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A Systems Approach to Biomechanics, Mechanobiology, and Biotransport

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ABSTRACT (136/250 words)

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, Bioengineering, 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.

INTRODUCTION

The human body is a collection of interacting systems. These include a multitude of parts – molecules, macromolecules, cells, and multi-cellular 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 nanometer 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 billion cardiomyocytes that comprise the 7 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 2,000 gallons 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.

The objective of this review is to highlight systems approaches presented at the 2022 Summer Biomechanics, Bioengineering, and Biotransport Conference (SB3C 2022) in the areas of solid mechanics; fluid mechanics; tissue and cell engineering; biotransport; and design, dynamics, and rehabilitation. We showcase how systems approaches are 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.

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 [1]. 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. Reductionist approaches of the past, while valuable to our understanding, cannot fully explain structure-function relationships or the complex, dynamic mechanoelectrochemical relationships between cells and their environment that govern cell activity. Thus, much effort in the solid mechanics community has focused on examining mechanical relationships across size scales – from cells, tissues, and organs to the whole body. 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.

This section highlights natural groupings of multiscale and systems biology solid mechanics research presented at SB3C 2022 and within related publications.

Subcellular-to-Cellular Interactions

Several studies examined the relationship between sub-cellular interactions and cell function, emphasizing how changes in gene expression and protein levels regulate cell biomechanical processes. For example, after a ligament injury, loss of collagen in the extracellular matrix (ECM) surrounding neural tissues in the joint can increase expression of the inflammatory mediator secretory phospholipase A_2 ($sPLA_2$), which, in turn, activates transcriptional changes that cause neural injury, inflammation, and tissue damage [2, 3]. Singh et al. created an *in vitro* tissue model that can be used to investigate the interaction between subcellular $sPLA_2$ and the cellular signaling cascade [3]. Their tests indicated that the inflammatory cascade could be dampened by inhibiting $sPLA_2$, protecting against the subcellular transcriptional changes that underlie neural injury and, ultimately, joint degeneration.

The recognition that subcellular processes are directly coupled to cell-level mechanical inputs is also 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 understand differences in aneurysm growth patterns [4]. Similar approaches that couple subcellular biochemical pathways and mechanical cues were used to simulate left ventricle growth by incorporating cell energy utilization [5] and the evolution of osteoarthritis using rate equations for cell hypertrophy and growth factor concentrations

192 [6]. In the latter study, the interplay between anabolic and catabolic processes with
193 mechanical overloading, normal loading, and immobilization accurately predicted the
194 compositional and volume changes observed experimentally (Fig. 2).

195 Computational models were also created to analyze how protein distributions,
196 whether they be structural or signaling proteins, combine to affect cellular response. For
197 example, reaction-diffusion equations and connectome models were used to model
198 diffusion of toxic proteins within the brain, simulating neurodegenerative disease [7].
199 The data show the ability to model increases in abnormal biomarkers in the brain and the
200 value of adjusting the models for anatomical factors to better describe disease
201 progression. Other work has incorporated 3D matrix fibril orientation and distribution
202 into simulations of angiogenesis [8]. In comparison to methods that only consider fibril
203 orientation, models that consider both orientation and distribution better match
204 experimentally measured microvessel guidance.

205 Other modeling frameworks, such as Sarc-graph and the Cellular-Potts model, use
206 subcellular structures, for example, Z-discs in sarcomeres and lamellipodia in the
207 mesendoderm, to measure the contraction of individual cells and synchronized
208 mechanical behaviors of cells in tissues [9, 10]. The Sarc-graph tool allows an enhanced
209 ability to automatically segment and track sarcomeres and facilitate new approaches for
210 analyzing beating human-induced pluripotent stem cell-derived cardiomyocytes (hiPSC-
211 CMs) [10, 11]. This approach allows more sarcomeres to be tracked than with other tools
212 [10, 11], permitting analysis of collective sarcomere contraction and spatial and temporal
213 irregularities in contracting hiPSC-CMs. Similarly, the Cellular-Potts Model represents a
214 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. The model's strength is apparent in cases when single cell behavior is expanded to include multi-cellular interactions that enable exploration of the emergent and stochastic nature of collective cell migration and tissue growth [9].

Multi-Cellular-to-Tissue Interactions

Multi-cellular systems were also used to explore complex tissue-level structure-function relationships. For example, Lamia et al. subjected transgenic mice to daily optogenetic stimulation to induce isometric contraction in the triceps surae muscle [12]. 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 [12]. 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 [13]. The study used these complementary approaches to identify changes in mechanosensitive microRNA 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 wave forms [14]. 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 [15], and cellular composition is thought to affect mechanical properties of thrombi [16, 17]. 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 [17]. 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 Interactions

The mechanical behavior of soft tissues is complex and dependent on the collective composition, organization, and distribution of the tissue microstructure. In other words, soft tissue is comprised of a “system” of microstructural elements that interact to achieve overall mechanical properties. 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 [18, 19]. Similarly, aging-related changes to tissue microstructure can lead to increased susceptibility to injury and failure, for example, in the skin. Recent advances in multiphoton microscopy enabled simultaneous observation of skin's macro scale mechanical behavior and micro scale collagen fiber reorganization during mechanical testing [20, 21]. Image-based multiscale mechanical models incorporating these data predicted how single collagen fiber failure can redistribute collagen fiber network loading and propagate tissue tearing [20].

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 [22]. 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 pre-specified 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 Interactions

Finally, at the macro-scale, there is a growing realization of the interdependence among individual tissues in a biological system. Research has increasingly focused on how distant tissues and organs affect a tissue's mechanical properties.

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 [23]. 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 [24]. 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 inter-tissue relationships [25]. This link between adipose tissue and osteoarthritis susceptibility may occur due to proinflammatory mediators released from adipose tissue, which trigger systemic inflammation that manifests locally in the joint, although the exact mechanisms have not yet been determined [25].

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 [26]. 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 [27]. 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 [28] has been ongoing over the last decade. The I-PREDICT human body model has been used to describe relationships between the tissues of the intervertebral discs 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 [28]. 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, we find that there is an increased awareness of systems biology and the need to consider the body as a system in solid mechanics. Research presented at SB3C 2022 integrated size scales from protein to tissue to whole body movement, allowing us to develop a more accurate understanding of biomechanics.

Fluid Mechanics

Simulation Realism

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. Such realism is often incorporated using physiologic boundary conditions in current-generation computational simulations. For example, Gupta et al. presented work at SB3C 2022 related to automated tuning of boundary condition values for a lumped parameter network (LPN) representation of the circulatory system [29]. The authors combined neural networks, scaling, and ventricular volume estimates to achieve target hemodynamic parameters from 500 data sets, presenting results within ~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 [30]. 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 [30]. 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 [31]. The SB3C presentation 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 manuscript with complete details has since been published [32].

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 [33]. 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 [34]. 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, many of which are linked intracellularly, the vascular response to fluid 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 interrelated 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) [35]. 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 [36, 37].

Artificial Intelligence and Machine Learning

Subject-specific FSI and/or CFD simulations require a 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 [38]. While errors in the pressure drop across stenoses peaked at only 4.14 mmHg, centerline coordinates for reconstructed images were on 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 heterogenous 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.

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 microenvironment, and, conversely, how cells influence the tissue microenvironment. The interplay among microenvironmental 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 microenvironment.

Mechanical Stimuli

Cells receive important biophysical and chemical cues from the microenvironment, 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. 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 [39]. 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.

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 2D cultures, revealing that tensile-loaded cells increased expression of HSP27 [40]. 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 [41]. 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 [42]. 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 [43]. 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 microenvironment. For example, a novel computational model that couples both cell migration and division with volumetric growth was used to explore brain development [44]. 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 [45-48] and growth factor supplementation. Transforming growth factor-β (TGFβ) is the most widely used growth factor for cultivating engineered cartilage [49-51]. However, exogenous TGFβ delivery may help or hinder the cells' ability to produce its own TGFβ, depending on delivery dose and timing, thereby impacting matrix production [50]. TGFβ 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 TGFβ dose and TGFβ binding kinetics [52]. This systems approach showed that the common dose of 10 ng/mL delivered exogenously resulted in TGFβ penetrating the scaffold by less than 500 μm, but that heparin affinity domains in a scaffold can increase TGFβ retention, especially at higher doses. These findings are exciting, as development of larger engineered constructs (i.e., > 5 mm diameter) are hindered by greater matrix production on the construct periphery, reducing nutrient flow to the center of the construct [53].

Csordas et al. further showed that TGFβ 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 hours of TGFβ exposure, which supports the hypothesis that TGFβ induces a fibroblast-to-myofibroblast transition [54]. The *in vitro* culture system moves beyond traditional cells in a scaffold by embedding a 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 non-destructive 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 fiber alignment and matrix architecture [55], 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 microenvironment [56]. 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 [57]. 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 mid-region 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 [58, 59]. 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 [60]. 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-κB 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 [60]. The increase in inflammation on softer substrates conflicts with previous observations, which may be due to the relatively small range in substrate stiffness (50kPa difference rather than orders of magnitude) [61].

554 Chen et al. evaluated the effect of mechanics on wound healing using an *in vivo*
555 mouse model [62]. A mechanical loading device applied uniform static tension to the
556 mouse dorsum after an incisional wound was created. Tensile loading increased fibrotic,
557 inflammatory, and migratory myeloid cells, resulting in fibrotic tissue formation with
558 highly aligned collagen fibers. The role of focal adhesion kinase (FAK) during the wound
559 healing process was assessed using a knockout mouse model and by inhibiting FAK at
560 the wound site. FAK knockout or inhibition reduced pro-inflammatory markers and
561 promoted a more regenerative myeloid cell population. These findings show promise that
562 mechanical modulation in early wound healing can trigger a mechano-
563 immunomodulation of the early responding cells, with a downstream effect on
564 regenerative healing.

565 The powerful synergy between computational models and experimental data
566 provides a true systems biology approach to address complex biologic questions. Indeed,
567 cell-microenvironment interactions have been explored *in silico* using models informed
568 by *in vitro* experimental data. For example, Flanary and Barocas created a computational
569 model to predict 2D contractile behavior of vascular smooth muscle cells when subjected
570 to a variety of environmental stimuli [63]. Their model integrated cell contractile signal
571 generation, actomyosin stress fiber interaction, and elastic substrate deformation to
572 provide a novel way to interpret traction force microscopy data. By incorporating prior
573 systems biology advances for modeling cell signaling networks [64], along with
574 experimental tribology data, they were able to model ECM deposition and phenotype
575 switching to predict actomyosin activation and substrate displacement. Their novel
576 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 [63].

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 may also induce 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 brain transport 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 total body's 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. As local consumption of metabolites occurs with neuronal activation, cerebral blood flow increases to supply oxygen and metabolites to the

600 depleted area, a process called functional hyperemia, which displaces cerebrospinal fluid
601 (CSF) and drives flow in the space around cerebral arteries [65]. These flows are thought
602 to couple to the glymphatic system, which facilitates waste clearance. Gan et al. used a
603 whisker stimulation model in mice to investigate its effects on glymphatic fluid transport,
604 finding the first direct evidence that functional hyperemia accelerates CSF influx to the
605 brain, as well as waste clearance from the brain space. Further, they demonstrated that
606 changes in arterial diameter, rather than neuronal activation, may be responsible for
607 increased CSF influx. Through this study, Gan et al. quantitively demonstrated key
608 interactions between the glymphatic system and the vasculature during functional
609 hyperemia that drive transport of nutrients and metabolic wastes [65].

610 Data-driven approaches were also reported at SB3C 2022. For example, Messou
611 et al. employed a deep learning approach to systematically identify the differences in the
612 cardiovascular systems of cannabis smokers with respect to those of non-smokers using
613 both cannabis use survey data and cardiovascular magnetic resonance (CMR) images in
614 the large-scale biomedical database UK Biobank [66]. In this study, the authors applied a
615 machine learning algorithm to establish that mapping images of the left ventricle
616 myocardium from regular (self-reported) cannabis users and images from non-users could
617 be distinguished with their deep learning approach. This tool has potential future use in
618 non-invasive image-based approaches for identifying links between the use of cannabis
619 and cardiovascular health.

620 *Multi-Cellular and Multi-Tissue Interactions*

621 Systems approaches at tissue and cellular level interactions were employed in two
622 studies to develop microfluidic tumor models. Chi et al. presented a three-layer

623 microfluidic cell array co-culturing breast cancer cells with immune cells to reconstruct a
624 tumor-stroma microenvironment. The array is integrated with a recirculation circuit
625 designed to study T cell infiltration and cytotoxicity. [67]. The design allows for study of
626 tumor-endothelium and tumor-stroma interactions in cancer drug responses, in a scenario
627 that closely mimics real physiological conditions [67]. The model successfully provides a
628 testbed for screening cancer immunotherapeutic agents paired with a high throughput
629 adaptation.

630 The role of cancer-associated fibroblasts (CAFs) in pancreatic cancer has been
631 one of the most actively studied interactions between tumor and stromal systems.
632 Interestingly, the stromal system has been considered pro-tumorigenic due to its
633 promoting tumor cell growth and invasion, augmenting chemoresistance, and posing a
634 drug delivery barrier. Several recent studies reported that the stromal system may also
635 play an anti-tumor role by suppressing growth and invasion of tumor cells [68, 69]. Thus,
636 systems approaches to delineate these two competing roles can be of potential
637 significance. Moon et al. used a 3D microfluidic tumor-stroma model of pancreatic
638 cancer to systematically screen analogs of inhibitors of Ref-1, a signaling protein known
639 to regulate redox metabolic activity of cancer in hypoxia and induce drug resistance and
640 metastasis. This investigation of the efficacy of Ref-1 inhibition on pancreatic cancer
641 cells while sparing the anti-tumor role of the fibroblasts helps demonstrate the tumor
642 microenvironment on-chip model's capability to provide a test bed for studying potential
643 cancer-selective drugs in the complex and heterogenous tumor microenvironment [70].

Design, Dynamics, and Rehabilitation

When understanding and investigating human dynamics, a system-based approach is critical to understanding the underlying behavior of human performance. 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 that are responsible for the intended human performance (Fig. 4). In rehabilitation, a system-based approach helps us understand how variables such as initial conditions and perturbations 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 design holds the most promise for not just addressing health delivery challenges but also yielding significant improvements in both patient and service outcomes. In these sections, the work presented at SB3C 2022 that employed a system-based approach in the study of design, dynamics, and rehabilitation is highlighted.

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 [71]. Studies have observed a reduction in coordination variability and flexibility in diseased and injury-related subjects as compared to healthy controls [72-76]. Gomez, et al. studied

667 the effects of regular and irregular surfaces on whole-body angular momentum in people
668 with Parkinson's disease (PD) by investigating changes in whole-body angular
669 momentum when walking from a regular to an irregular surface, comparing outcomes in
670 persons with PD with healthy, age-matched controls [77]. By analyzing adjustments in
671 angular momentum about the three principal body axes (sagittal, frontal, and transverse)
672 during terrain transitions, the team observed that persons with PD adopt a distinct and
673 specific strategy for crossing the surface transition, with alterations to their sagittal and
674 transverse angular momentum that differ from what can be expected in a healthy
675 individual. These findings are critical to developing safe movement strategies for
676 individuals with PD when moving through irregular walking surfaces [77].

677 A systems-based approach while accounting for the environment in which the
678 task is performed is critical to the outcomes of rehabilitation intervention. Schwartz et al.
679 investigated the correlations between wheelchair fit (environmental variable) and hand
680 rim biomechanics metrics (biomechanics) during propulsion (human performance) in
681 pediatrics [78]. Currently, adult wheelchair user recommendations, which include
682 minimizing force and rate of force application to the wheel hand rim, maximizing contact
683 phase time, and reducing push frequency, are applied to pediatric users. Furthermore,
684 adult wheelchair fit setting recommendations, such as wheel axle position with respect to
685 the shoulder and elbow, are extrapolated for pediatric populations. The study found only
686 a moderate correlation between elbow angle and time spent in the contact phase, and no
687 correlation between frequency and propulsion technique, axle position and force, and
688 wheelchair fit measures and hand rim metrics, indicating that these adult
689 recommendations may not translate to children [78]. These findings highlight how using

690 a dynamical systems approach that accounts for environmental variables can correlate
691 upper extremity biomechanics metrics with propulsion to create recommendations for
692 pediatric wheelchair users.

693 *Physiological Systems Approach to Predicting Human Movement*

694 Because biological tissues, including nerves, tendons, and ligaments, exhibit non-
695 linear behavior, the dynamic behavior of human movements that involve spatio-temporal
696 evolution can be studied using a non-linear systems approach when accounting for time
697 and frequency responses/behavior [79]. Furthermore, the spatio-temporal evolution of
698 the system dynamics can be accounted for by incorporating related physiological
699 responses and controls. Coker et al. confirmed that a non-linear input-output time series
700 neural network could predict joint angles during gait at 50-200 milliseconds into the
701 future by observing current joint angles, torques, and surface electromyography (sEMG)
702 data of pertinent muscle groups [80]. Boyea and Canino evaluated the capability of this
703 method in predicting knee joint angles and torques at varying walking speeds [81].
704 Utilizing the dataset of Moriera et al. [82], they demonstrated a system that could
705 accurately estimate knee joint torque and angle 150 milliseconds into the future, with
706 accurate predictions of knee joint kinematics over various walking speeds. This robust
707 system may have applications in assessing post-acute injury rehabilitation therapy and
708 creating assist-as-needed exoskeleton devices.

709 In another study, Trapp et al. used experimental data obtained from neonatal
710 piglet brachial plexus to develop a computational neonatal human model. Simulations
711 using such computational models can address neonatal brachial plexus palsy birthing
712 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 were 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 [83-86]. These and other presented studies at the conference support the application of the physiological system approach to better understand and predict human movements and aim to contribute to our understanding of injury prevention, prognosis and treatment.

Systems Approach to Device Design

A systems approach for healthcare improvement is essential to integrated, patient-centered, effective, and efficient care [87]. Inacio et al. used patient-specific finite element modeling to virtually measure the interfragmentary strain environment in clinically realistic fractures [88]. 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 [85, 89, 90]. 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 [91]. 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.

Education

SB3C 2022 included multiple studies that moved bioengineering 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 multi-level dynamics of the entire system are critical to advancing knowledge. In educating the next generation of biomechanical engineers, we should integrate the multi-level 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 [92]. This modeling approach is a basis for systems approach papers presented

759 at the conference [88, 93]. Singh and Balasubramanian presented in-person and virtual
760 classroom laboratory approaches to give students hands-on experience in mechanical
761 tissue property testing, which also formed the basis for several system-based research
762 studies [20, 23, 94]. Singh examined the pandemic adaptations necessary to teach this
763 material during remote learning.

764 Across the breadth of multi-scale papers at the conference, many experimental
765 techniques were presented that could be incorporated into coursework. In addition to
766 being able to prepare cells and tissues and perform common biology and chemistry
767 techniques such as ELISA, western blot, and immunostaining, skills that might prepare
768 future multiscale researchers include imaging (from atomic force microscopy to magnetic
769 resonance imaging), microfluidics, micropattern fabrication, cellular and tissue level
770 mechanical testing, motion capture, electromyography, electrocardiography, and
771 statistics. Statistical methods are noted across a wide breadth of systems approach-based
772 papers.

773 Modeling is another essential tool in the systems approach. Modeling methods
774 used across the breadth of the multi-scale papers include image segmentation, 3D
775 computer aided modeling, finite element modeling, computational fluid dynamics,
776 lumped parameter modeling, and deep learning methods. The challenge to presenting
777 such modeling in the classroom is to address the complexity of multi-scale systems while
778 keeping it simple enough to be achievable by students in a limited time. One such
779 approach might be to follow a characteristic such as stiffness from the ECM and
780 intracellular fibers in cellular models to whole tissue or movement characteristics [22, 60,
781 88, 95]. For example, stiffness in the intracellular mechanics of muscle contraction in

turn plays a role in the dynamic stability of whole-body motion. A number of papers presented at SB3C 2022 investigated intracellular contractile mechanisms and stiffness on a molecular/cellular level, [96-101] which also play a role in whole body dynamics [99]. Textbooks that support this systems approach to muscle mechanics include *Animal Locomotion* by Andrew Biewener and *Muscles, Reflexes, and Locomotion* by Thomas McMahon [102, 103].

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. Multi-scale and multi-system 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 at conferences like SB3C 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 permutated in a

804 controlled and reproducible manner. Yet it remains challenging to vary one experimental
805 parameter (e.g., substrate stiffness, shear stress) without changing other parameters (e.g.,
806 crosslinking density, biotransport). These models must also enable the dynamic
807 characterization of temporal and spatial intra- and extra-cellular interactions at the
808 molecular, cellular, and tissue levels in 3D, multicellular *in vitro* systems. Advances in
809 experimental measurements (e.g., microscopy and other non-destructive *in situ*
810 techniques) are needed to enable investigators to gain as much information as possible
811 from *in vitro* models.

812 Enhanced experimental models require advanced computational techniques to
813 analyze large datasets and increase throughput. Rich high-dimensional data sets (e.g.,
814 temporal and spatial transcriptomics, proteomics, and metabolomics) require *in silico*
815 models to probe biomechanics hypotheses. Machine learning and artificial intelligence
816 methods may provide solutions; however, collaborations with computational biologists
817 and computer scientists are needed to fully integrate these techniques into the
818 biomechanics community. These data sets will also need to be shared widely to enable
819 those without access to experimental models to participate in higher order inquiry.
820 Computational models, for example of cardiovascular fluid mechanics, require validation
821 of parameters imposed on the vascular network (e.g., growth and remodeling), validation
822 of outcomes from a single step in the subject-specific modeling process (e.g. vascular
823 reconstruction), and validation of overall outcomes. These validations become even more
824 challenging when working across size scales. Finally, the frequently reported indices
825 from these models, such as time-averaged wall shear stress are undoubtedly important;

826 however, additional indices are needed to account for spatial and temporal variability as
827 well as cellular and molecular impacts.

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

839 At SB3C 2022, investigators presented the many ways in which they were already
840 exploring how solid, fluid, and cellular biomechanics, dynamic systems, and biotransport
841 impact sub-cellular and cellular signaling, cell-microenvironment, and inter-organ
842 interactions to understand tissue development and disease progression and eventually
843 control tissue repair. The SB3C 2022 presentations show the importance of considering
844 multi-scale biological complexity when attempting to understand and predict the
845 mechanical response of cells, tissues, organs, and humans. This holistic approach creates
846 incredible opportunities to transform our understanding of biomechanics if we can create
847 effective and accessible *in vitro* and *in silico* tools. Additional conferences or research
848 symposia that bring investigators from biomechanics together with computational

849 biologists and computer scientists are needed to accelerate the application of systems-
850 based approaches in biomechanics.

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-kB	Nuclear factor-κB
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
$sPLA_2$	Secretory phospholipase A_2
TCA	Tricarboxylic acid
TGFβ2	Transforming growth factor beta2
VOC	Ventilator-on-a-chip
VSMC	Vascular smooth muscle cell
WSS	Wall shear stress
YAP	Yes-associated protein

CONFLICT OF INTEREST DECLARATION

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FIGURES

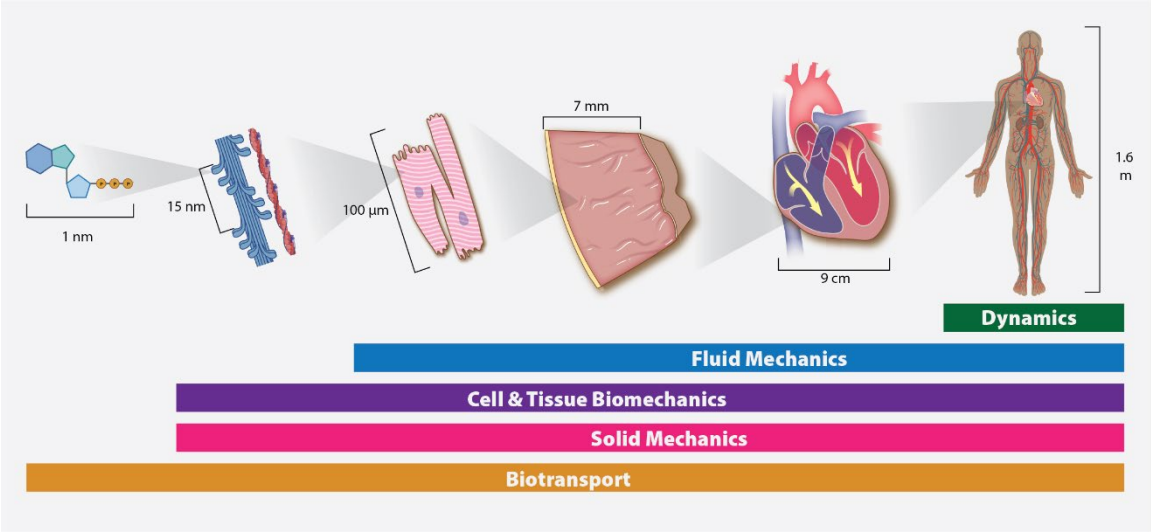
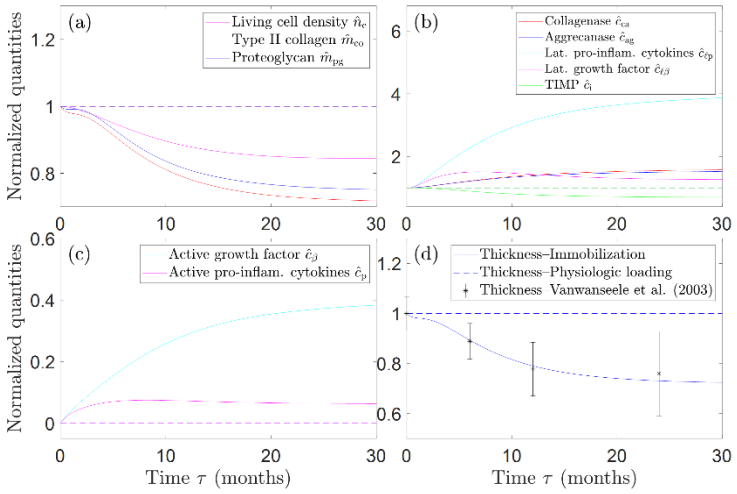


Fig. 1 A systems-level look at the components required to produce a heartbeat and pump blood around the body.



$$\Psi^{S} = (1-\nu)\Psi^{S}_{IM}(J_S, I_1) + \nu\Psi^{S}_{FN}(I_4, \mathbf{M}), \quad (1)$$

$$\Psi^{S}_{FN} = \frac{1}{\rho^{S}_{0S}}\int_{\tilde{\Omega}} \rho(\mathbf{M})\frac{k_1}{2k_2}\{e^{[k_2\langle I_4-1\rangle]^2-1}\}\mathcal{H}(I_4-1)\,d\Omega \quad (2)$$

$$\frac{d\hat{n}_c}{d\tau} = \left(r_1^c + r_2^c\hat{c}_\beta\right)\hat{n}_c - \left(r_3^c + r_4^c f(\sigma_{sh})\hat{c}_\beta + r_5^c\hat{c}_p\right)\hat{n}_c \quad (3)$$

$$\frac{d\hat{n}_{hc}}{d\tau} = r_1^{hc} f(\sigma_{sh})\hat{c}_\beta\hat{n}_c - r_2^{hc}\hat{n}_{hc} \quad (4)$$

$$\frac{d\hat{m}_{co}}{d\tau} = \left(r_1^{co} + r_2^{co}\hat{c}_\beta\right)\hat{n}_c - \left(r_3^{co} f(\sigma_1) + r_4^{co}\hat{c}_{ca}\right)\hat{m}_{co} \quad (5)$$

$$\frac{d\hat{m}_{pg}}{d\tau} = \left(r_1^{pg} + r_2^{pg}\hat{c}_\beta\right)\hat{n}_c - \left(r_3^{pg} f(E_3) + r_4^{pg}\hat{c}_{ag}\right)\hat{m}_{pg} \quad (6)$$

$$\frac{d\hat{c}_{ca}}{d\tau} = \left[\frac{r_1^{ca} + r_6^{ca}\hat{c}_p}{1 + r_2^{ca}\hat{c}_\beta}\right]\hat{n}_c + r_3^{ca}\hat{n}_{hc} - \left(r_4^{ca} + r_5^{ca}\hat{c}_i\right)\hat{c}_{ca} \quad (7)$$

$$\frac{d\hat{c}_{ag}}{d\tau} = \left[\frac{r_1^{ag} + r_6^{ag}\hat{c}_p}{1 + r_2^{ag}\hat{c}_\beta}\right]\hat{n}_c + r_3^{ag}\hat{n}_{hc} - \left(r_4^{ag} + r_5^{ca}\hat{c}_i\right)\hat{c}_{ag} \quad (8)$$

$$\frac{d\hat{c}_i}{d\tau} = \left(r_1^i + r_2^i\hat{c}_\beta\right)\hat{n}_c - \left(r_3^i + r_4^i\hat{c}_{ca} + r_5^i\hat{c}_{ag}\right)\hat{c}_i \quad (9)$$

$$\frac{d\hat{c}_{\ell\beta}}{d\tau} = \left(r_1^{\ell\beta} + r_2^{\ell\beta}\hat{c}_\beta\right)\hat{n}_c - \left[r_4^{\ell\beta} + r_5^{\ell\beta} f(\sigma_{sh})\right]\hat{c}_{\ell\beta} \quad (10)$$

$$\frac{d\hat{c}_\beta}{d\tau} = r_1^\beta f(\sigma_{sh})\hat{c}_{\ell\beta} - r_2^\beta\hat{c}_\beta \quad (11)$$

$$\frac{d\hat{c}_{\ell p}}{d\tau} = \left[\frac{r_1^{\ell p} + r_2^{\ell p}\hat{c}_p}{1 + r_3^{\ell p}\hat{c}_\beta}\right]\hat{n}_c - \left[r_4^{\ell p} + r_5^{\ell p} f(\sigma_1)\right]\hat{c}_{\ell p} \quad (12)$$

$$\frac{d\hat{c}_p}{d\tau} = r_1^p f(\sigma_1)\hat{c}_{\ell_p} - r_2^p\hat{c}_p \quad (13)$$

Fig. 2 A chemo-mechanical-biological model of evolving osteoarthritis showing cartilage evolving in homeostasis and immobilization. Left panel: 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 cytokines and growth factors, and (d) cartilage thickness. Dashed and solid lines represent moderate and reduced loading, respectively. Right panel: Model capturing the nonlinear mechanics of cartilage.

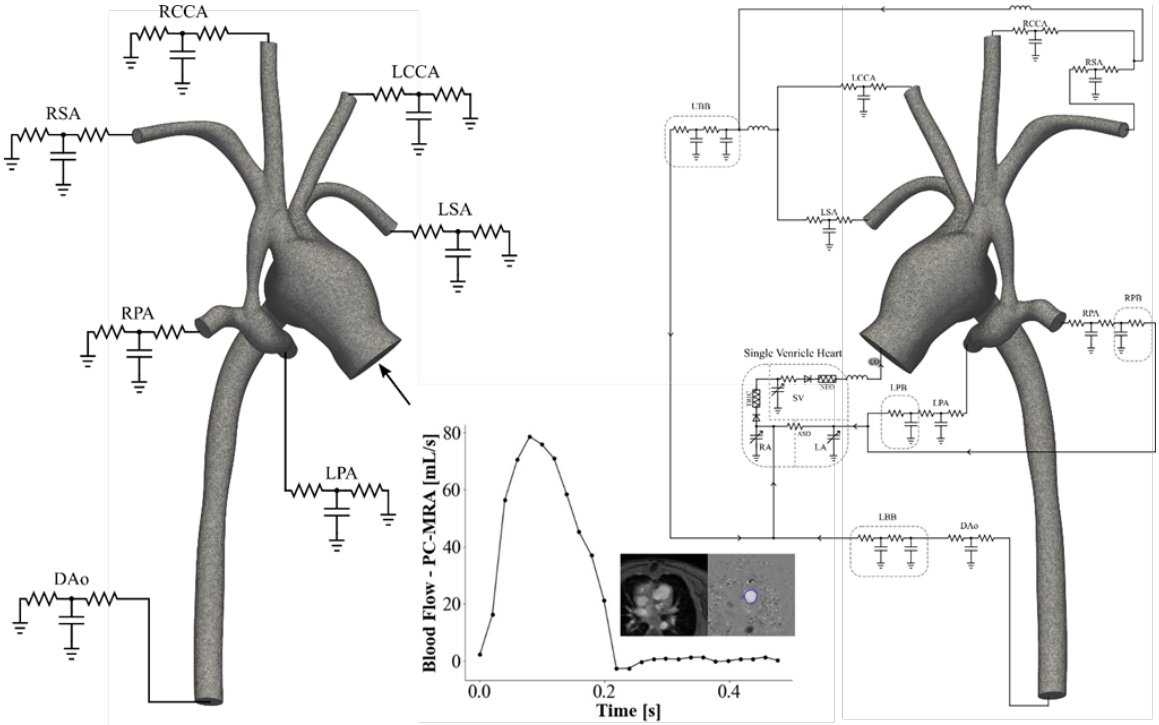


Fig. 3 An example of a systems approach to cardiovascular fluid mechanics through boundary conditions, growth, and remodeling.

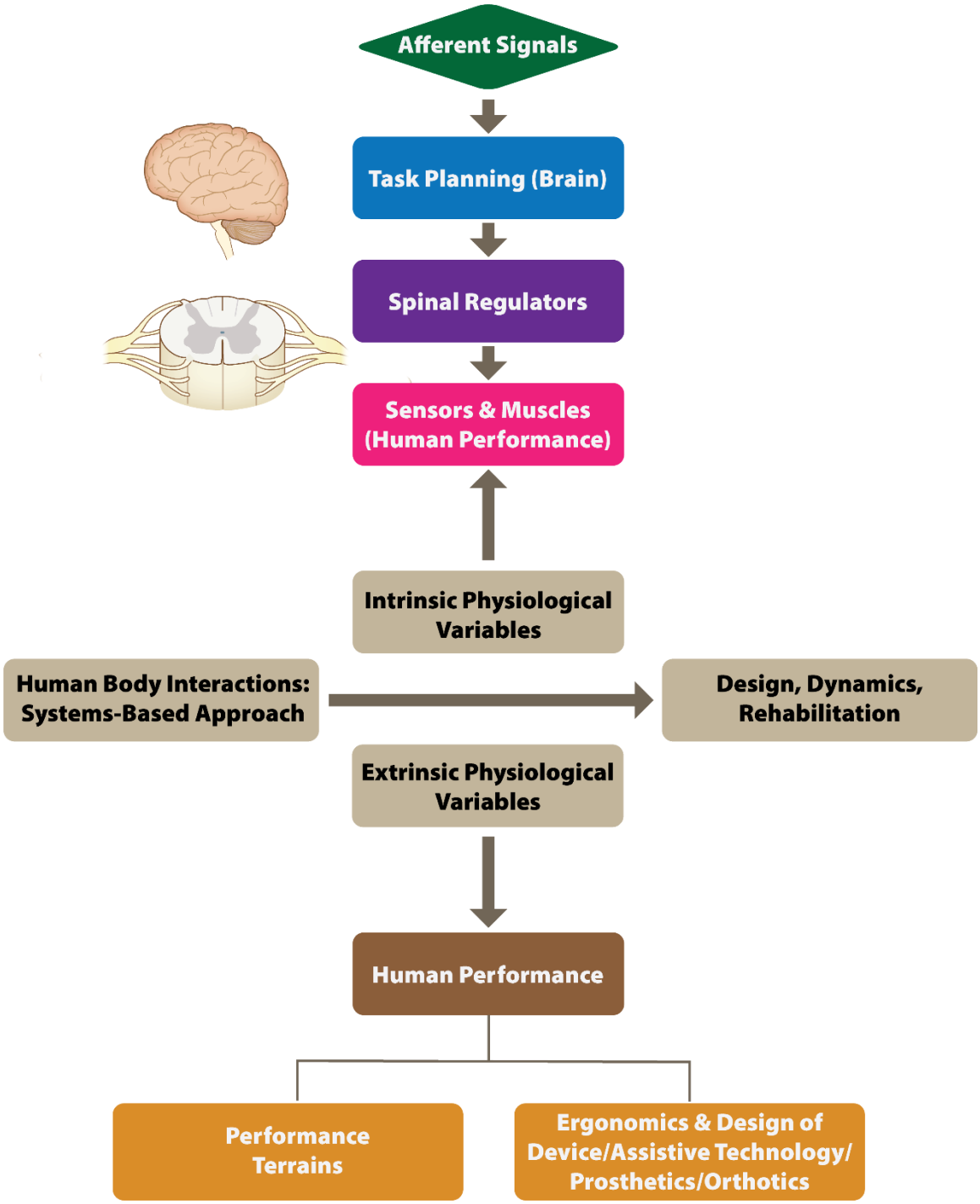


Fig. 4 Interactions to consider in a systems approach to design, dynamics, and rehabilitation.

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1246 **Figure Captions List**

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

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