MICROFLUIDIC KIT-ON-A-LID: A VERSATILE PLATFORM FOR NEUTROPHIL CHEMOTAXIS ASSAYS AND ASTHMA DIAGNOSTICS

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ABSTRACT

Improvements in neutrophil chemotaxis assays have enabled significant advances in understanding the mechanisms of neutrophil recruitment, however current methods are still limiting due to high sample volume requirements, low experimental throughput, or complex assay protocols. We report a microfluidic technology that performs neutrophil sorting and chemotaxis on-chip within minutes using nanoliters of whole blood and only requires a micropipette to operate. The platform was adapted to incorporate an endothelial cell monolayer; perform chemotaxis on mouse neutrophils; and human neutrophil chemotaxis in 3D. Finally, the platform was employed in a clinical setting to diagnose asthma in human patients.

KEYWORDS

Microfluidics, passive pumping, neutrophil chemotaxis, asthma diagnostics; FeNO;

INTRODUCTION

Chemotaxis, the ability of a cell to directionally migrate towards a chemical stimulus, is central to biological processes such as wound healing and cancer progression. Many assays have been developed to study chemotaxis such as the Boyden Chamber[1] and micropipette-based[2] assays; however, these techniques limit biological and clinical inquiry in certain areas. Here we present a comprehensive microfluidic solution, dubbed **K**it-**o**n-**a**-**l**id-**a**ssay (KOALA) for chemotaxis, that performs neutrophil purification from nanoliter volumes of blood in minutes; generates repeatable chemotactic gradients; and does not require specialized equipment to operate. The platform makes possible the study of neutrophil chemotaxis in multiple applications (Figure 1).

The user-friendliness; low sample volume requirements; and rapid setup time allowed for the use of KOALA technology in a clinical setting for asthma diagnostics. Asthma is currently diagnosed using a variety of quantitative and

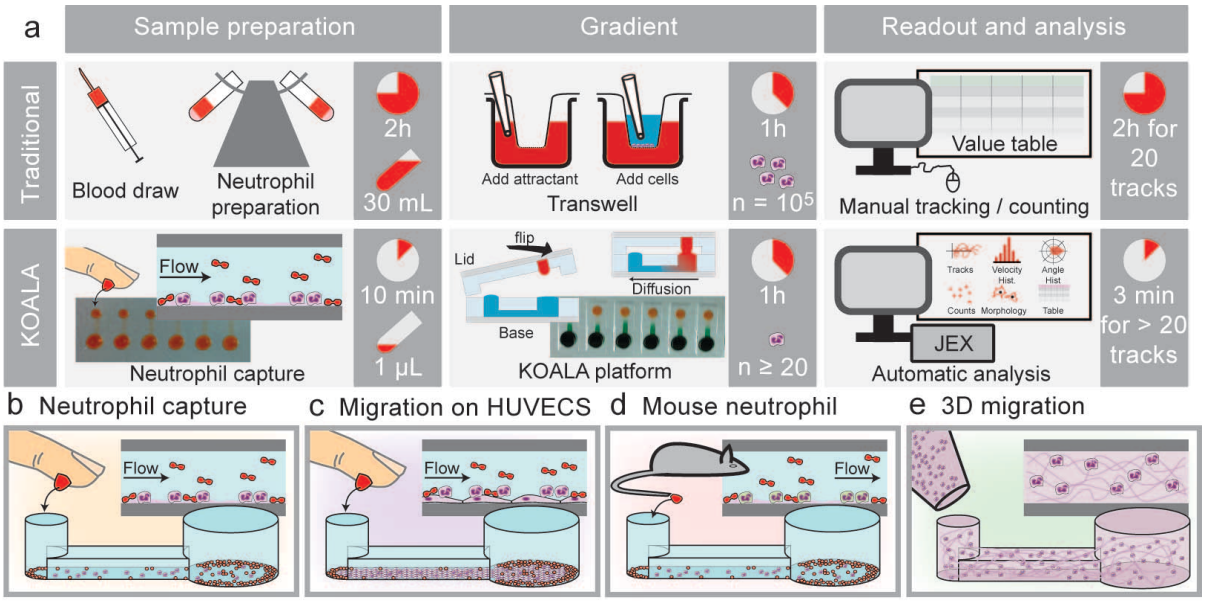

Figure 1. (a) Comparison of traditional chemotaxis assays and KOALA. (b-e) Applications of KOALA.

qualitative methods, although current methods still lead to over- and under-diagnosis for certain populations. Neutrophils are commonly associated with severe asthmatics and are known to be a primary effector cell in the pathogenesis of the disease. We assayed neutrophil chemotaxis for mildly asthmatic and non-asthmatic patients in order to determine whether neutrophil function could be used to diagnose asthma.

MATERIALS AND METHODS

We fabricated the KOALA platform using standard soft lithography methods with Poly-dimethylsiloxane (Sylgaard 164, Dow Corning, Salzburg, MI). First, multilayered molds were created using SU-8 negative photoresist (Microchem, Newton, MA). In brief, pattern designs were created using Adobe Illustrator (Adobe, San Jose, CA) and printed on film (Imagesetter, Madison WI). Microchannel dimensions for the asthma diagnostic assay, human 2D migration assay, and 3D assay were: 3 mm x 800 um x 80 um (LxWxH); 200 um height was used for endothelial cell substrate assay. Ports for all experiments were 1.5 mm (input) and 2.5 mm (output) in diameter. Chemoattractant was mixed into Matrigel in a 1:1 ratio to a final concentration of 4 mg/mL. Human umbilical vein endothelial cells were seeded at 4,000 cells/uL for 48 hours at 37C prior to experiments. Polystyrene was coated with 100 ug/mL mouse/human E/P-selectin (R&D Systems) for 30 minutes at 4C prior to the start of a chemotaxis experiment.

RESULTS AND DISCUSSION

The KOALA for chemotaxis device is initiated by placing a lid containing the reagents required for generating a gradient onto a base containing functionalized microchannels (purification technique first shown in [3]) where cell manipulation is conducted. Importantly, the low sample volume requirements and novel lid-based method of initiating the gradient of chemoattractant enabled applications that are difficult or impossible to achieve using traditional techniques, including: 1) human neutrophil chemotaxis on an endothelial cell substrate (ECS), 2) mouse neutrophil chemotaxis without sacrificing the animal for the first time, and 3) both 2D and 3D neutrophil migration. First, the neutrophil chemotaxis on the ECS revealed two distinct neutrophil phenotypes, showing that endothelial cell-neutrophil interactions influence neutrophil chemotactic behavior (Figure 3a). Second, we compared the adhesion and chemotaxis of neutrophils from chronically inflamed and wild-type mice, and observed

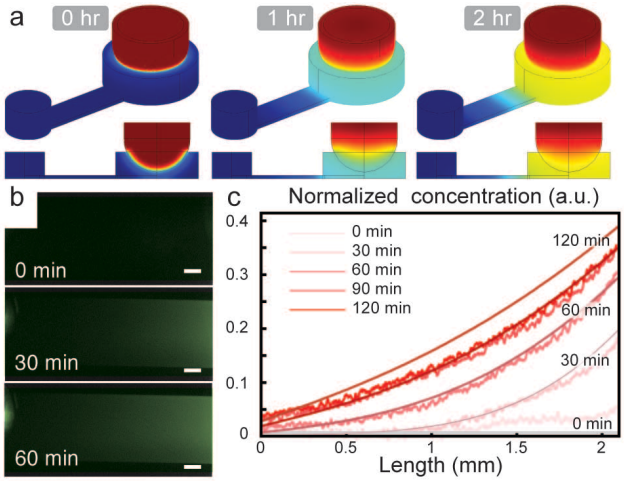

Figure 2. (a) Diffusion modeling of chemoattractant into microchannels. (b) Experimental imaging of AlexaFluor 488 in KOALA microchannels. (c) Comparisons of simulations and experiments.

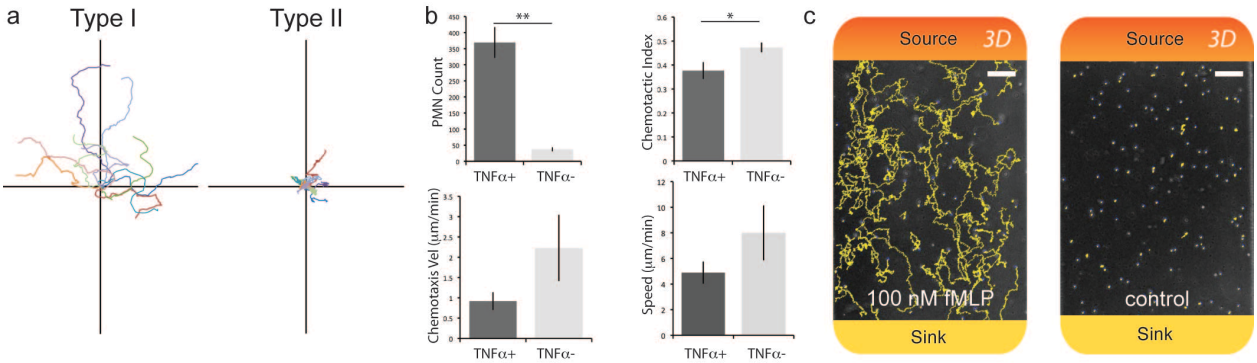

Figure 3. (a) Neutrophil tracks for Type I and II morphologies on ECS. (b) Comparison of neutrophil function of TNF transgenic and wild-type mice. (c) Example of human neutrophil migration in chemotaxis with cell tracking.

significantly higher neutrophil adhesion in blood obtained from chronically inflamed mice (Figure 3b). Third, we show that 3D neutrophil chemotaxis experiments are possible using our technique (Figure 3c). These methods allow for new

avenues of research while reducing the complexity, time, and sample volume requirements of neutrophil chemotaxis assays.

The handheld KOALA chip was employed in a clinical setting to assay 34 human patients (23 asthmatic and 11 non-asthmatic) to characterize the chemotaxis behavior of neutrophils from mildly asthmatic patients, and establish domains for diagnosis. This was made possible because of the technique's ease-of-use, fast pipet-based cell sorting, and low sample volume requirements. Neutrophil chemotaxis outputs were compared with typical clinical diagnostic measures such as fractional exhaled nitric oxide levels (FeNO) and spirometry measurements. First, we found that the neutrophils' average velocity towards the chemoattractant was significantly retarded for asthmatic patients compared to non-asthmatics (Figure 4a). Interestingly, changes in neutrophil function have been primarily linked to severe asthmatics, and eosinophils are more linked to mild asthma. This result identifies a new correlation between neutrophils and mild asthmatics. Second, the velocity increases for patients with low measures of FeNO (Figure 4b), with a significant increase in velocity near the cutoff point clinicians often use to help diagnose asthma. The other outputs we observed – speed and chemotactic index – did not provide definitive trends that could be utilized for diagnosis. Cross-validation was performed on the patient data and the optimal chemotaxis velocity for diagnosing asthma was determined to be 1.55 μm/s, which had diagnostic sensitivity and specificity of 96% and 72%, respectively. We propose that neutrophil chemotaxis may be used to diagnose and manage mild asthma in a clinical setting with KOALA.

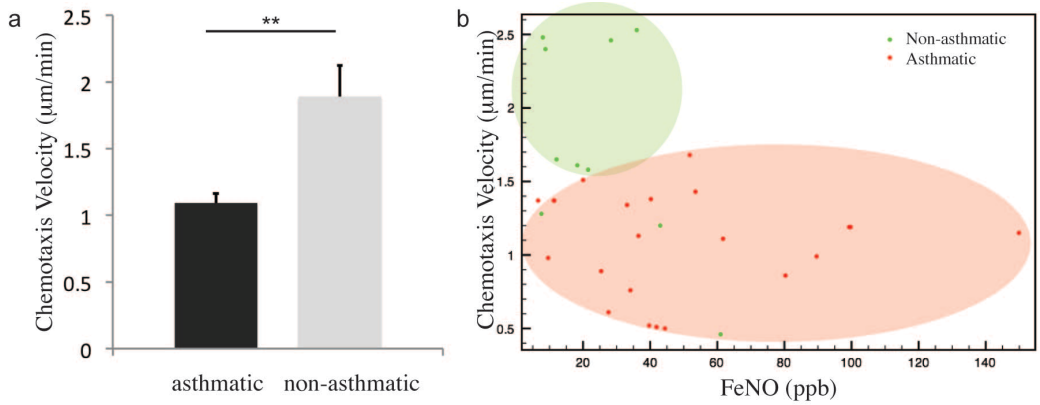

*Figure 4. (a) Difference in chemotaxis velocity for asthmatic and non-asthmatic patients; **p<.01. (b) Comparing chemotaxis velocity and forced exhaled nitric oxide (FeNO) for diagnosing asthma.*

CONCLUSIONS

We have developed a microfluidic platform that performs neutrophil chemotaxis assays for multiple applications, such as chemotaxis on an endothelial cell substrate; in small mammals; and in 3D suspension. Importantly, the comprehensive nature of our design solution -- the cell sorting; gradient generation; packaging; and automated cell tracking -- enabled us to assay a cohort of asthmatic and non-asthmatic patients in a clinical setting. Future studies are required to determine whether other inflammatory diseases would alter the specificity of the diagnostic chip.

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REFERENCES

[1] S. Boyden, *The chemotactic effect of mixtures of antibody and antigen on polymorphonuclear leucocytes*, J Exp Med, (1962).

[2] G. Gerisch and H. U. Keller, *Chemotactic reorientation of granulocytes stimulated with micropipettes containing fMet-Leu-Phe*., Journal of Cell Science, vol. 52, pp. 1–10, (1981).

[3] N. Agrawal and M. Toner, *Neutrophil migration assay from a drop of blood*, Lab Chip, vol. 8, pp. 2054–2061, (2008).

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