synthetase type I and alkyl hydroperoxide reductase, which happened to be detected as a virulence factor in VirulentPRED, other proteins were predicted to be avirulent.

8. Titania – interactions with T. forsythia

A total of six interconnected interactions were observed, the element most commonly reacted with proteins involved with metabolic and cellular process pathways, the oxide seem to have an interaction with glutaminase A, which was detected to be a virulent factor in VirulentPRED, other proteins were predicted to be avirulent.

Discussion

A cause and effect relationship between peri-implant biofilms and peri-implant mucositis has been observed (Pontoriero R et al.1994), the microflora on initial examination was thought to be similar to that of periodontitis more specifically the red complex bacteria (Heitz Mayfield et al. 2000), initial colonization of peri - implant surfaces by bacteria can occur in a matter of 2 weeks (Quirynen M et al 2006) and reports state that there is a difference in the total bacterial count between implants affected by the disease compared to that of healthy peri-implant tissue (Tallarico M et al 2017) and the microflora is distinct than that of periodontitis (Kotsakis et al 2021) . More recently due to improved sample processing techniques investigations revealed that the peri-implant biofilm is a complex ecosystem comprised of mixed, rather variable and in most cases dominated by gram negative anaerobic bacteria (Mombelli et al. 2011) (Ata-Ali et al. 2011)(Charalampakis G et al 2015), Based on available evidences the most common species found to be present are Porphyromonas gingivalis, Tannerella forsythia, Treponema denticola, Aggregatibacter actinomycetemcomitans, Prevotella intermedia, Fusobacterium nucleatum, Campylobacter species, and Bacteroides species (Zhuang LF et al 2016)(Perez - Chaparro et al. 2016) (Sanz-Martin I et al 2017), thus these strains were considered for this study. No metal or metal alloy is completely inert in vivo because of constant contact with tissues, body fluids which inturn acts as a source for electrochemical interactions and, mechanical loading of the implant leads to loss of ions by friction and electrochemical exchange, this process is referred to as biotribocorrosion (Mathew MT et al. 2009), it has also been suggested that long standing accumulation of biofilms and mechanical strain causes implant surfaces to deteriorate (Rodrigues DC et

al. 2013) and the ability to implant surface to re-passivate also tends to reduce as the inflammatory response (Mathew MT et al.2012) and mechanical wear persists (Bhola R et al. 2011)(Berbel et al. 2019). Presence of high levels of dissolved titanium was detected in submucosal plaque around implants when compared to intervention free sites, thus indicating an association between Ti dissolution and peri-implantitis (Safioti LM et al. 2016).The oxide corrosion products were found in newly formed trabecular bone and peri-implant vasculature and systemically distributed, the oxide particles tend to be cytotoxic having an effect on the cells of immunity and it has been noted that smaller the particle size greater the toxicity (Kumazawa R et al. 2002) (Noronha Oliveira M et al. 2018)(Messous et al. 2021), this particle also has an influence on host immunity causing activation of macrophage and consequently IL-1β release (Pettersson et al 2017) the cascade of events lead to osteoclastogenesis and osteolysis (Carmody EE et al. 2002) (St.Pierre CA et al 2010). Other workers have observed peri implantitis to be present in situations where the microbial threat is removed or under control through frequent supportive measures, there is evidence which proposes that titanium oxide debris causes immuno modulatory changes which bring about degenerative changes in osseous and periodontal tissues, this is because of the fact that immune cells around the implant that is the polymorphonuclear neutrophils, macrophages and monocytes recognize the implant as a foreign body, and release various signalling molecules such as reactive oxygen species, IL-8, TNFα, IL-6, IL-4, IL-10 which in turn affect the osteogenic capacity of the osteoblasts that adhere to that material surface(Vasconcelos DM et al 2016), it has been proposed that surfaces roughness of the implant has a significant immunomodulatory effects and that the macrophages tended to polarise towards a classical M1 phenotype which upon activation are known to secrete high levels of proinflammatory cytokines (Li X et al 2018) (Billing F 2021). However it is interesting to note that TiO2 has a continuous photocatalytic antimicrobial activity against pathogens (Suketa et al. 2006) (Ryo Shirai et al. 2016),this metal oxide alone or in combination with other metals like silver, copper or zinc is shown to have antimicrobial property and the same has been explored to a lesser extent (Khan ST et al. 2015), In the present study we observe a good number of interactions between TiO2 and common peri-implant pathogens the target was mostly enzymes involved in cellular nitrogen metabolism which inturn brings about alteration in protein synthesis hindering the ability of bacteria cause virulence , thus it can be taken that titanium modifies peri implant microbiome and has potential antibacterial activity much light has to be shed on this aspect and the same would be clinically useful in management of peri implant disease by modifying implant surfaces or and we can deduce that peri implantitis is a complex disorder which has multifactorial causation, and more experimental exploration on this aspect to be carried out to produce effective treatment outcomes.

Conclusion

Peri implantitis is a major concern which influences the success rate of dental implants, however effective the treatment plan be the occurrence of this inflammatory disease is unavoidable at times, from this observation we draw to a conclusion that titanium surface indeed undergoes degenerative changes and the by products of this process that is the titania has the potential to modify the peri implant microflora by interacting with the their metabolic processes and could potentially aggravate the auto-immune response that one could expect when a foreign object is inserted into the living system,

Bacteria	Virulent Protein	Location	Score
A. actinomycetemcomitans	**D7S_02015** (Glutamine synthetase, type I)	Cytoplasmic	9.97
B. fragilis	**BF2343** (Glutamine synthetase) **glsA** (Glutaminase) **sodB** (Superoxide dismutase)	Periplasmic Cytoplasmic Periplasmic	9.80 9.26 9.44
C. gracilis	**CAMGR0001_0503** (Antioxidant aphC family) **glnA** (Glutamine synthetase type I)	Cytoplasmic Cytoplasmic	9.26 9.97
F. nucleatum	**glsA** (Glutaminase A)	Cytoplasmic	9.97
P. gingivalis	**Sod** (Superoxide dismutase)	Periplasmic	9.44
T. denticola	**TDE_111** (Peroxiredoxin) **TDE_2512** (Alcohol dehydrogenase)	Cytoplasmic Cytoplasmic	9.26 9.97
S. exigua	**HMPREF0762_01947** (Glutamine synthetase, type I) **HMPREF0762_01621** (Alkyl hydroperoxide reductase)	Cytoplasmic Cytoplasmic	9.97 9.97
T. forsythia	**glsA** (Glutaminase A)	Cytoplasmic	9.97

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