MINI-REVIEW

Microfluidic systems toward blood hemostasis monitoring and thrombosis diagnosis: From design principles to micro/nano fabrication technologies

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Abstract
Perfusable endothelialized models of microfluidic systems that recapitulate unique biological and biophysical microvasculature conditions are improved with micro/nano engineering advances for monitoring of blood hemostasis and thrombosis treatment. Although bio-sensors and monitoring devices significantly advance in measuring platelet aggregation and thrombosis kinetics, currently platelet aggregation tests still do not meet the arising clinical requirements. Trying to seek new solutions for such a demanding from clinics, the present review provides an overview of design principles of microfluidic systems and micro/nano fabrication strategies in studying the platelet adhesion and aggregation. We critically sketch the characteristics of microfluidic systems to elucidate the role of platelets in the complex process of thrombus formation. The importance, benefits, and challenges of introduced principles and methods are discussed. The potential from various of basic research to clinical applications is also briefly discussed to help guide designing more versatile point-of-care devices for hemostasis monitoring and thrombosis diagnosis and treatment.

KEYWORDS
hemostasis monitoring, microfluidics, micro-nano fabrication, platelet adhesion and aggregation, thrombosis diagnosis and treatment
1 | INTRODUCTION

Accurate and rapid evaluation of blood hemostasis is vital for patient management by using extracorporeal devices and conducting anticoagulation therapy.\cite{1,2} Hemostasis monitoring is widely used in many clinical applications, for example, surgery, trauma, anticoagulation, and anti-platelet therapies. Therefore, serious disorders triggered by bleeding or thrombosis can be avoided by the management of hemostasis.\cite{3,4} Nowadays, since an increasing number of patients around the world in mortality is caused by catastrophic hemorrhage with the inability to manage anticoagulation dosage precisely and continuously, hence personalized anticoagulation dose monitoring in real-time becomes an important issue to be solved to maintain hemostasis in vivo.\cite{5,6} Reliable bedside hemostasis monitoring with low blood volumes is essential for patients with acute coagulopathy.\cite{4}

Various devices have been developed for platelet function tests in vitro and present useful knowledge for coagulation status or platelet function. However, they are less useful to predict thrombotic or bleeding risk in clinical applications.\cite{7} Tests such as the VerifyNow test, an aggregometry-based function test, and TEG-5000, are all closed-system designs, which are performed under static or unrelated flow conditions and fail in incorporation with necessary hemodynamic conditions.\cite{8,9} Light transmission aggregometry (LTA) is considered the “gold standard” for platelet function tests.\cite{10} However, the whole LTA procedure requires multiple steps of sample preparation to the final aggregation measurements. All these will lead to significant variations in tests between different laboratories.\cite{11} Consequently, the diagnostic value of the test becomes incomparable. The LTA test also requires a large volume of blood and presents significant challenges in pediatric patients with very limited blood sample. While PFA-100/200 analyzer is simple to operate and relatively inexpensive, and the kinematic information is involved with high shear stress. However, these tests are insufficient sensitive for further testing in patients with mucocutaneous bleeding, and nonspecific for platelet disorders confines their usefulness.\cite{12}

Up-to-date guidelines show that it was recommended not to perform template bleeding time as a screening test for defects in primary hemostasis.\cite{13} Furthermore, the high shear rate perfusion employed makes the device sensitive to disorders associated with von Willebrand factor (vWF).\cite{14} Therefore, developing a robust testing protocol that cover all aspects of platelet function can be very challengeable. Previously, quite a few excellent works review the commercial devices with their advantages and limitations.\cite{15,16} Unfortunately, until now, there are no standard recommendations access to external proficiency testing to clinical applications. Thus, new technologies that are reliable to illuminate the role of platelets in physiological and pathological thrombus formation are widespread anticipated to further guide the personalized anticoagulation dose monitoring in real-time.

As simple as plates, microfluidic systems can manipulate tiny fluids as well. Due to the transparency and closed characteristics of the microfluidic chip, once initiated, the blood does not exchange components with external environments, and hence cells to surface interaction is easy to be observed. Meanwhile, the microchannels can represent the classical ml scale batch reactor that facilitates liquid handling with robotics integrated with different size of well plates for the measurements of platelet function, coagulation biology, adhesion dynamics, pharmacology, and clinical diagnostics at μl scale without significant fluid flow or mixing.\cite{17} Furthermore, the material surface topography and chemistry have great impact on biological processes, for example, protein adsorption and conformation, cell behavior, blood contacting properties.\cite{18} Therefore, when thrombosis forms interfaces with biomaterials in vitro or in vivo, regulating platelets behavior can be in a predictable manner that has various applications for hemostasis monitoring and thrombosis diagnosis, for example, adhesion preference to specific substrates, patterning on predefined substrate regions, improved proliferation, controllable cell to cell communication, altered response to chemical or physical extracellular signals. Micro/nano engineering advances have enabled these applications integrated with microfluidic systems; therefore, thrombotic activity can be well investigated on specific surfaces, for example, biopolymers, matrix proteins, and tissue factor (TF), under designate structure and conditions, for example, microvasculature, stenosis, shear rate. By summarizing typical methodologies and micro/nano technologies in microfluidic chips for blood hemostasis monitoring and thrombosis diagnosis/treatment, this mini-review intends to inspire more researchers to devote themselves in designing new systems toward solving those problems under relevant hemodynamic conditions against blood-related diseases.

2 | PLATELET FUNCTION UNDER FLOW AND AGGREGATION BIOLOGY

Platelets play an important role not only in hemostasis and arterial thrombosis, but also in other physiological and pathophysiological processes.\cite{20} The onset formation of pathological arterial thrombus is typically caused by the rupture of an atherosclerotic plaque or an acute deterioration of endothelial layer.\cite{21} Subsequently, highly reactive subendothelial matrix proteins are exposed. Under high
shear rate, vWF binds to collagen and facilitates the platelets adhesion with a glycoprotein complex.\textsuperscript{[22]} It should be noted that interactions among GPVI, integrin, collagen, and fibronectin are requisite for stable platelet adhesion, Figure 1 right panel. Platelet activation is enhanced by releasing soluble agonists, for example, adenosine diphosphate, thromboxane $A_2$, and thrombin interacting with specific receptors, respectively.\textsuperscript{[23–25]} Consequently, the generated signals inside the platelet trigger the transition of GPIIb/IIIa from a low affinity to a high affinity state and enable binding to soluble plasma proteins, thereby facilitate stable thrombus formation.\textsuperscript{[26]} The arterial thrombus is enhanced by the amplification of platelet coagulation into a stabilizing fibrin mesh.\textsuperscript{[27]} Actually, experimental evidence shows that arterial thrombus formation is highly related with dynamic conditions, and developing thrombi are now known with two distinct regions,\textsuperscript{[28]} where fully activated platelets are close to the lesions and highly relying on soluble agonists.\textsuperscript{[29]} Meanwhile, the thrombus composed with low activation platelets is nearly non-sensitive to standard antiplatelet drugs.\textsuperscript{[30]} These experimental results provide useful information to distinguish between the hemostatic and thrombotic response regulating the growth of the thrombus formation, Figure 1 right panel.

It should be noted that platelet activation, adhesion, and secretion process can occur in different order.\textsuperscript{[31]} However, the processes finally are adjacent to platelet aggregation which will initiate the thrombus formation. Platelets are activated by biochemical agonists, or physical stimuli, for example, high shear stress. During hemostasis, platelets are rapidly enrolled and become highly adhesive to the injury sites with a sequence of biological processes to form thrombus.\textsuperscript{[32]} Similar processes may occur in arteries and veins, leading to myocardial infarction or stroke.\textsuperscript{[33]} Therefore, platelet adhesion and aggregation are the critical progression to mediate relevant physiological and pathological processes.

3 | DESIGN PRINCIPLES OF MICROFLUIDIC DEVICES FOR COAGULATION RESEARCH

The microfluidics systems promote the design to evaluate the complex processes of platelet aggregation. Therefore,
the hemodynamic factors and blood vessel injury can be generally engineered to mimic the pathophysiological environment of blood vessels for coagulation research, for example, contraction force during thrombosis formation, shear rate variation, stenosis formation (Figure 1 right panel), and microvasculature construction (Figure 1 left panel).

3.1 Platelet contraction force

The mechanical properties of blood cells and vascular tissues are closely correlated to the pathogenesis of various cardiovascular and hematological diseases, for example, sickle cell disease, bleeding disorders, and uncontrolled blood clotting in atherosclerosis or stroke. Therefore, the specific properties of blood and tissues, for example, blood vessel stiffness, platelets contraction forces, and stiffness, are capable to be treated as biomarkers for the diagnosis of cardiovascular and hematological diseases.

Platelet retraction forces can be studied by the elastic modulus of blood clots. The increase in contraction force is proved to be associated with disorders of clotting complications, such as chest pain, artery disease, and Buerger’s disease. However, it is technically difficult to directly measure the forces for contraction assays, and moreover, numerous processes influence the platelets biophysical properties. Therefore, the platelet function may alter in blood-related diseases compared with the controls. Several successful attempts have been pursued to measure the platelet contraction forces. Conventionally, atomic force microscopy (AFM) is used for precise high-resolution imaging at the nanometer scale using mechanical cantilever by probing the surface. Therefore, single platelet contractile force and elasticity can be measured using a modified side view AFM technique, Figure 2A. The technique enables kinetic measurements of platelet contraction even down to a single cell level. Moreover, platelets can also be settled on polydimethylsiloxane (PDMS) micropillars. When the platelets adhere and contact on the micropillars that act as springs, the deflections of the pillars and the resultant forces can be measured and calculated accordingly. Optical tweezers can also be applied to measure the binding strength and activation state of individual integrin-fibrinogen pairs for activated platelets. As platelets are physiologically response to the external environments, quantitative investigation of cellular mechanics of platelets responding to
the matrix microenvironment variation by using similar approaches may yield new biophysical insight in hemostasis and thrombosis.

### 3.2 Influence of shear gradient in thrombus formation

From a macroscale perspective, the mechanical properties of blood components, for example, blood viscosity, pressure and stiffness, fluid shear stress, and clots, are well balanced in healthy conditions, and it indicates that the circulatory system is maintained in a state of mechanical equilibrium. From a microscale perspective, as the mechanical equilibrium is changed, that is, plaque formation with increased shear stress,[47] the ligands trigger specific downstream signaling pathways by binding to specific receptors. For instance, the platelet adhesion at distinctively different shear rate by binding to specified platelet receptors is regulated by immobilized vWF and fibrinogen.[48] Therefore, to investigate platelets behavior in vitro, it is necessary to remodel the physiological microenvironment with biochemical and biomechanical cues to modulate cell behavior in vivo.[49]

In Figure 2B, a shear gradient-activated microfluidic device is designed to evaluate blood clotting with small sample volumes under relevant pathophysiological flow by mimicking the network of arterial vessels. The device is integrated with an extracorporeal circuit to study pig endotoxemia or heparin therapy models and produces ex vivo real-time readouts of variations for automated monitoring of whole blood hemostasis. The experiments show more reliable diagnostic results than the current standard clotting assays.[50] However, there are still some limitations for extracorporeal circuits in animal with real-time readouts. The clotting evaluation is not instantaneous, and hemostasis monitoring is not continuous since the devices have to be discarded after sequential measurements. Actually, instantaneous hemostasis assays are still not available, and the clinical decisions are usually delayed for several hours.[51] The introduced principles for point-of-care (POC) devices imply a new designing concept that the microfluidic chip with extracorporeal circuits can be functionally integrated. In recent years, research has focused on developing microfluidic flow cytometers that can be easily integrated with extracorporeal circuits.[52] These devices could ideally be ported, easy to be operated with less user training and utilized for POC diagnosis of platelets analysis in limited resource facilities.[53] Therefore, the coagulation monitoring and antithrombotic therapy using native blood in both clinical and home care settings can be exploited in a relatively simple and reliable way. By integrating micro-engineered pneumatic valve coupled with microfluidic system in vitro, mechanical injury bleeding model can be mimicked, and the interaction of different components of hemostasis can be monitored.[54] Moreover, real-time monitoring of blood clotting and measurement of hemostatic parameters can be achieved by a micro-fabricated film bulk acoustic sensor.[55] The device was made of an Au/ZnO/Si3N4 film stack exciting by a lateral electric field. It operated under a shear mode by recording the resonant frequency variation along with the change of blood viscosity to reveal the sequential clotting stages. However, the designing principle of the microfluidic system should fulfill the criteria of high throughput analysis, automation and portability, without sacrificing performance and testing accuracy in relatively low price.

Platelet thrombosis associated with serious hemorrhage or arterial occlusion is extensively affected with high shear rates combined with agonists or artificial surfaces. The analysis of shear rate from physiological to pathological flow conditions is of great help for evaluating and predicting native cardiovascular lesions and promoting new medical device design and testing. Understanding the fundamental mechanism of rapid thrombosis under different shear rates will help the clinicians, device engineers, and researchers for improving the therapies in conjunction with numerous factors affecting atherosclerotic disease.

### 3.3 Influence of stenosis in microvascular environment

Thrombosis usually occurs in pathologic arterial stenosis with absolute cessation of blood flow in vessel.[56] When the hemodynamic shear rates reach up to 5000 s\(^{-1}\) that represents pathological conditions, thrombosis associated with hemostasis, arterial pathology, and medical device occlusion usually happens with shear-induced platelet aggregation.[57]

In vascular flows, the shear rate at the vessel wall is the most interest for study of the platelet adhesion and aggregation. Since the fluid mechanics influences the biological processes, including the physiologic signaling, pathophysiologic process such as atherosclerosis and thrombosis. The wall shear rates generated from about 10 s\(^{-1}\) in large veins to 1000 s\(^{-1}\) in arteries.[58] Under pathological conditions, shear rates can rise up to 5000 s\(^{-1}\). The shear rates of stenotic disease are in wide range from a value of ~500 s\(^{-1}\) to 200,000 s\(^{-1}\).[59] Since the wall shear rate is proportional to the third order of the vessel diameter. Experimentally, the shear rates remain exceptionally high (>200,000 s\(^{-1}\)) when the stenosis is in high degree with reactive hyperemia, and the flow rate is reduced. Large scale of the thrombus formation occurs even without a reduction in flow.[60]
In Figure 2C, a stenosed vessel geometry of microfluidic chip is presented, and the interrelation between flow rate changes, and platelet aggregation is investigated. The experiment results reveal that initial platelet recruitment occurs at the stenosis apex, while the following platelet aggregation happens in the downstream expansion zone. The studies demonstrate a central role of shear micro-gradients in initiating the formation of stabilized discoid platelet aggregates in vivo and challenge the well-known theory about the platelet aggregation which is typically caused by the interaction of soluble agonists and platelets at the sites of injury. The experimental results show that discoid platelets aggregation in vivo is independent of sustained calcium signaling. Moreover, the granule release and P-selectin expression on the platelets surface occur relatively late during the thrombotic process. Understanding the fundamental interaction between blood rheology and platelet aggregation in localized flow changes will develop more effective targeted antithrombotic therapies than the existing approaches. Figure 2D shows a typical stenosis region and section views inside microfluidic chip. The influence of different shear rates and efficacy of antiplatelet therapy doses are investigated. Shear rates were found to have magnificent effects on thrombosis/dose-response curves. Therefore, the quantitative and statistic study of different shear rate and antiplatelet therapy dosages should be further modeled for optimizing patient treatment.

3.4 Influence of vascularized microfluidics

The biological and physical characteristics of the microenvironment within tissues are highly related to microvasculature that plays an important role in the initiation and progression of many thrombotic pathologies, that is, disseminated intravascular coagulation and thrombotic microangiopathies. A leaky endothelium of microvasculature is a feature of atherogenesis. Current understanding of blood-endothelium interface has been predominantly from two-dimensional in vitro models and complex in vivo animal models. However, these models are limited by their reductionism as they are not designed to accomplish complete recapitulation of biological complexity. To address these limitations, vascularized microfluidics have been becoming an important platform to recapitulate the unique biochemical and biophysical conditions of the microcirculation with the advanced microengineering development of perfusable, endothelialized models.

Figure 2E shows a flexible non-protein microfluidic platform for the platelet adhesion and aggregation study. A one-step, cost-effective strategy is proposed to tuning the physico-chemical characteristics of the microfluidic channel surface. The hemodynamic conditions can be mimicked to investigate the platelet-endothelium interface interaction in vitro in physiopathological process. The results indicate that the platelet adhesion and aggregation can be manipulated by engineered surface microtopography and charges. Figure 2F shows engineered micro-vessels of complex geometry down to 300 μm to investigate the pathological responses to endothelial activation. The results show that the secretion of vWF and its capacity depend on vascular geometry and fluid flow. Especially, with high shear stress, strong flow acceleration, and sharp turns, the vWF-induced thrombosis is in the greatest level. All these microfluidics models prove a set of biophysical parameters that influence microvascular thrombosis-vessel diameter, vessel geometry, vessel surface microstructure, and fluid shear rate, therefore have the potential to serve as an important model for the study of thrombotic-related disease. Recently, a microvasculature model integrated with a valve is proposed to mimic wound creation for bleeding that complements existing approaches to the hemostasis and thrombosis analysis. Another approach is to create living microvascular networks in three-dimensional tissue scaffolds utilize cellular self-assembly technique. These methods facilitate the fabrication of networks to test permeability and endothelial cell (EC) remodeling for the study of angiogenesis and thrombosis.

Microfluidic chips with microvascular geometry can be obtained by various microfabrication techniques, for example, casting gels around a needle, glass channels, or metal wire. Solid polymers, for example, PDMS, polyester and hydrogels, such as collagen, fibrin, alginate, gelatin, and polyethylene glycol (PEG) are all can be used for fabricating microfluidic chips. An important issue for constructing microvasculature model is the creation of robust inorganic-organic interface between microfluidic channels and the soft gels. Therefore, taking the advantage of the increased viscosity and surface tension of the micropatterned materials, the gel can be injected between pillars or similar structures to trap the gel and enable microfluidic access.

4 Fabrication Technologies in Microfluidic-Based Thrombosis Diagnosis and Treatment

Microfabrication techniques are widely applied to construct different model in vitro to mimic the macroenvironment in microfluidic chips. The extracorporeal
devices can be integrated into the microfluidic chip to control different parameters, for example, shear rate, stenosis, and microvascular structure for assessment of blood hemostasis and conducting anticoagulant therapy. However, to investigate platelet activation, adhesion, and aggregation in vitro, reconstruction of a physiologically relevant microenvironment that modulates cell behavior in vivo with biochemical and biomechanical cues is the most important issue to be solved. Surface chemistry and topographies have been demonstrated effective on numerous cellular responses. Therefore, controlling scale and pattern in chemical and topographic substrate pattern would help significantly to develop purpose specific platelet regulating cues in various biomedical applications. Nowadays, micro-nano technology applied in microfluidic devices is becoming a vital strategy to study the thrombogenicity of materials in spatially confined areas for thrombosis diagnosis and antiplatelet drugs testing.

### 4.1 Conformal coating

As microfluidic chips can mimic the atherosclerotic process, numerous studies have examined human whole blood with extracellular matrix (ECM) surface coatings. Figure 3A depicts a special channel geometry coated with different collagen and TF. The thrombogenic surface can be easily made to promote thrombus growth, while blood could flow freely through the other. However, some heterogeneity in the protein coated area can be observed. The phenomenon is due to the ECM proteins flushing away by the high shear rate. The observation implies that the approach is lack of uniformity and stability. In order to solve this problem, the coated ECM protein can be seeded on (3-aminopropyl)triethoxysilane-treated (i.e., silanized) glass. The results show that silanized glass is more suitable and stable for cell culture substrate. The platelets can also be served as anchoring points for the formation of a fibrin coating. Therefore, procoagulant platelets can be characterized by high Ca2+ dependent signals. Interactions at the blood-material interface of blood-contacting devices, such as vascular stents, catheters, artificial heart valves, and biosensors, are another important issue to be solved for treating thrombotic diseases. Intensive efforts in developing low-activating device coatings have been paid. However, the humoral responses and subsequent cellular adhesion lead to incompatibility reactions which limiting the device performance. ECs as biological surface coatings are an effective way to overcome unfavorable materials-related responses for continuous anticoagulation of patient. The design of hemocompatible coatings highly depends on the knowledge of material-blood interactions. General factors that influence hemocompatibility reactions applying coating method can be divided into several categories: wettability, functional groups, roughness topography. Future strategies of mimicking the thrombus formation or improving the hemocompatibility require both in depth understanding of the physiological activation pathways and its interaction with specified surfaces. Feedback control of the physiological properties might be the promising strategies for future developments.

### 4.2 Microcontact printing

Using microcontact printing, the distinct thrombotic regions can be successfully mimicked. The method is similar to stamps and inkpads, but at the micro/nanoscale. The PDMS stamps with randomly distributed pixels are fabricated by standard photolithography techniques. Then, different protein patterns can be printed covalently on reactive substrate. Figure 3B shows a basic microcontact printing strategy, and the method is used to immobilize human fibrinogen covalently in randomly placed, micron-sized islands at different surface coverage. Therefore, platelet adhesion and morphology on different substrates are systematically assessed. The microcontact printing of collagen and varying amounts of TFs are used to reveal a steep threshold for fibrin generation under whole blood flow under different shear rates in a parallel microfluidic chip. However, high shear rate flow can wash formed enzyme complexes or activated enzymes from the vicinity of the TF-exposed site. The diluted concentration of enzyme will be prohibited for the formation of fibrin perpendicular to flow. Using printed microarrays facilitates different proteins and TFs on the microfluidic channel to develop purpose-specific platelet regulating cues in relevant pathophysiological environment.

### 4.3 Ultraviolet photolithography

Ultraviolet (UV) photolithography is widely applied as an important microfabrication technique in biomedical applications. Figure 3C shows that patches of TF have been patterned into microcapillary flow models using deep-UV photolithography. The relationship between initiation of coagulation and shear rate can be investigated. Microfluidic live-cell microarrays show substantial promise for various biomedical research since cell signaling, cell to cell, and cell to substrate dynamic responses can be conveniently studied. As mentioned above, the geometry and precision of microarrays are guaranteed by UV photolithography. Platelet interaction with the physical and spatial cues in the microenvironment
FIGURE 3 Micro/nano technologies of surface engineering in microfluidic devices. (A) An eight-channel microfluidic device with a protein stripe patterned perpendicular to the parallel eight-channels region. Open access 2014, Wiley-VCH GmbH. (B) Schematics of microcontact protein printing process with the covalent immobilization of fibrinogen and albumin for platelet adhesion and morphology analysis. Adapted with permission, Copyright 2011, American Chemical Society. (C) Patches of tissue factor (TF) patterned into microcapillary flow models using deep-ultraviolet (UV) photolithography. Open access 2008, American Heart Association. (D) Platelet interacted with different interspacing of microarray using UV photolithography. Reproduced with permission, Copyright 2010, Elsevier. (E) Structure of patterned surface and platelet-adhesive sites on patterned styrene-block-(ethylene-co-butylene)-block-styrene (SEBS) using UV radiation. Open access 2013, Wiley-VCH GmbH

can be studied by applying microarrays. Furthermore, the study of different protein microarray or microtopography that influences the platelet behavior can be easily conducted. Figure 3D shows a typical microarray interacted with platelet using UV photolithography. The effect of surface topography on fibrinogen and adsorption was investigated with high aspect ratio. The study demonstrated that topographical parameters were found to induce low levels of fibrinogen adsorption, while platelet adhesion and aggregation are highly affected with aspect ratio and interspacing or density. Although the procedure of the UV photolithography is complicated, and certain skills are required, the homogeneity and reproducibility of micro/nano patterning are guaranteed.
4.4 | UV radiation

UV radiation is a facile way to construct versatile surfaces with environmentally friendly process. Figure 3E shows a competition between polymerization and degradation that make the platelet adhesion on styrene-block-(ethylene-co-butylene)-block-styrene to be switched on and off by using UV radiation technology.\cite{114} The adhesive sites of the platelets can be scaled to single cell level with dysfunctional platelets detection. Until now, various approaches have been developed with surface chemistry and micro/nanotechnology to study the platelets adhesion and aggregation. A common approach for constructing platelet-pattern surface is by introducing bioactive ligands on the surface to enhance the platelet repellency or adhesion. Bioactive ligands, for example, fibrinogen,\cite{115} collagen,\cite{116} fibrinogen,\cite{117} and vWF\cite{118} are developed to construct platelets-patterned surfaces for measuring platelet function and antiplatelet drug efficacy. However, most of these approaches consist of multi-step chemical grafting treatments or polymerization technologies that are generally time-consuming and complicated. Hence, it leads to limit the usage in clinical practice. What’s more, bioactive proteins are often expensive, easily deactivated, and specific to different platelet receptor.\cite{119} Therefore, the development of a physical, facile method of non-protein platelet-pattern surfaces in microfluidic chips is a feasible way to transform such a technology into a standard technique for clinics.\cite{120} However, it still remains challengeable since almost all the platelet-pattern surfaces are complicatedly constructed.

However, there are still unmet clinical needs for blood hemostasis monitoring and thrombosis therapy, and significant challenges to be addressed. For instance, complex blood rheology is critical to thrombosis during clot formation; however, currently microfabricated stenosed geometries in hemostasis monitoring device are predefined and cannot be changed during the study. While the thrombosis might be growing over time, as it always happens in vivo. Therefore, local shear gradients are changed all the time, and the local shear conditions cannot be mimicked with predefined structural design. Soft materials that can be manipulated to respond controllable and reversible way by modifying some of their properties as a result of external stimuli such as temperature, magnetic field or mechanical stress, and so on are perfect candidates for structural deformation. Combining with 3D printing and micro/nano fabrication technologies, those smart materials can be integrated in the microfluidic system forming desirable stenosed area variations. What’s more, blood samples are usually citrated and transferred to the testing devices for further platelet function diagnosis. However, citrated blood may alter platelet aggregation during transferring and interfere with thrombin generation. Therefore, measuring the coagulation status of patients in real time within blood flowing is another important issue for frequent hemostasis monitoring. Integration of improved hemocompatibility of tube surface with microfluidic system is a viable solution. Future strategies to improve hemocompatibility require in depth understanding of physiological activation pathways and the biology of the blood-materials interface. Thus, the microstructure and functional design of material surface will be guided enhancing the stability and accuracy of the microfluidic system to meet the clinical applications. Furthermore, currently used platelet function tests have many limitations and do not faithfully reflect in vivo situations. Large patient to patient variations make the strategy of adjusting antiplatelet medication inaccurate, according to the results of platelet function tests in cardiovascular events. To accurately reflect an individual difference to arterial thrombosis, real-time thrombotic status monitoring should be developed to improve cardiovascular risk prognosis and guide pharmacotherapy in practice. Hence, development of therapeutic approaches to treat vascular dysfunction and thrombosis at disease and patient specific levels using organ-on-chip techniques is a promising direction to solve those problems. A multicell culture system in which fluid flow is controlled in microfluidic channels between the single organ compartments can be fabricated using micro/nano techniques. These microdevices could simulate the complex interplay of different patients between multiple organ types with an intact circulatory system. Consequently, pharmacotherapy could be guided more accurately.

5 | CONCLUSION AND OUTLOOK

There has been flourishing research progress in the study of platelet function and thrombosis kinetics using microfluidic-based microfabrication technologies. Exploiting the advantages of microfluidic platforms, vascular environments can be mimicked through the patterning of subendothelial proteins with defined surfaces, stenosed geometry, and controllable shear flow. More accurate detection of dysfunctional platelets and cheaper, rapid assay for antiplatelet drugs in patients with cardiovascular disease are developed. Nowadays, microfluidic devices can successfully mimic a network of stenosed arteriolar vessels, permitting evaluation of thrombus formation, coagulation. Even platelet function can be accurately measured in vitro in patients’ blood samples integrated with an extracorporeal circuit in animal endotoxemia or heparin therapy model. The real-time readout of alterations in coagulation ex vivo is proved to be more reliable than the standard clotting essays.
Innovations in implantable material synthesis and microfabrication technologies have led to the development of various biosensors and bioelectronics. Integration of bioelectronic devices with microfluidic-based micro/nano technology may have great potential for the next generation of POC devices in hemostasis monitoring and thrombosis treatment. The testing results can be integrated with the data cloud platform to form the wearable module in Internet of Thing (IoT) devices. Personalized antiplatelet medication treatment with universal basic data monitoring and big data analysis can be achieved without going to hospital for further medical check-up. Cross infection can be avoided especially during the severe virus infection period, for example, coronavirus infection. However, accurate assessment of blood hemostasis for the management of patients who receive anticoagulation therapy or experience coagulopathies should be the most essential problems to be solved. We expect that the continuous development of this multidisciplinary field will bring more microfluidic-based testing methods and devices to the clinics and contribute to the fight against blood-related diseases.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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