Viability of Co-Cultured Mycobacterium smegmatis and THP-1 Cell-Derived Macrophages Following Infection in vitro

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Since 2013, there has been a 14% increase of patients diagnosed with multidrug-resistant tuberculosis, causing a need to further study the tuberculin bacteria. Transmission of tuberculosis occurs via aerosolized Mycobacterium tuberculosis (M.tb) bacilli, and thus the initial host-M.tb interaction likely plays a determinate role in whether or not infection is established. Detecting M.tb-macrophage interactions requires quantitative single cell measurement. Therefore, a 3D infection model was created to establish bacterial viability alone following infection. Mycobacterium smegmatis (M.smeg), rotating at 140rpm in an anaerobic environment of 37°C, was loaded with 5-chloromethylfluorescein diacetate dye. THP-1 cells, growing in 37°C and 5% CO2, was loaded with Deep Red dye and treated with Phorbol 12-myristate 13-acetate over 3 days to differentiate into macrophages. M.smeg and macrophages were mixed into a silk-collagen hydrogel and incubated for 24 hrs. in 24-well plates with the following groups: 100% bacteria, 100% cells, MOI 1:1 (1 bacteria=1 cell), and MOI 10:1 (10 bacteria=1 cell). Confocal microscopy was performed before and after two 45-min. osmotic lysis treatments. An alamarBlue quantification assay was performed 2 and 16 hrs. after the osmotic lysis treatment. In conclusion, osmotic lysis significantly reduced viability of 100% cells compared to 100% bacteria. The fluorescent images confirm that not all macrophages were successfully lysed. A longer water incubation time may be required to lyse all mammalian cells in order to track bacterial viability alone. In future steps, a 3D tissue model that recapitulates human granuloma morphology and structure will be created to study early stages of M.tb pathogenesis using the established bacterial viability method.

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