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A Microfluidic Platform for Modeling Metastatic Cancer Cell Matrix Invasion

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Abstract

Invasion of the extracellular matrix is a critical step in the colonization of metastatic tumors. The invasion process is thought to be driven by both chemokine signaling and interactions between invading cancer cells and physical components of the metastatic niche, including endothelial cells that line capillary walls and serve as a barrier to both diffusion and invasion of the underlying tissue. Transwell chambers, a tool for generating artificial chemokine gradients to induce cell migration, have facilitated recent work to investigate the chemokine contributions to matrix invasion. These chambers, however, are poorly designed for imaging, which limits their use in investigating the physical cell-cell and cell-matrix interactions driving matrix invasion.

Microfluidic devices offer a promising model in which the invasion process can be imaged. Many current designs, however, have limited surface areas and possess intricate geometries that preclude the use of standard staining protocols to visualize cells and matrix proteins. In this work, we present a novel microfluidic platform for imaging cell-cell and cell-matrix interactions driving metastatic cancer cell matrix invasion. Our model is applied to investigate how endothelial cell-secreted matrix proteins and the physical endothelial monolayer itself interact with invading metastatic breast cancer cells to facilitate invasion of an underlying type I collagen gel. The results show that matrix invasion of metastatic breast cancer cells is significantly enhanced in the presence of live endothelial cells. Probing this interaction further, our platform revealed that, while the fibronectin-rich matrix deposited by endothelial cells was not sufficient to drive invasion alone, metastatic breast cancer cells were able to exploit components of energetically inactivated endothelial cells to gain entry into the underlying matrix. These findings reveal novel cell-cell interactions driving a key step in the colonization of metastatic tumors and have important implications for designing drugs targeted at preventing cancer metastasis.

Introduction

Cell invasion of the extracellular matrix (ECM) is an important step in many normal and malignant processes in the body. For example, the wound healing cascade and inflammatory response both require well-controlled matrix invasion of fibroblasts and leukocytes, respectively.[1–3] In human disease, cancer metastasis provides an example of abnormal and

damaging tissue invasion, where cancer cells in circulation extravasate out of blood vessels to invade organ tissue and colonize a metastatic tumor.[4,5]

Recent research in the field of metastatic cancer has aimed to identify important drivers of matrix invasion during extravasation. While chemokine gradients are well known for their role in guiding invasion, cancer cells are also thought to interact directly with capillary endothelial cells via various adhesion molecules to gain entry to the underlying tissue.[6–11] These interactions may not even require active communication between the cells, as studies have shown that live fibroblasts are able to recognize and respond to fixed cells in culture.[12,13] In addition to direct cell-cell interactions, endothelial cells may also influence cancer cell invasion indirectly through matrix deposition. It has been shown that endothelial cells in culture deposit a significant layer of matrix on a culture surface and that gel invasion can be influenced by the gel protein composition and matrix fiber density.[14–17]

Currently, there is relatively little quantitative *in vitro* evidence of the physical interactions between cancer cells and capillary endothelial cells or cell-secreted matrix related to metastatic cancer matrix invasion. This dearth of knowledge is due, in large part, to the lack of an appropriate tool to study these interactions, which require high-resolution imaging of the invasion process to generate conclusive and statistically justifiable results. The Transwell assay is the standard tool for studying matrix invasion *in vitro*. In this platform, cells and underlying protein gels are supported by optically opaque, porous polycarbonate membranes and suspended above a culture plate well containing growth medium.[18] While the Transwell gel invasion geometry is well-designed for studying matrix invasion in a diffusive chemoattractant gradient, the construct is poorly suited for imaging, making the assay ineffective for investigating non-chemokine contributions to metastatic cancer cell matrix invasion. In addition, gel invasion in a Transwell insert is primarily quantified in terms of the number of cells that migrate through the gel to reach the membrane after a finite time period, giving no indication of more detailed metrics of invasion.[18]

Microfluidic devices offer a unique alternative geometry to Transwell inserts that allows for imaging the matrix invasion process. While current *in vitro* models have shown that cancer cell extravasation shares some similarities with well-characterized leukocyte extravasation, known differences in the mechanisms of arrest and adhesion as well as experienced shear stress have emphasized a need for cancer-specific extravasation models.[19–25] For example, recent work has demonstrated the power of microfluidic platforms in studying cancer cell invasion of a matrix designed to replicate the microenvironment of a metastatic lesion in bone tissue.[26,27] Other microfluidic platforms have been designed to facilitate imaging of the invasion of tumor cell aggregates rather than individual cells.[28] While these studies have shown the utility of microfluidic devices in providing information about speed and depth of matrix invasion, the platforms often contain few gel interface regions on which to quantify invasion, yielding a small sample size for drawing conclusions about physical cell-cell or cell-matrix interactions at the gel surface. In addition, the intricate geometries of many platform designs preclude the use of standard immunohistochemistry staining protocols, which are necessary for identifying physical aspects of the cancer cell-endothelial cell interactions involved in matrix invasion. Two recent models have addressed many of these issues in design and have even narrowed focus to imaging cancer cells crossing the

wall of a model blood vessel, but the investigations were centered on intravasation rather than extravasation.[6,29]

In this study, we report the use of a microfluidic device to isolate contributions of inactive endothelial cell bodies and endothelial cell-secreted matrix proteins to the matrix invasion behavior of metastatic breast cancer cells (MDA-MB-231). Our platform has been designed to selectively model cancer metastasis in the condition of static fluid flow characteristic of vessels blocked by tumor cells or cell aggregates.[23–25,30] This condition is commonly observed in cancer cell extravasation specifically and has been the fundamental assumption in the design of similar cancer metastasis platforms.[6,27–29,31] Our platform includes large gel surface areas and contains media channels of appropriate size and geometry for effective immunohistochemistry analysis. Our methods reveal that, in absence of a chemoattractant gradient, metastatic breast cancer cells require a surface monolayer of endothelial cells for invasion of a type I collagen gel.

To probe the mechanism of increased invasion in the presence of endothelial cells and to demonstrate the power of the microfluidic device in visualizing physical contributions to gel invasion, we studied cancer cell matrix invasion under conditions of decellularized endothelial cells as well as an endothelial monolayer treated with sodium azide to energetically halt cell activity while leaving cell membrane proteins intact. Sodium Azide is a common inhibitor of mitochondrial respiratory function *in vitro* that preserves organelle and plasma membrane integrity.[32–34] In applications where membrane protein integrity is critical, sodium azide treatment is a valuable alternative to aldehyde fixation, which cross-links membrane proteins and requires complex (and not entirely successful) antigen retrieval techniques.[35] Through immunohistochemistry staining, our results confirm that decellularized endothelial cells leave behind a fibronectin-rich matrix on the surface of a collagen gel but that the matrix alone does not promote cancer cell gel invasion to a degree that is physiologically relevant. Sodium azide-treated endothelial cells, however, were confirmed via imaging to remain on the collagen gel following treatment and resulted in significant matrix invasion of cancer cells. The combined results point to a mechanism of matrix invasion that does not rely entirely on active cell-cell communication to drive cancer cell entry in a gel matrix in the absence of a chemokine gradient. In the future, specific cell surface integrins involved in this communication could be probed using this same device and pharmacological techniques. The results presented in this study demonstrate the power of our microfluidic matrix invasion platform over current Transwell and microfluidic gel invasion platforms in visualizing cell-cell and cell-matrix contributions to metastatic cancer invasion in the absence of a chemokine gradient.

Methods

Cell Culture and Reagents

The invasive breast cancer line MDA-MB-231 (MDA-231; ATCC, Manassas, VA) was cultured in phenol red-free Dulbecco's Modified Eagle's Medium (DMEM) supplemented with antibiotics (Gibco, Grand Island, NY) and 10% heat inactivated fetal bovine serum (HI FBS; Invitrogen, Carlsbad, CA; 56°C for 30 min). Human umbilical vein endothelial cells (HUVEC; Chan Lab, Nanyang Technological University, Singapore) were cultured in

endothelial cell medium with optimized HUVEC supplements (Lonza EGM2-MV). Cells were cultured at 37°C and 5% CO₂ on plasma-treated tissue culture dishes and were detached from the surface using 0.05% trypsin (Gibco).

Microfluidic Gel Invasion Model

A microfluidic device was constructed by curing polydimethylsiloxane (PDMS) on a silicon wafer mold containing a positive relief of a straight rectangular channel with five inlets and outlets and hexagonal posts that extend the height of the channel and divide the main channel into five equally spaced lanes (Fig. 1a). The resulting PDMS channel was fully encapsulated upon binding to a glass microscope slide using a plasma etcher (Harrick Plasma, Ithaca, NY). Dimensions of the main channel are 4mm × 1.4mm × 100µm (L×W×H). The completed devices were stored at 80°C for 24hr prior to use in experiments to completely dry the central chamber, as hydrophobicity is critical for selective patterning of gels in the device. Collagen gels were prepared over ice by diluting rat tail type I collagen (Corning, Corning, NY) to a final concentration of 3mg/mL with 10% by volume 1× PBS and deionized water. The pH was adjusted to pH 8.0 with 1N sodium hydroxide (NaOH), and the gels were polymerized by incubation at 37°C for 30min. Gels were selectively polymerized in the 2nd and 4th lanes of the main channel by carefully injecting gel solution through the 2nd and 4th inlets and relying on surface tension between the hexagonal posts spaced 100µm apart to contain the solution in the desired lane (Fig. 1b). Following polymerization, the remaining lanes were filled with endothelial cell medium and stored at 37°C. HUVECs were suspended in normal culture medium at a concentration of 4×10⁶ cells/mL, and a pipette was used to inject 10µL of cell suspension through the central channel of a microfluidic device containing collagen gel lanes and media. The device was oriented with the horizontal channel cross-section perpendicular to the ground to promote cell seeding along exposed sections of collagen gel separated by PDMS posts. Following a 30min incubation at 37°C during which the remaining cell solution was kept on ice, cell seeding was repeated in the central channel. The device was again oriented perpendicular to the ground to promote seeding on the opposite wall of exposed collagen sections and incubated for 30min at 37°C. The device was returned to normal orientation, all inlets and outlets were covered with endothelial cell media, and the device was incubated 48h with no applied flow. Media was changed every 24h.

Immunohistochemistry Staining for Vascular Endothelial (VE)-Cadherin and Deposited Fibronectin

To prepare cells for VE-cadherin staining, endothelial cells cultured on the collagen gel were washed with PBS and fixed in 4% paraformaldehyde (PFA) in PBS for 12min at room temperature. Device channels were flushed with PBS to wash out PFA, and cell membranes were permeabilized with the addition of 0.1% Triton X-100 for 10min at room temperature. Alternatively, cells in devices prepared for fibronectin staining were not fixed but were instead decellularized by incubation with a solution of 20mM ammonium hydroxide and 0.5% Triton X-100 in PBS for 3min at room temperature. Following either decellularization or fixation/permeabilization, device channels were thoroughly washed with PBS and incubated in a blocking solution of 5% BSA-PBS overnight. The blocking solution was replaced with either a 1:400 dilution of primary VE-cadherin antibody (Cell Signaling

Technologies, Danvers, MA) or a 1:200 dilution of primary human fibronectin antibody (Sigma-Aldrich, St. Louis, MO) in 5% BSA-PBS and stored at 4°C for 18hr. Primary antibody was removed with a PBS wash, and cells were stored in a 5% BSA-PBS blocking solution for 8hr at 4°C. Blocking solution was then replaced with a solution containing 2µg/mL Hoechst 33342 (Molecular Probes, Eugene, OR) along with secondary antibodies with appropriate host species reactivity in 1% BSA-PBS for 1hr at room temperature. In devices prepared for VE-cadherin staining, cells were incubated with a 1:200 dilution of a fluorescein-conjugated secondary antibody (Jackson ImmunoResearch, West Grove, PA); for fibronectin staining, a 10µg/mL dilution of Alexa Fluor 488-conjugated secondary antibody was applied (Invitrogen, Carlsbad, CA). Antibody-labeled devices were stored at 4°C in PBS and imaged using an Olympus FV1000 scanning confocal microscope with 405 and 488 nm excitation lines using a 60× water immersion objective.

Preparation Sodium Azide-Treated HUVECs

To prepare a sodium azide-treated endothelial monolayer, HUVECs cultured on the collagen gel within the device were washed with PBS and incubated with 5% sodium azide in deionized water overnight at 4°C. A concentration of 5% azide has been shown to be sufficient for inhibiting ATP synthesis and inducing cell death in mammalian cells including HUVECs.[36–40] Device channels were washed thoroughly and stored overnight in PBS at 4°C before addition of cancer cells.

Quantifying Metastatic Breast Cancer Matrix Invasion

MDA-231 cells were stained with 10µM CellTracker Green CMFDA (Molecular Probes, Eugene, OR) prior to use in experiments. Stained cells were suspended in normal culture medium at a concentration of 1×10^5 cells/mL, and a pipette was used to inject 10µL of cell suspension through the central channel of the device. Optimal seeding concentrations for both cancer cells and endothelial cells were determined through referencing related microfluidic invasion literature and through real-time imaging of the seeding with a benchtop light microscope.[27,29] Cancer cells were seeded using the same technique used for HUVECs. To summarize, cancer cells were seeded in two rounds, with each seeding followed by a 30min incubation at 37°C. During both incubation periods, devices were oriented with the horizontal channel cross-section perpendicular to the ground to facilitate cancer cell seeding against vertically-oriented gel walls. Cancer cells were seeded in 4 different device conditions: 1) directly on the type I collagen gel surface; 2) on top of the endothelial monolayer cultured on type I collagen gels; 3) directly on type I collagen gels following decellularization of endothelial monolayers cultured on the gel; and 4) on top of sodium azide-treated endothelial monolayers. Decellularization and sodium azide treatment methods are described in detail above. Cancer cells and endothelial cells (depending on the condition being studied) that remained viable and anchored to the collagen wall following washing and media replacement were imaged with the Olympus IX81 microscope both immediately after seeding and again after 24h of incubation at 37°C. Images were captured using green fluorescent protein (GFP) and red fluorescent protein (RFP) filter sets. For analysis, the collagen gel lanes were divided into regions of interest (ROIs) that each include a volume of the 3D gel matrix corresponding to 3 adjacent gel surfaces separated by 100µm-wide PDMS posts. Each ROI was imaged multiple times while adjusting Z-plane focus

depth to capture invading cells at all heights along the vertical collagen gel wall. Images captured immediately after cancer cell seeding were used to qualitatively confirm homogeneity of seeding density in ROIs along the length of the central channel. Gel invasion in each ROI was measured as the maximum change in net displacement in a direction directly perpendicular to the gel surface. Invasion distance was quantified by directly comparing 0h and 24h images in ImageJ.

Statistical Analysis

In studying magnitude of matrix invasion, a total of $n = 36$ ROIs were compiled from 3 independent devices (each with a maximum of 12 ROIs) for each of the 4 device conditions. Pairwise comparisons between device conditions were tested for significance using Matlab to perform a two-tailed t test with significance cutoffs of $P = 0.01$ or $P = 0.05$.

Results

Design and Characterization of the Microfluidic Matrix Invasion Platform

Evenly spaced pillars in the main channel of the microfluidic platform (Fig. 1a) enable flexibility in matrix gel patterning and a large collective gel surface area on which to quantify invasion. In applying the model to study cancer cell matrix invasion from a central channel into two separate lanes of type I collagen gel (Fig. 1b), this microenvironment design resulted in 36 separate $100\mu\text{m} \times 100\mu\text{m}$ gel surfaces grouped into 12 equally-sized regions of interest (ROIs) per device for statistical analysis (Fig. 2a–b).

Endothelial cells cultured for 48h in the device were stained for VE-cadherin to investigate confluency along the vertically-oriented collagen gel wall. Confocal Z-stacks of endothelial cells following immunohistochemistry (IHC) staining for VE-cadherin revealed positive VE-cadherin staining at junctions between individual HUVEC nuclei (visualized with Hoechst) along the full height and width of exposed collagen gel surfaces (Fig. 1c,e). Confocal imaging also indicated that the HUVECs primarily grow on the vertical collagen gel surface but also extend from the gel to cover adjacent sections of PDMS and glass substrates forming the ceiling and floor of the microfluidic channel, respectively (Fig. 1d).

Type I Collagen Gel Invasion of Metastatic Breast Cancer Cells is Enhanced in the Presence of a HUVEC Monolayer

Top-down fluorescence images of cancer cells cultured on the collagen gel walls for 24hr indicated a significantly decreased magnitude of matrix invasion in the absence of HUVECs (Fig. 2e–f). In fact, a quantitative analysis of matrix invasion distances revealed that MDA-231 cells invade on average less than $1\mu\text{m}$ past the collagen gel surface ($0.61\mu\text{m} \pm 0.59\mu\text{m}$) in the absence of endothelial cells (Fig. 3). In the presence of a HUVEC monolayer, however, the cancer cell invasion distance increased significantly to an average of $26.7\mu\text{m} \pm 6.93\mu\text{m}$ ($P = 3.54 \times 10^{-4}$, Fig. 2c–d).

Breast Cancer Gel Invasion is Enhanced in the Presence of Sodium Azide-Treated Endothelial Cells but not Fibronectin-Rich Matrix Deposited by Decellularized HUVECs—Confocal Z-stacks of the collagen gel following HUVEC

decellularization and IHC staining for fibronectin revealed a dense fibronectin-rich matrix remaining on the gel surface in addition to sparse fibronectin throughout the 3D gel bulk (Fig. 4a–b). In contrast, confocal images of a Hoechst nuclear stain (not shown) applied in conjunction with the IHC secondary antibody resulted in no significant signal above background intensity. MDA-231 cells seeded on top of this deposited fibronectin matrix exhibited significantly less collagen gel invasion ($2.96\mu\text{m} \pm 0.58\mu\text{m}$) than in devices containing the living HUVEC monolayer ($P = 1.05 \times 10^{-3}$; Fig. 3). The mean invasion distance was, however, statistically greater than that of the “Gel Only” control condition lacking either HUVECs or fibronectin matrix ($P = 5.88 \times 10^{-3}$).

In studying the effect of present but physiologically inactive HUVEC cell bodies on cancer cell matrix invasion, fluorescence imaging of the microfluidic platform prior to cancer cell seeding revealed that HUVEC cells expressing red fluorescent protein (RFP) remained on the gel surface following sodium azide treatment and sufficient washing (Fig. 4c and Fig. S1). Seeding MDA-231 on top of sodium azide-treated HUVECs in the device resulted in an average magnitude of cancer cell matrix invasion ($14.0\mu\text{m} \pm 3.90\mu\text{m}$) that was significantly greater than that of either the “Gel Only” control condition ($P = 1.10 \times 10^{-3}$) or the condition of decellularized HUVECs ($P = 6.42 \times 10^{-3}$; Fig. 3).

Discussion

In this study, we have presented a microfluidic platform to facilitate staining and imaging of cell-cell and cell-matrix interactions that drive matrix invasion in absence of a chemokine gradient. As with other microfluidic models, our platform enables comparison of micron-scale invasion distance rather than the percentage migration typically reported for Transwells. This more detailed metric allows researchers to consider the relevance of invasion during specific physiological time periods.[41,42]

One advantage of our microfluidic device is that it relies solely on surface tension between adjacent PDMS posts to contain matrix gels within individual channels, a design aspect that allows researchers to polymerize matrix protein gels in any of the 5, 4mm-long parallel and connected channels. Selective patterning of a gel in two separate internal lanes, as we have demonstrated, leaves both length-wise sides of each gel lane exposed for seeding and imaging of attached cells or deposited protein matrix and results in an expansive surface area on which to image extravasation-related invasion compared to similar microfluidic devices. The novel flexibility in gel and cell patterning also allows this device to be easily modified to suit a variety of experimental applications. For example, alternative gel patterning in the outermost lanes creates a wider central channel, which may be of use in studying large-diameter tumor cell aggregates.[43,44] Gel width can also be increased to study larger invasion depths by polymerizing matrix gels in multiple parallel lanes. Another advantage of our system is its straight, parallel channel design that facilitates straightforward immunohistochemistry (IHC) staining protocols. With the ability to selectively stain for structural proteins, our device may be applied in the future to investigate a variety of deposited matrix proteins and cell surface adhesion molecules involved in extravasation. [45,46] Additional validation of our model involving syringe pump-driven fluid flow (Fig.

S2) and fluorescent dextran diffusion (Fig. S3) has also demonstrated the utility of the platform in studying cell response to shear stress and soluble signal gradients.

Physical cell-cell and cell-matrix interactions involved in metastatic cancer cell extravasation have been challenging to study because of the lack of an appropriate tool to both label cells and proteins and image the matrix invasion process. Here, we demonstrated these capabilities in our microfluidic platform through a novel investigation of physical interactions between metastatic cancer cells and endothelial cells that line capillary walls. The platform configuration used in this study resulted in complete lanes of polymerized collagen gel surrounded by channels of media on either side. This design choice mimics the configuration of gel invasion studies on Transwell membranes in which gels are suspended vertically between two media reservoirs. IHC staining of endothelial cells cultured on the gel revealed VE-cadherin located at junctions between neighboring endothelial cells. The presence of VE-cadherin at cell junctions is characteristic of an endothelial monolayer and is an important component of microvessel integrity *in vivo*. [47,48] These results suggest that our system models cancer cells encountering a monolayer of endothelial cells before invading an ECM protein gel. Furthermore, as endothelial cells in a monolayer have been shown previously to be quiescent in their own invasion and remodeling of the extracellular matrix, our VE-cadherin staining supports the assumption that measured cancer invasion in our system is representative of cancer-endothelium and cancer-matrix interactions rather than an artifact of matrix deformation. [49–52]

Compared to cancer cells in a device lacking endothelial cells, cancer cells seeded on top of the endothelial monolayer showed significant invasion of the collagen gel over 24hr, which is the time frame under which most metastatic cancer cells in circulation are assumed to complete extravasation *in vivo*. [29,53] This evidence of endothelium-facilitated cancer invasion is consistent with studies of invasion in Transwells and other 3D invasion platforms, which report increased invasion (related to both intravasation and extravasation) for cancer cells co-cultured with HUVECs or treated with HUVEC-conditioned media. [54–58] To confirm the relevance and consistency of our findings with the literature, invasion speeds computed from the reported invasion depths over the 24hr observation time were all within the range of accepted speeds for MDA-MB-231 cells in related *in vitro* matrix invasion studies. [17,29,53,59] In the investigation to reveal non-chemokine-driven mechanisms for this finding, positive IHC staining for fibronectin on the gel surface following endothelial decellularization confirmed previous reports that endothelial monolayers deposit a fibronectin-containing matrix. [14] The lack of positive stain following incubation with Hoechst (not shown) confirmed the removal of endothelial cell bodies. [60] A previously published gene expression profile study from Minn *et al.* suggests that the MDA-231 cells used in our study express both more integrins and matrix metalloproteinases for the deposited fibronectin than for the underlying type I collagen gel in our model. [61,62] However, while the larger mean invasion depth of a fibronectin-coated gel compared to the endothelial cell-free control was statistically significant with $P < 0.01$, the $\sim 2\mu\text{m}$ difference accounts for less than 20% of an average breast cancer cell diameter and likely does not represent a physiologically relevant magnitude of cancer cell matrix invasion. [63]

Sufficient cancer cell matrix invasion of at least 1 cell diameter was seen, however, when cancer cells were seeded on top of endothelial cells treated with sodium azide to terminate cellular activity while leaving endothelial cell bodies attached to the gel surface.[64] HUVEC membrane integrity and retained cell-cell and cell-matrix adhesion were confirmed with RFP images (Fig. S1) of HUVECs along the collagen gel wall following azide treatment, washing, and cancer cell addition.[65,66] Compared to common fixation methods involving crosslinking reagents, sodium azide energetically inactivates cells without the need for antigen retrieval techniques to expose membrane proteins.[67] Our results suggest that metastatic cancer cells are able to exploit these exposed membrane proteins or other aspects of the energetically inactivated endothelial cell membranes to gain entry into the collagen matrix without active and reciprocal interaction from the endothelial cells. This finding may have important implications for developing drugs targeted at metastatic cancer matrix invasion, as our results have shown that the process may involve structural cell-cell interactions that do not rely on soluble signal gradients or active endothelial cells.[68,69]

While the difference in invasion between the conditions of azide-treated cells and live endothelial cells was shown to be statistically insignificant ($P > 0.05$), previous reports that cancer cells can induce upregulation of matrix-metalloproteinases (MMPs) in live endothelial cells suggest MMP-induced matrix degradation and invasion as an interesting point of future study.[70] In considering the impact of data variability in assessing the significance of this data, we recognize that similar microfluidic invasion platforms have selectively eliminated regions of interest to improve statistical power.[27,29] Our decision to consider all 12 regions of interest from each platform, despite the inherent variability in including all data points, reflects our intent to demonstrate the power of this platform in providing an unbiased and low-maintenance tool for identifying novel trends in matrix invasion. Future applications of this platform will include a detailed mesh analysis and high-resolution time-lapse imaging to identify biologically reliable regions of interest to reduce statistical variance. In addition, this platform is well-suited to carry out future studies to identify the specific endothelial cell membrane proteins that cancer cells utilize in gaining entry to the ECM.[54,71] While current work on this subject has revealed important and complex mechanisms, we believe that the research would benefit from translation to a robust 3D microenvironment with the capability of real-time imaging.[30,72,73]

Conclusion

In this work, we demonstrated a novel platform that facilitates the isolation and imaging of cell-cell and cell-matrix interactions driving cancer cell matrix invasion in the context of extravasation. The expansive gel surface areas, the ease with which IHC protocols can be adapted for use, and the flexibility in microenvironment patterning lend this device to a variety of migration and invasion-based applications and make it a novel alternative to Transwell assays and the majority of currently available microfluidic invasion platforms. Applying the model to metastatic breast cancer cell matrix invasion revealed that important interactions exist between cancer cells and endothelial cells to facilitate extracellular matrix invasion in the absence of chemokine gradients or actively communicating endothelial cells. Our platform is well suited for use in future studies to image and target membrane proteins

driving these interactions and to expand the application of this work to other highly metastatic human cancers.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.

Microfabricated matrix invasion model, (a) Top-down view of microfluidic device containing 5 inlets and outlets and 4 rows of hexagonal posts. Central channel dimensions $4\text{mm} \times 1.4\text{mm} \times 100\mu\text{m}$ (L×W×H). (b) COMSOL rendering of the matrix invasion microenvironment built into the microfluidic device. Invasion model includes two lanes of type I collagen gel (blue), three lanes of endothelial growth medium containing 10% fetal bovine serum (FBS, gray), and endothelial cell monolayers (green) cultured on the vertically oriented inner faces of the collagen gels. (c) VE-cadherin (green) and Hoechst (blue) staining to show the confluency of the endothelial monolayer cultured on a section of the collagen gel between PDMS posts (outlined in white). (d–e) 3D rendering of a complete Z-stack (step size: $0.46\mu\text{m}$) of endothelial cells cultured on a section of collagen gel. (d) Endothelial cells are confined to collagen gel surface and adjacent PDMS and glass boundaries. (e) VE-cadherin staining at cell junctions along the full height of the gel.

Figure 2.

Region of Interest (ROI) analysis of metastatic breast cancer matrix invasion. (a) Top-down view of gel invasion platform showing patterning of 2 collagen gels lanes and a central lane containing HUVECs. (b) A magnified section of one of the two collagen gel lanes containing a single ROI. ROI boundary extends across the width of 3 consecutive PDMS posts and collagen gel walls containing seeded cells, (c–f) MDA-231 cells (green) and HUVECs (red) seeded along the collagen gel (white dashed line) imaged immediately after cancer cell seeding (c,e) and again 24hr later (d,f). The magnitude of MDA-231 matrix invasion in a platform containing a HUVEC monolayer along the gel (c–d) was compared to MDA-231 matrix invasion in a platform lacking HUVECs (e–f). (Scale bar: 100µm.)

Figure 3.

Magnitude of MDA-231 invasion of a type I collagen gel matrix over 24hr in varying HUVEC conditions. Compared to a platform lacking endothelial cells (“Gel Only” condition), the average distance of cancer cell matrix invasion was significantly larger in platforms containing live HUVECs, decellularized HUVECs, and sodium azide-treated HUVECs. Platforms containing live HUVECs and sodium azide-treated HUVECs also resulted in significantly larger magnitudes of cancer cell matrix invasion compared to platforms in which HUVECs had been decellularized prior to cancer cell seeding. Average values for $n = 36$ regions of interest (ROIs) from 3 independent devices for each condition. Error bars indicate standard error of the mean; **, $P < 0.01$ compared to “Gel Only” condition; ◆◆, $P < 0.01$ between indicated pairs by Student's t test.

Figure 4.

Confocal imaging to confirm conditions of HUVEC decellularization and treatment with sodium azide prior to addition of MDA-231 cells within the microfluidic platform. Outlines of PDMS posts indicated by dotted white lines. (a) Immunohistochemical (IHC) staining for fibronectin (green) to show a fibronectin-containing matrix remaining on the collagen gel surface following decellularization. (b) Top-down view of 3D Z-stack to show sparse fibronectin deposition throughout the collagen gel and dense fibronectin staining along the gel surface. (c) Top-down view of endothelial cell bodies (red) remaining along the surface of the collagen gel following sodium azide treatment and washing. (Scale bar: 50 μ m.).