ORT.20 - Screening Immunotherapies in Polymer Solution Microreactors: System Evaluation and Optimization

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Introduction: Immunotherapy agents are broadly defined as molecules that modulate the immune system, thereby amplifying or suppressing immune responses. This important category of drugs, which includes vaccines and various other biologics, has been used for prevention and treatment of several diseases. However, conventional techniques for assessing immunotherapies have some drawbacks, including the need for substantial amounts of both cells and reagents, and a demand for complex and expensive procedures.

Objective: To develop a polymer-based screening to efficiently identify and evaluate novel immunotherapies in which small amounts of immune cells and reagents are confined together in microreactors.

Methodology: A small library of polymer solutions including albumin, alginate, dextran (Dex), Ficoll, gum arabic, hydroxypropyl cellulose, hydroxypropyl methylcellulose, methyl cellulose, poly(2-ethyl-2-oxazoline), poly(ethylene glycol) (PEG), and poly(ethylene oxide) (PEO), were initially screened and selected in terms of handling characteristics (i.e., viscosity, phase-separation, and compatibility with salts used in culture medium). After selecting the most appropriate candidate systems, cell viability (calcein-AM/propidium iodide staining), phenotype (aggregation and cell morphology), cytokine secretion (ELISA assay), proliferation (Ki-67 immunofluorescence), and metabolic activity (ATP production) were assessed. Polymer solutions above the critical concentrations required for phase-separation were incubated with various types of immune cells for up to 7 days. The following immune cell lines were used: K-562 (human lymphoblast), RPMI 8226 (human B lymphocyte), and Jurkat (human T lymphocyte). The polymers that met the criteria were selected for optimization in terms of handling, cytotoxicity, and biomolecule confinement (cytokines, antigen, and vaccine adjuvants).

Results: Overall, the phase-separating polymers PEG/PEO, Dex, and cell culture grade BSA (Albumax) performed best with cells. Jurkart T cells cultured in Albumax BSA over 72 hours resulted in 98% cell viability against < 40% cell viability of either technical grade BSA from Sigma or HyClone. When the cells were cultured in Dex solution, cell viability was slightly lower compared to Albumax BSA (~ 97%). Although PEOs exhibited superior cell viability compared to PEG, the latter had more appropriate handling properties. These results suggest that PEG-BSA system is comparable to PEG-Dex system in terms of maintaining cell viability over 72 hours. Jurkat T cell activation was determined by IL-2 secretion. Overall, the polymer solutions did not stimulate IL-2 secretion over 24 hours, which suggests that PEG/PEO, Dex, and BSA do not activate the cells and can be used as a screening platform for vaccine adjuvants.

Conclusion: This study demonstrated that both PEG and Dex solutions can confine immune cells within polymer solution microreactors without significantly compromising cell viability, proliferation, and metabolic activity. Using this system to screen immunotherapy agents may facilitate the development of more effective vaccine adjuvants formulations and other immunotherapies.

Keywords: immunotherapies; screening; aqueous two-phase systems