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

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**Introduction**

In vivo models to study the metastatic progression of ovarian cancer have traditionally focused on monolayer and single-cell 3D spherical models. Recent studies have shown that cellular-extracellular matrix (ECM) interactions lead to the remodelling 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 cancers.

**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 versus non-activated stromal cells. Spheroids were treated with two surface-modified NP groups to assess differences in NP penetration compared to cellular components 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 smaller, yet more invasive after 5d, relative to spheroids without PMX or activated MRC5s. Hypoxia significantly reduced NP penetration for MPG-NPs compared to non-activated controls. Analysis of spheroid invasion and NP transport as a function of the 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 NP penetration compared to cellular components and hypoxic conditions.

**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 hypoxic conditions.

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