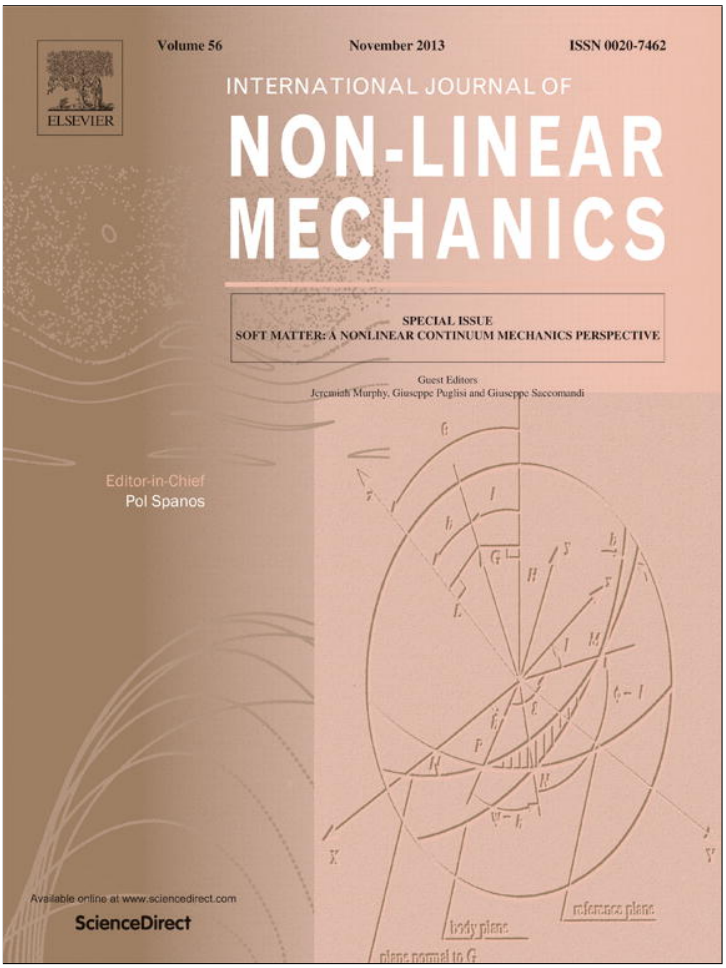

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Problems in (nano)medical mechanics

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ABSTRACT

Three major biomedical problems in non-linear mechanics are stated, and several sub-problems are derived from simplifications that correspond to substituting model nanotechnology-based systems for their biological counterpart. Strategies for the solutions to these problems are briefly proposed. The medical implications of the solutions to the general and simplified problems are discussed, and perspectives on the deep transformation in health care these solutions would engender are presented.

1. Introduction

The overall philosophy of this paper encompasses the following:

1. To *illustrate mathematical problems in biomedical mechanics, by stating them in general terms and discussing their significance*. Three such problems will be presented: The transport of nanoscale mass through the body; The transport of mass through nanoscale environments, with particular emphasis on signaling pathways inside of cells; and The development of predictive anatomy. For ease of reference, the General Problems (GP) will be referred to as GP1, GP2 and GP3 in what follows. GP1 and GP2 are related but different, since GP1 primarily addresses transport conditions through non-nanoscale biophysical domains.
2. *To identify simplifications to these problems, which are amenable to solutions that offer insights into solutions of the general problems in biomedical mechanics*. These simplifications are based on currently available nanotechnology platforms, so that these can be used for experimental verification. For GP1, the simplification arises from the use of synthetic nanoparticles of known properties, to model the nanoscale objects being transported throughout the body. For GP2, the simplification is the use of synthetic nanochannels of known properties as models for the nanoscale environments. The GP3 is simplified by reference to the optimal homogenization of biohybrid composites (biological and synthetic components) comprising nanoscale phases of known properties. For each GP, several additional problems arise from the discussion, which involve the above-listed simplifications. These shall be referred to as Simplified Problems (SP) 1–11. To enhance continuity in the narrative of the paper, the introduction of each GP is followed by the statement and discussion of the SP that pertain to it.
3. *To illustrate the significance of the solutions to the SP for securing advances against cancer and other diseases, as well as developing novel perspectives over the basic understanding of these maladies, and the nature of their differences from "health"*. Comments toward these goals are inter-dispersed within the paper's narrative, and an overarching discussion is presented in the section following the presentations of all the GP and SP.
4. *To discuss the common characteristics of the problems introduced*. This will be done in a section following the discussion of the three general problems, and their simplified counterparts.
5. *To identify and discuss further classes of "Super" General Problems, for future reference*. These problems include the mass transport of nanoscale objects through nanoscale environments; and the design of systems that optimize transport properties. This discussion will conclude the paper.

Before the statement of the general problems, however, the balance of this introductory section will be dedicated to some current aspects of nanotechnology and nanomedicine. Familiarity with these will aid in obtaining the simplified problems from the general problems, and in the discussion of the implications of their solutions in the clinic.

In view of the broadly interdisciplinary nature of the subject matters presented herein, an accurate survey of the literature would have much exceeded the assigned scope of this work. My apologies to those who have provided major contributions to the many subject areas discussed in this paper, and whose work is not properly cited.

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1.1. A primer of some aspects of the field of nanomedicine

Nanotechnology has gained wide acceptance in contemporary life, with thousands of commercially available products [1,2], including the complete overhaul of the electronics industry [3–8]. An extraordinary amount of attention is dedicated to nanotechnology and nanoscience by the scientific community [9,10], including the awarding of several Nobel prizes in Physics (1986) and Chemistry (1996) for discoveries and inventions that are foundational for the nano-disciplines. Long before words with the "nano" prefix had become common parlance in science, pharmaceutical preparations had been approved for clinical use, which employ vectors that meet the mathematical definition of nanoparticles, and thereby most logical, and even many “official” definitions of nanotechnologies.

The first nanovectors to gain regulatory approval were liposomes, i.e. globules composed of mostly fatty substances (cholesterol), which mimic the membranes surrounding biological cells. The first nanomedicines (or also "nanodrugs" in what follows) were liposomes of dimensions in the 100 nm order, comprising the anticancer drug doxorubicin [11,12] or the antifungal agent amphiteracin B [13], also used in clinical oncology. These drugs already were, and still are used quite widely, and with substantial benefit, in cancer clinics in their "naked" form (i.e., without encapsulation in nanoparticles). However, their nanoformulations offer the advantage that they localize in greater percentage at the tumor or target site, where they can provide enhanced therapeutic effects. At the same time, they present a reduction in the relative concentrations in non-target tissues or organs, where the drugs can create undesired, adverse collateral damage. This combined notion of enhanced therapy and reduced adverse effects is summarized by the expression “improved therapeutic index”. Another factor that contributes to the enhancement of the therapeutic index is the “PEG-ylation” of the liposomes, that is, the coating of their external surfaces with polyethylene glycol (PEG) to make them more hydrophilic, and therefore able to delay sequestration by the “trapping” and filtering organs of the body, which comprise the “Reticulo-Endothelial System” or RES. The main organs of the RES are the liver, the spleen, the bone marrow and the lungs: Nanoparticles without PEG-ylation or some alternative method of evasion from the RES are typically trapped within the liver and spleen within seconds or minutes from injection, without the ability to reach the target tumor. Different forms of PEG-ylation may extend circulatory half-life to hours and days, and thereby enhance tumor-site accumulation.

The reasons why liposomes are successful in concentrating with some degree of preference at the tumor site are their ability to penetrate preferentially the blood vessel walls that feed the tumors, in combination with the extended circulatory time. The enhanced penetration is true especially in the high growth, angiogenic phase of tumor development, when the tumor grows and recruits novel blood vessels to sustain its continued growth. The walls of the tumor-associated new blood vessels are hyperpermeable because of the presence of a large number of architectural defects – essentially large openings or pores (fenestrations) that permit the passage of suitably sized nanoparticles, which normally are impermeant through the vascular walls (endothelium) [14,15]. This selection process is termed “Enhanced Permeation and Retention" or EPR [16,17].

Thus – and this is the important lesson to be learned from the history of the development of liposomes – preferential concentrations of therapeutic substances at desired target sites can be secured, by designing nanoparticles that exploit the differences in biological barriers (such as the vascular endothelium) that accompany the growth and presentation of cancers [18]. A similar interpretation actually applies to the role of PEG-ylation in the temporary evasion of the RES: It is a means to advantageously negotiate a biological barrier. Today there are several classes of nanodrugs in clinical use [19,20], which are used in thousands of patients worldwide, achieving several billions of dollars in sales, and derive largely their localization preferences from the EPR effect. One major step forward was achieved with the clinical approval of albumin nanoparticles [21,22], which also take advantage of the enhanced transport properties of albumin molecules across the endothelium. No nanodrug was ever approved that bases its improved therapeutic index on the ability to recognize the target cancer cell, through molecular recognition agents (e.g. antibodies, aptamers, peptides) conjugated on its external surface, though this is an extremely active area of research worldwide [23–26].

Multistage vectors (MSV) were recently introduced by our laboratory [27–29] and others [30]. These comprise systems of drug-carrying nanoparticles nested within larger particles, so that each "stage" of the delivery system can traverse a suitable number of sequential biological barriers. Fig. 1 summarizes the three basic types or "generations" [31] of nanoparticles, and the mechanism of EPR.

A second nanotechnology platform of interest for this paper is the "nanochannel", which gave origin to the field of "nanofluidics" [32]. These nanochannels are formed with exquisite control over the key dimension, as small as 2.5 nm [33], and the channel wall chemistry. They are fabricated on silicon wafers, employing a method (Sacrificial Layer Technique) which is in some ways germane to the manufacturing protocols used for the electronics industry, and therefore easily amenable to large-scale manufacturing with exceptionally precise quality control methodologies. Nanochannel Delivery Systems (nDS) are capsules for subcutaneous implantation, which release therapeutic molecules to the body through membranes that contain nanochannels of predetermined shape and channel wall surface chemistry. The nDS can act as therapeutic "nanoglands" in three ways (Fig. 2): (1) By releasing therapeutic molecules into the surrounding tissues at constant release rate for prolonged periods of time (weeks to months, and even years) [34], based on diffusive mechanisms alone; (2) By releasing therapy with electrokinetic "active" controls, that allow for the variation of release rates by preprogramming, or by external activation, or by "intelligent" feedback loops [35]; and (3) By acting as an in vivo bioreactor. In the third method, live cells or transplanted tissues are encapsulated within the nDS, and the nanochannel membranes act both as immunisolators against rejection phenomena, and as molecular transport-metering ports. The nDS systems are in preclinical validation for different medical contexts such as cancer, analgesia, diabetes, endocrine and infectious diseases [34,36].

The third example of nanotechnologies in medicine is the class of "bio-nano scaffolds" (BNS) for implants to be employed for medical tissue regeneration, such as the regrowth of bone and the associated soft tissues destroyed by trauma or disease [37–42]. The BNS are composite materials that comprise both biological components and synthetic phases. Among the former are stem cells, which are required for any and all forms of "regrowth" in the body, and biological molecules, such as bone morphogenetic proteins, angiogenic factors, and antibiotics. In order to survive, properly differentiate into various lineages of cells, and remain in the desired location, stem cells for regenerative medicine must be co-implanted with an environment which can aid these necessary functions [43]. This can be accomplished by cell-surrounding scaffolds, typically composed of synthetic polymers and drug-delivery components [37–48]. For reasons of structural integrity and mechanical strength, reinforcing phases must also be dispersed within the synthetic polymer matrix, thus forming the complete definition of the BNS for orthopedic applications.

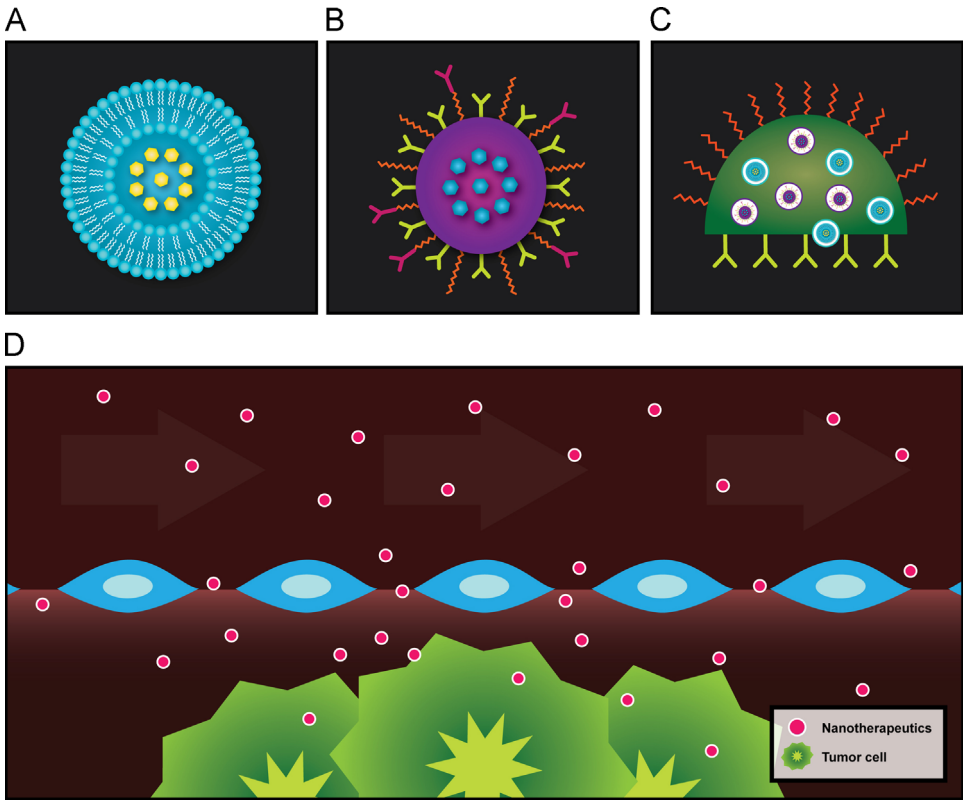

Fig. 1. Generations of nanoparticles and the EPR effect. (A) The first and simplest model of nanovectors comprises an enclosed "vehicle" (i.e., liposomes) encasing the therapeutic or active agents. These nanoparticles concentrate at the tumor or target sites via enhanced permeation and retention (EPR, see Fig. 1 panel D); (B) The next generation of nanoparticles exhibits more nuances such as the ability to selectively concentrate at tumor sites using biomolecules (e.g., antibodies), enable remote activation of payloads, or otherwise respond to the biological environment; (C) The most complex (third) generation yet offers sophisticated features such as the sequential and timely release of therapeutic payloads, often across multiple formidable biobarriers to different subcellular target sites; and (D) The EPR mechanism is primarily exploited by the first-generation nanoparticles for "passive" tumor concentration. Angiogenic vessels of the neovascular endothelia typically present with large fenestrations, through which nanoparticles can readily traverse to reach tumor sites.

2. The transport of nanoscale mass through the body

When an agent (e.g., conventional or biological drug, toxin, nanoparticle, imaging contrast agent, naturally secreted molecule, metabolic products, etc.) enters into the systemic circulation, in a very short time it faces a sequence of biological barriers. The agent's ability to penetrate across them, and gain access to different body compartments, governs largely the injected agent's distribution, final disposition, and its ability to have a beneficial or adverse health effect.

It is important to note that biological recognition at the molecular level plays two major roles in the transport of mass across the body. The first is that recognition may give free access to certain body compartments. For instance, penetration across the blood vessel walls is granted to certain white blood cells (WBC) that are needed to fight infection in the tissue of the body, but not to red blood cells (RBC), which need to remain in circulation despite the fact that they are smaller than WBC. It is the recognition of surface molecules that gives WBC free passage to extravasation. The second is recognition within a body compartment, e.g., to target a specific cell population dispersed within a tissue containing several other cell types. It must be noted that this type of selectivity can only be realized if penetration across a sufficient number of biological barriers, and toward the target tissue compartment, is warranted. As will be discussed later, however, these two types of recognition-based mechanisms are frequently in opposition to each other.

A comprehensive listing of biological barriers (biobarriers for short) would prove of encyclopedic length, if full detail was desired. For the purpose of reference throughout this paper, attention will be limited to several fundamental biobarriers, with a broad definition of what constitutes a "barrier", extending beyond the simple notion of barriers as biophysical surfaces. They are listed below, with their names *italicized* for clarity:

Enzymatic degradation [49] may be carried out by different molecules that act in different compartments. For instance, systemically circulating nucleases rapidly degrade potentially therapeutic nucleic acids. This is the major reason why an extremely promising class of targeted therapeutic agents such as siRNAs (Nobel Prize in Physiology or Medicine 2006) [28,50,51] never entered clinical use, though their efficacy is demonstrated countless times, on a daily basis worldwide, in laboratory cell cultures.

Opsonization occurs immediately upon injection of a nanoparticle or foreign agent into the body, with different types of proteins (opsonins) covering the surface of injected particles [52–54]. The recognition of the dominant opsonins over the surface of the nanoparticles then determines their final fate, such as *sequestration by the RES* in the liver and spleen: Resident macrophages in these organs have conjugate molecules to the opsonins, which bind to them and cause the nanoparticles they cover to be sequestered.

Blood flow dynamics are largely responsible for the ability of circulating agents to be in contact with the surface of blood vessels [55], and from there enter into body tissues by extravasation through the *vascular endothelium* (enhanced by molecular recognition, or through fenestrations), or be sequestered by phagocytosis within macrophages lining the blood vessels in the RES.

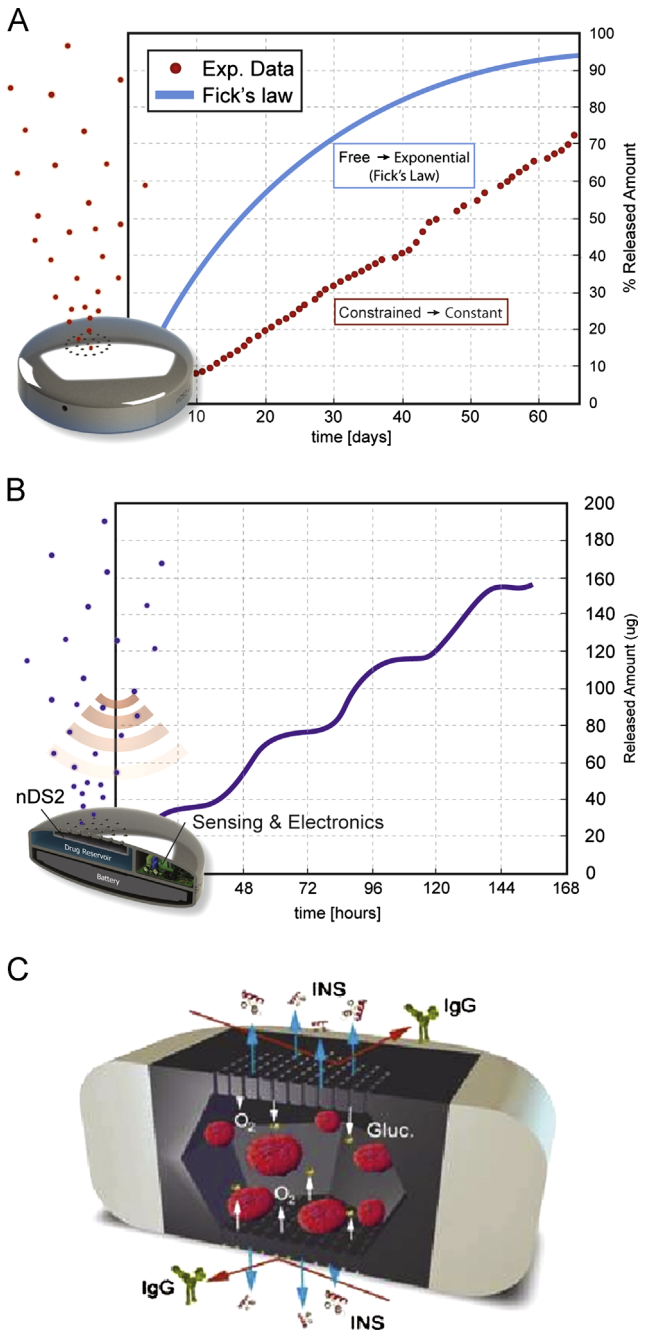

Fig. 2. "Nanogland" designs. (A) One version of the nanochannel Delivery System (nDS) is engineered to release therapeutic agents in constant and sustained fashion, over extended periods of time (weeks to months, or perhaps even years), via diffusion. This release profile deviates from Fick's law of exponential increase; (B) The nDS2 can be remotely controlled to release therapeutic molecules in waves, dictated to a remarkable degree of precision by the mode of regulation (external activation, preprogramming, etc.); and (C) The schematic depiction of yet a third model of nDS illustrates its use as an in vivo bioreactor, combining biological (and live) components such as cells or transplanted tissues incubated within. The nanochannel membranes (grid-like structures) in many ways act as "guardians" or molecular sieves for immunorejection phenomena and molecular transport, respectively.

The *organ and interstitial tissues* are themselves formidable barriers to mass transport [56–58]. The concentration-driven transport of mass is obviously impacted by factors that combine to form a spatially variable diffusion coefficient. Among these are the tissue density, the distribution of charges, the presence of molecules with recognition specificities, and local concentration of molecules such as Collagen IV, which give rise to true filtering surfaces such as the basement membrane. Transport in the convective regimen is affected by the *hydrostatic pressure states* (*oncotic*, *osmotic*, *interstitial*) [59], which in different cases result in driving forces that promote or oppose transport toward the target organ. In particular, extravasation may be opposed, as well as transport away from the blood vessel walls. In cancer, this is frequently the case: The overall driving force pushes from the cancer tissue into the blood vessel, and thus effectively opposing the transport of systemically injected drugs to the cancer cells. Even more dramatically, hydrostatic pressures can rapidly clear the cancer lesions of drugs injected directly into them. When convective transport opposes drug traffic to the cancer, the only remaining favorable transport modality is diffusion, which is less effective with increasing mass and size. Thus, therapeutic agents with molecular recognition capabilities, which are much larger than simple small molecule chemotherapy drugs, are at a major transport disadvantage, despite their ability to preferentially "recognize" cancer cells.

Cell membranes themselves are a very selective barrier to penetration, based on molecular recognition. Transport from the extracellular environment into the cells may take very different forms, in addition to simple diffusion and convective processes. Active transport arises from the recognition of molecules, nanoparticles, and substances by surface receptors embedded in the cell membrane. Receptor-mediated recognition triggers uptake by the cell through endocytocis, or phagocytic processes combine to engulf the transported species or nanoparticle in vesicles such as endosomes. These mature over time, acquiring different transport and processing specificities, and are transported actively with their cargo, by molecular motors along microtubules that act like rail tracks across the cells. The transported particles may be released into the cytoplasm, or at different stations corresponding to different subcellular organelles – which are themselves protected by perm-selective membranes, as for instance the nucleus is, and most remarkably so. Cargo that is not recognized for release by the vesicle molecular logics is stored into vesicles such as late endosomes or phagosomes, where it is attacked by acids to attempt its disintegration [60,61]. Thus, the *membranes of transport vesicles, and subcellular organelles* comprise a set of formidable and "intelligent" biobarriers. Even cargo that is released into the cytoplasm is subject to at least one more "barrier" to its stable deployment into a cell: As a protective mechanism, cells possess selective *molecular and ionic pumps* (*a.k.a multidrug resistance mechanisms*) that employ molecular motors to drive unwanted toxins and substances out of the cell and into the extracellular space [62,63]. These are especially present in cancer "stem cells", which are typically protected against chemotherapy and form the reservoirs to regrow cancers after the bulk population of cancer cells has been depleted by therapy.

At this time it is important to introduce a fundamental observation, which will be the basis for additional developments further below: *The biobarriers are frequently modified in the presence of disease.* The paradigmatic example is the above-cited hyper-permeability of the vascular endothelium in tumor-associated angiogenic (TAA) blood vessels, which is generated by the presence of architectural abnormalities and fenestration. Again, it is exactly because of these pathological presentations that nanopharmaceuticals have reached the clinic. The concept may be generalized to hypothesize that novel pharmaceutical agents may be constructed, which exploit *by design* the pathological presentations of biobarriers in disease, to create preferential concentrations at desired target sites. Even further, one may recognize that the inter-patient variability of these biological presentations may yield the opportunity to personalize treatment by specifically employing

pharmaceuticals that exploit the particular characteristics of these presentations, in individual patients. In this context, the term "pharmaceutical" is intended to mean both the "traditional active principles", and *the specific characteristics of the carrier vector*. For example, the observation that the fenestrations in TAA change over time and the course of treatment, and are different in different cancer types, may yield insight into the use of various size liposomes for assorted cancer lesions – a first foray into the notion of personalizing therapy by personalizing the vector, and (not only) the drug. There are many other examples of how biobarrier disruptions are associated with pathologies, or are their very causative factor, in a diversity of medical fields spanning neurology, immunology, metabolic diseases, and ophthalmology. Actually, it is difficult to identify any disease that is not related to biobarrier transport pathologies.

A biological model problem that exemplifies the complexity of the transport and processing of naturally occurring nanoparticles is found in the context of lipid metabolism. Low density lipoproteins (LDL) available in the body conjugate to their receptors and form a complex on the cell surfaces, which is successively internalized in a coated pit, or molecularly functional invagination of the cell membrane. The pit thus forms an endosome, which contains the LDL-receptor complex. The endosome is a multifunctional nanoparticle itself, which is further transformed into another nanoparticle inside of the cell, by fusion with a lysosome that contains a population of degradation enzymes, and a highly acidic environment. Within the lysosome, the receptors are cleaved from the LDL complexation, are granted free passage across the membrane of the endosome–lysosome, and travel by diffusion to reach the cell membrane. There they are irreversibly stabilized until they are contacted by another LDL nanoparticle, and are triggered into transport through the endosome–lysosome pathway once again. The cholesterol esters, once separated from their receptors in the lysosomes, are then enzymatically hydrolyzed to form free cholesterol, which then inhibits the synthesis of the enzyme HMG-CoA reductase and the LDL receptors, or causes their degradation. This negative feedback ensures proper balance of the cholesterol level. Fatty acids re-esterify the cholesterol inside the cell, for the purpose of storage. A deviation in any of the listed steps in the processing of the LDL results in pathologies of cholesterol metabolism, such as the formation of atheroma in blood vessels walls, which may in turn create strictures that prevent the proper flow of blood. This causes angina pectoris, if the site of occurrence is the endothelium of the coronary arteries. In more severe pathologies of the same origin, the coronary atheroma may become unstable, resulting in myocardial infarction. Remarkably, the most frequent symptom associated with the first presentation of coronary artery atherosclerosis, a disease of lipid nanoparticle transport, is actually sudden death [64–66].

The case of the body's own transport management of natural lipoprotein nanoparticles in atherosclerosis is but an illustration of a much more general reality, which is based on the recognition that, ultimately, protein and nucleic acids are biological nanoparticles. Cells are functional entities, which integrate and coordinate the operations of many subcellular organelles, such as the nucleus, mitochondria, ribosomes, transport vesicles, and many others – all of which are biological nanoparticles. The general statement that arises, with a benign modicum of provocation, is then as follows: *Life is a manifestation of nature's excellence in nanoparticle processing.*

Conversely, where this excellence faults, pathologies arise. The field of "*Transport Oncophysics*" [18,67] actually views cancer as a proliferative disease of mass transport dys-regulations, which is primarily brought about by the pathological modifications of its biobarriers (e.g., the angiogenic vascular endothelia).

Remembering that here, and throughout this paper, the term "nanoparticle" includes synthetic, biological, and otherwise natural objects with nanoscale dimensions, the first general problems can now be stated:

2.1. General problem 1 (GP1): To formulate the governing laws of transport of nanoscale mass through the body.

This general problem naturally induces a set of fundamental, related problems, of the form: *To identify the differences in nanoscale mass transport in health and* (*a given*) *pathology* – a class of problems for which Transport Oncophysics is the obvious prototype.

The importance of GP1 is difficult to overstate, for both its basic science and its medical implications. What could be the form of the "master governing equation" of nanoparticle transport in the body? The nanoparticle properties that are independent variables in the master equation are, in a first approximation, the nanoparticle geometry *g* (necessarily inclusive of both size and shape), the surface charge distribution *scd*, the particle mechanical properties *mp* (both elastic and time-dependent), its hydrophobicity *h*, its chemical stability in different environments *sde*, and its specific recognition properties *srp*. Given the evident impossibility of constructing an actual, deterministic system of continuum-level equations to govern the nanoparticle transport through the system of biobarriers, as a function of these governing parameters, one may want to consider alternative approaches. In one such, the independent variables may be integrated within probability functions P*j*, $j=1\ldots m$, which describe the likelihood that the nanoparticle will penetrate across, or otherwise "survive" the *i*-th biobarrier, say BB*i* in a sequence of biobarriers BB*1*…BB*n*.

The "master equation" then expresses the probability of a nanoparticle reaching compartment *Ck*, $k=1\ldots p$, as function of P*j* (*g*, *scd*, *mp*, *h*, *sde*, *srp*) – still an extraordinarily complex statement, in its broadest generality.

Perhaps the stochastic methods of Markov chains could be helpful to identify the master equations or some of its properties, though it is doubtful that each "state" would be independent from the prior configuration, in the sense that nanoparticles may acquire entirely different properties following interactions with one of the biobarriers – an example being the process of opsonization, which leaves a protein corona on the nanoparticles, thus increasing their size, reactivity, and specificity.

Another conceivable, though perhaps extreme, approach would be to define the ability of particles to penetrate across a biobarrier as a "distance" from the body compartment enveloped by the biobarrier. The metric to be used for measuring the distance could be reflective of the match or mismatch of the properties of the biobarrier and the particle. Then, the governing equation would be a mathematical statement of the evolution of the metric over time, and its effect over the nanoparticle trajectory.

The difficulties with any of these approaches are overwhelming. Thus, the need arises for simplifications of the governing problem. Not only are mathematical simplifications necessary, but so are the technological approaches that allow for the independent, controlled and reproducible variation of the nanoparticle parameters NP={*g*, *scd*, *mp*, *h*, *sde*, *srp*}, so that any assumptions on the form of the general master equation and its subcomponent systems may be suitably tested. This is exactly the approach followed below, with the introduction of the Simplified Problems – an approach that is finally conceivable now that suitable nanotechnology platforms are available, after many years of research and development.

In a first simplified approach, one may employ literature data to compile a sort of "periodic table" of nanoparticulates, with respect to their ability to penetrate across individual biological barriers.

These datasets may be integrated with novel experimental evidence, designed to fill the knowledge gaps. An annotated compilation of nanoparticle penetration data would be extremely useful, even for some of the crucial biobarriers, and could be used to generate "bio-distribution probability maps". To gain scientific insight and predictive power, in a simplified setting, it is useful to consider the notion of employing "test nanoparticles" of synthetic origin, manufactured with exquisite control over as many as possible of the nanoparticle parameters NP={*g*, *scd*, *mp*, *h*, *sde*, *srp*}. Toward this goal, Donald Tomalia and collaborators [68,69] employed a special type of polymeric particles known as dendrimers, since these can be synthesized with exquisite control over size and surface charge, with a basically spherical nanoparticle shape. In our group, we developed silicon micro- and nanofabrication techniques to manufacture particles with exquisite control over (non-spherical) geometry, density, surface charge, and stability [70,71]. Different degrees of hydrophobicity and biochemical specificity can be obtained by covering these particles with functional groups, though the biomolecular targeting approaches suffer limitations in vivo, as discussed above.

With the silicon particles we pursued the study of a fundamental system of biobarriers that pertains to their transport through the blood stream, their preferred localization on tumor-associated blood vessel walls, and their penetration into the vascular endothelium. This analysis is further broken down into the processes of margination in the blood stream, toward the vascular vessel wall, firm adhesion on the endothelium, and uptake by its cells. We employed a combination of mathematical modeling, microfluidic testing in the laboratory, and animal experiments.

In collaboration with Paolo Decuzzi, we have developed an integrated approach for the rational design of delivery systems for intravascular injection, which serves as the first stage of the MSV system described further below [71–75]. The Decuzzi methodology combines mathematical modeling, with in vitro microfluidic assays and in vivo small animal imaging.

This methodology afforded the understanding of the relative importance of size, shape, and the surface properties of the first-stage vectors, and any other nanoparticle of synthetic or biological origin. It is through this understanding that it became possible, for the first time, to proceed to a true rational, engineering design of the delivery vectors, which had always been developed through an initialized trial-and-error approach. In the Decuzzi approach, the fundamental design parameters are the size, shape and surface properties. The fundamental governing equations with respect to which these parameters are optimized include those that pertain to margination (motion toward the blood vessel wall, from any point in the vessel cross-section; this is of primary importance to foster adhesion, recognition of surface moieties that signify for instance tumor-associated angiogenesis, and for penetration across the endothelium through fenestrations), adhesion, and the intertwined phenomena of cellular uptake and transcytosis [55,70,76-79].

In the above studies, the pathological transport properties of biobarriers are associated with the properties of tumor-associated angiogenic vessels, which are typically chaotic in architecture, stagnant in flow, hyper-permeable, and present with many connections (anastomoses) between veins and arteries.

These studies were actually motivated by the desire to maximize the concentration of therapeutic nanoparticles at tumor sites, by the “Rational Design” of the particle size, shape, density, stability and surface properties [71,78], and to verify their enhanced efficacy in animal models. Using this approach, we were able to produce unprecedented results in the treatment of various diseases in animal models, which are uniformly lethal in their human clinical counterparts. Among these are: Metastatic and locally advanced ovarian cancer [28,80]; and Triple-negative breast cancers with metastases to the lung [81]. In addition, the Rational Design approach succeeded in yielding unprecedented concentrations of therapeutic nanoparticles in the bone marrow [82], where proliferative pathologies of blood cells originate, and micro-metastases frequently hide, before they resurface to generate lethal recurrences of neoplastic disease. These engineered vectors were designed to mimic platelets, in their ability to access and retain position in blood vessel walls, and thus were termed “plateloids”. More recently, methods of Rational Design were combined with cell biology, yielding the “leukolike” vectors [83]. These are particles that "wear the uniform" of the body cells that attempt to trap and destroy them; i.e. they mimic leukocytes, or white blood cells (WBC). The leukolike vectors are manufactured to be enveloped by the cell membranes of leukocytes on their external surfaces, thus providing a "biomimetic" approach to successfully negotiate at least two biological barriers: The uptake by the resident macrophages of the mononuclear phagocyte system, and the transport across the vascular endothelium, which is naturally and actively facilitated for leukocytes in inflamed regions of the body.

The reported successes in the treatment of cancer in animal models validate the dual nature of the approach we are advocating: The use of synthetic nanoparticles may be used as "probes" to aid in the understanding of the fundamental transport laws across biobarriers, and their differentials between health and disease. At the same time, the probes that demonstrate the greatest ability to reach intended targets in the body can then be employed as superior agents of therapy. Hopefully, the above-listed encouraging results in animal models of cancer may serve to reaffirm the importance and potential medical benefits of the approach of Rational Design of nanoparticles, and the utility of deriving transport laws for synthetic nanoparticles through biological barriers. With this motivation, the first classes of simplified problems may be stated as:

> *Simplified Problem 1* (*SP1*): *To formulate the governing laws of transport of* (*synthetic*) *nanoscale particles across a biological barrier of the body*, *or a system thereof.*

> *Simplified Problem 2* (*SP2*): *To design synthetic nanoscale particles to penetrate preferentially across a biological barrier of the body*, *or a system thereof.*

Modeling of transport of cells and particles within small blood vessels remains a challenge, despite advances in computational methods and computer technology. Milos Kojic and his collaborators at The Methodist Hospital Research Institute (TMHRI) in Houston and the R&D Center for Bioengineering in Serbia investigated various methods in this field [84,85]. They found that, for solving the solid–fluid interaction problems, the most robust and reliable is the method of strong coupling with a remeshing procedure. They then implemented this method to solve biological problems [86–88]. The fundamental balance equations of linear momentum for the solid and fluid domain, expressed in a finite element incremental-iterative solution scheme, can be written as

$$(\mathbf{K}_{fluid}^{(i-1)} + \Delta t \mathbf{K}_{solid}^{(i-1)})\Delta \mathbf{V}^{(i)} = \mathbf{F}_{fluid}^{(i-1)} + \mathbf{F}_{solid}^{(i-1)}, \quad (1)$$

where $\mathbf{K}$ are element matrices, $\Delta\mathbf{V}$ are nodal velocity increments, and $\mathbf{F}$ stands for unbalanced nodal forces for the equilibrium iteration i and time step Δt. Also, a simple concept was introduced for solving interactions among moving solids and solids and the walls, which is general since it includes repulsive and attractive forces, both deterministic and stochastic in character. This computational approach and the developed software provide a solid foundation for solving problems in biomedicine and bioengineering.

Two additional observations are introduced here, to motivate the statement of the final two simplified problems in this section. First, it is noted that imaging modalities that are routinely used in the clinic may serve to identify the characteristics of transport across biological barriers in health and disease. For years, for instance, radiologists have diagnosed brain and pancreatic cancers based on the fact that their blood vessels "leak" contrast agents that are visible by imaging modalities such as computed tomography (CT) scans or magnetic resonance imaging (MRI). It has recently been observed that the very same imaging procedures can be used to predict the uptake of systemic therapy by adenocarcinomas of the pancreas, and even predict patient response and survival [89]. This work is the first clinical demonstration of the use of biophysical markers for cancer treatment – a validation of the "Transport Oncophysics" framework, and an approach that may be predicted to have a great future impact in medicine. Just observation of the imaging results is not enough for prediction of response and survival: Quantitative measurements must be entered into a suitable mathematical formulation for the laws of transport.

Vittorio Cristini (publication pending) identified the necessary governing equation for the convective transport in the vasculature and resulting levels of tissue perfusion, which are described by a double-exponential:

$$y(t) = Y_{max} \cdot r \cdot \frac{e^{-r_c \cdot t} - e^{-r \cdot t}}{r - r_c}, \tag{2}$$

Here, the parameters describe qualities of the tissue and its surrounding vasculature, including the rate of exchange between the vasculature and tissue (r), the rate of clearance from the vasculature (r_c), and the maximum perfusion level of the vasculature (Y_{max}). Through a clinical study of the model Eq. (2) applied to 173 pancreatic cancer patients at MD Anderson in collaboration with Eugene Koay, and under the guidance of Jason Fleming [89], we have derived patient-specific parameters by fitting model Eq. (2) to CT data during 5-minute perfusion studies with a contrast agent and testing model predictivity against direct pathologic assessment of treatment outcome. We have demonstrated staggering differences in transport efficiency and efficacy between carcinomas and their normal pancreas counterparts in all patients and, as a consequence, *very significant correlation of the model parameters with drug delivery* (*gemcitabine*), *outcome of chemoradiation treatment and patient survival*. Further application of this model to thoracic, liver, colorectal and pancreatic cancer patients is underway.

In parallel, we have further developed the model description of transfer of molecules and drugs from the vasculature to the tumor cells to produce rational-design guidelines to improve delivery, e.g., by employing nanocarriers as "vascular depots" whose payload and release time scale are fine-tuned to overcome the tissue diffusion barrier. We have demonstrated (publication pending) that the fraction of tumor cells killed by a "free drug" is described by the following function:

$$f_{\text{kill}} = 2 \cdot \frac{\text{BVF}}{1-\text{BVF}} \cdot f^{\text{M}}_{\text{kill}}(\sigma_0) \cdot \frac{K_1(r_b/L) - K_1\left(\frac{r_b/L}{\sqrt{\text{BVF}}}\right)/\sqrt{\text{BVF}}}{r_b/L \cdot K_0(r_b/L)}, \tag{3}$$

which quantitatively links tumor kill to relevant measurable transport qualities from patient histopathology and in vitro cytotoxicity experiments, including: the diffusion penetration distance L of the drug; the blood volume-to-viable tumor volume ratio or "blood volume fraction" BVF; the equivalent radius r_b of the drug source (e.g., blood vessels); and the fraction $f^{\text{M}}_{\text{kill}}$ of cells killed in the absence of diffusion gradients, e.g., in a monolayer experiment (K_0 and K_1 are modified Bessel functions of the second kind of orders 0 and 1, respectively). We have retrospectively applied model Eq. (3) to colorectal cancer (CRC) metastases in the human liver by comparing measurements from histopathological samples to the model prediction of the fraction of tumor cells killed in each patient. *The mathematical model is statistically significant and in very good agreement with the patient outcome data*. This work can be translated to clinical applications to predict the effects of chemotherapy on patient outcomes and rationally designed individualized treatments. A comprehensive and retrospective study of model Eq. (3) applied to 57 patients with cancers in the liver is underway in collaboration with Steven Curley, also at MD Anderson.

We find that the significant diffusion barrier typically constrains chemotherapy outcome to $f_{\text{kill}} \ll 1$. We have extended model (3) to the case of nanocarrier-based delivery of drug molecules. The hypothesis is that by employing nanoparticles as vascular depots, permanence and continued release of drug molecules from the vasculature surrounding a tumor could be established, in contrast with the typically very brief residence of drug molecules in the system during traditional "bolus" delivery. The mathematical model modified to account for this different boundary condition predicts on average a three-fold increase in f_{kill} based on currently available nanocarrier payload and release time technology.

In order to place Eqs. (2) and (3) into perspective, it must be recognized that over 20 years ago Rakesh Jain [90] and Linda Simpson-Herren [91,92] had already proposed the idea that the tumor microenvironment leads to variation in tumor response to chemotherapy. Yet, research had been primarily directed toward in vitro experiments on cultured cells in monolayers and how drugs affect the individual cell, failing to include the additional biological factors involved in drug delivery through a disorganized vasculature and penetration through tissue to the tumor cells [57]. Building on the recently presented concept of "mathematical pathology", [93] we have then developed a quantitative model of the spatio-temporal mass transport physiome in humans as part of a cross-disciplinary, multi-institutional collaborative effort across TMHRI, MD Anderson and the University of New Mexico, under the umbrella of the National Cancer Institute-funded Center for Transport Oncophysics.

It is noted that the parameters used in Eqs. (2) and (3) are not part of the terminologies routinely used in radiology at this time, nor in the pharmaceutical sciences. In the latter, the kinetic of transport of pharmaceutical agents is typically expressed in terms of classical pharmacokinetic parameters such as AUC (Area under the Curve), and the Volume of Distribution. These terms of trade have proven useful in medicine to date, but in the future perhaps will be integrated, or even supplanted, by a novel nomenclature, such as the Cristini parameters, that is more aligned with an improved understanding of the laws of mass transport in the body.

With this background, the next statement is:

Simplified Problem 3 (*SP3*): *To determine and express the laws of transport across biological barriers for a given nanoparticle probe* (*contrast agent*) *in terms of quantities that can be observed and measured* in vivo, *using the* (*radiological*) *modality that is associated with the given nanoparticle probe* (*contrast agent*).

The associated clinical challenge is obviously to employ the solutions of SP3 to identify the transport differentials that can be employed to diagnose disease, and provide guidance in its treatment.

Finally, it is recalled that frequently the strategies that are employed to increase specificity of recognition of body target actually hinder the transport across the biobarriers that must be traversed to reach that target. The paradigmatic example here is the use of antibodies decorating a nanoparticle surface: Upon contact with the conjugate antigen expressed on the surface of a

cancer cell, the nanoparticle will adhere and be internalized; however, the presence of the antibody coating renders the nanoparticle more impermeant through the vascular endothelium and the tumor interstitium. Considering the heterogeneity of the cell populations and surface antigen expression within a cancer lesion, a true biobarrier to specific transport, this sets up the fourth class of simplified problems:

> *Simplified Problem 4* (*SP4*): *To identify the governing laws for specific transport of a nanoparticle across* (*a system of*) *biobarriers*, *as a function of the nanoparticle parameters*. *When* (*a subset of*) *these have an opposing effect*, *to identify the "borderline" conditions under which successful transport is possible*, *and therapeutic efficacy may be expected*.

The clinical significance of SP4 cannot be overstated: By identifying the conditions under which a therapeutic strategy has a chance to yield a positive response in a patient, it will be possible to prescribe the most appropriate therapeutic regimen. By contrast, current therapies only have a 10–30% likelihood of positive response in the patient populations for which they are approved. Their efficacy is normally determined by radiological imaging observation of growth or regression of tumor masses, weeks-to-months after multiple cycles of therapy, which themselves exact a grave toll on the health of the patient and limit the chances for trying a different therapeutic selection. It may be stated that these "borderline" hypersurfaces separate life and death for cancer patients.

3. The transport of mass through nanoscale environments

Mass transport in non-biological systems often reduces to convective and diffusive phenomena, with the possibility of chemical reactions. In biological systems, however, the dynamics of mass transport are rendered dramatically more complex by the concurrence of active transport mechanisms [94], that is, modalities of transport that require the expenditure of energy through complex interactions of organized component parts. The transport of biological molecules such as proteins and nucleic acids is of fundamental importance for all biological processes, and is governed by exceptionally complex dynamic processes. The biological lexicon employs the pictorial term "signaling" as shorthand for a cascade of mass transport processes at the nanoscale, and with key events happening during transport through nanoscale environments. A first example of nanoscale transport was the previously presented dynamics of processing of LDL. As a further example to illustrate this statement, the dynamics of transforming growth factor beta (TGFβ) signaling are summarized, next.

Even a cursory review of a major signaling pathway in cell biology may help introduce some general considerations. By way of example, the case of TGFβ is presented here. TGFβ is a cytokine that contributes in many ways to the development of the organism. If the TGFβ signaling pathways suffer malfunctions, adverse consequences that arise include multiple mechanisms that drive tumorigenesis. Among these is the reduction or elimination of the body tumor-suppression capabilities, and the stimulation of malignant progression through the acquisition of increased motility and methods for evasion of the immune surveillance. In addition, TGFβ dys-regulation promotes the transition of a primary cancer to the metastatic phenotype, for instance through enhancement of the cell's ability to extravasate, modify its surrounding environment, and the acquisition of mesenchymal characteristics. For these reasons, the world of molecular oncology dedicates extraordinary attention to TGFβ signaling [95]. It may be helpful to recapitulate the multiple molecular steps through which this signaling pathway operates, to shed light on the connection between this (and all signaling processes in the cell) and the concepts of mass transport at the molecular scale.

Briefly, TGFβ signaling starts with the cytokine being conjugated with a trapped molecule while diffusing in the pericellular space, and then diffusing to receptors located on the cell surface. These receptors are trans-membrane proteins, which collaborate with co-receptor molecules that diffuse along the cell membrane, and when in the immediate vicinity of the receptor offer a high-specificity binding site for the TGFβ-trap complex. Upon binding, the receptors activate certain transcription factors (RSmads) inside of the cell, which in turn bind the molecule SMAD4 and – in extreme simplification – through this action cross the nuclear membrane (diffusion+active transport), and are thereby in position to control the expression of hundreds of genes in the cell. The "molecular signal" is therefore expressed through a complex series of steps involving molecular recognition and activation (phosphorylation), together with random, concentration-driven transport processes in different media (pericellular and intracellular fluids), guided transport processes that are still concentration-driven (diffusion along the cell membrane), but biased by non-symmetrically reversible recognition events (e.g., receptor and co-receptor interactions) and active transport processes such as the encapsulation of molecules within vesicles that are actively transported by molecular motors (e.g., actin–myosin "walking" along microtubules). In molecular biology, immense attention is dedicated to the events in this cascade that have high specificity, such as recognition, activation, perm-selective passage, and active transport. From a transport perspective, key events are also diffusional transport as a function of the composition of the carrier fluids, constraint effects on diffusion, as brought about by constraints on the three-dimensional free agitation of the molecules, and the effect of asymmetry of recognition in "random walks"– all aspects where the molecular biologist might require collaboration with the physical scientists and engineers for a complete solution.

These aspects of transport are rarely studied, by comparison, and yet they may hold the key to explain crucial bases for health and disease: Carrier fluid composition or cell membrane thickness may prove to be as important as molecular selectivity, in a derangement of the TGFβ signaling pathway that generates malignant growth and metastatic proliferation. "Molecular Transport Oncophysics" has not been born yet – but we may need it in the joint fight against cancer, soon.

While the understanding of TGF-β signaling is of substantial importance per se, the establishment of an overall formalism for the study of molecular signaling would have a transformational impact on biology and medicine. Suffice it to mention that cancer is currently defined as a family of different diseases with many diverse presentations, but that all have in common six major characteristics, the so-called Hallmarks of Cancer [96,97]. All of these are based on deviant molecular signaling processes, which, in the current orthodoxy, are attributed solely to the over- and under-expression of genes associated with cancer. However, genes are the blueprint for the synthesis, and can only control the abundance of their protein products. The transmission of the molecular signals that drive the cancer hallmarks can be impacted by a multiplicity of factors, which may or may not be associated with genetic abnormalities. For instance, the availability and density of transporter molecules or surface receptors may be directly controlled by genes, while the environmental conditions that facilitate or oppose transport may be controlled by epigenetic or non-genetic factors, such as those that influence the physics of transport. Transport through the cell is in part diffusion through the cytoplasm, and as such governed by suitable versions of Fick's laws. However, it is fundamentally affected by the modalities of transport in and across nanoscale environments, such as the ionic and molecular channels on the membranes of cells and organelles,

and in active transport in the endosomal–lysosomal pathways, along microtubules powered by molecular motors.

With this background, the second general problem is stated as follows:

3.1. General problem 2 (GP2): To formulate the governing laws of transport of mass through nanoscale environments within biological cells.

Of particular importance are the laws that govern the nanoscale transport phenomena involved in signaling pathways in health and disease.

In accordance with the general philosophy of this paper, a simplified, nanotechnology-based platform is now introduced, which can be used to study transport at the nanospace, en route to the fuller understanding required for GP2. This is the “sacrificial layer nanochannels (SLNC)” approach, developed in our laboratory in Berkeley during the mid-Nineties [98], and is the foundational cornerstone for the field of nanofluidics. Briefly, SLNC are obtained by depositing a sacrificial layer of controlled nanometer-scale thickness between two structural layers made of different materials, and then removing the sacrificial layer by use of a chemical that does not degrade the structural layer materials. This creates a passageway of typically rectangular cross-section, with the height being of dimensions equal to those of the sacrificial layer [99]. The methodology is somewhat similar to the "lost wax" technique employed by sculptors in ancient Greece. A typical structural layer material is silicon, and a typical sacrificial layer material is silicon dioxide; thus, the processes for nano-manufacturing the SLNC are related to those employed for electronic chips, where a very thick and tightly controlled sacrificial oxide layer is employed for “gating”, the essential function for transistors. Recent forms of SLNC manufacturing have been demonstrated to have the ability to control nanochannel heights as small as 2 nm, with precisions in the order of angstroms, and the ability to simultaneously manufacture very large numbers of identical copies of the nanochannels in diffusion control membranes [100], with extraordinary quality control.

Initial applications of SLNC in medicine included the immunoisolation of cell transplants [32,101,102], the elimination of viruses from biological fluids [103], and the encapsulation of implanted biomolecular sensors, with the purpose of avoiding the fouling of the sensing surfaces [104,105]. By far the dominant application of SLNC in medicine so far has been in the field of controlled-release drug delivery from implants. With the establishment of SLNC methodologies, it was promptly recognized that transport of molecules within nanochannels does not necessarily follow the governing laws that pertain to non-nanoscale domains, both in the diffusive regime, and in active transport where the driving mechanisms are electrokinetic, that is, under the forcing of an applied electrical potential across the nanochannels. To illustrate, it is recalled that classical diffusive transport is governed by the basic mass balance equation:

$$-\frac{\partial c}{\partial t}+\frac{\partial}{\partial x}\left(D\frac{\partial c}{\partial x}\right)+q=0, \tag{4}$$

where c is concentration at a spatial point with coordinates x and at a time t, q is a sink term, and D is the diffusion coefficient. However the classical approach may not be directly applicable to nanoporous or nanochannel systems, because solid surfaces may affect diffusing particles in a fluid domain [106,107]. Theoretical and experimental studies suggested that D may be altered near the fluid–solid interface [108,109]. Recent studies of diffusion transport showed that nanoscale constraints effects should be accounted for in nanochannels and may help in the understanding of diffusion transport beyond classical approaches [110,111].

A first set of opportunities for transformational progress in medicine through the SLNC stems from the recognition that the nanochannel properties can be tailored to the drug molecules to be released from the implants, so that the release profile is zero-order (i.e., constant rate of release, regardless of concentration). This regime is not attainable under "conventional" diffusive transport dynamics, governed by Fick’s laws. The ability to release drugs at constant rates, from implanted "nanoglands", for periods of time as long as weeks, months and years, has extraordinary benefits in medicine: It increases patient compliance; it affords the reduction of dosages with the corresponding reduction in adverse drug-related side effects; it increases therapeutic efficacy in a broad spectrum of medical settings; and it allows for patient treatment modalities that do not require hospitalization [112].

Exceptional progress has been recorded in the applications of the SLNC to drug delivery under the leadership of Alessandro Grattoni at TMHRI, and the nano-pharmaceutical company Nanomedical Systems (NMS). Grattoni has developed an algorithm that allows for “personalized delivery approaches”, that is, the rational determination of the SLNC characteristics and design implant properties required to release at the desired rate, for the desired time interval for the individual patient and medical indication. The design parameters are the nanochannel heights, their density per unit area, and the overall volume of the container nanogland. With this approach, Grattoni and coworkers have demonstrated long-term constant release of a large number of nanotherapeutic agents for clinical applications (Table 1).

Active (non-diffusional, or driven by forces beyond concentration gradients) mass transport through nanochannels occurs by a multiplicity of mechanisms. In contrast with the macroscopic world, however, the application of mechanical pressure is not a dominant factor: The inverse proportionality with nanochannel dimension rapidly brings the required pressures to wholly unsustainable ranges, both in biology and human artifacts. Biology addresses transport requirements through molecular motors and biochemical process, as we have seen, compounded with recognition specificity and the action of local electrical fields. Nanofluidics offers the opportunity to gain insights into simplified versions of these phenomena [113]. Most significant progress has been recorded in the determination of the governing laws of electrokinesis, and in particular electro-osmosis, which is typically very relevant at the nanoscale, but not so at larger dimensions. Electro-osmosis is caused by the formation of electric double layers (EDL) at the walls of nanochannels, which move toward the opposite

Table 1
Sustained release of drugs and other agents by nanoglands.

Clinical indications	Delivered molecules	Reference
Cancer infectious diseases	Interferonα-2b	[33]
Pharmaceutical formulation excipient	10 kDa Dextrans	[100]
Pharmaceutical formulation excipient	Bovine serum albumin	
Antibiotic	Cefazolin	
Cancer	TGFβ-1 Thioaptamer	[161]
Pharmaceutical Formulation Excipient	Lysozyme	
Cancer	Leuprolide	[34]
Antioxidants	DF-1 fullerene	[33]
Hormone Replacement	Testosterone	[36]
Hormone Replacement	CyclodextrinD Testosterone	
Cancer	Octreotide	
Cancer	Letrozole	
Cancer	Docetaxel	
Cardiovascular	Resveratrol	
Cardiovascular	Atorvostatin	
Cancer	Lapatinib liposomes	
Hormone replacement	Human growth hormone	

polarity when a bias is applied. In nanochannels, the EDL may occupy a large part of the fluid, or even its totality, for dimensions in the range of 1–10 nm. In microfluidics, by contrast, the EDL typically comprise a very small (< 0.01%) portion of the fluid volume, and therefore mass transport primarily occurs by electrical attraction of the solutes and suspended species or particles, in the classical phenomenon of electrophoresis.

A "first order" of approximation in modeling diffusive mass transport within tissue or intra-and inter-cellular space is to consider the biological environment as a complex composite medium. Diffusion in this biological medium depends not only on internal microstructural geometry, but also on the biochemical interactions between the transported substance and the solid phase material of the microstructure. To take into account the complexity of diffusion under such conditions, a new multiscale diffusion model has been introduced. The model relies on coupling the molecular dynamics (MD) and continuum finite element (FE) method [111–115]. The effects of the interactions between the solid microstructural surfaces and the moving molecules or particles are evaluated by the detailed MD analysis, and these effects are expressed in a form of scaling functions for correction of the diffusion coefficients in the domains of retarded diffusion. The spatial field of the diffusion coefficient tensor is used in the detailed microstructural FE model. Additionally, a novel numerical homogenization procedure has been introduced to determine continuum parameters for FE modeling of large diffusion domains. The procedure is robust and general since it overcomes limitations of usual analytical approaches [116,117]. The continuum parameters include (for three coordinate directions x_i) equivalent diffusion coefficients D_i, as well as equivalent distances h_i from imaginary solid surface to account for the surface effects within the microstructure.

Against this background, a spectrum of Simplified Problems arises, in harmony with the spirit of this paper, which builds on each other to form progressive layers of complexity, toward the biologically significant:

> *Simplified Problem 5* (*SP5*): *To determine the laws of* (*diffusional*, *electro-osmotic*, *magnetic*, *other… combined and multiphysical*) *mass transport in nanopores and nanochannels*, *as function of the nanoscale geometry*, *and the chemical/physical properties of the nanopore/nanochannel walls*, *of the transported species and of the fluid*(*s*) *through which transport takes place;*
>
> *Simplified Problem 6* (*SP6*): *Same as SP5*, *with the addition of time dynamics of chemical transformation such as degradation of the transported species*, *and their effect on the surfaces of the nanochannels/pores* (e.g., *changes in charge*, *thickness*, *hydrophobicity*);
>
> *Simplified Problem 7* (*SP7*): *Same as SP6*, *with the addition of phenomena at the nanopore/nanochannel wall that are specific to the molecules/particles of the transported species*.

Examples of biologically significant problems in the family of SP7 include the understanding of conditions of reversible blockage: The transported species particles degrade, and their degradation products modify the chemistry of the nanochannel walls, to the point that transport is reduced and becomes impossible at critical threshold of charge, viable cross-sectional dimension, and hydrophobicity. The wall modifications are themselves unstable and, under no-transport conditions, degrade and release species that subsequently transport away, creating the opportunity for further transport of the original species – thus creating a "transport oscillator". Different forms of transport oscillators may pertain under regimes of simple diffusion, electro-osmosis, or different forcing functions. A yet more intriguing set of problems arises by adding the ability of the nanochannel walls to be modified "from/to the outside": The simplest such case is by allowing them to be generally permeable, or perm-selective, but "active walls" with an external feedback loop from the transported species to the external surfaces is of particular interest in biology, where the system may then model the transport of signaling molecules that trigger the biological production of molecules that in turn act on the channel walls.

A methodological observation may be warranted, at this point, with specific reference to diffusional transport, but with straightforward extension to other forms of mass transport: Fick's laws of diffusion are not fundamental laws of nature, in the sense that they do not apply in the classical forms to nanoscale transport, as discussed above in the case of zero-order release from nanochannels. What is a fundamental tenet of nature, indeed, is the thermal agitation of molecules. From this, Fick's laws can be deduced, through methods of statistical mechanics first demonstrated by Albert Einstein [118], and *under specific assumptions*. In particular, the assumption that thermal agitation is fully unconstrained yields Fick's laws. The reason why transport in nanochannels can take the non-Fickian forms that allow for zero-order release is that thermal agitation in the nanochannel is constrained in at least one direction, as long as the channel height is of the same order of magnitude as the dimension of the molecule [119–121]. Bi-directional constraints are applied when the transport vessel geometry transitions from nanochannel to nanopore, or when the density of the transported species in the nanochannels is such that interactions between the transported molecules become important. "Single-file diffusion" [122–124] is the combined case, which pertains to nanopores at high density of transported particles, and is also dramatically non-Fickian.

I suspect that the process for obtaining the non-Fickian transport laws that pertain to nanoscale geometries can also be obtained in a manner that is similar to Einstein's mode of derivation of Fick's laws from Brownian motion. I further suspect that the ability to do so would open novel avenues of scientific investigation, and great impulse to technologies in medicine and beyond. Thus, Simplified Problem 8 is stated, with a great endorsement for its significance:

> *Simplified Problem 8* (*SP8*): *To derive the laws of transport at the nanoscale*, *starting with Brownian motion*, *employing methods of statistical mechanics*, *with the addition of constraint conditions*.

My final suspicion in this setting is that the constraint conditions can be integrated in Einstein's method by means of Lagrange multipliers. Alternatively, methods of calculus of variations can be employed, or Emmy Noether's approach, requiring an energy formulation and the analysis of its symmetries.

I hope that a small digression at this point will not inconvenience the reader: I was privileged to be asked for a definition of nanotechnology at the launch of the major journal in the field, in 2005 [125]. At that time, I aligned with the orthodoxy in the field in some parts of the definition, that is, the obvious requirements of being a "techne" (i.e., man-made) and of nanoscale dimensions (still itself a notion of some controversy in its specifics across different worlds of science, administration, and politics). I also agreed that to qualify as nanotechnology, it is necessary for some "emerging property" to arise, which depends on the scale being nanoscopic, in a manner that does not present itself with the macroscopic counterparts. In the case of the nanochannels, such emerging property is zero-order diffusional release. The further component of the definition of nanotechnology I added – and has not generated any evidence of concurrence or any interest whatsoever by the scientific community, to be frank – was colloquially expressed as "it ain't nano if you don't have the math to back it up". More formally, this is the requirement that the emerging property be *predicted* from first principles. The model problem I had in mind for that requirement was exactly diffusion through

nanochannels. Thus, in some sense, SP8 was there at the very dawn of nanotechnology.

Vladimir T. Granik led the development of a new, predictive equation for osmotic pressure for a binary solution [126]:

$$\pi = \frac{RTm_2' V}{[1+(1+V)m_2'/m_1']}, \tag{5}$$

where

$R=$ gas constant,
$T=$ absolute temperature,
$m_1'=$ volume molal concentration of solvent
$m_2'=$ volume molal concentration of solute,
$V=$ volume of solvent

The unique features of this equation are that: (1) It is valid without restrictions on the concentration of the phases, while the classical equations [127,128] only hold in dilute conditions; (2) It is fully predictive, that is, it does not have "fitting parameters" to be experimentally determined or adjusted; (3) It allows for generalization to cases where the separation membranes are partially perm-selective, that is, have a non-integer reflection. The understanding of the pressure-dominated biological barriers that govern mass distribution in health and in disease might benefit from the predictive nature of this equation, for instance in the case of the interstitial pressure states that oppose mass influx into larger tumors [56]. Still, this equation was not derived from first principles, which poses questions on its absolute completeness, which at this point can only be answered with experimentation. Einstein's methods for the derivation of Fick's laws from Brownian motion simultaneously yielded an expression for osmotic pressure. By analogy, my hypothesis is that the solution of SP8 will also yield Eq. (5). *This is an implicit statement for SP9.*

4. Mathematical anatomy

Homogenization theory studies the effective properties of composite, multiphase, and otherwise structured materials, with the objective of predicting these properties in an "average" sense over a suitable spatial domain (representative volume element or RVE) where the spatial variation of these properties can be neglected in first approximation. The independent variables that are factored within the prediction of the effective properties may be the properties of the materials that comprise the respective phases, and the spatial distribution of the various phases in their physical domains.

For instance, the effective, fourth-rank elasticity tensor of a biphase composite material, comprising a matrix and an embedded population of inhomogeneous inclusions, can be expressed with a variety of literature models [129,130], each with its advantages and disadvantages. One of these models is the "Poly-inclusion Theory" (PT), which states [131–133]:

$$\mathbf{C} = \mathbf{C}^{\mathrm{I}} + \boldsymbol{\alpha}\langle(\mathbf{C}^{\mathrm{II}}-\mathbf{C}^{\mathrm{I}})[\mathbf{I}+(1-\alpha)\mathbf{E}\mathbf{S}^{\mathrm{I}}(\mathbf{C}^{\mathrm{II}}-\mathbf{C}^{\mathrm{I}})]^{-1}\rangle, \tag{6}$$

where

$\mathbf{C}^{\mathrm{I}}$ and $\mathbf{C}^{\mathrm{II}}$ are the stiffness tensors of the two phases,
$\alpha\equiv$ volume fraction of phase II,
$\mathbf{I}\equiv$ identity tensor of rank IV,
$\mathbf{E}=$ Eshelby's tensor,
$\mathbf{S}^{\mathrm{I}}=$ compliance tensor of phase I

In the above, the pointed brackets $< >$ denote averaging over all orientations of the inhomogeneities (assumed to be of identical shape and material) weighted by an orientation probability density function f(g) over the entire space spanned by three Euler angles. Thus, this approach yields a prediction for the overall elasticity as function of the elasticities of the two phases, and the shape and orientation distribution of the embedded inhomogeneities (phase II). The prediction model may agree with experimental verification at different degrees of accuracy, as the various assumptions that are embedded in it are reproduced in the physical sample being tested. As is the case for all homogenization methods, major deviations are typically observed from theoretical predictions as the concentration of the embedded phase increases, material interfaces are imperfect, and the elastic limits of the phases are approached in loading. The PT offers the advantage that the inhomogeneities can be arbitrarily anisotropic, and of any ellipsoidal shape including the limit cases of disk and cylinders. One major assumption in the PT is that the strain concentration in the equivalent eigenstraining inclusion problem can be expressed as follows, in terms of the homogeneous equivalent applied eigenstrain $\boldsymbol{\varepsilon}^*$:

$$\boldsymbol{\varepsilon}^{\mathrm{II}} = (1-\alpha)\mathbf{E}\boldsymbol{\varepsilon}^*, \tag{7}$$

Equivalently, the PT approach may be restated as postulating that

$$\hat{\mathbf{E}} = (1-\alpha)\mathbf{E} \tag{8}$$

where $\hat{\mathbf{E}}$ is the strain concentrator tensor, the main variable of this class of homogenization theories. While the PT formulation offers significant advantages and has proven accurate for broad classes of composites, the exact form of the strain concentrator tensor $\hat{\mathbf{E}}$ was never obtained in full generality, for composites with 2 or more phases. Thus:

> *Simplified Problem 10* (*SP10*): *To derive the closed form, exact expression for the strain concentrator tensor, for the general case of a bi* (*multi*)*phasic composite with arbitrary anisotropy, orientation distribution, and concentration of the phases.*

Once the equivalent inclusion strain concentrator $\hat{\mathbf{E}}$ is known, the effective elasticity can be predicted exactly as:

$$\mathbf{C} = \mathbf{C}^{\mathrm{I}} + \alpha\langle(\mathbf{C}^{\mathrm{II}}-\mathbf{C}^{\mathrm{I}})\left[\mathrm{I}+\hat{\mathbf{E}}\mathbf{S}^{\mathrm{I}}(\mathbf{C}^{\mathrm{II}}-\mathbf{C}^{\mathrm{I}})\right]^{-1}\rangle, \tag{9}$$

Biological evolution theory is predicated on the notion that competitive advantages are acquired with adapting improvements in the performance of crucial functions, and that these advantages determine the prospects for survival of the species. A predictive form of homogenization theory, such as the PT or the equations that would derive from the solution of SP 10, can be used to yield "optimal design", that is, the combination of factors that yield the best possible overall response, in the context of an application or function. In other words, one may formulate a response function (e.g., a certain elasticity), and then determine the combination of factors (e.g., phase elasticities and distribution) that yield the maximum or most desirable value. Optimization theory therefore meets homogenization – and they both describe biological evolution if the assumption is made that each successful evolutionary step corresponds to an increase in a property, toward an optimum and a combination of optima.

This perspective may be applied to systems and subsystems of a biological entity, for instance to ask what would be the ideal diameter and density of collagen in tendons in the legs of animals that perform a certain set of feeding and transport functions to thrive, that in turn correspond to certain loading conditions on their legs. This is the concept we have termed "predictive anatomy". The corresponding overarching problem is discussed in the next section.

4.1. General problem 3 (GP3): To determine the governing equation of predictive anatomy.

There are obviously innumerable special problems that derive from GP3, for different organs, tissues, and systems, probably organelles and cells too. And, just as obviously, they vastly exceed the domains of elasticity or more generally that of mechanical response. The above discussion focused on elasticity only for the sake of illustration of the concept – and to point to the fact that linear elasticity, the simplest possible mechanical response, is already enormously complicated, to be sure.

The nanotechnology-enabled, simplified forms of GP3 emerge from considering BioNanoScaffolds (BNS). These are multiphase composites, which are developed for regenerative medicine. In their first formulation, they were designed to regrow bone and the associated soft tissue (nerves, muscles, tendons, blood vessels) [134–137] following a catastrophic injury that would currently require amputation or the use of external fixation devices. The requirement for the BNS materials and implant structure are that they need to be a substitute for the shattered bone, cure in vivo rapidly enough to sustain ambulation within a few days, stimulate and protect the regeneration of the bone and soft tissues to full and sustainable load-bearing capacity, and then disintegrate in the body without causing harmful effects and requirement for additional surgeries (First call: DARPA-Broad Agency Announcement BAA-08–50; Second call: BAA number: DARPA-BAA-10–55) – a transformational vision, to be sure! The BNS materials developed under the leadership of Ennio Tasciotti and Bradley Weiner essentially comprise the following components: A biodegradable, polymeric matrix; biodegradable nanoporous silicon inhomogeneities embedded in the polymer, so to provide mechanical strength and the time-released delivery of antibiotics, biological growth factors, and analgesics; and biological stem cells, prepackaged with the materials, or recruited into it by factors released by the BNS components [38,39,42]. The stem cells necessary to regenerate the tissue originate from the patient [138–145].The implant may comprise several components, mimicking the structure and functions that it is designed to replace (Fig. 3). These components comprise different formulations of the BNS, with different proportions and distributions of the constituent phases.

This sets up exactly the problem of optimal design stated above: Under the expected loading condition, such as initially guarded ambulation, the mechanical fields in the implant must be such that mechanical failure does not occur – thus, the subsystems must be made sufficiently strong and stiff, by a sufficiently high concentration of embedded phases, and a suitable choice of polymer matrix. Yet, if the implant components are too stiff, the bone-forming cells do not experience sufficient mechanical stimulation to start regenerating the bone and associated soft tissue. This poses a design problem of extraordinary complexity, which must be solved by way of suitable mathematical tools, such as mechanical homogenization theory, since a trial-and-error approach is simply impossible.

Homogenization theory generally employs a “top-down” approach, which is, start with a continuum- or macroscopic-level description and then attempts to identify the micro- or nano-scale properties that comprise the phenomenological response, in the form of constitutive properties. This approach, though certainly justifiable for its viability, suffers from the lack of well-posedness in the inverse problem that seeks optimal distributions and properties of the phases. A "bottom-up" approach has the potential of being constructive in its format, but of course requires the management of extraordinary complexities at the atomic and molecular scales, and with the spatial distributions that occur at the scale of the inhomogeneities. A bridging perspective was developed by the scientific inspiration of Vladimir T. Granik. Termed “Doublet Mechanics” [132], it represents matter as an array of discrete points, with pairwise interactions. Transition from the discrete to the continuum level is rigorously derived through balance laws and thermodynamics. Full compatibility is achieved with continuum mechanics (at one limit scale) and lattice theory (at the atomic scale), while preserving an intrinsically greater richness at all intermediate scales. The connection between homogenization theory and Doublet Mechanics is in the equation:

$$\left[\mathbf{C}^{\mathrm{I}}+\alpha\langle(\mathbf{C}^{\mathrm{II}}-\mathbf{C}^{\mathrm{I}})[\mathbf{I}+\hat{\mathbf{E}}\mathbf{S}^{\mathrm{I}}(\mathbf{C}^{\mathrm{II}}-\mathbf{C}^{\mathrm{I}})]^{-1}\rangle\right]_{\mathrm{ijkl}}=\sum_{\alpha,\beta=1}^{\mathrm{n}}\tau_{\alpha i}\tau_{\alpha j}\tau_{\beta k}\tau_{\beta l}A_{\alpha\beta} \quad (10)$$

where $\boldsymbol{\tau}^{\alpha}$ is the unit vector for the α doublet, and $A_{\alpha\beta}$ is the constitutive axial elasticity matrix for the doublet assembly. Superscripts and subscripts are counting indices and have no vectorial significance. Thus, $\tau_{\alpha i}$ is the i-th vectorial component of the unit vector $\boldsymbol{\tau}^{\alpha}$, which corresponds to the α-th doublet (pair of points).

With this background, it is now possible to state:

Simplified Problem 11 (SP11). To solve Eq. 10 and for the strain concentrator $\hat{\mathbf{E}}$.

The yang of the benign biological evolution concept in biomechanics is, again, cancer. In Transport Oncophysics [18,67], cancer

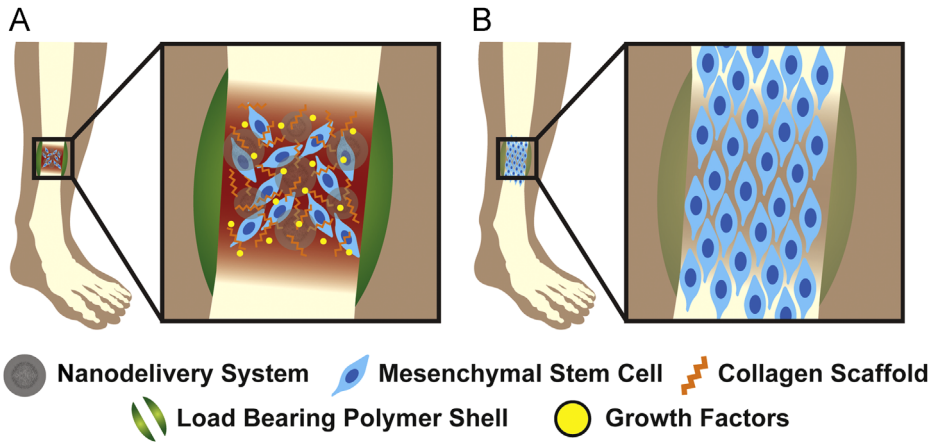

Fig. 3. Schematic representation of applied BioNanoScaffolds (BNS). (A) A broken, fractured or otherwise damaged long bone, for instance, can be encased with an implant structure (load-bearing biodegradable polymer shell) that contains a host of factors intended to help regrow the bone (cells, growth factors, drugs, etc.); (B) Over time, the combination of structural support and biological enhancements cooperate to bolster not only bone but surrounding soft tissue integrity and function. Also notable is the biodegradation of the polymer shell into components that can be easily and safely excreted from the body.

is defined as a proliferative disease of mass transport dys-regulation, which manifests itself primarily in the disruptions of the biological barriers that separate body compartments. From this perspective, it is perhaps natural to view cancer as a "parasitic organ" that grows uncontrollably exactly because of the competitive advantages it acquires in its mechanical functions. Most evident among these are: The ability to "push" its way into surrounding tissues (Cancer Hallmark: Invasion); The ability to move to remote locations (Hallmark: Metastasis); and The ability to modify its embedding environment and surrounding tissue, upsetting the balance of distribution of nutrient and disposition of metabolites (Hallmark: Angiogenesis). It is a deranged form of optimal design of mechanical properties, providing an unquestionable evolutionary advantage, but with most unfortunate outcomes for the host organism.

It is then perhaps not surprising that the theory of cancer growth and its response to treatment finds it central components in non-linear mechanics. Bernhard Schrefler and coworkers have used multiphase porous media mechanics to model tumor evolution in a three dimensional setting, using governing equations obtained via the Thermodynamically Constrained Averaging Theory (TCAT) [146,147]. A tumor mass is treated as a multiphase medium composed of an extracellular matrix (ECM); tumor cells (TC), which may become necrotic depending on the nutrient concentration and local mechanical pressure acting on the tumor phase; healthy cells (HC); and an interstitial fluid (IF) for the transport of nutrients. Existing blood vessels are modeled by line elements and blood flow is taken into account. The resulting set of equations involves second-order partial differential operators and is solved by a Finite Element method to predict the growth rate of the tumor mass as a function of the initial tumor-to-healthy cell density ratio, nutrient concentration, mechanical strain, cell adhesion and geometry. TCAT provides a rigorous yet flexible method for developing multiphase, continuum models at any scale of interest [148,149]. Differently from mixture theories applied in legacy models, TCAT considers the interfaces between constituents with interfacial properties throughout the domain. Interfaces are in fact a critical aspect when modeling tumor growth. Here, there is no need to trace sharp interfaces between constituents or to introduce computationally expensive phase field models which require higher order partial-differential operators, as in legacy models. Macroscopic interfaces arise naturally from the solution of an initial-boundary value problem that must be composed of the mass balance equations of all phases involved. The model has a modular structure, and further species and phases can be easily added. From a fuller understanding of the growth and response dynamics of cancer, one may indeed expect to identify promising clues for the development of more effective treatments.

5. Discussion

The fundamental approach of this paper was to state three general problems (GP) in mechanics and mathematics, and to reduce them to simplified problems (SP) that employ nanotechnology-based platforms of recent development, as means to validate and test the solutions of the SP, as well as gain insight into the solutions of the GP. This approach is only possible due to the development of the nanotechnology platforms, as solutions to clinical problems in medicine. Thus, nanomedicine grew to the point of warranting a place in the taxonomy of oncology [20], and more broadly of medicine, but it was through the discoveries that originated from these efforts that general problems in pathophysiology were identified, and a new field such as Transport Oncophysics [18,67] originated as a "daughter discipline".

Transport Oncophysics is "multiply multiscale", and in this sense it embodies general characteristics that are central to the vision behind this paper: It requires multiscale mathematical modeling of the mechanical properties and transport. Thus, the molecular scale needs to be integrated with the cell and tissue scales, bridging discrete descriptions of matter with the world of continuum and structural mechanics. An example of this is the mathematical theory of adhesion to vascular walls, which includes molecular recognition (in the domain, e.g., of Molecular Dynamics) and specific conjugation, and macroscopic descriptors of flow. Another example, discussed in Section 4, is the identification of the continuum-level, poly-inclusion strain concentrator tensor from the discrete representation of Doublet Mechanics. A third example is the development of diffusion and osmotic pressure laws from thermal agitation of molecules, as discussed in Section 3.

The second component of the multiscale nature is in the fact that the imaging methods for experimental verification of transport and bio-distribution inherently bridge multiple dimensions and modalities: Nuclear and optical methods for the scales bridging the molecular to the subcellular organelle to the individual cell and cell clusters; magnetic resonance, ultrasound and x-ray-based methodologies bridging to the levels of tissues, organs, and full organisms. The entire spectrum is necessary for answering the GP and SP. Finally, the "probes" for the tests of transports across biological barriers and into tissue are themselves multiscale, from the nanoscopic to the micron-scale, to the composition thereof such as in the case of the MSV. Again, all of these are necessary, and an operational beauty to this approach is the fact that the same probes demonstrating success in disease-related transport processes of interest can then also be employed as vectors of therapy.

While the GP present an exceptionally tall order of complexity, they still lend themselves to further generalization into their combination as “Super” General Problems, with the corresponding integrated nanoplatforms as technology support. Examples of these would include the use of electrokinetic SLNC to release synthetic, rationally designed nanoparticles that mimic the transport across cell membranes. Further integration is attained by considering the "mathematical physiology" counterpart of this problem, that is, the notion of optimizing this form of transport by optimizing the combination of its biological equivalents, and developing associations of this with biological evolution.

At this point in the paper, it may be opportune to retrace our steps, and focus on the beneficial impact that the GP, the SP, and the underlying nanotechnology platforms may have in the clinic.

5.1. Implications for medicine

The essential promise of nanomedicine is the “personalization” of all phases of health care, from screening and laboratory or imaging diagnostics, to multiple forms of therapy and measures to preserve the quality of life in extreme situations. The necessity for personalization is perhaps nowhere more apparent than in oncology: Following a centuries-old tradition, we continue to classify cancers by site of origin (breast, lung, colon, etc.). Further subdivisions of the cancer types exist, totaling about 200 "official" cancer types that are based on macroscopic characteristics of a particular neoplasm, and/or its cell type and molecular expressions. Yet, at the molecular profile level, no two cancers are identical, evoking the notion of "malignant snow flakes". To add to the complexity, each individual cancer lesion is an evolving biological entity, characterized by extreme genomic instability. Cancers always evolve in time, in the sense that new clones are spawned, locally or at distant sites (metastases), which have different characteristics from the primary form. One dramatic difference is observed in the characteristics of mass transport: A primary tumor will most

frequently originate metastases that have entirely different permeabilities to different therapeutic agents, mostly because of differences in the sequences and presentations of biological barriers. Thus, the drug that is effective at treating the primary cancer often fails to treat the metastasis, or treats a metastasis now but may not be able to do so in a few weeks. Worse yet, metastases from the same primary tumor to different sites (say, liver versus brain) normally have entirely different transport characteristics, and even when metastasizing to the same organ and in the same generation, major transport differences may be found. In front of this extraordinary, and continuously heterogeneity of presentation, resort to an "individualized" approach is necessary, if we are ever to achieve major successes in the fight against metastatic disease. By now it is ominously apparent, that "individualization" here refers not only to the individual patient at a given moment in time, but certainly to the individual cancer lesion in the patient, and probably even deeper, to tissue level and cell subtypes. The individualization of cancer medicine is not only a good idea, it is an absolute necessity.

A simple definition of individualization, in the context of therapy, articulates into three operational requirements, for each patient at each moment in time: To provide treatment at the right location in the body, with the right time profile of intervention, and to engage the local and systemic biology in a joint effort to heal. These requirements map exactly onto the above-stated GP, and their progeny of SP enabled by nanotechnology platforms. Thus, the engagement of the local biology in the healing process is exemplified by the regenerative medicine nature of the BNS (Section 4), while the optimized temporal control of the release of therapeutic agents is the very *raison d' etre* of the nanochanneled implantable “glands” (Section 3).

As for the requirement of reaching the right location: The notion of *personalization of the therapy through the personalization of the carrier vector* [18] emerges from the described approaches, with application to cancer, cardiovascular disease, and other pathologies. In particular, Decuzzi and collaborators developed a multiscale, multiphysics computational model for the vascular deposition of systemically injected nanoconstructs combining macroscale, patient-specific data and micro/nanoscale information [150,151].

In another application of the MSV technology platform, it was demonstrated that geometrical confinement of MRI contrast agents, both T1 and T2, into mesoporous structures can enhance magnetic relaxivity [152,153].

This approach allows for imaging of a very small number of cells of interest, such as the initial phase of a primary cancer, or the movement of cancer cells toward the establishment of a metastasis, with exceptional potential for novel and early diagnostic tests. In addition, it is possible to co-load the contrast agents and therapeutic substances in the MSV, thus forming a true "theragnostic" (therapeutic and diagnostic) system, which allows for the monitoring in real-time of the distribution of the therapy-carrying vectors. An example of the theragnostic approach arises upon consideration of the fact that the same agents that can serve for diagnostic imaging contrast, can also be activated by exogenous energy, to perform thermal ablation of the lesions of interest, or enhance transport of therapeutic agents. For example, gold nanoparticles are excellent contrast agents for CT scans and can be triggered to perform thermal ablation by radiofrequency or laser light activation, especially in the near-infrared regimen [154]. Iron oxide nanoparticles are outstanding T2 contrast agents for MRI and can be activated to thermal therapy by magnetic fields as well [155]. The notion of using imaging to monitor distribution of therapeutic agent, and then base the localization treatment on the information derived from imaging, opens unprecedented vistas for medical therapeutics: Nothing of the sort is possible in contemporary medicine, and this is a major reason for the unnecessarily omnipresent adverse side-effects in the course of treatment, and the difficulty in selecting the therapeutic regimens among multiple options in patients with progressing neoplastic disease.

The emerging therapeutic methodology is several quantum leaps beyond current practice, yet it is based on science and technology platforms that are currently available, to a large extent. This vision encapsulates the following, again with metastatic cancer as an example, and certainly one of extraordinary importance in its own right:

First, imaging protocols are used to localize the lesions, and map their transport properties, using the Cristini parameters (Section 2) and possible further refinements thereof. It is noted that Kenji Yokoi has identified and validated a set of soluble markers that are themselves predictors of transport properties to cancer lesions and can be derived from simple blood tests (publication to appear). These could be used to supplement the transport properties identified through imaging. The use of molecularly targeted contrast agent might allow for the molecular mapping of the vascular endothelia that are associated with the target lesions [156,157].

Second, the transport properties for each lesion, or family of lesions, are entered into the rational design algorithms, starting with those developed by Decuzzi and coworkers, to determine the optimal design (size, shape, temporal degradation profile, surface properties and affinity) for the first-stage vectors in a MSV platform.

Third, the design prescriptions are entered into the manufacturing protocols for particles with the properties required by the mathematical optimization algorithm. Obviously, this requires a fabrication capability that affords exquisite control over the particle geometry and chemistry, with the further requirements of biocompatibility of the constituent materials, and the possibility of manufacturing scale-up with quality controls in harmony with the mandates of the Food and Drug Administration (FDA) and other regulatory bodies. The only strategy that we could find that would meet this set of extraordinarily demanding manufacturing requirements was to employ nanoporous silicon and photolithographic techniques [158,159], which we have pioneered and refined over 20 years.

Fourth, the first-stage particles must be loaded with therapeutic or contrast agents, or combinations thereof. For each of these, it is necessary to develop an intermediate “second-stage” nanoparticle that contains the agents of therapy or imaging contrast, and in turn be contained in, and released from, the nanopores within the first-stage particles. As described in Sections 1 and 2, our group has demonstrated this approach for a broad variety of second-stage particles (nanoliposomes, polymer, gold, and carbon nanoparticles, micelles, iron oxide particulates, among others) and therapeutic agents (doxorubicin, Taxol, different siRNAs, etc.). It stands to reason that the more "sophisticated" and targeted the drug is, the better the clinical outcome will be. However, from the experience gathered over many years, my inclination is to be fairly agnostic as regards to the drug: Any that is cytotoxic is just as good, including the simplest and cheapest possible drugs, as long as the delivery vector is accurate. This is a major divergence from cancer pharmaceutical orthodoxy, which looks for molecularly targeted agents, of extraordinary sophistication, specificity to tumor-associated signaling processes, and frequently extravagant cost.

This four-step methodology ensures that maximal tumor microenvironment concentration of the therapeutic molecules is attained. Further refinement of frontier optical imaging techniques such as intravital microscopy might allow for the direct imaging of the interactions between the vectors and the cancer cells, and the cells and tissues of the tumor-associated microenvironment, as is currently possible in animal models [160],

yielding further opportunities for the refinement of rational designs protocols.

In clinical oncology, molecular "scores" are associated with decisions about therapeutic regimens. Specifically in breast cancer, a score of 3+ (high abundance) of the Her/2neu cell membrane receptor warrants use of a very successful molecularly-targeted drug, a monoclonal antibody named trastuzumab. A low abundance score (such as 1+) indicates the likely inefficacy of this therapy, which is then normally deselected in favor of other approaches, such as aromatase inhibitors and/or hormonal therapy for breast cancers with high estrogen and progesterone receptor scores.

In view of the developments in transport oncophysics, it is not inconceivable that this molecular signature scoring system for therapeutic guidance may be supplemented, or supplanted in the future by a system that is based on transport properties, and is therefore to some extent agnostic as to the drug being delivered. This novel scoring system envisions the identification of the pathologies on the basis of their mass transport properties, and in particular of the transport properties across the pathologically modified barriers that pertain to the lesions of interest: A primary or metastatic cancer lesion could be classified by a series of numbers that identify its accessibility to a molecule, nanoparticle, or multistage vector of given characteristics, with each number referring to one in a sequence of biobarriers. For example, in a scale of 1–5 rating highest penetration as 5, a tumor with respect to a (naked, nanoencapsulated, or MSV-formulated) therapeutic agent could be scored (5, 3, 3, 4, 5, 5) with respect to (local vascular endothelium, systemic macrophage system, tumor hydrostatic pressure state, cell membrane, lysozyme escape, multidrug efflux pump) indicating a good likelihood of deployment of the therapeutic agent in the cytosol of the target cells, while a scoring string with any numerical entry of 1 or 2 would indicate the probable inefficacy of the delivery. Similar approaches could be devised for any non-oncological pathology where transport is an essential consideration.

The roots and foundation of this new world in medicine are in mathematics, in mechanics, and in the solution of the general and simplified problems stated above.

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