Nanoencapsulation of mycolic acids as a deliverable to macrophages

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INTRODUCTION

Asthma is a reaction to environmental antigens causing a chronic inflammatory disease by a Th2 response. Mycolic acids (MA), depicted in **Figure 1**, which are part of the cell envelope of *Mycobacterium tuberculosis*, were shown to have potential as immunotherapeutic agents in a mouse model of asthma[1]. Due to the extremely hydrophobic nature of mycolic acids, a suitable vehicle is needed to introduce them into the body and guide them to their natural macrophage targets. Previously liposomes were used, but a more stable vehicle was desired.

The **objective** of this project was to explore the encapsulation of mycolic acids into vehicles other than liposomes for delivery into macrophages and addressing the challenge of low solubility. As vehicles nanoparticles (NP) have the advantage over liposomes in that they can increase the stability of the drug as well as effect controlled release of the agent as a function of *in vivo* particle degradation[2].

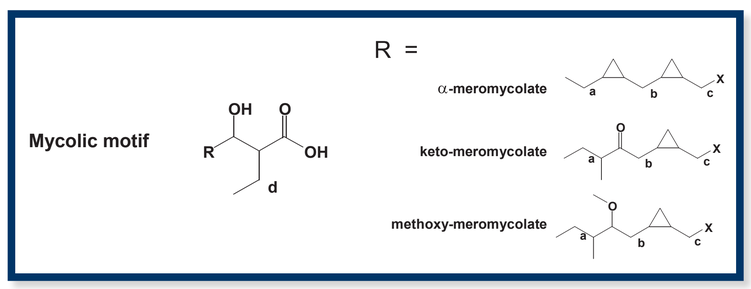

Figure 1: Generalised structures of MA from *Mycobacterium tuberculosis*. Hydrocarbon chain lengths are indicated as a, b or c, to a total of around 90 carbons per mycolic acid

EXPERIMENTAL AND RESULTS

In liposomes composed of egg phosphatidylcholine and cholesterol and that were loaded with mycolic acids, transfer of the labeled MA to unloaded liposomes occurred very rapidly (within 5 minutes) at 37 °C as determined by flow cytometry. The process could not be slowed down at 2 °C to an extent that would allow elucidation of the mechanism of MA redistribution within the first five minutes after mixing the loaded and unloaded liposomes. This called for an alternative delivery vehicle other than liposomes to ensure focused delivery of the MA to the macrophages, without dilution of the MA content in the general circulation.

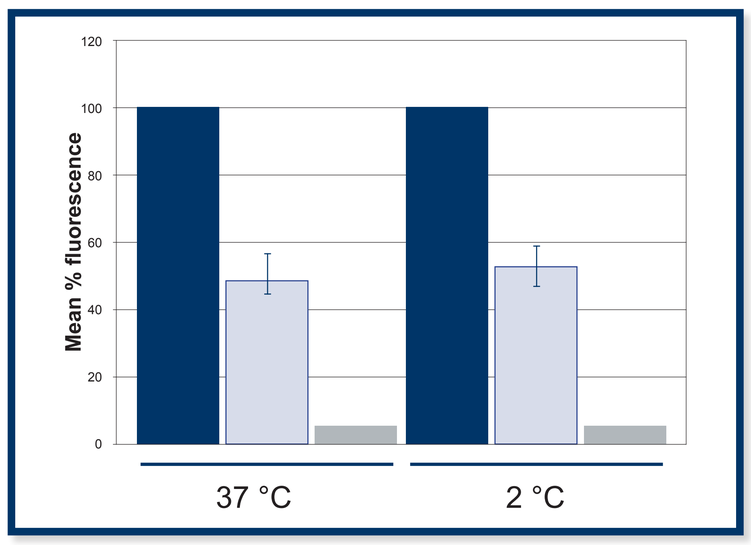

Figure 2: The mean % fluorescence distribution of liposomes at 37 °C and 2 °C. Original labeled MA (dark blue), unlabeled MA (grey), the mixed population (light blue). The value of the mixed population is a mean value for 7 (SD 8.39; 37 °C) and 3 (SD 6.17; 2 °C) measurements respectively. All liposomes contained the same amount of PC (90 μl) and cholesterol (45 μl)

To prepare nanoparticles, Poly lactide-glycolide (PLGA) was used to prepare a multiple nano emulsion with fluorescently labeled MA[3] and the emulsion was subsequently freeze dried. The resulting nanoparticles were characterised based on size, zeta potential and morphology. The SEM image (**Figure 3**) indicated the formation of nanoparticles. Because only the MA and not PLGA co-polymer is fluorescently labeled with 5-Bromomethylfluorescein[4] in the nanoparticle, fluorescent images (**Figure 4**) confirmed that the MA was indeed part of the nanoparticle matrix.

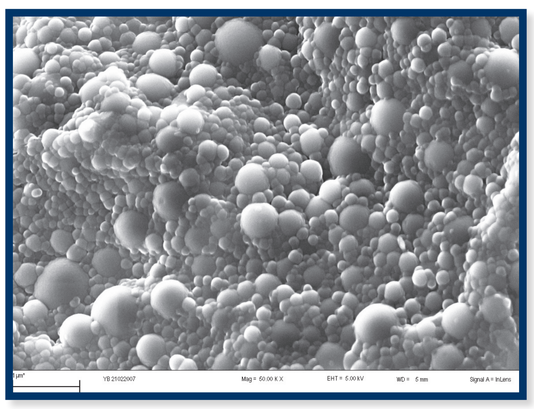

Figure 3: SEM of MA PLGA nanoparticles with an average size of 490-509 nm measured by a zeta sizer

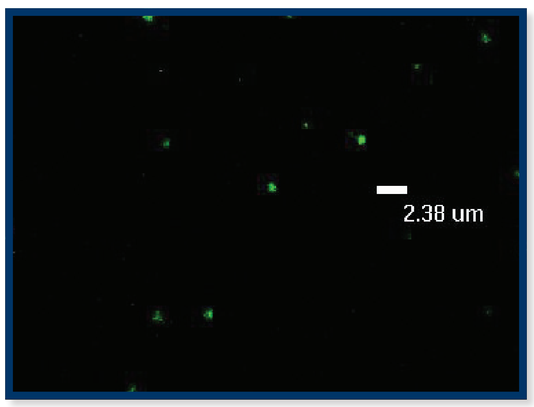

Figure 4: Fluorescence measurement excited by a 488 nm laser indicating labeled MA as part of the PLGA nanoparticles

THP-1 and U937 monocyte-macrophage like cell lines which are often used in nanoparticle uptake studies[5,6], were used to determine whether the labeled MA nanoparticles would be taken up by the cells. The MA containing nanoparticles were incubated with the respective macrophage cell lines. The particle uptake was analysed over a period of time via confocal microscopy. The data as presented in **Figure 5** illustrated that labeled MA nanoparticles were successfully taken up into the macrophage cells. This demonstrates that nanoparticles may serve as a suitable carrier for MA to reach their target sites.

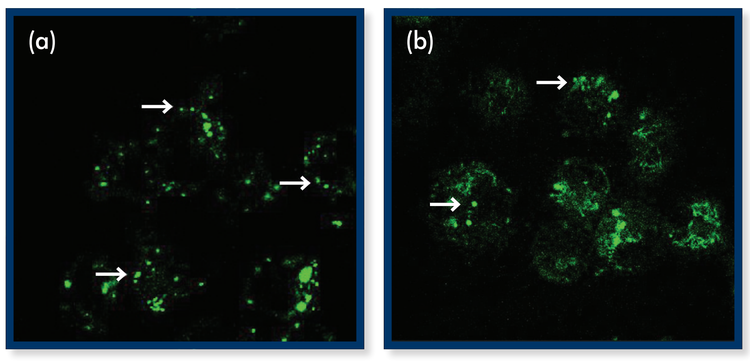

Figure 5: Live cell image of labeled MA nanoparticles taken up by (a) THP-1 and (b) U937 macrophages

DISCUSSION AND CONCLUSION

This study is the first to report successful encapsulation of MA into solid matrix like PLGA nanoparticles. The study aimed to assess the potential of PLGA nanoparticles as alternative carriers of pharmaceutical agents to be taken up in the body and processed in macrophages to deliver their beneficial biological effects to patients. In particular, MA was investigated, a highly hydrophobic molecule, as pharmaceutical agent that may be beneficial for the cure of asthma. Current research is focused on uptake and localisation studies within macrophages.

ACKNOWLEDGEMENTS

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More effective delivery of pharmaceutical agents to specific sites within the human body can help improve health. CSIR researchers have shown that nanoparticles may be suitable carriers or vehicles for immunotherapeutic agents used to fight asthma and tuberculosis.

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