15

Nanotechnology in Cancer Drug Therapy: A Biocomputational Approach

Hermann B. Frieboes[1], John P. Sinek[2], Orhan Nalcioglu[3], John P. Fruehauf[4], and Vittorio Cristini[1,2,*]

Departments of Biomedical Engineering[1], Mathematics[2], Radiological Sciences and Tu & Yuen Center for Functional Onco-Imaging[3], Medicine—Hematology/Oncology[4], University of California, Irvine.

[*]Corresponding author: Department of Biomedical Engineering, REC 204, University of California, Irvine, CA 92697-2715.

15.1. INTRODUCTION

15.1.1. Challenges with Chemotherapy

Although the clinical arsenal in treating cancer has been greatly extended in recent years with the application of new drugs and therapeutic modalities, the three basic approaches continue to be (in order of success) surgical resection, radiation, and chemotherapy. The latter treatment modality is primarily directed at metastatic cancer, which generally has a poor prognosis. A significant proportion of research investment is focused on improving the efficacy of chemotherapy, which is often the only hope in treating a cancer patient. Yet the challenges with chemotherapy are many. They include drug resistance by tumor cells, toxic effects on healthy tissue, inadequate targeting, and impaired transport to the tumor. Determination of proper drug dosage and scheduling, and optimal drug concentration can also be difficult. Finally, drug release kinetics at the tumor site is an important aspect of chemotherapy.

In this chapter we consider each of these hurdles and examine how nanotechnology can help to address them. The role of biocomputation will be explored as a means to specify

cancer drug therapy, with the goal of applying the results in the clinical setting, especially the modeling of drug delivery via nanoparticles. Biocomputation could save lives and enhance the quality of cancer treatment by making it possible to tailor therapy to the individual patient and reduce the time and costs involved. With these goals in mind, we will look in more detail at the system-level biocomputation of tumor growth and cancer therapy, and raise considerations for future research. We begin by briefly reviewing the advantages of nanotechnology, its application to cancer chemotherapy, and its challenges in a biological setting.

15.1.2. Possibilities of Nanotechnology

Nanotechnology applied to cancer treatment may offer several promising advantages over conventional drugs. Nanoscale devices are two orders of magnitude smaller than tumor cells, making it possible for them to interact directly with intracellular organelles and proteins. Because of their molecule-like size, nanoscale "tools" may be capable of early disease detection using minimal amounts of tissue, even down to a single malignant cell [60]. These "tools" may not only prevent disease by monitoring genetic damage, but also treat cells *in vivo* while minimizing interference with healthy tissue. By combining different kinds of nanoscale "tools" on a single device, it may be possible to run multiple diagnostic tests simultaneously [56]. In particular, it is hoped that cancer drug therapy involving nanotechnology will be more effective in targeting malignant cells and sparing healthy tissue. In this regard, the role of nanoparticles loaded with chemotherapeutic drugs has been receiving much attention. Research and development in this area is expected to dramatically increase in importance in the coming years.

15.1.3. Chemotherapy via Nanoparticles

In general, nanoscale drug delivery systems for chemotherapy can be divided into two categories: polymer- and lipid-based [46]. Polymers, which are usually larger than lipid molecules, form a solid phase, such as polymeric nanoparticles, films, and pellets, while lipids form a liquid (or liquid crystalline phase), such as liposomes, cubersomes, micelles and other emulsions [22]. While polymer-based systems are considered biologically more stable than lipid-based systems, the latter are generally more biocompatible. Polymer-based systems might possess good drug targeting ability because their uptake may be different for cells in different tissues [53]. In fact, Feng and Chien [22] have suggested that a combination of polymer- and lipid-based systems could integrate their advantages while avoiding their respective disadvantages. An example of such a nanoparticle would be a liposomes-in-microspheres (LIM) system, where drugs are first loaded into liposomes, and then encapsulated into polymeric microspheres. This way both hydrophobic and hydrophilic drugs can be delivered in one nanoparticle. The bioactivity of peptides and proteins would be preserved in the liposomes, whose stability is protected by the polymeric matrix [22].

Chemotherapy using nanoparticles has been studied in clinical trials for several years and numerous studies have been published in this regard ([43], pp. 283–290). Two liposomally delivered drugs are currently on the market: daunorubicin and doxorubicin [51]. These encapsulated drugs can be formulated to maximize their half-life in the circulation.

For example, a "stealth" version of liposomal doxorubicin, coated with polyethylene glycol to reduce its uptake by the reticuloendothelial system, can extend its half-life in blood for up to 50–60 hours [10].

15.1.4. Challenges of Nanotechnology

The difficulties facing nanotechnology in the service of clinical medicine are numerous. These difficulties should be kept in mind when considering chemotherapeutic treatment involving nanotechnology and the potential role of biocomputation. First, there are basic physical issues with matter at such a small scale. Since matter behaves differently on the nano than it does at micro and macro levels, most of the science at the nanoscale has been devoted to basic research, designed to expand understanding of how matter behaves on this scale [56]. Because nanomaterials have large surface areas relative to their volumes, phenomena such as friction are more critical than they are in larger systems. The small size of nanoparticles may result in significant delay or speed-up in their intended actions. They may accumulate at unintended sites in the body. They may provoke unexpected immune system reactions. Cells may adapt to the nanoparticles, modifying the body's behavior in unforeseen ways [56]. The efficacy of nanoparticles may be adversely affected by their interaction with the cellular environment. For instance, the reticuloendothelial system (RES) may clear nanoscale devices, even "stealth" versions, too rapidly for them to be effective because of the tendency of the RES to phagocytose nanoparticles ([43], p. 259). Nanoparticles can be taken up by dendritic cells [18] and by macrophages [16]. RES accumulation of nanoparticles could potentially lead to a compromise of the immune system. On the other hand, larger nanoparticles may accumulate in larger organs, leading to toxicity [56]. Perhaps the biggest issue of all is that the physically compromised tumor vasculature may prevent most of the nanodevices from reaching the target cells by vascular transport or diffusion. Alterations in the tumor vasculature may adversely affect the convection of the nanodevices in the blood stream [9]. Local cell density and other stromal features may hamper drug or nanodevice diffusion through tumoral tissue. This topic will be examined in more detail when we consider the issue of chemotherapeutic drug transport and the system-level biocomputation of cancer therapy.

15.1.5. Biocomputation in Cancer Treatment

The challenges of nanotechnology may be better evaluated through the use of biocomputational methods that examine the fundamental physical principles that affect delivery and degradation of nanoparticles in cancer treatment. Biocomputation, in general, provides a means of mathematically modeling these physical principles so that basic truths about the interaction of nanotechnology and living tissue may be better understood. This knowledge could save time and resources by providing guidance to the experimentalist and the clinician, support a coherent framework for further research, and offer the potential to predict experimental outcomes. The main challenge of biocomputation is to be able to incorporate these physical principles into a biologically relevant model while retaining the capability to numerically solve for concrete results. It is difficult to model from the nanoparticle (10^{-9} m) to the tumor (10^{-3} m) scale, not only because matter behaves very differently in each, but because of the enormous computational cost associated with having to span six orders of

magnitude of length scales over a significant period of biological time. In fact, simulation may require integration of multiple hierarchies of models, each differing in several orders of magnitude in terms of scale and qualitative properties [40].

Modeling of drug delivery encompasses the formulation of quantitative descriptions for drug transport in biological systems to evaluate feasibility of new drug delivery methods, to estimate dose response and toxicity, and to speed experimental and clinical evaluation [61]. Modeling principles apply to both procedures and technologies. For example, local drug administration, targeted drug delivery, and controlled drug release polymers should all be considered [61]. In the treatment of cancer, it is hoped that biocomputation will facilitate formulation of optimal treatment models that enable administration strategies for chemotherapy that maximize benefit while minimizing side effects [22]. Biocomputation-based generation of theoretical results could potentially be validated by correlation of numerical predictions with *in vitro* and *in vivo* data of a particular patient's cancer response to chemotherapy. In turn, these experimentally and clinically validated biocomputation results may be used to design personalized therapy protocols *in silico* using computer simulations.

Biocomputation of targeted and controlled drug delivery via nanoparticles is not only expected to offer insight into *in vivo* drug delivery, but also simulate the therapeutic effects of the delivery device and stipulate its preparation specifications in order to better address the challenges of nanotechnology. This approach may offer a means to optimize existing products and enhance new product development for cancer chemotherapy and disease treatment. The types of drug, excipient, and composition of the device could be essential components of a model [22]. Since there are no encompassing mathematical models that can apply to all conceivable physical and chemical processes in product development, it is important to develop an adequate theory grounded in physical considerations for specific systems. For instance, physical considerations that apply to polymer devices include drug delivery and diffusion, polymer swelling and degradation/erosion. It may also be necessary to consider osmotic, steric, magnetic, and charge effects [22].

15.2. ISSUES WITH CHEMOTHERAPY: HOW NANOTECHNOLOGY CAN HELP AND THE ROLE OF BIOCOMPUTATION

15.2.1. Drug Resistance

One of the major challenges that prevents most patients from benefiting from chemotherapy is the presence of tumor cell mechanisms that cause drug resistance. A tumor may evolve mechanisms to avoid damage by chemotherapeutic agents via the acquisition of mutations that confer a drug-resistant status. Nanoparticles with an appropriate surface coating could possibly overcome some mechanisms of cellular drug resistance, thereby improving the value of chemotherapy [22]. In fact, multidrug resistance (MDR) might be treatable with liposomes that enhance molecular MDR modulating strategies in addition to improving therapeutic activity through pharmacological optimization [54]. However, constant release of drug by nanoparticles at a tumor site could potentially exacerbate cellular resistance by exposing cells to a predictable (steady) level of stimulation. In fact, there is evidence that a single drug exposure can induce cellular resistance [80]. Biocomputation

could help to quantify a nanoparticle drug release regimen that minimizes drug resistance. For example, Jackson and Byrne [33] proposed a mathematical model that described the chemotherapeutic response of a spherical vascular tumor containing two cell species. They contrasted the tumor response to continuous intravenous drug infusion versus intravenous bolus injection, and found that bolus injection decreased the time to cure when the drug resistant cell population was present. Biocomputation might also help to identify drug-resistant tumors via nanotechnology. Dordal *et al*. [17] analyzed fluorescent drug uptake by tumor cells using a three-compartment model in which rapid diffusion from extracellular fluid into a cell was followed by uptake into a non-exchangeable pool (where the drug bound with its intra-cellular target). By using a flow cytometric assay of drug uptake, the kinetic parameters of drug transport may be specified. The model could thus identify the presence of drug-resistant cells in a tumor by the reduced cellular uptake or increased cellular efflux of drug. This model could be a starting point to study the effects on drug resistance of drugs delivered with nanoparticles.

15.2.2. Drug Toxicity

Another challenge in chemotherapy is the use of potentially toxic side-groups that enhance the hydrophilicity of typically hydrophobic drugs. The addition of such side-groups may not be necessary with nanoparticles of biodegradable polymers that are small enough to allow intracapillary or transcapillary passage, and that possess a surface coating that evades macrophage uptake [22]. Thus, nanoparticles could be used to deliver traditional chemotherapy without toxic adjuvants to cancerous cells, and to treat conditions that may arise over time with anticancer therapy.

Toxicity could be considered in a biocomputational model as a constraint to preserve the white blood cell (leukocyte) number at a certain level while maximizing the reduction of the tumor cell population [4]. The goal would be to optimize the nanoparticle drug regimen under this constraint. On the other hand, Parker and Doyle [68] point out that through modeling of leukopenia, optimal delivery profiles could be constructed to minimize toxic effects. The method of delivery (e.g. bolus or continuous infusion) should also be considered, as it can lead to differences in toxicity.

15.2.3. Drug Targeting

Another issue with chemotherapy is that the drug may be delivered to tissues other than the tumor, affecting organs such as the heart and liver. Nanoparticles could provide a controlled and targeted means to deliver encapsulated drugs, resulting in lower side effects and higher efficacy [47]. A purely "chemical" strategy that relies on the molecular recognition of unique surface signatures of tumor tissue by chemical ligands (such as antibody-drug conjugates and immunoliposomes) may not work well with tumors because other tissue could also bear these signatures [71]. Controlled delivery may be achievable instead via a "physical" strategy because macromolecular transport across tumor microvessels can occur via fenestrations, vesicular vacuolar organelles, and transendothelial channels and inter-endothelial junctions [22]. The pore cutoff size of many tumor vessel models is between 380 and 780 nm, so nanoparticles in this size range should preferentially extravasate from tumor vessels [29, 89]. Since nanoparticles could also exit the circulation through the liver

and bone marrow, the amount of particles needs to account for extravasation at these areas. A more unique signature of cancer cells is their abnormal DNA. Nanoparticles capable of screening DNA sequences of individual cells could recognize and kill cancerous ones.

Biocomputation could quantify the various means to target nanoparticles to specific sites within the body under various treatment scenarios. In fact, a combination of "chemical" and "physical" strategies may work best. One way of targeting is to conjugate cell-targeting agents on the nanoparticle surface [23, 25, 41]. Research is underway on ligand-targeted "stealth" liposomes that utilize moieties attached to the liposome surface to selectively bind the liposome to specific cancer cells [72]. A targeting ligand is chosen based on the ability of the target cell to internalize the liposome. Another way of targeting is through a magnetic field by using magnetite nanoparticles [32].

15.2.4. Drug Transport

A principal barrier to chemotherapy delivery can occur at the level of the compromised tumor vasculature. This barrier may prevent the delivery of adequate doses of drug to tumor cells [35]. Blood flow in tumor vessels is abnormal, since the flow is intermittent, periodically abating and reversing. These effects are caused by a chaotic arterial organization and impaired venous and lymphatic drainage [27]. The full consequences of an abnormal tumor vasculature on drug transport are not well understood. In the past some researchers (e.g. [37]) believed that it might take from days to months for a macromolecule to diffuse into the center of a tumor, mainly due to the high tumoral interstitial pressure and the collapsed tumor vessels. The high hydrostatic pressure in the tumor interstitium-see also Jain and Baxter [36] and the mathematical model by Sarntinoranont *et al*. [73]—would create an outward convective interstitial flow and cause drug resistance [38]. Recently researchers of this same group [67] have found evidence that proliferating cancer cells can cause intratumor vessels to compress and collapse, especially vessels without supportive stromal structures. Interstitial fluid pressure, on the other hand, is about the same as the microvascular pressure in the tumor, which makes it unlikely that the collapse of intratumor vessels is due to fluid pressure. It's important to note that this vessel collapse and transport limitation occurs on a timescale of hours and days, based on the rate of cell proliferation. However, the pharmacokinetics of a drug can be effected on a timescale of seconds, especially in highly perfused tissues such as the central nervous system, and as evidenced by radiographic scans showing drug delivery throughout brain tumors [7, 90]. Perhaps the drug is cleared from a tumor site on a timescale that precludes a full effect on all tumor cells, especially quiescent cells, and this effect, rather than a compromised tumor vasculature, is the main reason for the inadequate dosing. The tumor extracellular matrix assembly and composition could also be factors limiting drug transport [59].

The extent that a compromised tumor vasculature affects the bioavailability of larger molecular agents into the interstitium may depend on tumor type [81, 82]. By using contrast agents of different molecular weights in dynamic contrast enhanced MRI, it was shown that interstitial availability of macromolecular agents in different animal tumor models may be a function of tumor growth rate. (Fig. 15.1, top). In a fast growing tumor (top left), there was sufficient amount of macromolecular contrast agents in the interstitium within the measurement window of 16 minutes. In fact, the larger molecular weight agent started reaching equilibrium at the end of this period and resided in the interstitium for a

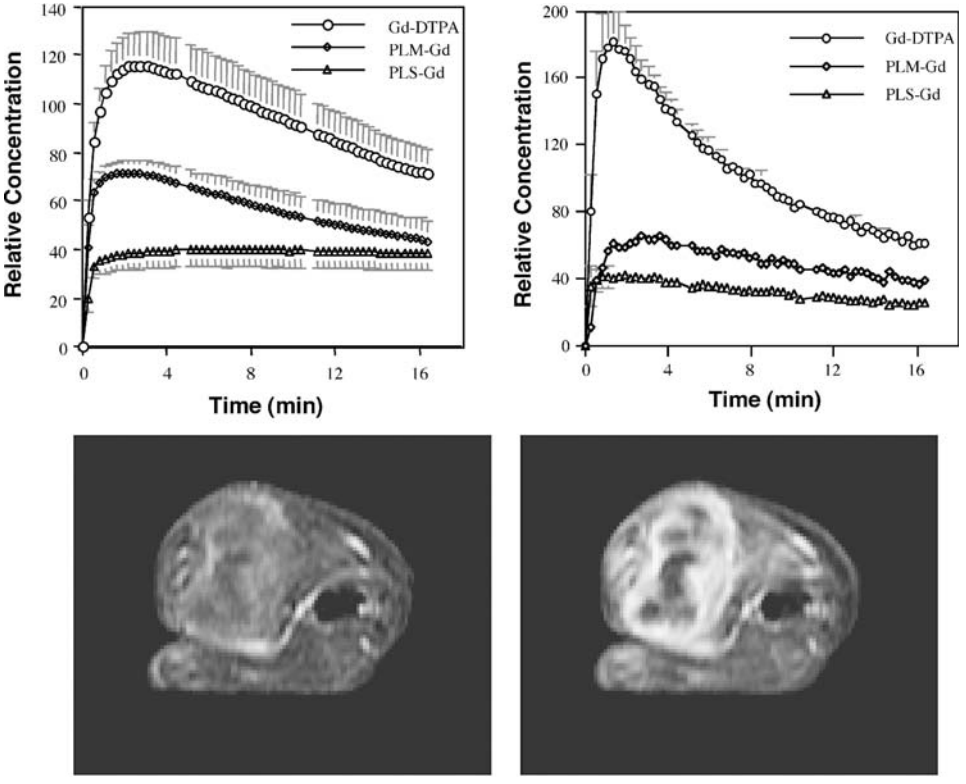

FIGURE 15.1. Top: contrast enhancement curves for different contrast agents (labeled) with hydrodynamic diameters 1-2.3 nm from a fast (left) and a slow (right) growing tumor. Bottom: Albumin-Gd-DTPA enhanced image at 10 minutes (left), and Gd-DTPA enhanced image at 30 sec (right). The former has higher molecular weight and 3 nm hydrodynamic diameter. Adapted from Su *et al.*, [81, 82], Magnetic Resonance in Medicine Vol. 34 and Vol. 39. Copyright © 1995 and 1998. Reprinted with permission of Wiley-Liss, Inc., & subsidiary of John Wiley & Sons, Inc.

considerable duration. Su *et al*. [81] showed that an even larger molecular weight contrast agent could have considerable interstitial uptake after 10 minutes (Fig. 15.1, bottom). The slower growing tumor demonstrated a very different behavior. In particular, the larger molecular weight agent did not leak into the extravascular space (Fig. 15.1, top right). In the faster growing tumor, vascular permeability was determined to be larger, resulting in higher accumulation of larger molecular weight agents in the extravascular space. It is conceivable that the behavior observed with macromolecular weight MR contrast agents also applies to similar size therapeutic drugs or nanoparticles.

Regardless of the potential mechanics that may affect drug delivery, very recently computer simulations in two spatial dimensions have demonstrated that nanoscale drug delivery systems could in principle be affected by similar limitations as traditional chemotherapy [79]. Nanoparticles first have to be transported in the blood stream to the vicinity of the tumor and extravasate from the blood vessels into the interstitial space; then the drug needs to be released and diffuse through or around the tumor cells [34]. In addition, nanoparticles must avoid protein binding in serum and in the extravascular space, metabolism in the blood,

and phagocytosis by the reticuloendothelial system. Finally, the irregularity of the tumor vasculature with its abnormal blood flow and the timescale of the pharmacokinetics as a function of tumor type may present major obstacles. These limitations will be considered further when we address system-level modeling of cancer therapy.

Modeling of tumor vasculogenesis could be used as a possible angiogenesis assay to study the impact of an altered tumor vasculature on chemotherapy delivery via nanodevices. Chaplain and Anderson [11] recently reviewed a number of mathematical models that have been used to describe the formation of tumor capillary networks through angiogenic stimuli. They concentrated on a specific model that employed mathematical techniques to generate both two- and three-dimensional vascular structures. The model focused on the main events in angiogenesis, i.e., the migratory response of endothelial cells to cytokines secreted by a tumor, endothelial cell proliferation, endothelial cell interactions with extracellular matrix macromolecules, and capillary sprout branching and anastomosis. They presented numerical simulations of the model, using parameter values based on experimental data, and the theoretical structures thus generated were compared with the morphology of actual *in vivo* capillary networks.

The heterogeneity that may exist within the tumor (e.g. blood flow and pressure variation) complicates the modeling of chemotherapeutic agent delivery as a free drug or encapsulated in nanoparticles. The mathematical representation of vessel trees that do not adhere to normal diameter and branching patterns can become very complex. As a result, flows and pressures inside an abnormal vasculature become more difficult to calculate. McDougall *et al*. [52] used a discrete mathematical model to specifically study tumor-induced angiogenesis that described how the endothelial cell proliferative and migratory chemotactic responses led to the formation of a capillary sprout network of abnormal structure. They analyzed fluid flow through this network by considering the effects of fluid viscosity, blood vessel size and network structure on the rate of fluid flow, the amount of fluid present in the complete network at any given time, and the amount of fluid reaching the tumor. The incorporation of fluid flow through the generated vascular networks identified transport issues that may have implications for both nutrient supply and drug delivery to a tumor, echoing the earlier results of Jain [37]. In fact, under some conditions, the model showed that an injected chemotherapy drug could bypass the tumor altogether (Figure 15.2). Whether this effect would occur *in vivo* is unclear, since, as we have seen, there is evidence that drugs can be delivered throughout certain tumors. In general, though, as these and other researchers have noted, the normalization of the tumor vasculature could enhance the flow to a tumor mass, and thus aid the delivery of nanodevices as well.

15.2.5. Drug Dosage and Scheduling

Another determinant of drug efficacy is delivery of the optimal drug dosage. Considerations in this regard include tumor type and size, and the patient's physical parameters (e.g. body surface area, m^2). Chemotherapy drug dosages are selected in part based on the competing goals of maximizing death of malignant cells while minimizing damage to healthy cells. Because of more precise targeting by nanoparticles, drugs in nanocrystalline form may require smaller doses for equal effect. Since they could be delivered directly to the desired tissue while minimizing uptake by other tissues, the harm to healthy tissue

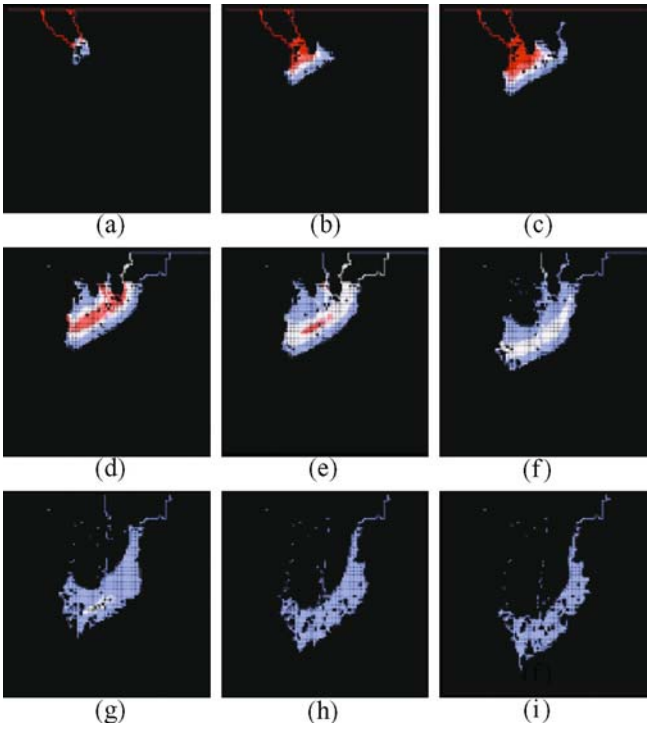

FIGURE 15.2. Effects of bolus injection through a computer simulated vasculature showing how most of the drug does not reach the tumor. Snapshots of drug concentration as it flows from parent vessel (situated at top edge of each picture) through the vascular network towards a tumor (situated at middle of bottom edge of each picture) over a physiological time duration (a)–(i). Colors: red = highest concentration; dark blue = lowest. Reprinted from Bulletin of Mathematical Biology, Vol 64, McDougall *et al*., page 697, Copyright (2002), with permission from Elsevier.

would be reduced, although uptake by the liver and bone marrow might remain an issue. Better targeting also allows for more precise doses because the drug delivery will fluctuate less. For instance, future nanoparticles could achieve precise control over drug release via nanopores that act as particle membrane channels [3].

The fact is that the determination of drug doses and delivery schedules for a particular patient is a difficult process that relies on a series of trial-and-error procedures to determine the maximum tolerable dose and effective treatment regimen [68]. The variation of tumors in individual patients compounds the difficulty in determining an effective treatment based on the partial knowledge about the pharmacodynamics of the drug. The frequency of chemotherapeutic treatment has generally been based on the interval of time required for the myelopoietic cells to regenerate adequate numbers of lymphocytes, platelets, and erythrocytes, rather than being based on the effects on tumor cells, which may continue to proliferate faster than the recovery time of healthy tissue. A better understanding of the issues affecting chemotherapy dosage determination is needed to formulate dosages and schedules for drugs delivered by nanodevices.

From a biocomputational viewpoint, cancer growth has traditionally been defined as exponential (based on work by Skipper and Schabel in the 1970s). This tradition led to the log-kill hypothesis as the underpinning of current drug dosage and scheduling principles [66]. These principles include simultaneous combination chemotherapy, maximum tolerated dose within the combination, and equally spaced cycles of equal intensity. For example, chemotherapy has been routinely administered in 3-week intervals for metastatic breast cancer patients. Biocomputation could enable a more systematic approach to defining the drug treatment regimen [68]. A biocomputational model based on a patient's cancer characteristics could be defined, leading to treatment acceleration and less damage through ineffective dosages.

The Gompertzian growth curve [24] applied to a tumor shows that cell gain is greater than cell loss in the early part of the curve but slows down as the tumor gains mass [44, 88]. The curve is applicable to normal and malignant growth and has its origin in the molecular regulation of mitosis, tissue geometry, and apoptosis [66]. The Norton-Simon hypothesis [63], which is based on the application of Skipper and Schabel's therapy to Gompertzian computation, is that the rate of tumor volume regression is proportional to the rate of growth. The log-kill is greater when the tumor is treated at a smaller size, and its growth rate is higher if the cells are not destroyed. Since two drugs at single-agent dose could be toxic to a patient, Norton and Simon [63] determined that the alternatives were full dose, reduced dose, sequential dosing, and alternating dosing. The mathematical model thus allowed the development of dose density and sequential therapy, based on the theory of combination chemotherapy [65].

Dose density refers to administration of drugs with a shortened inter-treatment interval. It is based on the observation that in experimental models, a given dose always kills a certain fraction, rather than a certain number, of exponentially growing tumor cells [12]. Regrowth of cancer cells between cycles of chemotherapy is quicker in volume-reduced Gompertzian cancer models than in exponential models. The Norton-Simon model predicted that dose density would improve therapeutic results, and that sequential chemotherapy that maintains dose density would preserve efficacy while reducing toxicity [65]. The model explained how cancers that follow Gompertzian kinetics (e.g. breast cancer) respond to treatment, and how they differed from the exponentially growing models often used in the laboratory [64]. These considerations indicated that therapeutic results should be the same, even if the sequential pattern was less toxic [66].

Indeed, Citron *et al.* [12] recently reported that dose density can considerably improve clinical outcomes, and that sequential chemotherapy can be as effective as concurrent chemotherapy. As predicted by the model, sequential chemotherapy was better compared to a strictly alternating pattern [8, 62]. Various dose-dense drug regimens have been under investigation in recent years [56]. Clinical trials have further confirmed the model's prediction, leading to early breast cancer treatment that is shorter, less toxic, and more effective [66]. Future research into the biologic etiology of Gompertzian growth and the molecular mechanisms of its perturbation could generate new hypotheses for dose-schedule regimens that are empirically verifiably [12], and that could take into account drugs delivered by nanodevices.

Quantification of optimal chemotherapy profiles, usually assuming continuous drug delivery, can motivate the development of tumor growth models. An understanding of

these models may be useful when considering cancer therapy via nanoparticles. Parker and Doyle [68] classified and described modeling approaches to cancer growth into two major groups: lumped parameter models and cell-cycle models. Lumped parameter models define tumor growth macroscopically in terms of cell count and selected tumor- or patient-dependent parameters, whereas cell-cycle models describe tumor behavior based on the number of cells in a given cell-cycle stage. Examples of lumped parameter modeling include logistic, Malthusian, Bertalanffy, and Gompertz equations. Each model is based on a growth function that is a continuous, monotonically rising function, describing increase per unit time in tumor cell count or tumor size. One major benefit of lumped parameter models is their advantage for controller design purposes due to their low order and monotonic cell growth behavior. However, the assumption of a homogeneously growing tumor cell population may not match real life, and the inability to account for cells in different growth stages prevents the use of lumped parameter models to study the effects of certain chemotherapeutic agents.

Although cell-cycle models may provide superior insight into the behavior of the tumor at the cellular level, they are very complex because each cell-cycle stage needs to have its own mathematical specification. In order to specify the exact number of cells in the various cell-cycle stages, direct measurements of the tumor cells would be necessary. Parker and Doyle [68] suggested that in the case when model parameters cannot be identified, approximate models of cell-cycle behavior can be constructed, and these models may be useful for analysis purposes. For instance, models of this type can handle the effects of cell-cycle specific drugs. Thus, if good estimates of model parameters are available, this model structure, with its additional detail, can provide a substantial advantage. Intermediate levels of model complexity and more detailed tumor growth models are also possible. In general, however, both lumped parameter and cell-cycle models share a number of shortcomings when it comes to their application to chemotherapy either as free drug or encapsulated in nanoparticles. For example, the assumption of continuous drug delivery may not be valid, since a metronomic regimen may not be appropriate. Moreover, the models are usually one-dimensional in space, disregarding the physical effects that the tumor three-dimensional heterogeneity can have on drug dosage and scheduling.

15.2.6. Drug Concentration

The therapeutic efficiency of a pharmaceutical product is determined by the proper concentration of drug at the lesion site, and biocomputation can describe the relevant pharmacokinetics, especially when considering devices on the nanoscale. Efforts in this area, for instance, have included the development of a mathematical model describing the microscopic profiles and biodistribution of drugs using enzyme-conjugated antibodies as part of a two-step method for cancer treatment [6]. The monoclonal antibodies by themselves may lead to heterogeneous uptake within the tumor, while the use of a low molecular weight agent may allow deeper penetration into the tumor. This mathematical model was used to describe concentration profiles surrounding individual blood vessels within a tumor, which allowed determination of the area under the curve and specificity ratios. Average tissue concentrations were determined by spatial integration and compared with experimental results. The model showed that the effective clearance of antibody inside the tumor

is less efficient than outside the tumor, which may be due to the antibody accumulation at the tumor. The conclusion was that enzyme-conjugated antibodies could help to achieve a more uniform distribution and higher concentrations of the active agent, as well as greater specificity.

In another study of drug concentration at the tumor site, Quian *et al*. [71] developed a mathematical model that could specify the physical dimensions of polymer millirods, composed of PLGA (poly(lactic-co-glycolic acid)) microspheres, inserted directly at the ablation boundary of thermally ablated solid tumors. Based on the rod dimensions, the model showed how an initial loading dose of chemotherapeutic drug followed by a sustained release can provide optimal drug concentrations at the tumor site. Without a loading dose, it would take several days to attain a desired therapeutic concentration via a zero-order (constant) release device. This model may have relevance when establishing the optimal drug concentration via constant-release nanodevices.

Recently, Eliaz *et al*. [19] developed a cell kinetic model showing that the potency of a chemotherapeutic drug (doxorubicin) encapsulated in liposomes was 5 to 6 times higher than free drug. Targeted liposomes delivered more drug into the cell than the free form. In fact, drug delivery via targeted liposomes was more efficient in killing the cells per amount of intracellular drug. This efficiency may be due to the vascular trapping of liposomes in the peritumoral space generating a constant release of drug and creating a more uniform drug concentration. Clinical testing has confirmed that the plasma distribution and elimination half life of liposomal doxorubicin can be much longer than that of free drug, and response rates can be significantly higher [51].

In vivo, tumor response to therapy is governed by the pharmacodynamics and pharmacokinetics of the chemotherapy drug. The effective drug concentration in the tumor model is generally assumed to be equal to that in blood plasma because it is difficult to measure drug concentration within a tumor. However, given the possibility that transport limitations through the abnormally constituted tumor vasculature may cause the drug concentration to be lower within the tumor [37, 48, 67, 79], this assumption may not hold. Thus, the specification of optimal drug therapy is very complex, principally due to a poor understanding of the response of the tumor system to drug therapy [68], which can include variability in drug concentration. The concentration of drug delivered by nanoparticles would depend on the concentration of nanoparticles, which would also be subject to this complexity.

It is important to note that the clinical effect of a drug ultimately depends on the drug concentration inside individual tumor cells, not just the extracellular concentration in the tumor interstitium. For instance, to aid introduction of highly charged or macromolecular drugs into the cytoplasm, pH-sensitive liposomes have been developed that deliver their contents through penetration of the endosomal membrane [78]. Inner and outer cell space can be represented with a two-compartment model of drug concentration. Lankelma [48] described two bounds on the intracellular (inner compartment) concentration: low cellular drug influx with rapid efflux in a sparse cell cluster, and high cellular drug influx with low drug efflux in a tightly packed cluster. The former will lead to homogeneous drug distribution without a gradient, while drug gradients will last longer in the latter. Gradients thus depend on cellular influx and efflux, and on blood concentration as influenced by the tumor vasculature. Gradients of nanoparticles would depend on similar factors. Local drug or nanoparticle concentration could vary considerably due to intercapillary distances and heterogeneity in the tumor cell population.

15.2.7. Drug Release

In this section we review some fundamental concepts regarding drug release from nano- and micro-particles, and examine the role of biocomputational modeling in this area. Drugs can be designed for programmed release *in vivo* by encapsulating them in particles from a few nanometers to microns in size. Particles are usually ingested or implanted, and designed to deliver a controlled release of drug that may last for an extended period of time (weeks or months). In general, drug kinetics can be studied as material fluxes between conceptual units, called compartments [5]. Holz and Fahr [30] reviewed two main groups of biological compartment models, namely, physiological and mechanistic models. On the other hand, Veng-Pedersen [85] reviewed non-compartmentally based models. These utilize systems analysis, such as linear systems analysis (LSA). The wide array of available LSA-based kinetic analysis tools may offer an alternative to traditional kinetic modeling. Both compartmental and non-compartmental models, however, do not usually describe the complexity of multiple spatial dimensions that exist in the tumor environment.

Mathematical modeling can help to optimize the design of a therapeutic device to yield information on the efficacy of various release methods [22]. For example, Wang *et al*. [86] compared two types of drug formulations, namely, controlled release from polymers and systemic administration, to predict spatial and temporal variations of drug distribution at the tumor level in two dimensions. In contrast with bolus injection, polymer-based delivery imparted a longer exposure time, a higher mean concentration, and a reduced systemic toxicity. Drug release from a polymer nano- or microparticle has been traditionally classified based on the material erosion mechanism: surface or bulk erosion [45]. For either type of erosion, models developed to characterize the kinetics of drug release from spherical microparticles were described by Zhang *et al*. [91]. They pointed out three mechanisms that combine to control the overall drug release process: dissolution of drug from the solid phase, diffusion of dissolved drug, and erosion of the polymer matrix. These models can be solved under either a finite or infinite mass transfer condition. For bulk erosion of both hydrophobic and hydrophilic polymers, the models showed a reasonable match with experimental results reported in the literature. Results also indicated that the surrounding environment had a profound effect on the drug release pattern under a finite mass transfer condition. For various surface-eroding polymers, it was observed that the radius of the microsphere followed an approximately linear profile of reduction with respect to time. In some cases, erosion and dissolution appeared to be dominant factors for drug release patterns. For better application of these models, the proportion of amorphous and crystalline polymer, and free chain and rigid chain could be investigated to justify the corresponding parameter values. Furthermore, physical property data (such as diffusivity and porosity) for drugs and microspheres should be determined experimentally to improve simulation results. In particular, quantitative analysis on the experimental diffusivity coefficient, dissolution constant, and erosion constant might help in this regard.

Feng and Chien [22] provided a comprehensive list of mathematical models that have been developed to study drug release at the nanoparticle level. As Siepmann and Goepferich [74] pointed out, the modeling of bioerodible delivery systems is more complex than the modeling of diffusion or swelling-controlled devices. Chemical reactions (e.g. polymer chain cleavage) in bioerodible systems have to be taken into account in addition to physical mass transport phenomena. These reactions continuously change the conditions for mass

transfer processes, complicating the modeling of erosion-controlled drug release. Siepmann and Goepferich [74] classified erosion-controlled drug release models into two categories: empirical models that commonly assume a single zero-order (constant) process to control rates of drug release, and models that consider physicochemical phenomena (such as chemical reaction processes or diffusional mass transfer). The latter category includes simulation of polymer degradation as a random event using direct Monte Carlo techniques (i.e., using computer-generated random numbers). The actual physics of the polymer dissolution process and the consequences for drug release have been modeled. For example, Narasimhan [55] described the main modeling contributions in this area using two broad approaches; phenomenological models and Fickian equations, and anomalous transport models and scaling law-based approaches.

Despite the phenomena complexity involved in drug release from nano- and microparticles, the two mathematical models commonly used to describe drug release kinetics from a large variety of devices are the Higuchi model [28] and the power model [69]. The Higuchi model is:

$$M_\mathrm{t}/\mathrm{A} = (D\,(2c_\mathrm{o} - c_\mathrm{s})c_\mathrm{s}\,t)^{1/2}$$

where M_t is cumulative amount of drug released at time t, A is surface area of the controlled release device exposed to the release medium, D is drug diffusivity, and c_o and c_s are initial drug concentration and drug solubility, respectively [76]. In general, the Higuchi model is valid for systems where drug concentration is much higher than drug solubility, whereas with the power model, the geometry of the system can be related to the drug release mechanism [42]. The power model is:

$$M_\mathrm{t}/M_{oo} = kt^\mathrm{n}$$

where M_t and M_{oo} are absolute cumulative amounts of drug released at times t and infinity, respetively, k is constant incorporating structural and geometrical device characteristics, and n is the release exponent, indicative of the mechanism of drug release [76]. For comparison to these two models, we note that drug release from a traditional matrix, as a result of a diffusion process that assumes excluded volume interactions between drug molecules, can be described by the Weibull function [87]:

$$M_\mathrm{t}/M_{oo} = 1 - \exp(-a\,t^\mathrm{b})$$

where a and b are empirical constants respectively defining the scale and shape of the response.

Various drug release/dissolution models were compared by Costa and Lobo [13]. They pointed out that models that in general describe drug release phenomena best are the Higuchi model, zero order model (as a special case of the power model), Weibull model and Korsmeyer-Peppas model. The Higuchi and zero order models represent two limit cases in the transport and drug release phenomena, while the Korsmeyer-Peppas model can be a decision parameter between these two models. Whereas the Higuchi model has a large application in polymeric matrix systems, the zero order model can be useful in describing membrane controlled dosage forms or coated dosage forms. Costa and Lobo [13] also

suggested using the adjusted coefficient of determination (R^2_{adjusted}) to compare models with different numbers of parameters:

$$R^2_{\text{adjusted}} = 1 - \frac{(n-1)}{(n-p)}(1 - R^2)$$

where n is number of dissolution data points, p is number of parameters in the model, and R^2 is the (unadjusted) coefficient of determination.

As Siepmann and Peppas [76] pointed out, there are several important assumptions when applying the Higuchi model to controlled drug delivery systems. We will briefly mention them and consider how they apply in the case of drug release at a tumor. The first assumption is that initial drug concentration is much higher than drug solubility, which justifies a pseudo-steady state modeling approach. This assumption may not hold for a tumor undergoing intermittent exposure to chemotherapy, which means that a pseudo-steady state approach may not be sufficient. The second assumption is that the mathematical analysis is based on one-dimensional diffusion. Although the simplification afforded by one-dimensionality can lead to valid insights, it seems that the physical heterogeneity of the tumor environment would be better represented by a multi-dimensional analysis [79]. The third assumption is that the diameter of the suspended drug particles is much smaller than the thickness of the system, which would be true for chemotherapy via nanoparticles. The fourth assumption is that dissolution or swelling of the carrier system is negligible. This assumption may or may not hold in the tumor stroma. The fifth assumption is that drug diffusivity is constant, which may not necessarily hold in the case of chemotherapy. The final assumption is that perfect sink conditions are maintained. This assumption probably applies to the tumor environment because the flow of extracellular fluid carries the drug away.

Having discussed some of the fundamentals of nano- and micro-particle drug release and its associated modeling, we will now consider modeling efforts in system drug release kinetics. The pharmacokinetics and distribution of a drug can change substantially by encapsulation into nanoparticles. The drug will assume the pharmacokinetics of the carrier until its release [72]. Thus, from a system viewpoint, the use of nanoparticles changes the drug release so that it primarily consists of two phases. First phase involves delivery of nanoparticles to the tumor site, and the second phase involves drug release by the collection of nanoparticles. In fact, analysis of nanoparticle release profiles will usually display such a biphasic release pattern ([43], pp. 258–259). This behavior can be quantified in terms of drug release parameter values that become an input to the biocomputational modeling at the much larger millimeter-scale of the tumor.

Important work has been done on modeling system drug release kinetics of microparticles, whereas the modeling of nanoparticles has not been as extensive. A small selection of studies and review papers will be noted in this section to illustrate some of the main modeling aspects in this area. The hope is that most of this work could, with some further research, be extended to the nanoscale. In fact, this effort would aid in bridging the gap from the nano- to the macroscale, by providing a quantitative link that could serve as an input parameter for the modeling at the tumor scale.

In the area of drug release kinetics from microparticles, Siepmann *et al*. [77] described an applied mathematical model, considering drug diffusion with non-constant diffusivities (to account for polymer degradation), which was able to quantitatively describe

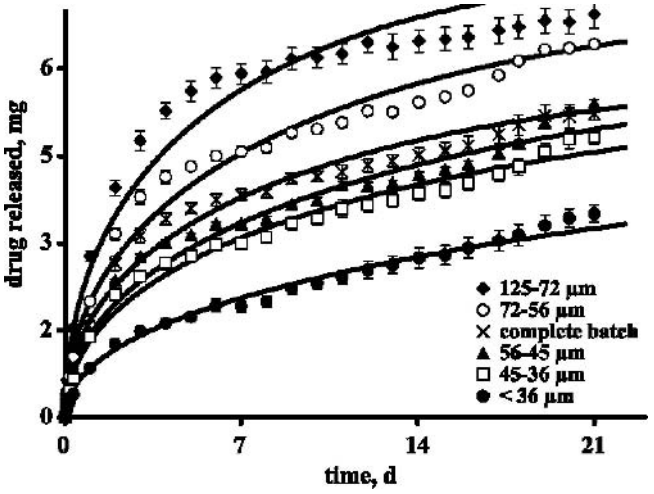

FIGURE 15.3. Fit of a mathematical model taking into account drug diffusion and polymer degradation (solid curves) to experimentally determined drug release from PLGA–based microparticles (symbols) in phosphate buffer pH 7.4. Particle size is given in the legend. Reprinted from Journal of Controlled Release, Vol 96, Siepmann *et al*., page 32, Copyright (2004), with permission from Elsevier.

experimentally observed drug release patterns. An exponential relationship was established between the diffusion coefficient and the initial loading of drug, allowing resulting drug release kinetics for arbitrary microparticle sizes to be predicted in a quantitative way. Drug release was found to be independent of particle size (in the range of <36 μm to 125 μm), and drug transport was primarily controlled by diffusion (Figure 15.3). Hombreiro-Perez *et al*. [31] modeled drug release by non-degradable microparticles, proposing a means to predict the effect of different formulation parameters on resulting drug release patterns (such as the effect of microparticle size). Siepmann *et al*. [75] proposed a model quantifying drug release from bioerodible microparticles using Monte Carlo simulations. The model was able to describe observed drug release kinetics accurately over the entire period of time, including initial "burst" effects, subsequent zero-order drug release phases, and second rapid drug release phases (Figure 15.4). The evolution of drug concentration profiles within the microparticles could then be calculated. Finally, Faisant *et al*. [21] described a mathematical model that enabled a quantitative description of drug release patterns of PLGA microparticles. The release was biphasic (initial burst, followed by a zero-order phase) and mainly driven by drug diffusion. Coefficients for drug diffusion increased as the polymer absorbed water and the average molecular weight of molecules decreased. The polymeric network breakdown did not affect the release process because it occurred after the drug was depleted.

15.3. BIOCOMPUTATION AT THE SYSTEM LEVEL

15.3.1. Modeling at the Nanoscale

We will now examine the system-level biocomputation of cancer therapy and its relationship with nanotechnology by first evaluating system modeling at the nanoscale. In

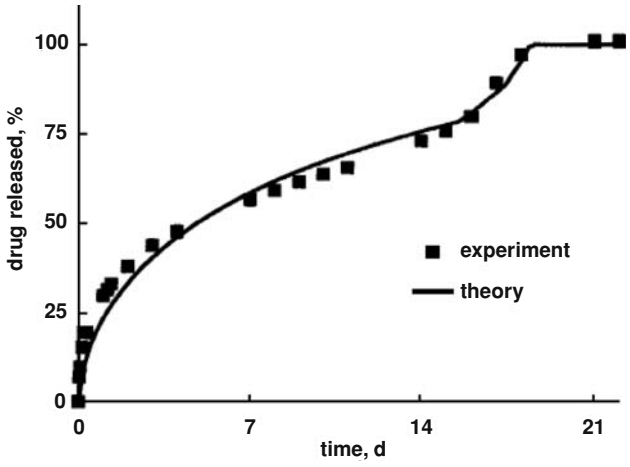

FIGURE 15.4. Triphasic drug release kinetics from PLGA-based microparticles in phosphate buffer pH 7.4: experimental data (symbols) and fitted theory (curve). Adapted from Pharmaceutical Research, Vol 19, 2002, p. 1887, Siepmann *et al*., Figure 1, © 2002 Plenum Publishing Corporation. With kind permission of Springer Science and Business Media.

general, modeling requires a solid theoretical framework in order to produce results that can deliver insights into the phenomena under study (NINT, 2004). Materials modeling underscores most of nanoscience research so that the performance and characteristics of novel materials (such as polymers) may be predicted. General areas of research in fundamental nanoscience include the development of new theories (such as many-body quantum theories and mesoscopic theories) and modeling strategies such as multiscale modeling and multiphysics, data processing and analysis, and comparing theory with experimental results (EPSRC, 2004). These approaches may provide solid tools for modeling materials at the nanoscale. For example, Sumpter *et al*. [83] describe recent developments in the formation, characterization and simulation of nano- and micro-scale particles of amorphous polymer blends and semi-crystalline polymers, including the modeling of structural characteristics, thermal and mechanical properties, particle-surface interactions, and particle-particle interactions.

Nanoparticle technology is based on the physics of materials at the molecular level. In fact, mathematical and computational modeling of systems at the nanoscale requires a blend of quantum with classical mechanics. Since quantum mechanical models computed from first principles (i.e., without any empirical input) require a large amount of computational power, the size of a system that can be described by accurate quantum mechanical models is limited to about 50 atoms (using current computer technology) [84]. Classical models, on the other hand, can neglect important quantum effects that give nanoscale devices their unique properties [84]. These constraints make the modeling of systems at the nanoscale very challenging.

Although fundamental nanoscience biocomputation is not easy, it could be argued that modeling at the tumor scale should not be burdened by this complexity when considering nanotechnology in cancer treatment. The reason is that the bulk behavior of nanodevices is what matters the most at the tumor level—not the behavior of individual particles of

sizes on the order of 10^{-9} m. It takes millions of nanoparticles and their combined effect to influence a tumor of size order 10^{-3} m. Thus, modeling of cancer drug treatment via nanotechnology could stay focused at the tumor scale by considering the collective behavior of nanodevices.

15.3.2. Modeling at the Tumor Scale

The foundation for models of nanoparticle delivery of cancer therapy depends on an accurate physico-chemical description of the tumor microenvironment, the parameters of which are derived from the study of cancer biology. Fundamental facts regarding tumor behavior have been well described ([1], pp. 1313–62). At a cellular level, tumor cells are transformed cells that have evaded natural cell senescence. All cells require a supply of energy to live and they produce metabolic waste. Cancer cells can survive where normal cells would die, such as under hypoxic, hypoglycemic and acidic conditions. They can adapt to changing micro-environmental conditions to develop resistance to therapy. They can sometimes thrive outside their natural environment, leading to metastases.

At a tumor (system) level, aggregates of cells will affect each other through mechanical forces in three-dimensional space in such a manner that some cells will have more access to nutrients than others. At first glance, the cell aggregate would be expected to expand as a perfect sphere if all cells on the periphery experienced the same mechanical forces. Experimental and clinical observation has shown that such perfect symmetry is usually not the case. The reason is that from a molecular perspective, the extracellular environment that each cell experiences (e.g. nutrient concentration) can vary quite dramatically, leading to favored cells entering the cell cycle more often than cells whose extracellular cues are more adverse ([1], pp. 985–6). Nutrient competition leads to the selection of cells favored for maximal proliferation in certain regions. Cells on the periphery of a tumor are favored and tend to proliferate faster than cells that are surrounded by other tumor cells. The morphology of a tumor can be seen as a function of response to various environmental fluctuations, including this nutrient diffusional instability [14, 15, 79, 92]. The goal of cancer modeling is to describe the actual detailed behavior of a cell aggregate as predicted by the proper physical formulation. The underlying hypothesis is that if the main components of this physical formulation are identified and abstracted to a mathematical level, then this formulation can be represented as an *in silico* system capable of predicting and shedding insight into the behavior of real tumors.

The history of the study of tumor biology via physical formulations has been long and insightful. For an excellent review, refer to Araujo and McElwain (2005). Cancer growth, angiogenesis, metastasis, etc. have all been abstracted to a mathematical level. A recent biocomputational implementation by Zheng *et al.* [92] encompassed some of the main physical characteristics of cancer growth and created an *in silico* system that exhibited combined two-dimensional tumor growth and angiogenesis. This system captured the complicated morphology and connectedness at the tumor/tissue interface, including invasive fingering, tumor fragmentation, and healthy tissue degradation. Implementation allowed for simulation of tumoral lesions through the stages of diffusion-limited dormancy, localized necrosis, vascularization and rapid growth, and tissue invasion in multiple spatial dimensions. Angiogenesis was included as a continuous feedback process involving tissue

growth and nutrient demand. An application of this simulator to chemotherapy is described in the following section.

15.3.3. Modeling of Cancer Therapy

There are currently few biocomputational models that specifically consider nanotechnology as part of cancer treatment, especially taking into account the physical multidimensionality of the tumor mass. Recently, Sinek *et al*. [79] have studied nanoparticle mediated drug delivery and tumor response using the tumor simulator of Zheng *et al*. [92]. Their multi-scale and multi-dimensional simulations demonstrated the potential increased efficacy of nanoparticle-based therapy as well as its potential weaknesses, due principally to transport limitations. They assumed a best-case scenario involving an homogenous tumor with one cell type that was also assumed to be drug-sensitive, low host tissue toxicity due to targeted drug delivery, and a constant nanoparticle drug carrier delivery at levels calibrated to be lethal to *in vitro* cell culture on a time scale of hours. Two ends of a spectrum were considered: therapy involving very small (1–10 nm) nanoparticles that extravasate from tumor vasculature, diffuse within the interstitium, and target cells, and therapy involving large (100 nm) nondiffusing nanoparticles that are assumed to remain at their point of extravasation from the vasculature and to function as a constant source of drug. In both cases nanoparticles were assumed to be delivered only to the tumor due to large vasculature openings. Because of lower toxicity, larger and more uniform drug concentrations were delivered to tumor cells over longer time periods in comparison to traditional free-drug administration protocols.

However, their simulations also showed that nanoparticle-based chemotherapy could suffer from the same fundamental transport limitations as free-drug administration. Competition between vasculature density, which favors nutrient and nanoparticle extravasation, and intratumoral pressure, which may oppose it, could result in non-uniform delivery. Diffusion of nutrient molecules and drug carrier within the tumor interstitium may further contribute to this inhomogeneity. Figure 15.5 shows non-uniform intratumoral distributions in simulated chemotherapy involving a continuous blood-serum concentration of 1–10 nm nanoparticles.

Sinek *et al*. [79] also simulated the effects of antiangiogenic therapy on tumor vasculature. It had been previously proposed that this therapy could "normalize" tumor vasculature through more efficient and uniform delivery of molecules and particles [38]. In this simulation, larger 100 nm particles were assumed to extravasate uniformly along the normalized vasculature and to release drug at a constant rate. Although Figure 15.6 shows that tumor regression was significantly higher than in the previous simulation, Figure 15.7 indicates that there was drug concentration inhomogeneity. As a result of non-uniform delivery of chemotherapy, tumor regression was likewise non-uniform, being highest around areas of maximum drug extravasation. Average *in vivo* cell death rates as simulated *in silico* were several orders of magnitude lower than those calibrated *in vitro*. Perhaps more importantly, non-uniformity of tumor regression consistently led to fragmentation (Figure 15.7) and new stable tumor mass at significant levels (Figure 15.6).

In order to be more complete, modeling of chemotherapy using nanoparticles should include other factors that affect tumor growth. These include hypoxic cycling cells and a heterogeneous population of genotypes, some of which are resistant to the drug. Also, the

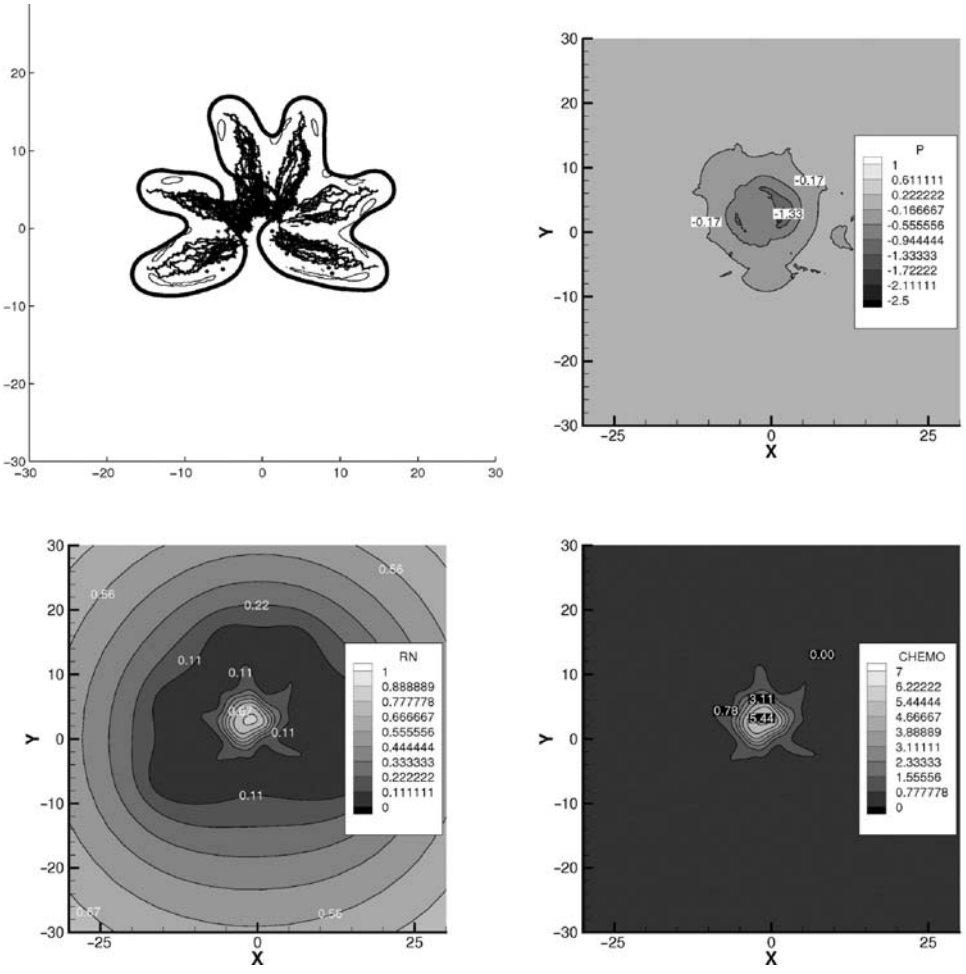

FIGURE 15.5. Top left: Stable highly perfused tumoral lesion proliferating around blood vessels. Solid thick perimeter: tumor boundary; solid thin perimeter: necrotic areas; solid: blood vessels. Top right: Tumor pressure distribution. Bottom left: Nutrient concentration. Bottom right: Nanoparticle distribution. All variables are dimensionless. Adapted from Biomedical Microdevices, Vol 6, 2004, p. 306, Sinek *et al*., Figure 4b, © 2004 Kluwer Academic Publishers. With kind permission of Springer Science and Business Media.

contribution to pressure within the tumor by the mass of necrotic cells may not be negligible [79]. The nanoparticles themselves could be better modeled by augmenting the knowledge of how they work *in vivo*, considering issues of vessel extravasation, clustering, interstitial diffusion, interaction with tissue, and erosion at the tumor site.

For instance, the pH of the microenvironment is a significant aspect controlling the degradation kinetics of many pharmaceutically relevant polymers, since hydrolysis rates can vary by orders of magnitude at different pH values [49]. The tumor extracellular environment is more acidic than normal tissue because of lactic acidosis from glycolysis [26]. The poorly perfused tumor vasculature maintains the acidic environment as well. Polymer nanoparticle degradation will thus be affected. Furthermore, as these polymers degrade into acids, the pH

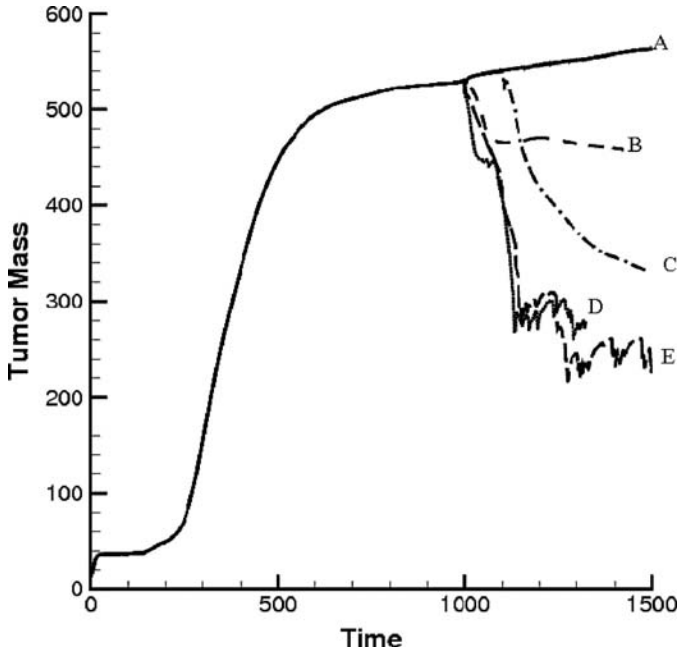

FIGURE 15.6. Simulation of tumor mass growth and regression as a function of time. The non-dimensionalized time unit is ≈3.3 days. (A) Without chemotherapy; (B) With chemotherapy via small, diffusing nanoparticles (Fig. 15.5); (C) With chemotherapy via large, non-diffusing nanoparticles and adjuvant anti-antiangiogenic therapy (Fig. 15.7); (D), (E) Simulations corresponding to cases (B) and (C) but assuming higher blood vessel mobility. Adapted from Biomedical Microdevices, Vol. 6, 2004, p. 303, Sinek *et al*., Figure 3, © 2004 Kluwer Academic Publishers. With kind permission of Springer Science and Business Media.

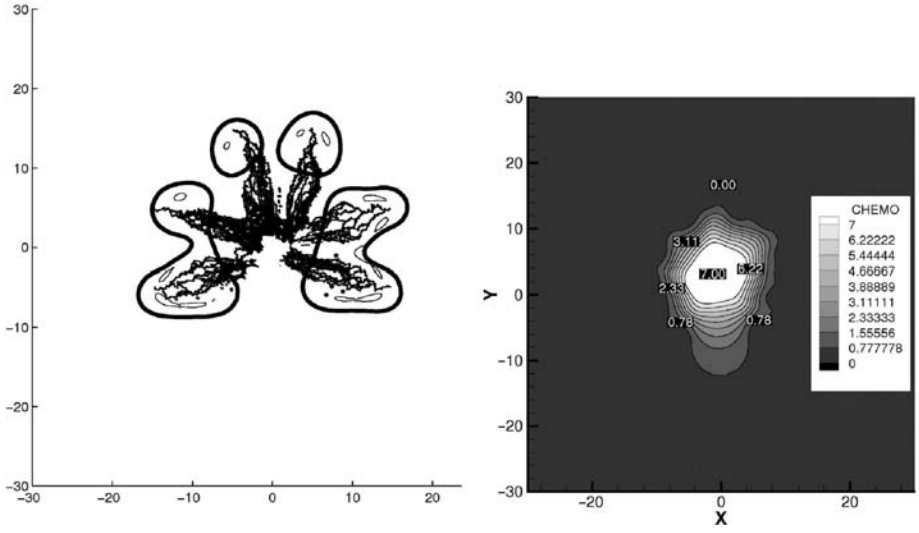

FIGURE 15.7. Left: Tumor regression with undesirable mass fragmentation after application of chemotherapy plus antiangiogenic therapy via nanoparticles. Right: Drug distribution. Adapted from Biomedical Microdevices, Vol 6, 2004, p. 307, Sinek *et al*., Figure 5, © 2004 Kluwer Academic Publishers. With kind permission of Springer Science and Business Media.

of the device microenvironment will be lowered as drug is released, inducing an autocatalytic effect that could lead to accelerated polymer degradation.

The specific nanoparticle device characteristics, such as material type, shape and size, as well as encapsulated drug, determine the mass transfer processes and chemical reactions controlling drug release. A biocomputational model should not only take these factors into account, but also drug release characteristics *in vivo*, such as cellular tissue reactions and osmotic pressure, which may bear significantly on particle degradation and resulting drug release kinetics [74]. The characteristics of drug release for collections of nanoparticles may then yield input parameters that can be used in continuum models at the tumor scale.

15.4. OUTLOOK ON MODELING

Biocomputation at the service of cancer treatment via nanotechnology may be of value in dealing both with the issues of chemotherapy delivery and in the design and manufacture of nanodevices that could be effective in cancer prevention, early detection and diagnosis. At a system level, modeling at the tumor scale could be enhanced by taking into account cell genetic characteristics, such as mutations of oncogenes and apoptosis-suppressor genes. Models of angiogenesis could incorporate the co-option of existing vessels, and perhaps even the morphology, flow, and pressure of vessels at specific sites in the body affected by tumor growth. Drug release characteristics of nanoparticles could be quantified in terms of drug release parameter values that become part of the model at the tumor scale. The ultimate goal, and "holy grail" in this field, would be to enable the clinical application of biocomputation to design cancer treatment via nanotechnology based on a particular patient's physiological conditions. The results of the *in silico* model could then provide valuable diagnostic, prognostic, and therapeutic information.

We conclude by noting that biocomputation predicts that the transport of nanoparticles, aimed at therapy at the individual cell level, should be expected to be ruled by the same type of physical phenomena as similarly sized molecules in the human body. For example, the laws of diffusion along a concentration gradient and convection along a pressure gradient still apply. It thus seems that delivery of nanoparticles through the circulation to a tumor would encounter similar issues as traditional chemotherapy, and that these issues may challenge nanodevices of the future. It may be noteworthy to mention how the body may react against cancer: some tumors can be heavily infiltrated by macrophages [50, 70], in a natural endogenous response to a wound that is growing faster than it can heal. Macrophages not only reach the tumor by passive transport, but can also diapadese into it. Perhaps nanodevices and nanoparticles that are engineered to behave like macrophages will be able to reach and stay at the tumor site in significant numbers to completely dispose of malignant cells. Future work in biocomputation can be expected to help formulate the details of such an active transport homing, as well as other mechanisms, in order to achieve a clinically successful response in the treatment of cancer.

REFERENCES

[1] Alberts et al. *Molecular Biology of the Cell*. Taylor & Francis Group, New York, 2002.

[2] R.P. Araujo and D.L.S. McElwain. A history of the study of solid tumour growth: the contribution of mathematical modeling. *Bull. Math. Biol.*, June 2004. In press.

[3] The A to Z of Nanotechnology at website www.azonano.com.
[4] D. Barbolosi and A. Iliadis. Optimizing drug regimens in cancer chemotherapy: a simulation study using a PK-PD model. *Comp. Biol. Med.*, 31:157–172, 2001.
[5] L.A. Bauer. *Applied Clinical Pharmacokinetics*. Mc-Graw Hill, New York. pp. 26–45, 2001.
[6] L.T. Baxter and R.K. Jain. Pharmacokinetic analysis of the microscopic distribution of enzyme-conjugated antibodies and prodrugs: Comparison with experimental data. *Brit. J. Canc.*, 73(4):447–456, 1996.
[7] K.L. Black and N.S. Ningaraj. Modulation of brain tumor capillaries for enhanced drug delivery selectively to brain tumor. *Cancer Control.*, 11(3):165–173, 2004.
[8] G. Bonadonna, M. Zambetti, and P. Valagussa. Sequential or alternating doxorubicin and CMF regimens in breast cancer with more than three positive nodes: ten year results. *J. Am. Med. Assoc.*, 273:542–547, 1995.
[9] I. Brigger, C. Dubernet, and P. Couvreur. Nanoparticles in cancer therapy and diagnosis. *Adv. Drug Del. Reviews.*, 54(5):631–651, 2002.
[10] CCO Formulary, Liposomal Doxorubicin, October 2003.
[11] M. Chaplain and A. Anderson. Mathematical modelling of tumour-induced angiogenesis: network growth and structure. *Cancer Treat Res.*, 117:51–75, 2004.
[12] M.L. Citron, D.A. Berry, C. Cirrincione, C. Hudis, E.P. Winer, W.J. Gradishar, N.E. Davidson, S. Martino, R. Livingston, J.N. Ingle, E.A. Perez, J. Carpenter, D. Hurd, J.F. Holland, B.L. Smith, C.I. Sartor, E.H. Leung, J. Abrams, R.L. Schilsky, H.B. Muss, and L. Norton. Randomized trial of dose-dense versus conventionally scheduled and sequential versus concurrent combination chemotherapy as postoperative adjuvant treatment of node-positive primary breast cancer: first report of intergroup trial C9741/cancer and leukemia group B trial 9741. *J. Clin. Oncol.*, 21(8):1431–1439, 2003.
[13] P. Costa and J.M.S. Lobo. Modeling and comparison of dissolution profiles. *Eur. J. Pharm. Sci.*, 13:123–133, 2001.
[14] V. Cristini, J. Lowengrub, and Q. Nie. Nonlinear simulation of tumor growth. *J. Math. Biol.*, 46:191–224, 2003.
[15] V. Cristini, H.B. Frieboes, R. Getemby, S. Corenta, M. Ferrari, and S. Sinek. Morphological instability and cancer inussion. *Clin. Cancer Res.*, (in press) 2005.
[16] Z. Cui, C.H. Hsu, and R.J. Mumper. Physical characterization and macrophage cell uptake of mannan-coated nanoparticles. *Drug Dev. Ind. Pharm.*, 29(6):689–700, 2003.
[17] M.S. Dordal, A.C. Ho, M. Jackson-Stone, Y.F. Fu, C.L. Goolsby, and J.N. Winter. Flow cytometric assessment of the cellular pharmacokinetics of fluorescent drugs. *Cytometry*, 20:307–314, 1995.
[18] P. Elamanchili, M. Diwan, M. Cao, and J. Samuel. Characterization of poly(D,L-lactic-co-glycolic acid) based nanoparticulate system for enhanced delivery of antigens to dendritic cells. *Vaccine*, 22(19):2406–12, 2004.
[19] R.E. Eliaz, S. Nir, C. Marty, Jr. F.C. Szoka. Determination and modeling of kinetics of cancer cell killing by doxorubicin and doxorubicin encapsulated in targeted liposomes. *Canc. Res.*, 64:711–718, 2004.
[20] EPSRC Research Priorities and Opportunities at http://www.epsrc.ac.uk/ContentLiveArea/Downloads.
[21] N. Faisant, J. Siepmann, and J.P. Benoit. PLGA-based microparticles: elucidation of mechanisms and a new, simple mathematical model quantifying drug release. *Eur. J. Pharm. Sci.*, 15(4):355–366, 2002.
[22] S.S. Feng and S. Chien. Chemotherapeutic engineering: application and further development of chemical engineering principles for chemotherapy of cancer and other diseases. *Chem. Eng. Sci.*, 58:4087–4114, 2003.
[23] X. Gao, Y. Cui, R.M. Levenson, L.W. Chung, and S. Nie. In vivo cancer targeting and imaging with semiconductor quantum dots. *Nat. Biotechnol.*, 22(8):969–76, 2004.
[24] B. Gompertz. On the nature of the function expressive of the law of human mortality, and on a new mode of determining the value of life contingencies. *Philos. Trans. R Soc. Lond.*, 115:513–583, 1825.
[25] R. Gref, P. Couvreur, G. Barratt, and E. Mysiakine. Surface-engineered nanoparticles for multiple ligand coupling. *Biomaterials*, 24(24):4529–4537, 2003.
[26] C.J. Gulledge and M.W. Dewhirst. Tumor oxygenation: a matter of supply and demand. *Anticancer Res.*, 16:741–750, 1996.
[27] H. Hashizume, P. Baluk, S. Morikawa, J.W. McLean, G. Thurston, S. Roberge, R.K. Jain, and D.M. McDonald. Openings between defective endothelial cells explain tumor vessel leakiness. *Am. J. Pathol.*, 156(4):1363–1380, 2000.
[28] T. Higuchi. Rate of release of medicaments from ointment bases containing drugs in suspension. *J. Pharm. Sci.*, 50:874–875, 1961.

[29] S.K. Hobbs, W.L. Monsky, F. Yuan, W.G. Roberts, L. Griffith, V.P. Torchilin, and R.K. Jain. Regulation of transport pathways in tumor vessels: Role of tumor type and microenvironment. *Proceedings of the National Academy of Sciences of the United States of America*. vol. 95, pp. 4607–4612, 1998.
[30] M. Holz and A. Fahr. Compartment modeling. *Adv. Drug Del. Rev.*, 48(2–3):249–264, 2001.
[31] M. Hombreiro-Perez, J. Siepmann, C. Zinutti, A. Lamprecht, N. Ubrich, M. Hoffman, R. Bodmeier, and P. Maincent. Non-degradable microparticles containing a hydrophilic and/or a lipophilic drug: preparation, characterization and drug release modeling. *J. Control. Rel.*, 88(3):413–428, 2003.
[32] A. Ito, Y. Kuga, H. Honda, H. Kikkawa, A. Horiuchi, Y. Watanabe, and T. Kobayashi. Magnetite nanoparticle-loaded anti-HER2 immunoliposomes for combination of antibody therapy with hyperthermia. *Cancer Lett.*, 212(2):167–175, 2004.
[33] T.L. Jackson and H.M. Byrne. A mathematical model to study the effects of drug resistance and vasculature on the response of solid tumors to chemotherapy. *Math. Biosci.*, 164:17–38, 2000.
[34] R.K. Jain and L.E. Gerlowski. Extravascular transport in normal and tumor tissues. *Crit. Rev. Oncol. Hematol.*, 5(2):115–170, 1986.
[35] R.K. Jain. Determinants of tumor blood flow: a review. *Canc. Res.*, 48:2641–2658, 1988.
[36] R.K. Jain and L.T. Baxter. Mechanisms of heterogeneous distribution of monoclonal antibodies and other macromolecules in tumors: Significance of elevated interstitial pressure. *Cancer Res.*, 48:7022–7032, 1988.
[37] R.K. Jain. Physiological barriers to delivery of monoclonal antibodies and other macromolecules in tumors. *Cancer Res.* (Suppl.), 50:814s–819s, 1990.
[38] R.K. Jain. Delivery of molecular medicine to solid tumors: Lessons from in vivo imaging of gene expression and function. *J. Control. Rel.*, 74:7–25, 2001.
[39] R.K. Jain. Normalizing tumor vasculature with anti-angiogenic therapy: A new paradigm for combination therapy. *Nature Med.*, 7(9):987–989, 2001.
[40] H. Kitano. Computational systems biology. *Nature*, 420:206–210, 2002.
[41] N. Kohler, G.E. Fryxell, and M. Zhang. A bifunctional poly(ethylene glycol) silane immobilized on metallic oxide-based nanoparticles for conjugation with cell targeting agents. *J. Am. Chem. Soc.*, 126(23):7206–7211, 2004.
[42] K. Kosmidis, P. Argyrakis, and P. Macheras. A reappraisal of drug release laws using Monte Carlo simulations: the prevalence of the Weibull function. *Pharm. Res.*, 20(7):988–995, 2003.
[43] J. Kreuter. Nanoparticles. In J. Kreuter, (ed.), *Colloidal Drug Delivery Systems*, Marcel Dekker, Inc. New York, Basel, Hong Kong, 1994.
[44] A.K. Laird. Dynamics of tumor growth. *Br. J. Canc.*, 18:490–502, 1964.
[45] R. Langer and N.A. Peppas. Chemical and physical structure of polymers as carriers for controlled release of bioactive agents: A review. *Rev. Macromol. Chem. Phys.*, C23:61–126, 1983.
[46] R. Langer. Drug delivery and targeting. *Nature*, 392:6679, 5–10, 1998.
[47] R. Langer. Biomaterials in drug delivery and tissue engineering: One laboratory's experience. *Acc. Chem. Res.*, 33:94–101, 2000.
[48] J. Lankelma. Tissue transport of anti-cancer drugs. *Curr. Pharm. Des.*, 8:1987–1993, 2002.
[49] K.W. Leong, B.C. Brott and R. Langer. Bioerodible polyanhydrides as drug-carrier matrices. I: Characterization, degradation and release characteristics. *J. Biomed. Mat. Res.*, 19:941–955, 1985.
[50] A. Mantovani. Biology of disease. Tumor-associated macrophages in neoplastic progression: a paradigm for the in vitro function of chemokines. *Lab. Invest.*, 71:5–16, 1994.
[51] U. Massing and S. Fuxius. Liposomal formulations of anticancer drugs: selectivity and effectiveness. *Drug Resis. Updat.*, 3:171–177, 2000.
[52] S.R. McDougall, A.R.A. Anderson, and M.A.J. Chaplain et al. Mathematical modelling of flow through vascular networks: Implications for tumour-induced angiogenesis and chemotherapy strategies. *Bull. Math. Biol.*, 64(4):673–702, 2002.
[53] K. Maruyama. In vivo targeting by liposomes. *Biol. Pharm. Bull.*, 23(7):791–799, 2000.
[54] L.D. Mayer and J.A. Shabbits. The role of liposomal drug delivery in molecular and pharmacological strategies to overcome multidrug resistance. *Can. Metas. Rev.*, 20:87–93, 2001.
[55] B. Narasimhan. Mathematical models describing polymer dissolution: Consequences for drug delivery. *Adv. Drug Del. Rev.*, 48(2–3):195–210, 2001.
[56] National Cancer Institute (NCI) website at http://press2.nci.nih.gov/sciencebehind/nanotech.
[57] National Cancer Institute website at http://www.cancer.gov/clinicaltrials/results/dose-dense0604.

[58] National Institute of Nanotechnology (NINT) of Canada at www.industrymailout.com/industry/do/news/view?id=9003&print=1.
[59] P. Netti and R.K. Jain. Interstitial transport in solid tumors. In L. Preziosi (ed.), *Cancer Modeling and Simulation*, Chapman & Hall/CRC, Boca Raton, London, New York, Washington, pp. 62–65, 2003.
[60] NIH Publication No 04-5489 by National Cancer Institute. cancer *NANOTECHNOLOGY—Going Small for Big Advances. Jan. 2004*.
[61] National Institutes of Health (NIH) website at http://press2.nci.nih.gov/sciencebehind/nanotech.
[62] L. Norton. Implications of kinetic heterogeneity in clinical oncology. *Semin. Oncol.*, 12:231–249, 1985.
[63] L. Norton and R. Simon. The Norton-Simon hypothesis revisited. *Cancer. Treat. Res.*, 70:163–169, 1986.
[64] L. Norton. A Gompertzian model of human breast cancer growth. *Cancer. Res.*, 48:7067–7071, 1988.
[65] L. Norton. Theoretical concepts and the emerging role of taxanes in adjuvant therapy. *Oncologist*, 3 (suppl):30–35, 2001.
[66] L. Norton. Karnofsky Memorial Lecture. ASCO, 2004.
[67] T.P. Padera, B.R. Stoll, J.B. Tooredman, D. Capen, E. di Tomaso, and R.K. Jain. Cancer cells compress intratumour vessels. *Nature*, 427:695, 2004.
[68] R.S. Parker and F.J. Doyle III. Control-relevant modeling in drug delivery. *Adv. Drug Del. Rev.*, 48(2–3):211–228, 2001.
[69] N.A. Peppas. Analysis of Fickian and non-Fickian drug release from polymers. *Pharm. Acta. Helv.*, 60:110–111, 1985.
[70] P.J. Polverini. How the extracellular matrix and macrophages contribute to angiogenesis-dependent diseases. *Eur. J. Cancer*, 32A(14):2430–2438, 1996.
[71] F. Quian, G.M. Saidel, D.M. Sutton, A. Exner, and J. Gao. Combined modeling and experimental approach for the development of dual-release polymer millirods. *J. Control. Rel.*, 83:427–435, 2002.
[72] P. Sapra and T.M. Allen. Ligand-targeted liposomal anticancer drugs. *Prog. Lipid Res.*, 42:439–462, 2003.
[73] M. Sarntinoranont, F. Rooney, and M. Ferrari. Interstitial stress and fluid pressure within a growing tumor. *Ann. Biomed. Eng.*, 31:327–335, 2003.
[74] J. Siepmann and A. Goepferich. Mathematical modeling of bioerodible, polymeric drug delivery systems. *Adv. Drug Del. Rev.*, 48(2–3):229–247, 2001.
[75] J. Siepmann, N. Faisant, and J.P. Benoit. A new mathematical model quantifying drug release from bioerodible microparticles using Monte Carlo simulations. *Pharm. Res.* 19(12):1885–1893, 2002.
[76] J. Siepmann and N.A. Peppas. Modeling of drug release from delivery systems based on hydroxypropyl methylcellulose (HPMC). *Adv. Drug Del. Rev.*, 48(2–3):139–157, 2003.
[77] J. Siepmann, N. Faisant, J. Akiki, J. Richard, and J.P. Benoit. Effect of the size of biodegradable micro particles on drug release: experiment and theory. *J. Control. Rel.*, 96(1):123–134, 2004.
[78] S. Simoes, J.N. Moreira, C. Fonseca, N. Duezguenes, and M. Pedroso de Lima. On the formulation of pH-sensitive liposomes with long circulation times. *Adv. Drug Del. Rev.*, 56:947–965, 2004.
[79] J. Sinek, H.B. Frieboes, X. Zheng, and V. Cristini. Chemotherapy simulations demonstrate fundamental transport limitations involving nanoparticles. *Biomed. Microdev.*, 6(4):297–309, 2004.
[80] T. Sonoda, H. Kobayashi, T. Kaku, T. Hirakawa, and H. Nakano. Expression of angiogenesis factors in monolayer culture, multicellular spheroid and in vivo transplanted tumor by human ovarian cancer cell lines. *Cancer Lett.*, 196:229–237, 2003.
[81] M.Y. Su, A.A. Najafi, and O. Nalcioglu. Regional comparison of tumor vascularity and permeability parameters measured by albumin–Gd-DTPA and Gd-DTPA. *Magn. Reson. Med.*, 34(3):402–411, 1995.
[82] M.Y. Su, A. Muhler, X. Lao, and O. Nalcioglu. Tumor characterization with dynamic contrast-enhanced MRI using MR contrast agents of various molecular weights. *Magn. Reson. Med.*, 39(2):259–69, 1998.
[83] B.G. Sumpter, D.W. Noid, and M.D. Barnes. Recent developments in the formation, characterization, and simulation of micron and nano-scale droplets of amorphous polymer blends and semi-crystalline polymers. *Polymer*, 44(16):4389–4403, 2003.
[84] Trends in NanoTechnology (TNT) 2001 at nanoword.net/library/weekly/ aa091201a.htm.
[85] P. Veng–Pedersen. Noncompartmentally-based pharmacokinetic modeling. *Adv. Drug Del. Rev.*, 48(2–3):265–300, 2001.
[86] C.H. Wang, J. Li, and C.S. Teo et al. The delivery of BCNU to brain tumors. *J. Control. Rel.*, 61(1–2):21–41, 1999.
[87] W. Weibull. A statistical distribution of wide applicability. *J. Appl. Mechan.*, 18:293–297, 1951.
[88] C.P. Winsor. The Gompertz curve as a growth curve. *Proc. Natl. Acad. Sci.*, 18:1–8, 1932.

[89] F. Yuan, M. Dellian, D. Fukumura, M. Leunig, D.A. Berk, V.P. Torchilin, and R.K. Jain. Vascular permeability in a human tumor xenograft: molecular size dependence and cutoff size. *Cancer Res.*, 55(17):3752–3756, 1995.

[90] H.S. Zaki, M.D. Jenkinson, D.G. Du Plessis, T. Smith, and N.G. Rainov. Vanishing contrast enhancement in malignant glioma after corticosteroid treatment. *Acta Neurochir.*, 146:841–845, 2004.

[91] M. Zhang, Z. Yan, L.L. Chow, and C.H. Wan. Simulation of drug release from biodegradable polymeric microspheres with bulk and surface erosions. *J. Pharm. Sci.*, 92(10):2040–2056, 2003.

[92] X. Zheng, S.M. Wise, and V. Cristini. Nonlinear simulation of tumor necrosis, neo-vascularization and tissue invasion via an adaptive finite-element/level-set method. *Bull. Math. Bio.*, 67:211–259, 2005.