

Original article

Review of an in vitro microfluidic model of sickle cell vaso-occlusion

Analyse d'un modèle microfluidique in vitro de vaso-occlusion drépanocytaire

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Abstract

Vaso-occlusive events are responsible for the majority of morbidity and mortality in sickle cell disease. Predisposing conditions are unclear, and proximal causes have not been established. Despite decades of intense study, until recently there has not been a well-controlled in vitro model of sickle cell vaso-occlusion. We have reported the development and initial use of such a model. Our experimental device relies on microfluidic technology and has allowed the initial analysis of the in vitro process of vaso-occlusion in terms of control parameters such as driving pressure, local oxygen concentration and flow vessel size. Our work demonstrates the potential of this type of device to lead to greater understanding of vaso-occlusive pathology including the role of adhesion molecules and inflammatory factors and possibly to improvements in monitoring and searches for new treatments.

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Résumé

Les événements vaso-occlusifs survenant dans la drépanocytose sont les responsables majeurs de la morbidité et de la mortalité de cette maladie. Les facteurs de prédisposition ne sont pas clairs et les causes déclenchantes n'ont pas été établies. En dépit de décennies d'études intenses, aucun modèle in vitro rigoureusement contrôlé de vaso-occlusion n'était disponible. Nous décrivons la mise au point et l'utilisation préliminaire d'un tel modèle. Notre dispositif expérimental repose sur la technologie microfluidique permettant une analyse in vitro du processus de vaso-occlusion, notamment par le contrôle de paramètres tels que la pression, la concentration locale d'oxygène et la taille des vaisseaux. Notre étude démontre le potentiel de ce type de dispositif pour aboutir à une meilleure compréhension de la pathologie vaso-occlusive, notamment le rôle des molécules d'adhésion et des facteurs inflammatoires et pouvant conduire éventuellement à des améliorations dans la surveillance et la recherche de nouveaux traitements.

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Mots clés : Drépanocytose ; Vaso-occlusion ; Modèle in vitro ; Mécanique des fluides ; Microfluidique

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1. Introduction

Since the identification of the molecular defect in sickle cell disease [1], it has been tempting to propose a simple mechanical explanation for the vaso-occlusive events which cause local tissue ischemia and lead to the devastating symptoms of sickle cell disease:

- sickle cells enter a deoxygenated environment;
- sickle hemoglobin polymerizes;
- sickle cells become stiff and occlude small blood vessels.

It is clear that this simple hypothesis is incorrect, because red blood cells enter deoxygenated environments every time they circulate through the body, and if a vaso-occlusive event occurred each time, sickle cell disease would be even more severe than it is. Further, there are many examples of patients with sickle cell disease whose cells are capable of this simple process but who nevertheless have very mild disease, some living beyond their sixties without any intense treatment [2]. There must therefore be additional environmental or genetic factors involved. Many such factors have been proposed and investigated [3]. In order to evaluate the contribution of these additional factors, it is important to develop a minimal system which recreates vaso-occlusion. Such a system can then serve as a testing environment for the role of these additional factors on their own and in combination with each other.

2. Method and results

We designed and manufactured a network of capillary-sized channels in a silicone polymer using soft-lithography or microfluidic methods [4,5] (Fig. 1). By juxtaposing channels for blood with channels for gas, it is possible to control the gas concentration in the blood channels by changing the concentration in the gas channels. Diffusion leads to equilibration over the course of tens of seconds. We have demonstrated that sickle cell blood steadily flowing through channels in this device will occlude following deoxygenation and will resume flow upon reintroduction of oxygen [6]. We have further demonstrated the ability to measure the effect of changes in pressure, channel size and oxygen concentration on the rate of occlusion.

Discussion

We believe that this experimental device will lead to insights into the pathophysiology of sickle cell disease. We have already characterized a phase space of vaso-occlusion in terms of pressure, oxygen concentration and channel size [6]. Additional “dimensions” can easily be added to this phase space, including hematocrit, hemoglobin F fraction and white

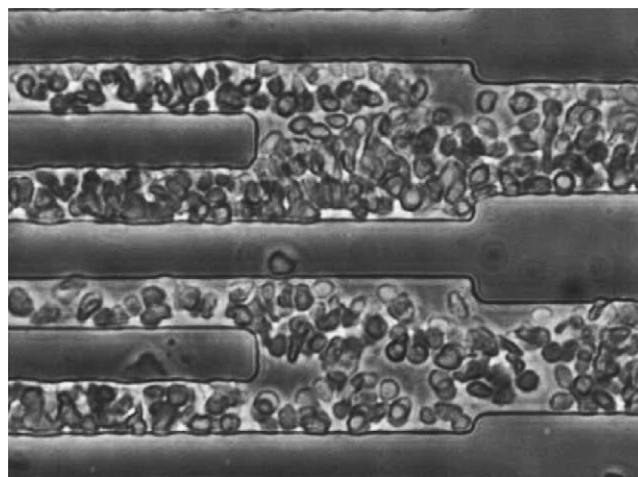


Fig. 1. Video micrograph of red blood cells in a microfluidic device.

blood cell count. Further, using surface chemistry techniques [7], adhesion molecules may be attached to vessel walls, and the effect of different surface densities of these molecules on the dynamics of sickle cell vaso-occlusion may be measured and compared. Such studies would provide useful information on the relative effect of different adhesion molecules and other factors on the dynamic process of vaso-occlusion. These multidimensional phase spaces can then be measured for individual patients over time, allowing finer resolution assessment of a patient’s disease state with potentially important medical implications. For example, it is reasonable to speculate that such measurements may justify early prophylactic transfusion to prevent painful occlusive events. Finally, this device may help assess the effect of novel experimental molecules on vaso-occlusion dynamics, perhaps speeding the search and evaluation for improved treatments.

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