An interdisciplinary computational/experimental approach to evaluate drug-loaded gold nanoparticle tumor cytotoxicity

Aim: Clinical translation of cancer nanotherapy has largely failed due to the infeasibility of optimizing the complex interaction of nano/drug/tumor/patient parameters. We develop an interdisciplinary approach modeling diffusive transport of drug-loaded gold nanoparticles in heterogeneously-vascularized tumors. **Materials & methods:** Evaluated lung cancer cytotoxicity to paclitaxel/cisplatin using novel two-layer (hexadecanethiol/phosphatidylcholine) and three-layer (with high-density-lipoprotein) nanoparticles. Computer simulations calibrated to *in-vitro* data simulated nanotherapy of heterogeneously-vascularized tumors. **Results:** Evaluation of free-drug cytotoxicity between monolayer/spheroid cultures demonstrates a substantial differential, with increased resistance conferred by diffusive transport. Nanoparticles had significantly higher efficacy than free-drug. Simulations of nanotherapy demonstrate 9.5% (cisplatin) and 41.3% (paclitaxel) tumor radius decrease. **Conclusion:** Interdisciplinary approach evaluating gold nanoparticle cytotoxicity and diffusive transport may provide insight into cancer nanotherapy.

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The complex spatial-temporal interaction between tissue, nanoparticle and drug parameters makes it difficult to evaluate nanotherapy performance solely through experimental effort. To this end, mathematical modeling and computational simulation have been applied to help elucidate the effects of transport and diffusion of nanotherapeutics in the tumor microenvironment [1–13]. In particular, the dynamic interaction between generic nanoparticle vascular extravasation, uptake and distribution with heterogeneous tumor interstitial, vascular and lymphatic conditions was recently studied [7].

Angiogenesis driven by uncoordinated stimuli by tumor and stromal cells leads to inefficient oxygen/nutrient delivery within tumors. Transport limitations coupled with increased interstitial fluid pressure (IFP)

within solid tumors [14] disrupt tissue homeostasis and generate hypoxic and necrotic tissue [15], and are also obstacles for systemically administered nanoparticles and chemotherapeutics. While circulating nanoparticles can preferentially exit fenestrated tumor capillaries [16] via the so-called enhanced permeability and retention effect (EPR), their diffusion is hindered beyond 3–5 cell diameters from point of extravasation [17,18]. Treatment failure results when chemotherapeutic concentration does not reach cytotoxic levels, or due to cycle-dependent drugs failing to induce death in quiescent cells in hypovascularized regions [14].

While 2D monolayer cell cultures are widely utilized for cytotoxicity evaluation, they lack key physiological and spatial features of the *in vivo* condition. For example,

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it is well known that drug inhibitory concentration to achieve 50% tumor mass regression (IC_{50}) typically increases substantially between 2D and 3D cell cultures based solely on the 3D effects, which represent physiological resistance. Although not the same as *in vivo* tissue, 3D cell cultures recreate an environment more closely resembling solid tumors, including extracellular matrix (ECM), diffusion gradients and transport limitations [19–23], and can therefore provide additional insight concerning nanoparticle and drug transport and effectiveness. Newer methodologies such as hanging drop arrays enable performing high-throughput screening of compounds with 3D cell cultures [24].

Nanotherapy can be effective in treating solid tumors, such as lung cancer [25], due to passive targeting leveraging the EPR effect [26], active targeting to avoid systemic distribution [27] and avoidance of intrinsic cellular resistance via distinct endocytosis mechanisms (e.g., [28]). Additionally, nanoparticle surfaces can be functionalized with polymers, drugs, or other compounds to enhance targeting capabilities, bioavailability and local cytotoxicity [29]. Cisplatin and paclitaxel are two commonly utilized chemotherapeutics for the treatment of non-small-cell lung cancer (NSCLC) [30], inducing DNA adducts and stabilizing microtubules, respectively, thus impairing cell proliferation and inducing apoptosis [31,32]. Studies *in vitro* have shown the efficacy of cisplatin- and paclitaxel-loaded nanoparticles for treatment of lung cancer [33–35].

Recently, gold nanoparticles were modified with hexadecanethiol (TL) and phosphatidylcholine (PC) to form a two-layer system, or with TL, PC and high-density lipoprotein (HDL) to form a three-layer system [36]. It was shown that such layered gold nanoparticles exhibit improved penetration in comparison to PEGylated nanoparticles in 3D cell cultures representing avascular tissue [36], and that they are uptaken into solid tumor tissue *in vivo* [37]. Both two- and three-layer gold nanoparticles could be loaded with cisplatin or paclitaxel, eliciting unique drug release kinetics [38]. Drug release experiments over a period of 96 h showed that paclitaxel followed a more sustained release in comparison to cisplatin, which experienced an initial release burst. In this study, we examine the efficacy of such cisplatin or paclitaxel layered gold nanoparticles in 2D and 3D cell cultures to determine cytotoxicity with a panel of NSCLC cell lines. We then employ the experimental data to further assess nanotherapy performance through computational simulation of heterogeneously vascularized tumor tissue and the associated diffusive transport barrier. By providing a platform in which complex interactions between drug, nano

and tumor parameters can be systematically analyzed with input from experiments, this interdisciplinary approach may help to bridge the gap from *in vitro* to *in vivo* performance of nanotherapy and, in particular, enable a more comprehensive evaluation performance of gold nanoparticles targeted to lung cancer lesions.

Materials & methods

Nanoparticle synthesis

Citrate-stabilized gold nanoparticles were synthesized using the method in which chloroauric acid is reduced by trisodium citrate [39]. In this process, 2.2–2.4 ml 1% weight/volume (wt/v) trisodium citrate (Fisher Scientific, MA, USA) is added to 200 ml of boiling 0.01% wt/v $HAuCl_4$ (Alfa Aesar, MA, USA), and the solution is allowed to continue boiling for 10 min. The solution is allowed to cool at room temperature before concentrating it to 20 ml at 20 OD using a rotovapor (Buchi Rotovapor System, BÜCHI Labortechnik AG, Flawil, Switzerland).

Nanoparticle functionalization

The first layer applied was 1-hexadecanethiol (TL; Sigma-Aldrich). The TL compound has a stronger binding affinity for the surface of the gold nanoparticles, thus displacing the citrate molecules. Previous work has shown that thiol compounds can displace surface-bound citrate from gold nanoparticles due to the strong binding affinity between thiol and gold in comparison to the electrostatic binding with citrate [40–42]; a comprehensive review concerning the covalent interaction between sulfur and gold can be found in [42]. This process creates a hydrophobic nanoparticle, with the hydrocarbon chains of the thiol compound pointing outward from the gold core. While stirring, 20 ml pure ethanol was placed in a beaker with 60 μ l TL dissolved in ethanol added secondly. While undergoing sonication, the TL solution was then slowly added to the nanoparticle solution over 10 min to reach a molar ratio between thiol and gold nanoparticles of 2500 : 1. The sample was then sonicated for 2 h, followed by 12 h on an orbital rocker (Boekel Scientific, PA, USA). The sample was spun down (5000 $\times$ g for 15 min), and the pellet was washed twice with ethanol and sonicated before resuspension in 9 ml chloroform. L- α -Phosphatidylcholine (PC; Sigma-Aldrich) was the second functionalization; it was solubilized in chloroform (2 mg/ml; Sigma-Aldrich), and 100 μ l (molar ratio 2000 PC: 1 nanoparticle) was added to the particles coated with TL and allowed to set for 12 h on an orbital rocker. The solutions were transferred to glass tubes and the chloroform evaporated at ambient temperature. This process completed the two-layer nanoparticles. Three-layered versions were created by

optimizing the ratio of HDL (Lee Biosolutions, MO, USA) to particle optical density (1 mg HDL per 20 OD nanoparticle), and allowed to react overnight after 2 h sonication.

Nanoparticle characterization

Nanoparticle identity was verified as follows: Extinction spectra were obtained using the Varian Cary 50 Bio Ultraviolet-Visible (UV-Vis) Spectrometer (McKinley Scientific; molar extinction coefficient: 3.07×10^{10} molar ext. $M^{-1} \text{ cm}^{-1}$); zeta potential measurements were obtained using the ZetaSizer Nanoseries ZS90 (Malvern Instruments, Worcestershire, UK); DLS (dynamic light scattering) was used to determine hydrodynamic size (intensity distribution) in solution (1 ml 2 OD in PBS); shape and size were further determined (1 ml 2 OD in PBS) using a Zeiss Supra 35VP (Carl Zeiss, Oberkochen, Germany) scanning electron microscope (SEM); presence of lipids on the particle cores was confirmed using a Fourier transform infrared (FTIR) instrument (Perkin Elmer Spectrum BX; Perkin Elmer, MA, USA).

Nanoparticle drug loading

The amount of drug loaded was chosen to achieve a molar concentration upon release typical for cell culture experiments with these drugs. Paclitaxel (Cayman Chemicals, MI, USA) was loaded after the sample completed 12 h on the orbital rocker (see section 'Nanoparticle functionalization'). After nanoparticle resuspension in 9 ml chloroform at 5 OD, an additional 1 ml of chloroform containing 5 mg paclitaxel was added to the solution. Nanoparticles were sonicated for 2 h before placing on an orbital rocker for 6 h. The solution was then further modified to add the second layer of PC as detailed above. Paclitaxel was thus loaded into the hydrophobic region created between the TL and PC layer. Cisplatin (Sigma-Aldrich) was loaded based upon the nanoparticle layering. For the two-layer system, the drug was added after the addition of PC. This was done by transferring the solutions to glass tubes and the chloroform evaporated at ambient temperature. Next, the nanoparticles were resuspended in 10 ml ultrapure H_2O (Purelab Ultra, Elga Labwater, UK) containing 7.5 mg cisplatin to accomplish a molar ratio of 350 cisplatin molecules per nanoparticle. For the three-layer system, cisplatin was added after the addition of HDL by synthesizing the particles as described above; 7.5 mg cisplatin was added and allowed to react for 2 h. Excess chemotherapeutic was removed by centrifuging ($5000 \times g$ for 25 min), removing the supernatant, and resuspending the particles in the corresponding solvent (1 ml). Washing was performed twice with ddH_2O .

Nanoparticle drug release

Evaluation of drug release from nanoparticles was previously performed [38]. In summary, drug-loaded nanoparticles were placed into dialysis tubes and submerged into beakers containing 500 ml 1X PBS (Hyclone [1X] with 0.0067M (PO_4) without Magnesium or Calcium) at pH 7.4. We chose to evaluate this release in saline solution [43–46] as the simplest system from which parameters could also possibly be extracted for the mathematical modeling. The dialysis tubing cellulose membrane had an average flat width of 25 mm and 12,000 MW cutoff (Fischer Scientific, MA, USA) and was clipped at both ends to particle leakage. The beaker was sonicated continually using a magnetic stirrer at 37°C and covered with Parafilm to minimize evaporation. At established time intervals, 3 ml samples of PBS containing drug were removed and replaced with fresh buffer to ensure a constant volume. Drug concentration of each sample was analyzed using high performance liquid chromatography (HPLC). Cumulative drug release was determined using the following equation (Equation 1):

$$\text{Cumulative drug release (\%)} = \frac{(\text{Drug})_t}{(\text{Drug})_{\text{total}}} \times 100$$

where $(\text{Drug})_t$ is the concentration of drug in the sample at time t and $(\text{Drug})_{\text{total}}$ is the total amount of drug loaded onto the nanoparticles [38].

Drug incorporation efficiency

Drug incorporation efficiency (IE; %) was expressed as the percentage of drug in the nanoparticles with respect to the drug amount initially used for synthesizing the nanoparticles [45]. The calculation was determined using HPLC as described in [38] in conjunction with the following equation (Equation 2):

$$\text{IE (\%)} = \frac{\text{Amount of drug in nanoparticle (mg)}}{\text{Initial amount of drug (mg)}} \times 100$$

Cell culture

A panel of human NSCLC cell lines, A-549, PC-9, NCI-H358, were maintained in RPMI-1640 medium (Cellgro, Corning Inc.) supplemented with 10% fetal bovine serum (Cellgro, Corning Inc.) and 1% penicillin-streptomycin-glutamine solution (Cellgro, Corning Inc.) in standard culture conditions.

Cytotoxicity of free drug in 2D cell culture

Cells were seeded into 24-well plates at a density of 2×10^4 cells per well, and incubated in standard conditions for 24 h. Media was removed from wells and replaced with 1 ml fresh RPMI-1640 media containing varying

concentrations of drug for 48 h. For cisplatin, cells were exposed to the following concentrations for 48 h: 1024, 256, 64, 16, 4, 1, 0.25, 0.0625 μ M. For paclitaxel, cells were exposed to the following concentrations for 48 h: 1024, 256, 64, 16, 4, 1, 0.25, 0.0625 nM. After incubation, media was removed and cells were washed with 1X PBS. Cells were detached using 0.05% trypsin and counted using trypan blue exclusion (Cellgro, Corning Inc.). Each experiment was performed n = 3.

Cytotoxicity of free drug in 3D cell culture

All cells were grown to 80% confluence before harvesting. Cells were seeded into 24-well ultralow cluster plates (Costar, Corning Inc.) at 1×10^5 cells per well, and lightly shaken for approximately 10 min to promote aggregation. Cells were then placed in standard culture conditions for 5 days for spheroid acclimation. After spheroid formation, the media was carefully removed. One milliliter of media containing either cis-

platin or paclitaxel at varying concentrations as in the monolayer experiments was added to the corresponding wells. Negative controls (without drug) were seeded and incubated under the same conditions. Spheroids treated with drugs were incubated for 48 h, at which time the drug-containing media was removed and the spheroids disaggregated with trypsin (0.05%). Cells were counted using trypan blue exclusion (Cellgro, Corning Inc.). Each experiment was performed n = 3.

Cytotoxicity of drug-loaded nanoparticles in 3D cell culture

Spheroids of A-549, PC-9, or NCI-H358 cells were created as detailed above. Negative controls (without nanoparticles) were seeded and incubated under the same conditions. The spheroids were exposed to varying concentrations of drug-loaded nanoparticles calculated by considering two parameters: the loading efficiency from HPLC data showing the exact con-

Table 1. Computational model main parameters and associated values.		
Parameter	Value	Ref.
Tumor proliferation rate	1 day ⁻¹	Measured
Tumor necrosis threshold	0.5700	[6]
Tumor hypoxic threshold	0.5750	[6]
Oxygen diffusivity	1 [†]	[47]
Oxygen transfer rate from vasculature	5 [†]	[47]
Oxygen uptake rate by proliferating tumor cells	1.5 [†]	[47]
Oxygen uptake rate by hypoxic tumor cells	1.3 [†]	[47]
Oxygen uptake rate by tumor microenvironment	0.12 [†]	[47]
Oxygen decay rate	0.35 [†]	[47]
NP extravasation from angiogenic vs normal vessels	10	Estimated
NP diffusivity	0.3 [†]	[36]
NP decay	12-h half-life	Estimated
CDDP diffusivity	0.6 [†]	Estimated
CDDP drug effect	40	Calibrated to experimental data
CDDP decay rate	0.5-h half-life	[48]
CDDP release from NP		Measured in [38]
CDDP <i>in vitro</i> IC ₅₀ (48 h) for A549 cells (spheroid)		Measured (Table 4)
PTX diffusivity	0.6 [†]	Estimated
PTX drug effect	270	Calibrated to experimental data
PTX decay rate	5.8-h half-life (for 6- to 24-h infusion)	[25]
PTX release from NP		Measured in [38]
PTX <i>in vitro</i> IC ₅₀ (48 h) for A549 cells (spheroid)		Measured (Table 4)
All other model parameters are as in [47]. [†] Value is rescaled by the square of the simulation system characteristic length (1 cm) and divided by the system characteristic time (1 s) multiplied by the oxygen diffusivity [49] (1×10^{-6} cm ² s ⁻¹). CDDP: Cisplatin; NP: Nanoparticle; PTX: Paclitaxel.		

centration of drug encapsulated onto the nanoparticles [38]; and the percent of drug released over the 48-h period. Each case was performed $n = 3$.

Computational modeling of tumor response to drug loaded nanoparticles

We build upon the model described in [6,7,47] to simulate the transport of nanoparticles and the release of drug in heterogeneously vascularized tumor tissue. The model represents viable, hypoxic and necrotic tumor tissue in a 2D Cartesian coordinate system; the initial condition is a small tumor (~50- μm diameter) in the middle of a preexisting vascular capillary grid. Conservation of mass equations describe tissue growth (proliferation as a function of cells cycling) and tissue death via necrosis as a function of low oxygen (hypoxia). The equations are combined with diffusion of small molecules (nanoparticles, drug, oxygen and cell nutrients) to a reaction-diffusion equation. The rate constants for proliferation and death depend on the availability of oxygen, nutrients and drug, and are thus spatiotemporally heterogeneous. The model main parameters are summarized in **Table 1**.

Tumor growth

The tumor component is based on [50]. Briefly, the tumor tissue is denoted by Ω and its boundary by Σ . The tumor tissue may have a proliferating region Ω_p (typically in the order of 100–200 μm) in which cells have sufficient oxygen and nutrients, a hypoxic region Ω_H in which oxygen and nutrients are sufficient for survival but not for proliferation, and a necrotic region Ω_N in which oxygen and nutrients are insufficient for survival. The tumor growth velocity (non-dimensionalized) is implemented via a generalized Darcy's law [50] (**Equation 3**):

$$\mathbf{v}_c = -\mu \nabla P + \chi_E \nabla E$$

where μ is cell-mobility representing the net effects of cell–cell and cell–matrix adhesion, P is oncotic pressure, χ_E is haptotaxis and E is ECM density. Definitions for χ_E and E are in [50]. By assuming that the cell density is constant in the proliferating region, the overall tumor growth can be associated with the rate of volume change (**Equation 4**):

$$\nabla \cdot \mathbf{v}_c = \lambda_p$$

where λ_p is the nondimensional net proliferation rate (see below).

Angiogenesis

The angiogenesis component simulates the model by [51] and is based on [47,52], representing blood flow,

vascular leakage and vascular network remodeling due to wall shear stress and mechanical stresses imposed by the tumor tissue. The angiogenesis model is described in detail in [47,52]. As the tumor grows within the vascular environment, the tissue experiences heterogeneous access to elements diffusing from the vasculature, which may depend on tissue pressure as well as distance from the nearest vascular source.

Transport of oxygen & nutrients

The transport of oxygen and nutrients σ through tumor tissue is simulated from the location of extravasation from the vasculature. Oxygen and nutrients are supplied from the neo- and preexisting vasculature with extravasation rates $\lambda_{ev}^\sigma = \lambda_{neo}^\sigma$ and $\lambda_{ev}^\sigma = \lambda_{pre}^\sigma$, respectively, diffuse with a coefficient D_σ , are taken up by both normal cells (with a rate λ_{tumor}^σ) and tumor cells (λ_{tissue}^σ in the proliferating region and q_s in the hypoxic region) and decay (with a rate λ_N^σ) in the necrotic region. Assuming steady-state conditions, the formulation is [6,7,50] (**Equations 5 & 6**):

$$0 = \nabla \cdot (D_\sigma \nabla \sigma) + \lambda_{ev}^\sigma(\mathbf{x}, t, \mathbf{1}_{vessel}, p_i, \sigma, h) - \lambda^\sigma(\sigma) \sigma$$

$$\lambda_p^\sigma = \begin{cases} \lambda_{tissue}^\sigma & \text{outside } \Omega \\ \lambda_{tumor}^\sigma & \text{in } \Omega_p \\ q_\sigma(\sigma) & \text{in } \Omega_H \\ \lambda_N^\sigma & \text{in } \Omega_N \end{cases}$$

where $\mathbf{x}$ is position in space, t is time, $\mathbf{1}_{vessel}$ is the characteristic function for vasculature (equals **1** at vessel locations and 0 otherwise), p_i is the interstitial pressure and h is the hematocrit in the vascular network which is related to oxygen extravasation (following [50]). The extravasation is modulated by the extravascular interstitial pressure p_i scaled by the effective pressure p_e , with k_{p_i} being the weight of the convective transport component of small molecules [6] (**Equation 7**):

$$\lambda_{ev}^\sigma = \bar{\lambda}_{ev}^\sigma \mathbf{1}_{vessel}(\mathbf{x}, t) \left(\frac{h}{\bar{H}_D} - \bar{h}_{min} \right) + \left(1 - k_{p_i} \frac{p_i}{p_e} \right) (1 - \sigma)$$

Constants $\bar{H}_D$ and $\bar{h}_{min}$ represent normal and minimum blood hematocrit required for oxygen extravasation, respectively, and $\bar{\lambda}_{ev}^\sigma$ is the constant transfer rate from both preexisting and tumor-induced vessels. The oxygen parameters are calibrated so that the growth rate as an avascular spheroid matches the experimentally observed rate.

Transport of nanoparticles

The transport of gold nanoparticles s through tumor tissue, like oxygen, is also simulated from the location

of extravasation from the vasculature. Nanoparticles are taken up both by both normal cells and tumor cells with a rate $\bar{\lambda}_{\text{uptake}}^s$. The formulation (Equation 8) is:

$$\frac{\partial s}{\partial t} = \nabla \cdot (D_s \nabla s) + \lambda_{\text{ev}}^s(\mathbf{x}, t, \mathbf{1}_{\text{vessel}}, p, s) - \bar{\lambda}_{\text{uptake}}^s s$$

Assuming a constant transfer rate $\bar{\lambda}_{\text{ev}}^s$ from both preexisting and tumor-induced vessels (with extravasation assumed ten times higher from neo- compared with preexisting vasculature due to the EPR effect), the nanoparticle extravasation is (Equation 9):

$$\lambda_{\text{ev}}^s = \bar{\lambda}_{\text{ev}}^s \mathbf{1}_{\text{vessel}}(\mathbf{x}, t) \left(1 - k_{pi} \frac{p_i}{p_e} \right) \left(\frac{C_t^s}{\bar{C}^s} - s \right)$$

where the diffusion in tumor tissue is assumed to be modulated by the interstitial pressure [6]. The nanoparticle concentration in the vasculature is initially $\bar{C}^s$, with the extravasation assumed to be of the form $C_t^s = \bar{C}^s e^{-\alpha t}$. This assumes first-order kinetics, for which the extravasation is mainly concentration dependent. The decay α is measured from previous *in vivo* experiments, for which the nanoparticle concentration is estimated to have a half-life of 12 h [37]. Although nanoparticle diffusivity can be dependent on charge [53] in addition to size, for this study we calibrate the diffusivity to the combination of these properties as measured from the *in vitro* data [36].

Transport of drug

The drug G is released at the location of extravasated nanoparticles and diffuses through the tissue with a coefficient D_G . The uptake by tumor and normal cells and the wash-out from the interstitial space are included as a combined effect in the rate $\bar{\lambda}_{\text{decay}}^G$, which reflects the drug half-life (assumed for simplicity to be similar as in plasma) (Equation 10):

$$\frac{\partial G}{\partial t} = \nabla \cdot (D_G \nabla G) + \lambda_{\text{release}}^G(t, s) - \bar{\lambda}_{\text{decay}}^G G$$

The drug release $\lambda_{\text{release}}^G$ from the nanoparticles (Equation 11) is:

$$\lambda_{\text{release}}^G = s C_t^G$$

The release C_t^G in time is fitted to follow the profile experimentally observed for cisplatin and paclitaxel obtained in [38]. Locally, the drug release rate thus combines the effect of the nanoparticle concentration and the drug release profile.

The boundary condition for all the diffusion equations is $\frac{\partial B}{\partial n} = 0$ (zero Neumann condition), where B is the diffusible substance.

Drug effect on the tumor

The drug only affects proliferating cells in order to simulate the cell-cycle dependent effects of paclitaxel and cisplatin. Accordingly, the drug effect is included into the proliferation term λ_p , where $\bar{\lambda}_{\text{effect}}$ is the rate of drug-induced cell death [7] (Equation 12):

$$\lambda_p = \begin{cases} 0 & \text{outside } \Omega \\ \lambda_M \sigma(1 - \bar{\lambda}_{\text{effect}} G) - \lambda_A & \text{in } \Omega_P \\ 0 & \text{in } \Omega_H \\ -G_N & \text{in } \Omega_N \end{cases}$$

where λ_M is the mitosis rate, λ_A is the apoptosis rate and G_N is the nondimensional rate of volume loss in the necrotic regions assuming that cellular debris is constantly degraded and the resulting fluid is removed [7]. The model assumes that the cellular proliferation and apoptosis rates are comparable prior and after therapy. For simplicity, cell death is assumed to be an instantaneous process.

Tumor response

Using the NSCLC tumor spheroid data obtained experimentally, we first calibrated the model parameters for tumor growth, oxygen and drug effect $\bar{\lambda}_{\text{effect}}$ to obtain a simulated 50% reduction in tumor size (the IC_{50}) for an avascular lesion. We then used this drug effect to simulate the therapy on a vascularized lesion. For consistency, and to focus on the effects of heterogeneous vascularization, we simulated a bolus with the same concentration of nanoparticles *in vivo* as needed *in vitro* to achieve the IC_{50} . Simulations were then run to evaluate the nanoparticle transport, drug distribution and corresponding tumor regression over the course of 250 h. A control (untreated) case was also simulated in order to compare the tumor responses.

Numerical implementation

Details of the numerical implementation are described in [47] and references therein. Briefly, to solve for the tumor oncotic pressure and the diffusible elements (oxygen and nutrients, nanoparticles, drug, as well as tumor angiogenic factors and matrix-degrading enzymes included in the angiogenesis model), the corresponding equations (Equations 3, 5, 8 & 10) are discretized in space using centered finite difference approximations and the backward Euler time-stepping algorithm. The discretized equations are solved using a nonlinear adaptive Gauss-Seidel iterative method [54,55]. A ghost cell method is used to implement the tumor pressure jump condition at the tumor-host interface [55]. This system of equations is itera-

tively solved together for the tumor oncotic pressure and the concentration of diffusible elements (as well as interstitial fluid pressure and blood vessel pressure in the angiogenesis component [47]) to steady state at each timestep, in other words, the equations are discretized implicitly in time. The level set method is used to update the tumor viable/necrotic region and the interfaces between the tumor-host and tumor viable-necrotic tissue regions. In the angiogenesis component, the vessel radii are discretized explicitly, and the hematocrit level is calculated every few iterations. This hematocrit is modulated by the blood flow and influences the extravasation of diffusible elements (oxygen and nanoparticles) from the vasculature. Further details regarding the numerical implementation are in [52] and references therein.

Results

Nanoparticle characterization

Diffusivity of two- and three-layer gold nanoparticles in 3D cell cultures was previously shown to be superior in comparison to PEGylated nanoparticles [36]. When the layered nanoparticles were loaded with cisplatin or paclitaxel, they displayed unique release kinetics, with cisplatin having an initial burst of drug release followed by a steady release and paclitaxel eliciting a sustained release curve over a period of 14 days [38]. Here, we examine the cytotoxicity of these two- and three layer drug-loaded nanoparticles in 2D and 3D cell cultures. The two-layer nanoparticles were synthesized by first adding TL and secondly PC, which created a hydrophobic region capable for loading water-insoluble compounds (Figure 1). The three-layer nanoparticle contained an additional modification of HDL on the outer layer (Figure 1).

Ultraviolet-visible (UV-Vis) spectroscopy was performed to determine maximum absorbance values. Two- and three-layer nanoparticles displayed similar spectra with a shift of ~ 4 nm, with the two-layer nanoparticles having a maximum absorbance peak of 539 nm and the three-layer nanoparticles having a peak at 535 nm (Table 2 & Supplementary Figure 1). The difference in the absorbance is not considered significant based on the heterogeneity in the nanoparticle size. Using scanning electron microscopy (SEM), sizing of two- and three-layer gold nanoparticles was shown to be 55 ± 8 nm and 62 ± 9 nm, respectively (Supplementary Figure 2). Characterization via zeta potential analysis showed that three-layer nanoparticles displayed a relatively neutral surface charge of -6 mV, while two-layer nanoparticles were more anionic at -21 mV (Table 2). The measurements were consistent with previous work [36–38]. Dynamic light scattering (DLS) provided further information regard-

ing size based upon Brownian motion when in solution [56]. The hydrodynamic diameter (z-average of the intensity distribution) for the two- and three-layer gold nanoparticles was 72 ± 10 nm and 80 ± 12 nm, respectively (Table 2). Previous studies have shown that nanoparticles sized approximately 50–60 nm are capable of recruiting enough receptors to trigger cell internalization [57]. In addition, gold nanoparticles sized ~ 60 nm have been shown to display high uptake in orthotopic tumors *in vivo* [58].

Fourier transform infrared (FTIR) spectroscopy confirmed the presence of PC and HDL onto the surface of nanoparticles. The spectra of pure PC and HDL were used for comparison [59,60]. Key peaks included a $([-CH_2]_n)$ rocking vibration at 720 cm^{-1} , PO_4^{3-} group vibrations at 900 cm^{-1} , a C–O–C stretch at 1100 cm^{-1} , and an asymmetric and symmetric $-CH_2$ at 2880 cm^{-1} and $-CH_3$ at 2950 cm^{-1} stretch and vibration (Supplementary Figure 3). Additional peaks were associated with the other chemicals used to synthesize the particles. HDL-coated nanoparticles exhibited several similar peaks to PC-coated nanoparticles, which were expected due to the layering process. The asymmetric and symmetric $-CH_2$ (2880 cm^{-1}) and $-CH_3$ (2950 cm^{-1}) stretch and vibration were still present from the PC-coating, yet several signature peaks of HDL also became visible. These peaks included a C=O from the lipid ester between $1700\text{--}1800\text{ cm}^{-1}$, an amide bond stretch between $1500\text{--}1700\text{ cm}^{-1}$, and a phospholipid $P=O_2$ stretch at 1250 cm^{-1} . These results were also consistent with previous work [36–38].

Nanoparticle drug release kinetics

Release of paclitaxel and cisplatin from the two- and three-layer nanoparticles was dependent upon the type of drug loaded and the number of layers. Paclitaxel was previously shown to release drug slower than cisplatin, which exhibited an initial burst (Table 3). The cumulative percent of paclitaxel released during the first 3 h was $1.42 \pm 0.12\%$ and $2.39 \pm 0.27\%$ for the two- and three-layer nanoparticles, respectively. For the cisplatin-loaded system, the two-layer nanoparticles released $33.8 \pm 2.6\%$ of drug within the first 3 h, while the three-layer system released almost twice this amount ($59.1 \pm 2.0\%$). At the end of 96 h, none of the nanoparticle systems had released the total amount of drug loaded. Results from a longer release experiment lasting 14 days have been published [38]. Three-layer nanoparticles loaded with cisplatin released the most drug ($78.9 \pm 2.1\%$) at the end of 96 h compared with the two-layer system ($49.7 \pm 0.70\%$). In the case of paclitaxel, the three-layer system released $5\times$ more drug as compared with the two-layer system ($55.7 \pm 4.7\%$ vs $11.9 \pm 0.90\%$).

Figure 1. Synthesis of two- and three-layered gold nanoparticles. Nanoparticles were functionalized by a layering process as depicted in the figure. (A) Before functionalization, gold nanoparticles were synthesized using method resulting in a citrate-stabilized nanoparticle. (B) Hexadecanethiol was added to the nanoparticle solution, which displaced the citrate molecules and formed water-insoluble nanoparticles. (C) Phosphatidylcholine was added to create a region suitable for loading of hydrophobic drugs, binding tail-to-tail with the thiol layer, thus creating a two-layer system. (D) A three-layer system was created by adding high-density lipoprotein to the phosphatidylcholine-coated system.

Since cytotoxicity measurements were performed at 48 h for cell culture experiments, the amount of drug release at 48 h from each of the nanoparticle systems was noted (Table 3) as measured previously [38]. The two-layer nanoparticles released $46.9 \pm 1.5\%$ of cisplatin and $8.20 \pm 0.09\%$ of paclitaxel at 48 h in PBS. The three-layer nanoparticles released $76.7 \pm 1.84\%$ of cisplatin and $23.1 \pm 4.2\%$ of paclitaxel at 48 h. The loading efficiency was determined by subtracting the amount of unbound drug from the amount of drug utilized to synthesize the nanoparticles. The loading efficiency of two-layer nanoparticles was $68.4 \pm 7.1\%$ of cisplatin and $99.1 \pm 0.7\%$ of paclitaxel. For the three-layer system, the loading efficiency was $78.9 \pm 0.7\%$ of cisplatin and $99.4 \pm 0.4\%$ of paclitaxel.

Nanoparticle cytotoxicity *in vitro*

The cytotoxicity of free cisplatin and paclitaxel was measured in 2D and 3D cultures of A549, H358, and PC9 NSCLC cells at 48 h. As expected, 2D cell cultures generally experienced higher levels of cytotoxicity at lower concentrations of drug in comparison to 3D cell cultures, as cells in monolayer are optimally exposed to drug that is unhindered by diffusive transport. For each cell line, the 3D cell cultures consistently showed higher cell viability at higher drug concentrations. Nanoparticle effectiveness compared with free drug treatment was evaluated by exposing 3D cell cultures to drug-loaded nanoparticles for the same time period. The drug concentration required by 48 h to achieve 50% inhibition (IC_{50}) with the 3D cell cultures for free as well as nanoparticle-administered drug

is shown in **Table 4**. For the nanoparticles, this concentration is reached at the 48-h timepoint, as the drug is incrementally being released during the time period of the experiment, whereas for the free drug a constant concentration is maintained. For each cell line and drug type, the nanoparticles were more cytotoxic compared with free drug.

To further compare free drug to nanoparticle cytotoxicity, we chose to plot cell viability as a function of area-under-the-curve (AUC; drug concentration for 48 h) (**Figure 2**), as the nanoparticles provided a variable drug exposure that is dependent on the release characteristics, while exposure to free drug essentially remained invariant during this time period. In all cases, the two- and three-layer drug-loaded nanoparticles were more cytotoxic than free drug in 3D cell culture, indicating enhanced cytotoxic performance by the layered gold system, but generally not as efficient as the free drug exposure in 2D cell culture. In particular, the two-layer system generally seemed to provide a slight advantage over the three-layer configuration at higher drug concentrations.

Simulation of nanotherapy in heterogeneously vascularized tissue

Figure 3 shows simulated tumor lesions prior to treatment. **Figure 3A** simulates an avascular spheroid in cell culture used for calibration of the tumor parameters and the drug effect. To illustrate the approach, we chose to match the IC_{50} drug effect for the A549 cell line when treated with three-layer particles. **Figure 3B** simulates a vascularized lesion *in vivo*, with **Figure 3C** highlighting the accompanying heterogeneous oxygen concentration profile due to diffusion gradients caused by heterogeneous vascularization. The lesions were exposed to nanoparticles diffusing either from the surrounding medium (**Figure 3A**) or from the vasculature via bolus injection (**Figure 3B**, with the flow in the vasculature being from bottom left to upper right for each panel). The treatment with paclitaxel-loaded particles is shown

graphically at specific timepoints in **Figure 4**. Initially (at 3.6 h), the nanoparticle concentration is very high while the drug concentration is low. Over the course of 24 h, the nanoparticles wash out of the system with the drug concentration peaking at ~12 h postinjection. During this process, the tumor lesion begins to shrink as a result of the drug effect. For cisplatin-loaded particles (**Figure 5**), the nanoparticle concentration follows the same profile as for paclitaxel, while the drug concentration peaks within minutes postinjection due to the burst release experimentally observed with cisplatin. Beyond ~7 h most of the drug has washed away while the particle concentration continues to decay. The simulated drug release from the three-layer system is shown in **Figure 6** for paclitaxel (**Figure 6A & B**) and cisplatin (**Figure 6C & D**) per time at the tumor site and cumulatively, respectively, with the latter fitted to the experimentally previously observed nanoparticle release profiles [38]. Paclitaxel has a steady release profile (**Figure 6B**), while cisplatin exhibits a burst release phenomenon (**Figure 6D**).

Figure 7 quantifies the simulated nanoparticle concentration decaying in time at the tumor site (A) along with the corresponding change in vascularized tumor lesion radius (B). The paclitaxel system achieved a 41.3% decrease in lesion radius compared with the untreated control by 250 h postinjection, while cisplatin attained a 9.5% decrease. These results highlight the enhanced performance of the paclitaxel system compared with cisplatin, suggesting that a longer drug half-life (5.8 vs 0.5 h for cisplatin, see **Table 1**) combined with steady drug release from the nanoparticles may provide a significant cytotoxic advantage. To further elucidate these effects, we simulated treatment in which a longer and shorter drug half-life were associated with a burst and steady drug release, respectively, that is, effectively swapping the paclitaxel and cisplatin half-lives while maintaining the same drug release profiles. The results indicate that the case with the longer half-life (and with burst release) would fare better

Table 2. Layered gold nanoparticle characterization using UV-visible spectroscopy (maximum wavelength), zeta potential analysis, and dynamic light scattering (hydrodynamic diameter representing z-average of the intensity distribution).

Nanoparticle type	Maximum wavelength (nm)	Zeta potential (mV)	Hydrodynamic diameter (nm)	Polydispersity index
Two-layer nanoparticles (hexadecanethiol/phosphatidylcholine)	539	-21 mV	72 (10)	0.08
Three-layer nanoparticles (hexadecanethiol/phosphatidylcholine/high-density lipoprotein)	535	-6 mV	80 (12)	0.11

Polydispersity index represents the relative variance in the nanoparticle size distribution, with a lower polydispersity index representing a more monodisperse sample. Parentheses denote standard deviation (n = 3).

Table 3. Cumulative drug release from layered gold nanoparticles at various time intervals with associated loading efficiency.

Nanoparticle type	3 h	12 h	24 h	48 h	72 h	96 h	Loading efficiency
Two-layer nanoparticles w/cisplatin	33.8 (2.6)	37.6 (2.7)	43.0 (1.1)	46.9 (1.5)	48.9 (0.5)	49.7 (0.7)	68.4 (7.1)
Two-layer nanoparticles w/paclitaxel	1.42 (0.12)	3.38 (0.13)	3.80 (0.11)	8.20 (0.09)	10.80 (0.11)	11.90 (0.90)	99.1 (0.7)
Three-layer nanoparticles w/cisplatin	59.1 (2.0)	70.2 (0.9)	72.8 (0.9)	76.7 (1.8)	77.7 (1.1)	78.9 (2.1)	78.9 (0.7)
Three-layer nanoparticles w/paclitaxel	2.4 (0.3)	3.9 (0.1)	7.5 (0.9)	23.1 (4.2)	36.5 (4.3)	55.7 (4.7)	99.4 (0.4)

All numbers are represented as percentages of cumulative drug release, with standard deviation in parenthesis (n = 3).
Data measured in [38].

compared with the untreated control, achieving a 48% decrease in tumor radius compared with the short half-life (and with steady release) case which basically had no effect.

Discussion

This study presents an interdisciplinary approach for evaluation of cancer nanotherapy, integrating data from experiments with computational simulations of heterogeneously vascularized lesions. We examined the cytotoxicity of novel two- and three-layer gold nanoparticles for the delivery of hydrophobic and hydrophilic chemotherapeutics, choosing cisplatin and paclitaxel due to their current application in treating NSCLC. To assess the effect of the diffusive transport barrier, the formulations were evaluated in both 2D (i.e., monolayer) and 3D cell cultures. The layering system was applied to the nanoparticle surface to enhance tumor targeting capability, while aiming to decrease possible immunogenicity and aiming for

reticuloendothelial system (RES) avoidance [61]. The RES includes macrophages of the lung and other organs known for removing foreign objects *in vivo* [62]. Two-layer nanoparticles were synthesized with a TL layer followed by a PC layer, which was critical for the formation of a hydrophobic region capable of loading water-insoluble drugs such as paclitaxel. This was accomplished as the tail groups of TL and PC bind tail-to-tail creating a water-soluble nanoparticle with similar characteristics to liposomal delivery systems (Figure 1). As liposomes cause less toxicity *in vivo* in comparison to other nanoparticle platforms, the PC layer also offers the capability of acting as a camouflage to decrease possible immunogenicity and increase bio-availability [63]. For the three-layer system, the HDL was added to the surface of the two-layer nanoparticles (Figure 1).

While both cisplatin and paclitaxel are commonly used in combination therapy for NSCLC, these compounds elicit different cytotoxic-inducing mechanisms

Table 4. Inhibitory concentration required to achieve 50% tumor reduction (IC_{50}) at 48 h in 3D cell culture for the indicated cell lines.

Treatment (3D cell culture)	Cell line	Paclitaxel IC_{50} (nM)	Cisplatin IC_{50} (μ M)
Free drug	A549	38.3 $\pm$ 4.2	26.6 $\pm$ 3.0
	H358	45.6 $\pm$ 8.1	32.9 $\pm$ 3.4
	PC9	30.7 $\pm$ 4.6	16.6 $\pm$ 4.5
Two-layer nanoparticle	A549	28.5 $\pm$ 6.9	14.8 $\pm$ 0.4
	H358	19.0 $\pm$ 1.0	17.4 $\pm$ 0.4
	PC9	20.0 $\pm$ 1.6	13.4 $\pm$ 1.4
Three-layer nanoparticle	A549	34.2 $\pm$ 2.6	15.9 $\pm$ 1.2
	H358	25.2 $\pm$ 3.3	10.8 $\pm$ 1.3
	PC9	24.4 $\pm$ 2.0	7.6 $\pm$ 2.4

For the nanoparticles, the value listed represents the concentration attained at 48 h due to the gradual release from the nanoparticles. Values denote mean $\pm$ standard deviation (n = 3).

Figure 2. Cytotoxicity measured for three non-small-cell lung cancer cell lines (rows A549, H358, and PC9). Cisplatin data is shown in the left column (A, C & E) while paclitaxel data is in the right column (B, D & F). Vertical axis: cell viability as a fraction of untreated control; horizontal axis: AUC indicating the product of drug concentration and time of exposure (48 h). Solid black (stars): free drug in 3D cell culture; dotted (triangles): three-layer nanoparticles; dashed (squares): two-layer nanoparticles; dashed-dotted (circles): 2D cell culture. In all cases, the layered gold nanoparticles showed higher effectiveness compared with free drug in 3D cell culture, while the drug concentrations required for cytotoxicity in 3D culture were generally higher than in 2D. Error bars denote standard deviation with $n = 3$.

AUC: Area under the curve; NP: Nanoparticle.

Figure 3. Simulated tumor lesions prior to treatment. (A) Avascular spheroid surrounded by oxygen and nutrients in cell culture, used for calibration of the drug effect. Red: viable (proliferating) tissue; blue: hypoxic (quiescent) tissue; brown: necrotic (dead) tissue. We chose to match the IC_{50} drug effect for the A549 cell line when treated with three-layer particles. (B) Vascularized lesion; color scheme as in (A). Existing capillary network is denoted by regularly spaced grid (brown), with vessels induced by angiogenesis shown as irregular lines growing towards the hypoxic tumor regions which act as a source of angiogenic stimuli. Normal tissue (not shown) surrounds the lesion. (C) Oxygen concentration profile due to diffusion gradients maintained by heterogeneous vascularization in (B) (with a maximum value normalized by the concentration in vasculature), highlighting the lower oxygen inside most of the tumor compared with the surrounding host tissue. Exposure was simulated to drug-loaded three-layer nanoparticles diffusing either from the surrounding medium (A) or from the vasculature via bolus injection (B). For all panels, bar: 250 μ m.

and can provoke distinct adverse effects. Cisplatin is a water-soluble drug delivered in saline with 5% dextrose, while paclitaxel is a hydrophobic compound commonly administered with castor oil [64]. Castor oil has been linked to severe toxicity in patients [64]. For this reason, nanoparticles capable of delivering paclitaxel are of particular interest. With paclitaxel loaded, it was hypothesized that nanoparticles would exhibit a slower release. This was confirmed by the drug release experiments. On the other hand, cisplatin was expected to experience a faster drug release due to weak non-covalent interactions with the outer layer of PC for two-layer nanoparticles or HDL for three-layer nanoparticles, which was confirmed with the experiments.

After synthesis of the nanoparticle systems, characterization was performed to ensure that desired nanoparticle surface modifications were present. The absorbance wavelength of both two- and three-layer nanoparticles (Supplementary Figure 1) was within the range for citrate-stabilized gold nanoparticles sized between 50–80 nm [65]. Nanoparticle size was measured using two different methods, SEM and DLS (Table 2 & Supplementary Figure 2). Sizing measurements by SEM and DLS can vary with nanoparticles experiencing a larger hydrodynamic radius through DLS in comparison to SEM measurements [66]. The nanoparticle surface charge (Table 2) can have significant effects on nanoparticle systemic travel, as proteins

and other body components have charges that may attract or repel the particles. For example, it is documented that nanoparticles possessing a neutral charge are less likely to be removed by the liver and spleen in comparison to highly cationic or anionic nanoparticles [67,68]. The two-layer nanoparticles with an outer layer of PC were expected to produce a slightly negative surface charge. Previously, it was shown that zeta potential measurements vary with the pH of the solution, with more acidic environments (pH ~5) producing a more positively charged surface area, and neutral pH (~7) producing nanoparticles with negative surface charges [69]. In comparison, HDL nanoparticles were expected to possess a neutral surface charge, as the HDL molecule is relatively neutral [70]. For storage, nanoparticles can be lyophilized into powder to improve longer term stability, as detailed in [71]. Finally, FTIR spectroscopy was utilized to confirm the presence of PC and HDL on the surface of the gold nanoparticles (Supplementary Figure 3).

Release of cisplatin and paclitaxel from nanoparticles was previously measured [38] to determine the necessary doses for cytotoxicity experiments (Table 3). Cisplatin-loaded nanoparticles displayed an initial burst of drug release, a common occurrence for compounds possessing weak interactions with nanoparticle surfaces. Paclitaxel-loaded nanoparticles elicited a steady release of drug, which can be attributed to the efficiency of loading within the hydrophobic layer as

Figure 4. Simulated treatment with paclitaxel-loaded three-layer gold nanoparticles. The vascular flow is from bottom left to upper right of each panel. Initially (at 3.6 h [A]), the nanoparticle concentration is high while the drug concentration is low. Over the course of 24 h (A–D), the nanoparticles wash out of the system with the drug concentration peaking at ~12 h postinjection (C). During this process, the tumor lesion begins to shrink as a result of the drug effect. Colors and scale as in Figure 3.

Figure 5. Simulated treatment with cisplatin-loaded three-layer gold nanoparticles. The vascular flow is from bottom left to upper right of each panel. The nanoparticle concentration follows the same profile as for paclitaxel, while the drug concentration peaks within minutes postinjection due to the burst release experimentally observed with cisplatin. (A–D) Show progression in time over the course of 10 h. Beyond ~7 h most of the drug has washed away while the particle concentration continues to decay. The tumor lesion shrinkage is transient and not as pronounced as with paclitaxel (Figure 4). Colors and scale as in Figure 3.

well as the hydrophobicity of the drug itself. For both drugs, addition of HDL augmented the total amount of drug released during the experiments. We hypothesize that this is due to disturbance of the PC-coating by the HDL components. It has been previously shown that albumin-loaded liposomes exposed to HDL effectively release most of their payload as the HDL components disrupts the membrane integrity of the liposome [72]. This also suggests that the HDL components may be integrating into the PC of the three-layer particles to create a hybrid PC/HDL layer.

Previous studies (e.g., [73,74]) have shown that 3D cell cultures can be superior to 2D cell cultures for cytotoxicity experiments. Newer and easier methodologies for creating and utilizing 3D cell cultures have been proposed (e.g., [74,75]). 3D cell cultures representing avascular tumor nodules consist of cells in the periphery optimally exposed to drug, oxygen and nutrients, while cells in the core region experience hypoxia, and even necrosis [76]. Additionally, cells in 3D establish more realistic contact with each other, thus promoting survival. A major limitation of 3D cell culture is the absence of essential elements found *in vivo* which affect nanotherapeutic efficacy, including an immune system, vasculature, and a fully developed extracel-

lular matrix. This limitation may make it difficult to compare results across cells originating from different tumors.

The *in vitro* experiments in this study show that the layered gold nanoparticles had higher effectiveness compared with free drug in 3D cell culture (Figure 2), with the best area under the curve (AUC) performance evinced by the system loaded with paclitaxel. We hypothesize that this effectiveness relies on the kinetics of the drug release from the particles as well as the drug half-life. The simulations of therapy of a vascularized lesion (Figure 7) suggest that a steady drug release combined with a longer drug half-life may provide a significant cytotoxic advantage. In contrast, the results in Table 4 primarily reflect the effect of the release kinetics, as these experiments were *in vitro* and therefore exposed to a constant drug concentration. In this (3D cell culture) scenario, the two-layer paclitaxel system seems slightly more effective than the three-layer version, while the three-layer cisplatin appears generally more effective than the two-layer formulation. This is also reflected by the AUC curves shown in Figure 2. In the cisplatin case, this may be due to the higher amount of (hydrophilic) drug released by the three-layer version within the time period of measurement (Table 3),

Figure 6. Simulated drug release kinetics. Paclitaxel (A & B) and cisplatin (C & D) release from the three-layer nanoparticle system is shown per time at the tumor site (A & C) and cumulatively (B & D), with the latter fitted to the previously observed release profiles measured in [38].

Figure 7. Simulated nanoparticle concentration and tumor radius change in time. Simulated nanoparticle concentration decaying in time at the tumor site (A) along with the corresponding change in vascularized tumor lesion radius (B). (B) Solid line: untreated (control) tumor; dashed: CDDP-treated lesion; dotted: PTX-treated lesion. The PTX system achieved a 41.3% decrease in lesion radius compared with the untreated control by 250 h postinjection, while CDDP attained a 9.5% decrease. CDDP: Cisplatin; NP: Nanoparticle; PTX: Paclitaxel.

which is expected to directly affect cytotoxicity. For paclitaxel, the lower release of the two-layer formulation may be more effective because smaller amounts of drug released by the particles (i.e., as in an emulsion) might be uptaken more efficiently by the cells in 3D culture than a larger amount that would aggregate due to hydrophobicity.

Conclusion

This study presented an interdisciplinary approach combining nano, drug and tumor experimental data with computational simulations to determine potential *in vivo* response to nanotherapy. In particular, the cytotoxicity of novel two- and three-layer gold nanoparticles was examined for future *in vivo* evaluation. These nanoparticles are expected to more likely accumulate in tumor lesions compared with freely circulating drug, as previously shown [37], and with potential cytotoxic efficacy, as evaluated by the computational simulations in this study (Figures 3–7). The interdisciplinary approach enables study of tumor-specific conditions hindering nanotherapy effectiveness, such as diffusive transport limitations resulting from heterogeneous vascularization. In addition, the approach helps to move the mathematical modeling effort beyond a theoretical exercise and along the path to practical application.

Future perspective

An interdisciplinary combination of experimental work with computational modeling to evaluate gold nanoparticle as well as drug performance based on dosage and tissue transport characteristics offers the possibility to rationally guide the design of such systems with patient tumor-specific data, especially to

bridge the gap from *in vitro* experimentation to *in vivo* performance. We note that the interaction between nanoparticles and cells, including penetration of biomembranes and intracellular transport [77], is a critical consideration in this regard, and would need further integration with the proposed approach. Future venues of inquiry include evaluating the mixing of paclitaxel- and cisplatin-loaded layered nanoparticles, thus allowing for dual chemotherapy, and possible combination with thermal therapy, which would require particles with light-absorbance transparent to tissue, such as near-infrared absorbing gold nanoparticles (nanorods, gold silica nanoshells or gold-sulfide aggregate nanoparticles). Evaluation of these and other therapy possibilities coupled with computational modeling that is updated to more faithfully represent patient-specific tumor characteristics is expected to significantly advance clinical translation of cancer nanotherapy.

Supplementary data

To view the supplementary data that accompany this paper, please visit the journal website at: www.futuremedicine.com/doi/full/10.2217/NNM.15.195

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Ethical conduct of research

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

Executive summary

Presented an interdisciplinary computational/experimental approach to assess nanotherapy

- Computational model includes tumor and angiogenesis components in two spatial dimensions.
- Tumor tissue and nanoparticle parameters are calibrated to experimental data, enabling projection of *in vivo* behavior based on experimental observations.
- Interdisciplinary approach helps to bridge the gap from *in vitro* experimentation to *in vivo* performance of nanotherapy.

Evaluated cytotoxicity of novel layered gold nanoparticles targeted for non-small-cell lung cancer treatment

- Substantial differential was shown for free-drug cytotoxicity between 2D (monolayer) and 3D (spheroid) cell cultures, demonstrating increased resistance conferred by diffusive transport.
- Layered gold nanoparticles had significantly higher efficacy than free drug, as measured by the IC₅₀.

Developed computer simulations of heterogeneously vascularized tumor lesions

- Enables analysis of growth and response to therapy as a function of diffusive transport within tissue.
- Simulations can be initialized to cancer-specific information to evaluate particular tumors.
- The approach helps to move the mathematical modeling effort beyond a theoretical exercise and along the path to practical application.

Simulated nanotherapy with model parameters calibrated to *in vitro* data

- A single layered nanoparticle treatment could potentially lead to substantial lesion regression, with the cisplatin formulation attaining 9.5% tumor radius decrease and the paclitaxel formulation achieving a 41.3% decrease compared with untreated control.
- A longer drug half-life combined with a burst or steady release may provide cytotoxic advantage.

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