

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

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### 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	<ul> <li>Injured platelets release PDGF, which acts as a potent chemo- attractant for various cells<sup>1</sup></li> <li>Stimulates angiogenesis, cell proliferation, and chemotaxis responsiveness</li> <li>Macrophage-mediated tissue debridement and granulation tissue formation<sup>2</sup></li> <li>Collagen synthesis and remodeling<sup>2</sup></li> </ul>				
FGF-2 or basic FGF	<ul> <li>Granulation tissue formation<sup>1</sup></li> <li>Re-epithelialization, induces angiogenesis, matrix formation<sup>1,2</sup></li> <li>Tissue remodeling<sup>1</sup></li> </ul>				
VEGF	<ul> <li>Formation of granulation tissue<sup>1</sup></li> <li>Endothelial cell migration, proliferation, and survival<sup>1</sup></li> <li>Stimulation of angiogenesis<sup>1</sup></li> </ul>				
IL-1	<ul> <li>Inflammation<sup>1</sup></li> <li>Re-epithelialization<sup>1</sup></li> </ul>				
IL-6	<ul> <li>Inflammation<sup>1</sup></li> <li>Re-epithelialization<sup>1</sup></li> </ul>	1	1		

Adapted from Barrientos, 2008.

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
MMP1 MVP	58.08	43.90	60.04
MMP-2 MVP	793214.47	105932.05	432610.31
MMP-3 MVP	1730.36	172.43	501.84
MMP-7 MVP	191.03	23.76	363.74
MMP-8 MVP	178740.47	532028.04	55254.84
MMP-9 MVP	115487.66	90524.51	48970.51
MMP-10 MVP	3993.68	2880.91	3833.51
MMP-12 MVP	6531.10	458.33	3933.62
MMP-13 MVP	102.86	31.79	119.52
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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 statistical 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).

A	Control			Study		12	Control						
	4 Weeks	8 Weeks	12 Weeks	4 Weeks	8 Weeks	12 Weeks	B	4 Wooks	8 Weeks	12 Weeks	4 Weeks	8 Weeks	12 Weeks
IL-b	55.48	23.01	32.72	116.36	209.03	389.71	IL-b	55.48	23.01	32.72	116.36	209.03	389.71
IL-1ra	77.82	60.04	114.53	418.51	328.30	512.10	IL-1ra	77.82	60.04	114.53	418.51	328.30	512.10
11-2	0.00	0.00	0.00	0.00	0.00	0.00	IL-2	0.00	0.00	0.00	0.00	0.00	0.00
114	0.76	0.90	0.68	1.81	0.77	0.60	IL-4	0.76	0.90	0.68	1.81	0.77	0.60
11-5	0.82	0.84	1.09	1.46	0.47	0.49	IL-5	0.82	0.84	1.09	1.46	0.47	0.49
11-6	27.64	5.99	2.91	3.80	11.74	1.77	IL-6	27.64	5.99	2.91	3.80	11.74	1.77
11.7	3.62	1.52	1 32	201	0.90	0.74	IL-7	3.62	1.52	1.32	2.01	0.90	0.74
11 - 8	1159.30	238 84	1161.74	00271	1621.52	682.89	IL-8	1159.30	238.84	1161.74	902.71	1621.52	682.89
11.9	3.61	2.89	1.90	4.04	2.81	3.09	IL-9	3.61	2.89	1.90	4.04	2.81	3.09
1.10	3.01	1.58	1.83	9.45	1.63	1.61	IL-10	3.01	1.58	1.83	3.45	1.63	1.61
11-12(070)	261	2.59	2 93	6.63	3.46	3.52	IL-12(p70)	2.61	2.53	2.93	6.63	3.46	3.52
11-13	2.75	1.05	0.88	0.71	0.63	0.28	IL-13	2.75	1.05	0.88	0.71	0.63	0.28
11-15	2.62	4.49	3.75	7.60	4.57	7.04	IL-15	2.62	4.49	3.75	7.60	4.57	7.04
11-17	426.90	20.65	10.58	37 15	13.51	9.95	IL-17	426.90	20.65	10.58	37.15	13.51	9.95
Entaxin	8.87	8.22	9.84	20.78	11.98	9.99	Eotaxin	8.87	8.22	9.84	20.78	11.38	9.99
EGE basic	28.51	73.49	43.26	113.65	66.51	30.60	FGF basic	28.51	73.49	43.26	113.65	66.51	30.60
G-CSF	9.55	3.97	4.93	15.83	20.56	15.60	G-CSF	9.55	3.97	4.93	15.83	20.56	15.60
GM-CSF	28.01	29.89	20.89	30.18	30.25	31.82	GM-CSF	28.01	29.89	20.89	30.18	30.25	31.82
IEN-a	8.03	9.68	10.45	26.35	11.08	10.34	IFN-g	8.03	9.68	10.45	26.35	11.08	10.34
IP-10	5.93	6.54	7.95	9.78	8 19	9.05	IP-10	5.93	6.54	7.95	9.78	8.19	9.05
MCP-1(MCAF)	19.37	7.20	13.11	16.63	7.33	7.29	MCP-1(MCAF)	19.37	7.20	13.11	16.63	7.33	7.29
MIP-1a	2.22	1.78	1.88	2.65	2.15	213	MIP-1a	2.22	1.78	1.88	2.65	2.15	2.13
PDGF-bb	292	2.36	2.69	6.25	3.79	1.89	PDGF-bb	2.92	2.36	2.69	6.25	3.79	1.89
MIP-1b	21.70	5.57	110.77	10.28	17.55	25.72	MIP-1b	21.70	5.57	110.77	10.28	17.55	25.72
RANTES	0.55	1.87	5.63	38.96	19.14	20.59	RANTES	0.55	1.87	5.63	38.96	19.14	20.59
TNF-a	12.23	6.10	6.75	7.09	6.40	4.65	TNF-a	12.23	6.10	6.75	7.09	6.40	4.65
VEGE	13.00	10.85	14.20	30.64	13.26	16.87	VEGF	13.00	10.85	14.20	30.64	13.26	16.87

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.

- 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

- 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



### Discussion

### Conclusion