

Supplementary Materials: Inhalable Antitubercular Therapy Mediated by Locust Bean Gum Microparticles

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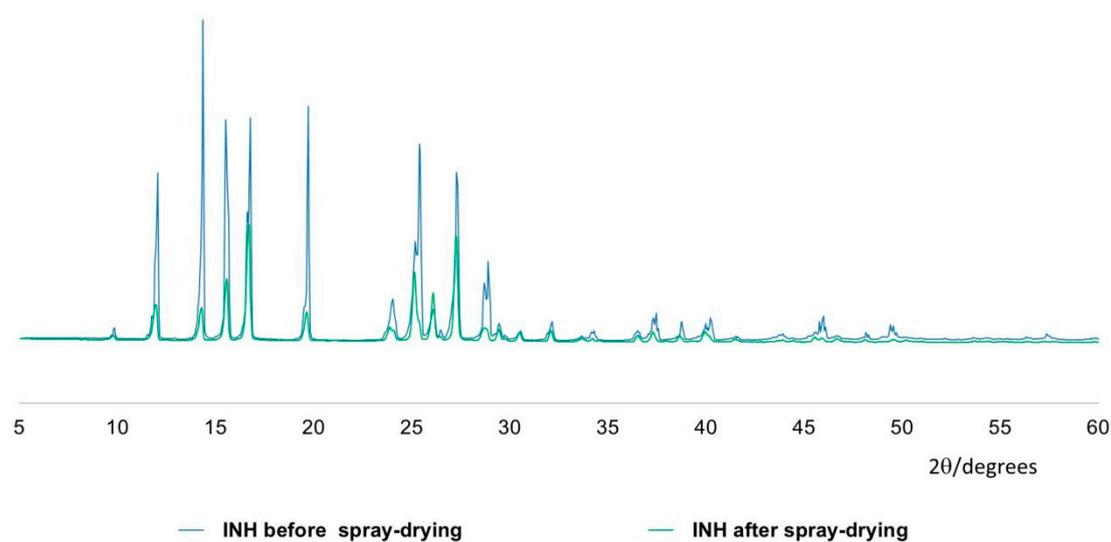


Figure S1. XRD spectra of isoniazid (INH) before and after spray-drying.

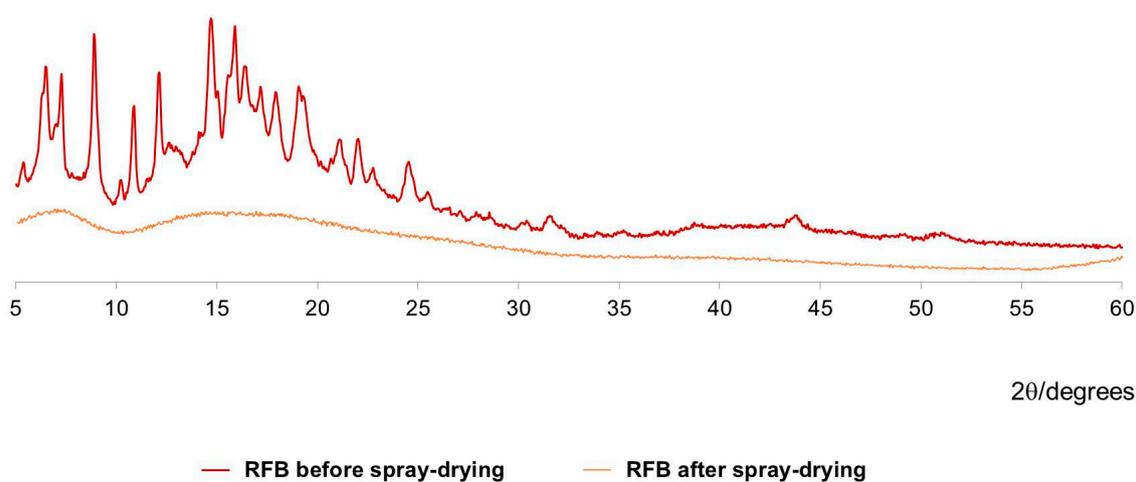


Figure S2. XRD spectra of rifabutin (RFB) before and after spray-drying.

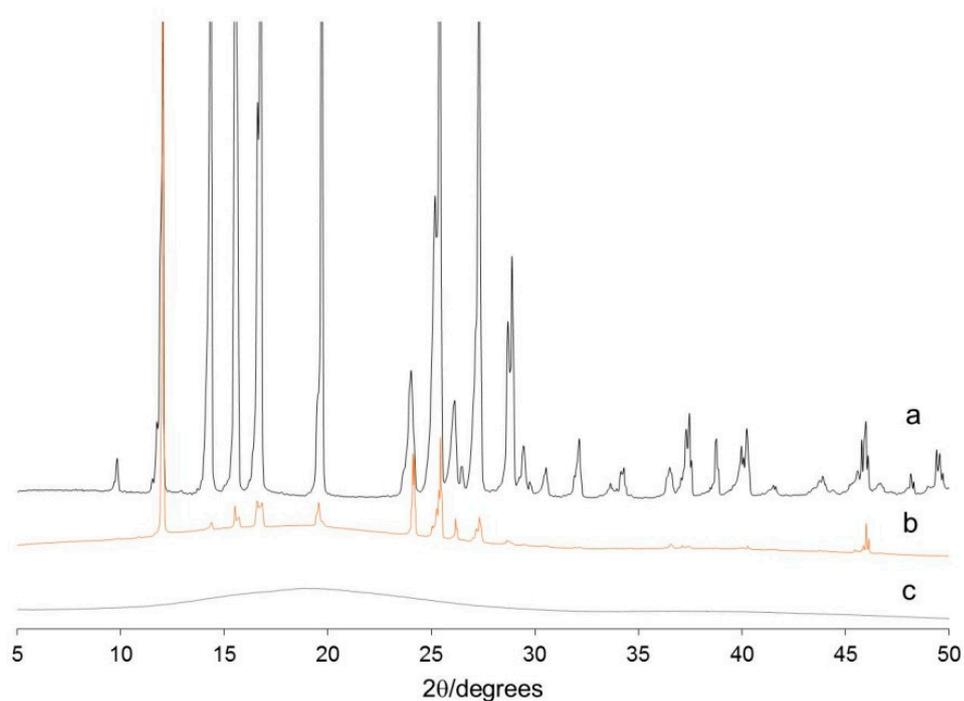


Figure S3. XRD spectra of (a) isoniazid (INH) in pure state; (b) physical mixture of locust bean gum (LBG) and isoniazid in mass ratio of 10/1; and (c) LBG.INH microparticles.

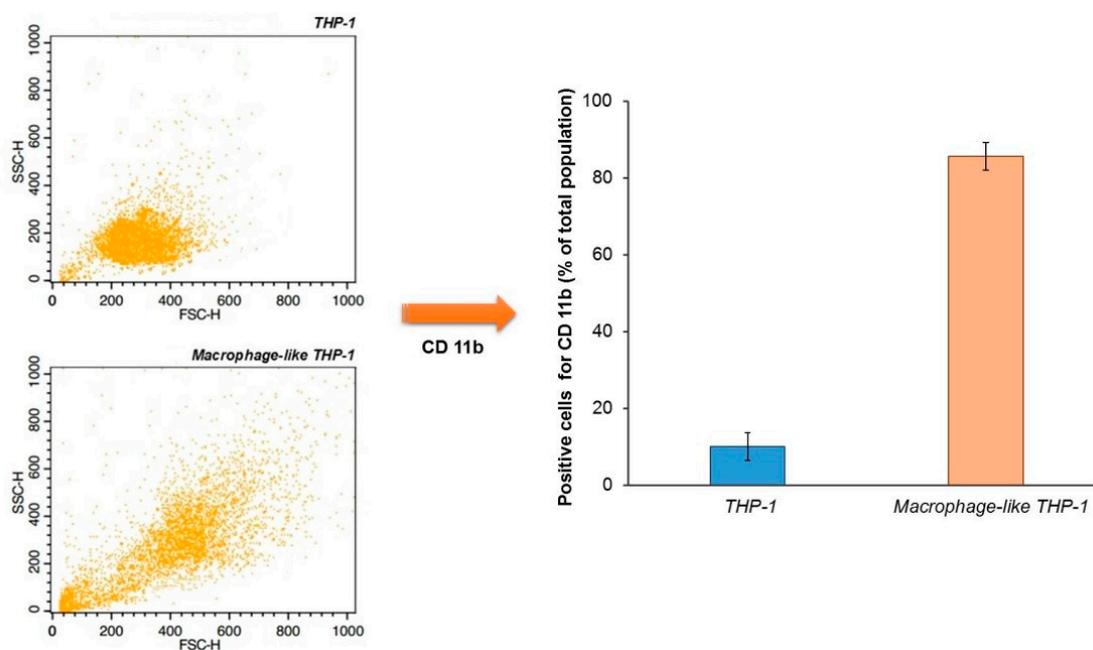


Figure S4. Immunocytochemical characterization of THP-1 cells and macrophage-differentiated THP-1 cells. Evaluation and comparison between size (axis FSC-H) and structure/complexity (axis SSC-H) of THP-1 and macrophage-like THP-1 cells. Macrophage-like THP-1 cells were differentiated by 48 h incubation with 50 nM of PMA. Marker anti-CD 11b labels the CR3 receptor present only in membrane of THP-1 differentiated cells. Results are expressed as mean \pm SEM.

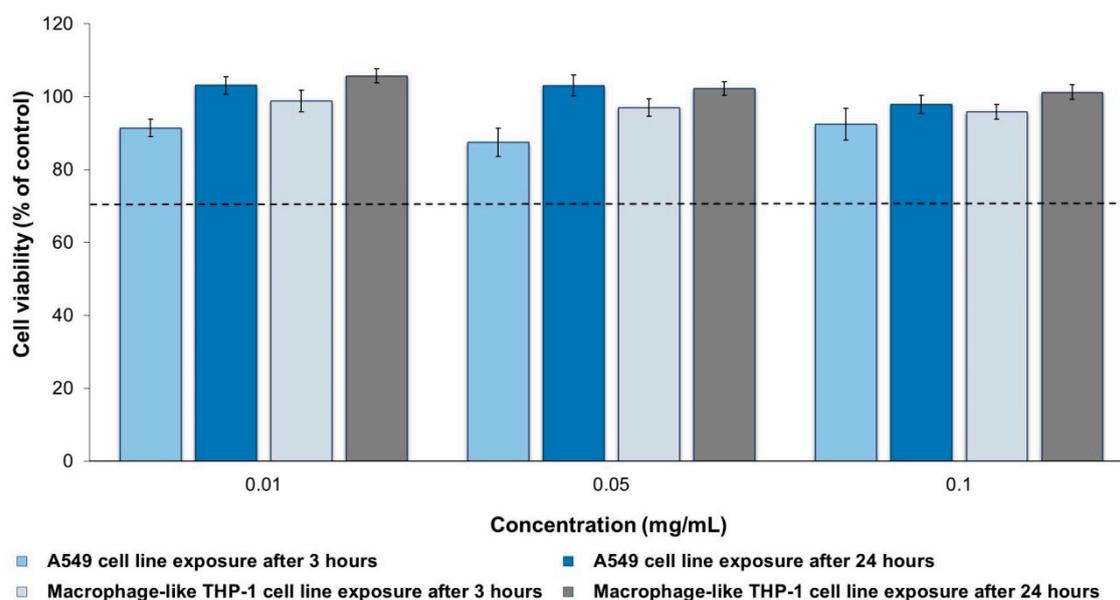


Figure S5. A549 (blue) and macrophage-differentiated THP-1 (grey) cell viabilities after 3 h (lighter colour) and 24 h (darker colour) of exposure to isoniazid. Results are expressed as mean \pm SEM ($n = 3$, six replicates per experiment at each concentration). Dashed line represents 70% cell viability.

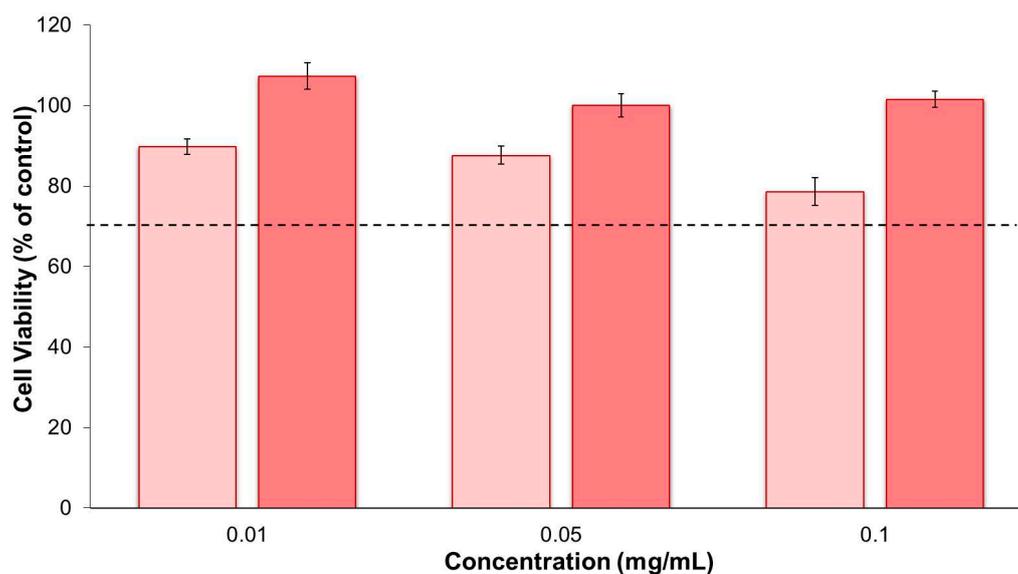


Figure S6. A549 (lighter colour) and macrophage-differentiated THP-1 (darker colour) cell viabilities after 3 h of exposure to rifabutin. Results are expressed as mean \pm SEM ($n = 3$, six replicates per experiment at each concentration). Dashed line represents 70% cell viability.

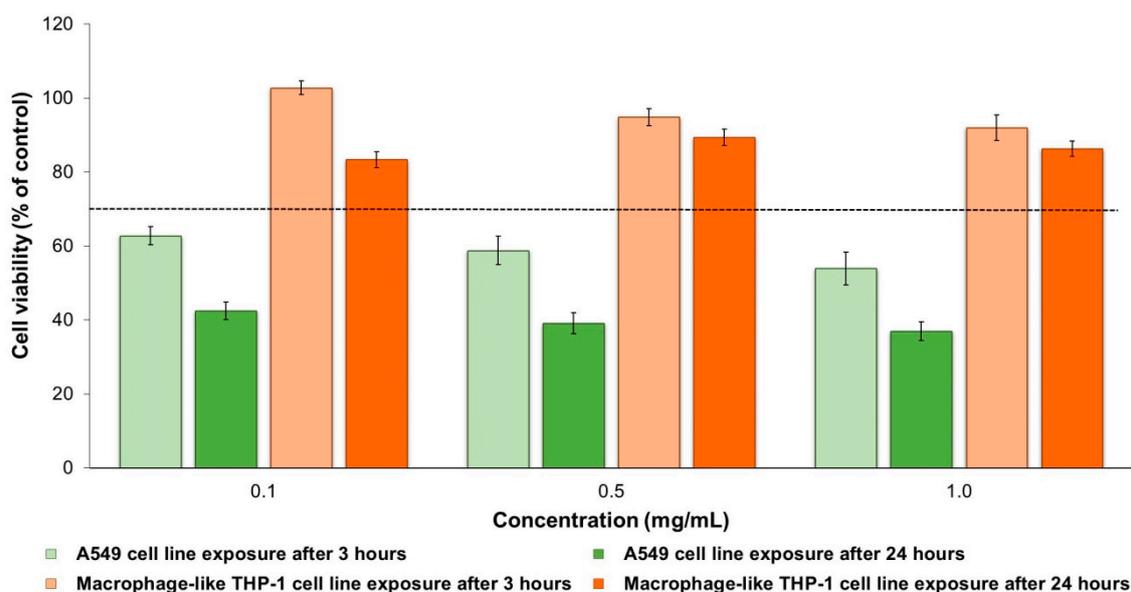


Figure S7. A549 (green) and macrophage-differentiated THP-1 (orange) cell viabilities after 3 h (lighter colour) and 24 h (darker colour) of exposure to raw material locust bean gum (LBG). Results are expressed as mean \pm SEM ($n = 3$, six replicates per experiment at each concentration). Dashed line represents 70% cell viability.

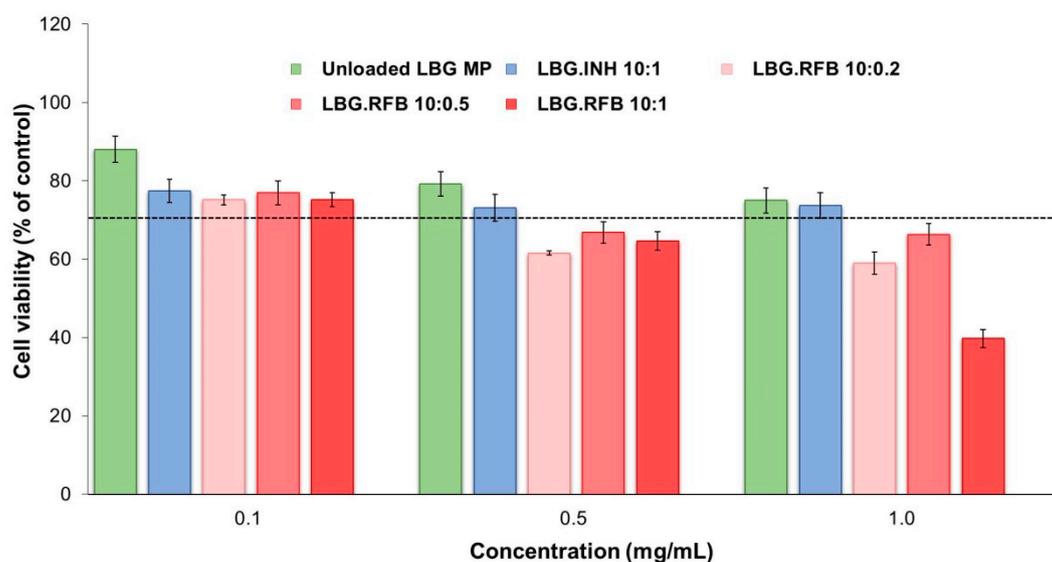


Figure S8. A549 cell viabilities after 3 h of exposure to LBG-based microparticle formulations. Results are expressed as mean \pm SEM ($n = 3$, six replicates per experiment at each concentration). Dashed line represents 70% cell viability (INH: isoniazid, LBG: locust bean gum, RFB: rifabutin).

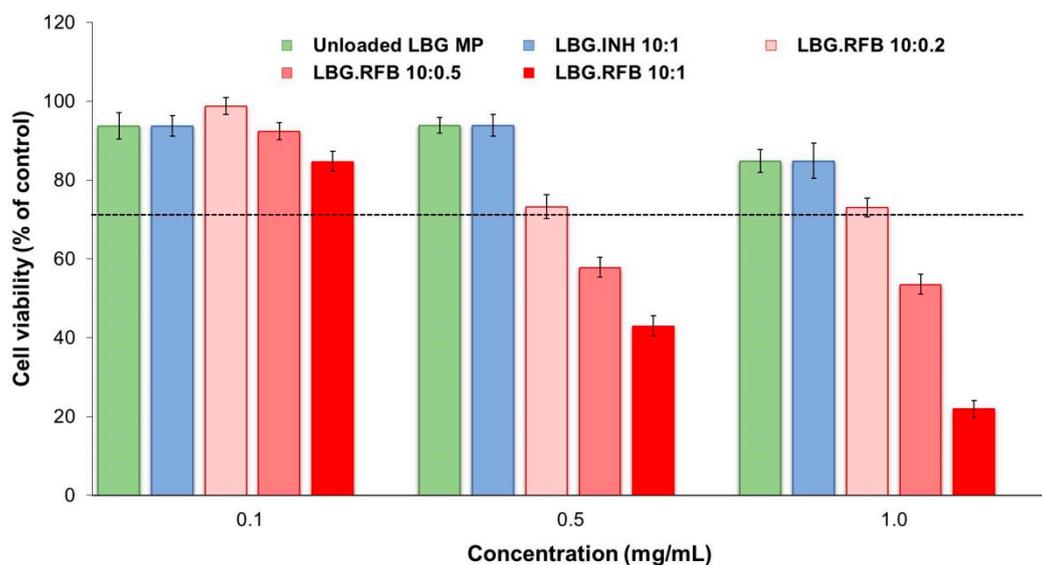


Figure S9. Macrophage-differentiated THP-1 cell viabilities after 3 h of exposure to LBG-based microparticle formulations. Results are expressed as mean \pm SEM ($n = 3$, six replicates per experiment at each concentration). Dashed line represents 70% cell viability (INH: isoniazid, LBG: locust bean gum, RFB: rifabutin).

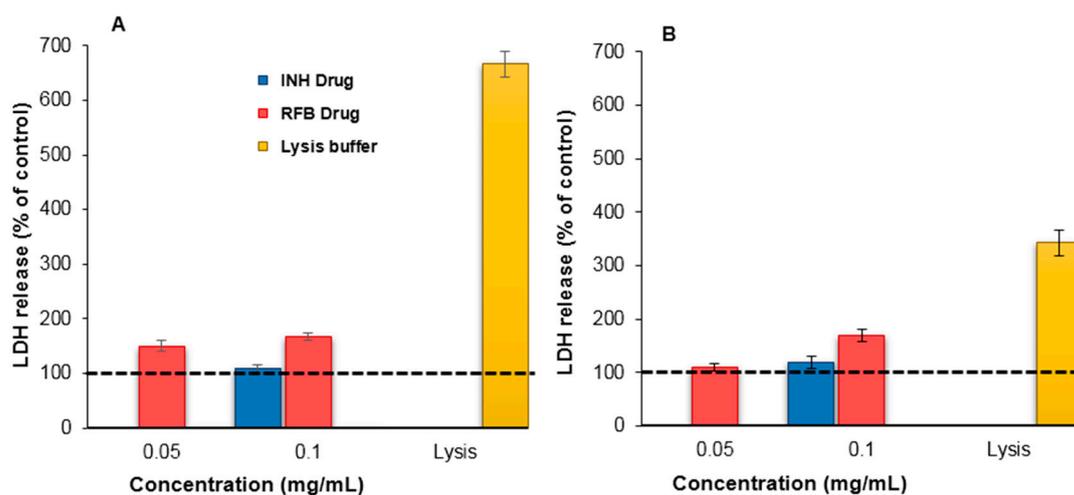


Figure S10. LDH released from A549 cells (A) and macrophage-like THP-1 cells (B) after 24 h exposure to free drugs (isoniazid: INH, rifabutin: RFB). Amount of LDH released from cells incubated with cell culture medium is assumed as 100% (dashed line). Results are expressed as mean \pm SEM ($n = 3$, six replicates per experiment at each concentration).

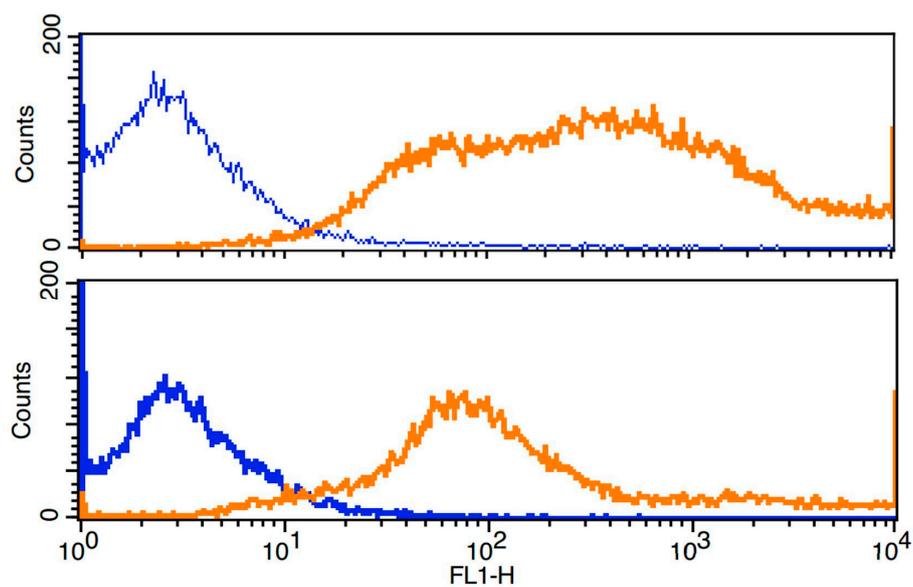


Figure S11. Fluorescent signal of macrophage-differentiated THP-1 (**upper** panel) and NR8383 cells (**lower** panel) upon 2 h exposure to $50 \mu\text{g}/\text{cm}^2$ of fluorescently labelled LBG microparticles (**orange** line) and unexposed cells, incubated with cell culture medium (**blue** line).