Nanoengineered Titanium as Protein-Releasing Implants: A Molecular Adjunct to Reduce Craniofacial Surgery

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ABSTRACT

Craniosynostosis is a developmental disorder characterised by the premature fusion of skull sutures in children, necessitating repetitive surgical interventions throughout infancy. A major goal of craniosynostosis research is to develop molecular adjunctive treatments to reduce the morbidity and complications associated with multiple craniofacial surgery. Recent progress in molecular biology has highlighted the regulatory effects of bone morphogenetic protein 2 (BMP2) antagonists, including glypicans (GPC1 and GPC3), on suture morphogenesis and cellular functions. Moreover, the availability of genetically-engineered murine models of human craniosynostosis and drug-delivery systems (DDS) has assisted towards investigation of the glypican-based therapeutics *in vivo.* However, the conventional DDS are limited by their uncontrolled release patterns and undesired pharmacokinetics. The development of clinically viable implantable DDS, prior to human trials, require preclinical studies to investigate their characterisation, efficacy, pharmacokinetics and toxicity both *in vitro* and *in vivo* (in animal models).

Medical Titanium (Ti) implants nanoengineered with Titania nanotubes (TNTs) have been recognised as a superior delivery platform in complex bone therapies (*i.e.* orthopaedics, cancer *etc*.) to localise the release of therapeutics in a controlled and sustained manner. This thesis presents the use of therapeutic-releasing TNT/Ti implant technology in a murine model, to address a key clinical challenge of delaying post-operative sutural bone growth in craniosynostosis. This interdisciplinary project has three aspects and specific aims including: (i) engineering and *in vitro* study: to fabricate and optimise TNT/Ti implants to study glypican release *in vitro* and bioactivity

in murine C2C12 cells, (ii) pre-*in vivo* cell study: to evaluate the biological response at TNT-cell interface of heterogeneous (human) suture mesenchymal cells (SMCs) and (iii) *in vivo* study: to assess *in vivo* implant biocompatibility and efficacy as a glypican delivery system in wildtype and Crouzon murine models.

TNT/Ti implants with controllable nanotube dimensions were fabricated via electrochemical anodisation process, and their protein-releasing capability and protein functionality were tested spectrophotometrically in physiological buffer and transfected C2C12 cells (BMP reporter cells), respectively. A metabolic activity assay was performed to investigate human SMC behavior at TNT-cell interface. The *in vivo* performance was assessed using micro-CT and histology in a surgical cranial defect model to verify TNT/Ti implant biocompatibility and glypican release efficiency.

A protein loaded, mechanically robust TNT/Ti implant (120 ± 10 nm pore-diameter) displayed a biphasic *in vitro* release profile, with high loading efficiencies and prolonged release durations, spanning across 1 to 4 weeks. The pharmacokinetic modelling, based on the protein release parameters, showed an anomalous burst release and a zero-ordered sustained release. GPC1 and GPC3 released from TNTs were biologically active and reduced the BMP2-osteogenic activity in C2C12 cells. A decrease in adhesion and proliferation of SMCs at the TNT-cell interface, rendered the implant nanotopography and surface chemistry suitable for craniosynostosis therapy. The murine studies confirmed the implant biocompatibility and reiterated the sustained delivery of glypicans *in vivo*, demonstrated by decreased bone volume and surface area in therapeutically-intervened cranial defects.

These findings confirm the potential of the nanoengineered TNT/Ti implants as an effective glypican delivery system to delay rapid post-operative bone re-growth in a murine model. This approach may evolve into a non-surgical molecular adjunct to minimise the need for recurrent re-operations in human craniosynostosis management.

PREFACE

This thesis is submitted as a “Combined Conventional Publication format” in accordance with “Specifications for Thesis 2015” of the University of Adelaide. It contains an introduction, a detailed literature review and six experimental chapters followed by conclusion and appendices. The research that was carried out during the three and a half years of this PhD program has resulted in successful publication and/or submission of two articles in reputed journals. Additionally, two other journal articles are under preparation. Also, the research findings of this PhD study have been presented at 7 national and international conferences. A complete list of publications is provided in following pages (p. xxii-xxv).

LIST OF PUBLICATIONS

Peer-reviewed Journal Articles Published:

1. **M. Bariana**, P. Dwivedi, S. Ranjitkar, J. Kaidonis, D. Losic, P.J. Anderson , "Biological Response of Human Suture Mesenchymal Cells to Titania Nanotube-Based Implants for Advanced Craniosynostosis Therapy", *Colloids and Surfaces: B*, 2017, 150, 59-67.

Journal Articles Submitted/In Preparation:

2. **M. Bariana**, P. Dwivedi, S. Ranjitkar, J. Kaidonis, D. Losic, P.J. Anderson, " Glypican-Based Drug Releasing Titania Implants to Regulate BMP2 Bioactivity as a Potential Approach for Craniosynostosis Therapy", *Nanomedicine: Nanotechnology, Biology and Medicine*, 2016. (Invited article under peer-review)

3. **M. Bariana**, S. Ranjitkar, J. Kaidonis, D. Losic, P.J. Anderson , " Titania Nanotube-based Glypican-3 delivery Implants inhibit Cranial Defect Healing in Crouzon Model of Craniosynostosis" 2016. (Under preparation for Nature Communications)

4. **M. Bariana**, S. Ranjitkar, J. Kaidonis, D. Losic, P.J. Anderson , "Assessment of *in vivo* Tissue Response to Titania Nanotube-based Cranial implants" 2016. (Under preparation for Journal of Biomedical Materials Research Part A)

Conference Presentations:

1. **M. Bariana**, P. Dwivedi, S. Ranjitkar, J. Kaidonis, D. Losic, P.J. Anderson , "Cellular response of Human Suture Cells on Titania Nanotube-based Implants for Craniosynostosis Therapy", International Conference on Nanoscience and Nanotechnology 2016, Canberra, Australia, February 2016. (Poster presentation)

2. **M. Bariana**, P. Dwivedi, S. Ranjitkar, J. Kaidonis, D. Losic, P.J. Anderson , "Nanoengineered Protein-Delivery System for Craniosynostosis Therapy", IADR ANZ Division 55th Annual Scientific Meeting, Dunedin, New Zealand, August 2015. (Poster and oral presentation)

3. **M. Bariana**, S. Ranjitkar, J. Kaidonis, D. Losic, P.J. Anderson, "Titania Nanotubes-based Protein-release Studies to Delay Suture Fusion in Re-synostosis Murine Model", 6th International Nanomedicine Conference, Sydney, Australia, July 2015. (Oral presentation)

4. **M. Bariana**, S. Ranjitkar, J. Kaidonis, D. Losic, P.J. Anderson, "A Nano-approach for Craniosynostosis Therapy" Asia Pacific Craniofacial Association 2014 Biennial Meeting, Adelaide, Australia, October 2014. (Oral presentation)

5. **M. Bariana**, S. Ranjitkar, J. Kaidonis, D. Losic, P.J. Anderson, "A Novel Treatment to Prevent Re-operation in Craniosynostosis" 2014 Joint Australian-New Zealand CRS Student Workshop Development of Pharmaceutical Therapeutics: From Biological Imaging to Delivery System Optimisation, Adelaide, Australia, October 2014 (Oral presentation)

6. **M. Bariana**, S. Ranjitkar, J. Kaidonis, D. Losic, P.J. Anderson, "Protein-eluting Titania Nanotube-based Implants for Craniosynostosis Therapy" 5th International Nanomedicine Conference, Sydney, Australia, June 2014. (Poster presentation)

7. **M. Bariana**, T. Kumeria, A. Santos, S. Ranjitkar, J. Kaidonis, D. Losic, P.J. Anderson, "Nanoporous Anodic Alumina as Protein-Delivery System for Localised Therapy: Controlling Release Characteristics by Structural modifications" International Conference on Nanoscience and Nanotechnology 2014, Adelaide, Australia, February 2014. (Poster presentation)

Awards:

1. International Association for Dental Research (IADR) ANZ Division: Joan Chong Award in Dental Materials 2014 for early career researchers (October 2014).

2. The Colgate Travel Award from the School of Dentistry, The University of Adelaide to present at the IADR ANZ Division meeting in Dunedin, New Zealand (August 2015).

3. Best Presentation Award at Research Day organised by the Faculty of Health Sciences, The University of Adelaide (July 2015).

Additional Publications:

Book Chapter:

1. M. S. Aw, **M. Bariana**, D. Losic, “Nanoporous Anodic Alumina for Drug Delivery and Biomedical Applications”, *Nanoporous Alumina: Fabrications, Structure, Properties and Applications* 2015, Springer International Publishing AG- Germany, Springer Series in Materials Science 219, DOI: 10.1007/978-3-319-20334-8.

Review Articles:

2. A. Santos, M. Sinn Aw, **M. Bariana**, T. Kumeria, Y. Wang., D. Losic, “Drug-releasing implants: Current progress, challenges and perspectives”, J*ournal of Materials Chemistry B*, 2014, 2, 6157-6182.

3. D. Losic, M. Sinn 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.

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 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. I acknowledge the support I have received for my research through the provision of an Australian Government Research Training Program Scholarship.

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MANPREET BARIANA

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"*Being a PhD student is like becoming all of the Seven Dwarfs. In the beginning you're Dopey and Bashful. In the middle, you are usually sick (Sneezy), tired (Sleepy), and irritable (Grumpy). But at the end, they call you Doc, and then you're Happy.*" (Adapted from Azuma, 2002, p.2)

I dedicate this thesis to these very special people in my life:

My Mum

My Dad

My Brother

And my fur-baby Dazzle.

I love you all dearly.