Evaluation of Surface-Modified Nanoparticle Transport and Metastatic Invasion Using a Novel Multicellular Ovarian Tumor Spheroid Model

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Introduction

Introduction: Until recently, *in vitro* models to study the metastatic progression of ovarian cancer were limited to monolayer and simple 3D spheroid models. Recent studies have shown that cellular-extracellular matrix (ECM) interactions lead to the reprogramming of the stromal environment and an increase in the metastatic potential of ovarian cancer.

Hypothesis/Objective: A novel 3D multicellular ovarian tumor spheroid model was developed to provide a more physiologically relevant platform to assess and relate nanoparticle (NP) transport to clinical therapy. We hypothesized that alterations to the tumor microenvironment (TME) induced by incorporating a peptide-based scaffold, in combination with stromal cell activation, would lead to enhanced cell migration and decreased NP transport, that may be more indicative of the challenging transport conditions encountered in clinical ovarian cancer.

Methods: Multicellular spheroids composed of ovarian cancer (SKOV3) and fibroblast (MRC5) cells were created using the hanging drop method. MRC5s were transformed to an activated phenotype by incubating with 20 ng/ml TGF-beta for 48 hr. Spheroids were subsequently introduced to a peptide-based scaffold (Puramatrix, PMX) to provide a more realistic TME. A co-cultured spheroid model without PMX was compared against the PMX model to investigate how cell invasion and NP transport were altered in the presence of PMX and/or activated stromal cells. Spheroids were treated with two surface-modified NP groups to assess differences in transport as a function of the TME.

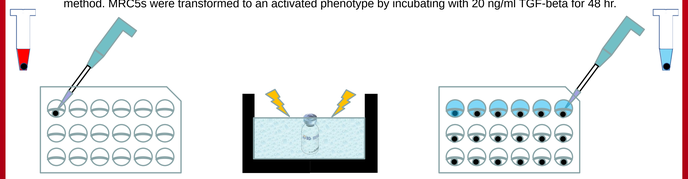
Results: Co-cultured spheroids composed of SKOV3 and activated MRC5s were significantly smaller, yet more invasive after 5d, relative to spheroids without PMX or activated MRC5s. Moreover, NP transport, regardless of surface-modification, was impeded in the presence of activated stromal cells. Results were statistically significant utilizing a one-tailed t-test ($p < 0.05$).

Conclusion: Increased invasion of epithelial cancers is a hallmark of epithelial to mesenchymal transition. Our observations of increased cell migration and diminished NP transport, as a function of TME and cell activation, suggests that this system may more accurately represent hurdles encountered in ovarian cancer therapy.

Methods

Multicellular Ovarian Tumor Spheroid Growth

Multicellular spheroids composed of ovarian cancer (SKOV3) and fibroblast (MRC5) cells were created using the hanging drop method. MRC5s were transformed to an activated phenotype by incubating with 20 ng/ml TGF-beta for 48 hr.

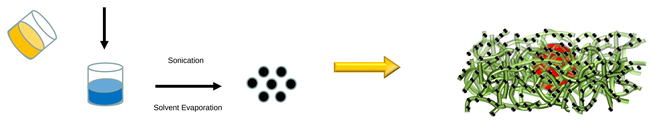


Introduction of Peptide Based Scaffold

Spheroids were subsequently introduced to a peptide-based scaffold (Puramatrix, PMX) to provide a more realistic TME. PMX was first sonicated and then introduced to the spheroids after 24 hr by removing 50% of the media and replacing with 2.5 mg/ml Puramatrix solution.

Poly(lactic-co-glycolic acid) (PLGA) Nanoparticle Synthesis

PLGA NPs were synthesized using a single emulsion oil-in-water technique, encapsulating the green dye Coumarin 6 (C6). The NPs were surface-modified with the ligands MPG and polyethylene glycol (PEG).



Nanoparticle Treatment and Transport Study

Spheroids were treated with both NP treatment groups after five days for 1.5 hr: no treatment (control), MPG NPs, PEG NPs.

Multicellular Spheroid Growth After 5 Days

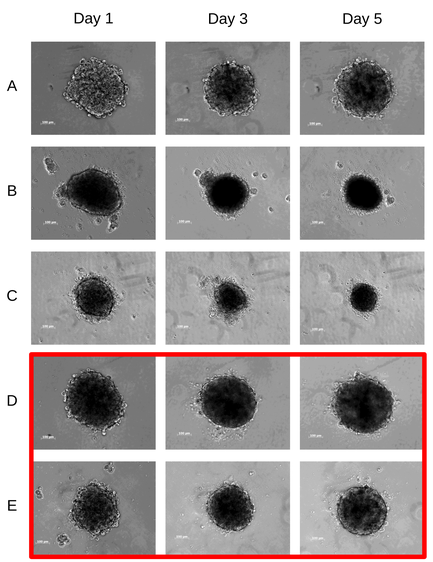


Fig 1. Spheroid Growth after 1, 3, and 5 days: A) SKOV3; B) MRC5; C) MRC5 (activated); D) SKOV3/MRC5; E) SKOV3/MRC5 (activated).

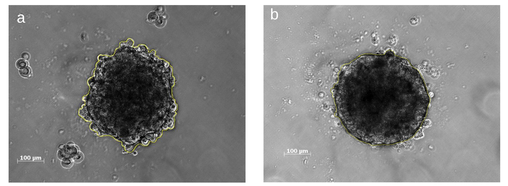


Fig 2. Multicellular Spheroid Growth Analysis for SKOV3/MRC5 (activated) after: a) 1 and b) 5 days of growth.

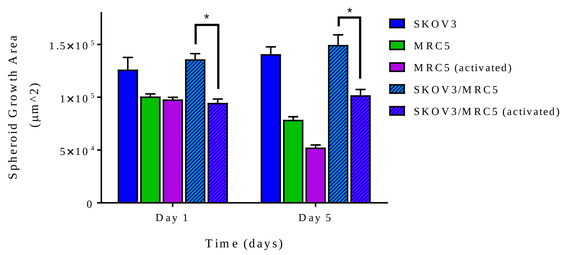


Fig 3. Multicellular Spheroid Growth after 5 days.
Data assessed using one tailed t test; $p < 0.05$ experimental groups D and E.

Multicellular Spheroid Invasion After 3 Days

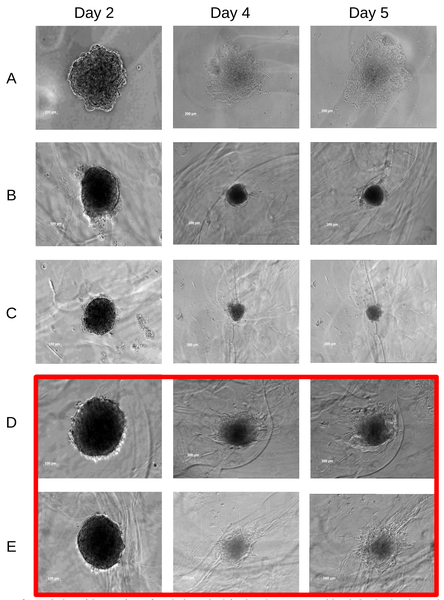


Fig 4. Spheroid Invasion after 3 days (t=0 is day 2 post growth): A) SKOV3; B) MRC5; C) MRC5 (activated); D) SKOV3/MRC5; E) SKOV3/MRC5 (activated).

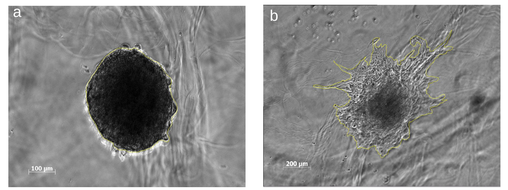


Fig 5. Multicellular Spheroid Invasion Analysis for SKOV3/MRC5 (activated) after: a) 1 and b) 5 days of growth.

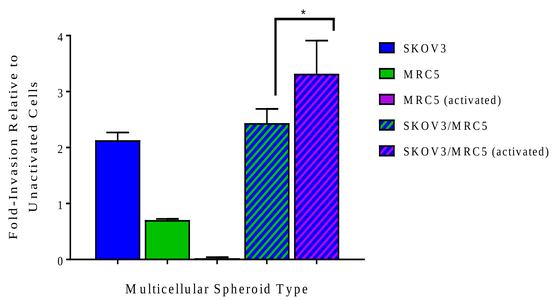


Fig 6. Multicellular Spheroid Invasion after 3 days.
Data assessed using one tailed t test; $p < 0.05$ experimental groups D and E.

Surface-Modified PLGA NP Transport

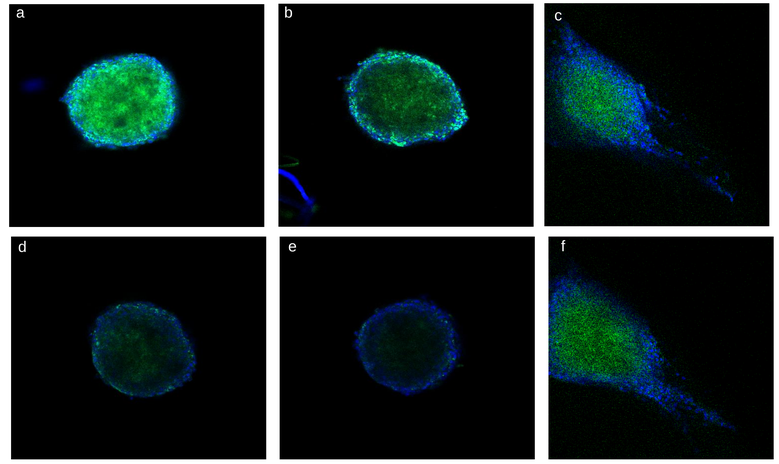


Fig 7. PLGA Nanoparticle Transport as a Function of Surface Modification and Stromal Cell Activation with and without PMX: a) PEG; b) MPG; c) PEG w/ MRC5 activation after 5 day invasion PMX (50 μm slice); d) PEG w/ MRC5 Activation; e) MPG w/ MRC5 Activation; f) PEG w/ MRC5 activation after 5 day invasion PMX (100 μm slice).

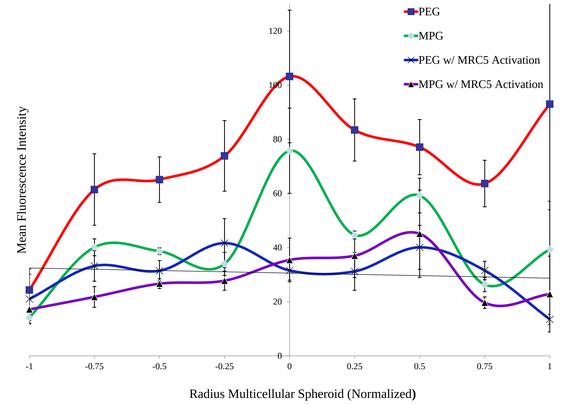


Fig 9. Distribution of Different Surface-Modified PLGA NP groups through Activated and Non-activated Tumor Spheroids, shown as a function of Mean Fluorescence Intensity of PLGA NPs and Distance through the Spheroid.

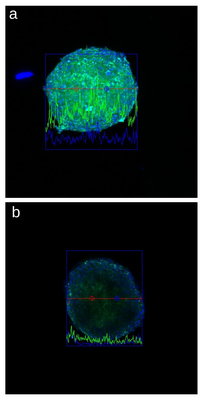


Fig 10. Fluorescence Intensity Map of: PEG NPs in a) non-activated and b) activated MRC5 spheroids.

Conclusions

Increased invasion of epithelial cancers is a hallmark of epithelial to mesenchymal transition. Our observations of increased cell migration and diminished NP transport, as a function of TME and cell activation, suggests that this system may more accurately represent hurdles encountered in ovarian cancer therapy. We look forward to assessing cancer-specific markers in future work to validate these findings.

Acknowledgements

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