USNCTAM PERSPECTIVES ON MECHANICS IN MEDICINE

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ABSTRACT

Over decades, the Theoretical and Applied Mechanics (TAM) community has developed sophisticated approaches for analyzing the behavior of complex engineering systems. Most of these approaches have targeted systems in the transportation, materials, defense, and energy industries. Applying and further developing engineering approaches for understanding, predicting, and modulating the response of complicated biomedical processes not only holds great promise in meeting societal needs but also poses serious challenges.

This report, prepared for the US National Committee on TAM, aims to identify the most pressing challenges in Biological Sciences and Medicine that can be tackled within the broad field of Mechanics. This echoes and complements a number of national and international initiatives aiming at fostering interdisciplinary biomedical research. This report also comments on cultural/educational challenges.

Specifically, the report focuses on three major thrusts in which we believe mechanics has and will continue to have a substantial impact: i) Rationally engineering injectable nano/microdevices for imaging and therapy of disease. Within this context, we discuss nanoparticle carrier design, vascular transport and adhesion, endocytosis, and tumor growth in response to therapy, as well as uncertainty quantification techniques to better connect models and experiments. ii) Design of biomedical devices including point-of-care diagnostic systems, model organ and multi-organ microdevices, and pulsatile ventricular assistant devices. iii) Mechanics of cellular processes, including mechanosensing and mechanotransduction, improved characterization of cellular constitutive behavior, and microfluidic systems for single cell studies.

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I. Introduction

For many years, the theoretical and applied mechanics community has addressed complex engineering problems, primarily in the fields of energy, materials, transportation, and defense. In addressing these problems, a broad array of tools, both experimental and theoretical, have been developed, tested and honed. These techniques allow for detailed characterization and behavioral prediction of the highly complex systems encountered in engineering applications. The traditional applications for which these tools were developed have large numbers of degrees of freedom and operate under unpredictable conditions. Yet by utilizing the framework of engineering and science, engineers and scientists can design these systems with a high degree of fidelity.

For most of its history, medical practice has been based on qualitative approaches often guided solely by external inspections and the analysis of the patients' vital signs, such as the body temperature, blood pressure, pulse, respiration rate, and weight. The interconnection of medicine and technology has led to the development of a series of instruments and equipment that are used daily in clinical practice to assist medical intervention and treatment planning. This includes the development of the imaging technologies, such as x-ray, ultrasound, magnetic resonance and nuclear imaging, and any combination thereof, for the non-invasive, internal inspection of a patient's body; external machines for assisting the natural function of the kidneys, heart and lungs; artificial implants for cardiovascular and orthopedic applications. The recent strides in computational engineering and sciences and nano/micro technologies present the field of medicine with new opportunities for developing personalized interventions and predictive tools and, possibly, for radically changing clinical practice. Mechanics, intended as the science concerned with the behavior of bodies and their interaction with the environment, can contribute significantly to this development.

In this report, prepared for the US National Committee on Theoretical and Applied Mechanics, we identify and discuss what we consider to be the most pressing challenges in Biological Sciences and Medicine for which mechanics can have a substantial impact. These challenges are the i) rationally engineering injectable nano/microdevices for imaging and therapy of disease, with discussions on nanoparticle (NP) carrier design, vascular transport and adhesion, endocytosis, tumor growth in response to therapy, and uncertainty quantification techniques to better guide models and experiments; ii) design of biomedical devices including point-of-care diagnostic systems, model organ and multi-organ microdevices, and pulsatile ventricular assistant devices; iii) mechanics of cellular processes including mechanosensing and mechanotransduction, improved characterization of cellular constitutive behavior, and microfluidic systems for single cell studies. In addition to these specific, scientific problems, we highlight the educational/cultural challenges and opportunities stemming from the interdisciplinary nature of mechanics in medicine.

II. Nanoparticle-mediated drug delivery

1. Overview

The history of applications of mechanics to medicine and biology is extraordinarily rich and diverse; suffice it to recognize its fundamental role in orthopedics, or the absolute reliance

of pharmacokinetics and pharmacodynamics on the equations of mass transport, or the centrality of fluid dynamics in the understanding of cardiovascular physiology and pathology. Beyond these traditional, yet still very productive and important areas, it is now possible to envision novel connections between mechanics and medicine in ways that were unimaginable until recent times, since they are made possible by the emergence of novel disciplines.

A first paradigm for this convergence is embodied by the triangulation of biology and medicine with mechanics by means of nanotechnology; a second, corollary example is the framework of Transport Oncophysics; yet another is the new field of Predictive Anatomy. By way of illustration one may consider the problem of transport of nano-scale objects (biological such as protein, or synthetic like a chemotherapeutic molecule, a radiological contrast agent, or an NP) inside the body. Though they obviously obey the general mechanical laws of transport, their distribution in the body is largely impossible to predict a priori, in view of the impossibility to establish general governing equations, which will take into account the various biological barriers of the body, which in turn ultimately determine the transport profiles. Yet, this is as central a problem as there can be in the field of biomechanics – life is predicated upon the ability of the body to succeed in transporting nano-scale objects in a very accurately controlled fashion. Though there is no current hope for a master equation of transport of an arbitrary biological nano-scale object, great simplifications can be attained by considering the body transport of synthetic NPs with precise engineering properties, so that the number of independent variables may be reduced to reason. Thus, nanotechnology enables the understanding of some fundamental aspects of biomechanics of mass transport in a manner that were unattainable before the development of precise methods for the manufacturing of NPs and their characterization. The additional beauty of this is that the very NPs that are developed for the study of the mechanics of body transport can then be employed as agents of therapy once the ability to distribute preferentially in target organs of the body is understood – this is the basis for new perspectives of cancer therapy and the personalization of treatment, based on mechanics and the rational design of vectors for the targeted delivery of drugs. A second example of nanotechnologies as models for otherwise intractable biomechanical transport problems is the use of synthetic nanochannels to replicate and study transport through nano-scale environments such as ionic and molecular channels on the surfaces of biological cells.

It is insightful to observe that the study of the body transport and selective accumulation of chemotherapeutic agents and nanomedicines has revealed that cancer is in reality a proliferative disease of mass transport dis-regulation, at multiple scales, from the local (tissue invasion by cells) to the systemic (distant metastases) and the molecular level (sub-cellular signaling pathologies). In this case, mechanics by way of nanotechnology yielded transport Oncophysics, i.e. the recognition of entirely novel perspectives on the nature of a major class of diseases such as cancer. Based on this paradigm, it may well be expected that further advances in mechanics could offer transformational insights into many other domains of medicine and biology.

Predictive Anatomy employs the mechanical tools of mathematical, multi-scale homogenization theory to predict the characteristics of body parts, as they are most likely to emerge in the course of evolutionary selection. The engineering counterpart of this novel discipline is the methodology of optimal design, which involves the statement of an objective function to be optimized, a space of design variables, and a set of constraints. Exactly the same

approach can be applied to yield “best designs” of body parts that serve mechanical functions such as the ability to bear loads, or transport mass and heat. Thus, recent methodologies such as the emergence of nanotechnology and advances in the mathematical theory of multi-scale homogenization can serve to join mechanics with medicine and biology in very innovative manners that provide new horizons for scientific advances, and opportunities for medical breakthroughs.

The expansion of nanotechnology over the past two decades has led to a paradigm shift in drug delivery. For most of the history of chemical therapeutics, delivery was non-specific and the single controllable parameter was concentration. Although targeting of specific pathways through drug design was first introduced in the 1950s [14], the control over molecular properties afforded by advances in nanotechnology has ushered in a new era in drug delivery. Whereas early targeting strategies relied on chemical changes to individual molecules, it is now possible to synthesize NPs with precisely controlled size, shape, stiffness, and surface chemistry to efficiently deliver drug molecules into diseased cells/tissues [9, 15]. Although researchers now have a greatly expanded set of knobs to turn when designing NPs, it is generally not clear how a change to a specific vehicle feature will alter the effectiveness of a drug, hence design of novel drug carriers require extensive and costly parametric studies that are generally specific to the system upon which the experiments were performed. Theoretical and computational modeling of the delivery process can greatly reduce the need for physical experiments and provide general design principles to expedite the design process [16-21].

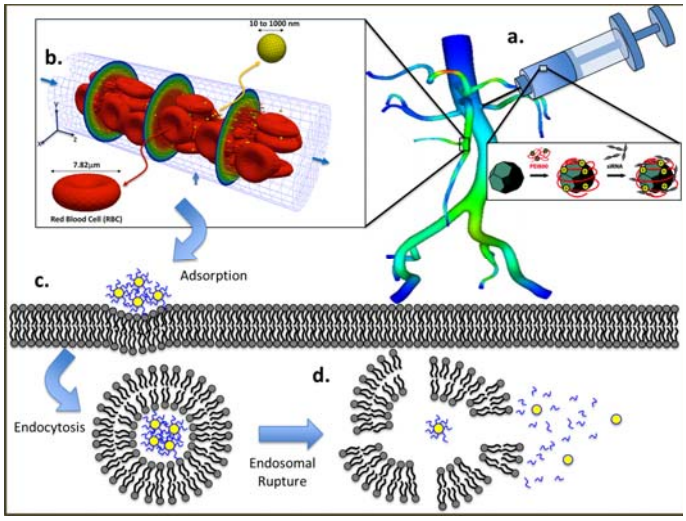

Figure. II.1. A schematic of the nanoparticle-mediated drug delivery process [3]. (a) A solution containing nanoparticle delivery platforms is injected into a patient’s circulatory system [4]. (b) In the microvasculature, nanoparticles are segregated from red blood cells, increasing their interaction with the endothelium, eventually leading to their removal from circulation [7]. (c) Nanoparticles diffusion through the extracellular matrix, eventually adsorbing onto the surface of a target cell. The nanoparticles are then endocytosed from the lipid membrane. (d) The endosome containing the drug-delivery complex ruptures, releasing the therapeutic agents into the cytoplasm. When released from the endosome, the nanoparticle cargo may be dissociated due to the local pH environment change.

Any modeling strategy that aims to predict a drug’s effectiveness based on the nanoscopic features of the delivery platform must account for processes across the disparate spatial and temporal scales traveled by a NP during delivery. Initially, a solution of drug carriers is introduced to the circulatory system, either through absorption or direct injection (Figure II.1 (a)). The circulation of the particles in the vasculature network can greatly affect the concentration of drug delivered to the area of diseased cells and depends on the geometry and chemistry of the particles. Modeling transport through the vasculature has presented significant challenges due to the vastly different length scales of the vascular network, which can range

from centimeters for the diameter of the ascending aorta to microns in the case of capillaries [4, 7, 22-24]. In the macrovasculature, particle transport can be modeled as an advection-diffusion process through complex networks. State-of-the-art techniques involve simulations through patient-specific vascular networks [22]. However near the endothelium and in the microvasculature, complex interactions between blood plasma, red blood cells (RBCs) and NPs require more detailed approaches that explicitly account for the interactions between red blood cells and NPs (Figure II.1(b)). These complex interactions can be studied by the immersed finite element technique [7, 24-42], accounting for the microcirculation behavior of NPs, under the influence of red blood cells and fluid flow. These models are proven to be capable of capturing the segregation of NPs and RBCs, as well as particle adhesion to the vascular walls [7, 23].

Upon adhesion, particles diffuse through the extracellular matrix, eventually reaching a cell possessing the targeted marker (Figure 1(c)). The adsorption of NPs onto the cell membrane is heavily influenced by the functionalization of the carrier surface, the local molecular composition of the membrane, and extracellular environmental variables such as pH and salt concentration [43]. Adsorbed particles are then wrapped by the cell membrane and endocytosed. The endosomes containing the NP-drug complexes can then either fuse into lysosomes, which can degrade the drug molecules in the process, or they can rupture, releasing their contents into the cytoplasm (Figure II.1 (d)). To understand the interactions between particles and cell membranes, it is necessary to model the systems at the molecular scale. Techniques such as molecular dynamics and molecular mean-field theory calculations are capable of illuminating the effect of particle size, shape and chemistry on cellular uptake rates [1, 44]. Although molecular calculations provide far more detail for specific systems, continuum theories can also provide useful guiding principles for NP design, even at length scales on the order of a single NP [45-49].

2. Nanoparticle-based drug carrier design

Nanoscale technologies can improve the bioavailability and biodistribution of systemically injected therapeutic and imaging agents [50, 51]. Nanoconstructs can navigate through the circulatory system and preferentially recognize the tumor neovasculature [52, 53] and encapsulate large amounts of different agents, for both therapy and imaging [54]. It is known that the blood vessel network in primary and metastatic tumors is different from the healthy vasculature [55, 56]. Endothelial cells form an imperfect lining with wide junctions (fenestrations), ranging in size from 100 to 1,000 nm. This leads to vascular hyper-permeability, lower mean blood velocity (1 – 10 μm/sec *vs* ~100 μm/sec in normal microvessels), and higher interstitial fluid pressure (up to 5 – 7 kPa). Furthermore, the surface density of inflammatory vascular molecules, such as ICAM-1 and E-Selectin, is 1 to 2 orders of magnitude higher on the tumor endothelium. Also, tumor specific vascular receptors, such as the $\alpha_v\beta_3$ integrins, are expressed at levels of 100 – 1,000 molecules/μm^2 [57]. Taking advantage of all of these differences between healthy and diseased vessels, a plethora of nanoconstructs with different sizes, surface properties, and more recently shapes have been developed over the last 20 years for delivering imaging and therapeutic agents preferentially to the malignant tissue. Indeed, the encapsulation of different, and multiple, agents into nanoconstructs has provided significant improvements in pharmacokinetics, toxicity, and biodistribution. Despite all of this, the

effective detection and treatment of malignant masses via the systemic injection of nanoconstructs is still limited by insufficient accumulation at the biological target (<< 10% injected dose per gram tumor – %ID/g) and non-specific sequestration by organs of the reticulo-endothelial system (RES) (tumor-to-liver < 0.1). Even the notion of targeting nanoconstructs to specific receptors, expressed on the tumor neovasculature or cells, has often led to contradictory results. The development of novel, clinically relevant delivery systems with high tumoritropic accumulation is critical for further improvement therapeutic outcomes and the early detection of solid tumors.

The size, surface properties, stiffness, and shape of the nanoconstructs affect their in vivo behavior and therapeutic efficacy. The importance of tuning the nanoconstruct size and surface properties has been recognized in the late '90s. In a series of seminal papers, Jain and collaborators [58] showed that liposomes and latex beads smaller than 300 – 400 nm in diameter would accumulate more efficiently in tumors than larger beads, via passive extravasation at the tumor fenestrations. This is the 'dogma' that has guided the field of nanomedicine since then and is known as the Enhanced Permeability and Retention (EPR) effect. Also, further studies [59-61] showed that the surface charge of lipid-based nanoconstructs can control accumulation in tumors, as well as in the liver and lungs. These were followed by many studies further elaborating on the role of nanoconstruct size and surface charge, for different material formulations and surface chemistry [62-64]. Molecular specific nanoconstructs have also been developed, where the particle surface is coated with ligand molecules capable of recognizing and binding to counter molecules (receptors) expressed on the target cells [65]. Despite its high in-vitro efficiency, this approach suffers in-vivo due to reduced binding affinity, lack of ligand immunogenicity, and the limited number of ligand molecules available, especially for the smaller particles. Because of this, data in the literature on specific tumor targeting of nanoconstructs are still highly controversial [66, 67]. Following the EPR dogma, a myriad of nanoconstructs have been developed with different surface properties and sizes, often presenting only minimal improvements in terms of tumor accumulation and liver escape. More recently, novel nanofabrication strategies have been presented for the synthesis of non-spherical nanoconstructs [68-71]. This fostered new theoretical [46, 47, 53, 72-74], in vitro [75-79] and in vivo [52, 80-84] studies demonstrating the importance of shape in controlling the vascular behavior, cellular uptake, and differential organ accumulation of the systemically injected nanoconstructs. Size, shape and surface properties can be envisioned as three independent variables in an optimization problem where the objectives are to maximize tumor accumulation and minimize the non-specific sequestration of nanoconstructs.

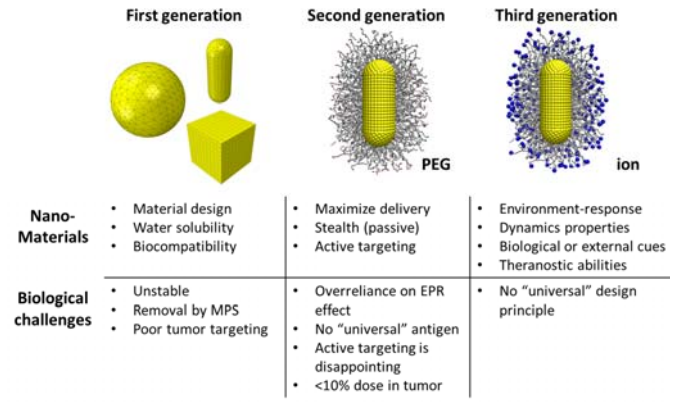

Figure II.2. Evolution of NPs design with their properties and biological challenges. The figure is taken and modified from Ref.[8].

The physiochemical properties of NPs, such as size, shape, surface charge, and stiffness, can affect their

biological clearance. Therefore, NPs can be modified in various ways to extend their circulation time. In recent decades, the design of NPs for biomedical applications has been advanced by studying their biological responses. The evolution of the NP carriers has followed advances in understanding of how size, shape, surface and stiffness affect efficacy. As shown in Figure II.2, there are three generations of NPs developed for biomedical applications [8]. In the first generation of NPs, the NPs are functionalized with basic surface chemistry (charges/ligands) and are evaluated through their biocompatibility and toxicity [85, 86]. However, these NPs are unstable and usually internalized by the immune cells during circulation. To overcome these problems in the second generation, the surfaces of NPs are grafted with polymer chains, improving their water solubility and allowing them to avoid aggregation and opsonization. Compared with the first generation, the second generation of NPs demonstrates improved stability and targeting in biological systems. However, the active targeting of these NPs to the tumor cells or other diseased cells is still disappointing. Thus, the third generation NPs shifts the design paradigm from stable materials to 'intelligent' and environmentally-responsive materials with improved targeting capabilities. Local environmental (i.e. pH value) changes cause the properties of these NPs to change in a prescribed way. Here we should emphasize that although the design of NPs shifts from the first-generation to the third-generation, the first- and second-generation NPs still have many applications in different areas, and their behaviors are still poorly understood. Moreover, a comprehensive understanding on the first- and second-generation NPs will help us to efficiently design the third-generation NPs.

3. Vascular dynamics and adhesion

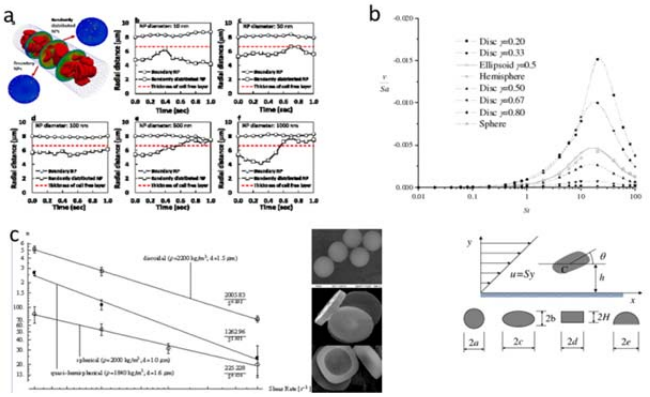

Figure II.3. Vascular dynamics of non-spherical nanoconstructs. a) Fast moving RBCs confine sub-micron sized nanoconstructs in proximity of the vessel walls, which favors the recognition of the diseased endothelium [7]. b) Thin discoidal nanoconstructs drift laterally, across the stream lines, with a higher velocity as compared to spherical, quasi-hemispherical or thick discoidal nanoconstructs [10]. c) In vitro parallel plate flow chamber experiments confirm that thin discoidal nanoconstructs would deposit and adhere more efficiently than spherical and quasi-hemispherical nanoconstructs, over a wide range of wall shear rates *S* [11].

The solution of such a problem requires the understanding and mathematization of the fundamental events in the journey of systemically injected nanoconstructs. In the case of nanoconstructs designed to adhere to the tumor microvasculature, these events are: i) transport within the vascular network; ii) firm adhesion at the diseased vessel walls; and iii) recognition/uptake by macrophages in the RES organs (mostly the liver, spleen, lungs). As per the vascular transport, small nanoconstructs (< 500 nm) tend to follow the streamlines moving parallel to the blood vessel walls with minimal interaction with the fast moving RBCs (Figure II.3(a)) [7]. However, sub-micron sized (and larger) nanoconstructs are pushed laterally by the RBCs and forced to move within the so called 'cell free layer' in proximity of

the vessel walls (Figure II.3 (a)). Note that this is similar to the behavior extensively documented for platelets and leukocytes [87]. Moreover, we have shown that thin discoidal sub-micrometer nanoconstructs are the most efficient to drift laterally across the streamlines (*margination dynamics*), which increases their likelihood of recognizing the diseased vasculature [53, 74, 75, 88]. This is evident in Figure II.3, where the margination performance of nanoconstructs with different size and shape combinations is presented. As per the vascular adhesion, the firm arrest of nanoconstructs at the vessel walls under flow is achieved only if the hydrodynamic forces are balanced out by sufficiently strong interfacial adhesive interactions. The latest could be specific, i.e. mediated by the formation of stable ligand-receptor bonds, and non-specific, i.e. resulting from colloidal interactions (van der Walls, electrostatic, steric forces) and non-specific molecular interactions, as mediated by plasma proteins adsorbed on the nanoconstruct surface. Theoretical predictions, in vitro analysis, and in vivo experiments have demonstrated that thin discoidal sub-micrometer nanoconstructs more firmly adhere to the vessel walls under flow as compared to spherical and slender cylindrical particles [13, 47, 53]. Furthermore, Figure II.4 (a) shows that for each particle shape there is an optimal size (or volume) that maximizes adhesion. In particular, thin discoidal nanoconstructs offer a larger surface of adhesion and oppose a smaller cross section to the flow thus reducing the dragging forces that would dislodge them away [47]. And for these reasons, thin discoidal particles are more efficient in adhering to the vessel walls. This has been confirmed also in vivo using two different orthotopic tumor models: melanoma and breast cancer (Figure II.4 (d)) [13, 83]. As per cell internalization, nanoconstruct geometry has been shown by several authors to dramatically affect the rates and mechanisms of uptake (see following sections). For instance, it is known that the rate of internalization of large spherical particles (> 500 nm) decreases as their diameter increases [53]. This can be predicted in terms of the geometrical and surface properties of the particles [46, 73]. Indeed internalization can be limited by increasing the density of PEG chains on the nanoconstruct surface, which leads to larger interfacial repulsive steric interactions. However, PEG chains tend to progressively detach and, consequently, nanoconstructs lose their shielding over time. On the other hand, it has been shown that elongated particles, laying with their major axes parallel to the cell membrane, can more efficiently resist cell internalization [89]. This has been demonstrated for elliptical particles and observed experimentally for micrometric and sub-micrometric particles exposed to macrophages and tumor cells [76, 77]. This has suggested the use of non-spherical nanoconstructs, in particular discoidal nanoconstructs with a sub-micrometer size to maximize accumulation at the diseased vasculature.

The above description demonstrates that nanoconstructs can be engineered by rationally selecting their size, shape, surface properties with the objective of maximizing their accumulation with the diseased tissue while limiting the sequestration in healthy organs. The rational design of nanoconstructs should integrate sophisticated computational modeling, accounting for the complexity of the blood flow, vascular geometry, and uncertainty in the quantification of the biological variables (receptor density, blood velocity, and so on); devices and apparatus for accurate in vitro characterization of vascular adhesion under complex flow patterns, internalization by endothelial cells and professional phagocytes; and eventually in vivo experiments through which quantify the circulation half-life and organ specific accumulation of the nanoconstructs over time. Such an endeavor requires the convergence of multiple disciplines and expertise pertaining to the field of computational mechanics, chemistry, physics as well as biology, immunology and biomedical sciences. This will eventually lead to the development of a new class of truly interdisciplinary scientists that would grasp the details of each individual field, facilitate constructive synergies, and be capable of synthesis towards the achievement of the common goal.

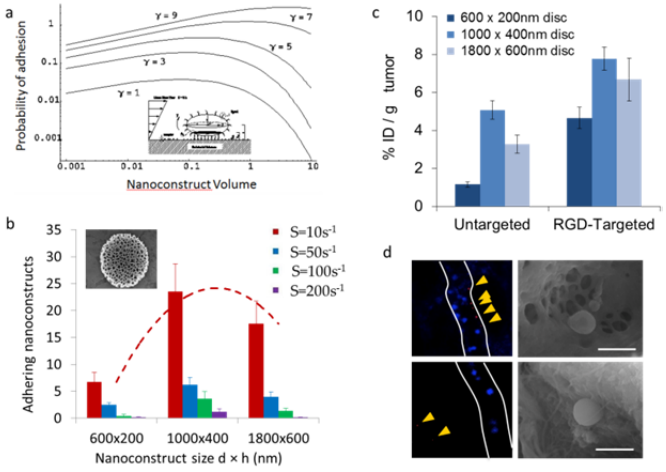

Figure II.4. Vascular adhesion of non-spherical nanoconstructs. a) The probability of vascular adhesion grows as the shape deviates from spherical. (γ = 1 – sphere; γ<< 1 – quasi-discoidal particles) [9]. b) Parallel plate flow chamber experiments showing a maximum vascular adhesion occurring for 1,000 x 400 nm discoidal nanoconstructs [12]. c) Tumor accumulation of untargeted and RGD-4C targeted discoidal nanoconstructs, demonstrating again a maximum accumulation for the 1,000 x 400 nm discoidal nanoconstructs [13]. d) Fluorescent images and SEM micrographs showing discoidal nanoconstructs (see yellow arrows) laying on the tumor neovasculature. 10% of the RBCs were stained in blue [13].

4. Endocytosis

i. Cell Uptake of One-Dimensional Nanomaterials

Various types of NPs, nanowires, nanofibers, nanotubes, and atomically thin plates and sheets have emerged as promising candidates for potential applications in next generation biosensors, drug delivery, and medical imaging. There is an urgent societal need for better understanding of both beneficial and hazardous effects of these nanotechnologies. Below is a summary of some recent work on the mechanics of cell uptake of one-dimensional nanomaterials such as nanotubes and nanowires. A combined study based on electron microscopy, theoretical modeling and molecular dynamics simulations shows that carbon nanotubes enter cells via a tip

recognition pathway that involves receptor binding, tube rotation driven by elastic energy at the tube–bilayer interface, and near-vertical entry.

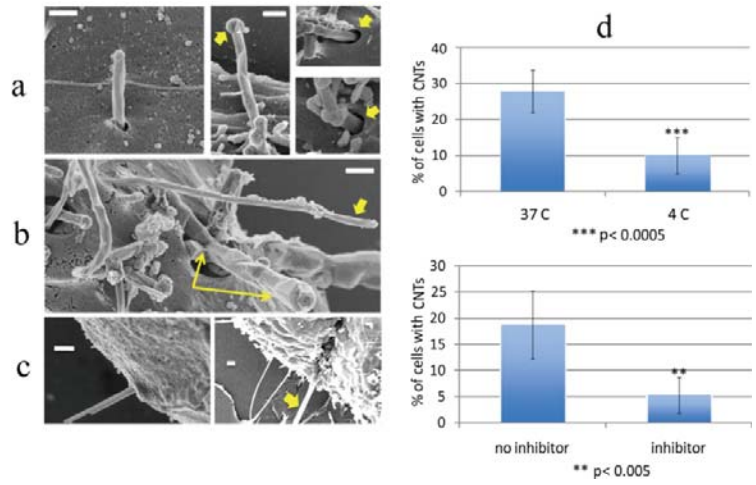

Figure II.5. Experimental observations of energy-dependent tip-entry of one-dimensional nanomaterials into cells. (a) Carbon nanotubes entering murine liver cells. Arrow in middle panel shows carbon shell at the tube tip that distinguishes the nanotubes from surface microvilli. Arrows in right panels show close views of membrane invaginations surrounding the tubes at the point of entry. (b) Examples of nanotube tip entry in human mesothelial cells. Both an isolated tube (single arrow) and a tube bundle (double arrow) are seen in the process of high-angle entry. (c) Examples of active tip-entry for other one-dimensional materials: 30 nm gold nanowires (left) and a 500 nm crocidolite asbestos fiber (right). (d) Effects of temperature and metabolic inhibitors on multiwalled CNT uptake as tests for active endocytic uptake. All images are obtained by field emission scanning electron microscopy following fixation and contrast enhancement with osmium tetroxide. All scale bars are 300 nm. Figure from Ref. [1].

Research on the mechanics of cell-nanomaterials interaction is of significance not only to the understanding of hazardous effects of viruses and nanomaterials in general but also to biomedical applications such as gene/drug delivery and medical imaging [90-92]. A current problem of immediate concern to society is that nanomaterials, which include various types of NPs, nanowires, nanofibers, nanotubes and atomically thin plates and sheets, could penetrate the membrane of human and animal cells. It is known that geometrical properties of NPs such as size [45, 48], shape [17, 46, 47, 93-98], elastic modulus [49] and surface microstructure [99, 100] can substantially influence endocytosis, phagocytosis, circulation [91] and targeting [92]. Below is a summary of some recent work at Brown University on the mechanics of cell uptake of one-dimensional nanomaterials [1]. Compared with other non-spherical NPs the cellular interactions of one-dimensional nanomaterials such as carbon nanotubes (CNTs) are particularly important for biomedical diagnostics and therapies [101, 102], and for managing health impacts of nanomaterials following occupational or environmental exposure [103-105].

Recently, we carried out a combined study by electron microscopy, theoretical modeling and molecular dynamics to elucidate the fundamental interactions of cylindrical one-dimensional nanomaterials with eukaryotic cell membranes [1]. Figure II.5 shows electron micrographs of common morphologies in the near-membrane region following in vitro exposure of murine liver cells or human mesothelial cells to different types of one-dimensional nanomaterials, including carbon nanotubes, crocidolite asbestos nanofibers and amine-terminated gold nanowires [1]. It can be seen that near vertical, tip entry is a common uptake pathway for geometrically similar but chemically very different nanomaterials. To determine whether the uptake of CNTs is

mediated by energy dependent endocytosis, murine liver cells were incubated either at 4°C or 37°C. Internalization of CNTs was significantly decreased at 4°C (Figure II.5 (d)). In the presence of metabolic inhibitors, the uptake of CNTs was also significantly decreased (Figure II.5 (d)) confirming that this uptake requires ATP.

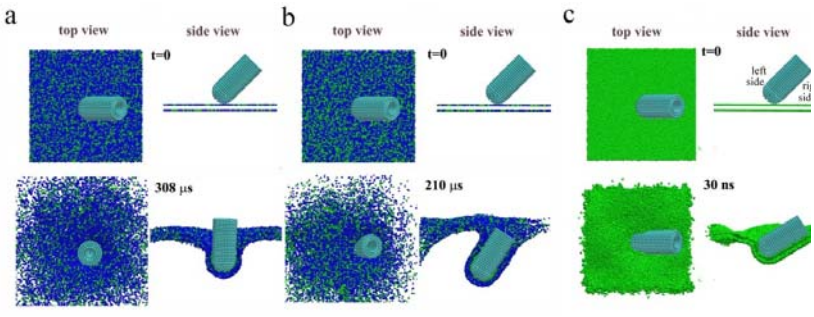

Figure II.6. Time sequence of coarse-grained molecular dynamics simulation results showing a multiwalled carbon nanotube penetrating the cell membrane at an initial entry angle of θ_0=45° as a function of receptor density. The receptor (green) densities are a: ϕ=0.25, b: ϕ=0.33, and c: ϕ=1. Figure from reference [1].

Why is tip-entry the preferred mode of cellular uptake of one-dimensional nanomaterials? This fundamental question has been investigated using coarse-grained molecular dynamics simulations under the basic hypothesis that nanotubes with closed, rounded caps can mimic particles and initiate endocytosis, and that elastic energy in the plasma membrane provides a driving force to rotate one-dimensional nanostructures from their initial angle of contact to high angles. In the simulations, a capped multiwalled CNT is initially positioned in close proximity above the surface of a patch of bilayer. The initial angle between the axis of the nanotube and the bilayer is pre-selected and a range of receptor densities, ϕ, are considered. The receptors diffuse along the bilayer and aggregate around the nanotube due to binding affinity. As receptors cluster and adhere to the nanotube surface, the tube is pulled into the bilayer and wrapped. In this process the tube is observed to spontaneously rotate to achieve an entry angle close to 90°, driven by membrane elastic energy minimization during wrapping (Figure II.6). Figure II.6 (b) shows that, at a higher receptor density of ϕ=0.33, the nanotube can become fully wrapped before it reaches the 90° entry angle. Generally, increasing receptor density tends to hinder the rotation towards 90° entry. In the extreme case of ϕ=1, in which the adhesion loses specificity, the membrane on the right side of the nanotube adheres to the tube much faster than that on the left side (Figure II.6(c)), and the nanotube adopts a very small entry angle. This can be understood from the fact that, for nonspecific adhesion, the right side membrane has the distinct advantage of being initially closer to the tube surface and dominates the early-stage receptor binding before rotation can occur. These simulations reveal two competing kinetic processes: rotation of the tube toward a 90° entry angle to relax elastic energy in the membrane; and wrapping speeds on different sides of the tube governed by receptor diffusion. If the former prevails, as would be expected at relatively low receptor densities, the final entry angle will be close to 90°. Note that the extreme case of nonspecific interactions shown in Figure II.6(c) is an interesting theoretical limit which is not expected to be important for a real cellular system.

Similar observations of tip entry and rotation toward 90º entry have been observed in simulations for different carbon nanotube diameters and lengths, receptor densities, receptor binding strengths and initial entry angles, and the results show that the tube still adopts a 90º

entry pathway [1]. Further simulations show that the tip-entry mechanism is essentially unchanged if the hemispherical caps are replaced by enlarged shells typical of catalytically produced carbon nanotubes, or if the nanotubes exist in suspension as small bundles. Interestingly, it is found that open-ended nanotubes do not undergo tip entry since they lack carbon atom sites for receptor binding on the cap in the early stages of wrapping. This suggests that oxidative cutting or other intelligent tip modification may be used to control the membrane interaction and cell entry of a subclass of hollow one-dimensional nanomaterials. Moreover, simple analytical models and coarse-grained molecular dynamics simulations show that the time scale for tip rotation is one or two orders of magnitude smaller than that for the overall wrapping of the NPs, and tip entry is expected to be a favorable pathway for cellular uptake of capped nanotubes and other one-dimensional nanomaterials [1]. This tip-entry mechanism is proposed as a key initiator of frustrated uptake and toxicity, since a vertical alignment provides no opportunity for the cell membrane to sense or anticipate the ultimate length of the fibrous target material.

The latest theoretical studies in molecular dynamics simulations show that the cell uptake of one-dimensional nanomaterials via receptor-mediated endocytosis is governed by a single dimensionless parameter, the normalized membrane tension $\overline{\sigma} \equiv 2\sigma a^2 / \kappa$, where a denotes the nanomaterial radius, σ the membrane tension, and κ the bending stiffness of cell membrane. As cell membrane internalizes one-dimensional nanomaterials, the uptake follows a near-perpendicular entry mode at small membrane tension but it switches to a near-parallel interaction mode at large membrane tension. This $\overline{\sigma}$ -dependent uptake behavior is also found to be ubiquitous in the interplay between cell membranes and one-dimensional nanostructures, and has broad implications on the different interaction modes exhibited by single nanotubes and nanotube bundles, tubulation of nanoparticles and bacterial toxins on cell membranes, control of the size of filopodia, and measurement of cell membrane tension [106].

In physiological situations such as endocytosis, adhesion bonds between biomolecules on NPs and cell surfaces usually operate cooperatively, and an initial phase of particle attachment or docking should, in fact, play a very important role in the overall process of endocytosis. The large surface area enables CNTs to achieve sidewall functionalization, to act as a template for cargo molecules such as proteins [107], small molecules [108], and nucleic acids [109]. It will be interesting to consider different functional groups on CNT walls, the kinetic process of receptor diffusion [110-113], interaction and docking of particles of different sizes, shapes and elastic modulus near a cell, extending recent study by Shi et al. [97]. It can be imagined that carbon nanotubes with different sizes, tip shapes, and patterns of functional groups can form a tunable platform for designing controlled cellular uptake. Substantial challenges exist when considering elastic nanofibers instead of stiff carbon nanotubes. This extension will require solving diffusion equations on curved deforming surfaces, and can therefore be extremely difficult especially in three dimensional modeling. Simulation studies will also play critical roles in the interaction mechanism between cell membranes and nanomaterials, especially in the case where NPs can penetrate into or destructively extract phospholipids [98, 114]. Overall, the cellular uptake of NPs is a multi-scale process both in spatial and temporal scales, which requires a coordinated study between experimental, theoretical, or atomic/molecular simulation approaches.

ii. Cell Uptake of Polymer-Coated Nanomaterials

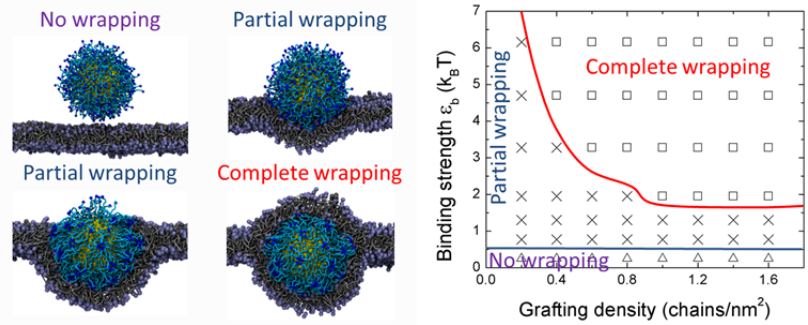

Figure II.7. Phase diagram of receptor-mediated endocytosis of the second-generation NPs. The figure is taken and modified from Ref. [2].

In the first-generation NPs, only ligands are attached to their surfaces. To improve the water solubility and avoid aggregation of NPs, the surfaces of the second-generation NPs are usually grafted with polymer chains, i.e. polyethylene glycol (PEG), which is a hydrophilic and biocompatible polymer. After PEGylation, the properties of the NPs are dramatically changed. For example, the surface charge of the NPs will be shielded by tethered chains and NPs with grafted chains can be well dispersed in the solution. More importantly, a 'stealth' shell will be formed by the grafted chains which can prevent clearance by the immune system (opsonization) [62, 115]. Therefore, PEGylated NPs display prolonged blood circulation time. To improve the endoctyosis of NPs, the free ends of grafted chains are conjugated with targeting moieties, e.g. cell–penetrating peptides (CPPs) [116-118], RGD peptides [119, 120], and anti-HER2 antibodies [121]. With the help of these specific ligand-receptor interactions, the cellular uptake of PEGylated NPs is tremendously enhanced. However, the interplay between NP core diameter, grafting density, and polymer chain length makes cellular uptake of the second-generation NPs distinct from the first generation NPs [2]. To understand these effects, we study the receptor-mediated endocytosis of polymer coated NPs through large-scale coarse-grained molecular dynamics simulations and self-consistent field theory [2]. A realistic coarse-grained model has also been developed to correctly reproduce the conformation of PEG polymers in the water [2, 122], based on the inverse Boltzmann method [123-125]. The following results are obtained through our simulations. First, the non-specific steric (repulsive) interaction between grafted chains and the cell membrane is found to have an effect which is comparable to, or even larger than, the bending energy of the membrane during endocytosis. By incorporating this non-specific steric interaction, the critical ligand-receptor binding strength for NPs to be internalized can be correctly predicted by a simple analytical equation. Second, an optimal grafting density of ~0.80 chain/nm^2, which can enhance the specific ligand-receptor interactions and reduce both non-specific steric repulsions and opsonization during blood circulation, is also identified through our simulations. Third, a phase diagram has been constructed according to the polymer grafting density and ligand-receptor binding strength, as described in Figure II.7. Three different phases, including no wrapping, partial wrapping and complete wrapping, have been identified through our simulations. These findings pave the way for designing new generation NP-based therapeutic carriers with improved cellular targeting and uptake.

5. Uncertainty Quantification of Drug Delivery Process

The NP-mediated drug delivery process involves many different spatial and temporal scales. These different scales interplay with each other with many uncertainties. To design efficient delivery platforms, these uncertainties cannot be ignored. For example, individual

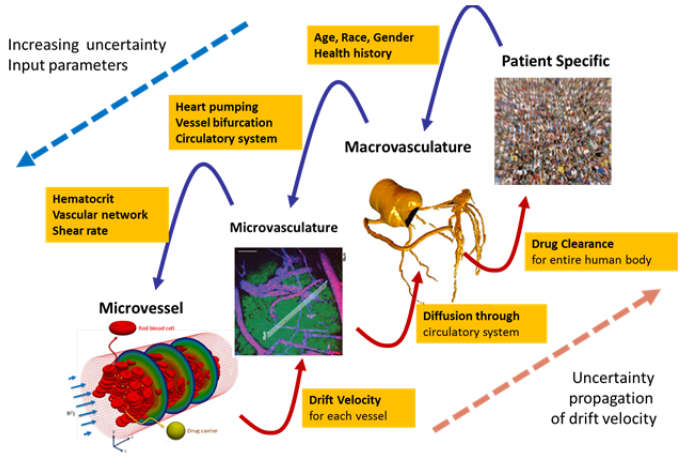

Figure II.8. Uncertainty of NP transport in the multiscale vascular system.

patients have different health histories, which may affect their immune systems. The heart pumping rate, vasculature network and circulatory network may also vary from one person to another. The circulation of NPs in the body can be greatly affected by these factors. The local-specific vessel characteristics, i.e. vessel diameter, flow rate and hematocrit, can change the near wall concentration of NPs tremendously during microcirculation. As we have seen, the endocytosis of NPs depends on cell types, ligand-receptor interactions, and particle sizes and shapes [17, 46]. Uncertainties in a model at one scale can propagate to the others when the inputs to simulations are informed by results of another, as described in Figure II.8. The transport of NPs in the circulatory network can change their concentrations in microvasculature network. The near wall concentration of NPs can influence the endocytosis process. Therefore, it is crucial to consider these uncertainties in the modeling and design of NP platform, especially connecting models across scales [24].

However, there is not a truly multiscale computational method connecting these different models together to bridge different spatial and temporal scales [3]. In the future, we expect that such a multiscale model will be available, allowing for the prediction of a drug-delivery vehicle's efficiency and specificity, and hence design based on the optimal performance across all stages of the delivery process, as opposed to optimization for specific portions of the drug-carrier's life. The flexibility of the method allows for rapid computational prototyping and testing of drug delivery complexes under realistic conditions within a short time, providing new insight into the interplay between molecular-scale interactions of intricate delivery vehicles and their transport within specific patient. Enhanced targeting will also alleviate patient suffering by reducing required dosage of highly toxic cancer drugs. In addition, medical expenses can be reduced as a result of shorter treatment durations and fewer serious side effects.

6. Application of patient-specific computational modeling to detect and/or treat cardiovascular disease

The foundations of science and engineering were rapidly, dramatically and irrevocably changed by the advent of the computer. Over the past decade in particular, the exponential growth of computing speed and capacity has transformed mankind's ability to assimilate immense amounts of data, analyze it, and apply it to solve global problems of extraordinary complexity [126]. Perhaps nowhere is the scientific revolution sparked by computational mechanics more promising than in the field of medicine.

This approach to problem solving employing theoretical and applied mechanics allows us to simulate physical events and thus take much of the guesswork out of scientific research

while simultaneously accelerating its pace. We can test our ideas in a virtual world and, with a high degree of accuracy, predict the outcomes. How will a medical treatment work on an individual patient? How will production of a particular energy resource affect the environment? Through the power of simulation and visualization, we can actually predict and see changes that are likely to occur. Thanks to these techniques, the holy grail of personalized medical treatments – those based on an individual's anatomy, genome, family history, and environmental history – is now realistically in sight. One such example is presented below.

Cardiovascular disease is the leading cause of death in the United States and represents more than a half-trillion-dollar business in research and treatment in the U.S. alone. Most people do not know that 70 percent of all fatal heart attacks are caused by rupture of plaques with large cores of lipid and necrotic debris encased by a fibrous cap, so-called vulnerable plaques (VPs), which often do not create significant narrowing of the lumen and therefore are not detected with standard medical imaging modalities, such as CT, MRI, coronary angiography and external ultrasound (Figure II.9). Both detection and treatment of vulnerable plaques present huge unmet clinical needs. It has been postulated that diseased arteries can be diagnosed and/or acutely treated with drugs delivered locally to rupture prone plaques using NPs in order to promote rapid plaque stabilization and/or passivation.

In addition to the size of the necrotic lipid core, the extent and location of plaque inflammation appears to be a key factor in determining plaque instability [127, 128]. Along with immune cell activation, inflammation contributes to the loss of collagen in the fibrous cap, a prelude to fibrous cap rupture. Inflammation is also known to induce differential surface expression of specific vascular molecules such as intercellular cell adhesion molecules (ICAM-1), intravascular cell adhesion molecules (VCAM-1) and selectins. Blood-borne NPs, conjugated with targeting ligands and loaded with therapeutic and/or imaging agents, can potentially recognize and use these molecules as vascular docking sites, thereby helping to detect vulnerable plaques and/or deliver site-specific acute therapy [128, 129].

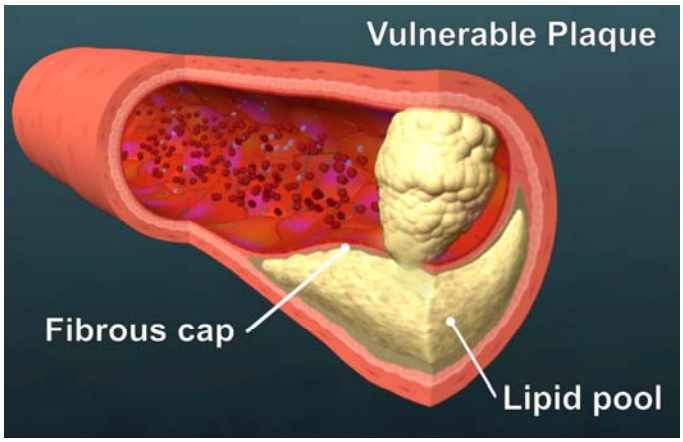

Figure II.9. A schematic of a typical vulnerable plaque with a large lipid pool and a thin fibrous cap that separates the thrombogenic components of the plaque from the lumen.

In a typical local drug delivery system, drug-encapsulated polymeric NPs are injected directly into the blood stream. These NPs are sufficiently small, 20 to 500 nanometers in diameter, to be administered at the systemic level. Carried by the blood stream, these NPs can reach any biological target. Some of the NPs marginate or drift toward the artery wall facilitating local interactions with the endothelium. To enhance the specific recognition of the biological target (NP docking sites), in this case receptors expressed in and around the vulnerable plaque

at the diseased site, the NP surface is covered with ligand molecules and antibodies through nano-engineering. Through the formation of ligand-receptor bonds, the particles firmly adhere to the vessel wall, withstanding the hydrodynamic forces that tend to dislodge them (Figure II.10 (a)). From this privileged position, the NPs can release the encapsulated drug (or even smaller drug-encapsulated particles) toward the extravascular space in the vessel wall (Figure II.10 (b)). The released molecules can then propagate through the artery wall to exert a therapeutic effect on the target region, the vulnerable plaque (VP).

It has been previously demonstrated how a patient's local blood flow features, such as wall shear stress (WSS), targeted receptor density, and physico-chemical properties of the NPs, including size, shape and surface characteristics can influence NP deposition pattern and consequently therapeutic efficacy[23, 130]. There is therefore an overwhelming need for mathematical models that can account for patient-specific attributes, along with NP design parameters, to ensure maximum NP targeting efficiency, thereby helping to personalize, and thus optimize, nanoparticulate therapeutic intervention in an individual patient.

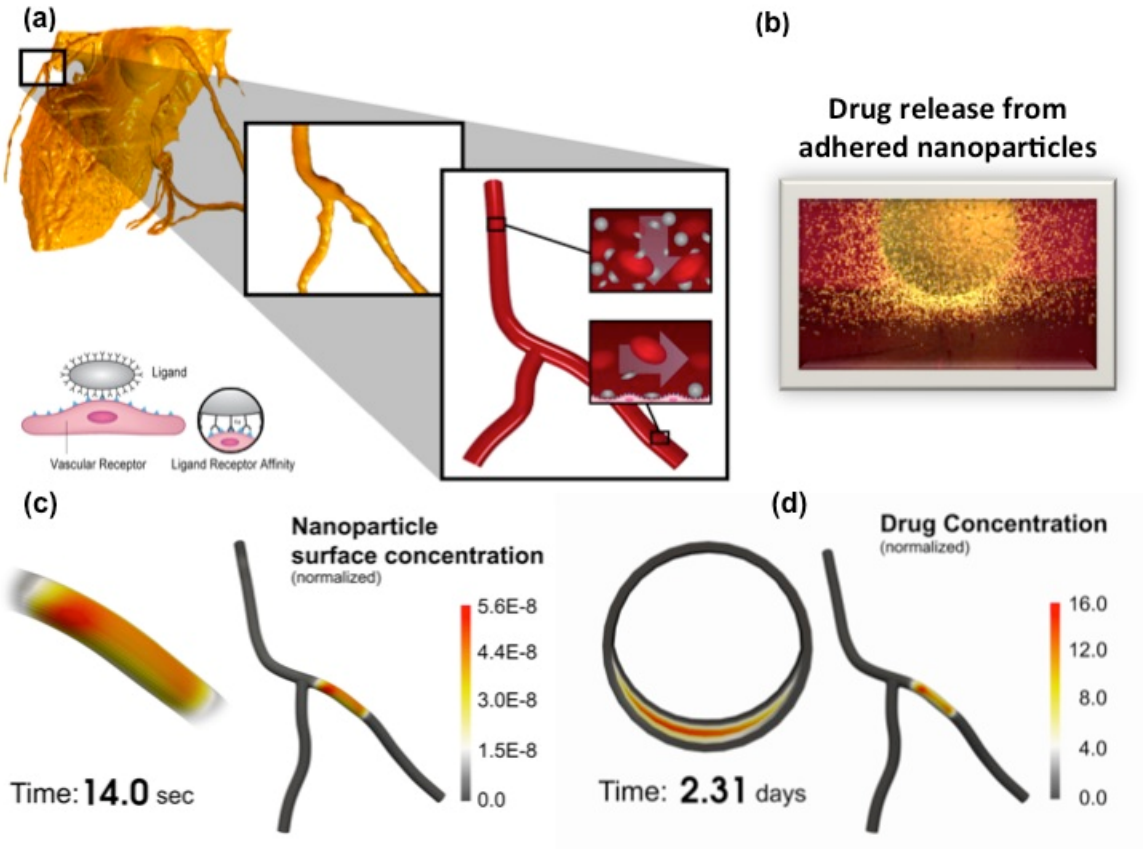

Figure II.10. (a) Patient-specific modeling of vascular deposition of nanoparticles from Ref. [19]. (b) Drug release from adhered nanoparticles. (c) Simulated results of nanoparticle distribution in the targeted region (near the vulnerable plaque) with a higher density of receptor expression. (d) The corresponding drug distribution pattern within an idealized vulnerable plaque.

To that end, a three dimensional (3D) computational tool-set has been developed that uses patient-specific information (e.g., 3D geometry, blood flow features, vessel wall

characteristics) as inputs to analyze and *predict* the vascular deposition of surface functionalized NPs within an inflamed arterial tree [23, 130]. The methodology allows the simulation of NP transport through the blood stream, their adhesion onto and penetration into the vessel wall, and the subsequent release and propagation of the encapsulated drug (or imaging agent) through the tissue in a patient-specific vasculature within an isogeometric analysis (IGA) framework [22, 23]. IGA is an improvement on the traditional finite element method, which has been shown to be particularly suitable for such cardiovascular applications because of its precise and efficient geometric modeling, capability to appropriately capture both laminar and turbulent blood flow regimes, and accurate representation of stresses and near-wall quantities. Figures II.10 (c) and II.10 (d) depict simulation results for NP deposition pattern and the corresponding drug distribution, respectively, within an idealized VP when using a catheter-based nanoparticulate drug delivery system.

The next step is to apply the computational tool in a clinical setting at Texas Heart Institute. The main goal is to validate the model in vivo, then collect data from a population of patients and incorporate the information into the model to predict the nanoparticulate drug (or imaging agent) delivery system that would be most efficacious. Successful realization of this goal will lead to a robust computational framework that can potentially answer critical questions such as, given a desired drug-tissue/imaging agent-tissue concentration in the targeted region (e.g., inflammation in vulnerable plaques), what would be the optimum NP delivery mechanism, NP shape, size and surface properties, and drug release rate, for maximum efficacy in a patient? This computational-clinical marriage will ultimately enable physicians to develop a personalized treatment for individual patients and potentially predict with a high degree of accuracy its effects on the patient before administering it.

7. Multi-phase computational modeling for predicting tumor growth and response to therapy.

In Transport Oncophysics [131, 132] cancer is defined as a proliferative disease of mass transport deregulation which manifests itself primarily in the disruption of the biological barriers that separate body compartments [133]. The most important consequences of this deregulation are invasion, the ability to “push” its way into host tissue; metastasis, the ability to move to distant locations; and angiogenesis, upsetting the balance of nutrient distribution and elimination of metabolites [133]. Tumor growth and connected nutrient and drug transport is a field of choice for numerical modeling. Many models related to this subject can be found in published literature, see for instance the review papers [134, 135]. Most models are fluid-fluid mixture models such as [136, 137], while fewer are solid-fluid models. The latter of which either consider the tumor as a solid, permeated by interstitial fluid [138] or are composed of an extracellular matrix (ECM) permeated by one or several fluids [139]. This last one seems to be the most versatile and will be considered in more detail. It can handle situations such as a melanoma growing on the skin or experiments like the one in [140] where cells are grown in an acellular ECM. The model comprises the following phases: (i) the tumor cells (TCs), which partition into living cells (LTCs) and necrotic cells (NTCs); (ii) the healthy cells (HCs); (iii) the ECM; and (iv) the interstitial fluid (IF) (**Figure II.11**).The ECM and IF pervade the whole computational domain, whereas the TCs and HCs are limited only to the subdomains with the tumor mass and healthy tissue, respectively. The ECM is modeled as a solid, while all other

phases are fluids. The TCs become necrotic upon exposure to low nutrient concentrations or excessive mechanical pressure. The IF, transporting nutrients, is a mixture of water and biomolecules as well as nutrients, oxygen and waste products.

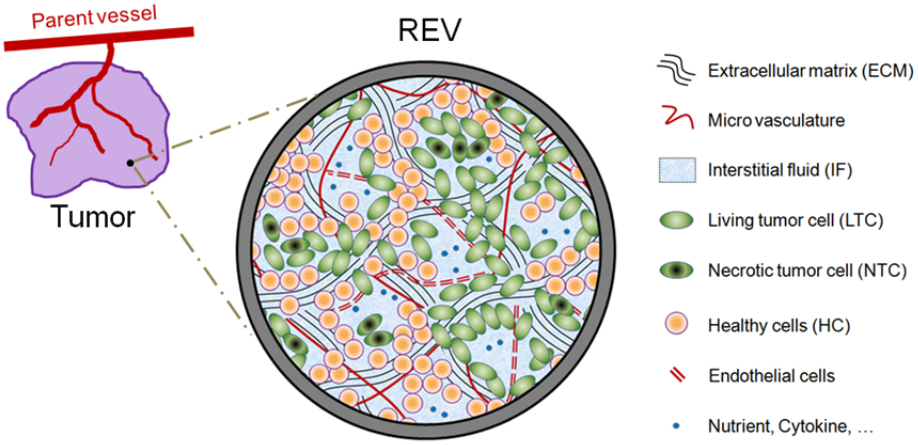

Figure II.11. The multi-phase system within a representative elementary volume [135].

Existing blood vessels are modeled by line elements and blood flow is taken into account. The governing equations are obtained via the Thermodynamically Constrained Averaging Theory (TCAT) [135]. The resulting set of equations involves second-order partial differential operators and is solved by the 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 [141].

Contrary to mixture theories applied in legacy models, TCAT considers the interfaces between constituents with interfacial properties throughout the domain and 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 ECM is treated as a viscoelastic solid material in the finite strain regime or as an elasto-visco plastic material if ECM remodeling has to be considered [142]. This paves the way to a better understanding of the tissue's mechanical properties on the development and growth of tumor masses [143]. The model has a modular structure and further species and phases can be easily added.

As an example of the TCAT model we show the case where tumor cells grow in proximity of two otherwise healthy blood vessels that are the only source of oxygen. The tumor cells are initially located around one vessel only. The volume fractions at 7 and 15 days of the healthy cell phase HC and of the living tumor cells phase TCL are shown in **Figure II.12.** The healthy cells are almost completely displaced by the tumor cells and necrosis occurs in locations within the tumor which are further from the left blood vessel.

From a more complete understanding of the growth and response dynamics of cancer, one may indeed expect to identify promising clues for the development of more effective treatments.

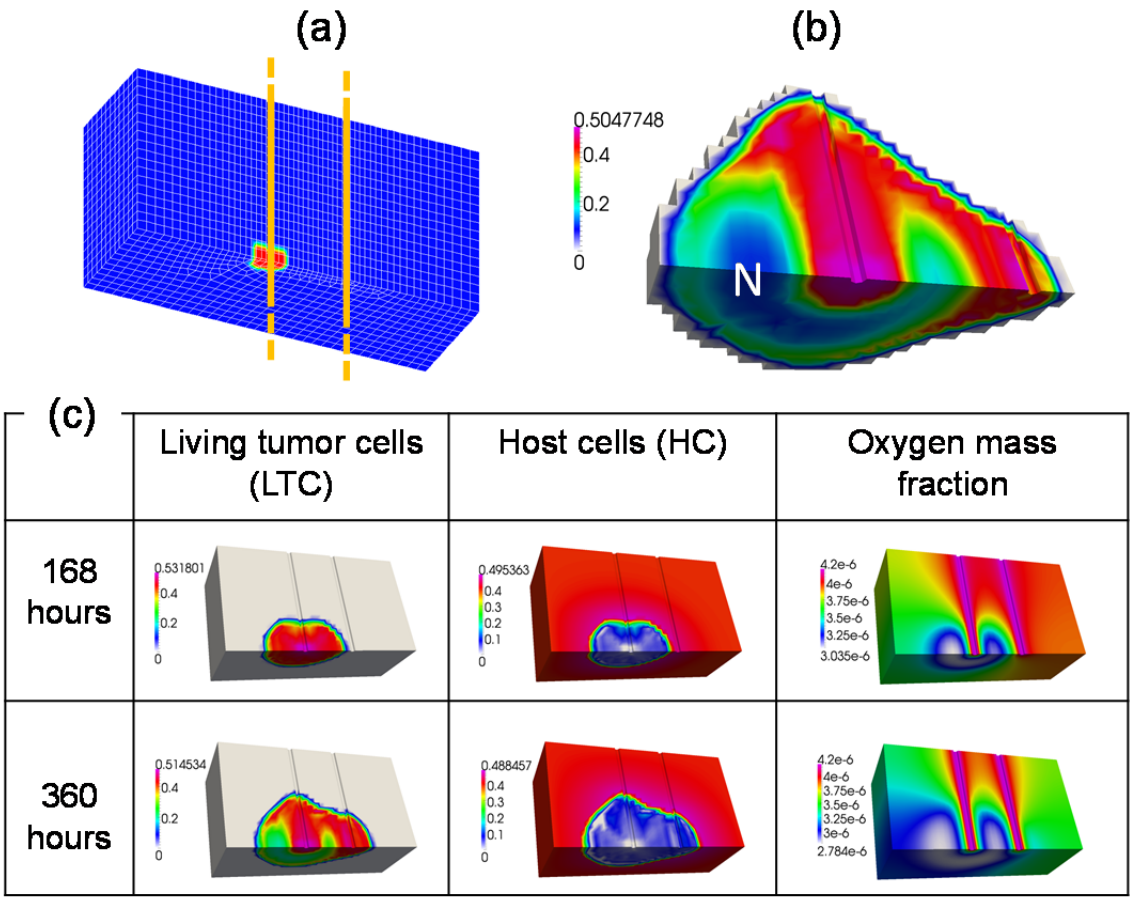

Figure II.12. (a) Geometry, yellow lines show the axes of the two capillary vessels. (b) Volume fractions of the LTC at 20 days; "N" indicates the necrotic areas. (c) Volume fractions of the living tumor cells (first column) of the healthy cells (second column) and mass fraction of oxygen (third column) [139].

III. Biomedical device design

1. Rapid and simple preparation of nucleic acids using micro and nanostructures

Rapid and simple preparation of nucleic acids is important for disease diagnosis, DNA sequencing, and forensic investigations. The challenge for rapid DNA preparation is to purify and concentrate DNA without compromising the performance of large laboratory equipment. The current methods are based on centrifugation, microfiltration, toxic buffers and skilled personnel. Microscale and nanoscale mechanics can offer an ample opportunity to replace the complex functions of equipment.

Electric fields can be combined with capillary action for preparation of microscale and nanoscale objects in liquid. When microscale or nanoscale tips are immersed in solution, the forces induced from capillary action and viscosity, in combination with an attractive electric-field-induced force, can capture or release the particles (Figure III.1 (a)). The size-selective capture

can purify DNA molecules in a sample matrix, replacing the function of centrifugation. Figure III.1 (b) shows a DNA extraction device designed for processing four DNA samples in one batch. Four chips are loaded onto a plastic coupon (Figure III.1c). Each individual chip has five microtips, which are made of a 1 μm-thick silicon nitride layer supported on a 500 μm-thick silicon layer. The top sides of the microtips are coated with a 20 nm-thick gold layer for electrical connection and preservation of DNA. Metallic rings are used to suspend sample solutions by surface tension (Figure III.1d). The device can yield similar performance to a commercial kit, but it can simplify the operation without toxic reagent. A nanotip also showed similar performance with a more straightforward operation, which demonstrates the application of microscale and nanoscale mechanics for novel bio-devices.

Toward commercialization of similar microscale and nanoscale devices, the remaining challenges are: (1) scalable production of microscale and nanoscale structures, (2) integration of such small structures into a device, and (3) quality control of the devices for uniform and reproducible performance. In the future, novel working principles in small-scale mechanics will lead to a revolution in the field of biomedical devices. The role of numerical simulation in commercial applications is to clarify the mechanics in the multiscale regime, which will explain the behavior of nanoscale objects and underlying physics for a novel mechanism of a device. In practice, numerical approaches have explained the sophisticated interaction of molecules in liquid [144], which will shorten the incubation time for device applications.

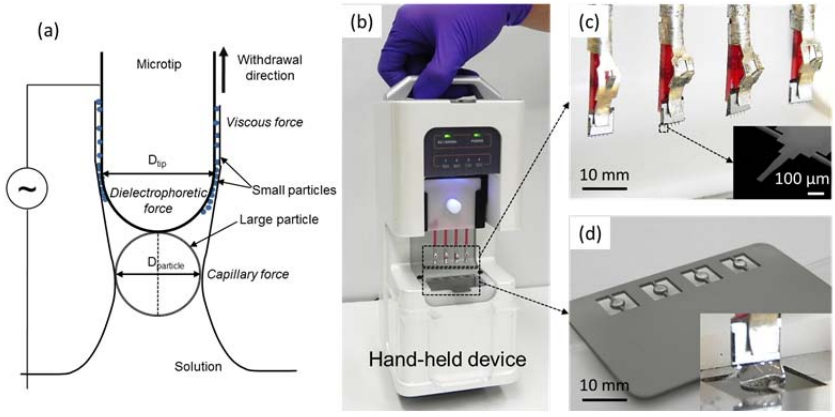

Figure III.1. (a) Force mechanics on a nanotip and microtip surface in the capturing process [5]. (b) a portable prototype device [6] (c) magnified view showing four chips in the holders. The inset shows the SEM image of a single microtip. (d) an array of wells containing the solution. The inset shows the immersion of the chip into the solution.

2. Point-of-care diagnostics using nanosensors.

Point-of-care (POC) diagnostic systems are a rapidly growing segment of biosensors that will eventually leadto home-diagnostic sensors. To date, many methods have been developed for POC diagnosis; assays based on polymerase chain reaction (PCR), immunoassays, etc. These methods are more sensitive and rapid than the traditional detection methods; however, the performance is still not satisfactory. In addition, due to the low analyte concentration in the actual samples, a pre-concentration step is critical. Currently available concentration methods employ centrifugation, microfiltration, or magnetic beads. However, the methods are limited by cumbersome preparation steps, low yield, and low throughput. To address the challenge, electric-field-induced concentration has the potential for application in highly sensitive detection of molecular biomarkers for disease diagnosis and drug discovery.

Using a nanostructured tip, a high-strength electric field can be generated to concentrate molecules larger than 2 nm in size with high efficiency. However, designing a tip that reliably concentrates a specific target molecule requires a detailed understanding of the physical interactions governing the separation process. Mechanics has already influenced the design process of mechanical and aerospace applications [145-149], as well as biosensors through the immersed finite element technique [29-32, 34, 150, 151], which is capable of accurately modeling the various forces experienced by a biomolecule during separation such as fluid-structure interaction of arbitrarily shaped structures, large structural deformations, electrokinetics, temperature dependent thermal fluctuations and molecular interactions [152]. The developed numerical tool accurately predicts the efficacy of preconcentration of molecular targets, in agreement with experimental results [153]. Once preconcentrated, the detection can be done by fluorescence and electrical measurement [154]. Such an amplification-free platform can show a high sensitivity equivalent to PCR but with a short assay time [155]. The nanostructured tip offers a simple configuration for POC diagnosis as well as a convenient home-diagnostic platform, and mechanical analysis plays an important role in rapid development of novel tip designs for specific problems. Furthermore, the numerical methods established in this context are ripe for further development and application to other important processes involving complex interactions with biomolecules, both in vitro and in vivo.

3. Developing model organ or multi-organ (in vitro) microdevices for the rapid and effective screening of pharmaceuticals.

During the past decade, the capabilities of microfluidics, enabled by the development of soft lithography [156], have expanded dramatically and now encompass a broad range of medical and biological applications ranging from single-molecule measurements to single-cell or cell-population studies. Indeed, the recent development of techniques that enable the co-culture of multiple cell types in 2- or 3-dimensional co-culture, have led to breakthroughs in our capability of recreating many aspects of organ function in a single microfluidic chip. This has numerous applications, among which are the abilities to replicate certain biological or pathological processes outside the body and to create model organ or tissue systems that can be used to screen for new therapeutics. Since these innovative microfluidic platforms are capable of capturing multi-cell-type interactions, they better mimic the real physiological situation. In addition, and importantly, they have the potential to take advantage of rapid advances in cellular reprogramming to produce human, induced pluripotent cells, or iPSCs [157]. By using the iPSCs from a particular patient, the prospect of patient-specific screening is brought one step closer to being a reality.

What are our current capabilities for producing these “organs-on-a-chip” technologies? Over the past several years, research has emerged demonstrating the capability to reproduce some functions of a variety of organs (Figure III.2) [158]. While these vary in their ability to truly reproduce or mimic organ function, they clearly reach far beyond the ability of single-cell-type drug screening in well-plate systems, which is still the standard approach employed by the pharmaceutical industry. One example is illustrated in the upper right of Figure III.2, the “lung-on-a-chip” [159]. Designed to simulate conditions in the gas exchange or alveolar region of the lung, it incorporates multiple cell types (e.g., both endothelial and epithelial cell monolayers),

allows chemical signaling between them, and subjects the cells to cyclic stretch by varying the air pressure in side channels, thereby stretching the elastic substrate on which the monolayers are grown. It also includes an air-liquid interface, which is necessary for the epithelial cells to take on the right morphology, and also stimulates the synthesis and secretion of pulmonary surfactant, which is critical for many lung functions.

Other systems incorporate the capabilities to grow cells on a 2-dimensional surface, appropriate for cellular monolayers, but in many cases also within 3-dimensional gels that mimic the microenvironment essential for natural function of cells embedded in the interstitial space. Examples of 3-dimensional gel microenvironments include the "liver bioreactors" produced by several groups [160] and model gastrointestinal tracts [161], blood-brain barriers, and muscles, both cardiac or skeletal. In these systems, simultaneous 2D and 3D cultures allow cells to interact in a natural way, exchanging signaling factors via the interstitial spaces of the gel, and allowing for a more realistic, cell-specific morphology. The enormous flexibility of these systems is only now being fully realized as the models become increasingly complex and more realistic. This poses new opportunities to the research community, especially in terms of creating computational models that capture the transport characteristics through channels, gels and within more complex tissues, while also incorporating the increased complexity of the biology.

Of particular note, efforts have recently been launched, under the support of substantial government programs from DARPA and NIH, to combine single-organ models of this type to produce "body-on-a-chip" systems in which multiple "organs" can interact in a realistic way. One of the major driving forces behind this effort is the need to understand and be able to anticipate off-target effects of drugs, deleterious effects on organs other than the one for which the drug is targeted.

An important feature of many tissue models is the capability to incorporate vascular perfusion and the exchange of various metabolites throughout the tissue space. Previous inability to do so has also been one of the major limitations in the development of engineered organs, with the exception of those tissues, such as cartilage or cornea, for which blood circulation is not essential. Recently, several groups have demonstrated methods in model systems to produce a vascular network that can be perfused. Two approaches have been developed. In one, the vessels are either etched onto the surface of the device, or cast into it [162] or created by other means [163], inside a 3D gel that may or may not be biodegradable. The channels produced are then seeded with endothelial cells that adhere to and form a confluent monolayer over the walls of the gel channels. These systems tend to be limited at present to channels that are larger than natural capillaries, but new methods are constantly emerging that are sure to reduce vessel diameter further. An alternative method that has been employed by several groups [120, 164, 165] is to induce the vascular cells to bore into the hydrogel from a monolayer and form new vessels by the process of angiogenesis [166, 167]. Another approach is to draw upon the natural capabilities of the cells to form networks when dispersed uniformly within the 3D matrix, termed vasculogenesis [120, 164, 165]. Either approach produces networks with morphologies that are both controllable and have dimensions closer to those of normal capillaries.

Numerous challenges exist in the design of systems that possess the same transport and mechanical properties of living tissue. Matrix materials are needed that replicate both the chemical and mechanical characteristics of normal human extracellular matrix. Abundant

evidence exists supporting the critical role of matrix mechanics in behaviors ranging from cell migration [168], to cytoskeletal functions [169], to stem cell differentiation [170], and the biomechanics community has made considerable progress in understanding these effects.

However, much of the design currently is based on trial and error, and a need exists to meld a fundamental understanding of biology with a sound approach to the mechanical issues. Computational approaches rely now primarily on agent-based models [171], in which the cell behavior is described by a collection of rules that are largely determined empirically. Few if any models can be found that are based on first principles, although the mechanical properties and transport characteristics of cell and extracellular matrix have been reasonably well characterized. Flows, both intravascular and interstitial, exert important influences on tissue function, and these can be modeled by conventional means. The greatest challenge is to meld these more traditional models with the intrinsic biology of the systems in order to create truly predictive simulations.

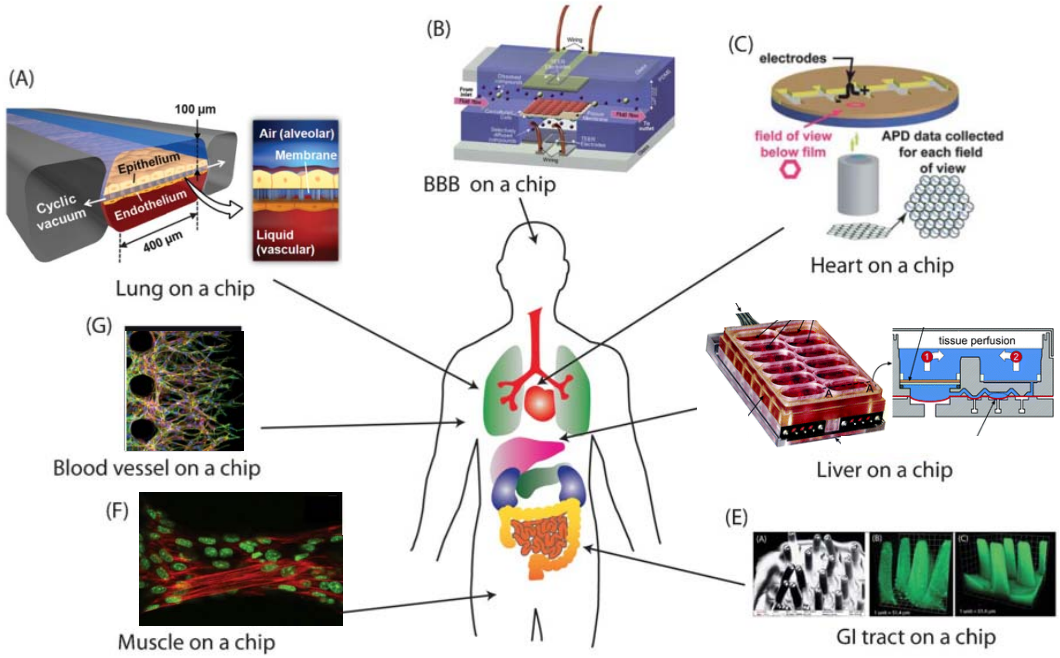

Figure III.2. The spectrum of model systems ("organs-on-a-chip") is being developed for drug screening purposes. Systems have been developed for (A) lung, (B) the blood-brain barrier, (C) heart tissue, (D) liver, (E) the gastrointestinal tract, (F) muscle, and (G) the microcirculation. [Reproduced from [158], adapted from [161] with permission.]

4. Fluid-structure-interaction modeling, simulation and optimization of pediatric Pulsatile Ventricular Assist Devices (PVADs)

Figure III.3. Left: The Berlin EXCOR PVAD. Right: Geometrical model of the PVAD containing the blood and air chamber with the corresponding inlets and outlets. The model is employed in fluid-structure interaction simulations in [172].

Heart failure is a common condition in the US, with more than 600,000 cases diagnosed annually [172]. Cardiac transplantation remains the preferred treatment, however a lack of suitable donors restricts this option for many patients, and the median survival with this condition is only two to three years after initial diagnosis. Ventricular assist devices (VADs) pump blood in parallel with the native heart function and provide full or partial mechanical circulatory support to one or both ventricles of the heart. They are used clinically in a range of adult and pediatric diseases, including congenital heart disease, cardiomyopathy, and post-infarction heart failure. They were first developed as a bridge to transplant, in order to prolong life of critically ill patients awaiting organ availability. However, as designs have evolved to become smaller and even fully implantable, they can now be used as destination therapy, supporting one or both ventricles. More recently, there has also been success, most notably in pediatric patients, with use of VADs in bridge to recovery scenarios, allowing sufficient offloading for myocardial remodeling and recovery.

The need for reliable and safe mechanical circulatory support is growing in the pediatric population as well [173]; however, the number of available donor hearts for this population has remained fixed at approximately 500 each year. VAD usage has thus increased in this population, although pediatric VADs have a notably poorer performance than available adult models. Development of VAD technology has taken place almost exclusively for the adult population. The Berlin EXCOR (see Figure III.3), a pulsatile device, remains the only FDA approved device for children. However, adverse clinical effects occur in these devices at an alarming rate. Particularly troubling is the consistently high rate of thromboembolic events (22%).

With the increasing prevalence of VADs in clinical use, there is now focus on improving design performance to reduce co-morbidities, reducing device size, and allowing patients a more active lifestyle. Computational-mechanics simulations, coupled with optimization techniques, can be used to accelerate the design process and optimize current and future designs. Simulations offer a promising means to cheaply and efficiently test and optimize competing device prototypes, thereby reducing time to market and identifying potential

performance enhancements. Computational-mechanics modeling and simulation is an integral part of the design process in many industries (e.g., aerospace, automotive). Almost every automobile manufacturer makes use of detailed, large-deformation structural-mechanics simulations to model car crashes in an effort to assess vehicle safety for the passengers. Aircraft manufacturers use computational fluid mechanics coupled to rigorous optimization algorithms as a crucial and cost saving part of the design chain for all major aircraft. However, despite its demonstrated success in these large-scale applications, adoption of simulation tools has lagged behind in the medical device industry. This is due in part to initial success with designs identified through trial and error and experimentation, as well as challenges associated with complicating factors of blood biochemistry, mechanobiology, and physiological response, which make simulations challenging. While simulations have been applied more recently, particularly in the design of the HeartMate II and HeartAssist 5 blood pumps, there remains a need for increased adoption of simulation technology and formal design optimization algorithms.

To model pulsatile VADs, dynamic interaction of air, blood, and a thin membrane separating the two fluids needs to be considered. Coupled fluid-structure interaction (FSI) simulations at full scale are essential for realistic and accurate modeling of pulsatile VADs. This is because the motion and deformation of the thin membrane depends on the flow in the device blood and air chambers, and the flow patterns, in turn, depend on the motion and deformation of the membrane. As a result, the fluid and structural mechanics equations need to be solved simultaneously, with appropriate kinematic and traction coupling at their interface. The computational challenges for FSI of pulsatile VADs include large, buckling motions of a very thin membrane, the need for periodic remeshing of the fluid mechanics domain (due to the large motions of the membrane, which induce very large changes in the blood and air flow domain geometry during the cycle), and the necessity to employ tightly coupled FSI solution strategies due to the very strong added structural-mass effect present in the problem.

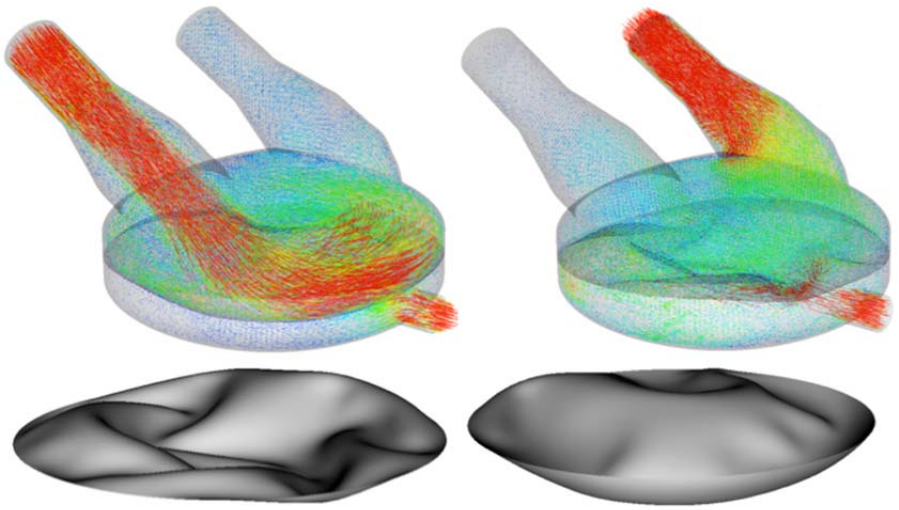

Figure III.4. Top: Snapshots of the blood flow velocity during the fill (left) and eject (right) stages of the PVAD operation. Bottom: Snapshots of the deformed configuration of the thin structural membrane during the fill (left) and eject (right) stages of the PVAD operation. The FSI simulations shown are from [174].

The state of the art in FSI modeling and simulation is able to address these challenges (see a recent book on FSI [175] and references therein), however, there is currently no readily available, off-the-shelf commercial software where these techniques are implemented and that may be robustly deployed for this class of problems. The successful, one-of-a-kind, physiologic FSI simulations of PVADs, as accomplished in the recent work (see [174] and Figure III.4), present an important first step toward computer-aided engineering design of these devices.

Thrombus formation (i.e., blood clotting) is the major problem in VADs and, in particular, PVADs. However, determining thrombotic risk factors in these devices is challenging. Thrombus formation is the result of a complex sequence of chemical reactions in the bloodstream, resulting in platelet activation and aggregation and the formation of fibrin networks around these aggregations. Thus it is desirable to not only model the (FSI) in PVADs, but also the process of blood coagulation in order to understand the source of the problem, and to propose device design modifications to mitigate it. Some research has been dedicated to this, although determining an appropriate blood coagulation model for our purposes is quite challenging. As a result, we explore other surrogates for thrombotic risk that may be directly computed from FSI simulation data. Long residence times and areas of blood recirculation or stagnation may lead to increased risk of thrombosis in PVADs [176]. A method for calculating particle residence time for flows in moving spatial domains was proposed in [177], and the developments for PVAD FSI and residence time computations were used to perform a shape-optimization study of a pediatric device, as in [178]. The optimization using a derivative-free surrogate management framework (SMF) [179] was carried out for a full-scale 3D device, with time-dependent FSI simulations performed under physiologically realistic conditions (see Figure III.5).

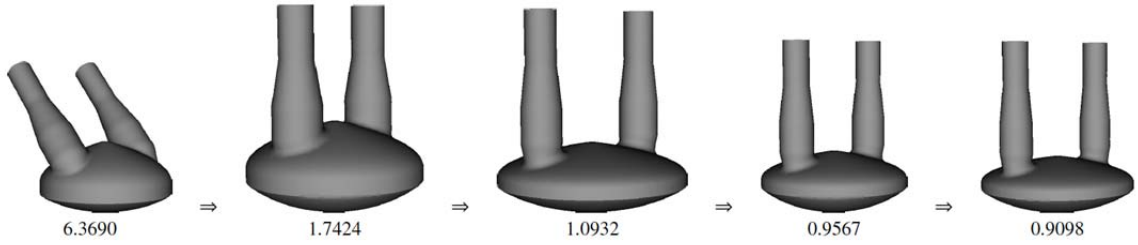

Figure III.5. Progression of the optimal design as determined by the FSI-based SMF optimization scheme. The optimization cost function value, which is derived from particle residence time in the blood chamber, is displayed under each model. The strong inclination toward vertically oriented arms is reasonable, as this configuration enables the incoming blood to move more quickly and uniformly toward the outlet than other designs. However, the idea to vertically mount the inlet/outlet arms is non-intuitive, a result that reinforces the value and importance of systematic design space optimization for PVADs. See [178] for more details.

Despite recent progress, challenges remain to increase the relevance and utility of VAD simulations in the device design process and in the clinic. First, complete modeling of blood biochemistry remains computationally intractable due to high computational cost. There is therefore a need for continued development and validation of reduced order models to measure the risks of thrombosis and hemolysis. Second, there is a need for integration of the advanced simulation and formal optimization methods outlined above to accelerate the design process in

the presence of constraints and uncertainties. As the optimization process identifies new designs, the need will also arise for rapid prototyping of simulation-derived designs for experimental testing. Third, as simulation methods mature, there is an increased need for validation of simulated risk of thrombosis and hemolysis against clinical data in VAD patients and animal models. Finally, one cannot ignore the underlying physiology of the patient, and VAD models should be coupled to lumped parameter network models of circulatory physiology to elucidate the interplay between the device and physiologic conditions. This is particularly compelling in pediatric cardiology due to the complex physiology and unusual anatomy in congenital-heart-disease patients.

IV. Cell Mechanics

1. Mechanosensing and Mechanotransduction

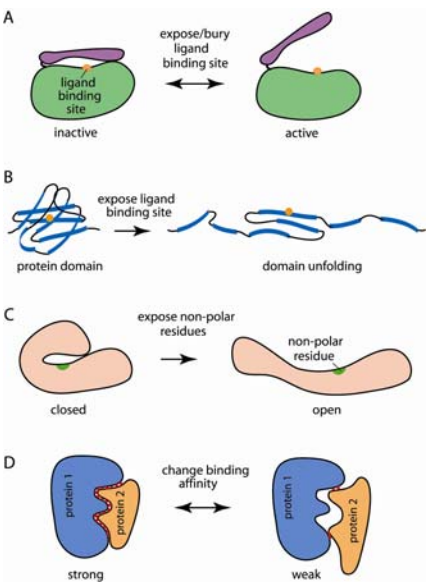

Figure IV.1. Examples of biological consequences of protein deformation. Mechanical forces can (**a**) switch a 'lid' in a protein from 'closed' to 'open' position, or (**b**) unfold a protein domain, thus exposing the ligand binding site. Protein deformation can also (**c**) expose the non-polar residues, causing non-specific interaction between the protein domain and other biomolecules; or (**d**) induce a change in binding affinity, altering protein-protein interactions.

As the basic unit of life, living cells perform an enormous variety of functions through synthesis, sorting, storage and transport of biomolecules; expression of genetic information; recognition, transmission and transduction of signals; and conversion between different forms of energy. Many of these cellular processes can generate, or be regulated by, mechanical forces at the cellular, subcellular and molecular levels. For example, during cell migration, contractile forces are generated within the cell in order for the cell body to move forward. These contractile forces, in combination with the adhesion of cells to extracellular matrix (ECM) through focal adhesion complexes, enable cells to sense the stiffness of the surrounding substrate and respond to it. Many normal and pathological conditions are dependent upon or regulated by their mechanical environment. Some cells, such as osteoblasts and vascular cells, are subjected to specific forces as part of their 'native' physiological environment. Others, such as muscle and cochlear outer hair cells, perform their mechanical function either by converting an electrical or chemical stimulus into mechanical motion or vice versa.

Of particular importance is the ability of cells to sense mechanical force or deformation and transduce these mechanical signals into a biological response. For example, endothelial cells can recognize the magnitude, mode (steady or pulsatile), type (laminar or turbulent) and duration of applied shear flow, and respond accordingly, maintaining healthy endothelium or leading to vascular diseases including thrombosis and atherosclerosis. Vascular smooth muscle cells in the arterial wall remodel when subjected to pressure-induced wall stress. Fibroblast cells 'crawl' like an inchworm by pulling the cell body forward using contractile forces. Bone alters its structure to adapt to changes in its mechanical environment as occurs, for example, during long bed rest. Stem cells sense the elasticity of the surrounding substrate and differentiate into different phenotypes accordingly. These and other examples demonstrate the ability of cells to sense and respond to their local mechanical environment. However, little is currently known about the fundamental molecular mechanisms by which cells sense mechanical force or deformation, and transduce the mechanical signal into a biological response. Answering this fundamental question in biomechanics will provide a quantum leap in our understanding of the essential roles of mechanical forces in biology and medicine.

A possible unifying mechanism for mechanosensing and mechanotransduction in living cells is protein deformation, broadly defined as protein conformational change under force. It has been well established that the three-dimensional conformation of a protein largely determines its function. However, the conformation of a protein can be altered by applied mechanical force, resulting in changes of the functional states of the protein and inducing down-stream biochemical and biological effects. Therefore, protein conformational change under mechanical force is an excellent candidate as the unifying molecular mechanism of mechanosensing and mechanotransduction in living cells. Shown in Figure IV.1 are some examples of the possible effect of protein deformation in a living cell. Many proteins have specific ligand binding sites buried initially by a protein domain or a peptide (a 'lid'). As illustrated in Figure IV.1(a), upon applying mechanical forces to such a protein, the 'lid' opens, exposing the ligand binding site. The reverse is also true: protein deformation can close the 'lid' that is initially open, thereby burying the ligand binding site. Alternatively, a protein globular domain can unfold under mechanical force, exposing the ligand binding site that is buried inside the globular domain (Figure IV.1(b)). Mechanical forces can also unfold a globular domain and thus expose the non-polar residues (Figure IV.1(c)), which may cause non-specific interaction between the protein domain and other biomolecules, and thus alter protein function. It is well known that proteins interact with each other based on conformational matches: good conformational match leads to high binding specificity and affinity between two proteins, while poor conformational match does the reverse. As shown schematically in Figure IV.1(d), when proteins 1 and 2 have good conformational match, they have strong interactions to realize their functions, for example, to activate a signaling cascade, or facilitate an enzymatic activity. However, when one of the proteins, say, protein 2, sustains a force-induced conformational change, the interaction between proteins 1 and 2 becomes weak due to the poor conformational match, thus altering the function of protein 2. The reverse is also true: deformation of a protein can increase its affinity to another protein that otherwise would not interact due to the poor conformational match in its native state. This concept is not limited to protein-protein interactions; protein-DNA, protein-RNA and protein-small molecule interactions can be altered by force-induced protein conformational change as well.

2. Deformation and Constitutive Behavior of Cells

Over the last few decades, extensive experimental and modeling/simulation studies have been performed to determine the deformation of cells and tissues under applied force, and their constitutive behaviors. Typical experimental set-ups for single-cell mechanical testing are shown in Figure IV.2. However, in most of the modeling studies and constitutive equations developed for living cells, the active feature of living animal cells has been either ignored or poorly captured. It has been well established that most of the living animal cells are 'active' materials and structures, i.e., their structure, morphology and thus constitutive behaviors change with applied mechanical load. Cell structural changes, including structural alterations in cytoskeleton and changes in density and/or distribution of local adhesion complexes, may happen within a few minutes upon loading. Therefore, it is likely that as mechanical measurement of cells is being conducted, significant changes in cell structure and/or surface contact occur concurrently, leading to an altered force-deformation response of the cell. The degree of changes in cell deformation behavior depends on both the magnitude and rate of applied force. Adding to the complexity is that certain cells also have force-generating functions, which should be considered in the constitutive behavior of cells as well. Thus, there is a critical need to develop better constitutive models for single-cell mechanical behavior, taken into account the active behavior of cells. However, it remains very challenging to quantify accurately the distribution of forces among various subcellular structures inside a living cell. It is well known that a significant portion of the forces is supported as well as generated by the cell cytoskeleton, but cells are active and the cytoskeletal structures are dynamic; they can undergo remodeling or re-organization in response to mechanical perturbations. Further, the measurement of mechanical behavior of individual cells may give rise to different results, which may depend on cell morphology, stage in the cell cycle, as well as how different subcellular structures respond to mechanical perturbation. This raises a fundamental paradox: How can we measure mechanical behavior of living cells if they react to our measurement tools? These issues are fundamental to the study of the mechanics of living cells.

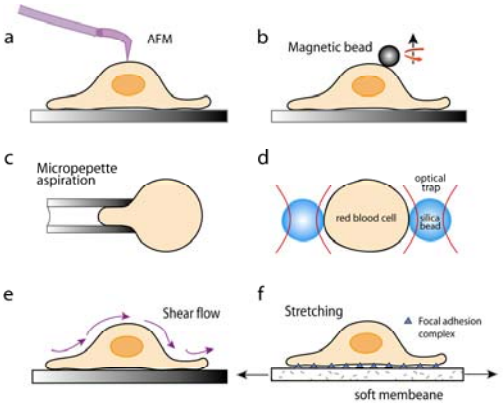

Figure IV.2. Schematic representation of the three types of experimental techniques used to probe living cells. Atomic force microscopy (AFM) (**a**) and magnetic twisting cytometry (MTC) (**b**) are Type A methods which can probe cell components at force resolution of 10^{-10} and 10^{-12} N, respectively, and displacement resolution of at least 1 nm. Micropipette aspiration (MA) (**c**) and optical trap (OT) (**d**) are type B techniques that can deform an entire cell at force resolution of 10^{-10} and 10^{-11} N, respectively. Shear flow (**e**) and substrate stretching (**f**) methods are capable of mechanical response evaluation of a population of cells.

3. Microfluidics systems for the single cell studies

Life-science researchers typically study cell behavior by performing experiments on populations of cells because the standard bulk methods are simple, available, and well established [180, 181]. Millions of cells are normally used for a bulk experiment, especially when robust and readily available cell lines are used. However, it is well known that cells in a seemingly identical environment can show heterogeneous behavior within a population [182, 183]. For instance, cells can be at different stages of the cell cycle or exhibit variations in gene expression due to the stochastic nature of biochemical processes. This biological variability is becoming increasingly recognized by the biological research community as an important factor [184, 185]. Indeed, potentially significant cellular behavior may not be captured by bulk techniques because the experimental ensemble average across the population can obscure an important subset within the data or lead to incorrect conclusions [180, 181]. In fact, cell heterogeneity has been posited as the cause of error in disease classification [186].

Unlike conventional bulk methods, single-cell studies can provide biochemical characterization of individual cells without the loss of specificity associated with ensemble averaging. This unique advantage is the key for capturing the effects of gene expression variations leading to different cell states, for enabling an understanding of the mechanisms inherent in biological noise, and for probing complex phenomena including cell differentiation and cancer proliferation. For example, cell signaling pathways — the link between inputs and outputs through interconnected molecular interactions — during stem cell differentiation display stochastic behavior within the pathways due to cross talk between multiple pathways, localization of reactions, and the low concentration of molecules involved in signaling [187]. To understand the complex intercellular input-output relationship and to develop mathematical descriptions of cellular behavior, it is essential to have tools for systematic single-cell analyses that can be performed with throughput that is statistically significant and practical with respect to research time per data point. Fundamental understanding of cellular variation will have a significant impact on biological studies and lead to advancements in our ability to predict input-output relationships in cells using mathematical models and predictive analyses. This ability is vital for understanding higher-level systems, such as tissues and organisms, and for developing therapeutic approaches [187].

Micro- and nano-fabricated devices are tools that possess great potential to address these needs. Microfluidic tools offer unique advantages for in vitro assays such as low-volume sampling and rapid analysis, due to short diffusion distances and small areas of interest for optical analysis [180, 181]. For example, microwell arrays [181, 188] have been used to acquire data from large sets of individual cells, offering statistically significant conclusions. In general, the miniaturization of tools used for biological applications is attractive because it reduces the volume of (often) expensive reagents, requires less space for replicates, allows automation and integration for sequential analyses, enables portability, and reduces waste [181]. Utilizing these advantages, many methods from single cell transfection, sampling, and analysis to on-chip cell manipulation and culture have been developed, which are critical toward the development of single cell studies.

For direct delivery of molecules into single cells, microfluidics systems are often integrated with micro-electrodes to achieve electroporation, which is the transient and reversible formation of nanometer pores, in the cell membrane, by application of an electric field. For

example, the Espinosa group has recently developed a microfluidic tool for single-cell electroporation using nanofountain-probe (NFP) technology (Figure IV.3). The NFP is a cantilever probe, with embedded microchannels, which allows the application of a local electric field when the probe and cell membrane are in contact. Unprecedented transfection efficiency and delivery of DNA, RNA, plasmids, and small molecules, where achieved with dosage control and very high viability [189]. Also, other groups demonstrated successful single-cell transfection using PDMS-based microfluidic devices, e.g., droplet and nanochannel electroporation [190, 191].

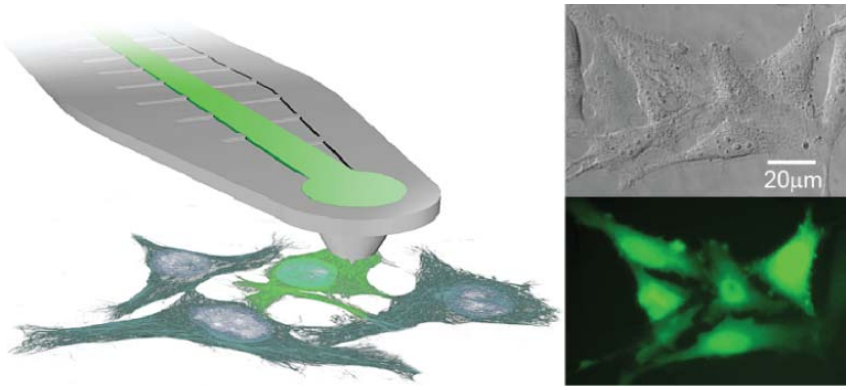

Figure IV.3. Transfection of single cells using nanofountain probe electroporation developed by the Espinosa group. HeLa cells were transfected with fluorophore-tagged dextran with 95% efficiency and high viability (>90%) after electroporation [189].

In addition to single cell transfection, reversed electroporation on a microfluidic chip has recently been shown to permit minimally invasive sampling of intracellular contents through transient and reversible nanopores on a cell membrane [192, 193]. By tuning parameters of the applied electrical input, i.e., polarity, voltage, frequency, and duration of input signal, during electroporation, precise and reproducible sampling of cells is possible while maintaining cell viability. These studies demonstrate the possibility of using electroporation for sampling. Such sampling must be followed by a robust bio-detection module. Indeed, biomolecular detection with up to atto-molar resolution was achieved by combining a microfluidic device with electronic or optical signal detection [194]. Goluch developed a bio-barcode assay (BCA) for single protein detection by employing functionalized NPs [195]. Gong developed integrated nano-electronic and electrokinetic devices for label-free atto-molar detection of proteins [196]. Jung utilized a capillary electrophoresis assay by combining on-chip isotachophoresis (ITP) with laser-induced confocal fluorescence detection [197]. The Heath group developed an integrated blood barcode chip, which can sample a large panel of plasma proteins from whole blood samples within 10 min of sample collection [198]. The Quake group has pioneered large-scale gene expression analysis from single cells that has been exploited in a wide range of applications such as whole-genome molecular haplotyping of a single human metaphase cell [199].

Microfluidics systems have become important tools for single-cell studies, yet many challenges remain. For example, microfluidic tools often operate separately for different applications, e.g., single cell manipulation, isolation, culture, transfection, sampling, or analysis. As a result, time dependent high-throughput study of individual live cells is still unattainable. This calls for efforts to integrate modular tools, with different functions, into one multi-functional microfluidics system. Moreover, the sensitivity limit of the detection scheme needs further enhancement, as small quantities of target biomolecules are often available in single cells. An example is the study of the role of low–copy number proteins (~1000 molecules per cell) in cell

functions such as signaling and regulation of gene expression. Finally, a method offering high throughput is very desirable to obtain statistically significant biological data from which meaningful conclusions can be drawn.

In order to establish a transformative engineering tool for single-cell studies, the Espinosa group and collaborators are pursuing the development of an integrated microfluidic system, see Figure IV.4. This effort spans fields ranging from mechanics to multiphysics computational analyses to nanotechnology and to systems biology. Such an interdisciplinary approach will provide unique opportunities to the mechanics community to explore complex systems with application to biological variability and input-output relationships that govern cell function. Moreover, emergent fundamental insights, enabled by such engineered system, will lead to advances in the understanding of regulatory pathways, complexity, and disease mechanisms, which are vital to explain higher-level biological systems, such as tissues and organs, and for developing early diagnostic and therapeutic approaches.

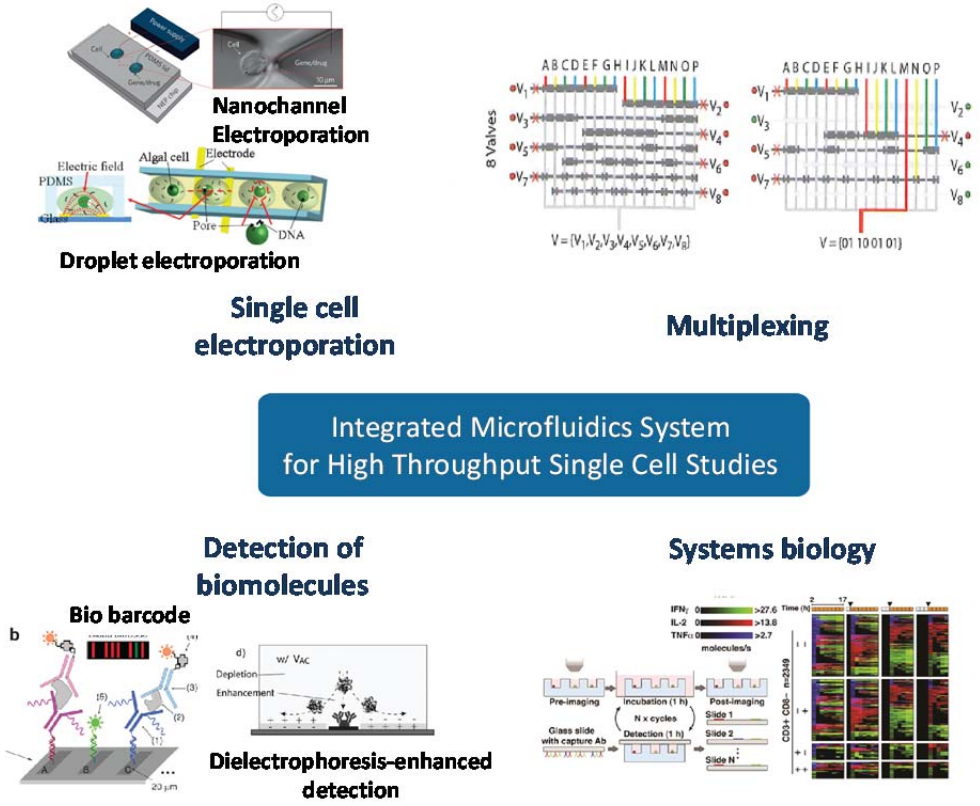

Figure IV.4. Integrated microfluidics system for single-cell studies. The system would consist of several modules for multi-functions including single cell manipulation, isolation, culture, transfection, sampling, and analysis [188, 190, 191, 196, 198, 199] in an automated and high throughput manner. Such a system would enable time dependent studies of cell response with single cell resolution.

V. Educational prospectus and summary.

The interdisciplinary nature of Mechanics in Medicine requires significant changes be made in the educational curriculum of mechanics students. The classical model for mechanics

education, consisting of fluid and solid mechanics course series, falls short of preparing students for interactions with biologists and medical doctors, which greatly hinders collaboration. A strong basis in mechanics is still required, but it must be augmented by additional courses designed to teach the essentials of biology and chemistry. Clearly, it is not reasonable to expect mechanics students to develop the level of expertise in biology and medicine as biologists or medical doctors, but a basic understanding of biological processes will greatly facilitate discussion with experts in the field. In addition to courses aimed at the basic science behind medicine and biomedical research, core mechanics courses should draw upon biological problems for illustrative examples. There is a long history of using biological examples to motivate mechanics problems, especially in fluid mechanics, but including modern case studies of mechanics guiding medicine would be greatly beneficial. Specific examples such as drug delivery platforms or model in vitro organs would expose students to archetypal medical applications while providing fascinating engineering systems in which to learn mechanics. Progress in this direction is underway. For example, Northwestern University has recently revamped a course series entitled *Multi-scale Modeling and Simulation of Solids and Fluids*, which now draws heavily on biomedical and biotechnological examples to illustrate modeling methods ranging from molecular dynamics to finite element simulations. In the future, we envision courses jointly taught by biologists or medical doctors and mechanicians, for students from both medicine and mechanics, with the aim of exposing each group to the capabilities and current research issues of the other. Courses intended to drive collaboration and the exchange of ideas between the two groups are essential for the full potential of Mechanics in Medicine to be reached. Training future researchers in Mechanics in Medicine requires a substantial effort to re-imagine mechanics education. Fortunately mechanics has proven itself capable of nimbly transitioning into new realms, keeping the field relevant in the face of rapidly changing research environments. With thoughtful changes and additions to the classical curriculum, mechanicians of the future will be capable of bringing the quantitative engineering principles to poorly understood areas of medicine.

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