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Award Number: W81XWH-11-1-0610

TITLE: Development of Novel Microfluidic Platform for Multiple Sclerosis Study

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REPORT DATE: August 2013

TYPE OF REPORT: Addendum to Final

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

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REPORT DOCUMENTATION PAGE				Form Approved OMB No. 0704-0188	
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1. REPORT DATE August 2013		2. REPORT TYPE Addendum to Final		3. DATES COVERED 15 July 2012- 14 July 2013	
4. TITLE AND SUBTITLE Development of Novel Microfluidic Platform for Multiple Sclerosis				5a. CONTRACT NUMBER	
				5b. GRANT NUMBER W81XWH-11-1-0610	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S) In Hong Yang E-Mail: iyang3@jhmi.edu				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Johns Hopkins University Baltimore MD 21205				8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012				10. SPONSOR/MONITOR'S ACRONYM(S)	
				11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
12. DISTRIBUTION / AVAILABILITY STATEMENT Approved for Public Release; Distribution Unlimited					
13. SUPPLEMENTARY NOTES					
14. ABSTRACT From petri dish to complex micro-devices, technological advances in microfluidic devices allowed us to study various disease cases in greater depth. Multiple Sclerosis (MS) is an autoimmune disease which occurs in the brain. The inflammation during MS is also known to occur at the wall of the tissue where Neural Progenitor Cells reside (NPC). These NPCs are recognized for their regenerative property; they can replace old or damaged neurons with newly formed neurons. Thus, in MS patients, it is difficult to maintain neurogenesis for restorative therapy as it is constantly inhibited due to the inflammation. Pathological studies reveal that the two microenvironments surrounding the inflammation site are different. This calls for a novel microfluidic device that mimics distinct microenvironments of the disease condition. Hence, we have developed a three-compartment system microfluidic system that can be used to study such disease. Using this device, the cellular and molecular signaling mechanism under MS in the NPCs may be elucidated for the first time.					
15. SUBJECT TERMS					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
U	U	U	UU	6	19b. TELEPHONE NUMBER (include area code)

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Introduction: Neural stem cells (NSCs) are multipotent cells isolated from striatal tissue and the subventricular zone (SVZ), which is one of a few neurogenic areas in the adult brain. NSCs in the brain have the potential to remyelinate damaged axons and restore neural function after nerve injury. Recent progress in our understanding of the biology of NSCs has inspired interest in exploring the roles of neurogenesis capable NSC in the pathology and therapy of neurodegenerative disorders including multiple sclerosis (MS) [1-2]. Evidence suggests that NSC proliferation and differentiation occur under physiological conditions and can be enhanced in certain pathological conditions following neural damage [3-4]. We hypothesize that in MS, soluble mediators released by inflammatory T-cells cause abnormal proliferation and differentiation of NSCs resulting in the impairment of neurogenesis of the brain. In the MS brain, NSCs experience spatially and temporally asymmetric levels of fulminant attacks by inflammatory T-cells. However, the communication between soluble factors of inflammatory T cells and NSCs that affect the proliferation and differentiation of NSCs remain unknown. The goal of this project is to develop a novel NSC culture platform that is capable of both compartmentalizing and fluidically isolating microdomains of NSC neurospheres. By building such a platform, aspects of the molecular and cellular signaling between micro-populations of NSCs and inflammatory T cells/macrophages can be elucidated for the first time in a novel in vitro system

Body: Preliminary design was created using AutoCAD. To create a microfluidic device of our goal, two-layer design was implemented: one for separate compartments, and one for the micro-channels that connect between compartments and allow molecular signaling. The height and width of the microchannel is 10um, the width of the middle chamber (where cells will be plated) is 800um, and the distance between the compartments is 400um.

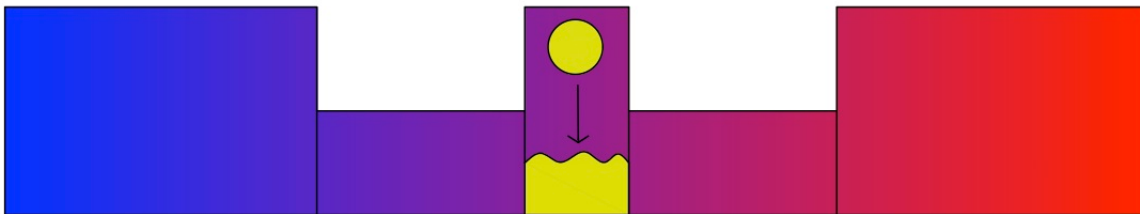


Figure 1. This is a 2D diagram of the device. Middle well is where the cells are plated. Microchannels connect the compartments.

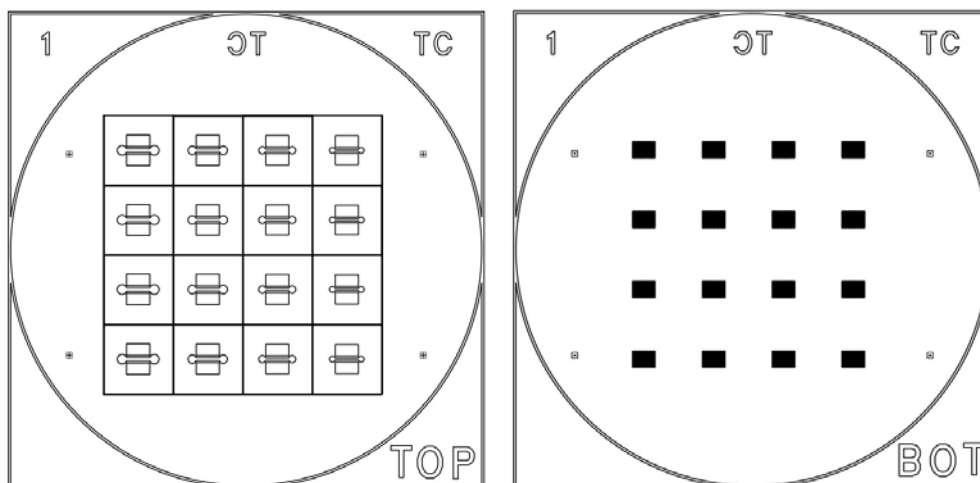
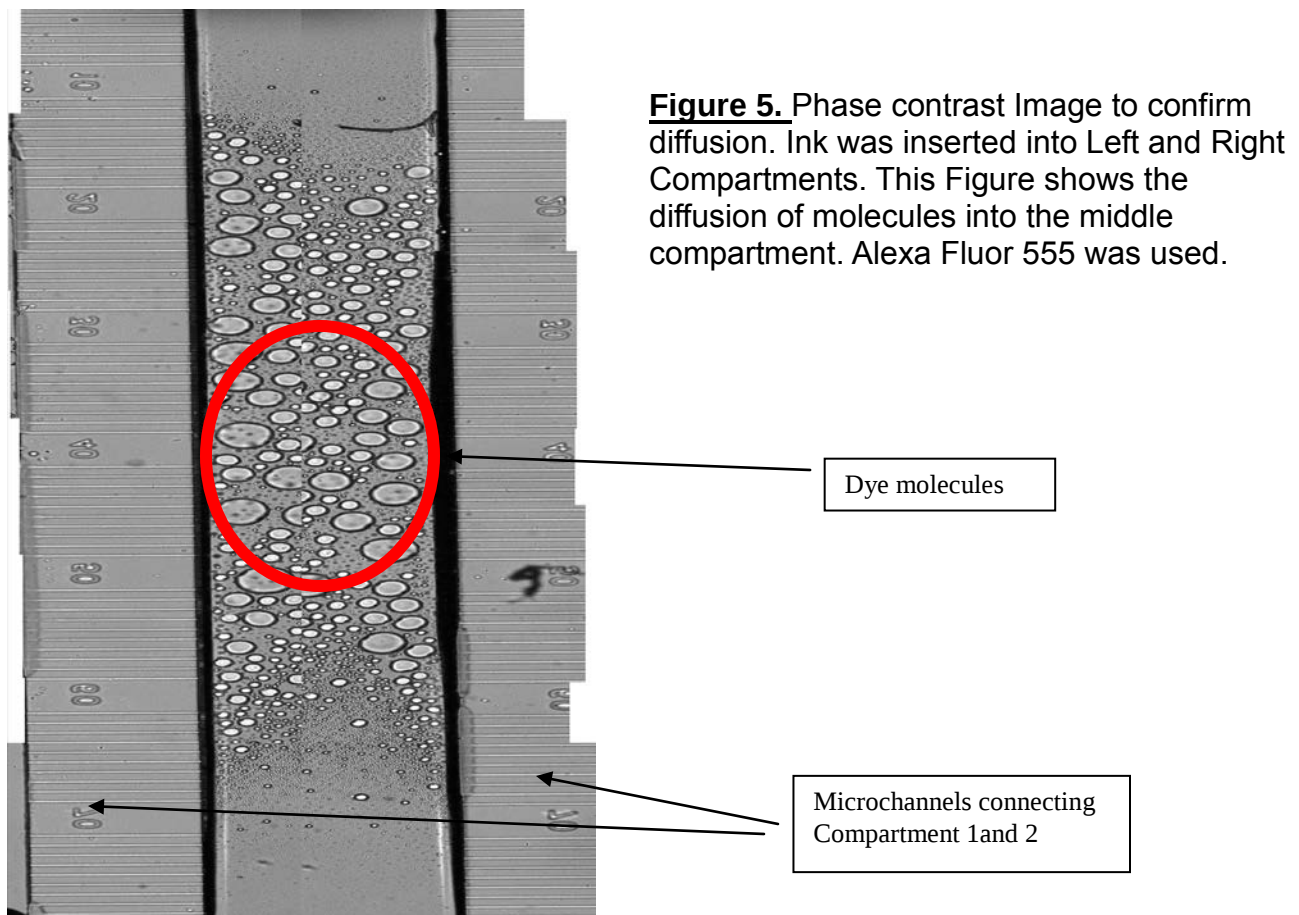
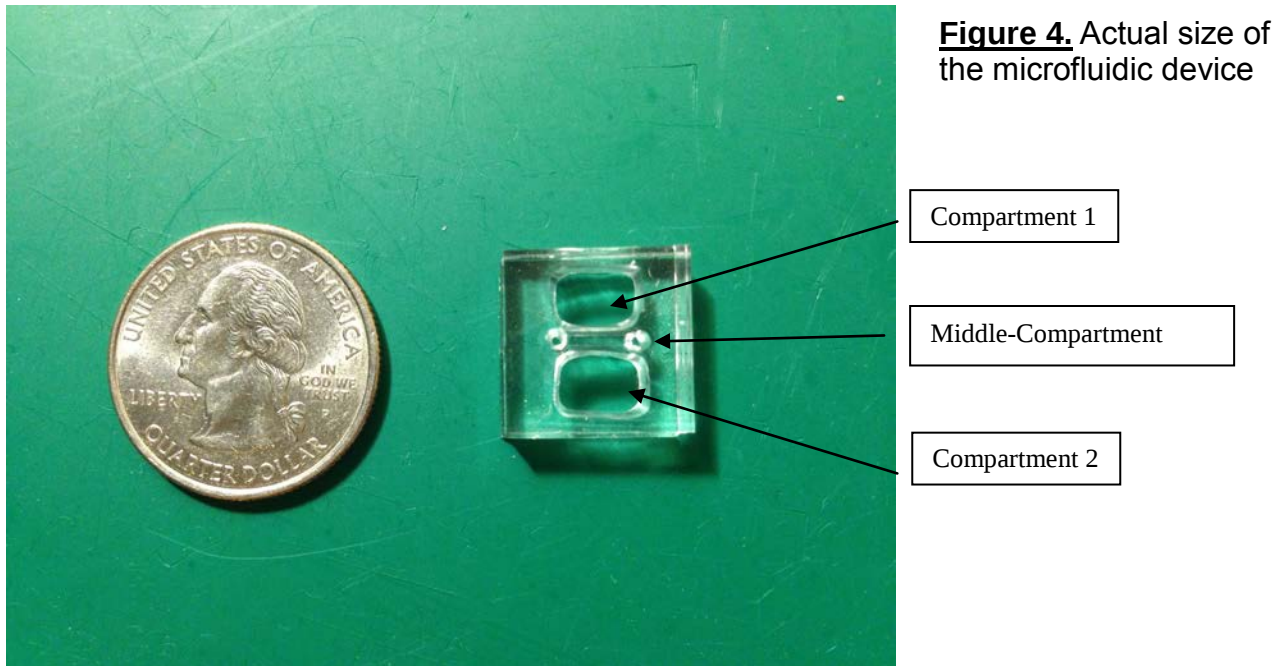


Figure 2. Left) layer for microchannels Right) layer for compartments. Cells are plated in the middle well which has circular ends and two compartments besides it mimic the pathological micro-environment.

Conclusion: Silicon wafer product was developed through soft lithography using Poly-Di-Methyl-Siloxane. Soft lithography technique is very useful in replicating the structures without causing damage to the original mold. PDMS is a superb material for developing micro-fluidic devices. The reason for such prevalent usage of PDMS is because it is inert, transparent, and easy to handle but at the same time robust. PDMS was mixed with curing agent and then degased to rid air bubbles. Then, it was poured on to the silicon wafer and individual device.



A multi-compartmental system was successfully created. Using this chamber, NPCs will be cultured and exposed to pathological conditions of Multiple Sclerosis. Granzyme B is known to inhibit neurogenesis in MS. NPCs will be exposed to Granzyme B in one compartment and proliferating media on the other. Fluorescence imaging will be used to elucidate cellular signaling pattern.

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