Measurement of Cytokines Collected from Lipopolysaccharide-Stimulated Monocytes

Robert J Schutte, Dean Wang, W. Monty Reichert
Department of Biomedical Engineering, Duke University, Durham, NC 27708

Statement of Purpose: Macrophage derived cytokines secreted in response to a foreign material orchestrate the actions of cells in the surrounding tissue throughout the lifetime of an implant [1]. Since this immune response varies with the composition of a material, it is expected that each material induces a unique set of intercellular signals. Thus, cytokine profiles secreted by monocyte cultures exposed to a biomaterial could be indicative of the in vivo immune reaction to that material.

Continuous in vivo cytokine sampling is possible from virtually any tissue using the microdialysis technique. Microdialysis probes are miniature continuous perfusion devices that acquire analytes from the surrounding medium via passive diffusion through a semi-permeable membrane. Because microdialysis sampling is dependent on molecular diffusion through a membrane, the analytes that are recovered are only a fraction of the available analytes in the surrounding environment. In vitro microdialysis sampling allows for an estimation of the recovery rates of analytes.

In this study, the profiles of several inflammatory cytokines secreted by monocytes in response to lipopolysaccharide (LPS) stimulation were examined and compared to the results of previous studies [2].

Cytokine	*MIP-1α*	*TNF-α*	*IL-10*	*IL-6*	*IL-1β*
Concentration (pg/mL)	120,000	25,000	120	60	0

Table 1 *Cytokine concentrations detected in monocyte culture medium after 4 hours of exposure to LPS [2].*

Samples collected via microdialysis were also examined for cytokine content and used to determine the recovery rates of inflammatory cytokines through the microdialysis probe membrane.

Methods: LPS (Sigma) was added at 10μg/mL to the culture medium of human monocytes (THP-1, ATCC) seeded at 250,000 cells/mL. Samples were directly removed with a pipet from the culture medium at 0, 1, 2, 3, 24, and 48 hours after the addition of LPS. Cytokines were also sampled through 100kDa molecular weight cut-off, polyethersulfone microdialysis probes (CMA Microdialysis) immersed in the monocyte culture medium. These probes were perfused with degassed RPMI medium at 1μL/min. Samples were collected at the probe outflow at the same time points as above. Concentrations of MIP-1α, IL-1β, TNF-α, and IL-6 were analyzed using an anti-cytokine protein microarray [3].

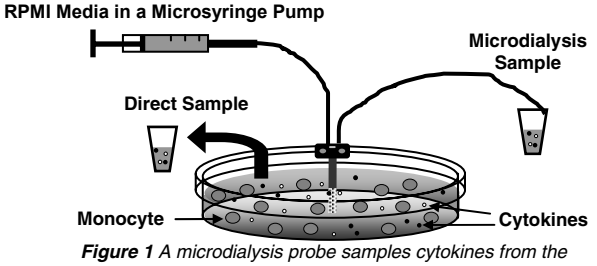

Figure 1 *A microdialysis probe samples cytokines from the surrounding LPS-stimulated monocyte media.*

Results / Discussion: MIP-1α concentrations in samples taken directly from LPS-stimulated human monocytes rose to 12ng/mL after 3 hours and continued to climb to 310ng/mL after 48 hours. Of the other cytokines only IL-1β, which was present at 6ng/mL after 48 hours, rose significantly above the baseline level.

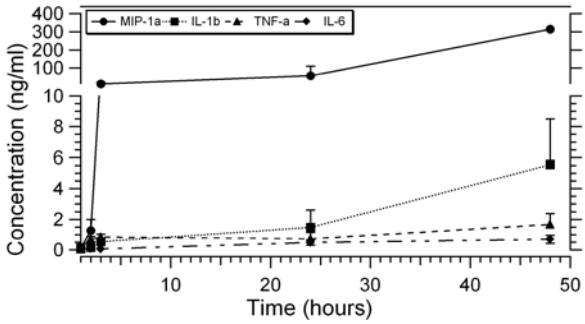

Figure 2 *Cytokines sampled directly from the surrounding LPS-stimulated monocyte media.*

The MIP-1α concentration in samples taken *via microdialysis* from LPS-stimulated human monocytes was 3ng/mL at 3 hours and 31ng/mL at 48 hours. Other cytokines were not detected significantly above baseline concentrations.

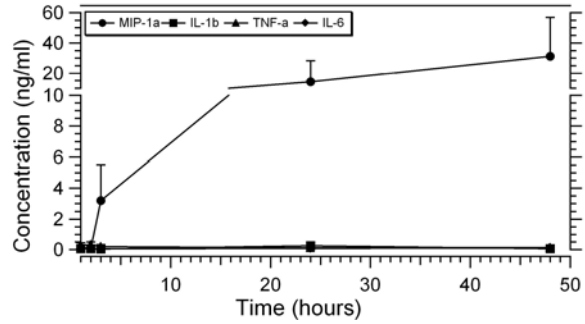

Figure 3 *Cytokines sampled via microdialysis from the surrounding LPS-stimulated monocyte media.*

The MIP-1α concentrations from samples taken directly from the monocyte culture medium were similar to the results of other researchers [2]. MIP-1α recovery rates from microdialysis probes ranged from 10-25%, which correlates well with previous protein microdialysis studies [4]. TNF-α was measured at lower concentrations than described in previous studies, which is likely due to the erratic performance of the TNF-α matched antibody pairs used with the anti-cytokine protein microarray.

Conclusions: LPS-treated monocytes secreted similar levels of MIP-1α to those found by previous researchers [2]. IL-1β was detected at 6ng/mL 48 hours after LPS stimulation. Microdialysis sampling collected a similar temporal profile of cytokines to the directly extracted samples.

References:

1. Anderson J. *Annu. Rev. Mater. Res.* 2001. 31:81-110
2. www.signaling-gateway.org
3. Li Y. et al. *Langmuir*, 2003. 19, 1557-1566
4. Schutte R. et al. *Anal. Chem.* 2004. 76, 6058-6063