Using Microfluidics to Model the Effect of Macrophages on Cancer Metastasis

Peyton A. Trauth, Geneva R. Doak, Kathryn L. Schwertfeger, David K. Wood
University of Minnesota, Twin Cities – Department of Biomedical Engineering – Living Devices Lab

Purpose

Background

- Microfluidics - controlled flow of liquid through micrometer sized channels; allow for accurate representation of the tissues as they occur naturally in the body
- Cancer metastasis - spread of cancerous tumors from the primary site to secondary sites within the body
- Macrophages – inflammatory or tissue resident cells that phagocytose debris, dead/damaged cells, break down fibrotic tissue; initiate and influence immune response
- Literature indicates that macrophages promote extravasation of cancer cells by:
  - Increasing affinity of tumor cells to the endothelium
  - Increasing endothelial permeability
  - Direct signaling and/or contact with cancer cells

Since these interaction between cancer cells and macrophages are intrinsically difficult to study, the model developed by Bischel et al. has been adapted to measure adhesion and transmigration of breast cancer cells and permeability of the endothelium in response to human macrophages and/or conditioned media to uncover macrophage mechanisms in extravasation.

It was hypothesized that conditioned media and macrophages will increase adhesion, invasion, and permeability alone, but treating macrophages with conditioned media will further increase this extravasation potential.

Methods

1. Channel was created through collagen using passive pumping as described by Bischel et al.
2. After collagen polymerization, HMVEC-Ls were added to create a confluent monolayer. Shear was applied for 24 hours using gravity-driven flow with or without conditioned media
3. Cancer cells were added and monitored for 48 hours to analyze affect of conditioned media and macrophages on metastatic potential
4. Extravasation events were quantified using ImageJ data analysis

(Shown in Figure 1)

Figure 1

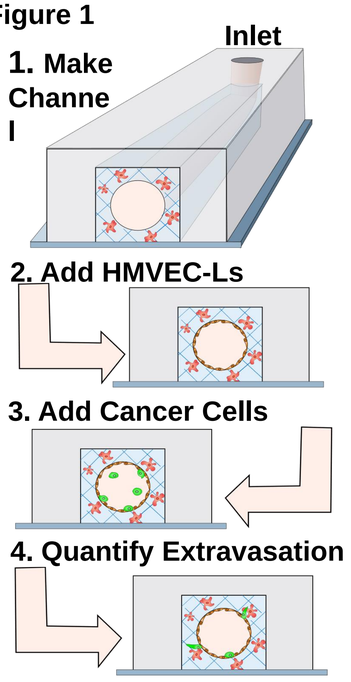
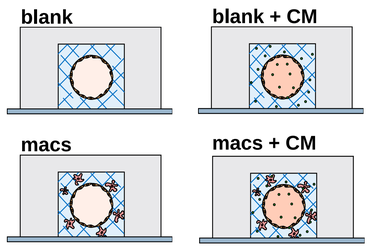


Figure 2



Overall, four different conditions were observed: no conditioned media and no macrophages (blank), conditioned media and no macrophages (blank + CM), no conditioned media and macrophages (macs), and conditioned media and macrophages (macs + CM). (Shown in Figure 2)

Influence on Extravasation

Cancer cell adhesion may be influenced by the combinatorial affects of both macrophages and conditioned media.

Figure 3

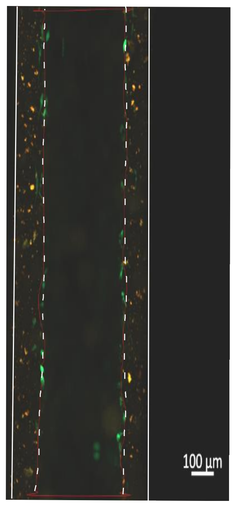
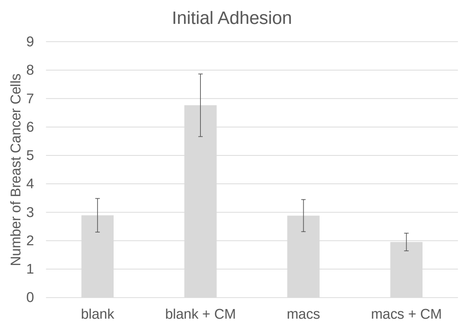


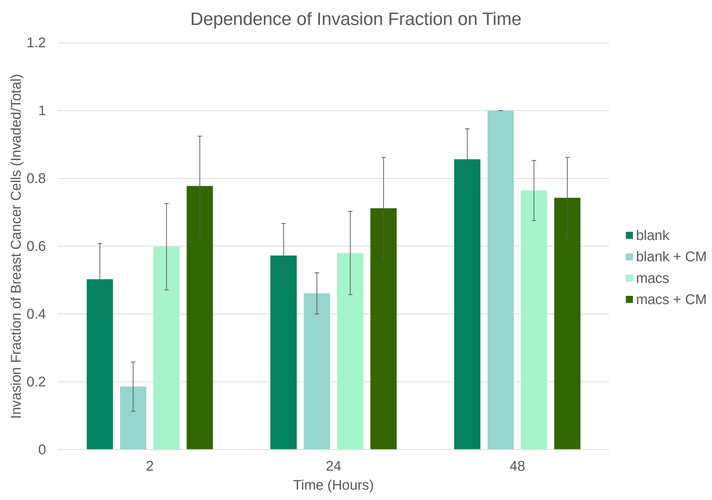
Figure 3: Fluorescent image of cross section of device. Cancer cells (green) have transmigrated endothelium (dotted white lines) into collagen space (orange beads).

Graph 1



Graph 1: Number of breast cancer cells in device after 2 hours. Conditioned media alone seems to increase initial adhesion significantly, whereas macrophages alone have similar initial adhesion as the blank condition. Macrophages treated with conditioned media, however, seem to decrease initial adhesion.

Graph 2



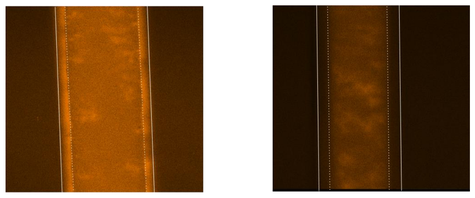
Graph 3: Invasion fraction was calculated for each device by dividing the number of cancer cells in the collagen space by the total number of cells in the device. In past experiments, the invasion fraction significantly increased when macrophages were treated with conditioned media, but not when either macrophages or conditioned media were alone. However, in this experiment, the invasion fraction for the conditioned media-treated macrophages remained fairly constant at initial measurement, day one, and day two. It is important to remember that this could be an artificial artifact of initial adhesion, for the invasion fraction is dependent on the number of cells in the device, and if there were few cells in the device to start with, invasion fraction increases significantly with few extravasation events.

Influence on Permeability

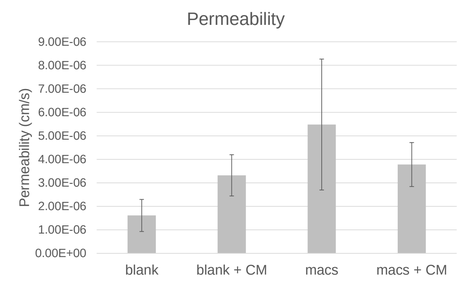
Macrophages and conditioned media increase endothelial permeability alone, and the combinatorial affect of macrophages and conditioned cell media has a greater affect on endothelial cell permeability than conditioned media alone, which may lead to increased extravasation.

Figure 4: The image on the left is a fluorescent cross section of 70 kDa TRICT-dextran perfusing a channel treated with EDTA to measure permeability of the matrix alone. After five minutes, the TRICT-dextran passes freely into the collagen. The image on the right is a fluorescent cross section of a channel with an endothelium that acts as a barrier to TRICT-dextran.

Figure 4



Graph 4



Graph 4: Quantification of permeability. The blank condition simulates *in vivo*-like permeability. When conditioned media and macrophages are added independently, permeability is increased. Additionally, when both macrophages and conditioned media are added together, the permeability is further increased.

Conclusions

In this study, a microfluidic model was employed to explore the role of human macrophages in the extravasation of breast cancer cells.
It was found that:

- Macrophages alone minimally affect extravasation
  - Increases permeability at 24 hours
- Conditioned media alters macrophage response to cancer cells
  - Decreases initial cell adhesion
  - Increases permeability of endothelium at 24 hours

These data support literature by indicating that the primary tumor influences macrophage behavior at the secondary site. The goal of using this microfluidic model is to analyze and systematically inhibit macrophage functions to investigate resulting cancer cell behavior. Ultimately, the objective is to identify potential metastasis-inhibiting therapeutic targets.
In both current and future experiments TNF-α, is used a positive control, for literature indicates that TNF-α activates endothelial cells to make them more adherent to breast cancer cells. Therefore, it's hypothesized that TNF-α treatment would increase breast cancer adhesion.

Acknowledgements

A special thank you to Geneva R. Doak for her mentorship on this project and in general.

This work was supported by NCI grants R21CA205455 (D.K.W.), R01CA215052(K.L.S.), and U54CA210190(D.K.W. and K.L.S.). G.R.D. is partially supported by a fellowship from Minnesota's Discovery, Research, and Innovation Economy. Portions of this work were conducted in the Minnesota Nano Center, which is supported by NSF through the National Nano Coordinated Infrastructure Network (award ECCS-1542202). We also would like to thank Paolo Provenzano for his generous gift of MDA-MB-231eGFP cells and Tanya Freedman for her generous gift of RAW 264.7 cells.

Reference: Bischel, et al. (2013) *Biomaterials* 34(5): 1471-1477