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**Novel polyhydroxyalkanoate beads for use as a vaccine
against tuberculosis**

A thesis presented in partial fulfilment of the requirements for the degree of

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Abstract

Tuberculosis was in 1993 declared as a re-emerging disease by the World Health Organization. The only vaccine currently available, BCG, an attenuated strain of *Mycobacterium bovis*, does not protect adults against the pulmonary disease, which is the form of transmission. New vaccine candidates are being developed to provide protection against tuberculosis. Subunit vaccines offer a safer alternative than whole cell preparations and provide the possibility of utilizing only the components that mediate protective immune responses. This thesis describes the production of bacterially derived polyhydroxyalkanoate (PHA) beads for use as a delivery system for *Mycobacterium tuberculosis* reverse vaccinology antigens and immune modulators.

In the first study, the immunogenicity of beads derived from an endotoxin-free host, *Clearest coli*, displaying *M. tuberculosis* antigens Rv1626, Rv2032 and Rv1789 was evaluated in mice. Beads displaying Rv1626 were selected for further studies based on the magnitude and specificity of the immune response elicited. In a final study, the immune modulators Cpe30, CS.T3₃₇₈₋₃₉₅ and Flagellin were co-displayed with Rv1626 antigen on beads and the immunogenicity of these functionalised beads evaluated in mice. Vaccinations with Rv1626 beads and the immune modulators Cpe30 and CS. T3₃₇₈₋₃₉₅ induced a Th1/Th17 skewed immune response. These beads were then assessed for their ability to protect mice against aerosol challenge with *Mycobacterium bovis*. Rv1626 beads reduced the bacterial loads in 0.48 log₁₀ compared with the negative control group but the inclusion of immune modulators did not enhance the immunogenicity or protection induced by Rv1626 beads.

This study has demonstrated the potential of PHA beads delivering a single reverse vaccinology antigen for protection against tuberculosis infection in mice. While the co-display of immune modulators did not improve the protection induced by the antigen, further studies are needed to determine optimal doses for delivery of immune modulators to enhance protective immunity.

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Preface

This thesis is written according to the Graduate Research School regulations for PhD thesis by publications. The list below presents the publication status of each chapter.

Chapter 1A

Basic concepts in immunology, vaccines and tuberculosis.

This chapter was written by Patricia Rubio Reyes as an introductory section of this thesis and is not intended for publication

Chapter 1B

Self-Assembled Protein-Coated Polyhydroxyalkanoate Beads: Properties and Biomedical Applications. Natalie A. Parlane, Sandeep K. Gupta, Patricia Rubio Reyes, Shuxiong Chen, Majela Gonzalez-Miro, D. Neil Wedlock and Bernd H. A. Rehm. Published in ACS Biomaterials Science & Engineering. Special Issue: PHA Biomaterials (2016).

This review was written by all the authors. Patricia Rubio Reyes made a contribution on the section describing biomedical applications of polyhydroxyalkanoate beads.

Chapter 2

Immunogenicity of antigens from *Mycobacterium tuberculosis* self-assembled as particulate vaccines. Patricia Rubio Reyes, Natalie A. Parlane, D. Neil Wedlock and Bernd H.A. Rehm. Published in International Journal of Medical Microbiology, 306 (2016) 624–632.

All experiments were carried out by Patricia Rubio Reyes except mice vaccinations and processing of mice samples that were co-carried out with Natalie A. Parlane.

Chapter 3

Immunological properties and protective efficacy of a single particulate mycobacterial antigen displayed on polyhydroxybutyrate beads. Patricia Rubio Reyes, Natalie A. Parlane, Bryce M. Buddle, D. Neil Wedlock, Bernd H.A. Rehm. Published in Microbial Biotechnology (2017).

All experiments were carried out by Patricia Rubio Reyes. Natalie A. Parlane helped with mice vaccinations and processing of mice samples and Bryce Buddle assisted with challenge experiment and lungs histology.

Chapter 4

Conclusions

This chapter was written by Patricia Rubio Reyes as conclusions of this thesis and it is not intended for publication

Appendix 4

***In vivo* polyester immobilized sortase for tagless protein purification.** Iain D. Hay, Jinping Du, Patricia Rubio Reyes and Bernd H. A. Rehm. Published in Microbial Cell Factories (2015) 14:190.

Patricia Rubio Reyes made a contribution on the preparation of the plasmid pET14:PhaC-SrtA-Rv1626, purification of Rv1626 antigen and in the demonstration of the functionality of PhaC-SrtA-MBP beads and only those parts are submitted for examination. The entire publication is included for a better understanding of the methods used.

Table of contents

Chapter 1: General introduction.....	1
Chapter 1A. Basic concepts in immunology, vaccines and tuberculosis.	1
Abbreviations:	1
1.1 Host Immune Responses.....	2
1.2 Vaccines	4
Production of recombinant proteins for use as antigens or diagnostics	5
Types of subunit vaccines.....	7
1.3 Tuberculosis (TB).....	8
Pathogenesis	9
Virulence factors.....	10
Role of the innate immunity in TB.....	12
Role of the adaptive immunity in TB.....	13
Prevention and control of TB.....	15
Development of improved vaccines for TB	18
Animal models in TB vaccine research.....	21
Challenges in the development of new TB vaccines	23
1.4 New approaches to vaccine development.....	24
Identification of vaccine antigen candidates by reverse vaccinology.....	24
New adjuvants formulations.....	25
New vaccine delivery systems	27
1.5 References	28
Chapter 1B: Self-Assembled Protein-Coated Polyhydroxyalkanoate Beads: Properties and Biomedical Applications	42
1.6 Introduction.....	44

1.7	Pha biosynthesis and self-assembly of PHA Granules	47
1.8	Composition and structure of PHA Granules	50
1.9	Granule-associated proteins.....	53
1.10	Biomedical applications of PHA-protein Assemblies	59
	Protein Production and Purification	59
	Diagnostic Applications of Engineered PHA Beads.....	62
	Vaccines for Infectious Diseases.....	65
1.11	Conclusions.....	67
1.12	References	69
	Thesis Scope	78
	Problem statement and approach.....	78
	Aims of the study.....	78
	Specific objectives.....	78
	Chapter 2: Immunogenicity of antigens from <i>Mycobacterium tuberculosis</i> self-assembled as particulate vaccines	80
2.1	Introduction.....	82
	Generation of plasmids for production of beads displaying Rv antigens.....	83
	Bacterial strains and growth conditions	85
	Bead isolation	85
	Bead characterization.....	85
	Vaccine preparation and mice immunization	86
	Preparation of mice samples and immunological assays	86
	Statistical analysis	87
2.3	Results	88
	Construction of plasmids containing mycobacterial rv genes and their ability to mediate polyester bead production in <i>Clear coli</i>	88

Bead properties	89
Vaccine formulation and mice immunization.....	91
Cytokine responses	91
Antibody responses	93
Specificity of IgG response	95
2.4 Discussion	96
2.5 References	102
Link to next Chapter.....	105
Chapter 3: Immunological properties and protective efficacy of a single particulate mycobacterial antigen displayed on polyhydroxybutyrate beads	
	106
3.1 Introduction.....	108
3.2 Materials and Methods	109
Generation of vectors for beads production.....	109
Production of PHA bead and soluble Rv1626	111
Analysis of fusion proteins	111
Preparation of vaccine and vaccination of mice	111
Samples preparation and immunological assays.....	112
<i>M. bovis</i> challenge trial.....	113
Culture of lungs and spleens.....	113
Statistical analysis	114
3.3 Results and Discussion.....	114
Development of <i>Escherichia coli</i> strains assembling PHA beads displaying various antigen Rv1626 and immunomodulators.....	114
Dose response study	115
Determination of immunogenicity of Rv1626 beads	116
Assessment of protective immunity induced by Rv1626 beads.....	119
3.4 References	126

Chapter 4: General discussion.....	128
4.3 References.....	133
Appendix 1:	135
Appendix 2:	147
Appendix 3:	148
Appendix 4 :	151
Statement of contribution to doctoral thesis containing publications.....	170

List of Figures

Chapter 1

Figure 1.1 Estimated TB incidence rates in the world in 2015.....	9
Figure 1.2. A ,PHA inclusions; B, Schematic of PHA granule.....	44
Figure 1.3. Representative constituents of PHAs.....	45
Figure 1.4. Biosynthesis and genetics of PHB production.....	47
Figure 1.5. Schematic of self-assembly of PHA granules.....	48
Figure 1.6. Proposed covalent catalysis mechanism for PHA synthesis.....	54
Figure 1.7. Topological model and engineering of PHA synthase (<i>Ralstonia eutropha</i>).....	49
Figure 1.8. Diagnostic applications of engineered PHA beads.....	62
Figure 1.9. Development of a new veterinary TB skin test reagent based on TB-antigen displaying PHA beads.....	65
Figure 1.10. PHA beads displaying vaccine candidate antigens are immunogenic and show properties suitable for applications as particulate subunit vaccines.....	66

Chapter 2

Figure 2.1. SDS-PAGE and Western Blot analysis of proteins attached to various polyester beads isolated from <i>Cleaver coli</i>	88
Figure 2.2. SEM of various PHA beads.....	90
Figure 2.3. Cytokine responses of mice splenocytes upon stimulation with PPDB and analysed by cytometric bead array.....	92
Figure 2.4. IgG1 and IgG2c titers expressed in EC50 values in response to various beads and PPDB analysed by ELISA for each immunized group.....	94

Figure 2.5. Immunoblot of beads associated proteins using pooled sera from mice immunized with the various beads.....95

Figure 2.6. Specific recognition of the Rv1626 antigen by sera of mice immunized with beads displaying Rv1626.....96

Chapter 3

Figure 3.1. SDS-PAGE analysis of proteins attached to various polyester beads isolated from *Clear coli*.....115

Figure 3.2. IgG1 and IgG2c titres expressed in EC50 values in response to Rv1626 of mice vaccinated with doses of Rv1626 displayed on beads and Wt beads analysed by ELISA..116

Figure 3.3. Serum IgG1 and IgG2c titres expressed in EC50 values in response to Rv1626 from mice vaccinated with different immune modulators.....117

Figure 3.4. Cytokine responses of mice splenocytes upon stimulation with soluble recombinant Rv1626 (recRv1626) and analysed by cytometry bead array.....118

Figure 3.5. IgG1 and IgG2c titres expressed in EC50 values in response to Rv1626 of mice vaccinated with BCG, recRv1626, Rv1626 beads, Cpe30-Rv1626 beads, CS.T3-Rv1626 beads and DDA analysed by ELISA.....119

Figure 3.6. Cytokine responses of mice splenocytes upon stimulation with recRv1626 or PPDB.....121

Figure 3.7. Histological appearance of lungs from mice after *M. bovis* challenge. Lungs sections were stained with H&E.....124

List of tables

Chapter 1

Table 1.1 TB vaccine candidates in clinical stage in the global pipeline.....19

Table 1.2. Summary of recent developments in PHA-bead-based biomedical applications..59

Chapter 2

Table 2.1. Strains, plasmids and oligonucleotides used.....83

Table 2.2. Concentration of proteins in beads.....89

Table 2.3 Size distribution of beads in vaccine formulations (μm) as measured by laser scattering.....90

Chapter 3

Table 3.1. Strains, plasmids and oligonucleotides used.....110

Table 3.2. Vaccine induced protection in lung or spleen after *M. bovis* aerosol infection...123