Collection of prostaglandin E_2 and leukotriene B_4 from implanted microdialysis probes

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Statement of Purpose:
Wound healing is a complex process involving an inflammatory phase followed by a remodeling phase. Macrophages are major cellular players in this process. They play both pro-inflammatory as well as anti-inflammatory roles depending on the cues provided by the microenvironment. Macrophages have genomic plasticity allowing these cells to exist in different phenotypic or "polarized" states. The M1 phenotype, or classically active phenotype, is present in response to LPS and/or IFN-γ. The M2 phenotype or alternatively activated phenotype, is produced in response to IL-4, IL-10, IL-13, TGF-β or glucocorticoids.[1] In addition to cytokines, macrophages produce arachidonic acid-derived signaling molecules including prostaglandins and leukotrienes. This research aims to understand how these lipid molecules differ between M1 and M2 polarized states to better understand the lipid chemistry of wound healing biology. Prostaglandin E_2 (PGE_2) and leukotriene B_4 (LTB_4) have been chosen as representations of the eicosanoids, and they are commonly used in different type of analysis. In this work, preliminary data illustrating the collected amount of LTB_4 and PGE_2 are presented.

Methods:
In vitro recovery experiments were performed with CMA 20 PES 100 (MWCO) microdialysis probes using a flow rate of 1 µL/min. The perfusion fluid consisted of Ringer's solution (150mM NaCl, 4mM KCl, 2.4mM $CaCl_2$) supplemented with 6% dextran and 0.1% BSA to prevent ultrafiltration. The concentrations of PGE_2 and LTB_4 external to the probe were respectively 1000 pg/mL and 500 pg/mL. The sample collections were taken each hour post-implantation, as denoted in the tables. Samples were analyzed the same day of the collection with ELISA (Cayman Chemical). The plates were read at a wavelength between 405-420 nm.

In vivo experiments were performed with male Sprague-Dawley rats (250-300 g) with dialysis probes implanted into the dorsal subcutaneous tissue. On day 1 and day 4 post-implantation, dialysate samples were obtained, stored on ice, and quantified the same day. Pooled wound fluid, from the subcutaneous pocket where the probe inlet and outlet lines were sealed in, was collected from each animal. These samples were stored at -20°C until analysis (3 days maximum).

Results:
PGE_2 had a concentration of 1000 pg/mL, and the average recovery of PGE_2 was 344 ± 85 SD pg/mL (N=4), which gives a 34.4% extraction efficiency. The LTB_4 average recovery was 240 ±18 SD pg/mL (N=5), which gives a 48% extraction efficiency.

Table 1. Microdialysis concentration for the PGE_2

PGE_2 (pg/mL)	Rat 1 Day 4	Rat 2 Day 4	Rat 3 Day 4
~6th Hr			25.4
7th Hr	26.4	8.9	17.7
8th Hr	14.3	30.3	
9th Hr	12.0		
10th Hr	15.2		

Table 2. Microdialysis concentration for the LTB_4

LTB_4 (pg/mL)	Rat 1 Day 4	Rat 2 Day 4	Rat 3 Day 1	Rat 4 Day 4	Rat 5 Day 4
~6th Hr			N.D. †	4.8 (duplicate N.D. †)	6.5
7th Hr	N.D. †	3.9	N.D. †	N.D. †	N.D. †
8th Hr	14.3	3.6			
9th Hr	N.D. †				
10th Hr	N.D. †				

Table 3. PGE_2 and LTB_4 concentration in wound fluid

PGE_2 (N=3) (pg/mL)	LTB_4 (N=3) (pg/mL)
26.7 ±15.2 S.D.	N.D. †
13.5 ±3.5 S.D.	6.1 ±0.6 SD

† N.D. Not Detected; S.D. Standard Deviation

Conclusions:
This preliminary study was based on the concentration of prostaglandin E_2 and leukotriene B_4 in two different biological samples: wound fluid and dialysates.

References:
1. Martinez, F. O., *et al*. (2008) Macrophage activation and polarization. *Front. Biosci., 13*, 453-461.