Microparticles, coagulation and inflammation
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Chapter 1

Introduction and outline of the thesis
Chapter 1

History

In 1946, Chargaff demonstrated that platelet-poor plasma contains a subcellular fraction that facilitates coagulation. High-speed centrifugation of the platelet-poor plasma prolonged the clotting time [1]. Some 20 years later, in 1967, Wolf showed by electron microscopy and functional tests that this subcellular fraction consists of small vesicles of platelet origin, the so called “platelet dust” [2]. The procoagulant activity associated with this material was designated platelet factor 3 (PF3).

Interest in microparticles subsequently increased steadily, as represented by the number of articles published per year in international journals (Figure 1).

![Figure 1. Published articles on microparticles of various cellular origin in international journals from 1960 - 2003.](image-url)
Platelet-derived microparticles

Storage, shear stress as well as direct platelet activation lead to the budding of small vesicles from the platelet surface membrane, usually called platelet-derived microparticles (PMP) [3,4]. Platelets, activated by agonists like α-thrombin plus collagen [5], the membrane-attack complex of the complement system (C5b-9) [6,7], and Ca\(^{2+}\)-ionophores as A23187 [8], cause the release of PMP. Also, small vesicles stored in secretion granules are secreted during the platelet activation. These vesicles, called exosomes, are not the subject of this thesis. At present, it is unclear whether the shedding of PMP is solely due to activation. In several studies it has been suggested that in platelets activation of apoptosis-associated pathways occur that may be linked to vesiculation under certain conditions [9].

PMP not only occur in vitro but also in vivo in both physiological and pathophysiological conditions. Compared to healthy humans, elevated numbers of PMP are observed in blood from patients suffering from paroxysmal nocturnal hemoglobinuria [10], sickle cell disease [11], acute coronary syndrome [12], diabetes [13-15], heparin-induced thrombocytopenia [16-18], severe stenosis [19], myocardial infarction [20], unstable angina [12,21], ischemic brain disease [13,22], uremia [23], idiopathic thrombocytopenic purpura [24,25], thrombotic thrombocytopenic purpura [26-28], hemolytic uremic syndrome [27], meningococcal sepsis [29] and disseminated intravascular coagulation [30,31]. Also patients undergoing cardiac surgery [32-34], plasmapheresis [35], or coronary angioplasty [12,20,36] have elevated numbers of PMP in their blood. Most of these patient groups have an increased risk for thromboembolic events. This suggests that PMP are involved in coagulation activation and/or potentiation in vivo, as an underlying cause for this increased thromboembolic risk. A complete overview of the occurrence of microparticles in various diseases is provided in Table 1 of Chapter 8.

The procoagulant properties of in vitro generated PMP have been extensively characterized [37-39]. Activated platelets and PMP expose negatively charged phospholipids such as phosphatidylserine (PS) and phosphatidylethanolamine. In resting cells, including platelets, the outer leaflet of the phospholipid bilayer of the plasma membrane contains predominantly the uncharged phospholipids sphingomyelin and
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phosphatidylcholine (PC), whereas the PS and phosphatidylethanolamine are located almost exclusively in the inner leaflet [40]. The exposure of negatively charged phospholipids enables the binding of coagulation proteins, thereby supporting the formation of tenase (factors VIIIa/IXa)- and prothrombinase (factors Va/Xa) complexes [41,42]. Compared to activated platelets, PMP have a higher density of high-affinity binding sites for activated factors IX (IXa) [43] and V (Va) [6,44,45], and a continuous expression of high affinity binding sites for factor VIIIa [46]. As a consequence, PMP support the formation of tenase- and prothrombinase complexes and elicit tenase- [47,48] and prothrombinase activity [6,49].

The membrane that surrounds the PMP not only contains phospholipids, but also several glycoproteins (GP), such as GPIb and the integrin GPIIb-IIIa [3]. Whether or not these adhesion receptors, and others such as P-selectin, have the same function(s) as on platelets, is unknown but it may explain the ability of the microparticles to adhere to fibrinogen, fibronectin and collagen [50], or to cells such as monocytes [51,52].

**Microparticles of non-platelet origin**

Membrane vesiculation is not restricted to platelets. For instance, activation of erythrocytes results in phospholipid scrambling and shedding of microparticles [53,54]. In addition, endothelial cells [55-58], monocytes [51], fibroblasts [59], polymorphonuclear leukocytes [60], and smooth muscle cells [61] release microparticles upon appropriate activation. An overview is presented in Table 1 of Chapter 8. In general, microparticles from these cells expose negatively charged phospholipids and sometimes tissue factor (TF). Apart from the exposure of these phospholipids, the exposure of TF, believed to be the main initiator of coagulation in vivo, enables these microparticles to be procoagulant.

**Coagulation**

Because the major part of this thesis focuses on the involvement of microparticles in coagulation, a brief overview of the coagulation pathways is presented. As already mentioned on page 13 of this Introduction, microparticles can expose negatively charged phospholipids. The exposure of such phospholipids is a prerequisite for coagulation
factors to bind to the membrane and thus facilitate the formation of the coagulation factor complexes. This binding is calcium dependent.

Coagulation can traditionally be initiated by TF, i.e. via the extrinsic coagulation pathway, or by activated factor XII (factor XIIa), i.e. the intrinsic coagulation pathway (Figure 2). Under normal conditions TF is localized extravascularly, i.e. on the surface of smooth muscle cells in the subendothelium of the vessel wall. In a wound, blood contacts this TF and (extrinsic) coagulation becomes initiated. In vitro, endothelial cells and blood cells such as monocytes, and possibly granulocytes and platelets, synthesize and expose TF on their surface upon the appropriate stimulation [62-65]. Such TF exposure has also been observed on monocytes and possibly granulocytes under pathophysiological conditions in vivo [66,67].

TF, a transmembrane protein, is the cellular receptor for factor VII and facilitates autoactivation to factor VIIa. The TF/VIIa complex activates factor X directly or indirectly via factor IXa [68-71]. In turn, factor Xa associates with factor Va and calcium ions on the membrane surface, thus forming the prothrombinase complex, which catalyses the conversion of prothrombin (factor II) to thrombin (factor IIa) [72,73].

Very recently, a novel form of TF has been described. This alternatively spliced form of TF lacks the transmembrane domain. It is presently unknown which relation exists between the alternatively spliced TF form and the so called soluble TF, i.e. the TF present in plasma after removal of the blood cells [74].

The intrinsic pathway becomes activated when blood contacts negatively charged surfaces, the “contact activation pathway”. Factor XII becomes activated (factor XIIa), and in turn factor XIIa activates prekallikrein to kallikrein and factor XI to factor XIa. Factor XIa activates factor IX to IXa, which then forms the already mentioned tenase complex. It is important to note that factor XI is thought to play a central role in the amplification of thrombin formation. Trace amounts of active thrombin directly activate factor XI to factor XIa, thus promoting additional thrombin formation via factor IXa and Xa. Factor XIa can also be formed via autoactivation [75-78]. Part of this thesis is aimed at the elucidation of the pathways involved in the activation of the coagulation system by microparticles in various clinical conditions.
Recent developments

At the start of this thesis, virtually nothing was known about the presence of microparticles from platelets and other cells, or their function, in blood and other body fluids in vivo. More recently, it has become apparent that microparticles from platelets and other cells circulate in vivo and may be involved in functions other than coagulation. Amongst others, microparticles have been shown to induce endothelial dysfunction and to trigger cells to produce proinflammatory mediators such as cytokines. An overview of the various alleged functions of microparticles is provided in Table 2 of Chapter 8. In the General discussion and summary (Chapter 8), the findings presented in this thesis will be discussed in relation to these recent developments.

Structure of this thesis

The aim of this thesis was to investigate the presence, number and cellular origin of microparticles in human blood and synovial fluid, and to focus in particular on the ability of these microparticles to initiate and support coagulation and inflammation.

We studied the occurrence of microparticles and their relationship with coagulation in plasma samples from patients undergoing coronary bypass surgery (Chapter 2) or
suffering from meningococcal septic shock (Chapter 3). In Chapter 4, the possible involvement of microparticles in basal coagulation was studied in healthy human individuals. Subsequently, in Chapter 5 the presence of microparticles and their possible relationship with in vivo coagulation activation and inflammation were studied in patients with sepsis and multiple organ failure. In Chapters 6 and 7, not only plasma samples but also synovial fluid samples from (non-) rheumatoid arthritis patients were analyzed for the presence of cell-derived microparticles. In Chapter 6, the procoagulant properties of synovial microparticles were studied and in Chapter 7 the ability of these microparticles to stimulate the production of cytokines by fibroblast-like synoviocytes was determined.