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The Roles of Cellular Nanomechanics in Cancer

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Abstract

The biomechanical properties of cells and tissues may be instrumental in increasing our understanding of cellular behavior and cellular manifestations of diseases such as cancer. Nanomechanical properties can offer clinical translation of therapies beyond what are currently employed. Nanomechanical properties, often measured by nanoindentation methods using atomic force microscopy, may identify morphological variations, cellular binding forces, and surface adhesion behaviors that efficiently differentiate normal cells and cancer cells. The aim of this review is to examine current research involving the general use of atomic force microscopy/nanoindentation in measuring cellular nanomechanics; various factors and instrumental conditions that influence the nanomechanical properties of cells; and implementation of nanoindentation methods to distinguish cancer cells from normal cells or tissues. Applying these fundamental nanomechanical properties to current discoveries in clinical treatment may result in greater efficiency in diagnosis, treatment, and prevention of cancer, which ultimately can change the lives of patients.

Keywords

atomic force microscopy; cellular elasticity; cancer therapeutics; cellular mechanics; nanomechanical properties; cancer cells; anticancer drugs

1. INTRODUCTION

Cancer remains the second leading cause of death worldwide.¹ The American Cancer Society reports ~1,660,290 new cancer cases and ~580,350 cancer-related deaths occurred during 2013 in the United States alone.² In our opinion, it is important to develop

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CONFLICT OF INTEREST

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methodologies that improve understanding of disease condition and progression. Physicochemical forces drive proliferation, differentiation, and migration of cells in living tissue. Significant alteration of these forces or heterogeneity within the tissue implicates a role of nanomechanics in cancer diagnosis. Biomechanical or nanomechanical properties (nanomechanics) of ultracellular and subcellular components may provide the indications regarding onset and progression of carcinogenesis. Using scanning force microscopy, Lekka et al.³ initially studied the elastic properties of cells and determined that normal (Hu609 and HCV29) cells have a higher magnitude of Young's modulus than cancerous (Hu456, T24, BC3726) cells. Similarly, the elasticity function of cells distinguishes different cells using a microfluidic optical stretcher.⁴ Atomic force microscopy (AFM) has evolved into a multifaceted tool that can efficiently differentiate normal and cancer cells at a nanoscale level. Now these nanomechanical properties have significant implications in cancer research, including in cancer therapeutics.^{5–8} The measure of elastic behavior of the ultrastructural network of cells has also led to the development of drug molecules for cancer therapeutics, which is an attractive area of research in the pharmaceutical industry.⁹ Therefore, the aim of this review is to (1) outline the importance of AFM for measuring cellular nanomechanics and the factors that influence the measurements, (2) characterize the transformation of normal cells to cancer cells to metastasis, (3) examine the implications of indentation of nanoscale mechanics in cancer treatment, diagnosis, and therapeutics, and (4) assess the clinical validation of nanomechanics. This review also gives insight into evaluating the nanomechanics of cells and extrapolating for the estimation of carcinogenesis.

2. AFM AND CELLULAR NANOMECHANICS

The current review summarizes recent studies that link transformation of normal cells to cancer cells using nanomechanical properties of cells. The nanoscale measurements can be performed by employing various types of analytical techniques such as micropipette aspiration (typical force range, 1–100 nN), optical tweezers/optical traps (1–500 pN), magnetic bead microrheology (twisting, 10 pN to 1 nN; pulling, 100 pN to 10 nN), AFM (10 pN to 100 nN), shear flow (1–100 Pa), microindenter (1–100 nN), and so on.^{10, 11} In cell biology, typical force ranges are 1–10 pN in the case of kinesin and myosin, ~1–200 pN for protein–protein interactions, ~100 pN for partial protein unfolding, and ~1 nN to 10 μ N for migrating or contracting cells.¹¹ In many cases, the nanoscale mechanical measurements are related to the indentation and compression behavior of cells, which determine their overall consolidated ultra- to nanoscopic structures. AFM is widely used to investigate cell morphology through imaging and mechanical measurements in deflection control (Fig. 1A).^{5, 6, 12} AFM force spectroscopy experiments can measure deflection of the cantilever versus sample position, from which tip–sample interaction is determined. AFM force-based spectroscopy is used for molecular recognition mapping, single molecule force spectroscopy, and single-cell force spectroscopy to reveal the localized interactions of biological molecules at a single molecular level. From these distinct measurements, it may be possible to differentiate between malignant and nonmalignant mammalian cells.¹³ Cell height, surface roughness, nucleus morphology, cytoplasm area, microtubule networks, fibril

area, stiffness, and Young's modulus are commonly investigated components that reveal the nature of investigative cells.

AFM nanoindentation is capable of capturing time-evolved mechanical responses from structurally varied biological proteins, sugars, mineralized hard and soft tissues, and very complex composite tissues.^{14, 15} Nanoindentation techniques have been employed to study microbial,¹⁶ stem cells,¹⁷ brain,¹⁸ dental, and bone,^{19–22} other biological hard tissues such as seashells,^{23, 24} aging, and other diseases. The use of AFM to examine various aspects of cell(s), tissue(s), and cancer has been reported in detail (Fig. 1B). However, studies of nanoindentation or nanomechanics in cancer research are limited; therefore, significant opportunity exists to implement this technique. Nanoindentation studies on biological tissues conducted over the last decade suggest significant promise in the use of nanomechanics to differentiate cancer cells and normal cells. With improved technologies, nanoindentation techniques combined with in situ imaging capabilities provide new opportunities in cancer research. Significant research efforts directed toward experimental as well as modeling have been conducted to examine physicommechanical mechanisms of cellular functions including differentiation, growth, migration, and death.²⁵ The mechanical properties of all these stages of cells distinctly vary.²⁵ Such nanomechanical cellular information enhances our understanding regarding the complexity of cancer at the cellular and tissue level and thus can lead to development of advanced clinical solutions.

3. NANOINDENTATION FUNCTION AND FACTORS AFFECTING MECHANICAL PROPERTIES

A recent review article describes in detail the use of nanoindentation and its function, appropriate mechanical models used to test the indentation measurements at given test conditions, and suitable types of tip geometries helpful to measure indentations specifically on living cells.²⁶ In this section, we provide detailed information on conventional use of nanoindentation, function of nanoindentation, typical mechanical models used, and various factors that influence measurements.

3.1. Use of Nanoindentation

Nanoindentation has primarily been used to characterize ultrastructure and mechanical properties of inorganic stiff/hard materials such as metals at small scales.²⁷ Experimental nanoindentation studies enable monitoring of materials' discrete events such as plasticity, creep parameters, shear instability, phase transformation, crack, and delamination.^{7, 25} These measurements can be accomplished by determining the relaxation of the sample under load. The analysis examines the displacement of the indent just before the relaxation at a predetermined time, which is always much shorter than the characteristic relaxation time of the material. In biomedical sciences, it has been widely used to map and understand the gel structures in the human body to identify advanced poroelasticity. Cells or biological tissues consist of dynamically deformable rheology that is highly complex, rendering evaluation of nanomechanical behavior that is much involved.²⁸

3.2. Function of Nanoindentation

Typically, nanoindentation experiments involve recording force–displacement curves of cells under load-controlled or displacement-controlled modes (Fig. 2). The force–displacement curve (e.g., Fig. 2B) is converted to the force–indentation curve (e.g., Fig. 2C), which directly depicts the stiffness of the cell surface. The Hertzian model is frequently used to generate the quantitative data on the Young's modulus (E) of the cell surface (i.e., elastic modulus, E). Most of the force–displacement measurements unveil the inter- and intramolecular forces of cell–cell and cell-surface bimolecular associations.²⁹ This methodology is used to obtain a force–displacement curve in a three-step process: (i) approach: AFM probe/tip brought close to the cellular surface, (ii) contact or indent (compression step): indentation into the cell surface provides interaction between the cell-surface proteins and the tip, and (iii) retraction or separation (decompression step): retraction/separation from the surface of the cell allows extension of the bound macromolecule(s) as the tip–cell distance.³⁰ Nanoindentation data are highly influenced by the tip/cantilever type, substrate, mechanical model used, and in vitro/in vivo physiological condition.^{11, 26, 31} A spherical indenter is recommended for biological tissues/cells to enable small stress concentrations, while a sharp tip indenter suits relatively hard materials including dental and bone samples.^{28, 29} A pyramidal tip can be used for determining nanoscale mechanical properties of living cells.^{32, 33} Few reviews have been published that describe the mechanisms of indentation on hard materials and hard tissues^{25, 34, 35} and a comprehensive review at the cellular level with prospective cancer applications is not available.

3.3. Models Used to Measure Nanomechanics

Nanoindentation data are usually described in the form of Young's moduli or the equilibrium elastic modulus, which is calculated by converting force–displacement curves into force–indentation curves and fitting with the Hertz model.^{36–38} The appropriate Poisson ratio and half opening angle of the tip for cells and tissue samples are approximately 0.5° and 36° , respectively. However, in this model, measurements have been restricted to low force ranges resulting in <500 nm surface indentations in order to avoid cellular surface destruction. Many earlier studies have utilized the Hertz model for indentation on living cells, but living cell components such as cytoplasm/membrane/cytoskeleton structure often do not exhibit a linearly elastic behavior as approximated under Hertzian theory. Chen and Lu³⁹ developed a finite-element method that can be used to resolve the correction parameter observed in the Hertz model. This model uses viscoelastic integrals while ramping load and stress relaxation conditions through finite lateral and vertical dimensions. Additional new models and laws based on Newton, Maxwell, Voigt, Boltzmann, Tsay, Burgers, Weichert, and Kelvin can facilitate the prediction and interpretation of viscoelastic properties of cells for nanoindentation.^{40, 41} All models outlined above describe and evaluate AFM force–displacement curves, rather than relying on the classical Hertz model that does not allow for larger deformations and nonlinearity.⁴²

It is hypothesized that minute changes in cell volume and shape also contribute to important signaling for cell proliferation, death, and migration. Therefore, a new mathematical model developed by Jiang's group⁴³ considers cellular volume and pressure by incorporating

important parameters such as water permeation, mechanosensitive channels, active ion pumps, and active stresses in the cortex. The nanomechanical properties of live cells were first studied using AFM force curves in 1993.^{38, 44} Subsequent research successfully implemented this new model to study the differentiation between transformed (cancerous) and nontransformed (normal) cells.^{45, 46} In general, all these preliminary data suggest that tumors or cancerous cells are softer as well as highly deformable in terms of their elastic moduli compared to healthier counterparts or cells.²⁵ It is also possible to specifically detect glycans on a plasma membrane of cell lines using the AFM indentation method by modifying the AFM tip with three distinct lectins: concanavalin A, lectins from *Sambucus nigra*, and lectins from *Phaseolus vulgaris*.⁴⁷

3.4. Factors that Influence Nanomechanics

Validation of data obtained by nanoindentation experiments varies and primarily depends on the substrate, instrument condition, and environment used for experiments. Substrates such as glass cover slips,⁴⁸ tissue culture polystyrene,^{49, 50} chlorapatite single crystals,⁵¹ uncoated or coated with fibronectin/collagen I,⁵² poly(glycerol sebacate) elastomers,⁵³ and poly(lactic-co-glycolic acid) (PLGA),⁵⁴ are conventionally used in nanoindentation procedures. Human cells respond via alterations in their mechanics to the synthetic and biological substrate to which they adhere.⁵⁵ In biological applications, the substrate stiffness is always greater than the cells or tissue used for nanoindentation,⁵⁶ except in the case of tissue engineering scaffolds where the two are well matched. In general, variations in stiffness have been observed between cells in the different regions of the substrates. Under ideal conditions, the stiffness order of cells/tissues is as follows: substrate > dispersion > central regions > peripheral regions. In the case of fibroblasts, peripheral regions can be stiffer than the regions above the nucleus. Furthermore, nanoindentation results are greatly influenced by fixation and other processes used for cell measurements. Recent studies support that significant differences arise in cell stiffness due to fixation. Paraformaldehyde-fixed mouse embryonic fibroblasts (NIH/3T3) and human epithelial cancer cells (SW-13) exhibit at least ten times higher Young's modulus ($E > 5 \times 10^4$ Pa) over their living counterpart cells ($E \sim 5 \times 10^3$ Pa) during steady-state measurements.⁵⁷ Chemical fixation procedures also alter the elasticity, adhesion behavior, and membrane properties of cells. Additionally, substrate coating with artificial materials further interferes with cellular adhesion, thereby influencing AFM indentation results.³⁰ Hydrophilic groups such as hydroxyl, polyethylene glycol, carboxyl, amine, and hydrophobic alkyl and phenyl groups are commonly employed coatings. In an elaborate study, positively charged silica microspheres modified by a silane coupling agent of *N*-trimethylsilylpropyl-*N,N,N*-trimethylammonium chloride or *N*-methylaminopropyltrimethoxysilane showed a greater adhesion force with melanoma cells⁵⁸ due to the electrostatic attraction between the positively charged microsphere surface and the cell surface, while the unmodified polystyrene microsphere surface had no significant affinity. It has also been observed that the stiffness of cells is altered by the nature of the substrate that cells are exposed to.⁵⁹

4. TRANSFORMATION OF NORMAL CELLS TO CANCER CELLS

Transformation of healthy cells into cancerous cells arises from alterations in the barriers of cell membrane and the internal/external microenvironments.^{60, 61} In this process, cellular physiological factors include change in the overall energy conversion process, cell surface alterations and recognition, cellular transport, signal transduction, cell survival, and differentiation of cells. In many cases, significant contributions of mechanical properties of cells are largely governed by their cytoskeleton. The cell cytoskeleton is highly regressed and transforms into an irregular and compliant state under differentiated, highly mitotic, or cancerous phase.⁶² Additionally, many other internal cellular organelle that are associated with nucleus size, chromatin organization/condensation, and epithelial mesenchymal transition (EMT) are responsible for regulating cellular biomechanical measurements.⁶¹ Therefore, a significant variation in cellular nanostructures and mechanics can be directly influenced by the cancerous condition of cell.²⁸

Transforming growth factor- β (TGF- β) acts as a tumor suppressor by inhibiting proliferation and inducing apoptosis in early stages of cancer progression, while also accounting for EMT and cell motility when the disease progresses. The reason for this unusual characteristic was examined on normal murine mammary gland cells and kidney epithelial cells cultured on polyacrylamide gels. The results showed decreasing rigidity increased TGF- β 1-induced apoptosis.⁶³ β -catenin is a dual function protein, regulating the coordination of cell–cell adhesion and gene transcription. It has been found to play a significant role in tumorigenesis.^{64, 65} Single molecule force microscopy of β -catenin reveals low mechanical stability, and several armadillo repeat region repeats are observed to be unfolded and thus considered chemically active for its ligands.⁶⁶ This study proposed that β -catenin could serve as a mechanical safeguard under high forces to protect the links between cadherin and the actin cytoskeleton. A recent review indicates that various critical steps are involved in mechanical contributions of cancer cells and tissues to dysplasia, invasion of the extracellular matrix (ECM), and metastasis.⁶⁷

Cross et al.⁶⁸ demonstrated that live lung, breast, and pancreatic metastatic cancer cells obtained from body fluids of patients are apparently distinguished from normal ones by stiffness, although both cell types exhibit very similar morphologies. Another study from this group also confirmed that metastatic cancer cells promote elastic modulus up to 80% with a narrow distribution compared to normal cells.⁶⁹ This indicates that cancer cells show increased deformability compared to normal cells due to alterations in biochemical processes. Adipose-derived stem cells promote gastric cancer cell growth, migration, and invasion characteristics.⁷⁰ Deriving the mechanical properties from such stem cells will delineate important information. A clear enhancement (~ 10 – 17 times) in Young's moduli between living and dead undifferentiated adipose-derived stem cells was observed by Hu et al.⁷¹ Another study demonstrated that an increased compliance of cancerous and precancerous cells may behave in a “crumble and yield” manner.⁴⁵ This study revealed the order of Young's moduli at 1 nN stiffness of indentation force: normal squamous cells (EPC2) (4.7 kPa live and 9.9 kPa fixed) > metastatic (CP-A) (3.1 kPa live and 2.9 kPa fixed) > dysmoplastic esophageal cells (2.6 kPa live and 2.1 kPa fixed).⁷²

Nanoindentation of individual cancer or metastatic cell(s) may not describe the in vivo condition of cancerous cells, which are highly connected with stromal cells. To investigate the behavior of such proprietary cancer cells, Wilson et al.^{73, 74} implemented a coculture approach consisting of corneal stromal cells, fibroblasts, and type I collagen hydrogels constructs. Under such environment, nanoindentation results suggest that TGF- β 1 and wortmannin media supplementation significantly affect stromal cell behavior and differentiation. These findings also corroborate cell viability, phenotype, morphology, and protein expression. EMT potentiates the ability of cancer cells to migrate and invade. It is accepted that the majority of cancer mortality is due to metastatic disease. Defining the transition to metastasis by any biological and physical activity provides a foundation essential for improving cancer therapies. In an attempt to delineate pre-EMT to post-EMT advanced dendrimer, a modified tip was used in nanoindentation experiments.⁷⁵ This method enhances force sensitivity in measuring actin dynamics of ovarian cancer SKOV-3 cells upon binding of integrin α 5 β 1 to fibronectin. Post-EMT SKOV-3 cells induced by TGF- β produced a 41 nN adhesive mechanical force compared to 17 nN in pre-EMT SKOV-3 cells. This method provides a direct quantitation of faster migration characteristics of post-EMT cells. Elastic modulus of the enriched cancer stem-like cells/tumor-initiating cell population (0.32 ± 0.12 kPa) are about 46%, 61%, and 72% softer ($P < 0.0001$) than their aggressive late stage, intermediate, and nonmalignant early-stage murine ovarian surface epithelial counterparts, respectively (Fig. 3).¹⁰ Similarly, a decrease in elastic modulus was accompanied by epithelial–mesenchymal transition, F-actin, β -tubulin, nucleus size, and cytoplasm (N/C) ratio in the case of metastasis tongue squamous cell carcinoma cells.⁷⁶ Furthermore, to distinguish additional metastatic prostate cancer cell behavior, PC-3 (derived from bone marrow and LNCaP) lymph node derived, was proposed for adhesion nanoindentation experiments in the presence of bone marrow derived mesenchymal stem cells (SCP1 cell line) and collagen I.⁷⁷ The nanoindentation experiments were conducted using a tipless silicon nitride cantilever (with a spring constant of 0.01 N/m) to deposit cancer cells onto a monolayer of SCP1 cells or SCP1 cocultured with collagen I. The binding affinity determined from the force–distance curves was recorded while the piezo traveled in a closed loop up to 20 μ m at an approach velocity of 7 μ m/s until a trigger force of 100 pN and a retraction velocity of 3 μ m/s was reached. This study indicates that PC-3 cells demonstrate a greater binding affinity with SCP1 cells and collagen I than LNCaP cells. Yuen et al.⁷⁸ described a difference between normal and abnormal lymph nodes using pig lymph node tissues in agar–gelatin phantoms. This report suggested that there was no significant difference in the effective strain and Young's modulus at peripheral and middle regions of the lymph nodes, but this is distinctly different from observations in normal lymph node. Cumulative nanoindentation data have revealed that the mechanical properties of individual cells is valuable information that can delineate growth, motility, and metastasis phases of cancer cells and tissues.^{28, 79} Malignant thyroid cancer cells are either less elastic or less viscous than their normal counterparts, while metastatic cancer cells display significantly lower deformations. It was also observed that elastic moduli of cancer cells are significantly lower than that of normal cells on days 2 and 3 compared to day 1.⁸⁰

Current AFM nanoindentation techniques are highly suitable for measuring elastic properties of whole cells in a firmly adhered stage; however, it is difficult to probe

nonadherent cells. To overcome this issue, nonadherent cells were immobilized in specially designed micro-fabricated wells and measured at low deformation rate.⁸¹ In this setup, at a piezo extension rate of p 415 nm/s, myeloid (HL60) cells ($E = 855 \pm 670$ Pa) are 18 and 6 times stiffer than lymphoid (Jurkat) ($E = 48 \pm 35$ Pa) and human neutrophils ($E = 156 \pm 87$ Pa), respectively. The significant changes arise due to leukocytosis in human leukemia cells. Roberts et al.⁸² have demonstrated that the transformation of mouse ovarian surface epithelial cells from healthy to benign to malignant exhibits significant alterations in cytoskeleton integrity. Their investigation of cell elasticity suggests that latrunculin A (actin-targeting drug) significantly lowers elasticity and viscosity of cells in simple indentation and stress relaxation methods.⁸³ However, there was no significant change observed with microtubule affecting drugs such as suberoylanilide hydroxamic acid (SAHA, a histone deacetylase inhibitor) and nacodazole. Another investigation of MDA-MB-231 cells after SAHA treatment did not show a significant change in Young's modulus of cells as determined by the Strobl⁸⁴ test. The peak modulus of the cells was shifted from 0.2 to 0.24 kPa after treatment with SAHA. Interestingly, they also found that SAHA treatment increased circular and star microstructures by ~ 1.75 - to 2.5-fold in cell length and ~ 1.5 - to 2.8-fold in cell area due to increased tension within the actin cytoskeletal structure and increased microtubule network extension of the cells.

Adhesion of cells to the ECM is a key property of cells that has an important function in cell physiology. ECM is also responsible for sensing mechanical cues and offers possible engagement and clustering of integrins and formation of focal adhesions via the actin cytoskeleton. A key element in ECM is collagen I, which increases local stiffness up to 50 times. This observation is further confirmed by theoretical simulations and gives a better understanding of the interplay between fiber mechanics and the network organization.⁸⁵ Indentation experiments as well as molecular simulations have been performed on individual collagen filaments.⁸⁶ Keratin filaments are also responsible for mechanical properties of epithelial pancreatic cancer cells (Panc-1). The elastic modulus of living Panc-1 cells ranged from 100 to 500 Pa while the extracted in situ subcellular keratin intermediate filament network in Panc-1 cells show elastic modulus of only 10 Pa. The reason for this vast difference is due to the fact that keratin network extraction leads to removal of microfilaments, microtubules, membranes, and other soluble cytoplasmic components, which dissolve filamentous fibers and the viscosity from the cytoplasm. Concurrently, Sadeghi et al.⁸⁷ used three different strain energy models, namely, Ogden, Yeoh, and Polynomial, with hyperelastic properties of deflated lung tissue via an inverse finite element approach. The noncytoskeleton (lipid) region may also play a crucial role in determining cancer cell phenotype. Although there was not a significant difference in cytoskeleton and lipid region adhesive forces in MDA-MB-435 cells (0.291 ± 0.018 nN for cytoskeleton vs. 0.298 ± 0.024 nN for lipid), MDA-MB-435-BRMS1 expressed cells (transformed cells) were significantly different in their biophysical and biochemical homogeneity of the membrane surface, reflecting an increased adhesive force at lipid regions (0.477 ± 0.030 nN) compared to cytoskeleton regions (0.173 ± 0.025 nN).⁶² A study by Baker et al.⁸⁸ offered novel insights on mammary epithelial cell (MEC) transformation into breast cancer. This study was conducted in human-derived MCF10A. MECs overexpressed either ErbB2, 14-3-3z, or both ErbB2 and 14-3-3z, with empty vector as a control, which confirms that matrix

stiffness dictates the intracellular mechanical state of MECs. Integrated models suggest cancer-associated fibroblasts drive the progression of metastasis through cell population, paracrine, and mechanical dynamics.⁸⁹

5. CANCER TREATMENT AND DIAGNOSIS

Many current therapeutic agents slow the growth of potential cancer cells in the short term, but their long-term efficacy may be clouded by the cancer's development of de novo (intrinsic) and acquired (extrinsic) resistance to anticancer agents,⁹⁰ which leads to recurrence and relapse of cancer. Therefore, the pharmaceutical industry is now focused on developing drugs that can target multiple pathways instead of drugs that target a single pathway. In a similar context, in addition to utilizing conventional cytotoxicity evaluation methods such as ATP bioluminescence, cell viability or cell proliferation [(3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide, MTT or (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt, MTS], clonogenic, methylene blue, migration, or wound healing biological assays, etc., it is important and timely to study nanomechanical properties in order to define targets, design therapeutics, develop drugs, and monitor their efficacy.⁵ Although conventional methods provide substantial basic and cellular information, they fail to reveal microstructural interactions at the interface of the cellular membrane surface and drug molecule(s). A better understanding of the associated problems, such as recurrence and resistance, would enable the designing of experimental conditions to more closely mimic conventional chemotherapy. Therefore, nanoindentation experiments will potentially lead to improvements in drug effectiveness and accurate cytotoxicity estimations.

The frequency of chemotherapy use in oncology has increased extensively, where specific gene proteins or pathways that are disordered during tumorigenesis are targeted. Some of these drugs display greater efficacy than others in eradicating tumors. This signature can be determined by nanoindentation. Chemotherapy (doxorubicin and dexamethasone) induced cell death increased the stiffness of acute lymphoblastic leukemia and acute myeloid leukemia cells that were isolated from the blood of patients with newly diagnosed acute leukemia.⁹¹ The degree of cell death and associated increase in stiffness of leukemia cells depends on the microfluidic channels, which varies by cell population. The progression of cell death and increase of stiffness is attenuated by disruption of the actin cytoskeleton. In vitro examination of HL60 (myeloid) and Jurkat (lymphoid) cells exposed to daunorubicin and dexamethasone indicated the chemotherapy type dominates the kinetics of cell stiffness, not the leukemia cell type. The work by El Kaffas et al.⁹² also demonstrated that elasticity of MCF-7 breast cancer cells significantly increased upon paclitaxel exposure for 24 hr. Paclitaxel exposure increased the elastic and viscous moduli to 191.3 Pa ($>8000\times$) and 9 Pa ($\sim 9\times$), respectively, in low-frequency shear modulus measurements (1 rad s^{-1}). At higher frequencies (10 rad s^{-1}), these values are reduced to 188.5 Pa ($\sim 60\times$) and 1.7 Pa ($\sim 1.1\times$), respectively. Another interesting study correlated the biomechanical composition of the ECM as a critical regulator of cellular behavior.^{40, 93, 94} The developed in vitro system of “mechanically tunable” matrix-coated polyacrylamide gels promote hepatocellular carcinoma cell proliferation. After treating cells with cisplatin, survived cells proliferated to a significant number of colonies in a stiff microenvironment. This suggests that increased

matrix stiffness promotes proliferation and leads to resistance. Cytoskeleton-destabilizing agent (Cyt-B) attenuated the average elastic modulus of lymphocyte and Jurkat cells by the disruption of the actin filaments.⁹⁵ Cisplatin-resistant ovarian cancer cells (OVCAR5-CisR and SKOV3-CisR) respond to a combination of cisplatin and Cyt-B treatment by reducing the elastic modulus from ~1.75 kPa to ~ 7.5 kPa (Fig. 4).⁹⁶ Again, this study suggests chemoresistance is highly associated with extensive actin filaments in cytoplasm to peripheral regions rather than sensitive cells. Another drug, CB1a, showed dose-dependent toxicity toward NCI-H460 lung cancer cells while sparing normal MRC-5 lung cells.⁹⁷ This characteristic was also accompanied by a declining Young's modulus (~60%) within 5 min of CB1a treatment. In contrast, under mechanical forces the growth rate as well as effectiveness of clinically relevant drugs such as cisplatin, paclitaxel, doxorubicin, and KU-drug (experimental drug from the University of Kansas) (Tables I and II) against the A549 cell line increased the effectiveness of zactima against NCI-H358 cell lines.⁹⁸ Introducing such respiratory mechanical forces that mimic physiologically relevant conditions may help to develop anticancer drugs for successful clinical translation.

Natural chemopreventive agents such as curcumin, epigallocatechin gallate (EGCG), and their nanoformulations possess various biological photoelectric activities against cancer cells.^{99–108} For the first time, Watanabe et al.¹⁰² reported low cell stiffness with low Young's modulus values associated with high cell migration. These findings demonstrate elevated cell stiffness in B16 melanoma cell variants and in (2)-EGCG-treated cells. Another study also delineated the effects of EGCG on patients' metastatic tumor cells and A549 cells through biomechanical response.¹⁰¹ These data indicate that EGCG selectively targets human metastatic cancer cells but not normal mesothelial cells. The average Young's modulus of the untreated tumor and normal cells was found to be 0.41 ± 0.18 kPa ($n = 164$) and 2.53 ± 1.23 kPa, respectively. Treating these cells with EGCG resulted in a significant increase in stiffness of tumor cells to 2.54 ± 1.27 kPa ($n = 154$) while stiffness of normal cells, 2.48 ± 1.37 kPa ($n = 156$), did not change significantly. Another study aimed to test the real-time ultrastructural cellular drug interactions as evidenced by imaging and AFM nanoindentation.⁸ In detail, this study demonstrated substantially changed cell membranes and roughness of human cervical (HeLa), human hepatocellular liver (HepG2), and rat glioma (C6) cancer cells upon short-term exposure to colchicine or cytarabine. The results of this study provide a new rationale for the establishment of a real-time visual examination of toxicity. Similarly, immune therapy can also be evaluated by this novel indentation technology. For example, coculture of lymphocytes and K562 cells for 3 hr promoted protrusions on the cell surface and underwent apoptosis or necrosis. This is evidenced by significant alterations in the roughness of cell membrane due to damage and membrane lipid compositions. Another natural product, namely chrysin–organogermanium (IV) complex (Chry–Ge), was examined as an anticancer compound against MCF-7, HepG2, and Colo205 human cancer cell lines.¹⁰⁹ This compound exhibited enhanced anticancer effects on highly selective tumor cells by disorganized cytoskeletal network and fragmented morphology while sparing normal cells. This arises from reactive oxygen species (ROS)-dependent mitochondrial and apoptosis pathway. The toxicity on MCF-7 cells was also verified by force measurements that indicated a significant decrease in Young's modulus from 1.711 ± 0.864 MPa to 0.0245 ± 0.010 MPa upon treatment. Similarly, multifaceted effects of

phytochemicals, such as triterpene saponins and desglucoanagalloside B on human prostate cancer cells (DU-145 and PC-3 cells), were reported.¹¹⁰ This study further indicates inhibition of cell motility; CIL-1 (desglucoanagalloside B) attenuates the invasive potential of prostate cancer cells through an effect on their elasticity. Human lung cancer cells respond to phytohemagglutinin erythroagglutinating (PHA-E) (extracted from red kidney beans) by blocking the epidermal growth factor receptor (EGFR).¹¹¹ PHA-E and EGF bind to EGFR with a force of 207.14 ± 74.42 pN and 183.65 ± 86.93 pN, respectively, indicating that the binding affinity of PHA-E to EGFR was one order higher than EGF to EGFR.¹¹¹ Further, superior anticancer effects of PHA-E over EGF were confirmed in A-549 cells. Therefore, PHA-E could be a new target molecule for lung cancer treatment.

A prototypical example of the interactions a single molecule with the poorly understood agonist/receptor interaction, that is, leuporelin acetate's long-lasting effect on gonadotropin-releasing hormone (GnRH) (also known as Luteinizing-hormone-releasing hormone, LHRH) receptors of prostate cancer cells, is highly useful in cancer therapeutics.⁴⁷ Tumor necrosis factor related apoptosis-inducing ligand (TRAIL) is a promising apoptotic agent that is frequently used in cancer research due to its selectivity toward tumor cells. Targosz-Korecka et al.⁴⁸ proposed AFM nanoindentation combined with fluorescence methods to reveal the biomechanical aspects involved in this chemoresistance process. This study demonstrated that an increase in mechanical cell stiffness arises when the inhibition of apoptosis occurs by TRAIL in HEP2 cells. On the other hand, HEP2 cells treated with actinomycin D led to the depolymerization of F-actin and thus a perceived decrease in cell stiffness, which is associated with the Bcl-2 pathway. The stiffness of NIH3T3 cell membrane in response to serum conditions and nocodazole depends on the interplay between dynamically unstable microtubules, stable microtubules, and intermediate filaments, indicating a transient metastability.¹¹² Another finding suggests that sphingosine treatment on murine ovarian surface epithelial cells (cancer stem-like/tumor-initiating cells) led to an increase in the elastic moduli by more than 46% (0.47 ± 0.14 kPa, $p < 0.0001$).¹⁰ All these results provide evidence that cell environment conditions dictate the most desirable mechanical response.

The adhesion forces vary significantly between the polystyrene microspheres with different size or surface functional groups and the melanoma (B16F10) cells.³⁰ It was found that the contact area increases with the increase of sphere diameter. The adhesion force was found to be larger for the carboxyl-modified polystyrene microspheres compared to the unmodified polystyrene microspheres. However, the overall density of carboxyl groups and the zeta potential have no significant influence. The anticancer activity of selenium nanoparticles (Se NPs) in MCF-7 breast cancer cells have been investigated by nanoindentation and changes in cell membrane, CD44 molecule expression, and amounts of F-actin were compared.¹¹³ Control MCF-7 cells had a Young's modulus of 5.05 ± 2.43 MPa. When these cells were treated with Se NPs, Young's modulus was only 0.69 ± 0.31 MPa. This observation was consistent with many other drug treatments. An increased interaction of Arg-Gly-Asp peptide (integrin inhibitor) with melanoma cancer cells occurred after modification with carboxyl or hydrophobic-modified polystyrene microspheres.³⁰ These results improve the understanding of immunomodulation in a therapeutic setting.

There are several clinically improved drug-targeted NPs under clinical trials aimed at improving targeting efficacy and lowering side effects, which can potentially be the next generation of therapeutics using nanomedicine. Currently, polymer NPs are the most widely used formulations. Therefore, Vasir and Labhasetwar¹¹⁴ examined adhesive interactions of PLGA NPs and cell membranes using a modified AFM tip coated with NPs. The adhesion force quantification was distinctly different between unmodified (280 pN max and 20 pN min) and modified (1200 pN max and 50 pN min) NPs. The functionalized NPs exhibited a sixfold increase in adhesion events on cell membrane compared to unmodified NPs. This study serves to indicate that this technique can be extended to investigate NP–cell interactions under various biological conditions to uncover the role of nanomedicine in cancer therapeutics.^{5, 115–117}

6. CLINICAL VALIDATION

Nanoindentation techniques can measure the elastic response of cells and tissues, which is a subject of interest in cancer therapeutics and diagnosis.²⁸ Nanoindentation can provide time-dependent elastic deformation behavior of cells and tissues. The concept of imaging the spatial distribution of tissue elasticity is already used in medical applications. However, developing existing elastic modulus data as a clinical tool requires additional research. It is of great medical benefit to be able to differentiate normal cells from cancer cells and various states of cancer(s) cells by mechanical properties using nanoindentation. Furthermore, an expanded knowledge of elasticity of cancer cells is useful in experiments, drug–target interaction with cancer cells, ligand–receptor interactions, and adhesion force can be used to directly measure the individual near-physiological condition of cancer cells. A study conducted by Samani and Plewes has successfully measured elastic modulus of intact ex vivo breast cancer tissues.¹¹⁸ The proposed methodology is robust and accurate. Their preliminary results indicate that a benign lesion and malignant tumors are ~ 5 and ~10 times more rigid than normal breast tissues. An in vitro indentation test system was designed to assess the diagnostic capacity of prostate diseases.¹¹⁹ This study investigated viscoelastic properties of human prostate tissues at actuation frequencies between 0.5 and 30 Hz for comparison with normal tumor tissues. Validation of nanoindentation stiffness data of ex vivo prostate cancer tissue versus normal cells was further confirmed by the response of a resonance sensor-discriminating radical prostatectomy.¹²⁰ The accuracy of the stiffness curve is 0.941 for a loading force of 12.8 mN. Another study correlated robotic 60 nanoindentation data for five prostate specimens in combination with magnetic resonance imaging scans.¹²¹ A 90% (54/60) concordance rate was observed between the location mapping from the robotic indentation and imaging scans. The sensitivity and specificity of the robotic indentation system were discovered to be approximately 87.9% and 92.6%, respectively. More importantly, the indentation technique was able to determine invasiveness and malignant potential of pulmonary lesions when examining 114 ground-glass opacities (GGO).¹²² Based on this indentation data, 14 (12%) and 16 (14%) of these GGO lesions were categorized as invasive lung cancer and minimally invasive adenocarcinoma, respectively.

Nanoindentation in live and snap-frozen mammary tissues was used to examine the importance of tissue rigidity in cancer cells and contribution of the ECM and vasculatures.⁷⁰

This study suggested that isolated transformed MECs were intrinsically stiffer than their normal counterparts. At the same time, the malignant epithelium in situ was far stiffer than isolated breast tumor cells. Additionally, the vascular bed within the tumor core was substantially stiffer than the large patent vessels at the invasive front that are surrounded by the stiffest ECM. A simple analytical observation of nanoindentation confirms 84% of the variation in the spine bone stiffness and strength of all metastatic prostate, breast, lung, ovarian, or colon cancer cells received from 15 patients (41 specimens) when compared to 96 noncancerous specimens. This measurement can effectively be used as a tool to determine the load capacity of bones altered by metastatic cancer, osteoporosis, or both.¹²³ Screening for prostate cancer by serum prostate-specific antigen (PSA) and digital rectal examination (DRE) is widely used. The PSA and DRE approaches predict either high-grade or clinically significant tumors. The nanoindentation technique is able to provide kinesthetic feedback from regions of prostate cancer tissues (ex vivo examination).⁷² The elastic moduli are higher in tissues with a Gleason score of 8 or with a tumor volume $>5\text{ cm}^3$ compared to normal tissues. Another study reported the indentation loading response of a resonance sensor used to discriminate between prostate cancer tissue specimens from ten patients and normal tissue.¹²⁰ A recent finding¹²⁴ confirmed that normal and benign tissues of human breast biopsies exhibit uniform stiffness profiles obtained in a single distinct peak, while malignant tissues have a broad distribution due to tissue heterogeneity (Fig. 5). Similar correlative observations were found in systematic experiments on MMTV-PyMT (mouse mammary tumor virus-polyoma middle T antigen) transgenic mice, which mimic early to metastatic human disease.

The validation of nanoindentation experiments can further be extended to measurement of the stiffness of leukocytes as compared to the microbead-conjugated cancer cells (circulated tumor cells, CTC).¹²⁵ Combining CTC isolation technology and nanoindentation hybrid platform will result in high recovery rates as well as information on the state of cancers for superior diagnostics.¹²⁵ Another group investigated nanoindentation experiments and drug-target interactions directly on patient cancer cells, which mimic human in vivo conditions.⁷¹ The Gaussian fit of the histogram of nanoindentation binding force of CD20-rituximab on B-cell lymphomas was much higher than normal cells.⁶⁴ This protocol was extended to measure CD-20-antibody interaction on the pathological samples derived from the bone marrow of a follicular lymphoma patient.^{11, 65} However, in some cases, nanomechanical analysis of cancer cells and their counterparts reveal similar elasticity, which may not be sufficient to distinguish.¹²⁶ Therefore, analyzing obtained nanomechanical analysis data with other complementary investigative biological studies is necessary for a better understanding of cellular elasticity and cancer.^{6, 12, 75}

7. CONCLUSIONS AND FUTURE PROSPECTS

Basic knowledge of bionanomechanical profiles of normal, benign, and malignant biopsies brings together biology and new technologies for improved diagnosis and therapies. Cellular response is manifested in several biochemical expressions as well as through changes in mechanical response to loading. This review summarizes the various elastic modulus data on cells and tissues necessary to establish differences between normal and diseased mammalian cells. The viscoelastic property bridges the gaps between cellular behavior of normal cells

and cancer phenotype cells. The mission is to understand the response of cancer cells in terms of nanomechanical properties to drug treatments. Such information can potentially help identify selective and ideal drug(s) that can be useful in clinical settings. Studying nanomechanical properties in three-dimensional (3D) cell culture or scaffold cell culture provides ideal clinical relevance. This information is essential to translate drugs for therapeutic application against cancer and to assess their therapeutic value. 3D measurements of the environment will also open up possible undiscovered novel mechanisms, resistance, and synergistic actions of drug molecules. In such circumstances where delivery of drug is an issue, utilizing cutting-edge NPs offers safe and efficient delivery of the “drug or therapeutic agent.”

Extrapolating nanoindentation-derived data may be complex when identifying the type of cancer(s) and its progression; however, other fundamental biological characteristics of cancer will enhance the broad spectrum of its utility. A possible drug response in cell culture model will be helpful in screening and simultaneous cellular structural analysis will discover suitable drug candidates. Utilizing an antibody-coated tip is distinctly useful to identify a better technique for targeting of cancer cells and their specificity. This technique is a complex dynamic process and implementation builds on combining various signaling cascades and microenvironments based on new advances in nanomechanic instrumentation and theories. Another concern is while this technique is highly important for the development of potent anticancer drugs, current understanding of its physiological role on cancer-related immune systems remains unknown. However, nanoindentation is a “see, identify and treat” technique among many biophysical tools that provides a safe tool in biomedical research for possible human therapeutic application. Highly reproducible strategies of nanoindentation are required for such therapeutic evaluations.

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Biographies

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Kalpana S. Katti is a University Distinguished Professor at NDSU in the Department of Civil Engineering. She joined NDSU in 1997 after receiving a Ph.D. from the University of Washington in 1996. Her primary area of research is in tissue engineering and biomimetics. This project involves design of living–nonliving constructs for replacement of human tissue. She also conducts several research projects in the fields of nanocomposites for structural and barrier applications as well as bionanocomposites such as bone and seashells. Her research has won several awards from professional societies such as Microscopy Society of America and Federation of Societies for Coatings Technology, and she has over 145 publications in journals, conference proceedings, and book chapters in the field. She has received the most cited award from the journal *Colloids and Interfaces* for the years 2004–2007. She is also an NSF CAREER award grantee. At NDSU she is engaged in faculty and administrator search committees, promotion and tenure, teaching, NSF ADVANCE, and several other committees at department, college, and university levels. She received the Peltier award for innovations in teaching in 2007 and the NDSU 52nd Faculty Lectureship in 2011. Nationally, Dr. Katti has served as chair of the Academic Affairs Committee of Materials Research Society, served on the Publications Committee, and is the 2015 volume organizer of Materials Research Society. She is currently chair of the Biomechanics Committee of American Society of Civil Engineers Engineering Mechanics Institutes and also Vice Chair of the Properties of Materials Committee. She has given over 40 invited lectures at national and international conferences and institutions around the world.

Dinesh Katti is a Professor of the Civil and Environmental Engineering Department at North Dakota State University in Fargo, ND. Dr. Katti's area of expertise is in multiscale modeling of nanoscale materials. His group has made important contributions through discoveries and revelations of key mechanisms that describe the critical role of molecular scale interactions and nano/microstructure on macroscale properties of nanoscale materials. His group works on a variety of nanoscale materials of diverse origins that include geologic (clays and oil shale), synthetic (polymer clay nanocomposites), and biological (bone and seashells) origins. He is an author of three books, seven book chapters, and approximately 150 papers. He has received the John Booker Excellence Award for contributions to geomechanics from IACMAG and the Fred Waldron Award for Excellence in Research. He serves as an associate editor of two journals, *Journal of Engineering Mechanics* and *Journal of Nanomechanics and Micromechanics*. He has served as chair of several national committees that include ASCE Properties of Materials Committee, Poromechanics Committee, and Molecular Scale Modeling and Experimentation Committee. He has been invited to give plenary and keynote talks around the world. He is a licensed professional engineer in the state of Washington and has worked on about 125 consulting projects that include projects for Boeing and Microsoft. Dr. Katti received his Doctoral degree in Civil Engineering and Engineering Mechanics from the University of Arizona, Master's degree in Civil Engineering from Indian Institute of Technology, Bombay, and Bachelor's degree in Civil Engineering from National Institute of Technology, Srinagar. He currently serves as Interim Chair of the Civil and Environmental Engineering Department. He has previously

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Sheema Khan is currently a Postdoctoral Fellow at the University of Tennessee Health Science Center. Sheema obtained her Ph.D. at the Indian Institute of Integrative Medicine, India. She worked on the development of anticancer therapeutics from natural products, in particular identifying immune modifiers and targeting critical tumor promoting pathways. During her postdoctoral fellowship in Dr. Chauhan’s lab, she has been working on early diagnosis, biophysical evaluation, and therapeutic interventions for cancer(s). Her research studies particularly involve the tumor stromal interactions, mucin biology, and microRNAs. Her work has been published in 11 peer-reviewed journals.

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Figure 1.

Use of atomic force microscopy (AFM) in medicinal research. (A) AFM as a dual mode system for imaging and measuring force of cells and tissues at a nanoscale level. (B) Number of peer-reviewed publications using AFM for imaging and force (mechanical, nanomechanics, and nanoindentation) with cell(s), tissue(s), and cancer(s). Data collected using PubMed search (as of December 15, 2013) with key words: atomic force microscope (for imaging); and mechanical properties, biomechanical properties, nanomechanics, and nanoindentation (for force measurements).

Figure 2.

(A) Schematic representation of imaging/nanoindentation of cells using atomic force microscopy and (B, C) the fitting of the force-indentation curve by Hertz model to generate the Young's modulus of cellular elasticity. Scheme and graphs in this figure are produced with special permission from 11.

Figure 3.

Cell-elastic population distribution and elastic modulus of various stages of mouse ovarian surface epithelial (MOSE) and cancer cells. Population distribution responses of (A) early, (B) intermediate, (C) late MOSE cells and the MOSE cancer stem cells/tumor-initiating cells (CSC/TICs) in (D) control, (E) differentiated, and (F) treated conditions. (G) Elastic modulus of MOSE cancer cells and three variants of MOSE CSC/TICs. *Note:* The tumor-initiating phenotype matches well in the order of cancer malignancy-mechanics functionality profile. Graphs in this figure are reproduced with permission from 10.

Figure 4.

Biomechanical profiles of cisplatin-sensitive and cisplatin-resistant ovarian cancer cells treated with cisplatin or cytod/cisplatin. *Note:* Data indicate cisplatin-induced increase in cell stiffness of OVCAR5 cells is mediated via actin cytoskeleton and disrupting the actin cytoskeleton with cytod treatment can reverse this cell stiffness. The graphs in this figure are reproduced with permission from 95.

Figure 5. Stiffness distribution for normal mammary gland tissue, benign lesion, and malignant tumor tissue is unimodal, unimodal but broader stiffness, and heterogeneous stiffness distribution with characteristic soft peaks, respectively. The H&E-stained section confirms tissue identification for normal mammary gland tissue, benign lesion, and malignant tumor tissue. Scale bar applies to all images, 50 μ m. Graphs in this figure are reproduced with permission from 124.

Table I
Simulated In Vivo Forces Significantly Alter the Rate of Proliferation with and Without Investigated Drug

Treatment	Growth rates ^a				Change in growth rate ^b	H358		
	Without forces	With forces	Change in growth rate ^b	Without forces	With forces	Change in growth rate ^b	Without forces	With forces
No drug	0.621 (0.602, 0.641)	0.247 (0.222, 0.272)	↓ (<i>P</i> < 0.001)	0.402 (0.391, 0.412)	0.091 (0.065, 0.117)	↓ (<i>P</i> < 0.001)		
Cisplatin	-0.204 (-0.302, -0.105)	-0.211 (-0.309, -0.113)	↔ (<i>P</i> = 0.09)	-0.027 (-0.080, 0.026)	-0.233 (-0.339, -0.126)	↓ (<i>P</i> = 0.001)		
Paclitaxel	0.133 (0.035, 0.232)	-0.038 (-0.136, 0.060)	↓ (<i>P</i> = 0.02)	-0.005 (-0.058, 0.048)	-0.276 (-0.379, -0.174)	↓ (<i>P</i> < 0.001)		
Doxorubicin	-0.170 (-0.268, -0.071)	-0.268 (-0.367, -0.170)	↔ (<i>P</i> = 0.2)	-0.031 (-0.084, 0.022)	-0.357 (-0.460, -0.254)	↓ (<i>P</i> < 0.001)		
KU-drug	-0.390 (-0.489, -0.291)	-0.464 (-0.562, -0.366)	↔ (<i>P</i> = 0.3)	-0.105 (-0.158, -0.052)	-0.337 (-0.440, -0.234)	↓ (<i>P</i> < 0.001)		
Zactima	-0.055 (-0.141, 0.031)	-0.398 (-0.486, -0.311)	↓ (<i>P</i> < 0.001)	-0.163 (-0.209, -0.117)	-0.833 (-0.927, -0.739)	↓ (<i>P</i> < 0.001)		

Negative growth rate represents a reduction in total viable cell count. Up arrow indicates a statistically significant increase, down arrow indicates a decrease, and horizontal arrows indicate no change in growth rates. This table is reproduced with permission from 98.

^a 95% confidence intervals are in parentheses next to the corresponding growth rate.

^b *P*-value from a *t*-test, which tested for differences between the growth rates in the absence of mechanical forces and the corresponding growth rates in the presence of forces.

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