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

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Introduction

Introduction: *In vitro* models to study the metastatic progression of ovarian cancer have traditionally focused on monolayer and single-cell 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 in normoxic and hypoxic conditions.

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, NPs with MPG surface modification demonstrated the highest NP tumor penetration in the presence of peripheral hypoxic gradients in spheroids superimposed on a backbone of activated fibroblasts. Results were statistically significant utilizing one-tailed ANOVA ($p < 0.05$).

Conclusion: 3D multicellular ovarian tumor spheroid models incorporating TME components provide insight into surface-modified NP transport. ECM architecture may have larger impact on NP penetration compared to cellular components and hypoxia combined.

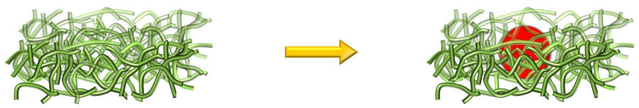
Methods

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.

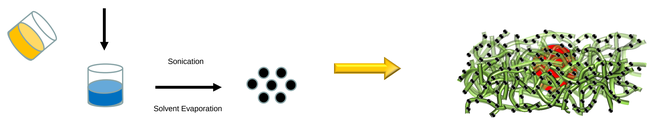
Introduction of Peptide Based Scaffold

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 24 hr: no treatment (control), MPG NPs, PEG NPs.

Multicellular Spheroid Growth After 5 Days

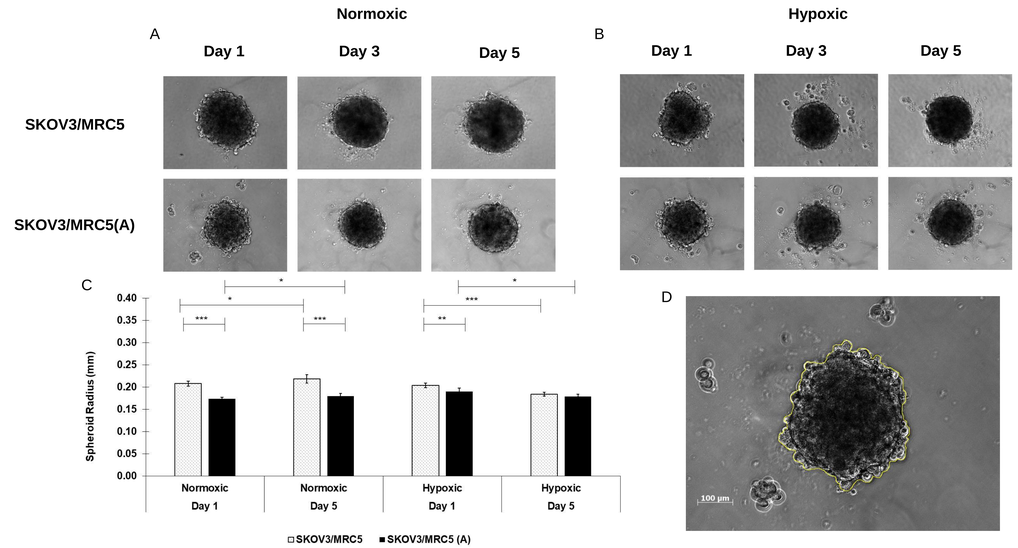


Figure 1. Non-PMX spheroids composed of activated SKOV-3/MRC-5(A) cells demonstrate decreased maximum cross-sectional growth after 5 days relative to a non-activated SKOV-3/MRC-5 control in both normoxic and hypoxic conditions. Representative phase images of (A) normoxic and (B) hypoxic non-PMX SKOV-3/MRC-5 spheroid growth. (C) Quantification of spheroid mid-cross-sectional area on days 1 and 5. (D) Multicellular Spheroid Invasion Analysis for SKOV3/MRC5 (activated) after 5 days of invasion One tailed ANOVA (* $p < 0.05$, ** $p < 0.005$, *** $p < 0.0005$). Error bars represent the mean ± standard deviation. Scale bars represent 100 um.

Multicellular Spheroid Invasion After 3 Days

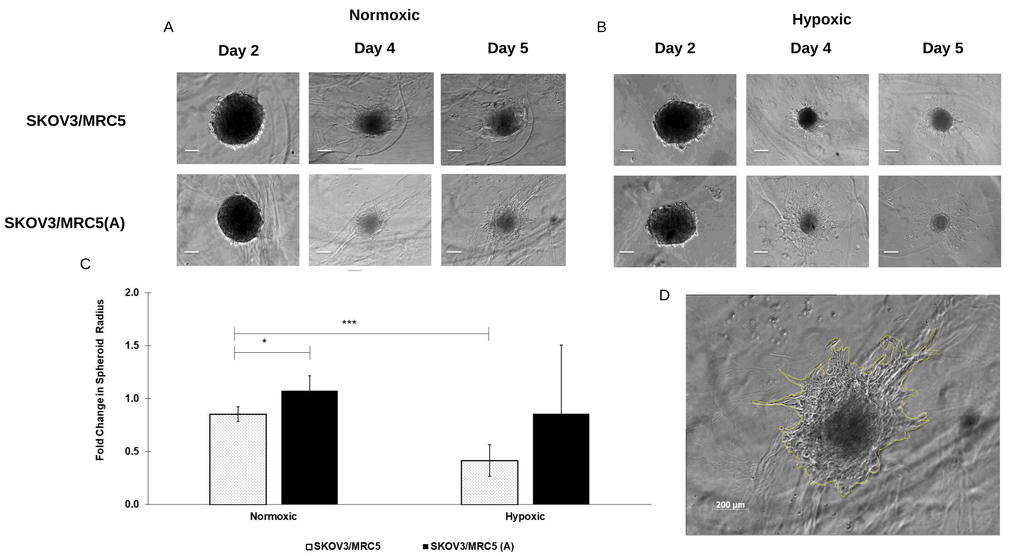


Figure 2. Spheroids composed of activated SKOV-3/MRC-5 cells demonstrate increased 3D invasion over 5 days relative to non-activated controls in both normoxic and hypoxic conditions. Representative phase images of (A) normoxic and (B) hypoxic combined SKOV-3/MRC-5 spheroid growth within PMX matrices. (C) Quantification of spheroid mid-cross-sectional area on days 2 and 5. (D) Multicellular Spheroid Invasion Analysis for SKOV3/MRC5 (activated) after 5 days of invasion. One tailed ANOVA (* $p < 0.05$, ** $p < 0.005$, *** $p < 0.0005$). Error bars = standard deviation. Scale bars represent 100 um.

Surface-Modified PLGA NP Transport

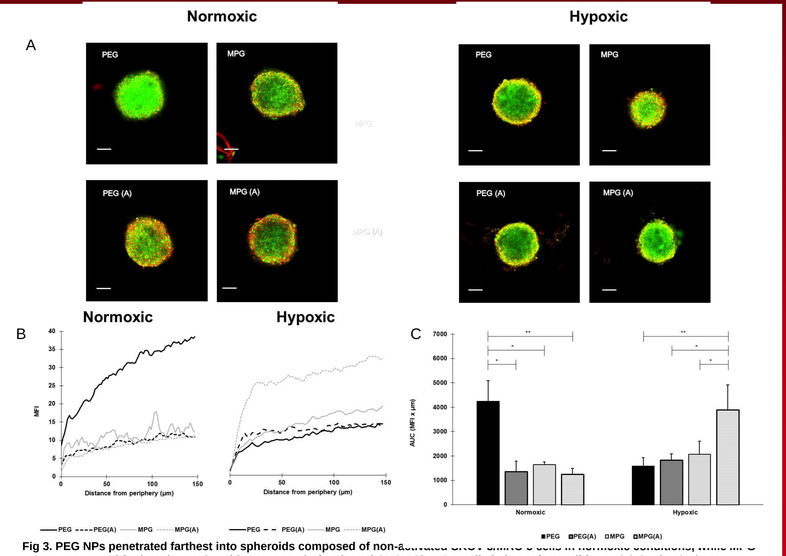


Fig 3. PEG NPs penetrated farthest into spheroids composed of non-activated SKOV-3/MRC-5 cells in normoxic conditions, while MPG NPs penetrated farthest into spheroids composed of activated SKOV-3/MRC-5 cells in hypoxic conditions. (A) Confocal microscopy images of nanoparticle transport in spheroids formed from non-activated and activated SKOV-3/MRC-5 cells grown in normoxic and hypoxic conditions. (B) Quantification of surface-modified PLGA NP transport into the tumor periphery as a function of mean fluorescence intensity (MFI). (C) Quantification of area under the MFI curves. One way ANOVA (* $p < 0.05$, ** $p < 0.005$, *** $p < 0.0005$). Error bars mean ± standard deviation.

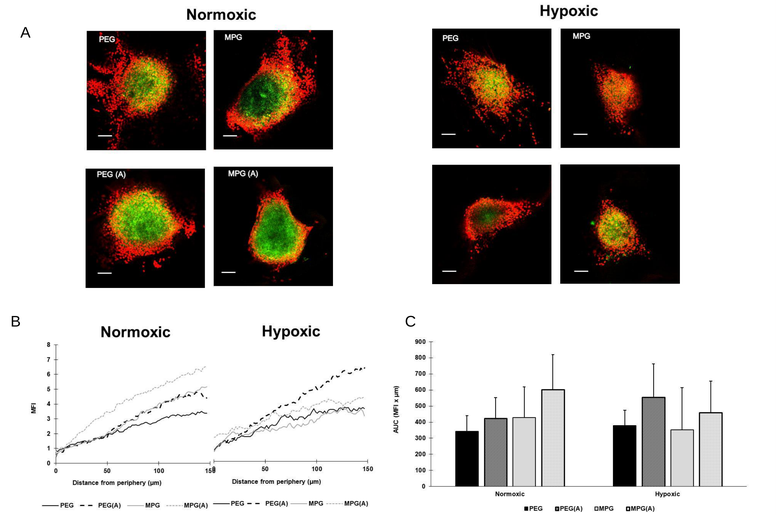


Fig 4. PEG and MPG-modified NPs demonstrate decreased tumor penetration in activated PMX spheroids after 24 hr incubation, relative to non-activated PMX sheroids in both normoxic and hypoxic conditions. (A) Confocal microscopy images of nanoparticle transport in PMX-encapsulated spheroids formed from non-actiated and activated SKOV-3/MRC-5 cells grown in Normoxic and hypoxic conditions. (B) Quantification of surface-modified NP transport into the tumor center as a function of mean fluorescence intensity (MFI). (C) Quantification of area under the normalized MFI curves. One way ANOVA; (* $p < 0.05$, ** $p < 0.005$, *** $p < 0.0005$). Error bars represent standard deviation.

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