ASSESSMENT OF MICROBIAL BIOBURDEN METHODOLOGIES FOR TISSUE BANK SPECIMENS

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A thesis submitted in accordance with the requirements for admission to the degree of Doctor of Philosophy

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2014

CERTIFICATE OF AUTHORSHIP / ORIGINALITY

I certify that the work in this thesis has not previously been submitted for a degree nor has it been submitted as part of requirements for a degree except as fully acknowledged within the text.

I also certify that the thesis has been written by me. Any help that I have received in my research work and the preparation of the thesis itself has been acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis.

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2014

ACKNOWLEDGMENTS

I would like to thank Professor Sydney Bell, Associate Professor Peter Taylor and Chinmoy Mukerjee for providing me with the opportunity to undertake post-graduate research. Peter, I am especially grateful for your guidance, assistance and encouragement. Thank you to Associate Professor Chris McIver for your expertise and patient instruction in molecular bacteriology, to Professor Ruth Hall for always having the time to talk to me, and to my supervisor Associate Professor Cynthia Whitchurch for helping me through my PhD journey.

I could not have completed my research without the information provided by the Biotherapeutics Association of Australasia and the tissue banks of Australia, especially the NSW Bone Bank who also provided me with musculoskeletal tissue samples. Thank you to the Australian TGA-licensed bacteriology laboratories who shared their bioburden testing methods with me.

Finally, my thanks go to my amazing family for their support and understanding. Joanna thanks for all the proof reading and your constant encouragement.

PUBLICATIONS ARISING FROM THIS THESIS

Varettas K. (2014) RT-PCR testing of allograft musculoskeletal tissue – is it time for culture-based methods to move over*? Pathology*. *In press.*

Varettas K. (2014) Evaluation of two types of swabs for sampling allograft musculoskeletal tissue. *Aust NZ J Surg*. Doi: 10.1111/ans.12661

Varettas K. (2014) Swab or biopsy samples for bioburden testing of allograft musculoskeletal tissue? *Cell Tissue Bank*. 15:613-618

Varettas K. (2013) Broth versus solid agar culture of swab samples of cadaveric allograft musculoskeletal tissue. *Cell Tissue Bank*. 14:627-631

Varettas K. (2013) Micro-organisms Isolated from Cadaveric Samples of Allograft Musculoskeletal Tissue. *Cell Tissue Bank*. 14:621-625

Varettas K. (2013) Culture Methods of Allograft Musculoskeletal Tissue Samples in Australian Bacteriology Laboratories. *Cell Tissue Bank*. 14:609-614

Varettas K. (2012) Bacteriology Laboratories and Musculoskeletal Tissue Banks in Australia. *ANZ J Surg*. 82:775-779

Varettas K & Taylor P. (2012) Fungal Culture of Musculoskeletal Tissue: What's the Point? *Cell Tissue Bank*. 13:415-420

Varettas K & Taylor P. (2011) Bioburden Assessment of Banked Bone Used for Allografts. *Cell Tissue Bank*. 12: 37-43

PRESENTATIONS AT SCIENTIFIC CONFERENCES

Varettas K. Bioburden of allograft musculoskeletal tissue from cadaveric donors. Poster Presentation. 21st Annual conference of the European Association of Tissue Banks (EATB). Vienna, Austria. November 2012.

Varettas K. Broth vs solid agar culture of cadaveric allograft musculoskeletal tissue samples. Poster Presentation. 21st Annual conference of the European Association of Tissue Banks (EATB). Vienna, Austria. November 2012.

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ABSTRACT

Musculoskeletal tissues form part of the skeletal and/or muscular system of the body, vital in providing support and mobility. Musculoskeletal tissue transplants outnumber all other organ and tissue transplants. The bioburden assessment of allograft musculoskeletal tissue must be performed as part of the assessment screening of living and cadaveric donors to minimise the potential risk of transmission of infectious diseases via the allograft to the recipient.

There are no guidelines or standard method for determining the bioburden assessment of allograft musculoskeletal tissue and microbiology laboratories may use different types of samples, culture media and methods. Determining the suitability of the allograft tissue sample and the sensitivity of the bioburden testing methods required investigation especially with the advent of nucleic-acid testing (NAT). Subsequently, this investigation highlighted the lack of information regarding microbiology laboratories and the tissue banking industry in Australia.

A questionnaire was sent to all Australian tissue banks to determine their current status and the types of allograft samples being collected for bioburden assessment. Another questionnaire was designed for Therapeutic Goods Administration (TGA) licensed clinical microbiology laboratories to establish what bioburden assessment methods were being used for allograft samples. The information obtained from these questionnaires guided the evaluations undertaken in this thesis to compare different allograft samples and methods for bioburden assessment.

The current practice of collecting a swab and biopsy sample of allograft musculoskeletal tissue appears optimal for bioburden assessment. Retrospective reviews of isolates recovered from allograft musculoskeletal tissue and from the literature found a wide range of aerobic and anaerobic micro-organisms with fungi infrequently isolated.

An evaluation of the Amies gel swab and the ESwab systems was performed to determine if bioburden recovery could be improved at the pre-analytical stage. Both swab systems were found to be suitable sampling devices for bioburden testing of allograft musculoskeletal tissue.

The most common bioburden assessment methods, agar and broth culture, were compared with a broad-range NAT method. Swab and biopsy samples were inoculated with known quantities of challenge organisms and the percentage recovery of the challenge organisms was compared. In this study, the NAT method was not more sensitive than the culture-based techniques evaluated with broth culture being the most sensitive.

Microbiology laboratories must continue to re-evaluate current methods and investigate new ones to improve sensitivity. Future directions must be cost-effective as the value of maintaining a TGA-licence has become uncertain for some laboratories. Ultimately, tissue banks, clinicians and, most importantly, the allograft recipient must have confidence in the pre-analytical sampling techniques and the testing methods used to determine the bioburden of allograft musculoskeletal tissue prior to transplant.