Nano-Engineered Titanium Implants for Complex Bone Therapies

A thesis submitted in fulfilment of the requirement for the degree of

Doctor of Philosophy

Engineering (Nanotechnology)

By Karan Gulati

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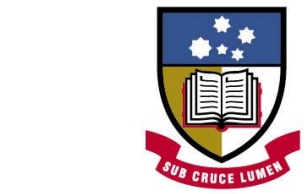

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ABSTRACT

A number of bone pathologies, such as fracture, infection or cancer, require drug therapy. However, conventional systemic drug administration is inefficient, wasteful, may not reach the target bone tissue in effective concentrations, and may cause unwanted side effects in other tissues. Ideally, drug should be delivered locally at the specific site, and in an optimal therapeutic concentration. Surface modification of the titanium implants can meet these challenges effectively by enabling effective delivery of therapeutics directly at the bone site for an extended period. Among the various suggested implant modifications, titania (TiO_2) nanotubes (TNTs), which can easily be fabricated on Ti surfaces *via* cost-effective electrochemical anodization, is emerging as a possible strategy for local drug delivery.

This thesis describes advances in TNT/Ti implant technology towards achieving effective therapeutic and cellular modulating action from the surface of Ti wire implants, which have been nano-engineered to fabricate TNTs. The concept was to design and optimize novel therapeutic features of TNTs, using simple and scalable technologies that can ensure easy integration into implants currently on the market. Specifically, in order to address complex bone conditions such as infection, inflammation, and cancers of bone, TNTs were fabricated on Ti wires that could be inserted into bone for 3D in-bone therapeutic release.

The main points of the thesis can be summarized as:

1. *Structural engineering of TNTs*: Periodic tailoring of the TNT structures using a modulated electrochemical anodization process in an attempt to enhance drug loading and releasing abilities of the TNTs.

2. *Fabrication optimization of TNTs on curved surfaces*: Optimization of anodization conditions was undertaken, with a special focus on defining the role of electrolyte ageing,

in order to fabricate a mechanically robust anodic layer (TNTs) on complex curved surfaces such as Ti wires. The purpose of this was to enable easy integration of TNT technology into the current implant market, which includes widely varied geometries (pins, screws, plates, meshes, etc.).

3. *Therapies for complex bone conditions*: Demonstration of TNTs/Ti wire abilities to meet a range of therapeutic needs was modelled, by determining the effect of local release of osteoporotic drugs from TNTs, when inserted into collagen gels containing human osteoblasts. This was followed by analysis of the therapeutic effect on cells, and cell spread/migration morphology on the TNT surfaces.

4. *Formation of chitosan-microtubes on TNTs in-situ*: Investigation of the fate of chitosan-modified TNT/Ti implants in phosphate buffer (isotonic to human blood). Chitosan degradation into micro-tubes on the surface of TNTs was investigated to elucidate the mechanism underlying the *in-situ* formation of these novel structures.

5. *Titanium (Ti) nanotubes vs titania (TiO_2) nanotubes*: Conventional titania (TiO_2) nanotubes were chemically reduced into titanium while preserving the nano-topography. The converted conducting titanium nanotube implants were proposed for electrical stimulation therapy and local drug delivery.

6. *TNTs on 3D printed Ti alloys*: Fabrication optimization of TNTs on a unique micro-rough 3D printed Ti alloy, to enable varied surface features, including irregular micro-roughness combined with nano-topography of TNTs. Comparison was then made of cell adhesion, attachment and modulation of osteoblast function by TNTs/Ti 3D implants with conventional smooth, micro-rough and TNTs/Ti flat foil surfaces.

The investigations presented in the thesis are expected to open doors towards the development of advanced in-bone therapeutic implants, in the form of easy-to-tailor nano-engineered Ti

wires, with superior 3D drug releasing abilities and enhanced bone healing functionalities. The emphasis has been on designing the simplest and most cost-effective methodologies to permit easy integration into the current implant market. Applications for these implants could be in the treatment of fractures, bone infections/cancers and ‘local’ osteoporosis in bones.

PREFACE

This thesis is submitted as a 'thesis by publication' in accordance with "Specifications for Thesis 2013" of The University of Adelaide. The PhD research spanning 3.5 years generated 1 book chapter, 15 peer-reviewed journal articles [9 published, 3 submitted, and 3 in final preparation for submission in August 2015], and 2 peer-reviewed conference publications. Furthermore the PhD research was also presented at 19 national and international conferences. Six research chapters included in this thesis were published (or submitted or in final preparation for submission) as research articles in highly ranked journals in the field. A complete list of publications is provided in the following pages.

LIST OF PUBLICATIONS

Published Book Chapter

K. Gulati, M. Kogawa, S. Maher, G. Atkins, D. Findlay, D. Losic "Titania Nanotubes for Local Drug Delivery from Implant Surfaces" in book *Electrochemically Engineered Nanoporous Materials: Methods, Properties and Applications* 2015, ed. by D. Losic and A. Santos (Springer International Publishing AG - Germany). Springer Series in Materials Science 220, **DOI**: 10.1007/978-3-319-20346-1_10

Peer-reviewed Journal Articles

Published/Accepted Articles

1. **K. Gulati**, A. Santos, D. Findlay, D. Losic "Optimizing Anodization Conditions for Fabricating Well-Adherent and Robust Titania Nanotubes on Curved Surfaces" *Journal of Physical Chemistry C,* 2015, **119**, 16033−16045. **DOI**: 10.1021/acs.jpcc.5b03383
2. **K. Gulati**, K. Kant, D. Losic "Periodically Tailored Titania Nanotubes for Enhanced Drug Loading and Releasing Performances" *Journal of Materials Chemistry B*, 2015, 3, 2553-2559.
3. **K. Gulati**, M. S. Aw, D. Findlay, D. Losic "Local Drug Delivery in Bone by Drug Releasing Implants: Perspectives of Nano-Engineereed Titania Nanotubes" *Therapeutic Delivery*, 2012, **3**, 857-873.
4. **K. Gulati**, M. S. Aw, D. Losic "Nano-Engineered Ti Wires for Local Delivery of Chemotherapeutics in Brain" *International Journal of Nanomedicine* 2012, **7**, 2069-2076.

5. V. S. Saji, T. Kumeria, **K. Gulati**, M. Prideaux, S. Rahman, M. Alsawat, A. Santos, G. J. Atkins, D. Losic. "Localized Drug Delivery of Selenium (Se) using Nanoporous Anodic Aluminium Oxide for Bone Implants" *Journal of Materials Chemistry B*, 2015 (accepted).
6. T. Kumeria, H. T. Mon, M. S. Aw, **K. Gulati**, A. Santos, H. J. Griesser, D. Losic "Advanced Biopolymer-Coated Drug-Releasing Titania Nanotubes (TNTs) Implants with Simultaneously Enhanced Osteogenic and Antibacterial Properties" *Colloids and Surfaces B: Biointerfaces*, 2015, **130**, 255–263.
7. D. Losic, M. S. Aw, A. Santos, **K. Gulati**, M. Bariana "Titania Nanotube Arrays for Local Drug Delivery: Recent Advances and Perspectives" *Expert Opinion on Drug Delivery*, 2015, **12**, 103-127.
8. T. Kumeria, **K. Gulati**, A. Santos, D. Losic "Real-Time and In Situ Drug Release Monitoring from Nanoporous Implants under Dynamic Flow Conditions by Reflectometric Interference Spectroscopy" *ACS Applied Material & Interfaces*, 2013, **5**, 5436−5442.
9. M. S. Aw, K. A. Khalid, **K. Gulati**, G. J. Atkins, P. Pivonka, D. M. Findlay, D. Losic "Characterization of Drug Release Kinetics in Trabecular Bone from Titania Nanotube Implants" *International Journal of Nanomedicine*, 2012, **7**, 4883-4892.

Submitted Articles

10. **K. Gulati**, M. Prideaux, M. Kogawa, L. Lima-Marques, G. J. Atkins, D. M. Findlay, D. Losic "3D Printed Titanium Implants with Combined Micro Particles and Nanotube Topography Promote Interaction with Human Osteoblasts and Osteocyte-Like Cells" *Biomaterials*, 2015. (Under Review)

11. **K. Gulati**, L. Johnson, R. Karunagaran, D. Findlay, D. Losic "*In-Situ* Transformation of Chitosan Films into Microtubular Structures: A New Bio-Interface for Bone Implants" *Biomacromolecules*, 2015. (Submitted)
12. **K. Gulati**, M. Kogawa, M. Prideaux, D. M. Findlay, G. J. Atkins, D. Losic "Titania Nanotube (TNT) Implants Loaded with Parathyroid Hormone (PTH) for Potential Localized Therapy of Osteoporotic Fractures" *Journal of Materials Chemistry B*, 2015. (Submitted)

Articles in Final Preparation (for submission in August 2015)

13. **K. Gulati**, S. Chandrasekaran, N. H. Voelcker, D. M. Findlay, D. Losic "Chemical Reduction of Titania (TiO_2) into Conductive Titanium (Ti) Nanotubes Arrays for Combined Drug-Delivery and Electrical Stimulation Therapy" *Chemical Communications*, 2015.
14. S. Rahman*, **K. Gulati***, M. Kogawa, G. J. Atkins, P. Pivonka, D. M. Findlay, D. Losic "*Ex-Vivo* Implantation of Nano-Engineered Implants: Investigating Drug Diffusion and Integration inside the Bone Microenvironment" *Journal of Biomedical Materials Research Part A*, 2015 (**Equal Contribution**).
15. G. Kaur, **K. Gulati**, T. Willsmore, I. Zinonos, S. Hay, D. Losic, A. Evdokiou. Nano-engineered Titanium Wire Implants towards Localized Anticancer Efficacy. *Biomaterials*, 2015.

Peer-Reviewed Conference Publications

1. H. Mokhtarzadeh, M. S. Aw, K. A. Khalid, **K. Gulati**, G. J. Atkins, D. Findlay, D. Losic, P. Pivonka "Computational and Experimental Model of Nanoengineered Drug Delivery System for Trabecular Bone". 11th World Congress on Computational Mechanics

(WCCM XI). July 2014 Barcelona, Spain. [5th European Conference on Computational Mechanics (ECCM V). 6th European Conference on Computational Fluid Dynamics (ECFD VI)]. E. Oñate, J. Oliver and A. Huerta (Eds).

2. **K. Gulati**, G. J. Atkins, D. M. Findlay, D. Losic "Nano-Engineered Titanium for Enhanced Bone Therapy". Proc SPIE Biosensing and Nanomedicine VI 2013: published online 11 September 2013, doi:10.1117/12.2027151

Conference Presentations:

1. **K. Gulati**, M. Prideaux, M. Kogawa, G. Atkins, D. Findlay, D. Losic "Nano-engineered titanium wires for enhanced in-bone therapeutic action" International Nanomedicine Conference. Sydney, Australia, July 2015 **(Oral Presentation).**
2. D. Losic, **K. Gulati**, G. Kaur, S. Maher, S. Rahman, G. J. Atkins, D. M. Findlay, A. Evdokiou "Drug Releasing Implants Based on Nanoengineered Titania Nanotubes for Localized Bone and Cancer Therapy" The 3rd International Translational Nanomedicine (ITNANO) Conference, Milocer, Montenegro, June 2015 **(Oral Presentation)**.
3. **K Gulati**, D. Findlay, D. Losic "Healing injured bones: nano-engineered drug releasing bone implants" Go8-C9 PhD Forum 2014 Global challenges of ageing populations: graduate perspectives from China and Australia, Sydney, Australia, Dec 2014 **(Oral Presentation).**
4. D. Losic, M. S. Aw, **K. Gulati**, Y. Wang, M. Bariana, G. Kaur, S. Rahman, A. Santos, D. Findlay, A. Evdokiou "Electrochemically nanoengineered drug-releasing implants for localized drug delivery and advanced bone and cancer therapies" Taishan Academic Forum, TAF-GNB 2014, Qingdao, China, October 2014 **(Oral Presentation).**
5. H. Mokhtarzadeh, M. S. Aw, K. A. Khalid, **K. Gulati**, G. J. Atkins, D. Findlay, D. Losic, P. Pivonka "Computational and experimental model of nanoengineered drug delivery

system for trabecular bone" 11th World Congress on Computational Mechanics (WCCM XI) Barcelona, Spain, July 2014 (**Oral Presentation, Publication**).

6. **K. Gulati**, S. Rahman, M. Kogawa, H. Mokhtarzadeh, P. Pivonka, G. J. Atkins, D. M. Findlay, D. Losic "Optimisation of nano-engineered drug-eluting bone implants" Australian Nanotechnology Network (ANN) Early Career Workshop, Sydney, Australia, July 2014 **(Poster Presentation, Travel Support).**
7. **K. Gulati**, S. Rahman, M. Kogawa, H. Mokhtarzadeh, P. Pivonka, G. J. Atkins, D. M. Findlay, D. Losic "In-bone therapeutic implants: concept, fabrication and drug release" NanoBio 2014, Brisbane, Australia, July 2014 **(Oral Presentation, Travel Support).**
8. H. Mokhtarzadeh, M. S. Aw, K. A. Khalid, **K. Gulati**, G. J. Atkins, D. Findlay, D. Losic, P. Pivonka "Experimental and finite element models of nano-engineered drug delivery system for trabecular bone" The eighth Clare Valley Bone Meeting, South Australia, Australia, March 2014 **(Poster Presentation).**
9. **K. Gulati**, G. J. Atkins, D. M. Findlay, D. Losic "Optimising the fabrication of titania nanotubes for enhanced bone implant therapy" ICONN 2014, Adelaide, Australia, Feb 2014. **(Poster Presentation).**
10. **K. Gulati**, G. J. Atkins, D. M. Findlay, D. Losic "Nano-engineered titanium for enhanced bone therapy" SPIE International Symposium on NanoScience + Engineering, part of Optics and Photonics 2013 event., California, USA, August 2013 **(Oral Presentation, Travel Support, Publication).**
11. **K Gulati**, D. Findlay, D. Losic "Healing traumatised bones: perspectives of nano-engineered drug-releasing implants" Australian Nanotechnology Network (ANN) Early Career Workshop, 25-26 July 2013, Flinders University, SA, Australia. **(Oral Presentation, Best Presentation Award).**
12. T. Kumeria, A. Santos, **K. Gulati**, D. Losic "Drug release Kinetics from nanoporous anodic alumina implant under dynamic flow conditions" 4th Australia and New Zealand

Micro/Nanofluidics Symposium (ANZMNF), Adelaide, Australia, April 2013 **(Poster Presentation).**

13. M. Kurian, **K. Gulati**, M. S. Aw, S. Hay, A. Evdokiou, D. Losic "Implants with titania nanotubes for human breast cancer therapy" 22nd Meeting of the Australasian Society for Biomaterials and Tissue Engineering, South Australia, Australia, April 2013. **(Poster Presentation).**
14. J. Kaiser, P. R. Buenzli, M. S. Aw, K. A. Khalid, **K. Gulati**, G. J. Atkins, P. Pivonka, D. M. Findlay, D. Losic "Computational and experimental characterization of drug release kinetics in trabecular bone from titania nanotube implants" Australian and New Zealand Bone and Mineral Society (ANZBMS) Meeting, Perth, Western Australia, September 2012. **(Poster Presentation).**
15. **K Gulati**, T. Altalhi, D. Findlay, D. Losic "Advanced drug-releasing implants for bone therapies composed of carbon nanotubes and titania nanotube arrays" Oz Carbon 2012, Adelaide, Australia, July 2012 **(Poster Presentation).**
16. D. Losic, M. S. Aw, **K. Gulati**, T. Kumeria "Self-organized nanopore and nanotube arrays for biomedical applications" International Nanomedicine conference, Sydney, Australia, July 2012 **(Invited Key Lecture).**
17. **K. Gulati**, M. S. Aw, G. J. Atkins, D. M. Findlay, D. Losic "Nano-engineered titania nanotube arrays as drug-releasing implants for advanced bone therapeutics" NT 12 Thirteenth International Conference on the Science and Application of Nanotubes, Brisbane, Australia. June 2012 **(Oral Presentation).**
18. **K. Gulati**, K. Kant, D. Losic "Titania nanotube arrays: improved drug loading and releasing characteristics by tailoring nanotube structures" NT 12, Brisbane, Australia, June 2012 **(Poster Presentation).**

19. **K. Gulati**, T. Altalhi, D. Losic "Characterization of titania nanotube arrays with carbon nanotubes for drug delivery applications" NT 12, Brisbane, Australia, June 2012 **(Poster Presentation).**

DECLARATION

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission in my name for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

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KARAN GULATI

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