

**Assessment of mycolic acids as ligand for
nanoencapsulated anti-tuberculosis drug
targeting**

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List of abbreviations

AD/ADD	4-androstene-3,17-dione/1,4androstadiene-3,17-dione pathway
AIDS	acquired immune deficiency syndrome
5BMF	5-Bromomethylfluorescein
Ca ²⁺	calcium
Caco-2	human colorectal carcinoma cell line
CIP	coronin interactin protein
CSIR	Council for Scientific and Industrial Research
DCM	dichloromethane
dddH ₂ O	double distilled deionized water
DIM	Phthiocerol dimycocerosates
DOT	Directly Observed Treatment
EE	encapsulation efficiency
EEA1	Early endosomal antigen 1
EIPA	5-N-ethyl-N-isopropylamiloride
EO	ethylene oxide
EPR	enhanced permeation and retention
ER	endoplasmic reticulum
FACS	fluorescence activated cell sorting
FAS	fatty acid synthetases
FCS	foetal calf serum
Fe ₂ O ₃	ferrous oxide

GI	bacterial growth index
HASMC	human arterial smooth muscle cells
HIV	human immunodeficiency virus
HPLC	High Performance Liquid Chromatography
IFN- γ	interferon-gamma
Igr	intracellular growth
IL-12	interleukin 12
INH	isoniazid
LAM	lipoarabinomannan
LD	lipid droplet
LpdC	lipoamide dehydrogenase C
LXR	liver X receptor
MA	mycolic acids
ManLAM	mannose-capped lipoarabinomannan
MARCO	macrophage receptor with collagenous structure
<i>M. avium</i>	<i>Mycobacterium avium</i>
MDR-TB	multidrug resistant tuberculosis
<i>M. leprae</i>	<i>Mycobacterium leprae</i>
MOI	multiplicities of infection
MS	mass spectrometry
<i>M.tb</i>	<i>Mycobacterium tuberculosis</i>
mV	milli-volts
MW	molecular weight



nm	nanometer
NMR	nuclear magnetic resonance
NP	nanoparticles
PAC	poly-alkyl-cyano-acrylate
PAMP	pathogen associated molecular patterns
PBS	phosphate buffer saline
PCL	poly- ϵ -caprolactone
PDI	polydispersity index
PEG	polyethylene glycol
PFA	paraformaldehyde
PIM	phosphatidylinositol-mannoside
PI(3)P	phosphatidylinositol-3-phosphate
PI(3)K/Vps34	phosphoinositide 3- kinase
PLA	polylactic acid
PLGA	poly, DL, lactic-co-glycolic acid
PMA	phorbol 12-myristate 13-acetate
PO	propylene oxide
PVA	polyvinyl alcohol
RIF	rifampicin
RSA	Republic of South Africa
rpm	revolutions per minute
RT	room temperature
SapM	<i>M. tuberculosis</i> effector molecule

SEM	scanning electron microscopy
S1P	sphingosine-1-phosphate
SiO ₂	amorphous silica oxide
SLN	solid lipid nanoparticles
TACO	tryptophane aspartate containing coat protein
TB	tuberculosis
TDM	trehalose dimycolate
THP-1	myelomonocytic cell line
TLC	thin layer chromatography
TLR	toll-like receptors
TMM	trehalose monomycolate
TNF- α	tumour necrosis factor alpha
TraSH	transposon site hybridization
U937	histiocytic lymphoma
USFDA	United States food and drug administration
WHO	World Health Organisation
w/o	water in oil
w/o/w	water in oil in water
WST	tetrazolium salt
w/v	weight/volume
XDR-TB	extensively drug resistant tuberculosis
ZnO	zinc oxide

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Summary

South Africa currently has the highest incidence of TB per 100 000 people in the world. In 2007 alone 112 000 people died of TB in South Africa, of which 94 000 were co-infected with HIV. Although TB treatments exist, poor patient compliance and drug resistance are challenges to TB management programs worldwide. Here, this challenge was addressed by the development of a polymeric anti-TB nanodrug delivery system for anti-TB drugs that could enable entry, targeting and sustained release for longer periods, hence reducing the dose frequency and simultaneously improve patient compliance. The aim was to prepare functionalised polymeric nano drug delivery vehicles to target TB infected macrophage cells. Successful nano encapsulation of anti-TB drugs was achieved and uptake of the antibiotics in the cells, demonstrated. A possible targeting agent, mycolic acids (MA) from *M. tuberculosis* was explored. The MA incorporated into nanoparticles could possibly serve as a ligand for cholesterol-rich areas, due to the cholesterol nature of MA and the fact that MA is attracted to cholesterol. In another targeting scenario, MA incorporated into nanoparticles may interact with the anti-mycolic acid antibodies that are anticipated to be present in higher concentrations at the infected areas. The cholesterol nature of MA was confirmed and how it related to the fine structure of the MA. The prepared MA containing nanoparticles were shown *in vitro* to be taken up in macrophage cell lines, without the MA hindering the uptake of the particles. In terms of toxicity, nanoparticles with or without MA were found to be acceptable for use, although MA did affect the viability of the cells more than poly, DL, lactic-co-glycolic acid particles alone in *in vitro* studies. This paves the way for testing MA as a ligand to target anti-TB drugs to the sites of infection in human TB patients.

Opsomming

Suid Afrika het tans die hoogste insidensie van TB per 100 000 mense in die wêreld. In 2007 het 112 000 mense gesterf van TB in Suid Afrika waarvan 94 000 mense geko-infekteer was met MIV. Alhoewel TB behandeling bestaan, is daar 'n groot uitdaging vir TB programme in die wêreld as gevolg van swak pasiënt-voldoening asook middel weerstandigheid wat ontstaan. Hierdie uitdaging is aangespreek deur die ontwikkeling van 'n polimeriese anti-TB nanomiddel-aflerwing stelsel wat opneembaarheid kan verhoog asook stadige vrystelling van die middel kan bewerkstellig wat dan die dosis kan verlaag en pasiënt-voldoening kan bevorder. Die doel van die projek was om die anti-TB nanomiddel aflerwing stelsel te sintetiseer en dit na TB geïnfekteerde selle te teiken. Die nanopartikels was suksesvol gesintetiseer met geïnkorporeerde anti-TB middels wat in selle opgeneem was. 'n Moontlike teikenmiddel, mikoosure van die *M. tuberculosis* bakterieë is ondersoek. Die mikoosure kon geïnkorporeer word in die nanopartikels wat as 'n ligand sou kon dien vir die cholesterol- ryke areas as gevolg van die cholesterioëde aard van die mikoosure, asook die feit dat mikoosure aangetrek word deur cholesterol. 'n Ander moontlikheid is dat die mikoosure met anti-mikoosuur teenliggame kan reageer wat waarskynlik in hoër konsentrasies teenwoordig sal wees in die geïnfekteerde areas. Die cholesterioëde aard van die mikoosure was bevestig, asook hoe die struktuur van die mikoosure daarmee verband hou. Daar is aangetoon dat die mikoosuur-bevattende nanopartikels *in vitro* in selle opgeneem word sonder dat die mikoosure die opname belemmer het. In terme van toksisiteit was die nanopartikels met en sonder mikoosure bevind om geskik te wees vir gebruik, alhoewel die mikoosure die lewensvatbaarheid van die selle meer geïnfekteer het as die poly, DL, lactic-co-glycolic acid-partikels alleen in *in vitro* studies. Die resultate maak die pad oop om mikoosure te toets as ligande om anti-TB middels te teiken na die geïnfekteerde loki van TB pasiënte.