ABSTRACT SUMMARY

A new tumor model is developed using microfluidics and tissue engineering technology in order to simulate the complex \textit{in vivo} tumor microenvironment including vasculature, interstitium and lymphatics. The developed model, referred to as the tumor-microenvironment-on-chip (TMOC), can mimic highly dynamic, interconnected and spatiotemporally varying tumor microenvironment and allows systematic characterization of nanoparticles (NPs) transport around tumors.

INTRODUCTION

Delivery of anti-cancer drugs and therapeutic agents using various NPs is emerging as a potential solution to achieve targeted drug delivery to tumor [1, 2]. Although promising, the NP delivery to target tumor is still significantly limited due to complex \textit{in vivo} transport barriers. These include (i) leaky and chaotic tumor vasculature, (ii) elevated interstitial fluid pressure (IFP), (iii) high cell packing density, and (iv) dense collagen matrix of tumor tissues [3-5]. In order to improve the \textit{in vivo} delivery efficacy, the NPs should be designed considering all these complex transport barriers. However, currently available tumor models are not adequate to simulate these transport barriers nor allow systematic characterization of the effects of these barriers on the \textit{in vivo} NP transport. In this study, we developed a new \textit{in vitro} tumor model, which simulates complex transport barriers around tumors and provides controllability to systematically study these transport barriers.

EXPERIMENTAL METHODS

In order to recapitulate \textit{in vivo} tumor microenvironment relevant to study complex transports of NPs around tumor, cell-fluid-matrix-NP interactions at several different tissue-tissue interfaces should be mimicked. As illustrated in Figure 1, these include blood flow driven NP transport, NP-endothelium interaction, transvascular transport, interstitial transport, cellular uptake of NPs, and lymphatic drainage of fluid and excess NPs [2, 3]. The conceptual design of the TMOC is also illustrated in Figure 1. The TMOC has a 3D structure formed by stacking two layers of PDMS microchannels with a porous membrane sandwiched between the layers. The top layer has a microchannel simulating the capillary of the tumor vasculature, and NP-suspended fluid will flow along the channel at a physiologically relevant velocity and pressure. The porous membrane mimics the capillary endothelium whose pore size determines the transvascular transport. The bottom layer has three channels, which are partitioned with periodic posts. The center channel simulates the tumor interstitium and the two side channels simulate the lymphatics. In the tumor channel, cancer cells grow within 3D collagen matrix, and the interstitial fluid flows through the matrix and exerts elevated IFP. The NPs are transported through this 3D tissue structure and reach the cancer cells. The excess NPs and interstitial fluid will be collected to the two side lymphatic channels.

Based on this design, a prototype was fabricated with PDMS and a polycarbonate membrane with 400nm pores. After fabrication of the TMOC, an artificial tumor tissue was constructed by filling the tumor channel with cancer cell-laden collagen solution, which was polymerized within the tumor channel. In this study, a human breast cancer cell line (MCF7) was mixed into type I collagen solution as described previously [6,7]. After gelation, the culture medium was filled along all the channels and the pressure of capillary, interstitium and lymphatic channel was set to 20, 20 and 5 mmHg respectively to simulate typical tumor environment [8,9]. Then, the tumor was
Figure 2. Tumor tissue with different microstructure on the TMOC. By varying the cell seeding density and collagen concentration, tumor tissues with three different microstructures were created. Top row shows at the time of seeding, and the bottom shows zoomed images for cellular morphology during 24 hour culture.

Figure 3. Transport of 100nm NPs in the TMOC model. (a) Time-lapse fluorescence images showing permeation of NPs into tumor channel, (b) Fluorescence intensity profiles. Gray area notes the capillary channel.

cultured at 37°C and 5% CO2 environment. While maintaining this condition, fluorescent polystyrene NPs with 100, 200 or 500nm diameter were introduced to the capillary channel as model drug carriers at a concentration of 10^{11}/ml. The spatiotemporal fluorescence intensity was monitored to characterize the NP transport.

RESULTS AND DISCUSSION

The growth of MCF7 cells on the TMOC during 24-hour culture is shown in Figure 2. In this model, the MCF7 cells were growing within the 3D ECM architecture in the presence of the interstitial fluid similar to in vivo environments [10]. When the tissue was polymerized (i.e., 0 hour), the cells were aggregated with distinct cell membrane boundaries. As the tumor tissue was cultured for 24 hours, the size of the aggregate increased and the cell boundaries were hardly distinguishable, which mimics the tumor tissue structure very well. At the same cell seeding density (i.e., 10^7 cells/ml), the MCF7 cells in the high collagen content gel (i.e., 6 mg/ml) grew much slower than their counterparts in the low collagen content gel (i.e., 3 mg/ml). As the seeding density increased to 10^8 cells/ml, however, the cells seemed to grow faster and form aggregates with cell-cell adhesion even at the high collagen content.

Figure 3 shows the time-lapse fluorescence micrographs indicating the spatiotemporal transport of the 100nm NPs in the TMOC. The changes of the NP concentration can be correlated to the fluorescence intensity, and the intensity profiles clearly show the spatiotemporal transport of the NPs. In the capillary channel, the fluorescence intensity rapidly increased within the first 1hr and remains constant. In the tumor channel, the concentration near the channel interface increased similar to that of the capillary channel due to the extravasation of the NPs, and subsequent interstitial transport of the NP was observed. Currently we are performing systematic investigation to study the effects of other environmental conditions including the pore size of the membrane, collagen concentration of the matrix, and the IFP. Moreover, different size and type of NPs with anti-cancer drugs are also being tested.

In summary, the TMOC model is capable of providing in vivo tumor-like environment with superior controllability. It will help to establish quantitative understanding of the in vivo transport processes, which is difficult to achieve using conventional in vitro or small animal models.

REFERENCES


ACKNOWLEDGMENTS

This work is partially supported by grants from NIH (R01 EB008388), NSF (CBET-1009465) and Purdue Research Foundation.