**Evaluation of nano drug carriers in 2D and 3D in vitro models**

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Nanoparticles have been used as promising drug carriers for cancer therapies. Multicellular tumor spheroids (MCTS) exhibit higher similarity to in vivo tumor tissues than monolayer cells, and therefore are considered as the first choice of tumor models to bridge the gap between 2D monolayer in vitro results and in vivo tests. More attempts have been made in using MCTS to investigate cellular responses to nano drug carriers. Interactions between nanoparticles and MCTS and the underlying mechanisms remain largely unknown. The multicellular tumor spheroids were prepared by culturing tumor cell lines with a hanging drop method or a liquid overlay method. Micelles were prepared by dialysis the block copolymers against water. Doxorubicin (Dox) encapsulated polymeric micelles, anti-metastatic drug (ruthenium-based, RAPTA-C) encapsulated micelles (RAPTA-C), and bovine serum albumin nanoparticles were prepared, respectively. The MCTS were treated with different drug carriers and the penetration, drug delivery, anti-tumor and anti-metastasis effects were investigated. The penetration of polymeric micelles depended more on transcellular transport than on diffusion through ECM between the cells. Stabilization via shell crosslinking improved the drug delivery efficiency of micelles in MCTS. RAPTA-C conjugated polymeric micelles showed improved anti-metastatic effects compared with free RAPTA-C for 2D cultured tumor cells. However, the anti-metastatic ability of RAPTA-C polymeric micelles exploited with MCTS showed contrary results to 2D cell culture models. BSA nanoparticle penetrated deeper into MCTS, while SPARC protein plays an important role in this penetration.

In addition to MCTS models, we have introduced tumor organoids into the evaluation of nano drug carriers. Organoids are miniaturized organ produced in vitro in three dimensions and have attracted much attention as a promising model for biomedical research. We have used a droplet-based microfluidic technology to generate high-throughput organoids, including human colon organoids and mouse mammary organoids. The organoids were embedded in various hydrogels as supporting matrices and showed high viability during the culture. These organoids models can be used for the screening of nanoparticles.

Native tumors are composed by heterogenous cells with different size, morphology, metabolism, phenotype and genotype et al. The change of cell size can change cell membrane tension and cytoskeleton arrangement. Thus, cell behaviors including adhesion, proliferation, differentiation and endocytosis can be influence by modifying cell geometry. Nanoparticles internalize into cells via endocytosis pathways. Therefore, it is hypothesized that the cellular uptake of nanoparticles will be influenced by cell geometry changes. The micro-patterns were used to control the topographical micro-environment surrounding cell in order to force the cells to alter their spreading and internal cytoskeleton. Both human cancer and normal cells, including pancreatic carcinoma AsPC-1 cells, lung carcinoma A549 cells, umbilical vein endothelial cells (HUVEC) and mesenchymal stromal cells (MSCs) were cultured on micro-patterned surfaces, formed by immobilizing photo-reactive polyvinyl alcohol on tissue culture plates through photolithography process. The uptake of polymeric micelles of the cells on micro-patterned surfaces were investigated and analyzed with laser scanning confocal microscopy.

**References**

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