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Institutionen för kliniska vetenskaper  
Danderyds sjukhus

# **CELL DERIVED MICROPARTICLES: METHOD DEVELOPMENT, AND CLINICAL AND EXPERIMENTAL STUDIES**

AKADEMISK AVHANDLING

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av

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# ABSTRACT

## Background

Cell derived microparticles (MP) are released from the cell membrane upon activation or apoptosis. They resemble their parent cell by exposing similar proteins or surface receptors. This enables identification of their cellular origin. MP are considered to facilitate cross-talk between cells, and to be involved in coagulation, inflammation and vascular function. Elevated circulating MP have been shown in previous studies. Assessment of MP is, however, difficult due to methodological issues.

## Aims

To evaluate pre-analytical procedures and a flow cytometric method for detection of microparticles. To study the effects of statin treatment and inflammation on phenotype and functional properties of microparticles.

## Methods and Results

In **Paper I** we describe a flow cytometric method for measurements of platelet derived microparticles (PMP) exposing CD62P or CD142. Mean fluorescence intensity measurements were more reproducible than concentration measurements. The presence of platelet fragments could be detected with the peptide phalloidin. This approach can be used as a quality control of samples. Samples frozen and stored as platelet-free plasma generated lowest number of platelet fragments upon flow cytometric analysis. Using our flow cytometric protocol we found two times higher exposure of CD62P and CD142 on PMP in plasma from type 1-diabetes patients compared to healthy controls.

In **Paper II** and **III** we investigated the effects of atorvastatin on MP. Nineteen patients with atherothrombotic disease were randomized to treatment with atorvastatin or placebo in a cross-over fashion. Thrombin generation and exposure of CD61, CD62P, CD142 and phosphatidylserine (PS) were assessed on PMP (**Paper II**). Endothelial derived MP (EMP) were assessed by CD144 or CD144<sup>+</sup> CD142<sup>+</sup> exposure (**Paper III**). During atorvastatin treatment both thrombin generation and exposure of CD61, CD62P, and CD142 on PMP decreased. No effect was seen on PS exposure. Furthermore, we demonstrated that MP enhanced thrombin generation through PS and CD142 exposure. Unexpectedly, circulating EMP measured as CD144 or CD144<sup>+</sup> CD142<sup>+</sup> increased significantly during atorvastatin treatment.

In **Paper IV** we investigated and characterized *in vivo* release of MP from 15 healthy volunteers administered lipopolysaccharide (LPS) in the presence of hydrocortisone with or without inhaled nitric oxide. MP from platelets (CD42a or CD41), endothelial cells (CD144 or CD62E) and monocytes (CD14) were studied. Nuclear content in MP was assessed (SYTO 13 binding) as well as HMGB1 exposure. Irrespective of treatment, LPS led to an increase in numbers of all MP, as well as the number of PMP and monocyte MP positive for anti-HMGB1 and SYTO 13.

## Conclusions

We describe a flow cytometric method to measure MP in plasma, and we demonstrate that MP from platelets and endothelial cells respond differently to statin treatment, reflecting the complexity of MP formation. Furthermore, we show that experimental inflammation leads to elevated circulating MP, and that MP may be a source of extracellular HMGB1. MP may be used as biomarkers, an idea that deserves to be investigated more extensively in future studies.