THE DYNAMIC MASS SPECTROMETRY PROBE (DMSP) – ADVANCED PROCESS ANALYTICS FOR THERAPEUTIC CELL MANUFACTURING, HEALTH MONITORING AND BIOMARKER DISCOVERY

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Spatially and temporally resolved *in situ* monitoring of biochemical cell culture environments, e.g., in application to therapeutic cell bioreactors, is of critical importance for facilitating the development of new and reliable quality control methodologies for cell therapies. Identifying and monitoring secreted biomolecular critical quality attributes (CQAs) to enable online feedback control will enable large scale, cost-effective manufacturing of therapeutic cells. These CQA biomarkers have varying concentrations within a bioreactor, both in time and space. Current methods for monitoring these diverse biomolecules are generally ex-situ, time consuming, destructive, provide bulk measurements, or lack the ability to reveal the complete secretome/metabolome composition. The Dynamic Mass Spectrometry Probe (DMSP) synergistically incorporates a sampling interface for localized intake of a small fluid volume of the cellular content, a micro-fabricated mass exchanger for sample conditioning and inline separation, and an integrated electrospray ionization (ESI) emitter for softly ionizing (i.e. preserved biochemical structure) extracted biomolecules for mass spectrometry (MS). ESI-MS via DMSP treatment enables both biomarker discovery and transient (~1 min) analysis of biochemical information indicative of cell health and potency. DMSP is manufactured using advanced batch microfabrication techniques, which minimize dead volume (~20 nL) and ensure repeatable operation and precise geometry of each device. DMSP treatment removes 99% of compounds that interfere with mass spectrometry analysis, such as inorganic salts, while retaining biomolecules of interest within the sample for ESI-MS analysis. DMSP has demonstrated the ability to substantially increase signal to noise ratio in MS detection of biomolecules, and to further enhance sensitivity for probing lower biomarker concentrations via introduction of ESI-MS enhancing molecules (i.e. proton donating chemicals, protein denaturing solvents, and supercharging agents) into the sample within the integrated mass exchanger. To exemplify the DMSP's unique capabilities, Fig. 1 demonstrates detection of multiple low-concentration protein biomarkers sampled from a biochemically-complex cell media solution serving as a proxy to samples taken directly from cell growth bioreactors [1].

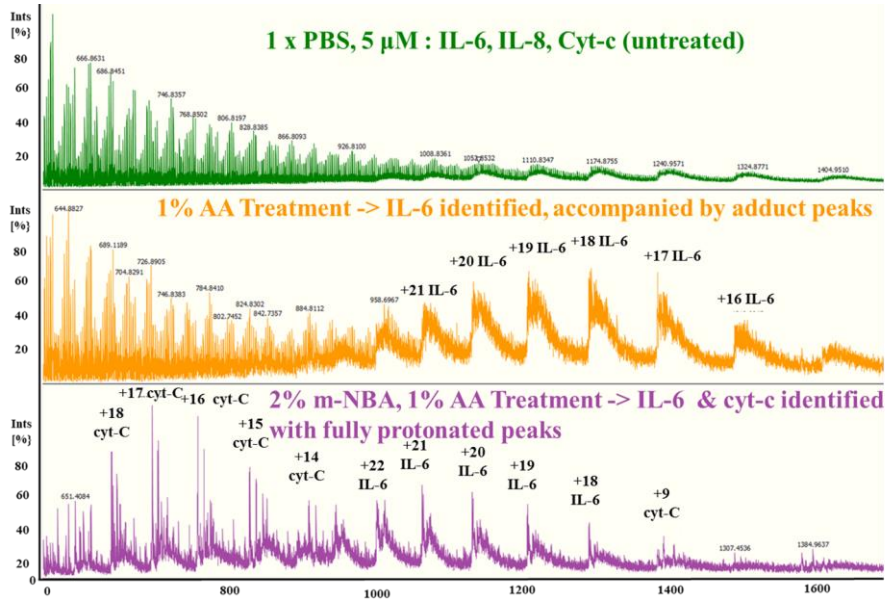

Figure 1 - Mass spectra produced via direct infusion ESI-MS through DMSP A. Untreated 1xPBS with 5 uM cytochrome-c (12 kDa), 5 µM IL-6 (21 kDa), and 5 µM IL-8 (8.4 kDa) shows no identifiable peaks associated with protonation of biomolecules. B. 1% AA treatment reveals multiple charge states associated with IL-6 only. C. 1% AA 2% m-NBA treatment reveals fully protonated charge states of cytochrome-c and IL-6.

[1] Chilmonczyk, M. A., Kottke, P. A., Stevens, H. Y., Guldberg, R. E., Fedorov A. G Dynamic mass spectrometry probe (DMSP) for ESI-MS monitoring of bioreactors for therapeutic cell manufacturing, *Biotech & Bioeng*. (subm).