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Using microfluidics to understand the effect of spatial distribution of tissue factor on blood coagulation

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Abstract

Initiation of blood coagulation by tissue factor (TF) is a robust, highly regulated process. Both the spatial distribution of TF and the geometry of the vasculature may play important roles in regulating coagulation. As this review describes, microfluidic systems provide a unique opportunity for investigating the spatiotemporal dynamics of blood coagulation *in vitro*. Microfluidic systems with surfaces of phospholipid bilayers patterned with TF have been used to demonstrate experimentally the threshold responses of initiation of coagulation to the size and shape of surfaces presenting TF. These systems have also been used to demonstrate experimentally that propagation of coagulation is regulated by the shear rate of blood flow in microcapillaries and microchannels. By understanding these and other aspects of the spatial dynamics that regulate blood coagulation, many new methods for treating clotting disorders, such as venous thromboembolism (VTE) and sepsis, could arise.

Introduction

Tissue factor (TF) plays an essential role in initiation and regulation of blood coagulation [1–3], and it is known to be present on surfaces of a wide variety of cells and microparticles [1,4]. Understanding how the spatial distribution of TF influences initiation and propagation of blood coagulation may yield insight into how coagulation is initiated and localized to only regions of significant vascular damage. Experiments and numerical simulations have shown previously that reactions in the coagulation network are regulated, in part, by a threshold response to the concentration of activators in solution [5–7]. On surfaces, however, both the size of a region, or patch, of surface containing TF and the rate of blood flow have been implicated as additional controlling factors of initiation of coagulation [8,9]. To experimentally characterize the effect of the spatial distribution of TF on surfaces on initiation and propagation of coagulation, we developed microfluidic tools to control the location and distribution of TF on the micrometer scale [10–16].

We have shown experimentally that both initiation and propagation of coagulation are regulated by a threshold response to the concentration of activators of coagulation, such as thrombin, and propose a physical mechanism to describe these dynamics. This threshold response is manifested by the initiation of coagulation only when the concentration of activator exceeds a threshold concentration. Therefore, initiation of coagulation is dependent on competition between the reaction, which produces activators, and transport, which removes activators by diffusion

Abbreviations: TF, tissue factor; VTE, venous thromboembolism.

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or fluid flow. According to this mechanism, initiation of coagulation is regulated by the size of a patch of surface stimulus, and propagation is regulated by the shear rate of blood flow at vessel junctions.

Initiation of coagulation is regulated by the size and shape of a surface patch of TF

When human plasma or whole blood was exposed to surface patches of TF in a microfluidic device in the absence of flow, coagulation occurred only on patches of TF larger than a critical (threshold) size (Figure 1A,B) [11,12]. Photolithography was used to pattern phospholipids bilayers with surface patches of TF of controlled size and shape in a microfluidic device [11–14,16]. Coagulation was indicated by the appearance of both thrombin, monitored by visualization of a blue fluorescent substrate for thrombin with fluorescence microscopy, and fibrin, monitored by bright-field microscopy. The size of the TF patch dictated whether the critical concentration of activator was reached due to differences in the rate of diffusion of activating species off of the patch. On small patches, diffusion removed activators from the patch faster than they were produced, and the critical concentration necessary to initiate coagulation was not reached. On large patches, removal of activators from the patch was less efficient – activators were produced faster than diffusion could remove them and the critical concentration of activators was reached. This threshold response of initiation of coagulation to patch size is a robust phenomenon under a wide range of conditions, including variations in temperature, the concentration of TF in the patch, and with variation in the concentration of individual components of the coagulation cascade (factor IIa, factor V, factor VIII, and thrombomodulin) [11]. Preliminary results showed that initiation of coagulation displayed a similar threshold response to the size of a patch of stimulus in the presence of flow. These results imply that patterned surfaces may be useful for assaying the ability of blood to coagulate, especially for point-of-care applications.

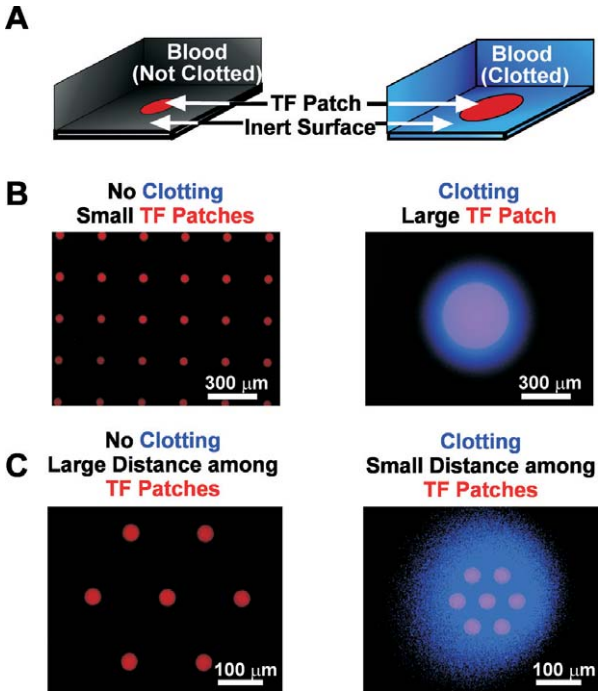

Fig. 1. Initiation of human blood plasma is regulated, in part, by the spatial distribution of tissue factor. (A) Schematic of an *in vitro* microfluidic system designed to expose human blood and plasma to patches containing TF reconstituted into phospholipid bilayer. Fluorescence (light grey in greyscale version, blue in online colour version) indicates thrombin production and coagulation. (B) Coagulation did not initiate on 50 μm patches (below the threshold size), but coagulation did initiate on a single large 400 μm patch (above the threshold size). (C) At the same total TF surface area, coagulation did not initiate on arrays of patches that were spaced far apart, but did initiate on arrays of patches that were spaced closely enough to allow cross-talk by diffusion. The TF patches appear as white/grey circles in the greyscale version and as red circles in the online colour version.

When human blood plasma was exposed to TF patches with the same total surface area, initiation was dependent on the shapes of the patches [13]. Coagulation initiated on circular, square, and wide rectangular patches, but not on narrow rectangles and star-shaped patches. The response of coagulation to the shape of a stimulus could be utilized in the design of particles for drug delivery with shapes that are resistant to coagulation.

Initiation of coagulation is regulated by the spatial distribution of surface patches of TF

The spatial distribution of individual patches of TF, not the total surface area of TF, regulates initiation of coagulation [12]. To understand the influences of the spatial distribution of TF on initiation of clotting, arrays of surface patches of TF of varying spatial density were patterned as described above. Within all arrays, the size of each individual patch was below the threshold size necessary to initiate coagulation, and each array contained the same total concentration and surface area of TF. Coagulation did not initiate when patches in an array were separated by a long distance. However, coagulation initiated when these patches were spaced closely together (Figure 1C). In this case, individual TF patches may "communicate" with other patches by diffusion. These results demonstrate that blood

can be exposed to active TF, as long as it is localized in small patches or particles that are below threshold size and separated from each other by a sufficient distance. This explains how blood could be exposed to a low concentration of microparticles expressing active TF with out clotting. These results also have implications for understanding coagulation by bacteria, and predict that localization of bacteria should be a controlling factor for direct initiation of coagulation.

Propagation of coagulation is regulated by shear rate

To understand the influence of blood flow on the spatial dynamics of initiation of coagulation by TF, microfluidic techniques have been developed to enable precise control of fluid flow as well as channel geometry and surface chemistry [15]. Micrometer-scale channels were coated with various phospholipids, including components of the blood-coagulation network such as thrombomodulin and tissue factor. In these microfluidic channels, clots propagated in a wave-like fashion with a constant velocity in the absence of flow. In the presence of flow, propagation of coagulation from an occluded channel to a channel with flowing blood plasma can be regulated by the geometry of the junction and the shear rate in the channel with flowing plasma. The mechanism responsible for regulating propagation of coagulation in the presence of flow also involves competition between the production of activated clotting factors from the clot in an occluded channel and the removal of these factors by flow. Initiation of coagulation by surface patches of TF may display a similar threshold response to shear rate of flowing blood. This mechanism also implies that initiation of coagulation in solution may be regulated by the rate of mixing.

These results demonstrate that some aspects of coagulation are regulated by flow and suggest a mechanism for why immobility and increased blood stasis are risk factors for venous thromboembolism (VTE). Under physiological shear rates, coagulation should not be initiated by sufficiently small injuries to the endothelium, or by TF present on isolated cells in flowing blood. Clots present in capillaries should remain localized. However, if shear rates are reduced below critical values, even very small injuries may initiate clotting, and clots formed in capillaries may propagate out of capillaries and into larger veins.

Conclusion

In conclusion, we have developed several microfluidic approaches for investigating the spatial dynamics of coagulation *in vitro*. We used these approaches to address fundamental questions about how spatial distribution of TF and the geometry of the vasculature can influence the initiation and propagation of coagulation. If these results and predictions are confirmed *in vivo*, we anticipate that new treatments for VTE and deep vein thrombosis could be developed based on careful control of shear rates in vessels. Further development of these microfluidic techniques should also be useful for new assays, especially point-of-care tests, for monitoring coagulation and determining drug dosage.

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References

[1] Mackman N. Role of tissue factor in hemostasis, thrombosis, and vascular development. *Arterioscler Thromb Vasc Biol* 2004;**24**:1015–22.
[2] Butenas S, Orfeo T, Brummel-Ziedins KE, Mann KG. Tissue factor in thrombosis and hemorrhage. *Surgery* 2007;**142**: S2-S14.
[3] Morrissey JH. Tissue factor: A key molecule in hemostatic and nonhemostatic systems. *Int J Hematol* 2004;**79**:103–08.
[4] Rauch U, Nemerson Y. Circulating tissue factor and thrombosis. *Curr Opin Hematol* 2000;**7**:273–77.
[5] Jesty J, Rodriguez J, Beltrami E. Demonstration of a threshold response in a proteolytic feedback system: Control of the autoactivation of factor XII. *Pathophysiol Haemost Thromb* 2005;**34**:71–9.
[6] van't Veer C, Mann KG. Regulation of tissue factor initiated thrombin generation by the stoichiometric inhibitors tissue factor pathway inhibitor, antithrombin-III, and heparin cofactor-II. *J Biol Chem* 1997;**272**:4367–77.
[7] Ataullakhanov FI, Pohilko AV, Sinauridze EI, Volkova RI. Calcium threshold in human plasma clotting kinetics. *Thromb Res* 1994;**75**:383–94.
[8] Beltrami E, Jesty J. The role of membrane patch size and flow in regulating a proteolytic feedback threshold on a membrane: possible application in blood coagulation. *Math Biosci* 2001;**172**:1–13.
[9] Jesty J, Beltrami E. Positive feedbacks of coagulation – Their role in threshold regulation. *Arterioscler Thromb Vasc Biol* 2005;**25**:2463–69.

[10] Runyon MK, Johnson-Kerner BL, Ismagilov RF. Minimal functional model of hemostasis in a biomimetic microfluidic system. *Angew Chem Int Edit* 2004;**43**: 1531–36.
[11] Kastrup CJ, Shen F, Runyon MK, Ismagilov RF. Characterization of the threshold response of initiation of blood clotting to stimulus patch size. *Biophys J* 2007;**93**:2969–77.
[12] Kastrup CJ, Runyon MK, Shen F, Ismagilov RF. Modular chemical mechanism predicts spatiotemporal dynamics of initiation in the complex network of hemostasis. *Proc Natl Acad Sci USA* 2006;**103**:15747–52.
[13] Kastrup CJ, Shen F, Ismagilov RF. Response to shape emerges in a complex biochemical network and its simple chemical analogue. *Angew Chem Int Edit* 2007;**46**:3660–62.
[14] Kastrup CJ, Ismagilov RF. A physical organic mechanistic approach to understanding the complex reaction network of hemostasis (blood clotting). *J Phys Org Chem* 2007;**20**: 711–15.
[15] Runyon MK, Johnson-Kerner BL, Kastrup CJ, Van Ha TG, Ismagilov RF. Propagation of blood clotting in the complex biochemical network of hemostasis is described by a simple mechanism. *J Am Chem Soc* 2007;**129**:7014–5.
[16] Kastrup CJ, Runyon MK, Lucchetta EM, Price JM, Ismagilov RF. Using chemistry and microfluidics to understand the spatial dynamics of complex biological networks. *Acc Chem Res* 2008, in press.