Development and characterisation of a 3D cell culture model of the tuberculosis granuloma

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ABSTRACT

Tuberculosis (TB) is a disease caused by *Mycobacterium tuberculosis* (Mtb) that results in 1.6 million deaths yearly. The TB granuloma is the hallmark cellular structure of latent TB that contains the spread of infection[1]. More comprehensive in vitro models of TB that better resemble the cellular and immunoregulatory complexity of the granuloma would facilitate the study of the interplay between the bacteria and the different immune system cells[2]. We aim to generate an in vitro, 3D cell culture model of the TB granuloma that can be easily implemented using readily available commercial reagents and materials. A commercial encapsulation system based on sodium cellulose sulphate (NaCS) and Poly (diallyldimethylammonium chloride) (PDADMAC)[3] was used to generate small capsules containing human peripheral blood mononuclear cells (PBMC) in the presence of GFP-expressing Mtb H37Rv and maintained in culture for several weeks. The 3D structure formed by the cells inside and outside the capsules was evaluated by fluorescence microscopy and flow cytometry to distinguish the different cell types, and how they are organised inside the sphere and to measure cell survival and bacteria replication. The results show that human PBMCs readily form 3D cellular aggregates around infected cells and that cells cultivated outside the capsules are attracted and surround the capsules in response to infection. The model could be maintained for several weeks before bacteria-induced cell necrosis. PBMC's viability remained stable, with more than 80 % live cells following two weeks of culture. Moreover, adding an exterior layer of cells helped control bacterial replication, suggesting relevant communication between cells inside and outside the capsules to control the infection.

METHODOLOGY

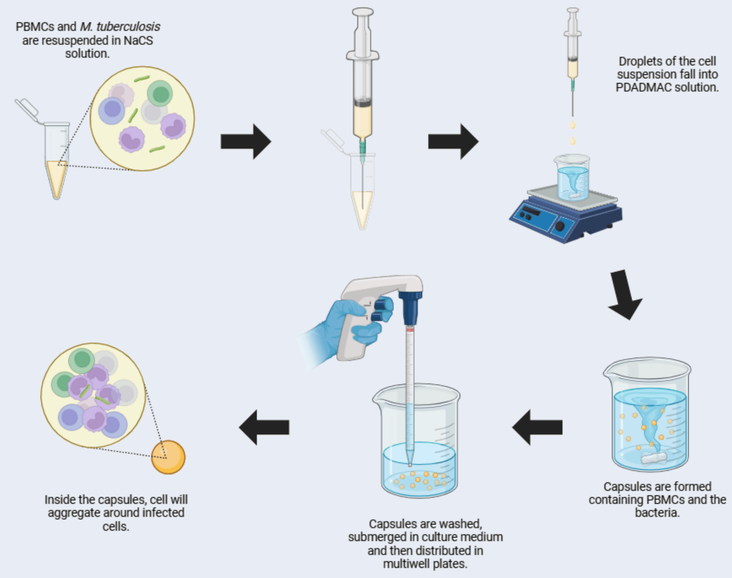

Figure 1. Workflow to generate encapsulated PBMCs infected with *M. tuberculosis*. Briefly, PBMCs were isolated from buffy coats of BCG-vaccinated healthy human donors by density-gradient. Approximately 0.1 Mtb H37Rv GFP per monocyte were added to the cells. The cell suspension was then resuspended in NaCs and aspirated into a syringe with a hypodermic needle. Applying constant force to the plunger, small droplets were generated and delivered into a PDADMAC solution under constant stirring, Capsules are formed upon contact with PDADMAC and subsequently washed three times with PBS and another three times with cell culture medium (RPMI1640 + 10 % FBS + NaPyr + HEPES + L-glutamine). Capsules are then ready for distributing and incubating as required.

RESULTS

A. Encapsulated and infected PBMCs or THP-1 cells form agglomerates around infected cells

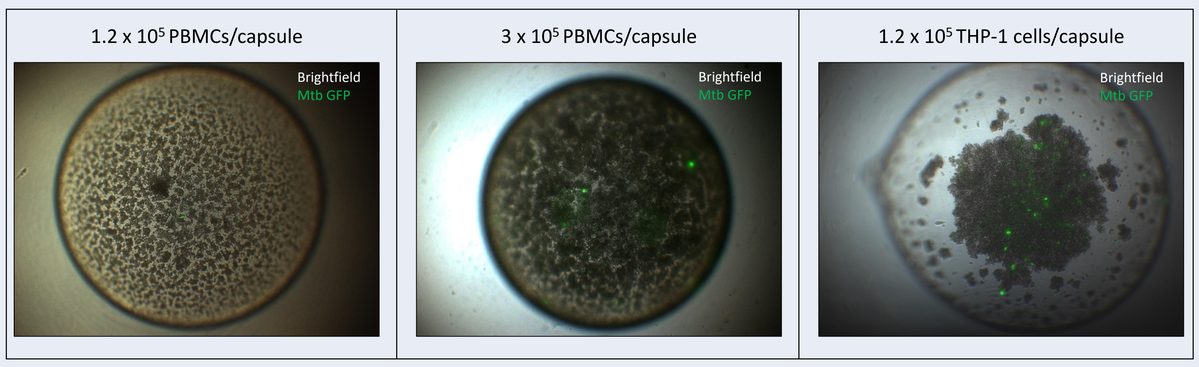

Figure 2. The visual aspect of encapsulated cells infected with Mtb-GFP according to the cell type and estimated number of cells per capsule. PBMCs isolated from human donors or THP-1 cells were infected with an MOI of 0.01 Mtb-GFP per cell (≈0.1 per monocyte) and simultaneously encapsulated. Images were taken after 5 days of culture and depict merged brightfield and GFP channels with a magnification of 40x. The diameter of the capsules is approximately 1.5 mm.

B. Encapsulated PBMCs maintain their viability while allowing the slow growth of Mtb

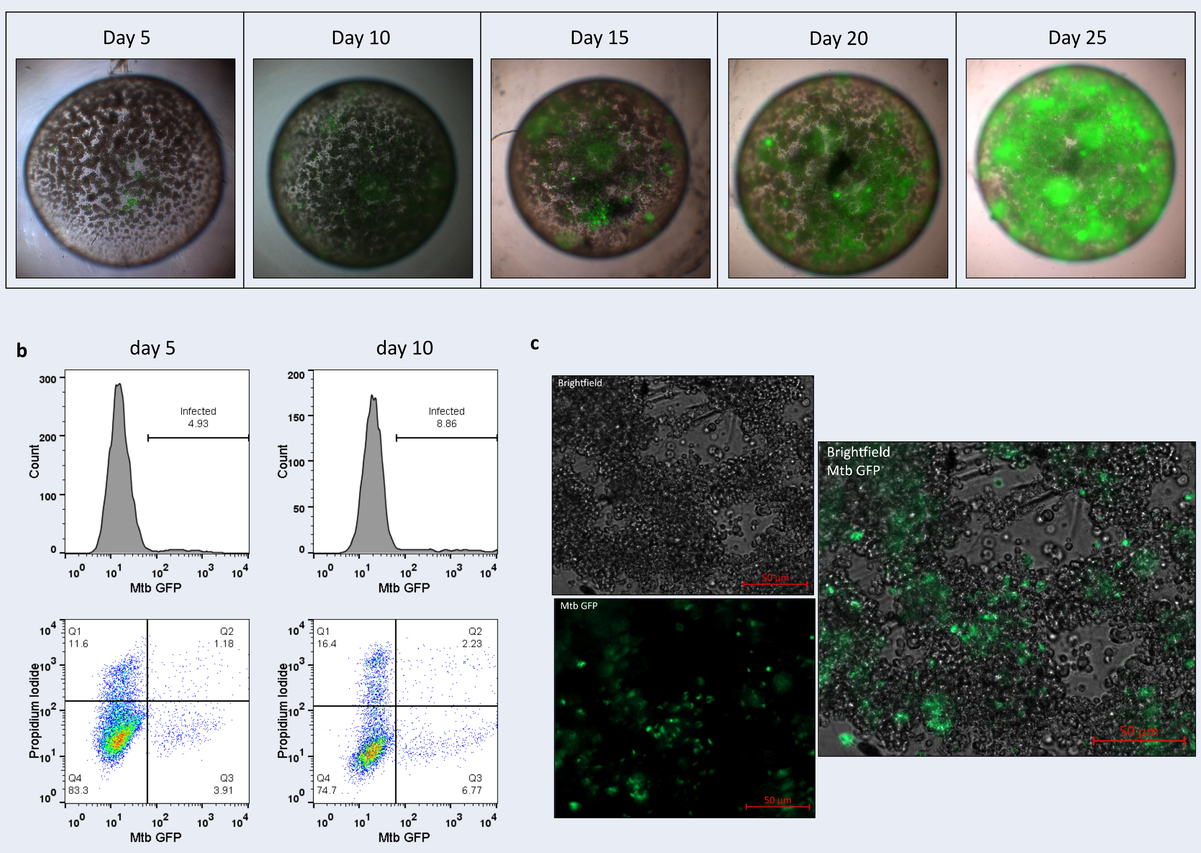

Figure 3. Characterization of encapsulated Mtb-infected PBMCs. (**a**) Growth of Mtb-GFP in infected and encapsulated PBMCs. PBMCs isolated from human donors were infected with an MOI of 0.01 Mtb-GFP per cell (≈0.1 per monocyte) and simultaneously encapsulated. Images were taken at selected time points following infection and encapsulation and depict merged brightfield and GFP channels. The diameter of the capsules is approximately 1.5 mm. (**b**) Analysis of infected PBMCs after decapsulation. Following selected time points the capsules were disrupted and the cells were recovered. Cells were stained with propidium iodide to assess cell death and analysed by flow cytometry. (**c**) Higher magnification image (40x) depicting the cellular aggregates formed around infected cells after 10 days of culture.

C. Addition of an outer cell layer slows Mtb growth inside the capsules

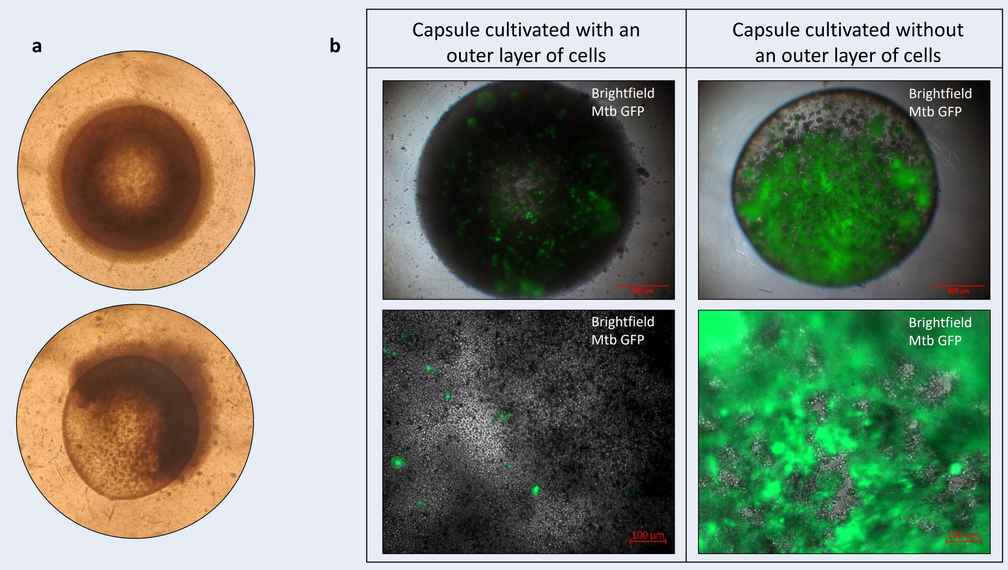

Figure 4. The addition of an outer layer of PBMCs helps control the infection inside the capsules. A mixture of 50 % $CD14^+$ monocytes and 50 % monocyte-depleted PBMCs was infected with an MOI of 0.05 (≈0.1 per monocyte) and encapsulated. Next, each capsule was cultivated with a 5:1 ratio of monocyte-depleted PBMCs. (**a**) Brightfield imaging of a capsule with a layer of surrounding cells after 10 days of culture. The bottom image depicts a tilted capsule. (**b**) Comparison of capsules cultivated with or without an outer layer of PBMCs following 15 days of culture.

CONCLUSIONS AND FUTURE PERSPECTIVES

The generated 3D infection model resembles some of the structural and cellular features of the TB granuloma and significantly improves the duration of infection experiments. Our preliminary results demonstrate the stability of this model for several weeks, which is fundamental for its potential future use in immunological and drug discovery studies. Further characterisation of the internal environment of the capsules and bacterial/immune cell gene expression will be required to assess if this model adequately reflects the features of the human granuloma.

REFERENCES

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