A 3D CELL CULTURE MODEL OF THE TUBERCULOSIS GRANULOMA THAT CAN BE APPLIED FOR HOST GENETIC STUDIES IN THE CONTEXT OF A MULTICELLULAR IMMUNOLOGIC RESPONSE TO INFECTION

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BACKGROUND AND OBJECTIVES

The granuloma is an inflammatory infiltrate of mononuclear cells. Some bacterial infections are characterized by the formation of granulomas as part of the immune response to contain the infection. Granuloma models have contributed valuable insights into the genetic basis of granuloma formation during infection. For example, IFNGR1 and IFNGR2 variants have been found to disrupt the immune response, resulting in impaired granuloma formation and increased susceptibility to diseases by *Mycobacterium* sp. More easily implemented comprehensive models would facilitate the study of the different immune mechanisms and help identify new disease-associated genes. Our objective is to generate an in vitro 3D cell culture model using human primary cells and microspheres to generate a stratified granuloma model for future use in genetic, immunological and drug discovery studies. A commercial system based on sodium cellulose sulphate (NaCS) and Poly (diallyldimethylammonium chloride) (PDADMAC)[1] was used to encapsulate human peripheral blood mononuclear cells (PBMC) infected with GFP-expressing *M. tuberculosis* (Mtb) and maintained in culture for several weeks. The cellular constituents of these granulomas and their organization were characterized by fluorescence microscopy and flow cytometry as well as the viability of the cells and the extent of bacterial replication in factor of time. The results demonstrate a ready recruitment of cells towards infected macrophages, leading to the formation of densely populated aggregates. These aggregates maintained cell viability for several weeks and displayed an enhanced control of bacterial replication compared to the more common monolayer infection models. Moreover, the capsules can be easily disrupted when required to isolate genetic material for further analysis.

METHODOLOGY

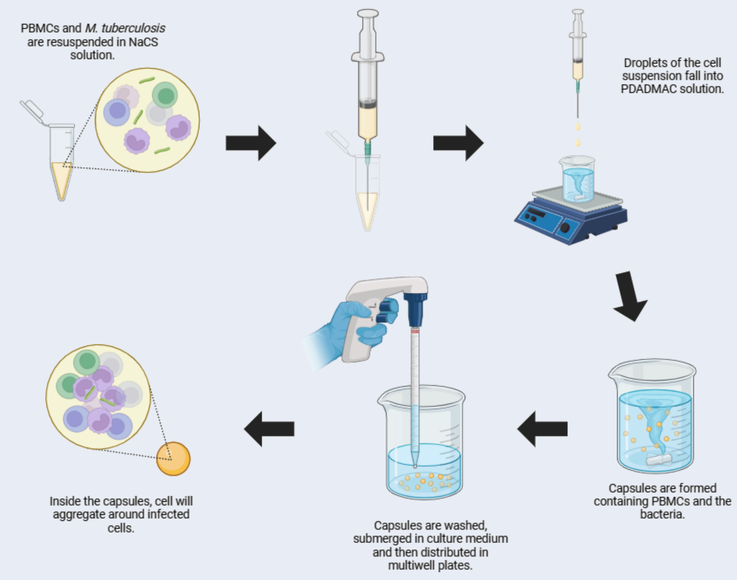

Figure 1. Workflow to generate encapsulated PBMCs infected with *M. tuberculosis*. Briefly, PBMCs were isolated from buffy coats of BCG-vaccinated healthy human donors by density-gradient. Approximately 0.1 Mtb H37Rv GFP per monocyte were added to the cells. The cell suspension was then resuspended in 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

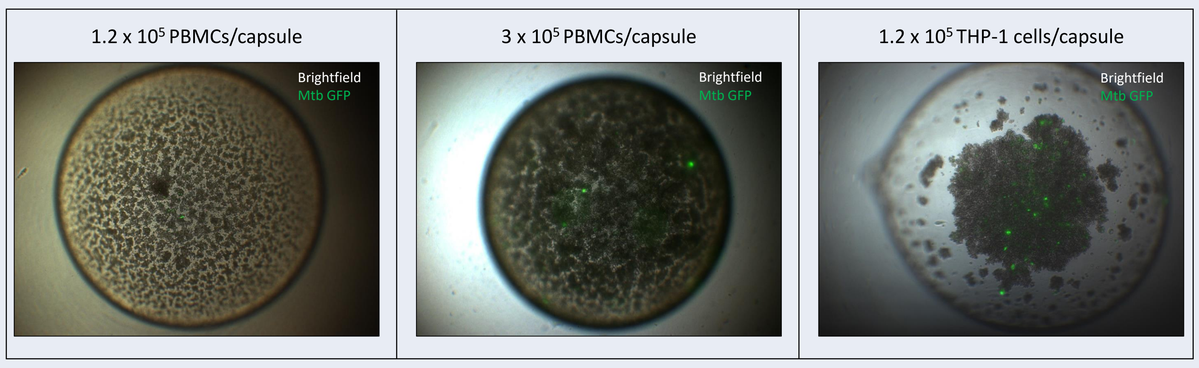

Figure 2. The visual aspect of encapsulated cells infected with Mtb-GFP according to the cell type and estimated number of cells per capsule. PBMCs isolated from human donors or THP-1 cells were infected with an MOI of 0.01 Mtb-GFP per cell (≈0.1 per monocyte) and simultaneously encapsulated. Images 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

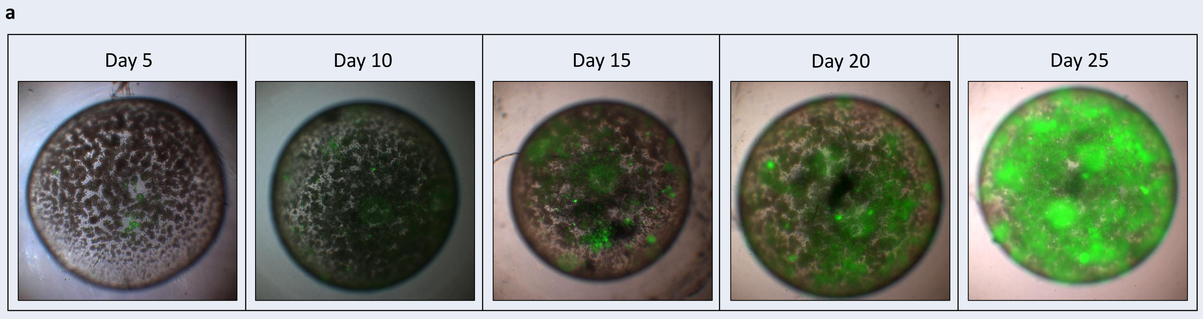

Figure 3. Characterization of encapsulated Mtb-infected PBMCs. (**a**) Growth of Mtb-GFP in infected and encapsulated PBMCs. PBMCs isolated from human donors were infected with an MOI of 0.01 Mtb-GFP per cell (≈0.1 per monocyte) and simultaneously encapsulated. (**b**) Analysis of infected PBMCs after 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 depicting the cellular aggregates formed around infected cells after 10 days of culture. Images were taken at selected time points following infection and encapsulation and depict merged brightfield and GFP channels with a magnification of 40x. The diameter of the capsules is approximately 1.5 mm.

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

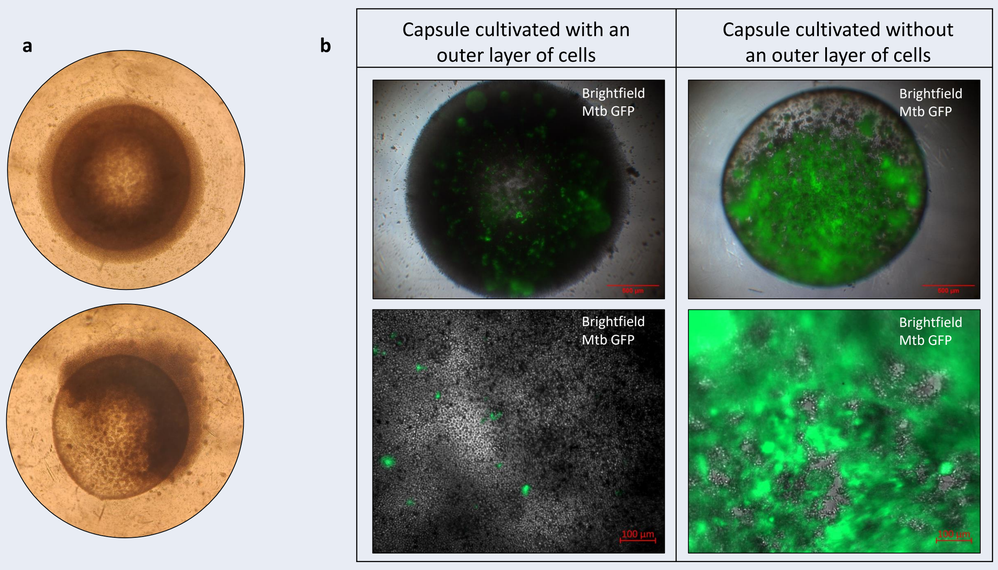

Figure 4. The addition of an outer layer of PBMCs helps control the infection inside the capsules. A mixture of 50 % $CD14^+$ monocytes and 50 % monocyte-depleted PBMCs was infected with an MOI of 0.05 (≈0.1 per monocyte) and encapsulated. Next, each capsule was cultivated with a 5:1 ratio of monocyte-depleted 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 proposed 3D model resembles some structural and cellular characteristics of the tuberculosis granuloma and maintains its stability beyond more common 2D models of infection. These preliminary results demonstrate that this model can be used to further explore the determinants of granuloma formation and host response to infection. Further characterization 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

[1] Dautzenberg, H., *et al.* (1999) *Ann. N. Y. Acad. Sci.*, 875: 46-63.

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