# MICROFLUIDIC DEVICES AS IN-VITRO MICROENVIRONMENTS FOR STEM CELL CULTURE

Amir Shamloo, Mohammad Abeddoust, Nastaran Mehboudi
Mechanical Engineering School, Sharif University of Technology
Tehran, Iran
Shamloo@sharif.edu, Abeddoust@mech.sharif.ir, nastaran.mehboudi@gmail.com

**ABSTRACT**
Many potential therapies are currently being studied that may promote neural regeneration and guide regenerating axons to form correct connections following injury. It has been shown that adult neurons have some limited regenerative capabilities, and the lack of connection formation between neurons is not an intrinsic inability of these cells to form axons after being damaged, but rather the inhibitory microenvironment of the injured tissue prevents regeneration. In this study, the polarization and chemotaxis of neuronal stem cells (NSC) in response to quantified gradients of nerve growth factor (NGF) was examined. To accomplish this, a microfluidic device was designed and fabricated to generate stable concentration gradients of biomolecules in a cell culture chamber within a 3D microenvironment. Numerical simulation was implemented to optimize the device geometry for generating a uniform concentration gradient of NGF which was found to remain stable for multiple hours. NSCs migration was studied within this microfluidic device in response to NGF concentration and within a 3D environment of collagen matrix. This device is expected to have wide applicability in the study of shear-sensitive cells such as NSC and non-adherent cell types as well as in the study of migration through three dimensional matrices.

**KEY WORDS**
Microfluidic Device, Cell Chemotaxis, Matrix Porosity, Concentration Gradient, Nerve Growth Factor.

## 1. Introduction

Cell migration is an indispensable part of many biological processes including wound healing, cancer developments, angiogenesis and the formation of new blood vessels [1-5]. During this migration process the cell respond to several environmental cues. The critical and basic step towards investigating the role of various cell migration mechanics is to study each of migration factors independently and quantitatively [5-7].

In recent years, application of mammalian cell culture has remarkably attracted the attention of researchers in modern biology. Microfluidic devices benefit from inherent advantages that make them strictly suitable to be used as mammalian cell culture platform [8, 9]. One of the main roles of microfluidic devices is to generate favourable (stable, uniform with minimum fluid flow shear stress) concentration gradients of biochemical factors. Therefore, developing microfluidic devices with specific characteristics of concentration gradient like stability and uniformity has been subjected to the study of many researchers in recent years.

Early microfluidic devices for concentration gradient generation used a serpentine-shaped inlet channels that would create a stable concentration gradient [10]. The main drawback of these microfluidic devices is the fluid convection through the cell culture platform that is an unfavorable effect in many cell culture systems. To deal with this limitation, many researchers proposed and designed new devices that were exclusively based on mass diffusion rather than fluid convection [11, 12] for generating a special concentration gradient. These new types of microfluidics platforms were more applicable in the study of bacterial and neutrophil chemotaxis [13, 14]. Despite their advantages, these devices are unable of maintaining the gradient concentration for long-term cultures. Besides, they need extra fabrication steps to add hydrogels or membranes into the device.

While many *in-vitro* stem cell studies are performed by utilizing the traditional cell culture techniques, but developing appropriate methods that can resemble natural *in-vivo* microenvironments can revolutionize our understanding of stem cell growth and differentiation mechanism. For example, as most of *in-vitro* stem cell research studies are performed on 2D tissue culture platforms, appropriate 3D *in-vitro* platforms are more physiologically relevant since *in-vivo*, and the cells are surrounded by the extracellular matrix (ECM) in a 3D microenvironment. Cell morphology and function as well as signal transduction and gene expression are known to be quite different in 2D versus 3D environments [15]. Also, many of the current 3D *in-vitro* techniques used for studying stem cell growth and differentiation are not capable of resembling the natural stem cell niches in a quantifiable manner. Here, we have designed a novel microfluidic device that is appropriate for neural stem cell migration in 3D microenvironment of collagen matrix. The study of this phenomenon can result in the development of new methodologies for treating neural system injuries.

Spinal cord injuries and traumatic brain injuries (TBI) are devastating because of the limited regenerative capacity of the central nervous system. Currently existing clinical treatments to damages of central nervous system (CNS) offer only minor symptomatic relief [16]. Employing

*DOI: 10.2316/J.2014.216.818-0067*

exogenous neural stem cells for healing brain damages is one of the inspiring areas of biomedical investigations.
It has been demonstrated that transplantation of neural stem cells can be a promising strategy to help recovery from TBI, but the therapeutic benefits of transplantation techniques are limited by a high degree of donor cell death [17]. To successfully make use of neural stem cells, their migration, differentiation, axonal pathfinding and network formation should be strictly controlled. Various biochemical, biomechanical and electrical cues has been found to influence neural progenitor cells (NPCs), function, but there is still no reliable therapeutic treatment for their application in patients. This is mainly due to the lack of understanding about the various environmental factors that can affect NPC differentiation and neuronal network formation.
It has also been shown that axons are guided along specific pathways by attractive and repulsive cues in the extracellular environment. Therefore, several groups have proposed introducing gradients of stimulatory factors into the wounded tissue to promote neural regeneration and guide the migration of regenerating axons. We have explored the neuronal axon responses to biochemical concentration gradients of neurotrophic factors within a microfluidic device.
Here a simple concentration gradient generator microfluidic platform has been proposed to study the polarization and migration of highly shear-sensitive neuronal stem cells. The steady-state diffusion of guidance cues generates a stable and quantifiable concentration gradient which is constantly replenished. According to Figure 1A the device is composed of four main parts. Source and sink channels, the cell culture chamber, the cell tank and connecting microcapillaries in order to allow Fickian diffusion of guidance cues from the source channel into the cell culture chamber and finally to the sink channel. Figure 1.B is the geometry that was used in our previous studies, while in this study we modified the geometry of the device as is shown in figure 1.C. The attached cell tank to the microfluidic platform in figure 1.C enables us to culture NSCs in a shear free environment required initially for these shear sensitive cells while we can study their migration towards the cell culture chamber. So the present simple design provides a precious opportunity to study NSCs polarization and chemotaxis in response to a continuous, measurable concentration gradient of NGF in the absence of effects of fluid shear stress.

## 2. Materials and Methods

### 2.1 Microfluidics device simulation

In order to simulate fluid flow and reagent diffusion, a Finite Volume Model was generated. By solving the Steady-State Navier-Stokes equation, flow field was obtained while the concentration distribution of reagents was obtained by solving transient convection-diffusion equation. The cell culture chamber was considered as a porous medium with relevant available properties for collagen type I to be best matched with the actual condition of cell migration in mammalian tissues. As the flow regime is laminar Re ~$10^{-6}$ so we neglected the inertia resistance constant in simulating the porous medium and the viscous resistance coefficient values was obtained using available correlation [18].

### 2.2 Microfluidics device fabrications

Standard soft lithography and micromolding techniques were used to fabricate the microfluidic chamber. A high-resolution printer was used to print a transparency mask. By patterning two layers of negative SU8 photoresist on silicon wafer, the master molds were fabricated and inlets and outlets were punched to the cell culture chamber using sharpened needles. After inserting tubings into the inlet holes of device, they were connected to 100 µL syringes mounted on a syringe pump. Digital imaging of fluorescent biomarkers was used to verify the reagents concentration distribution in the device.

## 3. Results

### 3.1 Microfluidics device simulation results

Using a finite volume model simulation, various design parameters were tested. Without any loss on the generality of the simulations, we set the concentration value for the source channel equal to 100 and that of sink channel equal to 0. The optimal flow rate was determined based on the diffusivity of the guidance cues. After running the simulation for injection rates from 2-60 $nL\,min^{-1}$, the optimum flow rate was measured to be 8 $nL\,min^{-1}$ in which the concentration gradient profile was more stable along the entire x-axis of the cell culture chamber. As shown in Figure 2, the fluid flow velocity through the cell migration chamber was measured not to exceed $2\mu m\,min^{-1}$. The corresponding induced shear stress on the cell surface can be determined using the following relation for the sear stress on the surface of a sphere in laminar flow regimes.

$$\tau = \frac{3\mu U}{2a}$$

Where U is the fluid velocity, µ is fluid viscosity and "a" is the average diameter cells [19]. Therefore the estimated maximum shear stress is ~ $10^{-6}\ dyn\ cm^{-2}$

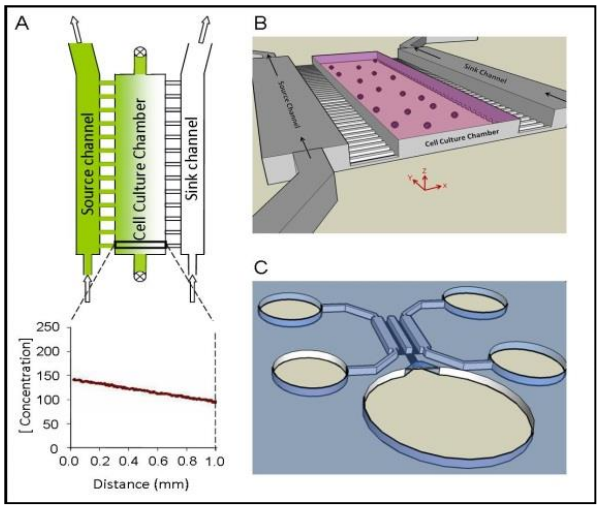


Figure 1. A. Microfluidic device with quantified concentration gradients of biochemical factors. B. 3D schematic of the microfluidic device C. The version of the device used for current neuronal axon guidance studies.

which is much below the allowable physiological shear stress on the cells, Figure 3.

Porosity of collagen matrixes is a key parameter in the study of cells' chemotactic behaviour in 3D microfluidic devices [20-22]. At the same time, there is limited related data on collagen type I porosity in the literature. In order to reach a general understanding of the effects of collagen matrix porosity on the NGF concentration within our microfluidics device we repeated the simulation for various porosity values.

Considering that the "0" porosity refers to a completely solid medium and "1" porosity is equivalent to a totally fluid phase, we reported the concentration profiles for three different vertical lines in the cell culture

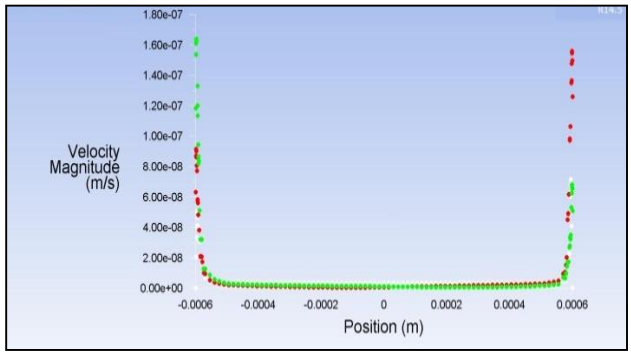


Figure 2. Velocity Diagram for the right (green), middle (black) and Left (Red) side of cell migration chamber in vertical direction.

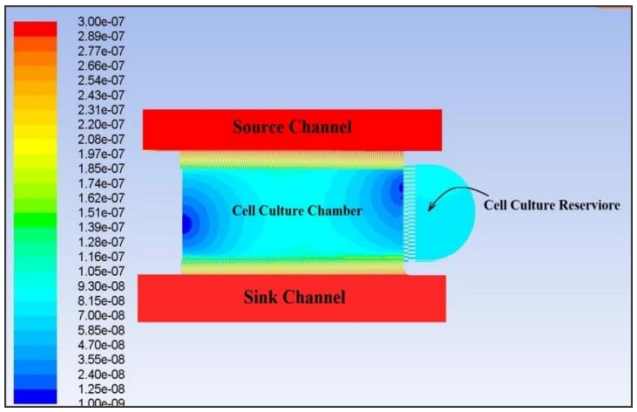


Figure 3. Contours of Shear Stress (Pascal) throughout the Cell Culture Chamber ($\tau_{max} < 3 \times 10^{-7}\ Pa$).

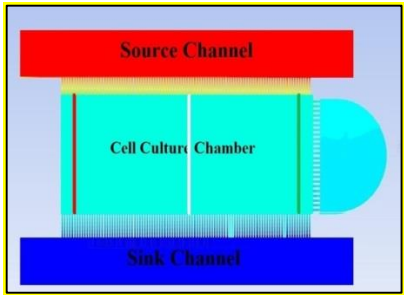


Figure 4. Three reference lines along which the velocity and concentration gradient profiles were plotted.

chamber; a line at the right side of the cell culture chamber, the other one at the left side and the last one at the middle as showed in the Figure 4.

Figure 5 shows the concentration distribution throughout the microfluidic cell migration chamber for porosity of 0.5. The uniformity of generated concentration in the horizontal direction can be observed in this figure.

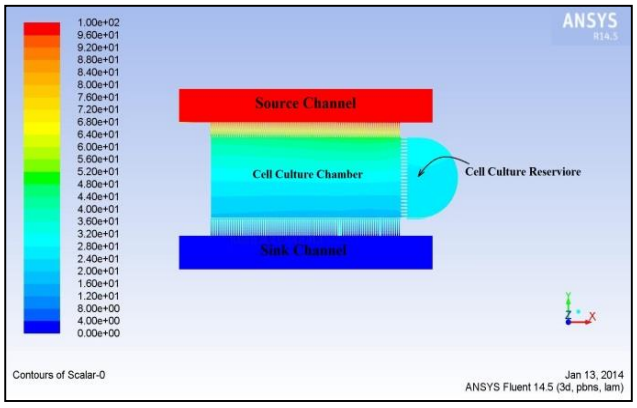


Figure 5. NGF Concentration Distribution within the Microfluidic cell migration chamber ($Hydrogel\ porosity = 0.5$).

Figure 6-9 show the concentration profiles for different values of porosity. According to Figure 6 the concentration profiles are remarkably similar for three vertical lines of interests. This guaranties uniform concentration along the horizontal direction and so satisfies one of our primary

goals in designing the current microfluidic device. The results of concentration profile for 0.3 and 0.7 porosities are showed in figure 7 and 8.

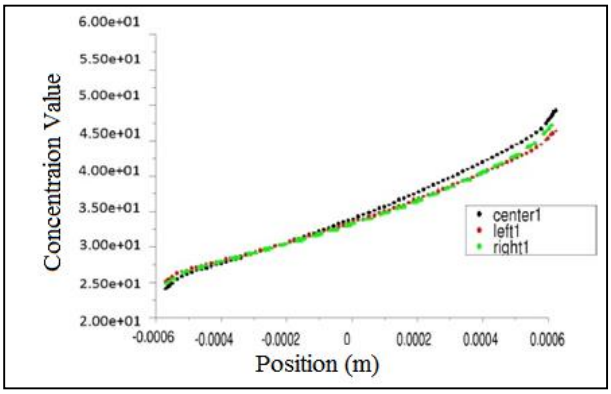


Figure 6. Concentration Profile for right (green), middle (black) and Left (Red) side of cell migration chamber in vertical direction (porosity=0.5).

The dependence of concentration gradient on the porosity of cell culture chamber is illustrated in the Figure 9. As it is evident, the value of concentration gradient decreases with reduction of porosity, inferring that, the more the cell culture chamber is filled with hydrogel matrixes, the less the guidance cues are able to diffuse throughout the media. As the porosity goes down beyond

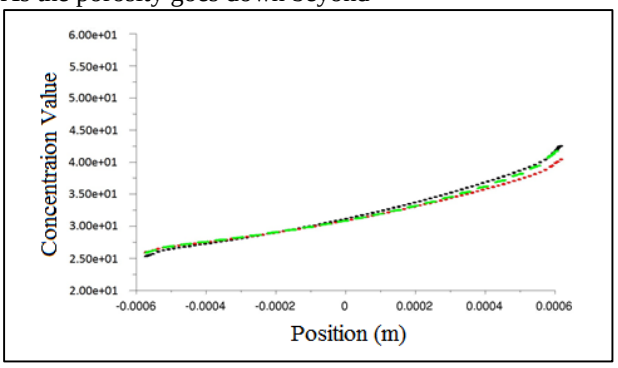


Figure 7. Concentration Profile for porosity=0.3 along three reference lines (see figure 4).

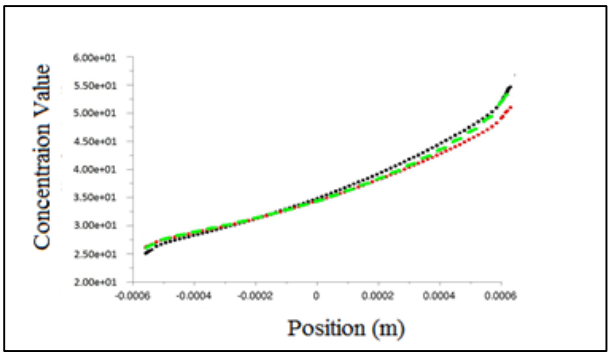


Figure 8. Concentration Profile for porosity=0.7 along three reference lines (see figure 4).

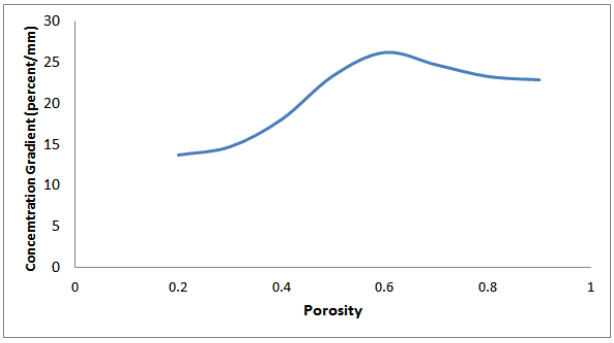


Figure 9. Dependence of generated concentration gradient on the porosity of hydrogel matrix. The steepest concentration gradient corresponds to porosity = 0.6 of hydrogel matrix.

0.2, the concentration profiles becomes highly unstable and non-uniform and so unsuitable for cell migration study purposes, Figure 10.

On the other hand, as the porosity increases from 0.2 to .06 the concentration gradient increases until it reaches its maximum value at 0.6. Beyond porosity values of 0.6 the concentration gradient decreases slowly until it becomes independent of porosity for porosity values more than 0.85 percent. Based on this graph, the porosity in which the steepest concentration gradient occurs is approximately 0.6. This value of porosity resulting in the steepest concentration gradient formation was helpful in determining the desirable range of collagen matrix density used within the microfluidic device.

| Case | Bottom concentration (ng/ml) | Top concentration (ng/ml) | Gradient (ng/ml/mm ) |
|---|---|---|---|
| 1 | 200 | 400 | 200 |
| 2 | 50 | 105 | 55 |
| 3 | 27 | 57 | 30 |
| 4 | 14 | 29 | 15 |

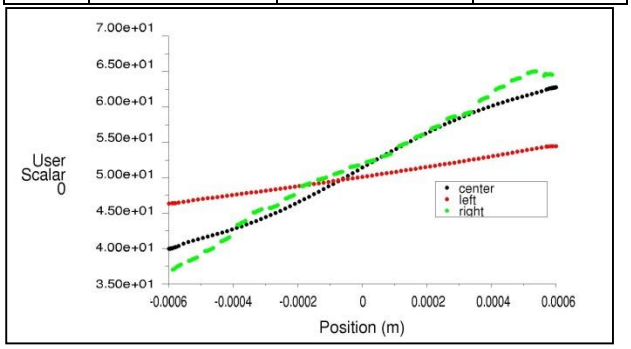


Figure 10. The generated concentration gradient becomes highly unstable for 0.2 porosity and lower.

### 3.2 Neuronal culture within the microfluidic device and in response to varying concentrations of NGF

NSCs were cultured as neurospheres within the cell tank of the microfluidic device. Adult neurons were also cultured initially in the cell tank and allowed to extend their axons into the cell culture chamber. The navigation of the cells

from the cell tank into the cell culture chamber were caused by producing concentration gradients of NGF within the cell culture chamber. Table 1 shows the quantified values of these concentrations.

Table 1. Varying concentration gradients of NGF were produced within the culture chamber of the device

### 3.3 NSCs chemotaxis in stable NGF gradient

Initially, the microfluidic device was implemented for neuronal cell migration, differentiation and path-finding studies (Figure 11). Preliminary results showed the differentiation and axon path-finding of NSCs in response to Nerve Growth Factor (NGF) concentration gradients using time-lapse imaging microscopy. Stock collagen was mixed with neurobasal media containing NPC neurospheres and transformed into the gel phase by the addition of sodium hydroxide (0.5 N stock solution (5% volumetric mixture with stock collagen). Different densities of collagen gel were made by altering the collagen concentration. Immediately after mixing, ~20 µL of the cell-hydrogel solution was injected into the cell culture reservoir of the microfluidic device; gelation occurred in 5-10 minutes.

It was observed that by increasing collagen matrix density, the entanglement of these fibers increases and the mechanical stiffness of the matrix becomes higher (measured by performing rheology test of collagen matrices). It was observed that within collagen matrices of intermediate density (0.9 m/ml), NPC migration and differentiation is higher compared to higher and lower matrix densities.

## 4. Conclusion

In the present study, a simple microfluidic device was proposed that relies solely on Fickian diffusion to generate a stable and quantifiable concentration gradient. The device also minimizes the induced shear stress by fluid convection on the shear-sensitive cells. It is also possible to study three-dimensional cell migration by filling the cell culture chamber with hydrogel matrix. To validate the microfluidic device, we studied NSCs polarization and migration in response to NGF stimulation quantitatively.
We also investigated the effects of porosity of collagen fiber matrixes on the concentration gradient. The maximum value of concentration gradient was observed to occur at porosities close to 0.6. Decreasing porosity from 0.6 has a dramatic effect on the concentration gradient and decreases it by about 50 percent. On the other hand, although increasing the porosity beyond 0.6 decreases the concentration again, the value of reduction in concentration gradient is much less than that of for porosities below 0.6. Moreover for the porosities lower than 0.2 it was observed that the generated concentration gradient becomes highly non-uniform and therefore it is not advisable to use the collagen matrix porosities lower than mentioned value. So this leads us to better selection of collagen matrixes porosity to reach the most favourable Concentration gradient.

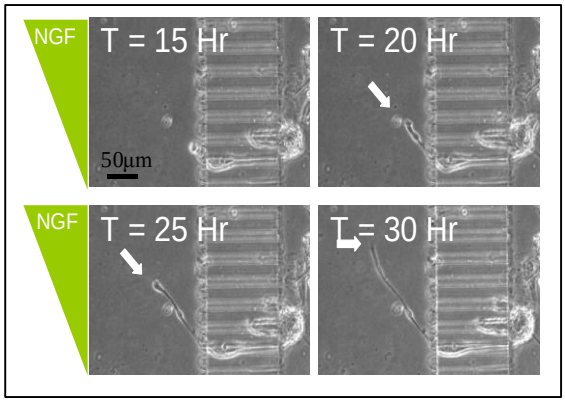


Figure 11. Time-lapse imaging of NPCs within the modified version of the microfluidic device. The image sequences show the axon growth cone path-finding in response to nerve growth factor (NGF) and within collagen matrix.

## References

[1] Lopezovoa, J. M., and Nieto, M. A., Inflammation and EMT, 2009. "an alliance towards organ fibrosis and cancer progression". *EMBO Molec Med*, 1, pp 303-314.
[2] Singh, A,. and Settleman, J., 2010. "cancer stem cells, and drug resistance: an emerging axis of evil in the war on cancer". *Oncogene* 29: 4741-51.
[3] Frederick, B. A., Helfrich, C. D. Coldren, D., Zheng, D., Chan, P.A., Bunn, J., and Raben, D., 2007. "Epithelial to mesenchymal transition predicts gefitinib resistance in cell lines of head and neck squamous cell carcinoma and non-small cell lung carcinoma" *Mol Cancer Ther* 6, pp 1683-1691.
[4] Thomson, S., F. Petti, I. Sujka-Kwok, D. Epstein, and Haley, J. D., 2008. "Kinase switching in mesenchymal-like non-small cell lung cancer lines contributes to EGFR inhibitor resistance through pathway redundancy". *Clin Exp Metastasis* 25, pp 843-854.
[5] Barr, S., S. Thomson, E. Buck, S. Russo, F. Petti, I. Sujka-Kwok, A. Eyzaguirre, M. Rosenfeld-Franklin, N.W. Gibson, M. Miglarese, D. Epstein, K.K. Iwata, and Haley, J. D., 2009. "Bypassing cellular EGF receptor dependence through epithelial-to-mesenchymal-like transitions". *Clin Exp Metastasis* 2008. 25, 685-693.
[6] Chakravarti, A., Loeffler, J. S., and Dyson, N., 2002. "Insulin-like growth factor receptor I mediates resistance to anti-epidermal growth factor receptor therapy in primary human glioblastoma cells through continued activation of phosphoinositide 3-kinase signaling" *Cancer Res* 62, pp 200-207
[7] Taube, J.H., Herschkowitz, J., Komurov, A.Y., Zhou, S., Gupta, J. D., Yang, K., Hartwell, T.T., Onder, P.B. Gupta, K.W. Evans, B.G. Hollier, P.T. Ram, E.S. Lander, J.M. Rosen, R.A. Weinberg, F., and Mani, S.A., 2010. "Core epithelial-to-mesenchymal transition interactome gene-expression signature is associated with claudin-low

and metaplastic breast cancer subtypes." *Proc Natl Acad Sci* 107, pp1544-1554.
[8] Irimia, D. and Toner, M., 2009. "Spontaneous migration of cancer cells under conditions of mechanical confinement," *Integr. Biol*. 1, pp 506-512,.
[9] Ching, T. K., Chiling, Ch., and Andrew, M., 2011. "Modeling of cancer metastasis and drug resistance via biomimetic nano-cilia and microfluidics". *Biomaterials* 35, pp 1562-1571.
[10] Dertinger, S. K., hiu, D. T., Jeon, N. L., and Whitesides, G. M., 2001. "Generation of gradients having complex shapes using microfluidic networks". *Anal. Chem* 73, pp 1240-1246.
[11] Shuko, S., Lisbeth, G., and Leavesley, D., 2005. "In vitro bioactivity of MOEP grafted ePTFE membranes for craniofacial applications" *Biomaterials* 26, pp 5303-5312.
[12] Eliceiri, B., and David, A. Ch., 2001. "Adhesion events in angiogenesis" *Current opinion in cell biology* 13, pp 563-568.
[13] Kahle, N. A., Brenner-Weiss, G., and Overhage, U., 2003. "Regulation of phosphatase activity in bacterial chemotaxis" *Immunobiology* 218, pp 254-265.
[14] Francis, L., Wajeeh S., and Noo, L. J., 2004. "Effective neutrophil chemotaxis is strongly influenced by mean IL-8 concentration" *Biochemical and Biophysical Research Communications* 319, pp 576-581.
[15] Yamada K. M. and Cukierman E., 2007. "Modeling tissue morphogenesis and cancer in 3D " *Cell* 130, pp 601–610
[16] Horner, P.J., Gage, and F.H., 2000. "Regenerating the damaged central nervous system". *Nature* 407, pp 963-970.
[17] Jagasia, R., Song, H.J., Gage, F.H., and Lie, D.C., 2006. "New regulators in adult neurogenesis and their potential role for repair". *Trends in Molecular Medicine* 12, pp 400-405.
[18] Marc, R. C., and Alexander, N., 2002. "Analytical solution of shear stress on round bodies". *Analytical Fluid Mechanics* 212, pp 220-231.
[19] Valerie, L. C., Ying, Z., and Nak W. C., 2010. "Dense type I collagen matrices that support cellular remodeling and microfabrication for studies of tumor angiogenesi s and vasculogenesis in vitro". *Biomaterials* 31, pp 8596-8607.
[20] Miguel, M. M., Joachim, S., and Frederick G., 2010. "The differential regulation of cell motile activity throug h matrix stiffness and porosity in three dimension al collagen matrices". *Biomaterials* 31, pp 6425-6435.
[21] John, A. P., Federica, B., and Melody, A. S., 2007. "Effects of extracellular fiber architecture on cell membrane shear stress in a 3D fibrous matrix". *Journal of Biomechanics* 40, pp 1484-1492.
[22] Yang, J., Mani, J.L., Donaher, S., Ramaswamy, R.A., Itzykson, C., Come, P., Savagner, I., Gitelman, A., Richardson, P., and Weinberg, R.A., 2009. "Twist, a master regulator of morphogenesis, plays an essential role in tumor metastasis Expression of the transcription factors Snail, Slug, and Twist and their clinical significance in human breast cancer." *Cell* 117, pp 927-939.