Directing Macrophage Polarization with Microdialysis Probe Implants: Perfusion Fluid and IL-4 Effects

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Statement of Purpose: Macrophages are highly versatile cellular players during the foreign body reaction (FBR) and exist in different phenotypic or "polarization" states. Pro-inflammatory, classically activated, M1 phenotypes are produced when exposed to bacterial endotoxin or type 1 cytokines. Alternatively activated, anti-inflammatory, M2 phenotypes are induced by different cytokines including interleukin-4 (IL-4), IL-10, and IL-13. There is significant recent interest in the polarized states of macrophages in the fields of regenerative medicine and biomaterials[1,2,3]. Our long-term goal is to direct the macrophages towards the M2 profile that leads to a decrease in the FBR and an increase in implant function.

This study aims to determine the macrophage phenotypic changes in response to localized IL-4 delivery via implanted microdialysis sampling probes, which serve as a positive control for the M2 phenotype. A prerequisite to this study was to determine the differences between perfused and non-perfused microdialysis probe implants. Pro-inflammatory cytokines IL-6 and IL-1β were targeted for quantification, as their downregulation would serve as markers to an M2 phenotype.

Methods: For all studies, CMA 20, 100 kDa MWCO, 10-mm PES microdialysis probes (Harvard Apparatus, MA) were implanted into the dorsal subcutaneous space in male Sprague-Dawley rats (250-300g). To determine perfusion fluid effects, Ringer's solution was perfused in one probe for 4 days at 0.5 µL/min. The control probe was not perfused. On day 7 post-implantation, the rats were euthanized and tissues surrounding the implanted probes were harvested for histological and immunohistochemical analysis.

For IL-4 delivery studies, the treatment probe included 50 ng/mL of recombinant rat (rr) IL-4 (RnD systems, Minneapolis, MN). Sampling was performed on the day of implantation (day 0) and on day 3 post-implantation with a flow rate of 1 µL/min for a total of 4 hours in awake and freely-moving animals. Samples were collected every hour. On days 1 and 2, a flow rate of 0.5 µL/min was used for delivery of rrIL-4. Luminex™ multiplex assays (Millipore, Bellerica, MA) were used for the quantification of cytokines (IL-1β, IL-6) in the dialysate samples. On day 3, the rats were euthanized and tissues surrounding the implanted probes were harvested for histological and immunohistochemical analysis.

Results:

Table 1: Microdialysis Concentrations of IL-1β (pg/mL) from control probe on day 3

Hour	1	2	3	4	5
Rat 1	130	200	110	19	50
Rat 2	257	38	177	86	35

Table 2: Microdialysis Concentrations of IL-6 (pg/mL)

Day 1	Control Probe			Treatment Probe	
Hour	Rat1	Rat 2	Rat 3	Rat 1	Rat 2
1	32	32	89	103	139
2	32	73	60	239	368
3	ND*	153	ND*	219	96
4	ND*	ND*	ND*	ND*	76

ND* = Not detected.

Probe failure in two animals and analyte concentrations below the assay LOD were the main constraints in comparative cytokine data analysis.

Figure 1: Masson's Trichrome staining of tissue sections surrounding the probes. Left: non-perfused probe. Right: perfused probe

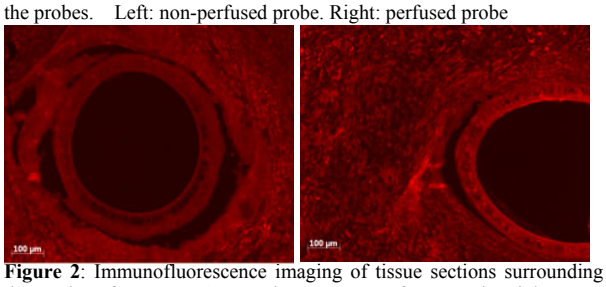

Figure 2: Immunofluorescence imaging of tissue sections surrounding the probes for CD-206 M2 phenotype. Left: control. Right: IL-4 treatment

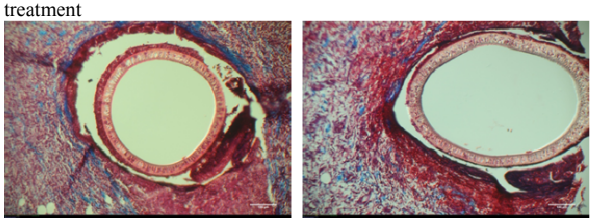

Figure 3: Masson's Trichrome staining of tissue sections surrounding the probes. Left: Control probe. Right: IL-4 treatment probe

Conclusions: Probe perfusion appears to cause more inflammation than just the probe implant. On day 3, the difference between control vs. IL-4 treatment is not noticeable. Future directions include comparing the cytokine profiles at day 7 post-implantation and obtaining data on the collagenous bag formation around the probe in response to IL-4.

References:

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2.Brown et al, Biomaterials. 2009 March; 30 (8):1482-1491
3.Higgins et al, Am J Pathol. 2009 July; 175(1): 161–170