Published in final edited form as:

Phys Biol. 2011 December ; 8(6): 066005. doi:10.1088/1478-3975/8/6/066005.

Role of carrier number on the procoagulant activity of tissue factor in blood and plasma

GW Tormoen¹, S Rugonyi¹, A Gruber^{1,2}, and OJT McCarty^{1,3}

OJT McCarty: mccartyo@ohsu.edu

¹Department of Biomedical Engineering, School of Medicine, Oregon Health & Science University, 3303 SW Bond Ave, Portland, OR 97239, USA

²Department of Medicine, Division of Hematology/Oncology, School of Medicine, Oregon Health & Science University, 3303 SW Bond Ave, Portland, OR 97239, USA

³Department of Cell and Developmental Biology, School of Medicine, Oregon Health & Science University, 3303 SW Bond Ave, Portland, OR 97239, USA

Abstract

Tissue factor (TF) is a transmembrane glycoprotein cofactor of activated blood coagulation factor VII (FVIIa) that is required for haemostatic thrombin generation at sites of blood vessel injury. Membrane-associated TF, also detected in circulating blood, has been shown to contribute to experimental thrombus propagation at sites of localized vessel injury. It remains uncertain how TF can circulate without initiating intravascular coagulation. This study was designed to assess whether the carrier number per unit volume of TF-inducible monocytic cell line U937 or synthetic TF-carriers affected procoagulant activity, hence thrombogenic potential. Experiments were performed to characterize the effects of TF-carrier number on the kinetics of clot formation in both open and closed systems. The procoagulant activity of TF carriers was found to correlate with carrier count. TF carriers enhanced the amidolytic activity of FVIIa towards the chromogenic substrate, S-2366, as a function of carrier count. These results suggest that TF-initiated coagulation by circulating TF is kinetically limited by diffusion path lengths between TF-bearing surface. Biophysical parameters of circulating TF such as carrier count, and flow parameters of the circulation are therefore critical determinants of the procoagulant activity of circulating TF.

1. Introduction

Tissue Factor (TF), the physiologic primary initiator of coagulation, complexes with factor VII (FVII) to activate coagulation factors IX (FIX) and X (FX). The surface-bound nature of TF [1], and the need for FVII to combine with TF to achieve physiologically relevant reaction rates [2], implies that the initiation of coagulation by TF is a surface phenomenon. Kinetics of TF-initiated enzyme activation have been found to depend upon physicochemical characteristics of the TF-bearing surface and its interface with blood [3–5]. The discovery of intravascular, circulating TF [6–11] has brought anew the importance of understanding kinetic limitations on TF-initiated thrombin generation, as the accessibility of circulating TF to blood is different in many aspects compared to immobilized TF. *In vivo* [12] and *in vitro* [13], circulating TF has been shown to be active in initiating coagulation and indeed contribute to experimental thrombosis at sites of vessel injury [14, 15]. However, healthy subjects have been shown to have a basal level of circulating TF without evidence for pathological initiation of coagulation [13, 16]. Therefore, a paradox emerges, where a coagulation initiator is exposed to plasma coagulation factors *in vivo* without initiating coagulation.

Studies into the ability of TF to initiate coagulation have shown that the critical TF surface density to result in surface deposition of fibrin is largely dependent upon flow parameters [4]. Generation of activated FX (FXa) was shown to depend upon the presence of phospholipid and FVII levels in an *in vitro* system of TF surface-catalyzed coagulation [3]. Studies with lymphoid cell lines support the notion that the membrane environment, rather than TF exposure, is the dominating factor in determining procoagulant activity of TF [5]. The relevance of circulating TF to disease pathology has been broadened in light of several situations where blood-borne cellular TF is present, either as circulating tumor cells, TF-expressing leukemias, models of endotoxemia, or TF-positive microparticles. Coagulation phenotypes for these various scenarios range from normal to disseminated intravascular coagulation for endotoxemia and acute promyelocytic leukemia, to venous thromboembolism in cases of metastatic cancer. Full understanding of the physiological implications of intravascular TF has yet to be achieved.

This study was designed to test the hypothesis that the procoagulant phenotype of intravascular TF is dependent upon the TF carrier count per unit volume. We characterized the procoagulant and prothrombotic phenotype of TF-inducible monocytic cell line U937 and synthetic TF-carriers in both an open and closed systems. Our results demonstrate that for circulating TF, carrier count plays a role in determining coagulation initiation kinetics, and suggest that flow parameters regulate coagulation enzyme activity.

2. Methods

2.1 Reagents

All reagents were purchased from Sigma-Aldrich (St. Louis, MO) or previously described sources [17] unless otherwise specified. Monoclonal antibodies (mAbs) to Factor XI were generated as previously described [18]. Anti-TF antibodies (clone D3H44) were obtained from Genentech (San Francisco, CA). Recombinant, lipidated TF (Dade® Innovin®) was purchased from Siemens Healthcare Diagnostics (Deerfield, IL). Fibrillar equine collagen was from Chrono-log (Havertown, PA). Biowhittaker® Premium fetal bovine serum was from Lonza (Basel, Switzerland).

2.2 Blood Donations

All donations from healthy human subjects were obtained in accordance with Oregon Health and Science University IRB approval. Blood was collected by antecubital venipuncture into a one-tenth volume 3.8% sodium citrate for occlusive thrombus assays, or 3.2% sodium citrate for coagulation and enzyme generation assays. Platelet poor plasma (PPP) for coagulation studies was prepared by centrifugation of citrated blood at 2150g for 10 minutes. Platelet rich plasma (PRP) was decanted from the spun blood, pooled with PRP from two other donors and subjected to centrifugation at 2150g for 10 minutes. PPP was then collected by decanting off the supernatant. Aliquots of PPP were immediately frozen at -80 °C until use.

2.3 U937 Cell Culture

U937 cells were purchased from ATCC (Manassas, VA). Cells were cultured in non-treated T25 flasks in a medium consisting of RPMI 1640 containing 10% fetal bovine serum and 1× Penicillin-Streptomycin and kept in an incubator at 37 °C containing 5% CO₂. Culture media was supplemented weekly with L-glutamine. Flasks were seeded at a concentration of $2 \times 10^5 \text{ mL}^{-1}$ and kept below $2 \times 10^6 \text{ mL}^{-1}$ with viability maintained above 90%. All experiments were performed between passages 4 and 7.

2.4 U937 Induction of TF

U937 cells were washed with $50 \mu\text{g mL}^{-1}$ anti-TF antibody to minimize the influence of constitutively expressed TF. Cells were then split, and resuspended in the normal culture media or media containing $10 \mu\text{g mL}^{-1}$ lipopolysaccharide (LPS) and placed in an incubator for 24 hours. Then, cells were pelleted by centrifugation at $130g$ for 10 minutes and resuspended in Hank's balanced salt solution (HBSS). Suspensions were diluted from a concentration of 1×10^7 to $1 \times 10^2 \text{ mL}^{-1}$ with HBSS as determined by a haemocytometer.

2.5 Microsphere Coating

Polymeric microspheres (diameter = $9.86 \mu\text{m}$) were purchased from Bangs Laboratories (Fishers, IN). Stock solution microsphere concentrations were created by diluting in H_2O (resistivity = $18.2 \text{ M}\Omega \text{ cm}^{-1}$) and counted with a haemocytometer. One million microspheres were then dispensed into a 1.7 mL vial. Tissue factor coating solutions were prepared by diluting TF stock to 1 nM in H_2O . 1 mL of coating solution was added to the microspheres and allowed to coat for 60 minutes at room temperature. Coated microspheres were pelleted by centrifugation at $16,100g$ for 10 minutes and surface blocked with 0.5% denatured, filtered bovine serum albumin (BSA) in phosphate-buffered saline (PBS). Coated and blocked microspheres were pelleted, and resuspended in HBSS.

2.6 Clotting Time Determination

TF-coated, BSA blocked microspheres and LPS-stimulated U937 cells, hereafter referred to as TFcarriers, were suspended in HBSS and carrier density counted with a haemocytometer. Suspension concentrations were then diluted from 1×10^6 to $1 \times 10^2 \text{ TF-carriers mL}^{-1}$. 50 μL of carrier suspension were added to 50 μL of PPP and allowed to mix for 180 s at 37°C , after which 50 μL of 25 mM CaCl_2 in H_2O were added and the clotting time recorded on a KC4 Coagulation Analyzer (Trinity Biotech, Bray, Co. Wicklow, Ireland). To determine the procoagulant mechanism of microspheres and U937 cells, anti-FXI mAbs were added to PPP and allowed to mix for 5 minutes at 37°C prior to addition of microsphere or cell suspensions and the clotting time recorded. Anti-TF antibodies were mixed with the carrier suspensions for five minutes at 37°C before mixing with the PPP, and the resultant clotting time recorded. Reported values represent the average value for a minimum of three experiments.

2.7 Occlusive Thrombus Assay

Ex vivo occlusive thrombus assay was performed as previously described [19]. Briefly, glass capillary tubes ($2.0 \times 0.2 \text{ mm}$, VitroCom, Mountain Lakes, NJ) were coated with $100 \mu\text{g mL}^{-1}$ equine fibrillar collagen in 10 mM acetic acid for 60 minutes at room temperature while rotating on a carousel. Next, tubes were washed with PBS, and surface-blocked with 0.5% denatured-filtered BSA in PBS for 60 minutes at room temperature while rotating on a carousel. Blocked tubes were then washed, and one end fitted with a 1 cm length of 0.40 inch internal diameter Silicone tubing and attached to a suspended 3 mL syringe while submerging the other end in PBS. TF carrier suspensions were mixed with citrated blood and recalcified immediately before subjecting them to flow. The time for the blood solutions to occlude flow in the collagen-coated capillary was recorded as the time to occlusion as described previously [19].

2.8 Enzyme Generation Assay

15 μL of 4 mM S-2366 substrate (Chromogenix, Milan, Italy) were combined with 50 μL of PPP and dispensed into individual wells of a 96-well plate. Next, 50 μL of TF-coated microsphere suspensions ranging from a concentration of 1×10^6 to $1 \times 10^3 \text{ mL}^{-1}$ were added to the wells and allowed to mix for 15 minutes at 37°C . Then, 50 μL of 25 mM CaCl_2

in H₂O were added followed immediately by measurement of absorbance of 405 nm wavelength light at 22 °C from each well at 60 s intervals for 1 hour in a spectrophotometer (Tecan, Mannedorf, Switzerland). Absorbance data exhibited a sigmoid relationship with time. The data was analyzed by first normalizing by the maximum and subtracting the baseline absorbances. Next, the initiation lag was recorded as time corresponding to the first measurable increase in absorbance. Then, the molar equivalent of S-2366 added to the plasma (600×10^{-12} moles) was divided by the difference between time points corresponding to 0 and 100% absorbance through extrapolation of the slope at 50% absorbance. The diffusion path length was calculated by taking the cube root of the reaction volume (0.165 cm^3) divided by the number of microspheres added. This gives a measure of the linear distance between microspheres assuming a uniform suspension.

2.9 Statistical Analysis

Data are presented as mean $\pm$ standard error (SEM). Statistical significance between means was determined with the one-tailed paired Student's t-test. Significance for all statistical tests required $P < 0.05$.

3. Results

3.1 Monocytic cell line-derived TF is procoagulant in a carrier number and TF-dependent manner

To verify that our assays were sensitive to haematopoietic TF, the ability of TF-expressing U937 cell suspensions to coagulate plasma was assessed. As shown in figure 1, our results indicate that LPS-stimulated cells had higher procoagulant activity than non-stimulated U937 cells (clotting times of 221 and 480 s, respectively, $P = 0.005$, $n=4$). The clotting times decreased with increasing counts of U937 cells from $1 \times 10^4 \text{ mL}^{-1}$ to $1 \times 10^6 \text{ mL}^{-1}$, and their ability to coagulate plasma was abrogated by the addition of an anti-TF antibody (clotting times of 221 s vs. 600 s, $P = 0.009$, $n=4$). No significant effect on clotting time was observed for U937 counts below $1 \times 10^5 \text{ mL}^{-1}$.

3.2 Synthetic TF carriers are procoagulant in a carrier number and TF-dependent manner

TF carrier suspensions from $1 \times 10^2 \text{ mL}^{-1}$ to $1 \times 10^6 \text{ mL}^{-1}$ yielded clotting times that varied upon the carrier count (figure 2(a)). The mechanism of coagulation initiation for these microspheres was abrogated with an anti-TF antibody. In contrast, clotting time was unaffected by the blockade of the contact pathway with the anti-FXI mAbs, 14E11 or 1A6, unless the extrinsic pathway was blocked concomitantly (figure 2(b)).

3.3 Enzymatic initiation time and reaction rate are carrier number dependent

Experiments were designed to investigate whether the time required for assembly of active enzyme complexes upon the TF-carriers, or the reaction rates of assembled complexes were dictated by carrier number. Cleavage of the chromogenic substrate S-2366 was performed to measure the initiation time lag (initiation time) for activated enzyme to form as well as the rate at which formed active enzymes cleaved substrate (enzyme reaction rate). Initiation time decreased as carrier counts increased (figure 3(a)). The rate of substrate cleavage, on the other hand, was found to increase with increases in carrier counts (Figure 3(b)). We also calculated a "diffusion path length" which represents a measure of the distance between carriers, and thus the distance substrate enzyme has to diffuse before reaching a carrier surface. We found that initiation time was approximately proportional to the diffusion path length (figure 3(c)), whereas enzyme reaction rates were slower for longer diffusion path lengths (figure 3(d)).

3.4 TF carriers promote occlusive thrombus formation in a TF and carrier number-dependent manner

Experiments were designed to determine the role of carrier count on the prothrombotic activity of TF-carriers in a flowing, open, whole blood assay by measuring the time required for occlusion of flow. Addition of TF-expressing U937 cells (figure 4(a)) or synthetic TF carriers (figure 4(b)) were prothrombotic in a carrier number-dependent manner. The prothrombotic activity for both U937 cells and synthetic TF carriers was found to be abrogated with the addition of an anti-TF antibody. Blockade of the contact pathway with 14E11 did not influence time to occlusion unless an anti-TF antibody was also included.

4. Discussion

This and other studies have shown that the addition of TF-expressing cells to plasma result in shortened clotting times in a cell concentration manner. The ability of TF-expressing cells to initiate coagulation has been shown to depend on physicochemical properties of the cell-plasma interface. In other words, the mere presence of TF or its concentration may not dictate its ability to initiate coagulation. In this study, we implemented a purified system utilizing a single, fully active form of TF adsorbed onto surfaces of polymer microspheres to determine if the sequestration of TF to a surface would impact its procoagulant activity. Specifically, this study aimed to determine the impact of TF carrier count on the procoagulant activity of TF. In a purified system, we determined that both the time to initiate enzyme activation and enzyme reaction rates were affected by the diffusion path length for suspensions of TF carriers. These findings are consistent with properties of surface bound reactions, where substrate needs to traffic from solution to the carrier surface before it can be cleaved by the surface-bound reactive complex. Carrier count directly impacts the diffusion path for substrate from bulk solution to the carrier surface, and by shortening the diffusion path through increasing the carrier count, we found that active enzymes were initiated sooner and cleaved substrate at a faster rate.

TF-initiated coagulation is a surface-bound enzymatic reaction, and therefore, subject to kinetic limitations inherent to surface reactive systems, e.g., these systems may be constrained by diffusion transport. For TF exposed at the site of an injured vessel, diffusion transport limitations are minimized due to convective transport of flowing blood. In this scenario, coagulation factors are continually supplied to the reacting surface. This is equivalent to minimizing the diffusion path length for substrate to reach the reactive surface. However, for circulating TF, where convective transport can be minimal, we hypothesize that the diffusion path length dominates the initiation of coagulation. The results of our study demonstrate that diffusion paths become a prominent kinetic limitation for intravascular TF under steady-state conditions. Immobilized TF has been shown to transition from diffusion-limited to convection-assisted reaction kinetics above wall shear rates of 120 s^{-1} [3]. A transition from diffusion limited reaction kinetics for circulating TF to convection-assisted kinetics for immobilized TF may explain how TF can circulate without initiating coagulation, yet contribute to thrombus propagation when incorporated into the growing thrombus. The diffusion-limited reaction kinetics for circulating TF would convert to convection-assisted kinetics once TF is immobilized. Additionally, the deposition of circulating TF upon the growing thrombus would concentrate TF carrier number per unit volume on the thrombus surface. Our data has demonstrated that TF carriers yielded increased procoagulant activity at higher carrier counts. Thus, we have demonstrated the potential for circulating TF carriers to increase procoagulant activity based solely on changes in spatial distribution. Additionally, turbulent flow would increase substrate transport through mechanically mixing the circulating TF with plasma. Anecdotally, regions of turbulent flow due to branching vessels in a baboon sepsis model were shown to undergo increases in TF-dependent coagulation [20].

In suspension, increasing the TF carrier count, and thus the accessibility of reacting TF surface to soluble substrate, increases the rate of formation of active enzyme complexes per unit volume. Increases in TF-carrier count in blood, such as due to an increase in the number of circulating TF-bearing microparticles could promote thrombosis. Here, in its simplest form, a parent cell sheds microparticles. These microparticles, being composed largely of the parent cell membrane, would not alter the concentration of circulating TF. However, the TF carrier count per unit volume of blood would be dramatically increased, resulting in plasma zymogens having greater access to TF surfaces, hastening their conversion to active enzyme. This may elevate the procoagulant activity in the blood. For instance, the induction of microparticles by monocytes in models of endotoxemia [7] and in patients with paroxysmal nocturnal hemoglobinuria [21] has been reported, both of which are associated with thrombotic complications. Similarly, a link between intravascular TF and cancer cell derived microparticles has been observed [22], perhaps explaining why cancer patients experience elevated rates of venous thromboembolism. Whether the procoagulant activity for a single TF concentration can be manipulated by dispersing onto different carrier counts has yet to be determined.

The case for physical parameters dictating the procoagulant activity of intravascular TF may highlight a novel therapeutic strategies for complications associated with intravascular TF. While reducing expression of TF could reduce the thrombogenicity of the microparticle, such as by inhibiting TF expression on LPS-induced monocytic cell lines by activated protein C [23] it is possible that inhibiting the formation of microparticles from the parent cell could also be effective at preventing thrombosis. For instance, IL-10 administration was found to reduce thrombin generation of LPS-stimulated monocytes, which resulted in concomitant reductions in microparticle formation as well as TF expression [24]. Moreover, blockade of the leukocyte receptor, PSGL-1, has been shown to reduce leukocyte-derived microparticles [25], while PSGL-1 ^{-/-} mice were unable to incorporate circulating TF into thrombi at sites of vessel injury [14]. Whether reductions in circulating TF activity is due to inhibition of TF expression or microparticle generation is difficult to know, as inhibition of one is often linked with inhibition of the other. Further, to our knowledge, isolation of microparticle generation from TF expression has not been performed or quantified.

This study aimed to determine the impact of TF carrier count on the procoagulant activity of TF. An inverse relationship on clotting time, time to occlusion, and enzyme initiation time was seen with carrier count, while a direct correlation of enzyme reaction rate was observed with carrier number. These findings were evaluated in light of diffusion transport limitations. These findings suggest for the first time that the determinants of prothrombotic intravascular TF activity, hence the risk of developing pathological clots or occlusive thrombi include the TF carrier count, such as the number of circulating TF-carrying microparticles and cells rather than the solely the whole blood concentration of TF. The paucity of thrombotic events with the presence of intravascular TF may be explained by coagulation reaction kinetic limitations, which can only be overcome when the carrier count per unit volume reaches a critical level, or a transition from diffusion-limited to convection-assisted reaction kinetics occurs.

Acknowledgments

This work was supported in part by the National Institute of Health (1U54CA143906-01, R01HL038779), and the Oregon Health and Science University School of Medicine MD/PhD program. The project described was supported by Award Number U54CA143906 from the National Cancer Institute. The content is solely the responsibility of the authors and does not necessarily represent the official view of the National Cancer Institute or the National Institutes of Health.

Abbreviations

TF	tissue factor
FVII, FX, FXa	coagulation factor VII, coagulation factor X, activated factor X, respectively
FBS	fetal bovine serum
BSA	bovine serum albumin
PBS	phosphate buffered saline
HBSS	Hank's balanced salt solution
PRP, PPP	Platelet-rich plasma, platelet-poor plasma, respectively
LPS	lipopolysaccharide
SEM	standard error of the mean

References

- Maynard JR, Heckman CA, Pitlick FA, Nemerson Y. Association of tissue factor activity with the surface of cultured cells. *J. Clin. Invest.* 1975; 55:814–824. [PubMed: 47334]
- Nemerson Y. The phospholipid requirement of tissue factor in blood coagulation. *J. Clin. Invest.* 1968; 47:72–80. [PubMed: 16695947]
- Gemmell CH, Turitto VT, Nemerson Y. Flow as a regulator of the activation of factor X by tissue factor. *Blood.* 1988; 72:1404–1406. [PubMed: 3262388]
- Okorie UM, Denney WS, Chatterjee MS, Neeves KB, Diamond SL. Determination of surface tissue factor thresholds that trigger coagulation at venous and arterial shear rates: amplification of 100 fM circulating tissue factor requires flow. *Blood.* 2008; 111:3507–3513. [PubMed: 18203955]
- Pickering W, Gray E, Goodall AH, Ran S, Thorpe PE, Barrowcliffe TW. Characterization of the cell-surface procoagulant activity of T-lymphoblastoid cell lines. *J. Thromb. Haemost.* 2004; 2:459–467. [PubMed: 15009464]
- Lerner RG, Goldstein R, Cummings G, Lange K. Stimulation of human leukocyte thromboplastic activity by endotoxin. *Pro. Soc. Exp. Biol. Med.* 1971; 138:145–148.
- Aras O, Shet A, Bach RR, Hysjulien JL, Slungaard A, Hebbel RP, Escolar G, Jilma B, Key NS. Induction of microparticle- and cell-associated intravascular tissue factor in human endotoxemia. *Blood.* 2004; 103:4545–4553. [PubMed: 14988149]
- Moosbauer C, Morgenstern E, Cuvelier SL, Manukyan D, Bidzhekov K, Albrecht S, Lohse P, Patel KD, Engelmann B. Eosinophils are a major intravascular location for tissue factor storage and exposure. *Blood.* 2007; 109:995–1002. [PubMed: 17003379]
- Ritis K, Doulas M, Mastellos D, Micheli A, Giaglis S, Magotti P, Rafail S, Kartalis G, Sideras P, Lambris JD. A novel C5a receptor-tissue factor cross-talk in neutrophils links innate immunity to coagulation pathways. *J. Immunol.* 2006; 177:4794–4802. [PubMed: 16982920]
- Rickles FR, Edwards RL. Activation of blood coagulation in cancer: Trousseau's syndrome revisited. *Blood.* 1983; 62:14–31. [PubMed: 6407544]
- Panes O, Matus V, Saez CG, Quiroga T, Pereira J, Mezzano D. Human platelets synthesize and express functional tissue factor. *Blood.* 2007; 109:5242–5250. [PubMed: 17347408]
- Pawlinski R, et al. Hematopoietic and nonhematopoietic cell tissue factor activates the coagulation cascade in endotoxemic mice. *Blood.* 2010; 116:806–814. [PubMed: 20410508]
- Giesen PL, Rauch U, Bohrmann B, Kling D, Roque M, Fallon JT, Badimon JJ, Himer J, Riederer MA, Nemerson Y. Blood-borne tissue factor: another view of thrombosis. *PNAS.* 1999; 96:2311–2315. [PubMed: 10051638]
- Falati S, Liu Q, Gross P, Merrill-Skoloff G, Chou J, Vandendries E, Celi A, Croce K, Furie BC, Furie B. Accumulation of tissue factor into developing thrombi in vivo is dependent upon

- microparticle P-selectin glycoprotein ligand 1 and platelet P-selectin. *J. Exp. Med.* 2003; 197:1585–1598. [PubMed: 12782720]
15. Chou J, Mackman N, Merrill-Skoloff G, Pederson B, Furie BC, Furie B. Hematopoietic cell-derived microparticle tissue factor contributes to fibrin formation during thrombus propagation. *Blood.* 2004; 104:3190–3197. [PubMed: 15280200]
 16. Cvirn G, Gruber HJ, Koestenberger M, Kutschera J, Wagner T, Ferstl U, Sedlmayr P, Juergens G, Gallistl S. High availability of intravascular tissue factor in neonates. *J. Pediatr. Hematol. Oncol.* 2007; 29:279–283. [PubMed: 17483700]
 17. White-Adams TC, Berny MA, Patel IA, Tucker EI, Gailani D, Gruber A, McCarty OJT. Laminin promotes coagulation and thrombus formation in a factor XII-dependent manner. *J. Thromb. Haemost.* 2010; 8:1295–1301. [PubMed: 20796202]
 18. Tucker EI, Marzec UM, White TC, Hurst S, Rugonyi S, McCarty OJT, Gailani D, Gruber A, Hanson SR. Prevention of vascular graft occlusion and thrombus-associated thrombin generation by inhibition of factor XI. *Blood.* 2009; 113:936–944. [PubMed: 18945968]
 19. Berny-Lang MA, Aslan JE, Tormoen GW, Patel IA, Bock PE, Gruber A, McCarty OJT. Promotion of experimental thrombus formation by the procoagulant activity of breast cancer cells. *Phys. Biol.* 2011; 8:015014. [PubMed: 21301066]
 20. Lupu C, Westmuckett AD, Peer G, Ivanciu L, Zhu H, Taylor FB Jr, Lupu F. Tissue factor-dependent coagulation is preferentially up-regulated within arterial branching areas in a baboon model of *Escherichia coli* sepsis. *Am. J. Pathol.* 2005; 167:1161–1172. [PubMed: 16192650]
 21. Liebman HA, Feinstein DI. Thrombosis in patients with paroxysmal nocturnal hemoglobinuria is associated with markedly elevated plasma levels of leukocyte-derived tissue factor. *Thromb. Res.* 2003; 111:235–238. [PubMed: 14693169]
 22. Langer F, Spath B, Haubold K, Holstein K, Marx G, Wierecky J, Brummendorf TH, Dierlamm J, Bokemeyer C, Eifrig B. Tissue factor procoagulant activity of plasma microparticles in patients with cancer-associated disseminated intravascular coagulation. *Ann. Hematol.* 2008; 87:451–457. [PubMed: 18292996]
 23. Shu F, Kobayashi H, Fukudome K, Tsuneyoshi N, Kimoto M, Terao T. Activated protein C suppresses tissue factor expression on U937 cells in the endothelial protein C receptor-dependent manner. *FEBS Letters.* 2000; 477:208–212. [PubMed: 10908722]
 24. Poitevin S, Cochery-Nouvellon E, Dupont A, Nguyen P. Monocyte IL-10 produced in response to lipopolysaccharide modulates thrombin generation by inhibiting tissue factor expression and release of active tissue factor-bound microparticles. *Thromb. Haemost.* 2007; 97:598–607. [PubMed: 17393023]
 25. Hrachovinova I, et al. Interaction of P-selectin and PSGL-1 generates microparticles that correct hemostasis in a mouse model of hemophilia A. *Nat. Med.* 2003; 9:1020–1025. [PubMed: 12858167]

Figure 1. Characterization of the procoagulant activity of monocytic cells in a closed system U937 cells were procoagulant in a tissue-factor (TF) dependent (a) and cell concentration-dependent manner (b). Human sodium citrate-anticoagulated plasma was pretreated with vehicle (–) or a neutralizing antibody to TF (anti-TF, $20 \mu\text{g mL}^{-1}$) prior to addition of cultured U937 cells (10^4 to 10^6 mL^{-1}) for 3 minutes at 37°C . U937 cells were stimulated with lipopolysaccharide ($10 \mu\text{g mL}^{-1}$ LPS) for 24 hours. Coagulation of plasma was initiated by recalcification using 7.6 mM CaCl_2 (final concentration) and clotting times were recorded on a coagulometer. Data are reported as mean $\pm$ SEM, from 3–6 experiments. * $P < 0.05$ versus non-LPS stimulated cells. # $P < 0.05$ versus the absence of cells.

Figure 2. Characterization of the procoagulant activity of TF microspheres in a closed system
 Tissue-factor (TF)-coated polymeric microspheres were procoagulant in a carrier concentration (a) and TF-dependent manner (b). Human sodium citrate-anticoagulated plasma was pretreated with neutralizing antibodies to TF (anti-TF, 50 $\mu\text{g mL}^{-1}$) and FXI (14E11 or 1A6, 10 $\mu\text{g mL}^{-1}$) prior to addition of BSA-coated (white bars) or TF-coated (black bars) microspheres (10^2 to 10^6 mL^{-1}) for 3 minutes at 37 °C. Data in (b) were obtained with microsphere additions of 10^6 mL^{-1} . Coagulation of plasma was initiated by recalcification using 7.6 mM CaCl_2 (final concentration) and clotting times were recorded on a coagulometer. Data are reported as mean $\pm$ SEM, from 3 experiments. * $P < 0.05$ versus BSA-coated microspheres.

Figure 3. Characterization of enzyme activation kinetics initiated with TF microspheres

Tissue-factor (TF)-coated polymeric microspheres promoted assembly of active enzyme complex in a carrier concentration dependent manner (a). The rate of substrate cleavage was dependent upon carrier count (b). Enzyme initiation time yielded a direct relationship with the calculated diffusion transport length between TF microspheres in suspension (c). Enzyme reaction rate yielded an inverse relationship with diffusion transport length for TF microspheres in suspension (d). Human sodium citrate-anticoagulated plasma was pretreated with 15 μL of S-2366 before addition of 50 μL of TF microspheres (10^3 to 10^6 mL^{-1}) and recalcification using 7.6 mM CaCl_2 (final concentration). Absorbance of 405 nm wavelength light was recorded with a spectrophotometer at 1 minute intervals for 60 minutes. Data are reported as mean $\pm$ SEM, from 4–7 experiments.

Figure 4. Characterization of prothrombotic activity of TF microspheres and U937 cells in an open system

LPS-stimulated U937 cells (a) and TF-coated polymeric microspheres (b) were prothrombotic in a concentration-dependent manner when added to recalcified whole blood in an ex-vivo occlusive thrombus assay. The procoagulant activity of 10⁵ mL⁻¹ TF-coated microspheres was abrogated with an anti-TF antibody. Blockade of the contact pathway of coagulation with 14E11 (anti-FXI) had no effect on time to occlusion for TF microsphere suspensions in the absence of anti-TF antibodies (c). U937 cells were stimulated with lipopolysaccharide (10 µg mL⁻¹ LPS) for 24 hours. U937 cells or TF microspheres (10³ to 10⁶ mL⁻¹ final concentration) were added to human sodium citrate-anticoagulated blood

prior to recalcification using 7.6 mM CaCl_2 (final concentration). Occlusive thrombus formation was initiated by flowing the pretreated blood at a constant pressure through a collagen-coated capillary tube and the time to occlusion was recorded when flow ceased. Data are reported as mean $\pm$ SEM, from 3 experiments. * $P < 0.05$ versus non-LPS stimulated cells. # $P < 0.05$ versus BSA-coated microspheres.

\$watermark-text

\$watermark-text

\$watermark-text