Polymer Chemistry Influences Uptake and Intracellular Integrity of Polyanhydride Microspheres in Dendritic Cells

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Statement of Purpose: To examine the relative affects of polymer chemistry on the uptake and intracellular fate of polyanhydride microspheres in murine dendritic cells.

Methods: Aqueous Suspension of Polyanhydrous Microspheres: Non-aqueous cryogenic atomization method was used (1) to prepare 3% (g/ml) FITC-dextran loaded MS formulations. Varying ratios of Sebacic Acid (SA), 1,6 bis(p-carboxyphenoxy)hexane (CPH) and 1,8-bis(p-carboxyphenoxy)-3,6-dioxaoctane (CPTEG) were used as polyanhydride chemistries. Aqueous suspensions of MS were prepared for each chemistry in PBS (pH 7.4), sonicated for 1 minute and kept on ice until added to DC cultures.

DC Isolation and Cellular Interactions with Microspheres: Murine dendritic cells (DCs) were derived from the bone marrow of C3H mice following 8 day stimulation *in vitro* (2) and plated at a density of $1x10^6$ / well onto glass coverslips in 24 well tissue culture plates. The following day, cellular interaction studies were initiated by adding equivalent amounts of MS (100µg/well), incubated for 30 minutes in 37^0C, CO_2 incubator, washed 3x with PBS to remove extracellular-nonattached MS, and incubated for either an additional 2h or 48h. At the indicated times, cells were fixed with 4% paraformaldehyde, washed and stained for either polymerized actin (Alexa564-conjugated Phalloidin; Invitrogen) or the lysosome associated membrane protein Lamp1 (Developmental Studies Hybridoma Bank, Iowa City, IA). The Lamp1 (ID4B clone) antibody was detected by subsequent incubation with TRITC conjugated anti-Mouse secondary antibodies (Jackson ImmunoResearch, West Grove, PA). Visualization of cellular interactions employed either an Olympus IX-71 epifluorescence microscope or a Leica NTS-LSCM equipped with 488 nm and 564 nm lasers. Image analysis was performed using NIH Image J analysis software. Intracellular particles were quantified from randomly selected fields of view using 100x NA 1.4 oil objective.

Results/Discussion: Incubation of MS with DCs for 48 hrs did not negatively impact cell health in any observable way. Internalized MS were examined for FITC-MS within the plasma membrane (delineated by polymerized cortical actin) (Figure 1). Incubation of DCs with MS for 30 minutes was sufficient enough time for individual cells to internalize MS. Quantification of intracellular particles was performed in ImageJ using images of at least 5 randomly selected, separate fields of view (Figure 3). Densities of DCs were equivalent across all chemistries. Comparing size distribution of internalized particles at 2 hrs revealed that the higher [SA]-MS were internalized better and retained initial microparticle size (20-50 µm) (Figure 1). In contrast, , only smaller 2-5 µm particles of CPTEG/CPH chemistries were internalized.

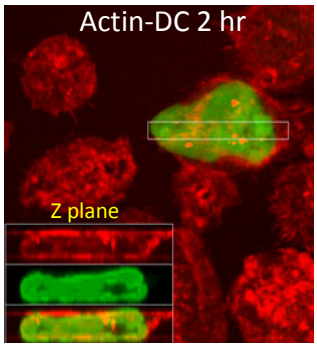

Figure 1. Poly-SA MS (green) inside MuDC (polymerized actin-red) at 2hr post-internalization. Confocal side Z-plane (insert) was generated from cropped area (yellow box) to highlight intracellular location of large MS within a single cell.

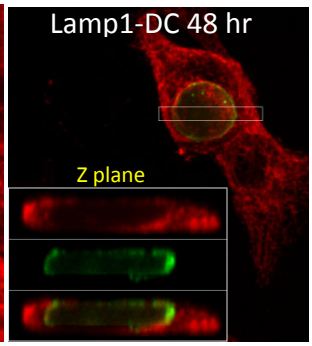

Figure 2. Poly-SA MS (green) inside MuDC (polymerized actin-red) at 48 hr incubation. Confocal side Z-plane (insert) generated from cropped region (yellow) shows poly-SA MS inside the late endosomal/lysosomal compartment of MuDCs.

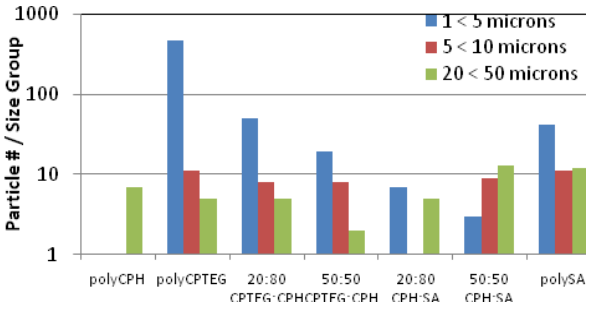

Figure 3. Size Distribution of intracellular MS at 2 hrs

Conclusions: Microspheres of different particle chemistries were internalized at different efficiencies whereby 20:80 CPH:SA MS represented the best combination of uptake efficiency and sustained intracellular integrity over 48hrs. Once inside DCs, MS were delivered to the late endosome/lysosomal compartment.

References:

1. Determan AS. Journal of Microencapsulation. 2006; 23(8): 832 – 843.
2. Lutz MB. Journal of Immunological Methods. 1999; 223:77-92.