CELLULAR UPTAKE OF BCG-LOADED CHITOSAN MICROPARTICLES AND IN VIVO EVALUATION OF IMMUNE RESPONSE FOLLOWING INTRANASAL IMMUNISATION

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INTRODUCTION

Attenuated *Mycobacterium bovis* bacillus Calmette-Guérin (**BCG**) is the only currently available **vaccine against tuberculosis.** It is highly effective in pre-exposure immunisation against TB in children when administered by subcutaneous route to newborns. However, it does not provide permanent protection in adults.

In this work, **polymeric chitosan-alginate microparticles** have been evaluated as potential **nasal delivery systems** and **mucosal adjuvants** for live attenuated BCG.

Chitosan (CS) has been employed as adjuvant and mucosal permeation-enhancer, and, together with alginate (ALG), as additive to enhance BCG-loaded microparticles (MPs) **cellular uptake** in a human monocyte cell line, by particle surface modification. The most suitable particles were used for vaccine formulation and evaluation of immune response following **intranasal immunisation** of BALB/c mice.

METHODS

Microparticles production:

- CS/ALG-BCG MPs were produced by by **ionotropic gelation.**[1]
- BCG (*) and TPP were dispersed in 0.1 % (w/v) CS (Sigma-Aldrich) solution, followed by 0.1 % (w/v) ALG (FMC BioPolymer) solution.[2]
  (*) *M. Bovis* BCG Pasteur (ATCC35734) and a recombinant *M. Bovis* BCG expressing Green Fluorescent Protein (GFP) were used.[3]

Characterization methods:

- **Particle size** → laser diffraction (Mastersizer2000, Malvern).
- **Zeta potential** → electrophoretic mobility (Malvern Zetasizer).
- **Cell uptake** → rBCG-loaded MPs were incubated in THP-1 cell cultures for 24h. Cells were observed under fluorescence microscopy. Multiplicity of Infection (MOI) = n rBCG cells /n THP-1 cells .[4]

Immunisation studies:

- Four groups of female BALB/c (n=4/group), 6-8 weeks old, were immunized with $1x10^7$ BCG Pasteur cells by subcutaneous or **intranasal administration**.
- **Specific IgG, IgG2a, IgG1** were determined using purified protein derivative (PPD) bovine tuberculin.

RESULTS AND DISCUSSION

Particle Size and Surface charge

CS/ALG MPs presented a size of 10.0–11.8 µm in diameter with narrow size distribution and zeta potential (ZP) ranging from -23.7 ± 0.2 to +12.1 ± 0.9 mV. In both cases, particles were spherical and non-aggregated. CS:ALG ratio 1:1 (L14) was chosen for BCG microencapsulation.

Table 1. *Particle size distribution and zeta potential of CS/ALG microparticles.*

Formulation	CS/ALG (w:w)	Mean particle size (µm)	Span	ZP (mV)	BCG Pasteur (CFUs/mL)	E.E. (%)
M13	1:0.8	11.8	1.6	+12.1 ± 0.9	Empty	--
L14	1:1	10.0	2.1	-23.7 ± 0.2	Empty	--
BCG/L14	1:1	22.2	6.5	-16.4 ± 2.1	1.7E+06	64%
BCG/CS	--	--	--	+90.6 ± 3.5	1.7E+06	--
BCG	--	--	--	-23.1 ± 11.5	1.7E+06	--

Span = [d (0.9) - d (0.1)] / d (0.5) ; E.E. - Encapsulation Efficiency

BCG-loaded MPS presented 22.2 µm in diameter, negative ZP (-14.4 ± 2.1 mV), and good encapsulation efficiency (64%).

BCG modification with CS turned ZP strongly to positive values (-23.1 mV to +90.6 mV). This could enhance microparticle uptake by THP-1 cells.

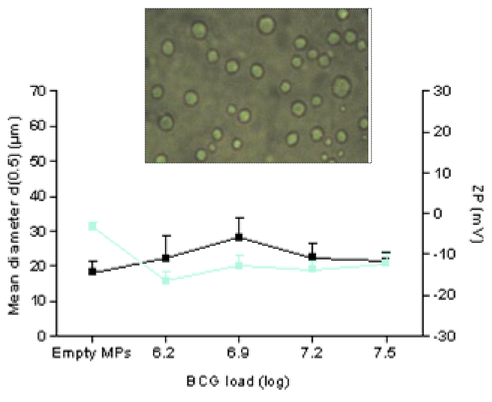

Figure 1. *Mean diameter, ZP and morphology of BCG-loaded MPs.*

THP-1 cell uptake

rBCG-loaded CS/ALG MPS were uptaken by THP1 cells. As expected, due to ZP, and surface area, monodispersed bacteria were more extensively internalized (MOI=10) than clumped bacteria (MOI=1).

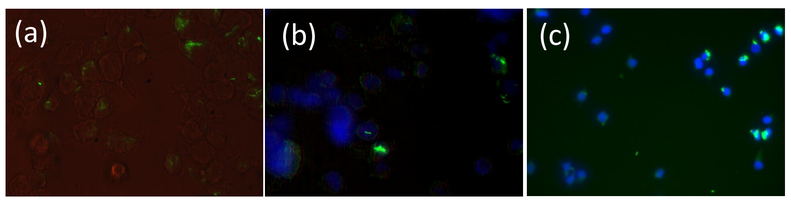

Figure 2. *Fluorescence microscopy images from THP-1 cells after 24h of incubation with: (a) plain rBCG; (b) rBCG-CS; (c) rBCG-CS/ALG MPs. GFP-expressing bacteria (green), Lysotrack labeled lysosomal compartments (red) and DAPI stained nuclei (blue).*

Immunisation results

Intranasal immunisation successfully elicited significantly higher IgG2a levels than in s.c. route. This predominant Th1 response indicates that intranasal immunisation is a suitable route for a cell-mediated immune response against TB. Mucosal stimulation was confirmed by increased sIgA levels in the lungs.

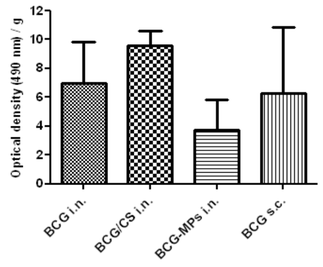

Figure 4. *Secretory IgA (sIgA) levels of lungs homogenates of immunised mice*

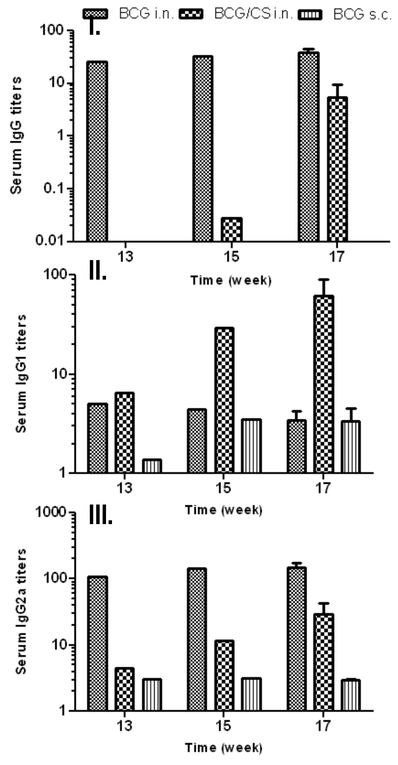

Figure 3. *Serum anti-M.tb specific IgG (I), IgG1 (II) and IgG2a (III) profiles of mice immunised by i.n. route with chitosan suspended BCG, compared to plain BCG and s.c. route.*

CONCLUSIONS

The developed particulate system allowed efficient whole live bacterial microencapsulation. BCG-loaded CS/ALG microparticles were efficiently uptaken by a human monocyte cell line, and improved Th1 cellular immune response in mice, as well as mucosal immunity. Thus CS/ALG MPs exhibit promising features potential as BCG vaccine carrier for mucosal immunisation.

References

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