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A Systems Approach to Biomechanics, Mechanobiology, and Biotransport

The human body represents a collection of interacting systems that range in scale from nanometers to meters. Investigations from a systems perspective focus on how the parts work together to enact changes across spatial scales, and further our understanding of how systems function and fail. Here, we highlight systems approaches presented at the 2022 Summer Biomechanics, Bio-engineering, and Biotransport Conference in the areas of solid mechanics; fluid mechanics; tissue and cellular engineering; biotransport; and design, dynamics, and rehabilitation; and biomechanics education. Systems approaches are yielding new insights into human biology by leveraging state-of-the-art tools, which could ultimately lead to more informed design of therapies and medical devices for preventing and treating disease as well as rehabilitating patients using strategies that are uniquely optimized for each patient. Educational approaches can also be designed to foster a foundation of systems-level thinking. [DOI: 10.1115/1.4064547]

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Introduction

The human body is a collection of interacting systems. These include a multitude of parts—molecules, macromolecules, cells, and multicellular assemblies—linked together by cause-and-effect relationships that span spatial scales ranging from nanometers to meters. For example, systems of molecules, proteins, cells, and tissues coordinate to produce a heartbeat (Fig. 1). Hydrolysis of adenosine triphosphate (ATP), a molecule approximately 1 nm in diameter, enables the protein myosin to bind with actin, a protein several microns long, which exerts a physical force that causes myofibril contraction. Coordinated contractions in the thousands of myofibrils bundled into each of the 2×10^9 cardiomyocytes that comprise the 5–10 mm-thick myocardium produce a heartbeat that pumps approximately 70 mL of blood into circulation. Through the dynamic interactions of these systems across spatial scales, chemical energy is converted into the biomechanical energy that enables a healthy heart to pump 7600 liters of blood per day. Mechanobiology,

biomechanics, and biotransport processes govern interactions between these systems to drive the heart's performance, and pathology ensues when their interactions are impaired. Hence, studying these fields from a systems perspective, that is, appreciating how the parts work together to enact changes across spatial scales, is necessary for achieving a deeper and more holistic understanding of how systems function and fail [1]. For example, in recent years the field of cancer research has embraced the use of systems approaches to study the underlying mechanisms of cancer progression and to design novel treatments [2]. By blending data-driven analysis of high-throughput “-omics” data with mechanistic computational modeling, cancer systems biology is uncovering new molecular pathways that drive tumor growth and metastasis and that can be therapeutically targeted with small molecules, immunotherapies, and individualized combination drug treatment strategies [3].

The objective of this review is to highlight systems approaches presented at the 2022 Summer Biomechanics, Bio-engineering, and Biotransport Conference (SB3C 2022) in the areas of solid

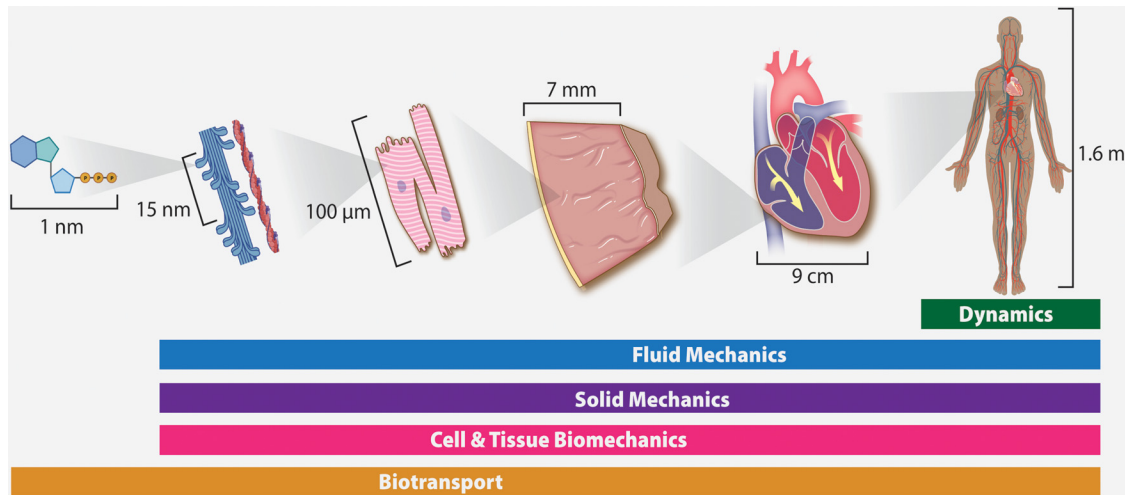


Fig. 1 A systems-level look at the components required to produce a heartbeat and pump blood around the body

mechanics; fluid mechanics; tissue and cell engineering; biotransport; and design, dynamics, and rehabilitation. While these areas of research have historically deployed systems approaches through the use of computational modeling and imaging, for example, [4–6], systems approaches are evolving because of the explosion of biological data across spatial scales, the emergence of tissue- and organ-on-a-chip technologies, and the advent of artificial intelligence (AI). In this review, we showcase how state-of-the-art systems approaches are now yielding new insights by leveraging high throughput “-omics” data, advanced imaging and mechanical testing techniques, tunable experimental models that replicate the complexity and diversity of biological tissues, and sophisticated computational modeling and simulations being advanced by high-performance computing and machine learning. The intended audience for this article includes students entering the field and researchers with experience in the field who are interested in a summary of highlights from the conference.

As a collection, the studies referenced in this article reveal how the integration of data produced by these state-of-the-art tools is laying the foundation for in silico replicas of the human body, a “digital twin”, that can uniquely depict and predict the complex anatomy, physiology, and pathophysiology of individual patients from the level of genes to the whole body [7]. The ideal outcome of these efforts is the more informed design of therapies and medical devices for preventing and treating diseases and rehabilitating patients in ways that are uniquely optimized and personalized to the individual. We also discuss educational innovations designed to infuse systems thinking into the classroom and teach experimental and computational tools for systems-level mechanobiology, biomechanics, and biotransport research. We conclude with a brief summary of the current challenges and future opportunities for systems approaches to push the boundaries of these fields.

Applications in Biomechanics, Mechanobiology, and Biotransport

Solid Mechanics. Solid mechanics research has increasingly shown that the complex interactions among individual constituents give rise to emergent behavior within a given system. In the past, reductionist approaches to mechanics focused within scales that limited the approaches to cellular mechanics [8–10], tissue deformation [11–14], or body kinetics [15–18]. While valuable to our understanding, these single scale approaches cannot fully explain multiscale structure-function relationships or the complex, rapidly changing mechano-electrochemical relationships between cells and their environment that govern cell activity. Thus, much effort in the solid mechanics community has focused on examining

mechanics across size scales and determining the relationships between cells, tissues, organ systems, and the whole body [19–26]. Furthermore, experimental and in silico techniques are being combined to extract new information about multiscale biomechanical relationships from biochemical assays, “-omics” approaches, multiscale imaging, and mechanical testing [27–30].

Subcellular-to-Cellular Scale Interactions. Historically, experimental and computational studies of cellular processes have failed to connect chemical, biological, and mechanical factors, leading to limited understanding of the complex interrelationships between them. However, recent studies presented at SB3C have made large steps forward in investigating these chemo-biomechanical interactions. The recognition that subcellular processes are directly coupled to cell-level mechanical inputs is driving their incorporation into mathematical models of growth and remodeling. Subcellular biochemical pathways can be represented with a set of kinetic equations akin to those used in systems biology approaches. Work by Sadrabadi et al. explored the use of one- and two-way coupled ordinary and partial differential equations to represent biochemical inputs and elucidate the ways in which these inputs affect aneurysm growth patterns [31]. This improved modeling enables the implementation of flexible coupling strategies between systems biology and continuum mechanics models of growth and remodeling. Existing models such as PyMyoVent were also expanded and used cell energy utilization principles to account for the effects of molecular signaling on the growth and deformation of cardiac valves [32]. This brings a new dimension to predicting valve growth in conditions such as aortic stenosis or mitral insufficiency. Still others were able to develop models incorporating the contributions from chemical, mechanical, and biological constituents using rate equations for cell hypertrophy and growth factor concentrations to predict volumetric changes in cartilage [33]. In the latter study, the interplay between anabolic and catabolic processes with mechanical overloading, normal loading, and immobilization accurately predicted the compositional and volume changes observed experimentally (Fig. 2). Together, these models represent improvements in the way we understand the chemo-biomechanical relationships in tissue growth and provide opportunities for medically relevant predictions.

Computational models have also been developed to more fully understand the relationships between proteins, whether they be structural or signaling proteins, and their effect on mechanical cellular responses. LaBelle et al. expanded on existing FEBio and Monte Carlo techniques to develop a model that predicts cyclical relationship between extracellular matrix alignment and cellular traction in angiogenesis [34]. Accounting for the feedback between cellular traction and ECM protein alignment more accurately

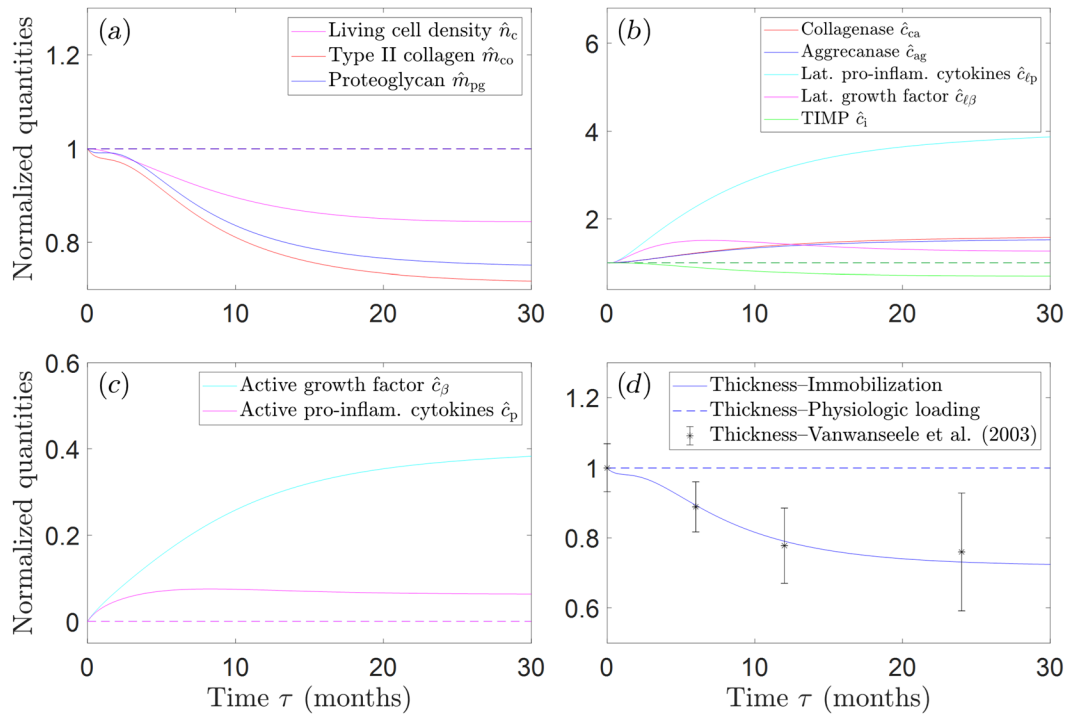


Fig. 2 A chemo-mechanical-biological model of cartilage evolving in health and disease [33]. Normalized quantities of (a) structural components (living chondrocytes, collagen, and proteoglycan), (b) cytokines and growth factors (collagenase, aggrecanase, TIMP, latent growth factor, and latent pro-inflammatory cytokines), (c) active growth factors and pro-inflammatory cytokines, and (d) cartilage thickness. Dashed and solid lines represent normal physiological loading and reduced loading/immobilization, respectively. The model response to mechanical overloading is not shown. Asterisks with error bars represent corresponding experimental data from Vanwanseele et al. [135].

replicated experimentally measured microvessel guidance. Other protein-cell interaction models used reaction-diffusion equations and connectome models to model diffusion of toxic proteins within the brain and its consequences on brain atrophy [35]. They showed that improved connectome measures are necessary to accurately predict protein kinetics in the brain, and therefore better predict the rate of neurodegenerative disease, which to date has been underestimated. Experimental models have also investigated this protein-cell interaction. For example, it has been shown that loss of collagen in the extracellular matrix (ECM) surrounding neural tissues in the joint increases expression of inflammatory mediator secretory phospholipase A2 (sPLA2), which, in turn, activates transcriptional changes that cause neural injury, inflammation, and tissue damage [36,37]. Singh et al. created an in vitro tissue model of neurons embedded in collagenous ECM and used it to investigate the response of cellular signaling cascades to ECM degradation via MMP-1 [37]. Their tests indicated that the inflammatory cascade could be dampened by inhibiting sPLA2, protecting against the subcellular transcriptional changes that underlie neural injury and, ultimately, joint degeneration. Together these computational and experimental models seek to elucidate how protein alignment, distribution, and degradation promote both biochemical and mechanical cellular responses.

Finally, at the cellular level there has been a growing interest in understanding not only the mechanical stimulation of a single cell, but of collective cell behaviors. Collective cell contraction and movement is essential for functions such as cardiac contraction and tissue organization. Computationally, the Cellular-Potts Model represents a novel methodology for modeling cell migration by allowing for representation of multiple interdependent subcellular mechanisms within a cell with a set of rules that are also impacted by the cell's environment [38]. The model's strength is apparent in cases when single cell behavior is expanded to include multicellular interactions that enable exploration of the emergent and stochastic nature of collective cell migration and tissue growth as seen in the

reorganization of the mesendoderm. Experimentally, new techniques are being applied that specifically image Z-disks in sarcomeres to measure the contraction of individual cells and synchronized mechanical behaviors of cells in tissues [39]. The Sarc-graph tool allows an enhanced ability to automatically segment and track sarcomeres and facilitate new approaches for analyzing beating human-induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) [39,40]. This approach allows more sarcomeres to be tracked than with other tools, permitting analysis of collective sarcomere contraction and spatial and temporal irregularities in contracting hiPSC-CMs.

Multi-Cellular-to-Tissue Scale Interactions. Beyond cellular signaling, there has been great interest in understanding how multicellular systems and interactions both respond to and modify mechanical signals. Numerous experimental and computational approaches have been recently developed to specifically explore these complex tissue scale structure-function relationships. For example, Lamia et al. subjected transgenic mice to daily optogenetic stimulation to induce isometric contraction in the triceps surae muscle [41]. Importantly, this approach allows the isolation of local skeletal muscle adaptations to exercise that are difficult to separate from systemic metabolic responses. Transcriptomic analysis via RNA sequencing revealed time-dependent enrichment of pathways related to the immune response, muscle regeneration, and matrix remodeling as a direct result of mechanical stimulation and independent from any systemic effects of exercise [41]. The combination of unique tissue stimulation models and "omics" data provides a more mechanistic understanding of tissue adaptation across the cellular, tissue, and systemic levels.

The past decade has seen a rise in "organ-on-chip" technologies. A "ventilator-on-a-chip" (VOC) study assessed cellular responses and interactions from alveolar epithelial cells, endothelial cells, and alveolar macrophages, along with in silico computational modeling and in vivo studies [42]. The study used these complementary

approaches to identify changes in mechanosensitive micro-RNA expression, which could be leveraged to mitigate ventilator-induced lung injury through the optimization of mechanical ventilator settings. Organ-on-chip studies are complemented by others that focus on cell to tissue interactions, such as Herrmann et al., in which isolated alveolar lobes, carefully dissected from pig lungs, were subjected to prescribed mechanical ventilation waveforms [43]. The collective deformations of individual alveoli were acquired via confocal microscopy and the use of a novel image registration algorithm that relies on periodicity to reconstruct alveolar motion. The relationships measured here could be applied to the VOC platform to help connect tissue-to-cell-to-subcellular processes and develop better ventilation strategies.

Multiple cell types, including red blood cells, platelets, and leukocytes are involved in thrombus formation [44], and cellular composition is thought to affect mechanical properties of thrombi [45,46]. Cruts et al. combined compression testing, histological analysis, and computerized tomography (CT) imaging of thrombi derived from healthy blood to examine the link between cellular composition and mechanical characteristics, including contraction [46]. Their finding that the ratio of red blood cells relative to other cell types in the thrombus had a significant impact on tissue-level compressive stiffness, contractile properties, density, and perviousness may aid in improving methods for treating acute ischemic stroke.

Extracellular Matrix-to-Tissue Scale Interactions. The mechanical behavior of soft tissues is complex and dependent on the collective composition, organization, and distribution of the tissue microstructure. For example, although tendons derive their tensile properties largely from fibrillar collagen, genetic deletion of small leucine-rich proteoglycans (SLRPs) can negatively impact tendon mechanics because of the role SLRPs play in collagen fibril diameter, spacing, and organization [47,48]. Thus, the individual microstructural elements of collagen fibers, SLRPs, and other proteins interact to control the mechanical behavior of the larger “system” that is the tendon. Similarly, aging-related changes to the tissue microstructure, such as the loss of proteoglycans and collagen fiber connectivity in the dermis [49], can lead to increased susceptibility to injury and failure. Precisely how changes to the properties of these individual elements in skin tissue and how they are connected contributes to the overall mechanical behavior and failure properties of the system remains unclear. Recent advances in multiphoton microscopy have enabled detailed tracking of microscale collagen fiber reorganization during mechanical testing to failure of mouse skin [50,51]. Image-based multiscale mechanical models that incorporated microstructural details on collagen fiber organization predicted how single collagen fiber failure can redistribute collagen fiber network loading and propagate tissue tearing [50]. The models reasonably predicted system level mechanical behavior and resistance to tear propagation due to the ability of collagen fibers to reorient before recruitment into the direction of tissue stretch. These results suggest that aged skin could be more susceptible to tearing because of diminished fiber recruitment.

Moreover, computational models featuring discrete networks of cell actin filaments and ECM collagen fibers were used to inform continuum-based finite element models that, in turn, described macroscopic growth and remodeling phenomena [52]. As fibers in the network reorganized in response to applied deformations, each individual fiber’s radius was updated (i.e., remodeled) based on the fiber’s current stress and a prespecified target stress state. Then a new deformation was found until the average network fiber stress was zero. This model was used to simulate growth in a cylindrical arterial blood vessel, where it predicted circumferential growth in response to increased pressure, similar to predictions from continuum-based growth models. The inclusion of networks, however, has the potential to provide additional insight into fiber-level events that lead to tissue failure, such as in aortic aneurysms.

Tissue-to-Organ System Scale Interactions. Although single tissue studies have long been the norm, there is a growing realization

of the interdependence between individual tissues in a biological system. These range from the effects of neighboring tissues to the consequences of distant signaling pathways in a whole-body response.

Joint tissues provide an excellent medium for highlighting tissue interdependence. For example, muscle tissue immobilization during development significantly reduces tendon stiffness and strength, possibly due to inhibition of collagen elongation within the ECM [53]. Similarly, surgical removal of certain tissues, such as the infrapatellar fat pad in the knee joint, can lead to replacement with fibrous tissue and significant changes to osteophyte volume and cartilage and menisci instantaneous and equilibrium moduli, presumably due to an altered loading environment in the knee [54]. The finding by Collins et al. that “fat-free” mouse strains that fully lack adipose tissue have less naturally occurring or post-traumatic osteoarthritis also highlights the complexity of intertissue relationships [55]. This link between adipose tissue and osteoarthritis susceptibility may occur due to pro-inflammatory mediators released from adipose tissue, which trigger systemic inflammation that manifests locally in the joint, although the exact mechanisms have not yet been determined [55].

Even seemingly distant tissues such as the renal sympathetic nervous system and aortic vasculature exhibit strong interconnections. The role of the renal sympathetic nervous system in regulating blood pressure through modulation of electrolyte balance, renin production, and renal blood flow has been reported [56]. Attenuating or inhibiting the activity of the renal sympathetic nervous system via renal denervation has emerged as a potential therapeutic approach to resistant hypertension. Gkousioudi et al. investigated alterations in the mechanical properties and microstructure of common carotid arteries in rats following renal denervation, finding that the procedure reduced arterial stress [57]. These findings suggest that relieving arterial stress prompts changes in the cells and components of the common carotid arteries, effectively reversing their pathological biomechanical behavior.

These studies indicate that to best predict the mechanisms responsible for bodily function, it is necessary to take a systems approach. The development of whole-body models that consider multi-organ interactions on biomechanics such as I-PREDICT has been ongoing over the last decade [58]. Recently, the I-PREDICT human body model was developed within FEBio to take advantage of the tissue damage and active muscle modeling capabilities [59]. Within FEBio, the human body model was used to describe relationships between the tissues of the intervertebral disks and torso musculature to predict the human body’s response to mechanically traumatic events, such as blunt trauma, as well as potential injuries associated with posture during long-duration flights [58]. The progress made thus far with tools like I-PREDICT suggests that future developments will greatly improve our ability to view the body as a complex system.

Overall, in the field of solid mechanics there has been increasing interest in understanding the relationship between chemical, biological, and mechanical contributions to health, especially across scales from proteins, to cells, to tissue, to the whole body. This desire to investigate the body as a complex system has led to the development of many computational and experimental approaches and discoveries that allow us to more accurately perceive the consequences of mechano-electrochemical signaling in disease.

Fluid Mechanics. A systems approach in fluid mechanics includes the collective aspects of a system that influence bulk fluid flow and local flow patterns. For example, systems-based approaches are used to increase the realism and predictive capabilities of subject-specific computational fluid dynamics models developed for cardiovascular pathologies. This can be represented in the network of downstream vasculature that imposes resistance to blood flow, resulting in local pressure within some physiological range under normal conditions, or pathological levels in the setting of disease.

Simulation Realism. The realism alluded to above is often incorporated using physiologic boundary conditions in current-

generation computational fluid mechanics simulations (e.g., Fig. 3). For example, Gupta et al. described automated tuning of boundary condition values for a lumped parameter network (LPN) representation of the circulatory system [60]. The authors combined neural networks, scaling, and ventricular volume estimates to achieve target hemodynamic parameters from 500 data sets, presenting results within $\sim 5\%$ (normalized root-mean-square error) of aimed values.

Another example of a systems approach for fluid mechanics involves consideration of the mechanisms that regulate vessel caliber in response to adverse stimuli resulting in stenoses or intimal hyperplasia that cause local flow perturbations. As alluded to in the prior section, growth and remodeling (G&R) algorithms apply system-based approaches to increase the realism and predictive capabilities of subject-specific computational models developed for cardiovascular pathologies. Bazzi et al. used a $FBLN4^{SMKO}$ mouse model of thoracic aortic aneurysms to study hemodynamically-driven stimuli mediating heterogeneous growth in the aortic wall [61]. The team applied fluid-solid-interaction (FSI) model simulations with longitudinal imaging data as part of a G&R framework meant to incorporate aortic adaptation to mechanical stimuli and differences manifesting from the $FBLN4^{SMKO}$ mouse model with realistic boundary conditions [61]. More specifically, mouse-specific material properties and wall thicknesses were incorporated into boundary conditions for FSI simulations and results for von Mises stress were used as input to solve a local stress-driven G&R model. Model results qualitatively matched the experimentally observed radial growth for ~ 10 weeks relative to the initial state for the cohort of mice presented.

Azarnoosh et al. similarly described their detailed approach to extracting image-based data for eventual use with G&R modeling of the aortic response to severities and durations of aortic coarctations (one of the most common congenital cardiovascular defects) observed in patients experiencing hypertension despite surgical correction at a young age [62]. They showed FSI results in which boundary conditions were altered in a physiologic manner based on adaptation of the local and downstream arterial system to match empirical results, and the accompanying paper with complete details has since been published [63].

A recent study by Chiastra et al. described a new index called the wall shear stress (WSS) topological skeleton. This index is calculated from the divergence of the normalized WSS vector field and provides additional characterization of forces that may be experienced by cells along a vessel surface [64]. De-Nisco et al. presented data from 49 subject-specific models of human coronary arteries designed to investigate whether the WSS topological skeleton index can predict longitudinal changes in local plaque burden [65]. The correlation between the index and plaque burden was consistent with that from elevated oscillatory shear index, low time-averaged WSS, and pronounced relative residence time. Nevertheless, the theory behind WSS topological skeleton as an index that provides a more nuanced measure of the variability of the contraction and/or expansion action exerted by flowing blood on endothelial cells makes it perhaps more useful in uncovering the link between stimulus and response from a mechanistic systems biology perspective.

Stimulus and Response. Because fluid mechanics stimuli are known to affect a wide range of individual intracellular pathways,

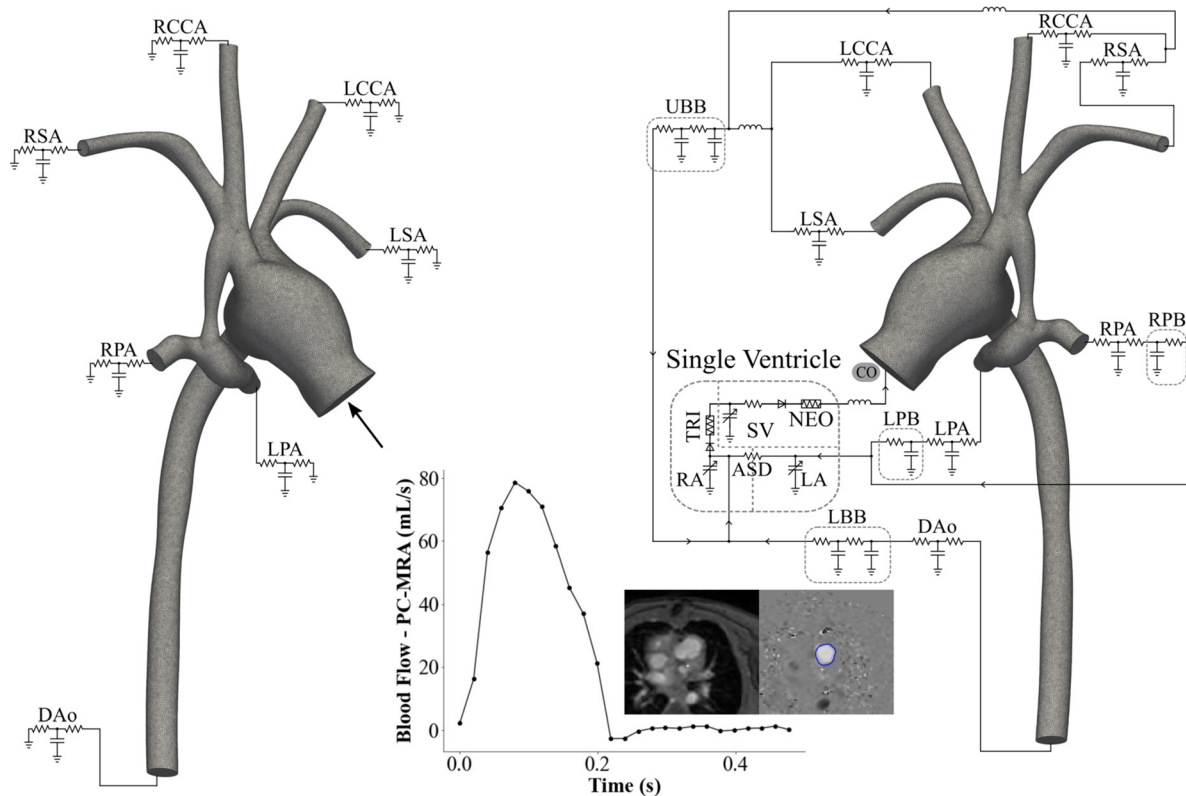


Fig. 3 Schematic illustration of a systems approach to cardiovascular fluid mechanics simulations via advanced boundary conditions often used with computational growth, and remodeling studies. Left: an open-loop approach is shown with discretized model geometry and an ascending aorta flow waveform extracted from patient phase contrast MRI (PC-MRI) data imposed at the inlet, and three-element Windkessel representations applied at outlets. Outlets shown include the right (RSA) and left (LSA) subclavian arteries, right (RCA) and left (LCCA) common carotid arteries, right (RPA) and left (LPA) pulmonary arteries, and descending thoracic aorta (DAo). Right: a closed-loop lumped parameter network is shown with a single ventricle cardiac model coupled to the discretized model geometry. In addition to model outlets, the closed-loop representation includes the left pulmonary bed (LPB), right pulmonary bed (RPB), lower body bed (LBB), upper body bed (UBB), as well as a single ventricle cardiac model. Adapted from Blanch-Granada et al. [136].

many of which are linked intracellularly, the vascular response to wall shear stress represents an area that may benefit from a systems biology approach. For example, endothelial cell metabolic changes may link endothelial cell dysfunction and cardiovascular disease. From a systems perspective, focusing on a single pathway, or single target within a given pathway, does not account for the inter-related nature of metabolic networks. Moiz et al. used computational isotope-assisted metabolic flux analysis (iMFA) to understand how glycolytic side branch pathway inhibition impacted interconnected metabolic networks using human umbilical vein endothelial cells (HUVECs) [66]. The iMFA model showed that inhibition of the polyol and pentose phosphate pathways induced systemic metabolic changes in HUVECs that extended beyond their stated targets. The cells adapted to these metabolic disturbances by altering tricarboxylic acid (TCA) cycle activity. The systems approach leveraged computational and empirical approaches and could be used in the future to limit off-target effects of novel pharmacological compounds [67,68].

Artificial Intelligence and Machine Learning. Subject-specific FSI and/or CFD simulations require a three-dimensional (3D) domain to be reconstructed from medical imaging data. This is an important step conducted prior to applying physiologic boundary conditions at the inlet and outlets of the computationally reconstructed domain. Image-based vascular reconstruction can often be enhanced by using a systems approach. Iyer et al. introduced a deep learning approach aimed at performing 3D coronary artery reconstruction using synthetically-generated angiography images from two projections [69]. While errors in the pressure drop across stenoses peaked at only 4.14 mmHg, centerline coordinates for reconstructed images were of the order of the artery diameter. Importantly, their approach did not require image calibration or knowledge of the projection angle from which images were acquired. Future work aims to use more heterogeneous data sets and include coronary branches. Nonetheless, this promising initial work will likely improve the accuracy of 3D reconstructions for use with G&R algorithms and boundary conditions employing a systems-based approach.

Heraclitus is famously credited by translation with the realization that “everything flows.” Theoretically this is manifested in governing equations describing the bulk flow of fluids as well as local velocity patterns. Fluid mechanics from a systems perspective further underscores a less literal meaning also attributed to Heraclitus’ quote: that everything is dynamic (in response to the complexity of a system). Emerging topics in systems analysis of fluid mechanics are rooted in both theoretical advancements as well as practical applications related to pathology. Simulation realization improvements, enhanced accuracy in predicting the system response to stimuli as a result of improved realism, and infusing of artificial intelligence and machine learning in seemingly every fluids analysis process are at the forefront of fluids mechanics research as the field aims to further understand how everything flows from a systems perspective.

Cell and Tissue Mechanics. Systems biology approaches have gained traction in cell and tissue engineering to study how cells are influenced by biophysical and chemical cues in their micro-environment, and, conversely, how cells influence the tissue micro-environment. The interplay among micro-environmental aspects and complex cellular signaling within and between disparate cell populations has led to higher-order questions with a potentially large parameter space. Moreover, because these numerous factors are often interconnected and time-dependent, a systems approach offers a more holistic view of multiparameter and temporal questions than a single or multiparameter reductionist approach. The experimental control afforded by in vitro engineered constructs or assays enables researchers to study these inherently “messy” problems, such as cell-cell and cell-matrix interactions during development, injury, degeneration, repair, or regeneration. This section highlights SBC3 2022 presentations that used systems

biology approaches to evaluate relationships between cells and the micro-environment.

Mechanical Stimuli. Cells receive important biophysical and chemical cues from the micro-environment, which, in turn, influence the cells’ biologic responses. These cues and responses are essential to understanding tissue dysfunction and disease progression, engineering tissue replacements, and developing novel therapeutics. For example, shear stress activates endothelial cells to increase reactive oxygen species (ROS) and generate a pro-inflammatory state, which is associated with cardiovascular disease. O’Hare et al. investigated the role of shear stress and healthy glycocalyx in deterring problematic endothelial activation. ROS probes and RNA-seq were used to study the dynamic expression of multiple genes associated with either endothelial redox rate or inflammation [70]. Their finding that shear stress resulted in a shift toward anti-oxidant and anti-inflammatory gene expression, while degradation of the glycocalyx layer induced a pro-inflammatory response, thereby suggesting a complex response to endothelial activation. Indeed, their analyses showed that the endothelial activation involved dozens of genes, highlighting its complexity, and illustrating the need for future high-throughput analyses to more completely understand the endothelial redox response [70].

Tensile loading has also been explored within a recent study of mechanotransduction in cancer to identify its influence on the activation and expression of heat shock protein 27 (HSP27), with implications in chemoresistance. Epithelial ovarian cancer cells were subjected to oscillatory tensile loading in two-dimensional (2D) cultures, revealing that tensile-loaded cells increased expression of HSP27 [71]. The mechanically-preconditioned cells were then cultured in a 3D microfluidic device with fully formed vasculature to create a new 3D microfluidic-based model of epithelial ovarian cancer. This model incorporates contributions from other relevant cells (i.e., microvascular epithelial cells and normal lung fibroblasts) without the confounding influence of soluble factors. With this new model established, through the use of mechanically-primed cells and their unstrained controls, future studies can explore the biomechanical relationships between tensile stress, heat shock protein expressions, and chemoresistance. In addition to the cell’s own response, mechanical stimuli can also influence how cells signal to neighboring cells, such as their release of extracellular vesicles (EVs) and EV cargoes. Sangha et al. explored this in red blood cells (RBCs) by subjecting normal RBCs to shear stress and analyzing resulting EVs [72]. Their findings suggest that shear stress may stimulate mechanosensitive healthy RBCs to generate EVs with different size, morphology, and biomarker expression than those EVs generated under pathologic conditions.

Chemical Stimuli. Cellular response to chemical stimuli can involve multiple integrated pathways, necessitating a systems biology approach. Using a combination of RNA-seq and pathway analyses on vascular smooth muscle cells, Mathieu and Clyne studied the role of glutamine on glycolysis [73]. They observed upregulation of both glycolytic and contractile genes and proteins, suggesting that glutamine plays a more complex role than simply promoting a “contractile” or “synthetic” phenotype and that glutamine is essential for both phenotypes.

Cell behavior can also be influenced by integrated mechanical and chemical stimuli. For example, tension in the plasma membrane disturbs endocytic membrane trafficking, with important implications for cancer cell migration. Chan et al. explored this in stationary triple negative breast cancer cells, which were genome-edited to express a fluorescently tagged endocytosis component, AP2-EGFP [74]. By combining confocal microscopy with optical tweezers housed within the same system, they were able to measure endocytic dynamics and plasma membrane tension, respectively. Additionally, the team utilized a computational model to simulate epidermal growth factor gradients that induce chemotactic migration and designed a microfluidic model to enable similar measurements in migrating cells. This integrated approach yielded experimental

results connecting spatial endocytosis dynamics to membrane tension, providing a foundation to study their influence on cell migration, including cell lines with different levels of malignancy.

Stimuli From Local Cells and Tissues. Systems biology approaches are well suited for investigating how cell behavior is influenced by other cells in their micro-environment. For example, a novel computational model that couples both cell migration and division with volumetric growth was used to explore brain development [75]. This model incorporated spatial-temporal development of multiple cohorts of neurons born at different times and showed good qualitative agreement with experimental data in ferret brain development and cortical folding. Such integrated in silico approaches provide a powerful platform for rapid mechanistic exploration, and to inform and guide future experiments, including investigations into atypical brain development and potential factors or mechanisms that drive aberrant development.

Engineered cartilage that recapitulates native healthy tissue has been widely researched, with significant improvements in tissue production through mechanical stimulation [76–79] and growth factor supplementation. Transforming growth factor- β (TGF β) is the most widely used growth factor for cultivating engineered cartilage [80–82]. However, exogenous TGF β delivery may help or hinder the cells' ability to produce its own TGF β , depending on delivery dose and timing, thereby impacting matrix production [81]. TGF β exists in an active or latent state, which can also alter tissue production. Dogru et al. used a combined computational and experimental approach to study the relationship between TGF β dose and TGF β binding kinetics [83]. This systems approach showed that the common dose of 10 ng/mL delivered exogenously resulted in TGF β penetrating the scaffold by less than 500 μ m, but that heparin affinity domains in a scaffold can increase TGF β retention, especially at higher doses. These findings are exciting, as development of larger engineered constructs (i.e., >5 mm diameter) is hindered by greater matrix production on the construct periphery, reducing nutrient flow to the center of the construct [84].

Csordas et al. further showed that TGF β supplementation increased α -SMA expression from fibroblasts cultured in a collagen gel (type I). This study used confocal imaging to assess protein expression within the first 25 h of TGF β exposure, which supports the hypothesis that TGF β induces a fibroblast-to-myofibroblast transition [85]. The in vitro culture system moves beyond traditional cells in a scaffold by embedding a piezo-electric milli-cantilever sensor in each scaffold to measure matrix stiffness changes. While additional model validation is needed to confirm stiffness measurements, this approach may present researchers with a nondestructive assessment of engineered tissue mechanical properties over time.

Microfluidic devices have been used to study how flow impacts cell-matrix and cell-cell interactions. Jewett et al. evaluated how microfluidic channel design alters extracellular matrix fiber alignment and matrix architecture [86], which is critical to recapitulating the highly organized collagen fibers located in specific tissue regions. In this study, flow-induced fiber alignment was achieved by decreasing the channel width. Fiber alignment was maintained even after constructs were removed from the microfluidic device, providing promise for developing more complex organ structures with microscale fiber architecture control. Shelton et al. used microfluidic devices to study cell–cell interactions in the tumor micro-environment [87]. Specifically, fibroblasts from tumors or normal tissue were embedded in a gel, and endothelial cells were applied as a monolayer to assess vascularization. The findings showed that cancer-associated fibroblasts increased sprouting angiogenesis and macrophage polarization.

A number of novel in vitro model systems incorporate mechanical loading to more closely replicate key mechanobiologic aspects of in vivo environments. Brown et al. developed a static loading culture system for ligament-to-bone culture [88]. Type I collagen sheets were constrained between two clamps, causing cells to experience tension between the clamps and compression under the clamps. The

loading system resulted in variations in tissue organization after 4 weeks. Specifically, cells that experienced tensile strains in the midregion produced collagen fibers aligned with the loading direction, while collagen fibers were more disorganized in the region that experienced compression under the clamps. This system highlights how complex tissue structures can be cultivated using more complex loading conditions or more localized mechanical stimuli. Moreover, improvements in tissue interface development, e.g., entheses and myotendinous junctions, may be important for successful implantation of engineered tissues and integration with surrounding native tissues.

In vivo tissue degeneration includes both inflammatory and mechanical changes, which may interact to accelerate tissue degeneration. A growing body of work indicates that macrophages are mechano-sensitive [89,90]. Kim et al. assessed baseline cell signaling from healthy and pathologic human synovial tissue, and then evaluated the effect of substrate stiffness and inflammation on macrophage response [91]. Synovial tissue from patients receiving total knee arthroplasty was more fibrotic, suggesting a stiffer tissue, and had a greater staining for CCR7 (M1 macrophage surface marker) and nuclear translocation of p65 (NF- κ B subunit containing the activation domain). The team then assessed interactions between matrix stiffness and macrophage response in an in vitro culture system with variable substrate stiffness (5 or 55 kPa). Macrophage adhesion to soft hydrogels had a greater inflammatory response than macrophages seeded on stiffer substrates. The inflammatory response by macrophages was further altered in the presence of biochemical cues, where TNF- α increased the expression of M1 markers and the increase in expression was even greater for softer substrates [91]. The increase in inflammation on softer substrates conflicts with previous observations, which may be due to the relatively small range in substrate stiffness (50 kPa difference rather than orders of magnitude) [92].

Chen et al. evaluated the effect of mechanics on wound healing using an in vivo mouse model [93]. A mechanical loading device applied uniform static tension to the mouse dorsum after an incisional wound was created. Tensile loading increased fibrotic, inflammatory, and migratory myeloid cells, resulting in fibrotic tissue formation with highly aligned collagen fibers. The role of focal adhesion kinase (FAK) during the wound healing process was assessed using a knockout mouse model and by inhibiting FAK at the wound site. FAK knockout or inhibition reduced pro-inflammatory markers and promoted a more regenerative myeloid cell population. These findings show promise that mechanical modulation in early wound healing can trigger a mechano-immunomodulation of the early responding cells, with a downstream effect on regenerative healing.

The powerful synergy between computational models and experimental data provides a true systems biology approach to address complex biologic questions. Indeed, cell-micro-environment interactions have been explored in silico using models informed by in vitro experimental data. For example, Flanary and Barocas created a computational model to predict 2D contractile behavior of vascular smooth muscle cells when subjected to a variety of environmental stimuli [94]. Their model integrated cell contractile signal generation, actomyosin stress fiber interaction, and elastic substrate deformation to provide a novel way to interpret traction force microscopy data. By incorporating prior systems biology advances for modeling cell signaling networks [95], along with experimental tribology data, they were able to model ECM deposition and phenotype switching to predict actomyosin activation and substrate displacement. Their novel integration of systems biology modeling with traction force microscopy data leverages feedback from both chemical and mechanical signals to enable unique investigation into the biologic aspects of cell-matrix interaction and cell contraction [94].

In summary, models that use a systems approach to study the role of biophysical and chemical cues on the interplay between cells and their micro-environment provide powerful tools for studying disease progression and treatment options. In 2023, new legislation

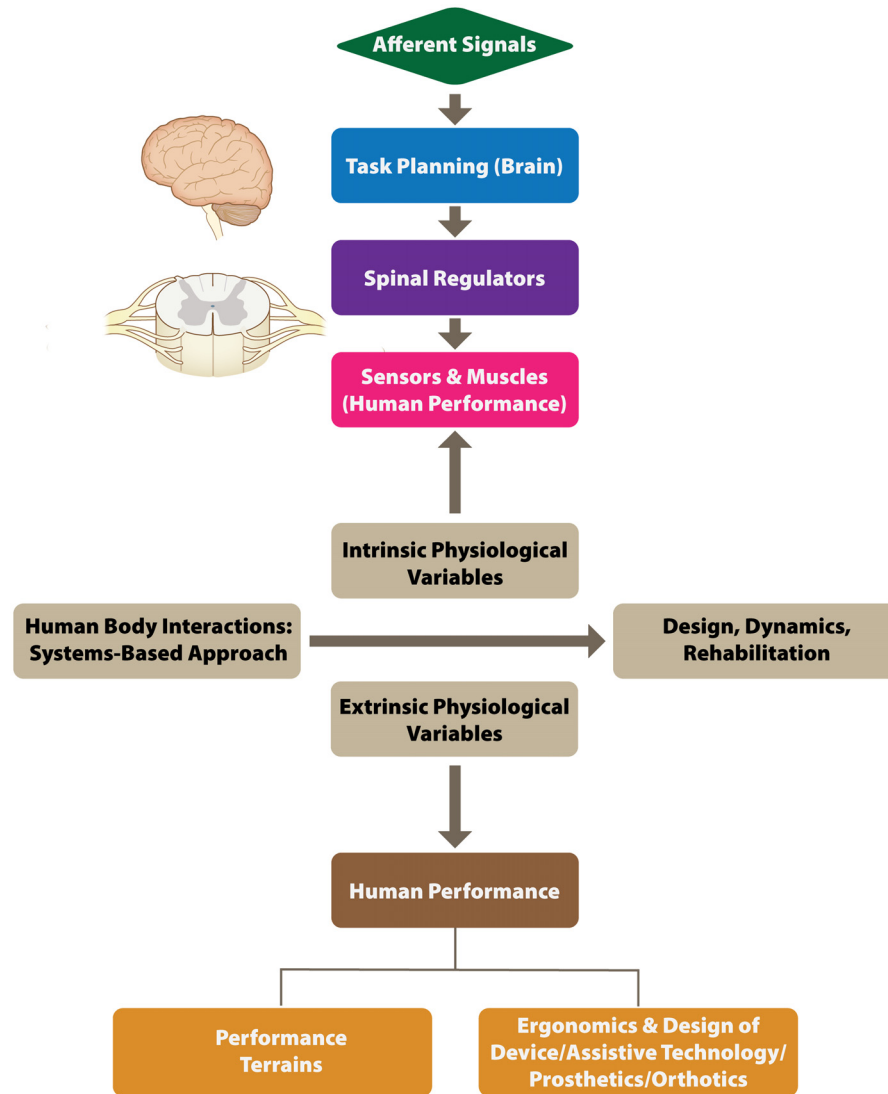


Fig. 4 Interactions to consider in a systems approach to design, dynamics, and rehabilitation [102,108–110]

altered the Food & Drug Administration (FDA) approval process by removing the requirement for animal testing prior to clinical testing, which greatly increases the importance of novel in vitro model systems, particularly those using human cells and engineered to closely mimic key aspects of the in vivo micro-environment. The aforementioned studies show how systems can be used to study cardiovascular disease progression, cancer treatment effectiveness, cancer migration and metastasis, organ development, tissue degeneration, and tissue regeneration.

Biotransport. Since biological systems interact by regulating the transport of mass, momentum, energy and chemical species, systems approaches in the biotransport area often aim to understand these interactions by measuring and controlling transport processes. Most actively studied interactions at the organ and tissue levels are transport processes by blood perfusion. Blood perfusion, which is often associated with thermoregulation and thermal therapies, functions to regulate the system temperature by mitigating the effects of either spatial or temporal temperature change of organs and tissues. Blood perfusion also induces interstitial fluid flow, thus transporting growth factors, nutrients and metabolic byproducts, crucial for regulation of cellular behaviors, therapeutic responses, and performance of biomedical devices.

Multi-Organ Interactions. Research on biotransport in the brain has been largely focused on the blood-brain-barrier, which tightly

regulates transvascular transport. The significance of the glymphatic system has recently been recognized, particularly its cyclic activation of lymphatic function associated with the circadian rhythm. As the brain is the most metabolically active organ, consuming 20–25% of the body's total energy expenditure, a functional lymphatic system is crucial to brain physiology. Gan et al. presented a systems approach to investigating the blood perfusion of brain [96]. As local consumption of metabolites occurs with neuronal activation, cerebral blood flow increases to supply oxygen and metabolites to the depleted area, a process called functional hyperemia, which displaces cerebrospinal fluid (CSF) and drives flow in the space around cerebral arteries. These flows are thought to couple to the glymphatic system, which facilitates waste clearance. The investigators used a whisker stimulation model in mice to investigate its effects on glymphatic fluid transport, finding the first direct evidence that functional hyperemia accelerates CSF influx to the brain, as well as waste clearance from the brain space. Further, they demonstrated that changes in arterial diameter, rather than neuronal activation, may be responsible for increased CSF influx. Through this study, Gan et al. quantitatively demonstrated key interactions between the glymphatic system and the vasculature during functional hyperemia that drive transport of nutrients and metabolic wastes [96].

Data-driven approaches are also essential to systems analysis of biotransport phenomena across multiple organs. For example,

Messou et al. employed a deep learning approach to identify differences in physiological cardiovascular transport in cannabis smokers as compared to nonsmokers using both cannabis use survey data and cardiovascular magnetic resonance (CMR) images from the large-scale biomedical database UK Biobank [97]. The authors applied a machine learning algorithm to establish left ventricle blood transport from regular (self-reported) cannabis users and could be distinguished from that of nonusers with their deep learning approach. This study demonstrates the feasibility of applying image-based machine-learning approach to correlate and predict system-level transports in disease development and diagnostics [97].

Multi-Cellular and Multi-Tissue Interactions. Systems approaches at tissue and cellular level interactions were employed in two studies to develop microfluidic tumor models. Chi et al. presented a three-layer microfluidic cell array coculturing breast cancer cells with immune cells to reconstruct a tumor-stroma micro-environment. The array is integrated with a recirculation circuit designed to study T cell infiltration and cytotoxicity [98]. The design allows for study of tumor–endothelium and tumor–stroma interactions in cancer drug responses, in a scenario that closely mimics real physiological conditions. The model successfully provides a testbed for screening cancer immunotherapeutic agents paired with a high throughput adaptation [98].

The role of cancer-associated fibroblasts (CAFs) in pancreatic cancer has been one of the most actively studied interactions between tumor and stromal systems. Interestingly, the stromal system has been considered protumorigenic due to its promoting tumor cell growth and invasion, augmenting chemoresistance, and posing a drug delivery barrier. Several recent studies reported that the stromal system may also play an antitumor role by suppressing growth and invasion of tumor cells [99,100]. Thus, systems approaches to delineate these two competing roles can be of potential significance. Moon et al. used a 3D microfluidic tumor-stroma model of pancreatic cancer to systematically screen analogs of inhibitors of Ref-1, a signaling protein known to regulate redox metabolic activity of cancer in hypoxia and induce drug resistance and metastasis [101]. This investigation of the efficacy of Ref-1 inhibition on pancreatic cancer cells while sparing the antitumor role of the fibroblasts helps demonstrate the tumor micro-environment on-chip model's capability to provide a test bed for studying potential cancer-selective drugs in the complex and heterogeneous tumor micro-environment.

In summary, biotransport examines how mass, momentum, energy, and chemical species move in physiological systems across multiple scales. A systems-level understanding of biotransport is crucial to improve our understanding of physiological and pathological processes, as well as to design and test new, innovative imaging agent and therapeutic delivery.

Design, Dynamics, and Rehabilitation. When understanding and investigating human dynamics, a system-based approach is critical to understanding the underlying intrinsic and extrinsic physiological variables that control the behavior of human performance (Fig. 4). The human body interacts at multiple levels, including the cell, neuron, motor unit, musculotendinous unit, joint, limb, or body, and an endless number of interactions are possible when studying human dynamics. A system-based approach allows one to understand the finer levels of complexity, including the afferent signals that engages the brain in task planning followed by spinal regulators that interact with the sensors and muscles to perform the intended human performance. In rehabilitation, a system-based approach helps us understand how variables such as initial conditions and perturbations on a performance terrain affect human performance while investigating the selection of an optimal trajectory for desired motor performance during rehabilitation interventions. Finally, a systems approach to design includes taking a team-based collaborative approach while engaging with stakeholders and staying patient- as well as physician-centric. Incorporating a system-based approach in ergonomics and design of

devices, assistive technology, prosthetics, and orthotics holds the most promise for not just addressing health delivery challenges but also yielding significant improvements in both patient and service outcomes.

Role of Human Biomechanics in Predicting Performance. A dynamical systems approach that fully explores how environmental variables impact integrated body movements is critical to predicting human performance [102]. Studies have observed a reduction in coordination variability and flexibility in diseased and injury-related subjects as compared to healthy controls [103–107]. Gomez et al. studied the effects of regular and irregular walking surfaces on whole-body angular momentum in people with Parkinson's disease (PD) by investigating changes in whole-body angular momentum when walking from a regular to an irregular surface, comparing outcomes in persons with PD with healthy, age-matched controls [108]. By analyzing adjustments in angular momentum about the three principal body axes (sagittal, frontal, and transverse) during terrain transitions, the team observed that persons with PD adopt a distinct and specific strategy for crossing the surface transition, with alterations to their sagittal and transverse angular momentum that differ from what can be expected in a healthy individual [108].

A systems-based approach while accounting for the environment in which the task is performed is critical to the outcomes of rehabilitation intervention. Schwartz et al. investigated the correlations between wheelchair fit (environmental variable) and hand rim biomechanics metrics (biomechanics) during propulsion (human performance) in pediatrics [109]. Currently, adult wheelchair user recommendations, which include minimizing force and rate of force application to the wheel hand rim, maximizing contact phase time, and reducing push frequency, are applied to pediatric users. Furthermore, adult wheelchair fit setting recommendations, such as wheel axle position with respect to the shoulder and elbow, are extrapolated for pediatric populations. The study found only a moderate correlation between elbow angle and time spent in the contact phase, and no correlation between frequency and propulsion technique, axle position and force, and wheelchair fit measures and hand rim metrics, indicating that these adult recommendations may not translate to children [109].

Physiological Systems Approach to Predicting Human Movement. Because biological tissues, including nerves, tendons, and ligaments, exhibit nonlinear behavior, the dynamic behavior of human movements that involve spatio-temporal evolution can be studied using a nonlinear systems approach when accounting for time and frequency responses/behavior [110]. Furthermore, the spatio-temporal evolution of the system dynamics can be accounted for by incorporating related physiological responses and controls. Coker et al. confirmed that a nonlinear input–output time series neural network could predict joint angles during gait at 50–200 milliseconds into the future by observing current joint angles, torques, and surface electromyography (sEMG) data of pertinent muscle groups [111]. Boyea and Canino evaluated the capability of this method in predicting knee joint angles and torques at varying walking speeds [112]. Utilizing the dataset of Moriera et al. [113], they demonstrated a system that could accurately estimate knee joint torque and angle 150 ms into the future, with accurate predictions of knee joint kinematics over various walking speeds. This robust system may have applications in assessing postacute injury rehabilitation therapy and creating assist-as-needed exoskeleton devices.

In another study, Trapp et al. used experimental data obtained from neonatal piglet brachial plexus to develop a computational neonatal human model [114]. Simulations using such computational models can address neonatal brachial plexus palsy birthing injuries, which are virtually impossible to study clinically in newborn humans. A systems-based approach of using physiological responses of neonatal nerve tissue obtained during stretch was utilized in the study to create models that can predict human responses to movement. The team created a 2D model of the neonatal brachial plexus that they used to investigate development

of stress and strain throughout the brachial plexus when various loads and displacements were applied. As such a model is enhanced and refined, it may provide the opportunity to assess the effect of the birth process and variations in individual physiologies on the risk of neonatal brachial plexus injuries [114–117].

Systems Approach to Device Design. A systems approach for healthcare improvement is essential to integrated, patient-centered, effective, and efficient care [118]. Inacio et al. used patient-specific finite element modeling to virtually measure the interfragmentary strain environment in clinically realistic fractures [119]. They showed that shear-dominated conditions could exist for the tissues in and around the fracture line, even without large gross shearing motions of bone fragments. These data suggest that shear-dominated strains may be much higher than previously appreciated, which does not necessarily predispose a fracture to nonunion. The findings of this study have significant implications for the development of orthopedic implants because the allowable fracture-site motion is a critical design input.

Simulation mannequins provide an immersive learning environment for medical and nursing students, which catalyzes the systems approach and helps address health delivery challenges, since students can directly observe, identify, and recognize the multiplicity of elements that interact to impact a healthcare outcome [116,120,121]. In a study by Eisele et al., a team of students used the system-based approach in design that accounts for stakeholders, which were the nursing professionals in their case. They identified the need for a temperature assessment component in their simulation mannequin to accurately portray the qualitative and quantitative components of body temperature and aid in the clinical training of healthcare providers [122]. The team designed and built a prototype standalone temperature assessment tool. Future versions of this tool could be integrated into a full simulation mannequin, incorporating clinical complexities to improve healthcare training approaches and, ultimately, patient care.

The presented studies used a system-based approach to understand the effects of extrinsic physiological variables to enhance our understanding of gait and upper extremity biomechanics, which are critical to developing safe movement strategies for patients of all ages. Physiological systems approaches will help us to better understand and predict human movements and improve injury prevention, prognosis and treatment. Finally, a system-based approach also enhances our understanding of intrinsic physiological factors and external factors in the design and development of novel devices.

Education. As described in the prior sections, SB3C 2022 included multiple studies that moved bio-engineering research from assessment of single components to a systems approach, recognizing the continuum of function from subcellular processes to cell, tissue, organ, physiological system, and eventually to human function. Biomechanical engineers recognize that none of these subsystems function alone and that understanding the multilevel dynamics of the entire system is critical to advancing knowledge. In educating the next generation of biomechanical engineers, we should integrate the multilevel perspective into the classroom.

In the education track, several authors examined how to use laboratory experiences to develop systems-level research skills. Ebenstein et al. created educational modules for patient-centered model development from 3D imaging to finite element analysis [123]. This modeling approach is a basis for systems approach papers presented at the conference [119,124]. Singh and Balasubramanian presented in-person and virtual classroom laboratory approaches to give students hands-on experience in mechanical tissue property testing, which also formed the basis for several system-based research studies [50,53,125]. Singh examined the pandemic adaptations necessary to teach this material during remote learning.

Across the breadth of multiscale papers at the conference, many experimental techniques were presented that could be incorporated

into coursework. In addition to being able to prepare cells and tissues and perform common biology and chemistry techniques such as ELISA, western blot, and immunostaining, skills that might prepare future multiscale researchers include imaging (from atomic force microscopy to magnetic resonance imaging), microfluidics, micro-pattern fabrication, cellular and tissue level mechanical testing, motion capture, electromyography, electrocardiography, and statistics. Statistical methods are noted across a wide breadth of systems approach-based papers.

Modeling is another essential tool in the systems approach. Modeling methods used across the breadth of the multiscale papers include image segmentation, 3D computer aided modeling, finite element modeling, computational fluid dynamics, lumped parameter modeling, and deep learning methods. The challenge to presenting such modeling in the classroom is to address the complexity of multiscale systems while keeping it simple enough to be achievable by students in a limited time. One such approach might be to follow a characteristic such as stiffness from the ECM and intracellular fibers in cellular models to whole tissue or movement characteristics [52,91,119,126]. For example, stiffness in the intracellular mechanics of muscle contraction in turn plays a role in the dynamic stability of whole-body motion [127–132]. Textbooks that support this systems approach to muscle mechanics include *Animal Locomotion* by Andrew Biewener and *Muscles, Reflexes, and Locomotion* by Thomas McMahon [133,134].

Current Challenges and Future Opportunities

The presentations at SB3C 2022 in solid mechanics; fluid mechanics; cell and tissue mechanics; biotransport; and design, dynamics and rehabilitation show the tremendous potential of systems-based approaches. Multiscale and multisystem analyses consider the biological complexity that is inherent in all lifeforms, enabling research studies that are more physiologically relevant and therefore more likely to alleviate morbidity and mortality. Sharing research advances across technical areas within biomechanics will create collaboration opportunities that could transform biomechanical engineering and medicine. However, significant challenges in both experimental and computational models continue to limit systems-based approaches in biomechanics.

As the complexity of research questions increases, so will the demand for experimental tools to study them. Complex studies create an expanding need for novel in vitro models that integrate biomechanical and biochemical parameters, in particular large-scale changes in solid or fluid mechanics with cellular and molecular responses. These models must enable multiple, individual parameters to be permuted in a controlled and reproducible manner. Yet it remains challenging to vary one experimental parameter (e.g., substrate stiffness, shear stress) without changing other parameters (e.g., crosslinking density, biotransport). These models must also enable the dynamic characterization of temporal and spatial intra- and extra-cellular interactions at the molecular, cellular, and tissue levels in 3D, multicellular in vitro systems. Advances in experimental measurements (e.g., microscopy and other non-destructive in situ techniques) are needed to enable investigators to gain as much information as possible from in vitro models.

Enhanced experimental models require advanced computational techniques to analyze large datasets and increase throughput. Rich high-dimensional data sets (e.g., temporal and spatial transcriptomics, proteomics, and metabolomics) require in silico models to probe biomechanics hypotheses. Machine learning and artificial intelligence methods may provide solutions; however, collaborations with computational biologists and computer scientists are needed to fully integrate these techniques into the biomechanics community. These data sets will also need to be shared widely to enable those without access to experimental models to participate in higher order inquiry. Computational models, for example, of cardiovascular fluid mechanics, require validation of parameters imposed on the vascular network (e.g., growth and remodeling), validation of outcomes from a single step in the subject-specific

modeling process (e.g., vascular reconstruction), and validation of overall outcomes. These validations become even more challenging when working across size scales. Finally, the frequently reported indices from these models, such as time-averaged wall shear stress are undoubtedly important; however, additional indices are needed to account for spatial and temporal variability as well as cellular and molecular impacts.

The advances in *in vitro* and *in silico* models that are needed to expand systems-based approaches in biomechanics research provide opportunities and challenges in educating the next generation of biomechanical engineers. Students will need broadened training opportunities and resources in systems-level approaches, which could be incorporated into courses, textbooks, conference-based workshops, and online certificate programs. Undergraduate students will need exposure to these concepts at a basic level, while graduate students, postdocs, and established investigators working in academia, government, and industry will need in-depth education and training. Key challenges include how to fit these complex concepts into an already packed curriculum, as well as developing educational resources (e.g., textbooks, instructors) to make the material accessible to learners from a wide variety of backgrounds.

Investigators are already exploring how solid, fluid, and cellular biomechanics, dynamic systems, and biotransport impact subcellular and cellular signaling, cell-micro-environment, and interorgan interactions to understand tissue development and disease progression and eventually control tissue repair. This research shows the importance of considering multiscale biological complexity when attempting to understand and predict the mechanical response of cells, tissues, organs, and humans. This holistic approach creates incredible opportunities to transform our understanding of biomechanics if we can create effective and accessible *in vitro* and *in silico* tools. Additional conferences or research symposia that bring investigators from biomechanics together with computational biologists and computer scientists are needed to accelerate the application of systems-based approaches in biomechanics.

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Data Availability Statement

No data, models, or code were generated or used for this paper. No data, models, or code were generated or used for this paper.

Nomenclature

2D = two-dimensional
 3D = three-dimensional
 ATP = adenosine triphosphate
 CAF = cancer-associated fibroblasts
 CCR7 = C–C motif chemokine receptor 7
 CFD = computational fluid dynamics
 CMR = cardiovascular magnetic resonance
 CSF = cerebrospinal fluid
 CT = computerized tomography

ECM = extracellular matrix
 ELISA = enzyme-linked immunosorbent assay
 EV = extracellular vesicle
 FAK = focal adhesion kinase
 FSI = fluid–solid interaction
 G&R = growth & remodeling
 hiPSC-CMs = human induced pluripotent stem cell derived cardiomyocytes
 HSCs = human Schlemm’s canal cells
 HUVECs = human umbilical vein endothelial cells
 iMFA = isotope-assisted metabolic flux analysis
 LPN = lumped parameter network
 MSC = mesenchymal stromal cells
 NF- κ B = nuclear factor- κ B
 RBC = red blood cell
 RNA-seq = ribonucleic acid sequencing
 ROS = reactive oxygen species
 SB3C = Summer Biomechanics, Bioengineering, and Biotransport Conference
 sEMG = surface electromyography
 SLRPs = small leucine-rich proteoglycans
 sPLA₂ = secretory phospholipase A₂
 TCA = tricarboxylic acid
 TGF β 2 = transforming growth factor beta2
 VOC = ventilator-on-a-chip
 VSMC = vascular smooth muscle cell
 WSS = wall shear stress
 YAP = yes-associated protein

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