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The gap between cell and animal models: nanoparticle drug-delivery development and characterization using microtissue models

“...a nanomaterial and 3D tissue model library becomes important ... in examining both therapeutic and diagnostic agents.”

Keywords: 3D tissue culture ■ drug delivery ■ high throughput ■ multicellular tumor spheroid ■ nanoparticles

Translating therapeutic agents from bench-top research to clinical application undergoes the incremental step from *in vitro* cell assays to *in vivo* observations. This large step overlooks many interactions that can be explored in an intermediate model of 3D tissue culture [1]. Improved *in vitro* models, in the form of 3D tissue culture, offer a greater insight into biological interactions and lead to improved design of new therapeutics. Many proof-of-principle nanotherapeutic experiments use intratumoral injection of agents to show possible efficacy of treatment. This form of investigation can be assayed in a similar environment to 3D cultures and has the benefits of avoiding the use of animal subjects. Over the last 60 years, 3D tissue culture and, more specifically, multicellular tumor spheroids have become an important part in understanding cell–cell and cell–matrix interactions. This research has culminated in understanding aspects of tissue formation, phenotypic expression, morphogenic properties, and cellular differentiation. While these 3D tissue properties are being rigorously understood, models that closely resemble *in vivo* conditions are being developed.

Nanoparticle systems offer multifunctionality for more specialized theranostic techniques [2]. They can be synthesized with controlled intrinsic properties and can be further functionalized with diagnostic, therapeutic or targeting abilities. Their ability to target selected biological characteristics is extremely beneficial for detection [3], greater efficient therapeutic delivery [4] and new routes of delivery [5]. Research in nanomedicine gives the promise of less systemically toxic therapy, early and enhanced detection, and tailored medicine. In this article, we will discuss the benefits and limitations of 3D tissue models for investigating nanoparticle drug delivery.

Current state

The microenvironment of tissues and cells in diseased states is markedly different than that of traditional *in vitro* cell culture. Cells adhered to a flat plastic surface enable the study of the direct cellular interaction with nanoparticles, while animal studies offer information on efficacy and toxicity. Neither model explores the interactions with tissue or tissue structures in a controlled manner. Underlying mechanisms of physical interactions with cells, the extracellular matrix (ECM) and concentration gradients are explored in 3D cultures. In addition to the physical interactions, cellular responses and phenotypic expressions change in response to the physical stimuli. For nanoparticle drug delivery, it is important to examine the microenvironment interactions of nanoparticles pertaining to diffusion, uptake and toxicity studies. 3D culture systems that are facile and highly controllable mostly come in the form of cells imbedded in a scaffold matrix [6] or grown in multicellular aggregates [7,8]. These systems have merits to explore nanoparticle design and also limitations to mimicking *in vivo* conditions.

Passive diffusion into 3D structures with cell–cell and cell–matrix interactions varies largely depending on tissue type and the properties of the agent. Initial interaction with the microenvironment depends on ligands or therapeutic agents on the outer surface of the nanoparticle. The ζ -potential and chemical properties of the nanoparticle surface determine the interactions with proteins, ECM and the varying cellular phenotypic expressions, such as receptors and cellular protrusions. In addition to passive diffusive rates, stability of particles can decrease after interactions with ECM and longer transit in interstitial paths, defined as tortuosity. Nanoparticle platforms with

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sizes ranging from molecular to viral are in the regime of 1–100 nm, and this size variation also creates differences in diffusion and surface contact dynamics. Scaffold matrix culture systems examine interactions with nanoparticles using controlled ratios of ECM, natural products, and biodegradable or degradable components with embedded cells. Multicellular aggregate-based systems can interrogate native ECM production and cellular interaction. For nanoparticle design, varying ligands or externally functionalized drugs alter passive kinetics and can be directly quantified. Diffusion of nanoparticles has been observed to be modulated by size, surface functionalization of collagenase on the surface of nanoparticles and collagenase treatment of multicellular tumor spheroids [9]. Measurement of diffusive ability can be accomplished by fixation and sectioning to measure penetration distance. Diffusion rates can be directly measured with fluorescent recovery after photobleaching and confocal microscopy [10]. Diffusion characteristics are the dominant factors in nanoparticle designs that rely on increased local concentrations due to the enhanced permeability and retention (EPR) effect [11,12]. The characterization of passive diffusion helps to understand basic microenvironment interactions and further optimize nanoparticle delivery.

Targeting ability is altered by phenotypic expression, 3D diffusion and matrix interaction, much like the diffusion processes. Greater resolution to specificity, cell interaction and ECM interaction can be examined in 3D tissue culture. With the customizable nanoscale delivery platforms, these molecule-sized agents can obtain both targeted diagnostics and targeted therapy. The addition of specific targeting agents alters the penetration depth and accumulation of nanoparticles. Targeting tumor vasculature has shown promise to inhibit the transport of oxygen and nutrients to cancerous cells as a mode of treatment. Endothelial cell-targeted iron-oxide nanoparticles have been found to associate specifically with newly formed endothelial cell coating on a multicellular tumor spheroid [7]. Mechanistic studies can optimize targeting and find underlying interactions that can maximize delivery of nanoparticles to desired targets. Targeting design and also further innovation into areas such as multi-stage delivery can benefit from understanding microenvironment interactions in 3D tissue culture.

Toxicity and therapeutic capacity of agents changes from traditional 2D cell culture and 3D tissue culture due to the diffusion gradients and

phenotypic expression in tissue. For example, hepatocytes have reduced functional ability to metabolize products and produce proteins in 2D cell culture, but regain this trait when grown in 3D [13]. Similarly, toxicity of agents can be altered by both phenotypic expression and diffusion of agents; an agent could be more or less toxic when changing *in vitro* and *in vivo* models. Cellular uptake is also altered as cell physical dimensions (i.e., cell–cell and cell–ECM contact) and phenotypic pathways for gene expression, uptake, metabolism and proliferation are modified [14]. Toxicity measurements in 3D tissue cultures have been found to more closely resemble results found with *in vivo* models [15].

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Theranostic application of nanoparticles uses the multifunctional ability to both deliver therapy and track progress of disease, allowing for interrogating and tailoring to certain disease states and individual patients [4]. Development of diagnostic-agent lifetime, in addition to efficacy of the therapeutic agent, can be monitored in 3D tissue culture. The degradation of the nanoparticle system, stability of diagnostics modes, and tracking of therapeutic location and efficiency can be defined in the microenvironment.

Advantages & limitations

Cost and ethical benefits of 3D tissue culture are from a reduced number of animal subjects. Greater mechanistic understanding of an agent's biological interactions improves design and provides greater insight going into *in vivo* studies. The economic cost to obtain and prepare animal models, and to have devoted animal care facilities, is an initial incentive to optimize nanoparticle therapy design before going into extensive studies that usually show markedly reduced effects. In addition, the ethical responsibility to minimize the use of animals in research makes optimizing therapeutic design a necessity.

3D tissue culture can replicate specific microenvironments and elucidate these interactions. Certain humoral responses can be introduced to incorporate some systemic responses, but it must be noted that some interactions can only be explored in an animal model. Protein and molecule interactions on the surface of nanoparticles

affect biological interactions. The surface of synthesized particles can be altered *in vivo* so that proteins coat the outer surface and this interaction must also be properly examined [16]. Applying appropriate proteins to cell culture media can mimic these interactions, but care must be taken as to not affect the 3D tissue model. Perfusion, flow and pressure are largely static for *in vitro* culture models. Beyond basic diffusion, EPR models, flow and pressure are implemented with bioreactors and, more recently, microfluidic systems. These systems have been used to examine changes in flow, mimic interstitial pressure and introduce environments such as those present in EPR that are found *in vivo* and affect delivery of agents [6]. While for complex cell–cell interactions, co-cultures can be used in 3D tissue culture to study controlled cellular interactions. The addition of other supporting cell types, such as fibroblasts, endothelial cells and astrocytes, gives greater similarity to the heterogeneous composition found in living tissues by adding both cellular interactions and signaling cues. However, co-cultures can become difficult to control and mimicking complex biological systems requires greater time for the development of these heterogeneous models. *Ex vivo* models offer examination of 3D tissue that is the exact biological environment of interest. Although these models still require animals, the intermediate phase of closely matching 3D tissue

culture systems to *in vivo* models would benefit from correlating results.

Conclusion

Once nanoplatforms have been well characterized in 3D tissue culture, combinations from optimal designs can be quickly implemented in brief 3D assays before continuing to an animal model. The idea of producing a nanomaterial and 3D tissue model library becomes important for sound scientific practice and efficient research [17]. Similar to the bank of animal models and high-throughput small-molecule discovery, resources for an array of 3D *in vitro* studies can be established for certain diseases in the context on nanoparticle drug delivery. These models can become important in examining both therapeutic and diagnostic agents.

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