Microscale Engineering of the Prostate Cancer Microenvironment for Therapeutic Targeting of Tumor-Associated Macrophages

David Kosoff[1], Jiaquan Yu[2], Joshua M Lang[1]

[1]Department of Medicine, University of Wisconsin Carbone Cancer Center, Madison, WI, USA, [2]Department of Biomedical Engineering, University of Wisconsin, Madison, WI, USA

National Institute on Aging

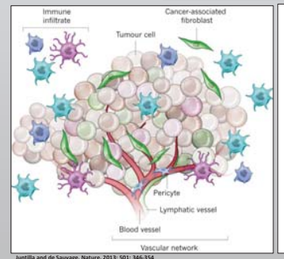
Carbone Cancer Center
UNIVERSITY OF WISCONSIN
SCHOOL OF MEDICINE AND PUBLIC HEALTH

Abstract

Despite continued advances in screening and treatment, prostate cancer continues to be a leading cause of illness and death for elderly men with 100,000 new cases and 23,000 fatalities anticipated in men over 65 this year. The development and progression of prostate cancer is greatly dependent on non-malignant cells within and around the tumor, collectively known as the tumor microenvironment (TME) [1-6]. Tumor-associated macrophages (TAMs) have been identified as critical components of the TME with key roles in disease progression and therapeutic resistance[7-10]. However, identification of the key pathways involved in TAM-directed tumor promotion, potential variation amongst individuals, and the contribution of age-related processes has been has been hindered by the complexity of the TME and the wide ranging functions of TAMs in cancer. To address these challenges we are utilizing a novel microfluidic culture platform. We have developed protocols that have successfully allowed for the culture of primary (derived directly from patient samples) and cell line epithelial, stromal, and immune cells, including macrophages within these devices. We have subsequently extracted cell-specific mRNA for evaluation of gene transcription along with concurrent analysis of cellular protein expression using fluorescence microscopy. Additionally, we have evaluated the impact of relevant TME variables, including the physical and chemical properties of culture surfaces, on cell growth, survival, and function. Future utilization of these microfluidic culture platform offers the potential for unprecedented analysis of the prostate cancer TME.

Background

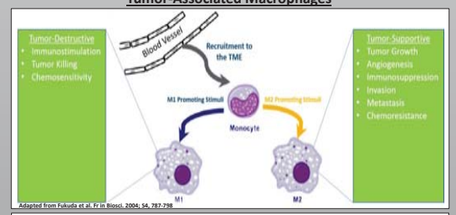
Tumor Microenvironment



Juntilla and de Sauvage. Nature. 2013; 501: 346-354

Illustration of the TME. The TME is comprised of the non-cancerous cells that reside within and around a tumor. These include immune cells such as T cells, B cells, neutrophils, and macrophages, as well as stromal cells such endothelial cells (blood vessels) and fibroblasts. These cells have roles in both cancer progression and eradication and have more recently become the focus of cancer therapies

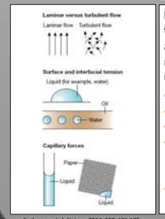
Tumor-Associated Macrophages



Adapted from Fukuda et al, Fr in Biosci, 2004; 56, 787-798

Illustration of the recruitment and polarization of TAMs. Many of the TAMs within the TME are derived from monocytes that are produced in the bone marrow and circulate within the blood. These monocytes are recruited to the TME by various tumor factors, where they differentiate into macrophages. Stimuli within the TME direct the TAMs to express unique gene sets and perform specific functions through a process known as polarization. M1-polarized TAMS support tumor eradication while M2-Polarized TAMs support tumor progression through involvement in a diverse array of processes.

Microscale and Microfluidics



Sackmann et al. Nature. 2014; 507; 181-189

Illustration of select physical properties that are altered at the microscale. At the microscale, fluid flow becomes laminar and forces like surface tension and capillary forces become more significant. Benefits of microfluidic platforms, which utilize the unique properties of fluid at the microscale include:

- Co-culture of multiple cell types in 2D and 3D
- Efficient utilization of primary cells for culture.
- Increased assay sensitivity
- Isolation of each cell population for multiplexed analysis:
  - Gene expression
  - Protein expression
  - Secretory factor production
- Ability to evaluate:
  - Distance dependent effects
  - Matrix remodeling

Results

Culture capabilities and Workflow of Stacks Platform

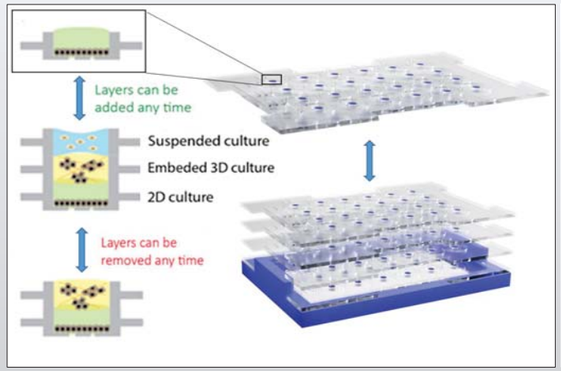
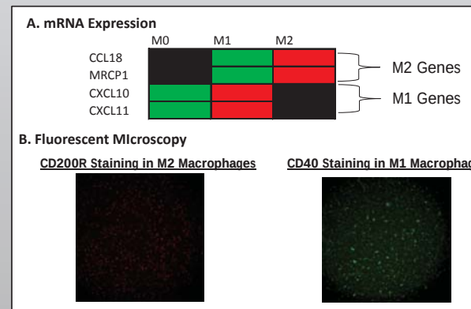


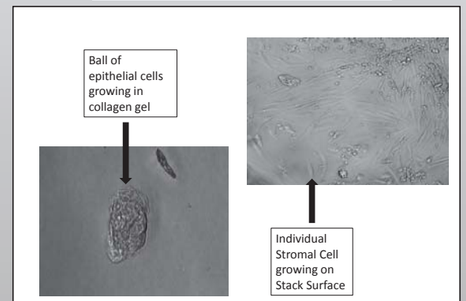
Illustration of the Stacks platform. The stacks platform is a reconfigurable open-microfluidic multi-cellular platform. Cells are cultured in wells located within milled polystyrene plates. Culture wells offer the potential of 2D (attach and grown on the bottom of the device) as well as 3D (within bio-scaffolds formed by matrix proteins such as collagen) cell culture. Diffusion ports within the wells allow for communication of cell populations in vertical alignment when plates are stacked on top of each other. In the devise illustrated to the left, the wells are 50um in height, allowing for culture of up to 6 layers simultaneously. Layers can be added or removed at any time to allow for evaluation of temporal variables. Wells with both 2D and 3D cultures can be added to co-cultures as needed to investigate the impact of matrix proteins.

Macrophage Culture, Polarization, and Expression Analysis



RNA and protein analysis of primary macrophages in stacks. Monocytes obtained from patient blood samples were differentiated into macrophages and polarized to M1 an M2 phenotypes within stacks. A. mRNA from cells was extracted and analyzed for expression of select M1 and M2-associated genes. Red indicates high expression. Green indicates low expression. Black indicates intermediate expression. B. Fluorescence microscopy images of cells stained for M1 and M2 markers. Left is M2-polarized cells stained for a M2 protein. Right is M1-polarized cells stained for a M1 protein.

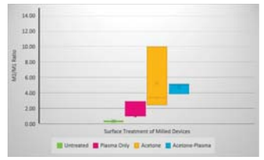
Culture of Primary Tumor and TME Cells



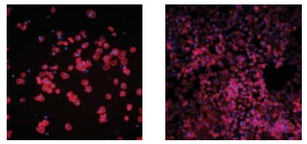
Brightfield images of primary cells cultured in stacks. Prostates removed from patients with prostate cancer were treated with a process designed to isolate specific cell types. These cells were then cultured within the stacks devices. Top Right: image of stromal cells growing in 2D culture. Bottom left: Image of epithelial cells growing in 3D culture(collagen matrix). Epithelial cells in 3D culture grow as an enlarging ball of cells.

Physical and Chemical Properties of the Microenvironment Affect Cell Growth, Morphology, Survival, and Gene Expression

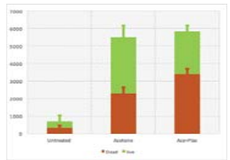
A. Surface properties affect macrophage gene expression



B. Surface properties affect prostate cancer cell morphology



C. Surface properties affect prostate cancer cell proliferation and viability



Evaluation of the impact of surface properties. on macrophages and prostate cancer cells. THP-1 macrophages (cell line) and DU145 prostate cancer epithelial cells (cell line) were cultured on four different surfaces, including milled, milled and acetone treated, milled and plasma treated, and milled, acetone, and plasma treated. Milling results in rough surfaces, acetone treatment produces a smoother surface, and plasma treatment produces a surface that is more hydrophilic. A. Relative expression of CCL18 mRNA in macrophages cultured on the four surfaces. B. Prostate cancer cells cultured on milled surface (left) and milled, acetone, and plasma treated surface (right) C. Average cell number and viability of prostate cancer cells in wells seeded with identical cell numbers and cultured for 3 days. Left column is data from milled surface, middle column is data from milled and acetone treated, right column is data from milled, acetone, and plasma treated.

Conclusions

- The stacks platform is a viable and advantageous modality for investigation of the TME and TAMs in prostate cancer.
- Within this platform, we can successfully:
  - Differentiate, polarize, and culture both primary and cell line derived macrophages
  - Culture additional primary and cell line derived TME cell populations
  - Culture primary and cell line derived tumor cells
  - Evaluate the impact of TME variables such as matrix proteins and general surface properties
  - Isolate and extract cell-specific mRNA for evaluation of gene transcript analysis
  - Fluorescently label and image cellular proteins for analysis of protein expression and cell morphology
  - Utilize computer software to quantify cell proliferation and viability using fluorescence microscopy images

Current and Future Directions

- Utilizing the stacks platform we aim to:
  - Identify key pathways utilized by TAMs to promote progression of progression of prostate cancer through:
    - Co-culture of macrophages and other immune cells, stromal cells, and tumor cells
    - Utilization of primary cells obtained from patient tissues and blood
    - Multiplexed analysis of RNA and protein expression, secreted factors, cell proliferation and viability
  - Evaluate pathways involved in TAM-mediated resistance to:
    - Hormone therapies
    - Chemotherapies
    - Radiation Therapy
  - Evaluate how age related processes, such as immunosenescence, contribute to macrophage support of prostate cancer progression
  - Evaluate patient-specific variability through culture of primary cells
  - Potentially evaluate new therapeutic targets and strategies

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Acknowledgements

This work was supported by a National Institute on Aging T32 Training Grant

We would like to express our deep gratitude to all of the patients who selflessly contributed to this research.