# Wound Fluid Extracellular Microvesicle Cytokine Patterns Determine Healing in Adults with Stage IV Pressure Ulcers

**Walid Mari, MD, MSc[1]; Madhulika Kastury, BS[2]; Lauren Fang, BS[2]; Sara Younes, MD, MSc[1]; Terry L Oroszi, MS, EdD[1]; William C. Grunwald, Jr., BS[1]; David R Cool, PhD[1]; Rajab Suliman, PhD[2]; Richard Simman, MD, FACS, FACCWS[2]**

**[1]Boonshoft School of Medicine, Wright State University, Dayton, OH; [2]University of Toledo College of Medicine and Life Sciences, Toledo, OH**

## Introduction

- Investigates wound fluid containing cytokines and matrix metalloproteinases (MMPs) within microvesicles to understand healing dynamics in stage IV pressure ulcers
- Microvesicle cytokine profiling explored as potential biomarker for wound healing

| Cytokines | Primary Function(s) | Level in Acute Wounds | Level in Chronic Wounds |
|---|---|---|---|
| PDGF | • Injured platelets release PDGF, which acts as a potent chemo-attractant for various cells[1]<br>• Stimulates angiogenesis, cell proliferation, and chemotaxis responsiveness<br>• Macrophage-mediated tissue debridement and granulation tissue formation[2]<br>• Collagen synthesis and remodeling[2] | ↑ | ↓ |
| FGF-2 or basic FGF | • Granulation tissue formation[1]<br>• Re-epithelialization, induces angiogenesis, matrix formation[1,2]<br>• Tissue remodeling[1] | ↑ | ↓ |
| VEGF | • Formation of granulation tissue[1]<br>• Endothelial cell migration, proliferation, and survival[1]<br>• Stimulation of angiogenesis[1] | ↑ | ↓ |
| IL-1 | • Inflammation[1]<br>• Re-epithelialization[1] | ↑ | ↑ |
| IL-6 | • Inflammation[1]<br>• Re-epithelialization[1] | ↑ | ↑ |

Adapted from Barrientos, 2008.[1]

**Table 1.** Important cytokines in wound healing, their functions, and their levels in acute versus chronic wounds.

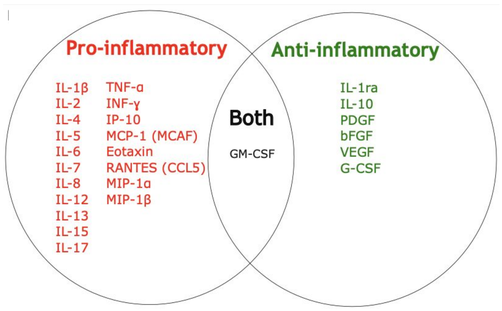


**Figure 1.** Cytokines analyzed by the Bio-Plex ProTM Human Cytokine Screening Panel, 27-plex. These include cytokines that are pro-inflammatory (red), anti-inflammatory (green), or both (black).

## Objectives

- Compare wound fluid microvesicle cytokine profiles between wounds treated with negative pressure wound therapy (NPWT) vs. NPWT plus Porcine Extracellular Matrix (PEM) dressing
- Analyze key cytokines and matrix MMPs within microvesicles at different stages of wound healing

## Methods

- Prospective, multi-centered, randomized, single-blinded clinical trial conducted
- Wound fluid samples obtained from 16 patients with stage IV trunk pressure ulcers, divided into control and study groups
- Microvesicles isolated and analyzed for cytokines and MMPs; ANOVA and paired/non-paired t-tests performed using R programming software to compare cytokine profiles and wound healing outcomes between groups

## Results

- Study group wounds treated with NPWT plus PEM expressed higher levels of pro-healing growth factors, such as fibroblast growth factor (FGF), at earlier time points compared to control group wounds
- Pro-inflammatory cytokines IL-6 and IL-8 decreased in the study group over time, suggesting enhanced inflammation resolution
- Anti-inflammatory cytokines IL-1ra and IL-10 were produced at higher concentrations in the study group, suggesting increased anti-inflammatory effects

| A | Control 4 | Control 8 | Control 12 | Study 4 | Study 8 | Study 12 |
|---|---|---|---|---|---|---|
| MMP1 MVP | 58.08 | 43.90 | 60.04 | 238.03 | 17.17 | 16.28 |
| MMP-2 MVP | 793214.47 | 105932.05 | 432610.31 | 7369.48 | 185507.39 | 56577.76 |
| MMP-3 MVP | 1730.36 | 172.43 | 501.84 | 67.38 | 18.60 | 47.53 |
| MMP-7 MVP | 191.03 | 23.76 | 363.74 | 7.40 | 1.12 | 7.24 |
| MMP-8 MVP | 178740.47 | 532028.04 | 55254.84 | 16935.83 | 5401.11 | 34107.22 |
| MMP-9 MVP | 115487.66 | 90524.51 | 48970.51 | 26848.63 | 16305.08 | 5515.96 |
| MMP-10 MVP | 3993.68 | 2880.91 | 3833.51 | 868.10 | 398.78 | 1892.31 |
| MMP-12 MVP | 6531.10 | 458.33 | 3933.62 | 206.20 | 184.57 | 561.67 |
| MMP-13 MVP | 102.86 | 31.79 | 119.52 | 9.89 | 5.53 | 10.39 |

| B | Control 4 | Control 8 | Control 12 | Study 4 | Study 8 | Study 12 |
|---|---|---|---|---|---|---|
| MMP1 MVP | 58.08 | 43.90 | 60.04 | 238.03 | 17.17 | 16.28 |
| MMP-2 MVP | 793214.47 | 105932.05 | 432610.31 | 7369.48 | 185507.39 | 56577.76 |
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**Figure 2.** Heat map analysis (HMA) compares cytokine concentrations between wounds treated with NPWT only (control group) vs. NPWT and PEM (study group) over 4, 8, and 12 weeks, using a color gradient from green (high values) to red (low values) for 27 cytokines. Panel (A) displays all cytokine concentrations, while Panel (B) focuses on group comparisons across time points.

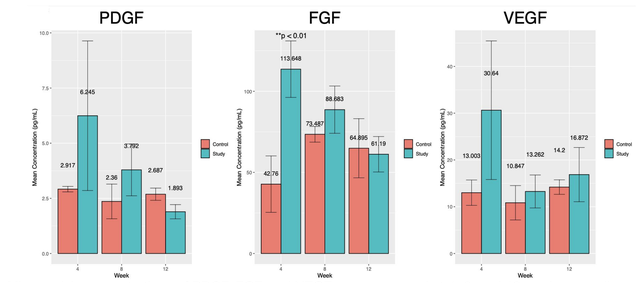


**Figure 3.** Microvesicle concentrations of PDGF, FGF, and VEGF were measured at 4, 8, and 12 weeks, revealing a statistically significant elevation in FGF levels at 4 weeks in the NPWT plus PEM study group compared to the NPWT alone control group ($p < 0.01$).

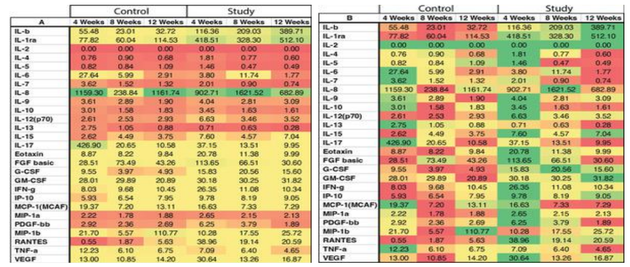


**Figure 4.** Heat map analysis (HMA) compares MMP expression in wounds treated with NPWT only (control group) versus wounds treated with NPWT and PEM (study group), with 9 MMPs ranked by mean concentrations using a green-to-red color range. Panel (A) illustrates all MMP concentrations, while Panel (B) highlights group comparisons across time points.

## Discussion

- Microvesicle cytokine profiling provides insights into wound healing dynamics, offering potential for early identification of wound healing
- Enhanced inflammation resolution and higher expression of pro-healing factors observed in study group support the effectiveness of NPWT plus PEM dressing in promoting wound healing
- Further research needed to validate microvesicle cytokine profiles as reliable biomarkers for wound healing outcomes

## Conclusion

- Microvesicle cytokine profiling offers promise as a tool for monitoring wound healing
- Combined NPWT and PEM dressing may accelerate wound healing by modulating cytokine expression
- Larger-scale studies warranted to confirm findings and assess clinical utility of microvesicle biomarkers in wound healing