Harnessing Biomechanical Force and Other Cellular Stressors in Extracellular Vesicle Biomanufacturing

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Extracellular vesicles (EVs) are rapidly gaining attention as key mediators of intercellular communication. These lipid bilayer-bound, submicron-size particles are produced by all cells and exert substantial influence over cellular phenotypes through the delivery of protein and nucleic acid cargo. EVs from a given cell type are often assumed to carry predictable cargo loads and exhibit consistent bioactivity. These assumptions, however, are deeply flawed. This dissertation forwards the thesis that EV biogenesis and properties vary significantly in response to several stressors, the most notable of which is biomechanical force. Standardizing EV production and understanding the interrelationships between EV properties and the production environment are thus vital for the realization of any large-scale, clinical implementation of EV technology.

This dissertation begins with a novel and comprehensive review of the current literature concerning the impacts of biomechanical force on EV biology. EV formation, composition, and bioactivity are individually examined, and implications for EV bioprocessing/biomanufacturing are discussed. Thereafter, specific relevant experimental findings are presented for large EVs from both megakaryocytes and Chinese hamster ovary (CHO) cells. Megakaryocyte-derived EVs (MkEVs), which promote the megakaryocytic differentiation of hematopoietic stem and progenitor cells, were observed to be highly sensitive to biomechanical force. Depending on the type, magnitude, and duration of force applied to parent megakaryocytes, MkEVs were either
more or less effective in promoting megakaryocytic differentiation and carried correspondingly variable levels of key microRNA cargo. Biomechanical force also promoted the formation of larger numbers of MkEVs per parent cell. Additionally, parent cell age and differentiation history were found to influence MkEV production rates. Among EVs derived from CHO cells (CHO EVs), both culture age and metabolite (ammonia, lactate, and osmotic) stress significantly influenced total and individual microRNA and protein cargo loads. Novel pro- or anti-apoptotic roles for highly abundant microRNA cargo were also identified. Moreover, microRNA concentrations in both MkEVs and CHO EVs were observed to be highly enriched relative to concentrations found in parent cells, suggesting deliberate (not passive) EV loading action by the cells.

In sum, this dissertation illustrates the ways in which EV quality is highly dependent on production conditions, especially where megakaryocytes and CHO cells are concerned. In some ways, these results provide tantalizing possibilities for the use of EVs as culture and clinical diagnostics, capable of detecting—and differentiating between—various stressors. In another sense, however, the findings of this dissertation warn of the challenges ahead for EV bioprocessing/biomanufacturing, where stressors—especially biomechanical force—represent a ubiquitous impediment to quality control and product homogeneity.