Key Developments That Impacted the Field of Mechanobiology and Mechanotransduction

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ABSTRACT: Advances in mechanobiology have evolved through insights from multiple disciplines including structural engineering, biomechanics, vascular biology, and orthopaedics. In this paper, we reviewed the impact of key reports related to the study of applied loads on tissues and cells and the resulting signal transduction pathways. We addressed how technology has helped advance the burgeoning field of mechanobiology (over 33,600 publications from 1970 to 2016). We analyzed the impact of critical ideas and then determined how these concepts influenced the mechanobiology field by looking at the citation frequency of these reports as well as tracking how the overall number of citations within the field changed over time. These data allowed us to understand how a key publication, idea, or technology guided or enabled the field. Initial observations of how forces acted on bone and soft tissues stimulated the development of computational solutions defining how forces affect tissue modeling and remodeling. Enabling technologies, such as cell and tissue stretching, compression, and shear stress devices, allowed more researchers to explore how deformation and fluid flow affect cells. Observation of the cell as a tensegrity structure and advanced methods to study genetic regulation in cells further advanced knowledge of specific mechanisms of mechanotransduction. The future of the field will involve developing gene and drug therapies to simulate or augment beneficial load regimens in patients and in mechanically conditioning organs for implantation. Here, we addressed a history of the field, but we limited our discussions to advances in musculoskeletal mechanobiology, primarily in bone, tendon, and ligament tissues. © 2017 Orthopaedic Research Society. Published by Wiley Periodicals, Inc. J Orthop Res 36:605–619, 2018.

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The German surgeon, Julius Wolff, M.D. (1836–1902), observed that trabecular bone forms load-bearing struts and models and remodels interstitially.[1–4] He was strongly influenced by the studies of the German civil engineer, Carl Culmann (1821–1881), who developed "graphical statistics" methods for depicting bridge trusses. The power and impact of Wolff's observations on bone remodeling are well known: Trabecular bone can remodel its geometry, post-fracture, along new lines of force in regions of both tension and compression. Interestingly, cellular activity with regard to bone remodeling was not mentioned in any of Wolff's reports. Ossification of bone in response to load bearing or unloading, as in rickets or resection, was addressed as a principle of geometric changes induced by mechanics.[1–4]

Wilhelm Roux M.D., a clinician, experimental embryologist, and developer of cell culture methods (1850–1924), advanced the idea of applying mechanics to biology by postulating that directed physical forces induced biological processes: Compression induced bone, tension induced dense and aligned connective tissues, and shear in conjunction with tension or compression induced cartilage.[5] Roux's major contribution to the mechanobiology field was the concept that "form follows function." However, these seminal thoughts laid dormant for almost 75 years. At the same time, the discovery of the primary cilium as an effector organelle in mammalian cells[6] (but really a mechanosensor) went unnoticed, most likely due to a consensus in the field by microscopists that bone cells, particularly osteocytes, were end-stage cells and that the primary cilium was an effete organelle.

A seminal treatise was published in 1941 by Friedrich Pauwels (1885–1980) in Germany.[7] In the chapter entitled "A New Theory Concerning the Influence of Mechanical Stimuli on the Differentiation of the Supporting Tissues," Pauwels emphasized that mechanical stimuli (i.e., hydrostatic pressure and tensile and compressive strains) were responsible for remodeling of bone and cartilage. In orthopaedic surgery, Pauwels introduced tensioned wires to place compressive loads on bone to assist in fracture healing.[7] Pauwels used extensive X-ray pictures of clinical case results and experimentation in animals to test his hypotheses about strain and tissue development. He took issue with Roux's observations on the definition of the forces that affected developing and remodeling tissues, but largely followed Roux's thesis concerning mechanics and tissue response overall.

Knowledge of strain measurements on the surface of load-bearing bone, of tooth movement with orthodontic jacks, and of computational modeling further advanced the field of orthopaedic sciences.[8–18] These advances, which began in the 1970s, along with the publications of the Mechanostat Theory by Harold Frost[19] and of theories on cell physical structure (tensegrity), cellular detection and response to mechanical stimulation,[20–22] and the availability of equipment to apply mechanical load to cells in vitro[23,24]

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reinvigorated the field to think about the effects of strain on musculoskeletal tissues and the subsequent cellular responses (Tables 1–2). Over 33,600 studies in the field of mechanobiology were published between 1970 and 2016 (Fig. 1A). Specific interest areas by field, including orthopaedics (8,231 reports), cardiovascular (6,144 reports), and pulmonary (2,031 reports) indicated that the orthopaedics field had over 50% of the publications of these three major fields represented and about 25% of the overall total publications (33,626; Fig. 1). This paper will review how some of these publications helped advance the field of orthopaedic mechanobiology.

BIOMECHANICS AND COMPUTATIONAL ANALYSIS

Biomechanicians have used computational analyses to test Wolff's law to produce constitutive equations that might predict how bone responds to applied loads.[12–16] Modern computational analyses of bone adaptation to load began with the work of Cowin and Hegedus' theory of adaptive elasticity for cortical bone.[12] Over a decade later, Carter and coworkers proposed that "the sequence of cartilage proliferation, maturation, degeneration, and ossification" could be "accelerated by intermittently applied deviatoric (shear) stresses (or strain energy) and inhibited or prevented by intermittently applied compressive dilatational stresses (hydrostatic pressure)."[13] They developed an osteogenic index based on computational analysis and the following equation:[13]

$$I = S + kD$$

where I is the osteogenic index, S is the octahedral stress, k is a constant, and D is the peak cyclic stress. This equation combined with the power of finite element analysis substantiated the earlier theories of

Table 1. Key Advances and Reports in Mechanobiology (1870–1985): Frequency of Citation

		Citation Frequency		
Year(s)	Advance/Publication	Google Scholar	PubMed	Reference
1870–1890	**Bone remodeled with applied force**			
1892	The law of bone transformation (Das gesetz der transformation der knochen)	2396		4
1890–1930	**In vivo studies and experimental embryology**			
1898	Beitrage zur kenntnis einiger Drusen und Epithelien	297		6
1940–1960	**Studies on muscle physiology/contraction**			
1954	Changes in the cross-striations of muscle during contraction and stretch and their structural interpretation	1619	228	124
1960–1970	**Studies on scar formation**			
1971	Contraction of granulation tissue in vitro: Similarity to smooth muscle	575	71	127
1975	Cyclic AMP and cyclic GMP: Mediators of the mechanical effects on bone remodeling	216	10	80
1975	Cyclic AMP levels in alveolar bone of orthodontically-treated cats.	133	3	28
1977	Collagen cross-linking alterations in joint contractures: Changes in the reducible cross-links in periarticular connective tissue collagen after nine weeks of immobilization	208	7	125
1975–1980	**Early computational models and studies on responses of cells to applied strain**			
1976	Bone remodeling I: Theory of adaptive elasticity	721		12
1977	Biochemical effects of stress on cultured bone cells	169	5	77
1980	Silicone rubber substrata: A new wrinkle in the study of cell locomotion	1239	231	79
1980–1985	**In vivo studies and further experiments applying strain to cells in vitro**			
1980	Ligamentous restraints to anterior-posterior drawer in the human knee. A biomechanical study	1092	83	70
1981	Stretch-induced growth of skeletal myotubes correlates with activation of the sodium pump	71	5	74
1982	Mechanically adaptive bone remodelling	446	27	8
1984	In vitro response of chondrocytes to mechanical loading. The effect of short term mechanical tension	158	9	73
1984	Regulation of bone formation by applied dynamic loads	1352	141	10
1984	Static vs dynamic loads as an influence on bone remodelling	819	67	9
1985	Regulation of bone mass by mechanical strain magnitude	1212	114	11
1985	An in vivo strain gauge study of elongation of the anterior cruciate ligament	287	17	38

Table 2. Key Advances and Reports in Mechanobiology (1985—Present): Frequency of Citation

Year(s)	Advance/Publication	Citation Frequency: Google Scholar	Citation Frequency: PubMed	Reference
1985–1995	**Commercialization of automated devices for stretching cells, development of theories on how cells sense load, and further computational studies**			
1985	Cells as tensegrity structures: Architectural regulation of histodifferentiation by physical forces transduced over basement membranes	245		20
1985	A new vacuum-operated stress-providing instrument that applies static or variable duration cyclic tension or compression to cells in vitro	433	46	23
1986	#Published studies in which a Flexcell® Tension or Compression System (Flexcell® International Corp., Burlington, NC) was used	2670		84
1986	Comparison of material properties in fascicle bone units from human patellar tendon and knee ligaments	426	31	68
1987	The mechanostat: A proposed pathogenic mechanism of osteoporoses and the bone mass effects of mechanical and nonmechanical agents	632	46	19
1988	#Published studies in which a Vitrodyne system (Liveco Inc., Burlington, VT) was used	175		180,181
1988	A computerized mechanical cell stimulator for tissue culture: Effects on skeletal muscle organogenesis	114	7	76
1988	The role of mechanical loading histories in the development of diarthrodial joints	209	20	13
1990	Cellular responses to mechanical loading in vitro	199	11	24
1991	Loading-related increases in prostaglandin production in cores of adult canine cancellous bone in vitro: A role for prostacyclin in adaptive bone remodeling?	160	14	81
1991	A noninvasive, in vivo model for studying strain adaptive bone modeling	224	29	31
1993	Cellular tensegrity: Defining new rules of biological design that govern the cytoskeleton	1071	126	21
1995	Proposal for the regulatory mechanism of Wolff's law	332	19	15
1995	Mechanoreception at the cellular level: The detection, interpretation, and diversity of responses to mechanical signals	399	37	22
1995	Integrins and signal transduction pathways: The road taken	3349	444	132
1990–2016	**Development of bioreactors for tissue engineering & further mechanotransduction studies**			
1997	#Published studies in which a STREX Cell Stretching System (STREX Inc., Osaka, Japan) was used	279		182,183
2000	Functional tissue engineering: The role of biomechanics	611	90	107
2003	Novel system for engineering bioartificial tendons and application of mechanical load	279	46	105
2007	Primary cilia mediate mechanosensing in bone cells by a calcium-independent mechanism	324	93	136
2008	Rapid signal transduction in living cells is a unique feature of mechanotransduction	286	128	131
2008	Functional tissue engineering for tendon repair: A multidisciplinary strategy using mesenchymal stem cells, bioscaffolds and mechanical stimulation	281	86	50
2014	Biomechanics and mechanobiology in functional tissue engineering	74	16	119

#Google Scholar search term (not including citations or patents)—Flexcell and one of the following: BioFlex, BioPress, Flexercell, StageFlexer, StagePresser, Tissue Train, UniFlex, Flex I, FX-5000, FX-4000, FX-3000, FX-2000, Strain Unit, tension system, FX5K, or compression system. Vitrodyne and one of the following: Liveco, V100, 1000 Universal Materials Tester, or V1000 Universal Tester. Strex and one of the following: Cell stretch, cell stretching, tension system, ST-140, STB-CH-10, Osaka, or STB-140.

Wolff that bone responds to forces and remodels. This computational analysis established a precedent in biomechanics for trabecular bone surfaces and confirmed that the action of physical forces could be calculated and used predictively to model bone accretion or removal.

The availability of increased computational power with computers having increased memory capacity made possible the ability to computationally model as well as simulate the response(s) of bone to applied load. Huiskes and colleagues used computational modeling to predict specific sites of bone remodeling

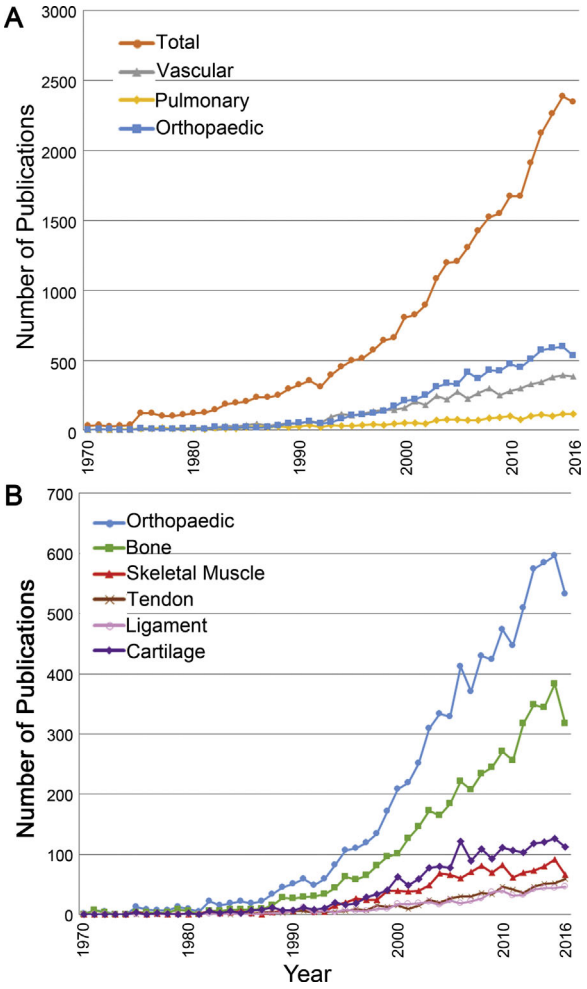

Figure 1. (A) The number of mechanobiology-related publications per year in general (total) as well as in the vascular, pulmonary, and orthopaedic fields (1970–2016). (B) The number of mechanobiology-related publications in orthopaedics per year according to the tissue or cell studied. Number of publications determined by a PubMed search, limited within the "text word" search field, in which one of the following terms appeared for the year of interest: Mechanotransduction, mechanotransducer, mechanobiology, mechanical biology, mechanobiological, mechanical load, mechanoresponse, mechanoresponsive, mechanosensation, cytomechanics, cell mechanics, cellular mechanics, cell mechanical, cell biomechanics, mechanical stimulation, mechanical stimuli, mechanoreception, mechanosensitivity, mechanosensitive, mechanosensory, mechanosensing, mechanical deformation, mechanoregulation, mechanical regulation, mechanotransmission, mechanical loading, mechanical signaling, biomechanical signaling, mechanical signal, mechanical detection, mechanoactive, mechanically active, mechanical compression, mechanical strain, stretch induced, mechanical tension, cellular strain, cyclic strain, mechanically induced, cell stretching, cell stretch, cell strain, cell compression, fluid shear, fluid shear stress, or flow-induced shear. Fields of study were determined by a similar method in which the articles found with the above method were further searched for using keywords related to the field or tissue of study.[179]

with respect to applied load and included not only the response of trabecular bone surfaces, but also the contribution of osteoblastic bone formation and osteoclastic resorption activities.[14–16] Loboa and colleagues extended such approaches to address new bone and other skeletal tissue formation in distraction osteogenesis and fracture healing.[17,18,25] The use of high-resolution images, such as magnetic resonance imaging (MRI) and computed tomography (CT) scans, has allowed for models to more accurately depict the natural anatomical geometries of tissues.[26,27] Taken together, biomechanicians showed that the kinetics of bone remodeling in response to applied forces could be predicted by computational modeling.

IN VIVO MODELS OF STRAIN ON BONE

In concert with the ability to computationally model how bone responds to loading, new animal models were developed in which the strain environment surrounding the bone tissue could be experimentally manipulated, and thus changes in bone signaling, form, and function could be observed. For example, Davidovitch[28] measured cAMP (cyclic adenosine monophosphate) amounts immunochemically in maxillary bone after orthodontic tooth movement in response to strain. This work showed that bone cells responded to applied strain by releasing a signaling mediator. Pioneering work by Lanyon et al.[8] used strain gauges bonded onto volunteer, human, patient tibias to assess strain magnitudes during walking or climbing with and without loads. His group showed that a minimum effective strain to induce bone remodeling was about 500 microstrain and that 3,000 microstrain could cause bone fracture. This work was followed by in vivo avian studies with load applied to the turkey ulna in a three-point bending paradigm, resulting in the finding that only short periods of cyclical loading of approximately 15 N peak load were required to maintain bone mineral density.[10,11] Although invasive, these tests established that living bone had measurable strains on the bone surface and that applied load of a limited magnitude and duration could stimulate or maintain bone mineral density. Lanyon further identified that short periods of loading on a quiescent bone surface were sufficient to promote bone remodeling within 7 days. In addition, his group demonstrated that indomethacin administered in vivo would abolish this response in the avian ulna.[29,30] The ablative effect of indomethacin on mineralization in bone was the earliest report of a drug inhibiting a load response, a precedent finding. Turner et al.[31] addressed new methods of applying loads to mouse long bone in vivo, using a four point bending device. Key findings from these studies was the accretion of bone on one side and resorption on the other with direct evidence of bone remodeling to the imposed loads.

IN VIVO MODELS OF STRAIN ON SOFT TISSUES

Numerous investigators have sought to determine in vivo load and deformation signals (i.e., magnitude, frequency, duty cycle, etc.) acting on musculoskeletal soft tissues, like tendon and ligament, for various activities of daily living (ADLs). Over the past three to four decades, kinesiologists, physiologists, bioengineers, biologists, and surgeons have used multiple device designs to measure, directly or

indirectly, in vivo ligament and tendon forces and deformations. The merits and challenges in using some of these devices have been well described in earlier reviews.[32,33]

While it would be ideal to directly measure axial ligament and tendon forces in living humans and animals using an in-line force transducer, such measurements would require disruption of the bone insertions (ligament and tendon) or actual detachment of the tissue to interpose a device along its axis.[34–37] Henning et al.[38] chose the first approach by inserting a "bone load gage" along the axis of a patient's anterior cruciate ligament beneath its distal insertion (while simultaneously measuring ACL elongation). However, calibrating the device to determine actual tissue forces proved problematic. To avoid the large surgical disruption, other investigators designed buckle gages through which the entire tendon or ligament could be woven.[39–46] These devices relied on strain gages to monitor the slight bending of the buckle as force developed in the tissue. The presence of the buckle could shorten the tissue and change its function, which proved a bigger problem in shorter ligaments than in longer tendons. Moreover, the tissue-device complex still had to be calibrated for the actual activities of daily living (ADLs) to relate device voltage to force in only that tissue. Other researchers chose to reduce complete tissue disruption by inserting modified pressure transducers (MPTs),[47–49] implantable force transducers (IFTs),[50–58] and arthroscopic implantable force probes (AIFPs)[59–61] between the collagen fiber bundles.

As an alternative to direct force measurement, investigators have also attached very compliant devices directly to and in parallel with the tissue fibers to record deformations and "relative" strains.[62,63] DVRTs (differential variable reluctance transducers) have offered the advantage of minimal stiffness and the opportunity to measure deformations following arthroscopic insertion in patients undergoing unrelated knee surgery.[32,64–67] Computing actual tissue strain (i.e., the ratio of deformation to initial tissue length) was more challenging, however, because of the difficulty in determining when the adjacent tissue first developed force. Consequently, the investigators chose to report a "relative" rather than actual tissue strain.

Taken together, research to date on ligaments and tendons has provided the field with valuable estimates of forces and strains for selected ADLs. (i) In animal models, studies have revealed that tendons are generally exposed to higher forces in vivo than ligaments. Research in goats has shown, for example, that the patellar tendon can sustain up to 40% of its failure force for selected ADLs while the anterior cruciate ligament rarely exceeds 7–10% of failure force.[50,55] (ii) Strains in these tissues, when referenced to their failure properties from cadaveric studies,[68,69] are typically in the "toe" or early linear regions for normal ADLs but under more vigorous activities, may result in serial failure of collagen fibers that can accumulate over time. (iii) Clinical tests can often produce forces and deformations that are too small to properly detect soft tissue injuries but are then revealed during more vigorous ADLs.[70]

Understanding what aspects of these force/deformation stimuli avert ligament and tendon injury[71,72] while still stimulating new tissue formation is topical. Recognizing those signals that induce tears and ruptures may be even more critical to crafting more effective strategies to diagnose, treat, and rehabilitate post-injury.

IN VITRO AND EX VIVO METHODS FOR STRAIN APPLICATION TO TISSUE AND CELLS

Beginning in the 1970s, researchers used orthodontic jacks on culture plates, elastic polymeric membranes, collagen, elastin, and even whole cell sheets as materials on which cells were grown and deformed by applied strain with weights, motors, and pneumatics.[23,73,74] Once the digital age began, improved and miniaturized instrumentation was developed. Digital read-out strain gauges, pressure transducers, and microprocessor-controlled cell stretching, compression, and shear stress devices that applied regulated strain or shear stress to substrata, and thus to the attached cells, could be used to more closely study the effects of applied loads on cells. These developments enabled research into how a given amplitude, frequency, and duration of applied strain could affect a given cell type. Early studies were more focused on if and how a cell responded to a physical force.[23,73,75–78] A report showing that cells could apply a traction force to their substratum and thus deform or "wrinkle" a "vulcanized" silicone oil surface confirmed the idea that cells had the capacity to apply a traction force to a substrate.[79]

Rodan and co-workers used a syringe with controlled pressure to the plunger to apply a hydrostatic pressure to a chick limb ex vivo.[78,80] This group reported that a pressure of 60 g/cm^2 on 16-day-old chick tibia reduced glucose consumption by 50% and reduced cAMP and cGMP secretion while increasing DNA synthesis.[80] Another report verified that strain applied to cultured mouse calvarial osteoblasts induced cAMP, calcium (Ca^{2+}), and prostaglandin E2 (PGE_2) secretion in vitro.[77] The experiment involved gluing an orthodontic jack to the reinforced bottom of a 100 mm diameter polystyrene culture plate, then turning the screw to drive the reinforcing blocks apart, thus applying a strain to the culture surface and hence to the attached cells. De Witt and co-workers also used a motor-driven cell sheet stretching device with chick chondrocytes to show that sulfate and glucosamine incorporation were increased, as well as DNA synthesis, in response to 5.5% strain at 0.2 Hz for 24 h.[73] In the late 1980s, a perfusion compression device was designed to study the mechanical responses of ex vivo canine trabecular cores. This device allowed the direct effects of varying load magnitudes on cells within their natural extracellular matrix and key mechanotransducer signals to be identified.[24,81] Further studies investigated these properties in fetal tissues, such as studies into the effects of load bearing on isolated ex vivo mouse bone applied in three and four point bending tests.[82] Vandenburgh developed a motor-driven push-up method for application of regulated strain to cultured striated skeletal muscle cells.[75,76] Other investigators have

used various techniques to apply strain to cells including the use of magnetic force.[83]

A pneumatic-driven, microprocessor-controlled, cell-stretching device was developed by Banes that controlled the magnitude, duration, and frequency of applied strain to rubber-bottomed culture plates.[23,84] The instrument was commercialized as a system in 1986 and included silicone elastomer-bottomed, 6-well culture plates covalently derivatized with a variety of matrix proteins to simulate substrates in a native environment.[84] The instrument allowed controlled, standard conditions for strain application to cultured cells so that repeatable dose response experiments could be conducted. Moreover, a mathematical expression was developed to investigate how a cell response (R) related as a nonlinear function of amplitude of the applied strain (A), the duration of A (t_1), the time between deformation events (t_2), the duration of a rest period after many deformation events (t_3), the number of cycles (C), the strain rate ascending to maximum A ($^*\varepsilon_1$), the strain rate descending from maximum A ($^*\varepsilon_2$), the shear stress if flow is present (τ), a substrate chemistry term (s), and a term indicating that the regimen can be repeated (n):[85,86]

$$R = \text{sum of}\left[A, (t_1, t_2, t_3), C, \left(^*\varepsilon_1, {}^*\varepsilon_2, \tau\right), s\right]_n$$

The terms in this expression, particularly frequency and amplitude of applied strain, gave early mechanobiologists the ability to use simple versus complex loading regimens to test how varying one parameter of a load regimen could alter a biological response (e.g., dose-response effect). The s (substrate term) gave users the opportunity to choose different substrate chemistries and stiffnesses to test for a biological result. At the time, it was recommended that users grow their cells on rubber-coated, matrix-protein derivatized plasticware (Surflex® culture plate with a soft rubber substrate over polystyrene) of the same chemistry and stiffness as the stretchable surface to reduce "substrate shock" when they shifted growth conditions from polystyrene to the softer, silicone elastomer bottom culture dishes.

Cell Responses to Strain and Pharmacologic Mediators

The first report of strain on tenocytes showed that changes in tenocyte behavior were time-dependent. Cell alignment and changes in expression of actin and tubulin were observed in tenocytes stretched at 0.25 Hz at 10% strain for 3 and 5 days.[23] Later, the first report of a growth factor synergy with applied load showed that tenocytes treated with pM amounts of platelet-derived growth factor-BB (PDGF-BB) and insulin-like growth factor-1 (IGF-1) increased DNA synthesis.[87] Almekinders et al.[88] later showed that indomethacin could block the secretion of PGE_2 by tenocytes in response to strain in vitro. Elfervig et al.[89] showed that tenocytes respond to strain and norepinephrine treatment synergistically with increased Ca^{2+} signaling. ATP is secreted in response to applied strain and may act as a strain effect modulator.[90,91] Changes in cell alignment in response to strain, collagen synthesis, and alkaline phosphatase expression (osteoblasts) were also noted in osteoblasts, smooth muscle cells, and endothelial cells.[92–96] Bone marrow stromal-derived progenitor cells (mesenchymal stem cells) responded to dynamic pulses of strain by expressing high levels of alkaline phosphatase and a bone cell phenotype one week later.[97] Osteoblast-like ROS 17/2.8 cells were used in an important drug-dose response experiment wherein mineralization, in vitro, was increased by mechanical load but decreased if cells were treated with 10 μM verapamil, a Ca^{2+} channel blocker, prior to day three of a stretch regimen.[98] These reports showed that both tenocytes and osteoblast-like cells could down-regulate their response to applied strain with pharmacologic mediators. The growth factor and norepinephrine publications were the first reports of anabolic effects of load on tenocytes with pharmacologic mediators.

Cell Responses to Fluid Shear Stress

In addition to strain (as tension or compression), an important contribution to the mechanobiology field was the concept that bone cells and other connective tissue cells were subjected to fluid shear stress.[99–102] Vascular researchers were ahead of those in the orthopaedics field in this area. But the observations that connective tissue cells, other than endothelial and smooth muscle cells, could respond to flow (laminar, pulsatile, and flow reversals), and the revelation that the primary cilium is a mechanosensor, again revolutionized the orthopaedics mechanobiology field and underscored the idea that mechanosensation is multifaceted. Detailing the importance of fluid shear in musculoskeletal cells is beyond the scope of this paper; however, recent reviews address fluid flow in musculoskeletal cells and in mechanotransduction.[103,104]

Tissue Engineering and 3D Cell Culture

With the advent of tissue engineering (TE), investigators developed novel methods to mechanically stimulate or "precondition" their TE constructs for both in vitro and in vivo applications.[105–110] The in vitro studies using cells cultured in geometrically defined constructs (e.g., linear, circular, off-axis) have shown that cell morphology modulates cellular responses to external mechanical stimulation. Studies have reported cellular induction of matrix compaction, contraction of the surrounding deformable environment,[50,111] and alterations in gene expression.[112,113]

Tendon and ligament tissue engineering represents a worthwhile example of these in vitro and in vivo studies.[105,106] The first reports of tenocytes grown in linear 3D collagen hydrogels (BATS or bioartificial tendons) and subjected to applied strain showed that tenocytes responded to strain with gene expression of matrix message and protein similar to levels expressed in 2D cells.[105] Moreover, human supraspinatus tenocytes grown in BATs and subjected to strain with an anabolic steroid, nandrolone, were biomechanically stronger than non-drug-treated controls.[106] This latter report was the first showing that a drug could have an anabolic effect on mechanically loaded tenocytes in 3D. Mesenchymal progenitor cells from rabbit tibiae, suspended in collagen gels, were found to contract around posts fixed in the wells of silicone dishes.[50] Mechanical stimulation of these tissue engineered constructs was found not only to increase their in vitro stiffness but also improved the structural integrity of patellar tendon defects filled with these constructs as compared to non-stimulated constructs,[50,114,115] particularly when the mechanical strain parameters mimicked native tendon loading in vivo. These mechanical stimuli could be optimized[116] and the effects of length, stiffness, and other construct factors could be identified.[117]

The in vivo applications have progressed one step further with surgeons implanting constructs that have been mechanically conditioned in vitro prior to insertion into wound sites.

Generally, mechanical pre-conditioning of connective tissue constructs improves tissue morphology and like confers potential benefits to the longevity of the construct and the healing process. Studies have also looked at the effects of mechanical loading in vivo on tissue regeneration and neovascular growth during wound healing in bone.[118] However, application of tissue engineered constructs in still extremely limited.

Taken together, cell biologists and engineers developed instrumentation and novel elastic culture surfaces with which the scientific community could apply regulated strain or shear stress to cells in 2D and 3D culture environments. With these new approaches, investigators could test varying magnitudes of strain or shear stress, frequencies, directions of fluid flow, and durations of applied force and measure a subsequent biologic response. An important outcome of the basic science in mechanobiology was to apply loads or shear stress to cultured cell-populated constructs for implantation in the body, a field known as Functional Tissue Engineering.[50,107,119]

BIOLOGY PRECEDENTS IN MECHANOBIOLOGY

Bacteria, plants, and animal cells have mechanosensors for detecting osmotic pressure changes and bending forces.[120–122] *C. elegans* has touch receptors for detecting surfaces.[123] Mammals have sensors for vision (photoreceptors), hearing (hair cells stereocilia receptors that transduce sound to the brain; stretch-activated channels), temperature (transient receptor potential channels; TRP channels), chemical (i.e., smell and taste; olfactory receptors, nociceptor, and pressure or touch (mechanoreceptors). Meissner corpuscles respond to light touch and adapt rapidly to changes in texture (vibrations around 50 Hz). Bulbous corpuscles (also known as Ruffini endings) and Merkel nerve endings (also known as Merkel discs) detect sustained pressure. The lamellar corpuscles (also known as Pacinian corpuscles) in the skin and fascia detect rapid vibrations (200–300 Hz). Mechanosensory free nerve endings detect touch, pressure, and stretching. Baroreceptors are a type of mechanoreceptor sensory neuron that are excited by stretch of the blood vessel.

A critical precedent for mechanical activity in biology is the act of muscle contraction. The sliding filament theory of contraction allowed for the mechanism of how muscle can shorten.[124] In the fields of general surgery, plastic surgery, and orthopaedics, there were questions about what was driving scar formation and contracture, limiting joint motion.[125] Contraction leading to contracture was believed to be cell-driven. Observations in patients with normal, hypertrophic, and burn scars and contracture underscored the idea that cells within a matrix could actively apply a "contraction" force on the matrix that pathologically shortens tissue and limits function.[126,127] The suspected mechanism was via a cell capable of contracting, compacting, and producing matrix, a cell termed the "myofibrocyte," (alpha-smooth muscle actin expressing myofibroblast; α-SMA-1). At the molecular level, the suspected mechanism of contraction was via the action of α-smooth muscle actin in concert with myosin, following the striated skeletal muscle paradigm.[127] Myofibroblasts develop in vitro and in vivo their highly contractile cytoskeletal apparatus only above a certain ECM stiffness threshold.[128] Tissue stiffness increases as a consequence of ECM-remodeling activities of fibroblasts and myofibroblasts. Contracting cells then generate the conditions that make them even more contractile in a detrimental feed-forward loop. Taken together, the clinical observation of cell-driven tissue contracture sets the scene for a search for the mechanisms responsible for the contracture and a means to prevent or control tissue contraction, adhesions, and limitation of range of motion in joints.

MECHANOTRANSDUCTION PATHWAYS: MECHANOSENSORY COMPLEX AND SIGNAL TRANSDUCTION

The question of how connective tissue cells, and indeed, all cells, sense and respond to strain in a "dose-dependent" manner to bring cells to homeostasis or equilibrium was and is a key question in the field. It was clear from the biomechanical and computational studies that bone could respond with accretion or resorption according to a mathematical expression.[12,13,16] An unanswered question in mechanobiology is, "what pathways do cells use to respond to deformation and how are the pathways regulated?" The initial work of Davidovitch,[28] Rodan,[80] Harell,[77] and co-workers demonstrated that osteoblasts and chondrocytes *could* respond to applied strain by secreting Ca^{2+}, PGE_2, and cAMP, but the engineering and scientific tools to test that hypothesis further were lacking in 1975. The tensegrity model of the cell introduced by Ingber was an advance in thinking about the cell from a structural viewpoint.[20]

The idea that the cell was a dynamic tensegrity structure was inspired by the observation of the similarity of the cytoskeleton to the R. Buckminster-Fuller geodesic dome, similar to the link that Wolff made to Culmann's bridge trusses.[20,21,129,130] A critical publication by Na et al.[131] that underscored the functional importance of integrins and the cytoskeleton in transducing a mechanical signal, outside-in, involved application of torque to a magnetic bead conjugated with fibronectin so that the engaging integrins could be activated. The kinetics of phosphorylation of Src by applied force were much faster compared to the rate achieved by EGF (epidermal growth factor) via its receptor. These results indicated that force alone transduced from the matrix through the cytoskeletal network could transduce a mechanical signal into a chemical signal, quicker than a ligand-receptor reaction acting through its pathway, a precedent finding! Data in Figure 2 indicate that there have been over 4,000 reports about actin, the cytoskeleton,

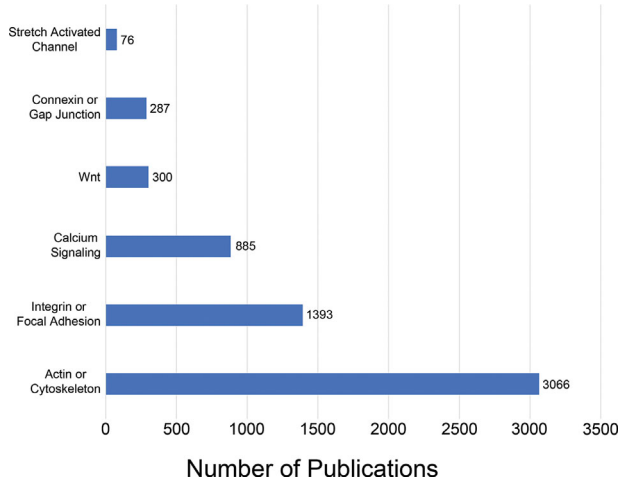

Figure 2. The number of publications in the mechanobiology field in which the charted keywords in mechanotransduction pathways were reported. Publication search conducted in PubMed in which the given pathways were found in a "text word" field. Publications limited to those articles found with the search terms given in Figure 1.

integrins, or focal adhesions within the mechanobiology field compared to approximately 1,400 reports about stretch-activated channels, connexins, gap junctions, Wnt signaling, or Ca^{2+} signaling combined. These greater number of reports pertaining to integrins and cytoskeleton versus other subject areas in mechanobiology underscores the impact and importance of the tensegrity idea linked to a mechanoresponse of the cell.

With all the technical advances in engineering, as well as biochemistry at hand, one could investigate at the molecular level, how cells might respond to strain.[22,132] Importantly, the assertion was made that cells could respond to strain with multiple mechanisms involving many pathways, and that mechanical signals were too important to be limited to a single pathway response.[22] The engineering principles of feedback control, equilibrium state, and even redundancy in regulation were postulated to be at work in a cell's response to applied strain.[22] Terms were coined to describe how cells responded to strain by application of strain to themselves by self-contraction (autobaric effect) or to other cells (parabaric effect).[22] The term, "mechanosensory complex," was coined to describe the cell's integrin-cytoskeletal machinery that could detect and respond to strain.[22] Presently, it is known that connective tissue cells detect and respond to an applied mechanical load through multiple and various mechanisms (Fig. 3).[133–135]

Primary Cilium in Mechanosensing

Mechanosensing in bone, both in vivo and in vitro, is strongly linked to the primary cilium and its associated proteins, including IFT88, and polycystins (polycystin 1 (PC1, Pkd1) and polycystin 2 (PC2, Pkd2)).[136–138] A largely overlooked structure, the primary cilium was first discovered on mammalian cells in 1898.[6] Emerging research over the past 20 years has revealed that a single primary cilium is present on nearly all somatic cells. In the context of connective tissue, primary cilia were initially observed in cartilage[139,140] and bone,[141] and later in the dense extracellular matrix of tendon and cartilage tissue.[142,143] They generally present characteristics of chemo and mechanosensitivity and are thought to, in part, coordinate mechanotransduction pathways, particularly in mechano-active cells derived from connective tissues.[141,144–148] A variety of important signaling pathways localize their signaling activity to the base and axoneme of the primary cilium, including proteins of the Hedgehog (Hh), Wnt, transforming growth factor-beta (TGF-β), and PDGF pathways across cell types.[149] These pathways have largely been studied under the context of the chemosensory properties of the primary cilium though the molecular mechanisms of ciliary mechanotransduction have remained elusive. PC1 and PC2 in conjunction with TAZ are thought to act as a sensor complex for osteocytes.[150] The Transient receptor potential vanilloid 4 (TRPV4) ion channel is suggested to control flow-induced Ca^{2+} influx into the primary cilium and flow-induced increase in Cox-2 gene expression, a prototypical osteogenic cell response.[151] However, a more recent report by Delling et al.[152] globally evaluated cilia-specific Ca^{2+} influx across tissue and cell types (including MLO-Y4 and Ocy454 osteocyte-like cells) using a transgenic ARL13b-mCherry-GECO1.2 mouse line in response to fluid flow, concluding that the mechanosensory activity in the primary cilium is not mediated by Ca^{2+} signaling. Though mechano-activated Ca^{2+} signaling has been proposed to be one of the primary mechanisms of primary cilia mechanotransduction, it remains a controversial area of study, in part due to the technical challenges of evaluating Ca^{2+} flux within the cilium and the variety of approaches and model systems used.

Ciliary mechanosensitivity has been largely demonstrated in changes in cilia-associated proteins in response to fluid shear stress,[136] but tenocytes and other connective tissue cells have also been shown to utilize the primary cilium to respond to strain[153,154] and even electric fields.[155] In cultured tendon explants under stress deprivation (i.e., culture of the explant in the absence of mechanical load) primary cilia elongate, presumably due to the absence of strain; however, further work suggests that cilia elongation may also be a consequence of biochemical degradation of the surrounding extracellular matrix.[156] This observation in tendon explants is somewhat in contrast to the observations in which cilia-depletion from cartilage in IFT88 confers a decrease in the mechanical integrity of the cartilage tissue.[157] The two observations are not necessarily contradictory as the experimental approach and tissues are very

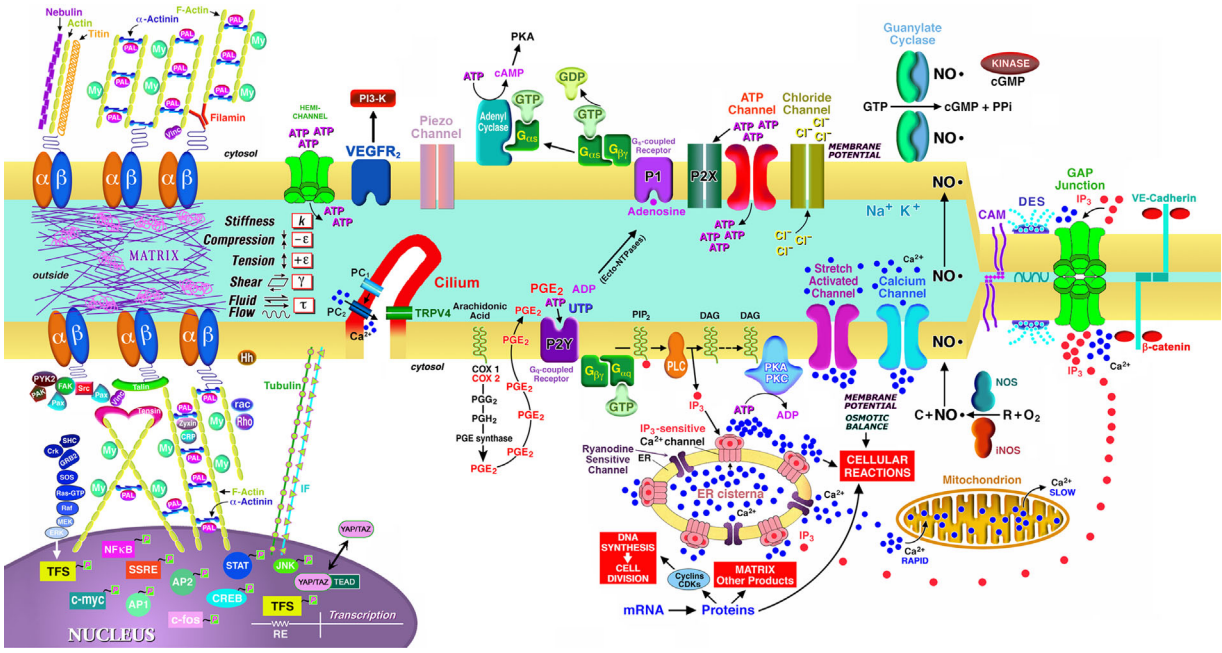

Figure 3. The detection of and response to external mechanical stimuli (i.e., compression, tension, shear, fluid flow) or changes in substrate/matrix stiffness involves multiple pathways and signaling mediators. A matrix-integrin-mechanosensory protein complex-cytoskeleton machinery is linked to a kinase cascade (tyrosine or nontyrosine kinase cascade or the JACSTAT kinase cascade) system. A mechanosensory protein complex contains talin, vinculin (Vinc), tensin, paxillin (PAX), Src, and focal adhesion kinase (FAK). In this model, a load deformation displaces matrix molecules tethered to clustered integrins at focal adhesions. The displacement is transduced to an integrin (β), to an integrin-binding protein, and then to associated proteins. Matrix-integrin-cytoskeletal interactions may also involve actin, myosin (My), nebulin, titin, α-actinin, filamin, palladin (PAL), tubulin, and intermediate filaments (IF). Activated extracellular signal-regulated protein kinases (ERK) enter the nucleus and up-regulate transcription factor expression (TFS, AP1, AP2, SSRE, CREB, c-fos, c-myc, STAT, JNK), and activate nuclear binding proteins, such as nuclear factor κB (NF-κB; P for phosphorylation). Polycistin-1 (PC_1) is co-localized with the primary cilium and activated when the cilium is deformed by fluid shear stress. The shear stress signal is transferred from PC_1 to polycistin-2 (PC_2) and induces the influx of calcium (Ca^{2+}) though PC_2, which in turn activates ryanodine receptors in the endoplasmic reticulum (ER) to release Ca^{2+}, resulting in Ca^{2+}-induced Ca^{2+} release. Changes in intracellular Ca^{2+} through the release of intracellular Ca^{2+} stores from the ER through or entry of extracellular Ca^{2+} through channels such as the store-operated, stretch-activated, mechanosensitive Ca^{2+} channels, and voltage independent or dependent Ca^{2+} channels. The release of adenosine triphosphate (ATP) and, at lower levels, uridine triphosphate (UTP), following the activation of ionotropic P2X and metabotropic, G protein-coupled P2Y receptors in an autocrine/paracrine fashion. ATP acts on $P2Y_2$ receptors, the primary ATP/UTP responsive receptor in tenocytes, activating the Gαq-protein, driving phospholipase C (PLC) and producing inositol trisphosphate (IP_3) and diacylglycerol (DAG). IP_3 acts on IP_3-sensitive Ca^{2+} channels in the ER to mobilize intracellular Ca^{2+}, and DAG activates a protein kinase C (PKC) pathway. Activation of adenyl cyclase activity yields cyclic adenosine monophosphate (cAMP), which stimulates cAMP-dependent protein kinase A (PKA), which may act at Raf in the kinase cascade. Initial action of ATP is terminated quickly by membrane-bound ecto-NTPases to its metabolites: ADP, AMP, and adenosine. Adenosine activates G protein-coupled P1 receptors, activating stimulatory (Gs) or inhibitory (Gi) signaling. Phosphoinositide 3-kinase (PI_3K) are activated by vascular endothelial growth factor receptor ($VEGFR_2$). Gap junctions pass IP_3, which propagates a Ca^{2+} wave from cell to cell after a mechanical signal is detected. Connexin hemichannels can pass ATP outside the cell. CAM, cell adhesion molecule; DES, desmosome; PPi, pyrophosphate; AP-1, activator protein-1; AP-2, activator protein-2; CREB, cAMP response element binding protein; MEK, MAPK/ERK kinase; NO, nitric oxide; PKB, protein kinase B; STAT, signal transducer, and activator of transcription; SHC, Src homology protein complex; Crk, Src homology adaptor protein that binds paxillin and C3G; GRB_2, growth factor receptor binding adaptor protein linking receptors to the Ras pathway through FAK and SOS (Son of Sevenless), a guanine nucleotide exchange factor; Ras, GTPase that regulates activation of Raf; IF, intermediate filament; YAP/TAZ, Yki transcription co-activators; TEAD, transcription factor; PYK2, a nonreceptor tyrosine kinase of the FAK family; PAK, p21-actived kinase; SSRE, shear stress response element; JNK, c-Jun N-terminal kinase; Hh, hedgehog; TRPV4, transient receptor potential vanilloid four channel; COX 1, cyclooxygenase 1; COX 2, cyclooxygenase.[134,135] (Used with permission from Flexcell International Corp.)

different; however, these data more broadly indicate that primary cilia may transduce signals, which contribute to how cells remodel their extracellular matrix (ECM) environment.

Further efforts to model the mechanical deformation of the cilia have mapped the areas of predicted strain. These computational studies have provided some insight into the likely localization of mechanosensitive protein structures and how the architecture of the cilium may affect transduction of signals from the surrounding environment.[158–160] There is a substantial body of work that loss of cilia in both experimental models[161] and naturally occurring genetic mutations and ciliopathic diseases[162–164] leads to reduced cellular mechanosensitivity and thus affects tissue homeostasis. However, identifying the specific mechanism by which this occurs is a major challenge in the cilia field overall.

Other Mechanosensing Mechanisms

Other mechanisms by which connective tissue cells can sense mechanical perturbations, include integrin receptors (α5β1), connexin 43 gap junctions and hemichannels, and Wnt signaling. Mechanical activation of the Frizzled receptor has been shown to lead to downstream signal up-regulation.[165] Osteocytes connect and communicate with each other in the canalicular system

in bone via gap junctions to sense mechanical signals and interact in a network to regulate osteoblasts and other osteocytes via paracrine factors.[166–170] Gap junctions are also involved in tenocyte mechanotransduction pathways.[171] The magnitude of the applied mechanical load can alter gap junction intercellular communication in tenocytes.[172] Furthermore, gap junctions modulate load-induced DNA and collagen synthesis and secretion.[173,174]

Lamins, intermediate filaments forming part of the nucleoskeleton, are yet another mechanosensitive structure within the cell which have garnered interest over the last 10 years. Expression levels of lamin-A,C can be modulated based on the Young's modulus of the culture substrate concomitant with changes in cell lineage specification in mesenchymal stem cells (MSCs).[175] Further, depleting or overexpressing lamin-A,C have profound effects on the ability of MSCs to undergo substrate directed differentiation toward osteogenic and adipogenic cell phenotypes.[176] Depletion of lamin-A,C in mouse embryonic fibroblasts leads to increased nuclear deformation and disrupted mechanotransduction when subjected to mechanical strain.[177] The LMNA gene encodes the lamin-A,C protein and mutations in this gene lead to human diseases such as Emery-Dreifuss muscular dystrophy. It is likely that lamins and other proteins of the nucleoskeleton will emerge another important component of mechanotransduction in mechanobiology.

Taken together, connective tissue cells can detect mechanical signals and, in turn, transduce a mechanical signal via the matrix and/or substrate upon which they are cultured. These cells accomplish this through both autobaric and parabaric effects[22,134,135] as well as utilizing cilia, multiple ion channels, signaling pathways, and matrix-integrin–cytoskeletal interactions in their responses to mechanical signals (Fig. 3).

FUTURE DIRECTIONS AND CONCLUSIONS

The NIH Decade of Bone and Joint (2000–2010) emphasized the need for prevention of osteoporosis and for all of us to exercise, particularly to load our skeletons in a healthy way to prevent disease. Understanding the underlying mechanisms of mechanosensation and developing therapeutic applications to combat osteoporosis, accelerate and/or drive a functional healing response, and regenerate tissues are current and future research goals from that effort. At this time, skeletal and soft tissue activation through known and controlled levels of exercise with or without anabolic steroid, growth factors, or other pharmacologic mediators, offers a positive therapeutic outcome for a patient. Given the redundancy and multiple pathways for sensing and responding to deformation at the tissue and cell levels, finding a single pharmacologic intervention that can regulate a body's response to mechanical signals will prove to be complex and diverse. The next phase of discovery will entail (i) modeling the cell from a biomechanical perspective[178] and investigating pharmacologic responses to strain, and (ii) modulating select genes and possibly finding a master gene controlling anabolic and catabolic responses to strain. Almost 150 years have passed since the observations of Wolff and Roux that forces dictate form and function in our tissues. We are narrowing down the candidates that control form and function via mechanotransduction pathways. We believe that continued collaboration among clinicians, engineers, and basic scientists will be essential to eventually solving this difficult problem.

AUTHORS' CONTRIBUTION

AB and MW contributed to all topics, organization, and drafting of the paper as well as the analysis of the citation frequencies. DB contributed to the drafting of the paper and critically reviewed the paper. EL, AE, and JB critically reviewed the papers. All authors have read and approved the final submitted manuscript.

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