The Effect of Titanium Dental Implants Surface Modification on the Macrophage Mediated Cell Response

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Submitted in fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

April 2013
Dedication

This thesis is dedicated to my mother’s soul and my father.
Abstract

**Background:** Titanium dental implants are currently one of the best treatment alternatives for replacing missing teeth. The direct bond that forms between the jawbone and titanium in a process defined as osseointegration has made titanium the material of choice for such devices. Whilst the underlying biological mechanisms responsible for the osseointegration process have not been fully elucidated, high demand by both patients and clinicians has driven the clinical application of titanium implants.

Modification of the surface properties of titanium has been proven to be effective in promoting osseointegration, and thus understanding the fundamental cellular and molecular mechanisms responsible for the clinically observed outcomes as a result of titanium surface modification is the focus of considerable research. Previous research in this field has, as expected, focused on osteoblasts as they are the bone forming cells. This however, ignores the possible fundamental role of early mediators of the healing process, such as platelets and macrophages, which may interact with the implant surface well ahead of osteoblasts. As platelets and macrophages have well documented roles in modulating the function of other cells including osteoblasts, it is reasonable to postulate that the events that occur during the very early stages of the healing process may modulate repair in later stages, and ultimately influence the final osseointegration outcome.

**Aims:** The primary aim of this study was to investigate the influence of titanium surface topography and chemistry on macrophages, a major early mediator of the
healing process. Further, this study also examined the influence of titanium surface modification on the way in which macrophages interact with upstream (platelets) and downstream (osteoblasts) wound healing cellular mediators, in order to better understand the very early phases of the osseointegration process. This may thus identify critical biological mechanisms that could be targeted to enhance osseointegration by engineering titanium implant surfaces.

**Materials and methods:** This project utilized titanium discs with polished (SMO), micro-rough sand blasted, acid etched (SLA), and hydrophilic modified SLA (SLActive®) titanium surfaces, which provide a range of titanium surface modifications that correspond to commercially available titanium implant surfaces. Scanning electron microscopy and X-ray photoelectron spectroscopy was used to assess the surface topography and chemistry of these titanium surfaces.

The response of primary human platelets to these surfaces was assessed by determining the degree of attachment to, and activation by, the surfaces using both enzymatic and ELISA methodologies to quantitate the release of the platelet proteins LDH, β-TG and PF4. The response of the human macrophage cell-line THP-1, cultured on these titanium surfaces was also assessed by comparing the relative expression of a wide range of pro-inflammatory cytokines and chemokine genes. Differences in gene expression were subsequently confirmed at the protein level using an immuno-blotting array.

A novel *in vitro* conditioned media/co-culture approach was used to examine the influence of titanium surface modification on the inter-relationships between
titanium surface driven platelet releasate, macrophage function and osteoblast differentiation. This co-culture approach aims to mimic the temporal sequence of cells arriving at the implant site.

First, the interaction between platelet released proteins and macrophage pro-inflammatory cytokine gene expression was assessed by PCR array following culture in conditioned media containing human megakaryocyte (MEG-01) derived platelet releasate. Second, the effect of macrophage cytokines released in response to the titanium surfaces on human osteoblast (hFOB) osteogenic gene expression (in particular those related to the TGFβ/BMP pathway) was assessed using a transwell co-culture system.

**Results:** While platelet attachment was significantly higher on the micro-rough SLA surface, the level of the activation markers β-TG and PF4 released into the media was similar from all surfaces, indicating a higher level of activation per platelet with the SLActive surface. In regard to macrophage function, the results showed that the hydrophilic SLActive surface attenuated the macrophage inflammatory cytokine response when compared to the other surfaces. Interestingly, subsequent experiments showed that the titanium surface-induced platelet releasate is also capable of modulating the macrophage inflammatory cytokine response. This was especially evident with the SLA surface whereby the initial enhanced macrophage pro-inflammatory cytokine response (when compared to the smooth polished surface), was attenuated when the titanium surface was pre-incubated with platelet releasate. Finally, this study also showed that in a surface-specific manner
(SLActive>SLA>Smooth), titanium surface-induced macrophage cytokines modulated osteoblast gene expression without any direct contact.

Conclusions: This study demonstrated that titanium surface modification is able to differentially modify both the molecular and proteomic response of early mediators of the healing process. Platelet activation is significantly increased by the SLActive surface, which has downstream effects on macrophage pro-inflammatory cytokine gene expression. Similarly, titanium surface modification is also able to directly modify macrophage pro-inflammatory cytokine expression and subsequent downstream osteoblast differentiation. These studies illustrate the importance of both platelets and macrophages during the very early stages of the host response to titanium surfaces. Further, the results demonstrate that subsequent engineering of the titanium surface to target these cells in order to improve osseointegration is feasible. Finally, this project demonstrated the importance of suitable co-culture in vitro models in order to mimic as closely as possible the in vivo environment in order to better understand the osseointegration process.
Statement of Originality

I, Mohammed A. Alfarsi declare that this work has not previously been submitted for a degree or diploma in any university. To the best of my knowledge and belief, the thesis contains no material previously published or written by another person except where due reference is made in the thesis itself.

Declared by

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Mohammed A. Alfarsi

Date
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Acknowledgements

The joy of completion can only be felt when looking back at the journey past and remembering all the loving family and friends whom help and support eased this long but fulfilling journey.

First and foremost I would like to express my heartfelt gratitude to my supervisors, Professor Saso Ivanovski and Dr Stephen Hamlet, who have patiently helped, supported and encouraged me throughout my study. The way you dealt with me gave me confidence and made my PhD a joyful journey.

My country, Kingdom of Saudi Arabia, who sponsored me through my employer, King Khalid University, which spared no expenses toward my education. Thank you, I pray God to bless you and help me to actively participate in your development for a prosperous future. I also thank Griffith University for funding my research, giving me amazing help and support and being the best place for me to undertake my PhD.

A very special thank you goes to my beloved wife, Dr Ashwaq Abdulghani, who scarified her professional career to help build mine, patiently supported me during my Master and PhD, selflessly raised our sons and always been the lovely girl I fell in love with. You darling are a gift from God; I love you and cannot live without you. As for my sons, Ra-ed and E-yad, who have missed their childhood at our hometown for me, tolerated a lot of my mood swings, worked even harder than me and been the best motive for me; you are the joy of my life and you make it all worth it. My family, friends and colleagues thank you for your continuous help and support.
Publications arising from this thesis


Related publications that the candidate contributed to during his candidature
