Development and Optimization of a P-selectin-Functionalized Microfluidic Platform for Modeling Adhesion Dynamics of Ovarian Cancer Cells

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Introduction

Ovarian cancer remains one of the most lethal gynecological cancers, largely due to its high tendency to metastasize, particularly through peritoneal dissemination (An and Yang, 2021; Mazidimoradi et al., 2022; Wolters-Eisfeld and Oliveira-Ferrer, 2024). Therefore, characterizing the mechanisms, particularly peritonal metastasis, is fundamental to understanding ovarian cancer progression.

P-selectin mediates cell-cell interactions under shear stress through recognition of specific carbohydrate ligands. In ovarian cancer, the sialyl Lewis X (sLe^x)-P-selectin cascade has been implicated in tumor-mesothelial adhesion, with highly metastatic cancer cells exhibiting enhanced P-selectin binding. In a previous study from our laboratory, Li et al. demonstrated that sLe^x-P-selectin-mediated adhesion occurs under ascitic fluid shear flow rather than static conditions (Li et al., 2019).

Various in vitro models that have been used to investigate ovarian cancer progression do not reproduce the dynamic fluid flow and shear stress experienced in the peritoneal cavity (Cortesi et al., 2024; Smit et al., 2023). Microfluidic platforms provide a promising approach by enabling precise control of flow conditions within defined channel geometries (Hassan et al., 2020). Previous work from our laboratory demonstrated that a microfluidic platform could reproduce physiologically relevant peritoneal shear stress (Ip et al., 2016), which was subsequently applied by Li et al. to investigate tumor-mesothelial adhesion (Li et al., 2019).

Building on these findings, there is a need for optimized microfluidic platforms that can reliably model ovarian cancer cell adhesion under physiologically relevant flow conditions. Consequently, this study aimed to investigate and optimize the P-selectin coating concentration for the adhesion of highly metastatic (HM) and non-metastatic (NM) ovarian cancer cells under microfluidic flow conditions and to compare the sensitivity and specificity of the optimized microfluidic platform using HM and NM ovarian cancer cell lines.

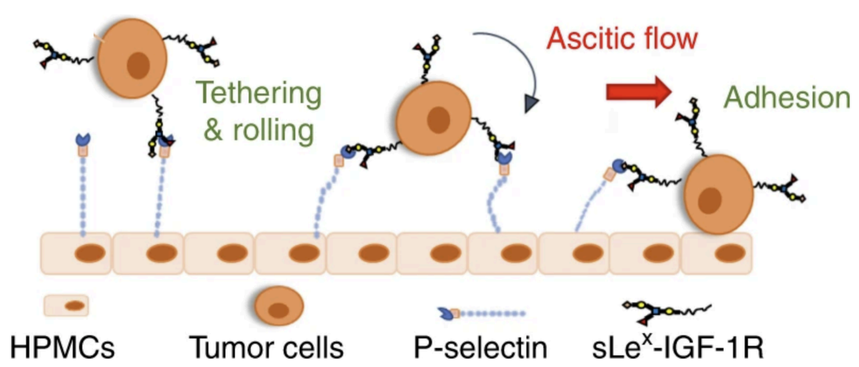


Fig 1. Schematic of sLex-P-selectin binding in peritoneal metastasis. HPMCs is an abbreviation for human peritoneal mesothelial cells. Reproduced from Li et al. (2019).

Objectives:

- Determine the optimal working concentration of P-selectin for studying the adhesion of HM and NM ovarian cancer cells under microfluidic flow conditions.
- Compare the sensitivity and specificity of the optimized P-selectin-functionalized microfluidic platform using HM and NM ovarian cancer cell lines.

Methodology

1. Microfluidic Chip Preparation and Protein A Coating: Each microfluidic channel was washed with ethanol and 1× phosphate-buffered saline (PBS) before coating with 20 µg/mL Protein A (60 µL/channel) for 1 h at room temperature, followed by blocking with 3% BSA for 30 min.

2. P-Selectin Concentration Optimization: Signal intensity and variance were evaluated for ten different P-selectin concentrations ranging from 0µg/mL to 40µg/mL (*Figure 2*).

3. P-Selectin Coating: The corresponding P-selectin solutions (60 µL/channel) were introduced and incubated overnight at 4°C in a moist chamber.

4. Preparation of the Coated Microfluidic Chip: Channels were washed with binding buffer (1× PBS, 1 mM $CaCl_2$, pH 6.5) and blocked with blocking buffer (3% BSA, 10% FBS, 1 mM $CaCl_2$, 1 mM $MgCl_2$) for 30 min at room temperature, followed by washing with binding buffer for three times.

5. HM and NM Cell Preparation: Kuramochi (HM) and OVSAHO (NM) cells were detached using EDTA, centrifuged at 1,000 rpm for 5 min, and resuspended in growth medium to a final concentration of 10^4 cells/ml. An equivalent volume of cell suspension was transferred to a 96-well plate to determine the corresponding initial cell number.

6. Microfluidic Flow Assay: HM and NM cells were introduced into the channels using 200-µL micropipette tips. The chip was operated in withdrawal mode using the LongerPump at 1,500 µL/h and 425 mm/min for 20 min, where the outlet is connected to the pump. The binding buffer level in the 200-µL micropipette tips at the inlet ends was monitored continuously.

7. Fluorescence Imaging and Cell Counting: Following staining, fluorescence images were acquired at FITC and TRITC wavelengths, and cells were quantified using QuPath.

8. Calculation of Cell Adhesion and Data Analysis: The percentage of cells retained within each channel following the flow assay was calculated as:

$$\textit{Attached cells (\%)} = \frac{\textit{Number of cells remaining in channel after flow}}{\textit{Initial number of cells}} \times 100$$

9. Sensitivity and Specificity Analysis: Sensitivity and specificity were calculated as:

$$\textit{Sensitivity (\%)} = TP / (TP + FN) \times 100$$
$$\textit{Specificity (\%)} = TN / (TN + FP) \times 100$$

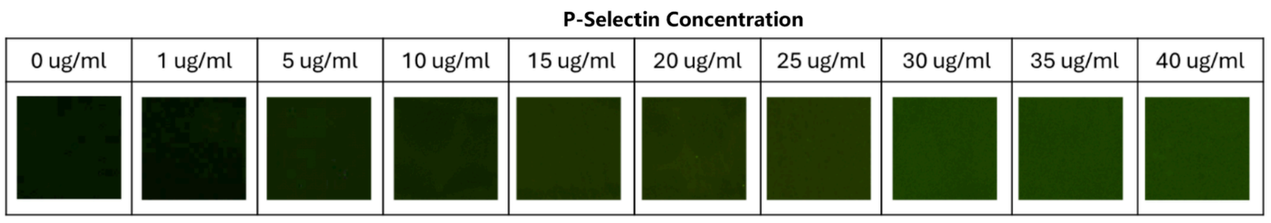
TP (true positive): HM Kuramochi cells correctly identified as HM.
FN (false negative): HM Kuramochi cells incorrectly identified as NM.
TN (true negative): NM cells (OVSAHO, OVCAR3, CaOV3) correctly identified as NM.
FP (false positive): NM cells incorrectly identified as HM.

Based on the results in *Figure 3A*, 25µg/mL P-selectin concentration was selected for downstream experiments in OVCAR3 and CaOV3 NM cells. Steps 1 and 3-8 were repeated for these NM cells by using 25µg/mL P-selectin concentration.

10. Data Analysis: The percentage of attached cells, sensitivity, and specificity were compared between HM Kuramochi and NM OVSAHO, OVCAR3, and CaOV3 cells. Graphs were plotted using GraphPad Prism.

Results

P-Selectin Concentration Optimization



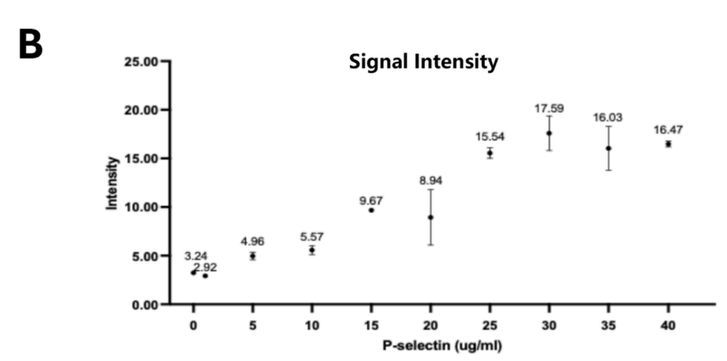


Fig 2. Results for P-selectin concentration optimization assays. ug/ml in the figures represents "µg/mL." Created using GraphPad Prism. Data was provided by Dr. Katie Sze Wai FUNG.

A. Fluorescence results for ten different P-selectin concentrations ranging from 0µg/mL to 40µg/mL.

B. Graph of intensity versus P-selectin concentration for fluorescence signal intensity analysis for ten different P-selectin concentrations used in A.

Sensitivity and Specificity Assays

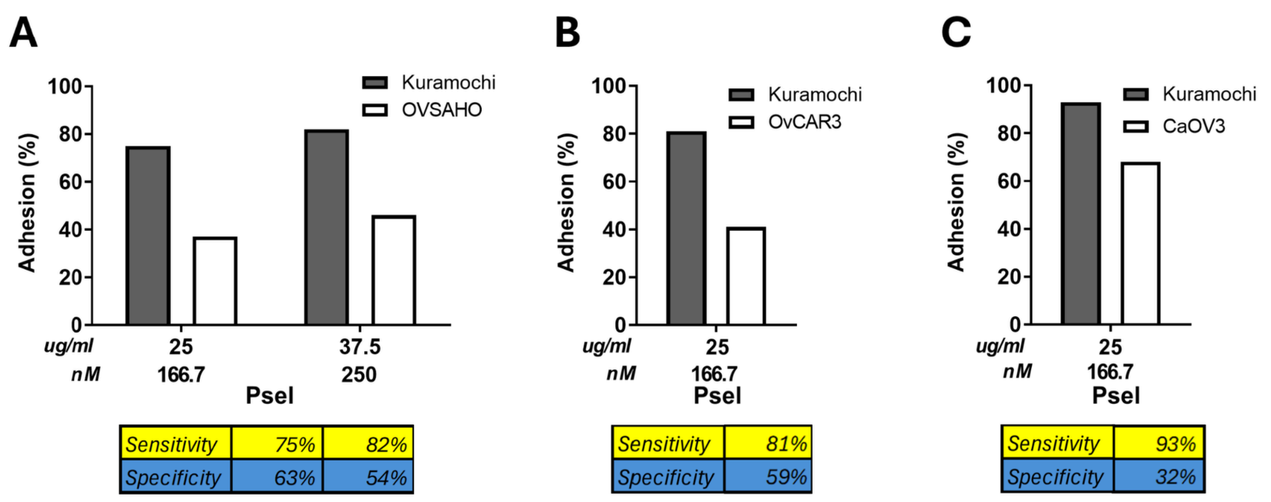


Fig 3. Boxplots for the HM cell line (Kuramochi) and NM cell lines (OVSAHO, OVCAR3, and CaOV3), with the corresponding sensitivity and specificity values shown below each plot. ug/ml in the figures represents "µg/mL." Created using GraphPad Prism. Data was provided by Dr. Katie Sze Wai FUNG.

Discussion

- 25 µg/mL P-selectin provided the most appropriate sensitivity-specificity profile.
- HM Kuramochi showed stronger adhesion than NM cells, supporting P-selectin-mediated adhesion as a model of metastatic adhesion phenotypes under flow.
- Variation among NM cell lines may reflect differences in P-selectin-binding properties and high-grade serous ovarian carcinoma (HGSOC) phenotypes.
- The optimized chip provides a simple, controllable platform for studying ovarian cancer adhesion under physiologically relevant flow.

Limitations and Future Directions

- **Cell lines only** → validate with additional cell lines and patient-derived cells.
- **Limited tumour microenvironment** → incorporate human peritoneal mesothelial cells and other relevant components.
- **Variable NM specificity** → further optimize HM/NM discrimination and sensitivity–specificity balance.
- **Mechanisms not directly assessed** → investigate sLe^x, CD24 and other P-selectin-binding molecules.
- **Potential application** → evaluate adhesion-modulating factors/therapeutic interventions under flow

Conclusion

This study investigated and optimized the P-selectin coating concentration for a microfluidic platform designed to study ovarian cancer cell adhesion under flow conditions. The optimized platform demonstrated differential adhesion between HM Kuramochi and NM ovarian cancer cell lines, supporting its potential for investigating metastatic adhesion phenotypes. Further optimization for NM cells and validation using patient-derived ovarian cancer cells may improve the sensitivity, specificity, and translational relevance of the platform.

ACKNOWLEDGEMENTS

This study was conducted in the laboratory of Prof. Alice S.T. WONG at the School of Biological Sciences, The University of Hong Kong, with Prof. Alice S. T. WONG serving as project supervisor, Dr. Katie Sze Wai FUNG as postdoctoral mentor, and Ms. Coco Man Yui LEE as PhD mentor. Data for Figure 2 and Figure 3 were provided by Dr. Katie Sze Wai FUNG. Support and funding were provided by the Laidlaw Foundation. Laboratory resources for all experimental assays were provided by Prof. Alice S.T. WONG's lab.

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