

Acoustic Tweezing Cytometry for Mechanical Phenotyping of Macrophages and Mechanopharmaceutical Cytotripsy

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Background, Motivation and Objective

Macrophages are immune cells responsible for tissue debridement and fighting infection. Drug treatment as well as pathophysiological states induce changes in macrophage mechanical property which in turn impact their phenotype and function. Clofazimine (CFZ) is an FDA-approved, small molecule drug and has been used to treat leprosy for decades as an effective antibiotic. It has also been investigated as a potential candidate to overcome resistant tuberculosis (TB), particularly if they can be targeted to the infection site. CFZ accumulates and precipitates as rod-shaped, crystal-like drug inclusions (CLDIs) within macrophage lysosomes. While soluble CFZ is cytotoxic, formation of CLDIs renders macrophages as massive drug depots that sequester the drug. However, it is unclear how these CLDIs alter mechanical properties of macrophages and affect macrophage function. In addition, little is known about how efficacy and safety profile of the drug are affected by its precipitation, self-assembly and intracellular accumulation within macrophages.

Statement of Contribution/Methods

We employed acoustic tweezing cytometry (ATC) as a new approach for *in situ* mechanical phenotyping of macrophages and for targeted macrophage cytotripsy for drug release. ATC applies targeted cyclic subcellular forces via integrin-targeted microbubbles (MBs). The acoustic radiation force of an ultrasound (US) pulse displaces the integrin-bound MBs without detachment. After US pulse, MBs retrack back toward their pre-US location. Application of a tone burst US exerted a controlled force to individual cells, enabling a creep test for measuring cellular mechanical property. Application of a series of US pulses generated cyclic forces to cells which may induce irreversible changes.

Results/Discussion

Our results revealed that macrophages with CLDIs became significantly softer with higher cell compliance, and behaved more elastic with faster creep and recovery time constants. On the contrary, phagocytosis of solid polyethylene microbeads or treatment with soluble CFZ rendered macrophages stiffer. Interestingly, actuation of the integrin-bound MBs by US pulses of longer duration and higher amplitude mobilized the CLDIs inside macrophages, turning the rod-shaped drug inclusions into intracellular microblender that effectively destructed the cells. Taken together, ATC provides a versatile platform for *in situ* measurement of cellular mechanical property, and cell stiffness may be used as a biomarker reflecting macrophage state and function. The phenomenon of acoustic mechanopharmaceutical cytotripsy may be exploited for US activated, macrophage-directed drug release and delivery.