Determining the Effect of Delivery Rate on Glucose Uptake by Cancer Cells

Luke Felton

University of Arkansas, Fayetteville

Follow this and additional works at: http://scholarworks.uark.edu/bmeguht

Part of the Bioimaging and Biomedical Optics Commons

Recommended Citation
Felton, Luke, "Determining the Effect of Delivery Rate on Glucose Uptake by Cancer Cells" (2016). Biomedical Engineering Undergraduate Honors Theses. 36.
http://scholarworks.uark.edu/bmeguht/36

This Thesis is brought to you for free and open access by the Biomedical Engineering at ScholarWorks@UARK. It has been accepted for inclusion in Biomedical Engineering Undergraduate Honors Theses by an authorized administrator of ScholarWorks@UARK. For more information, please contact scholar@uark.edu, ccmiddle@uark.edu.
Abstract

Radiation therapy is one of the most commonly performed cancer treatment therapies today. Radiation therapy can cause changes to tumor vasculature and affect blood flow rates. It is important to determine how changes in blood flow rates affect tumor glycolytic uptake to better understand tumor response to radiation therapy (1). The goal of this study was to use a fluorescent analog of glucose, 2-NBDG, to study the effect of varying flow rates on glucose uptake. A microfluidic device was constructed to flow 2-NBDG through. A murine metastatic breast cancer cell line was then cultured inside this microfluidic channel, and 2-NBDG was flowed through the device at .5 microliters/min for 20 minutes. After 20 minutes, the device was imaged using fluorescence microscopy. The 2-NBDG uptake was measured for only one flow rate. This is not sufficient evidence to determine the effect of varying flow rates on glucose uptake. Future studies should be conducted at increasing flow rates to determine this effect.
Introduction

One characteristic of cancer cells is heightened glucose uptake in comparison to normal cells. Quantifying the glucose uptake has been helpful in identifying cancers, and determining the effect of radiation therapy. Positron emission tomography has been a widely used imaging technique to quantify glucose uptake in the clinic. Though widely used, this method provides limited information concerning the metabolism of a tumor. This technique only provides the glucose uptake at a single point in time. The glucose uptake at that point in time cannot be indicative of tumor metabolic activity because glucose uptake can be affected by multiple factors. Glucose uptake can be perfusion dependent, influenced by varying flow rates, and dependent on the actual metabolic need for the tumor. Because the post-radiation tumor microenvironment can cause severe changes to the tumor microvasculature and hence affect the delivery of contrast agents, it is important to understand the effect of blood flow rates on the uptake of glucose analogs. The initial glucose uptake of the tumor provides insight into the metabolic demand of the tumor, helping to quantify tumor metastatic potential and tumor response to radiation therapy. It is important to determine the effect of varying flow rates to determine the actual metabolic demand of the tumor (1).
It has been shown in the figure above that 2-NBDG uptake is correlated with blood velocity. In this in vivo study, mice were injected with breast cancer cells to form a tumor. These mice were then subject to hypoxic conditions, and the blood velocity of the tumor and the glucose uptake (Rd) were measured before and after hypoxia. The study showed that as the blood velocity increased, the delivery rate of glucose increased, suggesting that 2-NBDG uptake is dependent on flow rate. This study has been the motivation for our study (2). The goal of our study was to isolate the effect of varying flow rates on glucose uptake so that more information on initial metabolic demand of tumors could be determined. We used a fluorescent glucose analog, 2-NBDG, to measure glucose uptake in murine breast cancer cells. This analog was chosen because it has shown to be a suitable molecule for measuring glucose uptake in previous studies. This study was sectioned into three main aims: to create a suitable device for 2-NBDG to flow, to model in vivo parameters by seeding cells inside the device and flowing 2-NBDG at flow rates similar to that of tumor vasculature, and to quantify 2-NBDG uptake through fluorescence imaging. The study of how this tracer is affected with varying flow rates will provide valuable
information concerning the identification of radiation resistance and radiotherapy response, and will serve as a valuable model for future in vivo studies.

Methods

Microfluidic channel fabrication

The microfluidic channels were fabricated using a silicone wafer constructed at the High Density Electronic Center, located at the University of Arkansas. This wafer was made using soft lithography techniques with a channel mold and photoresist grafting technique. This silicone wafer was designed to construct a channel with a width of 500 microns and a depth of 50 microns. (3). 65 grams of the PDMS elastomer base was mixed with 6.5 grams of the elastomer curing agent (10:1 ratio). The mixture was mixed vigorously for 10 minutes. After 10 minutes, the mixture was degassed and poured over the silicone wafer. The PDMS poured over the wafer was then placed in a 70°C oven for 3 hours to cure the PDMS. Once cured, the PDMS was peeled from the silicone wafer and cut into two rectangular shapes using a glass coverslip as a guide. The portholes were then made with a biopsy punch. The PDMS molds were then sonicated for 30 minutes in 100 ml of 50% pure ethanol and 50% deionized water and then dried with air. The molds and two glass coverslips were then placed inside an UV/Ozone machine for 15 minutes. After 15 minutes, the glass coverslips and PDMS were attached by gently pressing each PDMS mold onto the coverslip. To better seal the attachment of the glass coverslip and mold, PDMS was used to surround the coverslip. 15 grams of the PDMS
elastomer base was mixed with 1.5 grams of the elastomer curing agent. This solution was mixed for 10 minutes and degassed. This mixture was used to surround the entire parameter of the coverslip with a 1 ml syringe. After coated with the PDMS mixture, the device was placed in a 70°C oven for 3 hours to cure the surrounding PDMS. Once cured, the excess PDMS was removed so that the channel could be easily seen inside the coverslip.

![Microfluidic channel](image)

**Figure 1.** Microfluidic channel. The channel is formed by attaching a PDMS mold to a glass coverslip. The cells are then cultured on the coverslip.

**Cell culturing**

4T1 cells, a metastatic murine breast cancer cell line, were used for these experiments. 4T1 cells were cultured in DMEM with the addition of 10% fetal bovine serum and 1% antibiotics. Prior to channel injection, 20mM of Hepes was added to the cell suspension. Gelatin was injected inside the channel prior to injecting cells. The gelatin remained in the channel for 1 hour before injecting cells. The cells were injected inside the channel using a small piece of tygon tubing and a syringe. The cells were then incubated at 37°C for 90 minutes. After 90 minutes, the
cells were perfused with DMEM at 1 microliter/hour for 24-48 hours inside the incubator. After the incubation period, the cells were imaged with 2-NBDG.

**Imaging with 2-NBDG**

2-NBDG fluorescence was measured using traditional fluorescence microscopy. The image platform consisted of an inverted fluorescence microscope, the Olympus IX-81. The microscope consisted of a 100-watt halogen lamp, used for transmission microscopy. In addition, the microscope consisted of a 100-watt mercury lamp used for epifluorescent microscopy. The components of the microscope consisted of a reducer lens, two-piece collimating adapter, a liquid crystal tunable filter, and a CCD. 2-NBDG excites at 465nm, and emits at 540nm (4). 2-NBDG was excited with a FITC filter, and imaged with an Olympus camera. 300 microliters of 2-NBDG dissolved in glucose free media (10:1 ratio) was flowed through the channel at .5 microliters/min for 20 minutes. After 20 minutes, the cells were washed with glucose free media to remove any excess 2-NBDG before imaging. The cells were imaged with an integration time of 1s.
Results and Discussion

Smaller port size and PDMS coating decreases device leakage

Several adjustments to the device were made to inhibit leakage of fluid from the device. High amounts of leakage occurred at the inlet and outlet ports of the device, and at the bottom of the device. This leakage was due to high amounts of pressure occurring within the channel, especially at the ports. This leakage became problematic, and resulted in eventual coverslip detachment from the PDMS. Several structural changes to the device decreased device leakage, helping to promote device attachment, and preventing coverslip detachment from PDMS. First, the inlet and outlet port of the channel was decreased in size. The port size was decreased from 1/8 inch in diameter to .75mm in diameter. This decreased the amount of pressure due to accumulation of fluid at the ports. Second, PDMS was cured around
the glass coverslip to seal the bond between the coverslip and PDMS. This strengthened the adhesion of the coverslip and PDMS, and inhibited coverslip detachment from the PDMS. Third, a coverslip alcohol wash was removed from the protocol. This coverslip wash might have affected the attachment between the glass coverslip and PDMS. This process was replaced by cleaning the coverslip with air prior to coverslip attachment to PDMS. Lastly, the tubing at the entry and exit ports was sealed with PDMS. This increased the bonding between the tubing and PDMS, and decreased leakage at the ports. These structural changes led to an increase in device functionality. The devices could withstand higher flow rates, and could be reused without a loss in device performance.

*Functional flow rates increased for microfluidic device*

Functional flow rates for the device were determined by experimental and quantitative methods. This was completed by using PBS, in conjunction with a dye, and by using Poiseuille's law. The PBS and dye mixture was used to visualize leakage through the device. Structural changes in the device increased the functional flow rates for the device. Initially, the device had small amounts of success at low flow rates. The coverslip would detach from the device, or the device would experience high amounts of leakage. The functional flow rates increased from 10 microliters/min to flow rates of 50 microliters/min and higher. These experiments provided a qualitative representation to determine a range of functional flow rates for the device. These flow rates were then verified using quantitative calculations. Poiseuille's Law was used to calculate the maximum functional flow rate for the
channel based on channel parameters. Assuming a maximum pressure of 300kPa, the maximum flow rate was determined to be $1.23 \times 10^{13}$ microliters/min. These calculations were used to ensure that the qualitatively determined flow rates were less than the calculated maximum flow rate. After determining functional flow rates for the device, flow rates that model in vivo flow rates were determined. A range of in vivo flow velocities was given from a study conducted by Frees et al (2). This range was used to calculate viable flow rates based on the channel parameters. This range was determined to be from 0.75-3.75 microliters/min. These flow rates were determined to be used in 2-NBDG experimentation.

*Improvements in cell culturing allows for cell attachment within channel*

Several adjustments in cell culturing techniques initiated cell attachment within the channel. The cells were first cultured on a glass coverslip prior to attachment with the PDMS mold. This method of cell culturing had no success. It inhibited bonding between the coverslip and PDMS, and prevented formation of the channel. Higher success was observed when injecting cells inside the channel after the device had already been fabricated. This method did not interfere with the bonding of the PDMS and coverslip. To inject cells inside the channel, a syringe pump was used at a uniform flow rate. This method was problematic because no cells were observed inside the channel after pumping. The flow rates for the syringe pump were slow. This might have allowed for cell accumulation inside the tubing or in the bottom of the syringe. The second method for cell injection involved injecting cells manually with a syringe and a small piece of tubing inserted at the entry port.
Cells were visualized inside the channel after injection with this method. The cell count was increased to 6,000,000 cells/ml to improve cell visualization inside the channel, and gelatin improved cell attachment inside the channel. After injection, cells showed a tendency to exit the channel due to capillary action. Capillary action is the result of high pressure inside the channel. To keep cells from exiting the channel, the tubing was capped or adjusted. The cells could also be removed if the media was perfused to quickly. A slow perfusion rate was chosen to inhibit this effect. Incubation time also affected the results of this study. In one experiment, the cells were cultured inside the channel for 72 hours. Cells accumulated within the channel, and obstructed 2-NBDG flow during imaging. It was determined that the incubation time should be decreased to ensure proper flow mechanics within the channel.

**Figure 3.** a) Brightfield image of the microfluidic channel prior to cell injection (4X). b) Brightfield image of the microfluidic channel injected with 4T1 cells (10X).

2-NBDG fluorescence observed at .5 microliters/min
Fluorescence was observed as 2-NBDG was flowed through the microfluidic device. This flow rate was chosen so that the cells would not be removed upon fluid flow, and it modeled in vivo blood flow rates. An increase in fluorescence was observed as 2-NBDG entered the channel. The highest intensity of fluorescence is observed after 20 minutes of 2-NBDG flow. The intensity of 2-NBDG fluorescence decreased as the remaining 2-NBDG was removed with glucose free media. The remaining cells fluoresced visibly. The cells showed higher fluorescence than its surrounding environment. The concentration of 2-NBDG could have been increased to increase fluorescence intensity observed for the cells. Only one flow rate was tested for 2-NBDG imaging. To determine the effect of flow rates on glucose uptake, multiple flow rates should be testing in the future. These flow rates should be chosen based on the in vivo flow rates previously determined.
**Figure 4.** a) Brightfield image post 4T1 cell injection (10X). b) Image of cells 24 hours post incubation and prior to 2-NBDG flow (4X). c) Image of cells after 20 mins of 2-NBDG flow (10X). d) Image of cells after washing with glucose free media (10X).

**Conclusion**

Measurement of glucose uptake is important in determining tumor response to radiation. We have shown that the microfluidic device manufactured in this study is a suitable device in allowing 2-NBDG flow. We have determined in vivo flow rates for future experimentation. In addition, we have described valuable experimental techniques for the continuation of this study in applying varying flow rates and measuring glucose uptake. Future studies must be conducted with increasing flow rates to determine the effect on glucose uptake. Previous studies have shown valuable indications that 2-NBDG can be successful as a biomarker for measuring glucose uptake in the future. The continuation of this study will be important in determining initial metabolic demand, and thus, providing clinical significance in staging cancers and determining the response of radiation therapy.
References

Acknowledgements

I would like to acknowledge Dr. Rajaram and Dr. Puvanakrishnan for their mentoring and support throughout this project. I would also like to thank Dr. Balachandran and Josh Hutcheson for providing information in microfluidics. I would also like to thank the Honors College Research Grant for funding this project.