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Differential response to doxorubicin in breast cancer subtypes simulated by a microfluidic tumor model

Altug Ozcelikkale[a,1], Kyeonggon Shin[a], Victoria Noe-Kim[a], Bennett D. Elzey[b,c], Zizheng Dong[d], Jian-Ting Zhang[d], Kwangmeyung Kim[e], Ick Chan Kwon[e], Kinam Park[f], Bumsoo Han[a,c,f,g,*]

[a] School of Mechanical Engineering, Purdue University, West Lafayette, IN, USA
[b] Department of Comparative Pathobiology, Purdue University, West Lafayette, IN, USA
[c] Purdue Center for Cancer Research, Purdue University, West Lafayette, IN, USA
[d] Department of Pharmacology and Toxicology, Indiana University, Indianapolis, IN, USA
[e] Biomedical Research Institute, Korea Institute of Science and Technology, Seoul, South Korea
[f] Weldon School of Biomedical Engineering, Purdue University, West Lafayette, IN, USA
[g] Birck Nanotechnology Center, Purdue University, West Lafayette, IN, USA

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ABSTRACT

Successful drug delivery and overcoming drug resistance are the primary clinical challenges for management and treatment of cancer. The ability to rapidly screen drugs and delivery systems within physiologically relevant environments is critically important; yet is currently limited due to lack of appropriate tumor models. To address this problem, we developed the Tumor-microenvironment-on-chip (**T-MOC**), a new microfluidic tumor model simulating the interstitial flow, plasma clearance, and transport of the drug within the tumor. We demonstrated T-MOC's capabilities by assessing the delivery and efficacy of doxorubicin in small molecular form versus hyaluronic acid nanoparticle (**NP**) formulation in MCF-7 and MDA-MB-231, two cell lines representative of different molecular subtypes of breast cancer. Doxorubicin accumulated and penetrated similarly in both cell lines while the NP accumulated more in MDA-MB-231 than MCF-7 potentially due to binding of hyaluronic acid to CD44 expressed by MDA-MB-231. However, the penetration of the NP was less than the molecular drug due to its larger size. In addition, both cell lines cultured on the T-MOC showed increased resistance to the drug compared to 2D culture where MDA-MB-231 attained a drug-resistant tumor-initiating phenotype indicated by increased CD44 expression. When grown in immunocompromised mice, both cell lines exhibited cell-type-dependent resistance and phenotypic changes similar to T-MOC, confirming its predictive ability for *in vivo* drug response. This initial characterization of T-MOC indicates its transformative potential for *in vitro* testing of drug efficacy towards prediction of *in vivo* outcomes and investigation of drug resistance mechanisms for advancement of personalized medicine.

1. Introduction

Achieving effective delivery of therapeutics to the target tumor tissue and overcoming the drug resistance exhibited by cancer cells are the significant challenges of cancer management and treatment. The recent years have witnessed significant efforts in development of novel cancer nanomedicine aimed for improved delivery by fine-tuning their physiochemical properties such as size, shape, and surface functionalization [1,2]. As a result, various drug delivery systems, including nanoparticle (**NP**) formulations, have been proposed and developed [3–6]. While these novel formulations have shown promising outcomes in preclinical studies; their clinical benefits have often found to be diminished due to multi-faceted mechanisms of drug transport and resistance present at the tumor microenvironment (**TME**).

TME poses structural and pathophysiological barriers such as dense extracellular matrix (**ECM**) microstructure [7,8], high cell packing density [9,10], and an elevated interstitial fluid pressure (**IFP**) [11,12]. These barriers hinder transport of drugs and drug delivery vehicles at various stages of their *in vivo* journey, including blood circulation, transvascular transport (i.e., extravasation), interstitial transport and cellular uptake [3,5,6]. In addition to the hostile and adverse conditions within the TME affecting drug delivery, many cancers may acquire

* Corresponding author at: 585 Purdue Mall, West Lafayette, IN 47907, USA.
E-mail address: bumsoo@purdue.edu (B. Han).
[1] Present address: Institute for Physical Science and Technology, University of Maryland, College Park, MD, USA.

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multidrug resistance (**MDR**) by a group of membrane proteins that exclude cytotoxic molecules. These proteins belong to the ATP binding cassette superfamily of membrane transporters [13]. Other molecular mechanisms are also reported to cause the MDR by alteration of survival/apoptosis pathways, and increased DNA damage repair. For instance, eukaryotic translation initiation factor 3 subunit A (**eIF3a**) expression has been associated with poor clinical response to DNA-damaging drugs [14] and its reduced expression was shown to contribute to cisplatin and doxorubicin resistance possibly by inhibiting expression of DNA repair proteins and, thus, DNA repair activities [14]. Increased expression of 14-3-3σ protein has also been found in doxorubicin-selected and resistant cancer cells and was shown to contribute to doxorubicin resistance [2,15,16]. In addition to the over-expression of the MDR-associated proteins, cellular drug resistance also appears to be mediated by integrin signaling associated with adhesion of cancer cells to the ECM [17–19].

It is further complicated by the presence of tumor heterogeneity with different subtypes of cancer cells showing highly variable drug response [20]. For instance, multiple subtypes of breast cancer have been identified based on the expression of estrogen receptor, progesterone receptor, and human epidermal growth factor receptor-2 [20,21]. Each subtype has a different prognosis and response to chemotherapy, and several subtypes including luminal B, basal, claudin-low, and HER2-enriched subtypes show variable or poor response to chemotherapy [22–25]. Therefore, in order to develop personalized treatment strategies, drug response of breast cancers should be evaluated as a compounding consequence of all these mechanisms.

Currently available tumor models show significant limitations in screening drugs for efficacy and chemoresistance in a way predictive of their *in vivo* performance. This is mainly due to the complex drug transport and resistance mechanisms above that are present in the host environment but are not typically realized *in vitro* cell culture settings. Although conventional *in vitro* cell cultures are valuable tools for cancer research, their two-dimensional (2D) culture do not adequately represent the conditions present in *in vivo* three-dimensional (3D) TME. Therefore, the outcome of traditional culture of cancer cells often fails to be indicative of *in vivo* or clinical outcomes. Furthermore, while 3D culturing systems have demonstrated a step forward in applicability from 2D [26], few 3D *in vitro* culture systems have been empirically shown to approximate the *in vivo* TME for accurate drug response, and many of these are unique to individual laboratories with limited availability. A growing number of studies reported that the physical, chemical and mechanical microenvironment of cancer cells significantly affects their behaviors [27–30]. Multiple breast cancer cell lines have the same morphology when cultured in 2D, but show different morphologies and organized structures when cultured in a 3D matrix [29,30]. This difference depends on their subtypes [28]. Different drug sensitivity in 2D versus 3D culture is also reported [2]. Although small animal models can provide a more realistic tumor microenvironment, these only provide end results. It is not suitable to rapidly screen various chemotherapeutic agents with respect to heterogeneous subtypes of breast cancers. Thus, new tumor models are required to be capable of rapidly screening the efficacy of various drugs and drug delivery systems in the context of the *in vivo* tumor microenvironment where complex interactions occur among cells, ECM and interstitial fluid.

In order to address this critical gap, we developed a new 3D microfluidic *in vitro* tumor model, named tumor-microenvironment-on-chip (**T-MOC**), capable of simulating the complex transport at the *in vivo* TME and study cell-type-specific drug resistance of cancerous cells. The T-MOC is based on a microfluidic platform culturing cancer cells in 3D tissue architecture mimicking complex transport around tumors [31]. Specifically, the T-MOC is designed to mimic pharmacokinetics, extravasation, interstitial transport and cellular uptake of drugs and drug delivery systems. We previously utilized the T-MOC platform to investigate the transport of polymeric nanoparticles under different microenvironmental conditions by varying parameters such as cut-off pore size, interstitial fluid pressure, and tumor tissue microstructure [31]. In addition, we demonstrated the combination of T-MOC with preexisting *in vitro* cultures such as externally cultured multi-cellular spheroids that were transferred into the microfluidic device to simulate a dynamic *in vivo* microenvironment during drug testing [32]. We also investigated cell-type dependent drug uptake and efflux across the cellular membrane in the T-MOC platform [33].

In the present study, we used the T-MOC platform to characterize the transport and efficacy of doxorubicin (**Dox**) and dox-encapsulated hyaluronic acid nanoparticles (**Dox-HANPs**), in order to demonstrate its feasibility to rapidly characterize and predict drug transport, action and chemoresistance of breast cancers. Two human breast cancer cell lines (MDA-MB-231 and MCF-7) were introduced to the T-MOC platform, and were cultured in type I collagen matrices under an elevated IFP condition. These cell lines were selected to represent different molecular subtypes of breast cancers. MCF-7 is characterized as a member of luminal A subtype that expresses estrogen receptor and is typically responsive to chemotherapy and hormone therapy. On the other hand, MDA-MB-231 is a triple-negative breast cancer line identified by low expression of estrogen, progesterone and human epidermal growth factor 2 receptors as well as claudin-family tight junction proteins, exhibits markers associated with epithelial-mesenchymal transition and cancer stem cell and shows relatively poor response to chemotherapy [22–25]. Dox and Dox-HANPs were administered to T-MOCs in a way to simulate *in vivo* pharmacokinetics, specifically temporal clearance of drugs from plasma. During and after the administration, the transport and subsequent cellular response were characterized and compared with conventional 2D culture assay. The results were further validated against xenograft tumor models.

2. Materials and methods

2.1. Cells and reagents

Human breast cancer cell lines (MCF-7 and MDA-MB-231) were cultured following standard practices for adherent cell culture. Culture medium (DMEM/F12, Invitrogen, NY) was supplemented by 2 mM L-glutamine, 100 μg/mL penicillin/streptomycin, 5% v/v fetal bovine serum for MCF-7 and 10% v/v fetal bovine serum for MDA-MB-231. Cells were routinely harvested at 80% confluence using 0.05% trypsin and 0.53 mM EDTA, used for experiments or sub-cultured accordingly. Cells were cultured up to 20 passages before being cryopreserved or discarded. Care was taken to conduct T-MOC and 2D culture experiments in parallel to control for potential phenotypic variations during the culture period. For experiments involving 2D culture, cells were plated in a 24 well plate at an initial density of 4000 cells/well and cultured up to 5 days. For 3D culture on T-MOC device, cells were seeded in a type-1 rat tail collagen (BD Bioscience) matrix. For this purpose, first a neutralized collagen solution with 6 mg/mL collagen concentration was prepared as described previously [34] and cells were suspended in the collagen solution at 2×10^7 cells/mL concentration. Next, approximately 20 μL of cell-laden collagen solution was loaded in the T-MOC interstitial channel by surface-tension bounded flow initiated by applying suction pressure at one of device ports. Devices loaded with cell-collagen mixture were placed in a CO_2 incubator and maintained at 37 °C for an hour for polymerization of collagen matrix. Devices were perfused with culture media for 2 days before drug testing.

2.2. Design and fabrication of T-MOC

The T-MOC has a 3D structure formed by stacking two layers of microchannels with a porous membrane sandwiched between the layers as shown in Fig. 1A. The top layer has a 300 μm wide channel that is 100 μm in thickness, which simulates the capillary of the tumor