

# Proteomic Analysis of Synovial Fluid from Patients with Failed Hip Implants

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## Introduction:

Metal-on-metal (MM) implants have been considered as an alternative to conventional metal-on-polyethylene (MPE) implants because of their lower volumetric wear. However, metal wear products remain a cause for concern, in part because they can lead to early adverse tissue reactions [1]. Unfortunately, there is currently no generally accepted method for the diagnosis of these reactions. The hypotheses of this study are that: 1) clinically important protein biomarkers of adverse tissue reactions to metal wear can be identified in synovial fluid of patients with MM hip implants; and 2) the identification of such biomarkers can lead to a better understanding of the effects of metal wear on tissues at the molecular level. Because synovial fluid is in direct contact with the affected tissues, it represents an excellent source for the study of these biomarkers. Therefore, the objective of the present study was to identify proteins that are differentially represented in the synovial fluid of the following two groups of hip-implant patients: those with early MM implant failure associated with metal wear (metallosis and/or elevated ion levels) and those with late MPE implant failure.

## Materials and Methods:

This study has been approved by the Ottawa Hospital Research Ethics Boards. Synovial fluid samples were obtained by hip aspiration at the time of revision surgery from consenting patients with early MM implant failure associated with metal wear (metallosis and/or elevated ions) and late MPE implant failure. The mean patient age was  $54.2 \pm 11.0$  years for the MM implants (2 males, 3 females; mean time to implant failure:  $1.9 \pm 0.4$  years) and  $71.6 \pm 10.6$  years for the MPE implants (6 females; mean time to implant failure:  $17 \pm 7$  years). Samples were supplemented with protease inhibitors, filtered, and depleted of albumin by affinity chromatography. Proteins from each sample were dissolved in 8 M urea, then reduced and alkylated. Samples were then digested with trypsin and further fractionated with a strong cation exchanger Stage Tip. Proteins were analyzed by high-performance liquid chromatography/electrospray ionization tandem mass spectrometry (HPLC-ESI-MS/MS). The acquired MS/MS spectra were searched against the human International Protein Index (IPI) protein sequence database (version 3.85) using Maxquant with the label free quantitation (LFQ) option (2). The false discovery rate (FDR) was set to  $\leq 1\%$  on both protein and peptide level. Quantification was performed using normalized LFQ intensity. Statistical analysis was performed using a two-sided t-test and the Significance B test [2] with Benjamini-Hochberg correction at a FDR of 1%. A p-value  $<0.05$  was considered significant.

## Results:

Five hundred forty seven (547) distinct proteins were identified, 134 of which were unique to one of the two experimental groups. Of the 413 proteins present in both groups, 24 (5.8%) were differentially represented between the two groups. Fifteen (15) proteins were more abundant in the MM group and 9 were less abundant.

**Table 1.** Potential synovial fluid biomarkers – selected from the 134 proteins unique to either the MM or MPE patient groups and the 24 proteins present in both groups but differentially represented between them

IPI number	Protein name	Abundance	Unique*	Signif. B (p value)	t-test (p value)
IPI00000874	Peroxiredoxin-1	+		$8.67 \times 10^{-2}$	0.045
IPI00003817	Rho GDP dissociation inhibitor beta 2	+		$1.48 \times 10^{-3}$	0.183
IPI00003865	Heat shock 70kDa protein 8	+	✓	$2.76 \times 10^{-27}$	0.027
IPI00026199	Glutathione peroxidase 3	-		$6.85 \times 10^{-4}$	0.012
IPI00026272	Histone H2A type 1-B	+	✓	$1.87 \times 10^{-38}$	0.036
IPI00027508	Interleukin-1 receptor type 1	-	✓	$1.88 \times 10^{-24}$	0.083
IPI00219219	Galectin-1	+		$6.27 \times 10^{-2}$	0.029
IPI00645887	Integrin alpha-M isoform 1 precursor	-		$6.08 \times 10^{-9}$	0.402

\*Proteins unique to one of the two experimental groups and present in  $\geq 50\%$  of patients in that group. + over-represented in MM group; - under-represented in MM group.

As shown in Table 1, proteins differentially represented between the MM and MPE groups included: 1. Proteins involved in the immune response, such as Rho GDP dissociation inhibitor beta 2 (involved in phagocytosis by neutrophils and macrophages) and galectin 1 (involved in the regulation of T-cells [3]) that were more abundant in the MM group, as well as interleukin-1 receptor type 1 and integrin alpha M isoform 1 that were less abundant in the MM group; 2. Proteins involved in stress responses, such as peroxiredoxin-1 that was more abundant in the MM group and glutathione peroxidase 3 that was less abundant (both are antioxidant enzymes involved in oxidative stress responses [4]), as well as heat shock 70 kDa protein 8 (chaperone involved in oxidative stress responses) that was more abundant in the MM group; and 3. Nuclear proteins associated with nucleic acids, such as histone H2A type 1-B that was more abundant in the MM group.

### Discussion:

Recent advances have made the proteomic analysis of body fluids one of the most promising approaches to identify biomarkers for pathological conditions [5]. In the present study, we used HPLC-ESI-MS/MS to identify differentially represented proteins in the synovial fluid of patients with early MM implant failure associated with metal wear in comparison to patients with late MPE implant failure. The identification of such proteins can provide insights into the pathophysiology of tissue reactions to metal wear products compared to polyethylene wear, at the molecular level. Results show that some proteins involved in the immune response were differentially represented. The identification of these proteins may lead to a better understanding of the mechanisms that underlie the differences in the immune response to metal and polyethylene wear products. Other differentially represented proteins included proteins involved in oxidative stress responses, which can be induced by metal ions, and some proteins interacting with nucleic acids, which may indicate nucleic acid damage in the MM group. However, group sizes need to be increased to identify additional potential protein biomarkers, and to confirm that some of the proteins identified may be used as biomarkers for the diagnosis of adverse tissue reactions to metal wear products.

### References:

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