ASSESSING THE SECRETOME OF *IN VITRO* DIFFERENTIATED HUMAN MACROPHAGES TREATED WITH A PRO-INFLAMMATORY STIMULUS

Tricia Conti, MS and Nina Bionda, PhD iFyber LLC, Ithaca, NY

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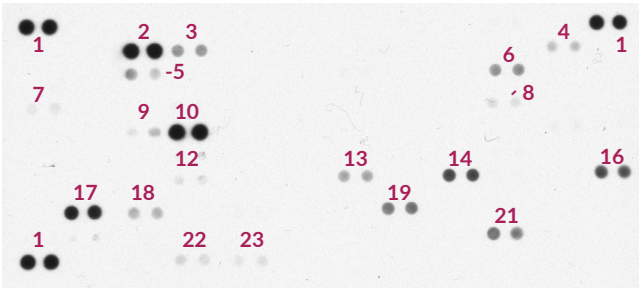
Predicting a positive or negative effect of a treatment on cytokine markers, *in vitro*, is a powerful tool for wound healing device developers as they optimize their product designs before moving to human clinical studies.

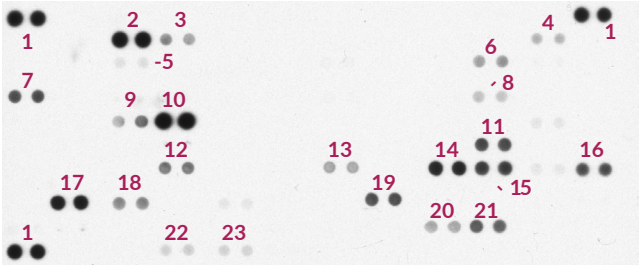
iFyber has implemented a membrane-based antibody array assay for the determination of relative levels of selected human cytokines. This assay allows for the simultaneous detection of 105 human cytokines in a single sample and is amenable to a variety of sample types including cell culture supernatants, cell and tissue lysates, serum, and plasma. The assay is a sandwich-based immunoassay that utilizes a chemiluminescent detection reagent and produces signal proportional to the amount of analyte bound. Signals are measured qualitatively or semi-quantitatively. Because this assay probes for a wide selection of cytokines in a single sample, it is both cost and time efficient. Additionally, it allows for subsequent down selection of the most relevant cytokines to focus on for further analysis.

Several cellular events have been associated with wound healing, and thus represent many candidate biological markers (biomarkers) with potential value in preclinical and clinical studies of wound care therapies. Of the cells involved in wound healing, macrophages are of particular importance. They release a variety of cytokines and growth factors that play a key role in healing[1]. It has also been shown that high levels of certain proteins, such as IL-1, IL-6, and MMPs, are present in non-healing, chronic wounds[2]. Therefore, it is essential to profile the biomarkers produced by macrophages when studying wound healing and wound care materials.

Untreated



LPS treated



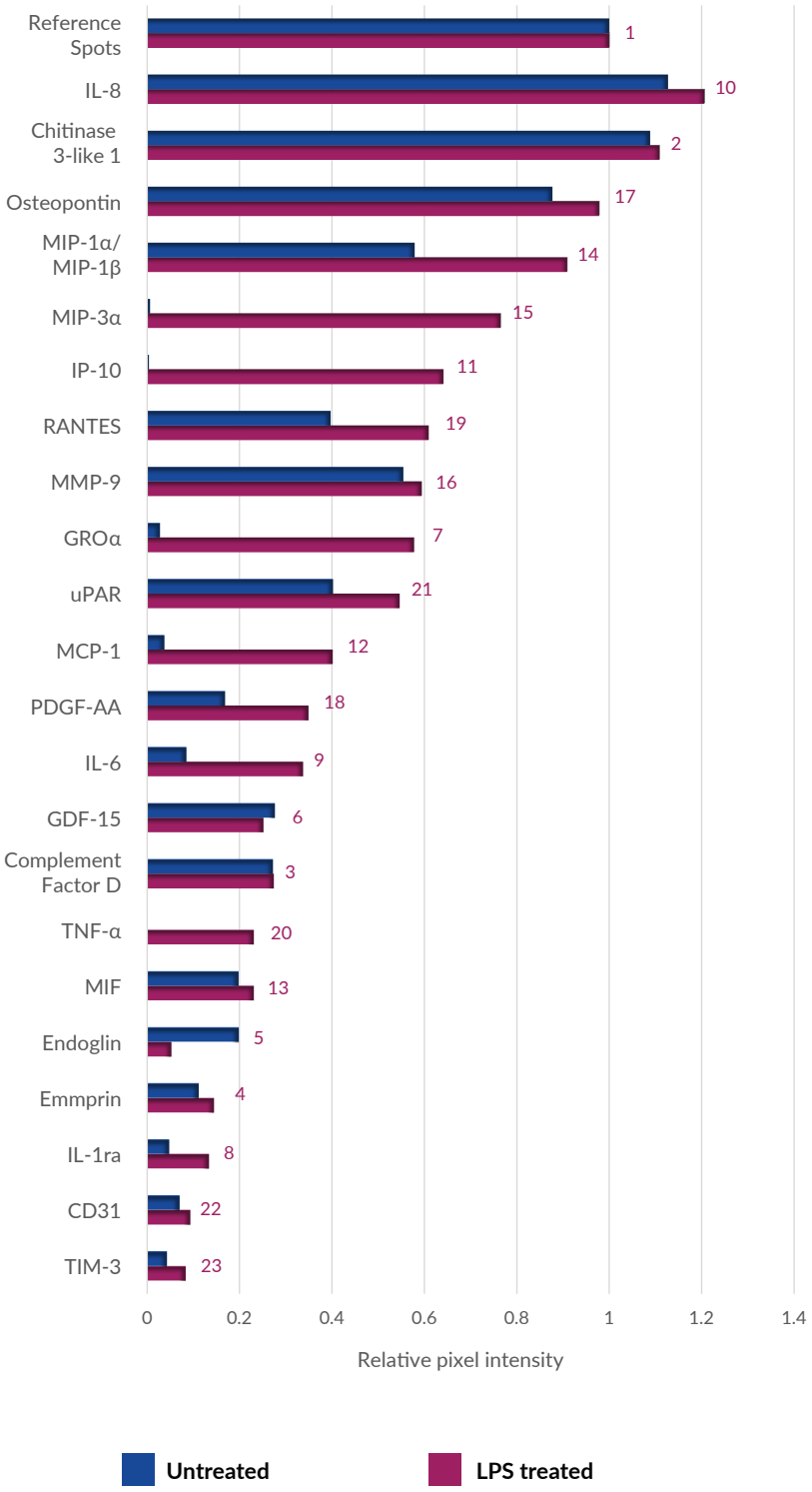


Figure 1: (Left) Comparative data for the biomarker expression for untreated and LPS-treated cells. (Right) Pixel intensities graphed for each biomarker untreated and LPS-treated cells.

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To assess the secretome of human macrophages, THP-1 human monocytes (ATCC TIB-202) were first differentiated to macrophages using phorbol 12-myristate 13-acetate (PMA)[3]. Once differentiated, the M0 macrophages were then seeded in a tissue culture plate with either cell culture medium alone, as a control, or cell culture medium plus 1 µg/mL lipopolysaccharides (LPS), as a pro-inflammatory stimulus – classical macrophage activation, M1 phenotype. Following the 24 hours incubation of both LPS-treated and untreated macrophages, under standard tissue culture conditions, the cell culture supernatant was collected, and cell debris was removed via centrifugation. The Human XL Cytokine Array (R&D Systems, ARY022B) was performed following the manufacturer's protocol[4]. Films were exposed using multiple exposure times, in order to obtain an optimal signal, and then developed. To determine the relative signal intensities, the films were scanned using a transparency scanner and the pixel density of the signals was measured using ImageJ.

Positive signals and their corresponding relative intensities for the 2-min exposed film are shown in Figure 1. Most notably, the cytokines IP-10, MIP-3 α, and TNF-α (numbered 11, 15, and 20) were not secreted by untreated macrophages but were in LPS-treated macrophages. These are pro-inflammatory cytokines known to be induced by LPS stimulation[3,5-7]. Other pro-inflammatory cytokines that were increased in the LPS treated cells include GROα, IL-6, MCP-1, and MIP-1α/MIP-1β.

iFyber has successfully implemented a membrane- based cytokine array assay as a tool to profile the expression of relevant cellular biomarkers. Shown here is the evaluation of the secretome of LPS-stimulated human macrophages. The results showed an increase in several pro-inflammatory cytokines over the control non-stimulated macrophages, which was in agreement with the literature[3-5-7]. This assay provides an efficient method for determining the most relevant proteins present in a sample to select for further analysis, making it a valuable tool for the study of biomarkers relevant to wound care.

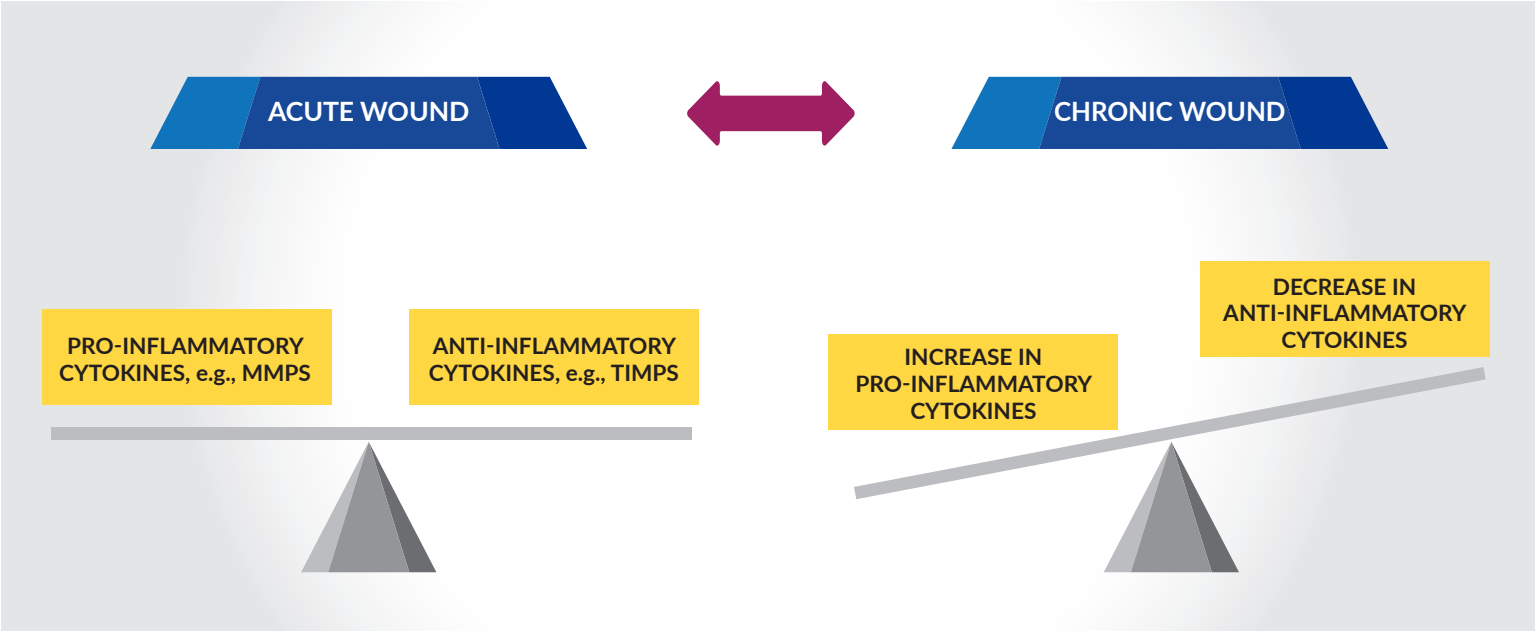

Figure 2: Balance between pro- and anti-inflammatory markers in wounds is one of the critical aspects in preventing progression of acute wounds into chronic wounds. Understanding the effect of a particular device, i.e., dressing, on this balance can assist in developing better products with more beneficial properties with respect to overall wound care.

[1]Krzyszczyk P, et al. Frontiers in Physiology. 2018 May; 9: 419. [2]Patel S, et al. Journal of Wound Care. 2016 Jan.; 25(1): 46-55. [3]Lund ME, et al. Journal of Immunological Methods. 2016 Mar.; 430: 64-70. [4]R&D Systems, *Proteome Profiler Antibody Arrays: Human XL Cytokine Array Kit Package Insert*. [5]Kopydlowski KM, et al. Journal of Immunology. 1999 Aug.; 163(3): 1537-1544. [6]Schutyser E, et al. Journal of Immunology. 2000 Oct.; 165(8): 4470-4477. [7]Ciesielski CJ, et al. European Journal of Immunology. 2000 Jun.; 32(7): 2037-2045.