Title: The Mechanics of Myeloid Cells

Authorship: Kathleen R Bashant[1,2,*], Nicole Topefner[3,4], Christopher J Day[5], Nehal N Mehta[6], Mariana J Kaplan[2], Charlotte Summers[1], Jochen Guck[7+], Edwin R Chilvers[8+]

Affiliations and Addresses: [1]Department of Medicine, University of Cambridge, Cambridge, UK. [2]Systemic Autoimmunity Branch, National Institute of Arthritis and Musculoskeletal and Skin Diseases, National Institutes of Health, Bethesda, MD, USA. [3]Center for Molecular and Cellular Bioengineering, Biotechnology Center, Technische Universität Dresden, Dresden, Germany. [4]Department of Pediatrics, University Clinic Carl Gustav Carus, Technische Universität Dresden, Dresden, Germany. [5]University of Sydney Business School ITLS, Sydney, Australia. [6]National Heart Lung and Blood Institute, National Institutes of Health, Bethesda, MD, USA. [7]Max-Planck-Institut für die Physik des Lichts & Max-Planck-Zentrum für Physik und Medizin, Erlangen, Germany. [8]National Heart and Lung Institute, Imperial College London.

[+]Individuals are co-senior authors and contributed equally to this work.

*Corresponding Author. Correspondence to Kathleen.Bashant@nih.gov. Telephone: (1)-240-535-3608

Abstract

The effects of cell size, shape, and deformability on cellular function have long been a topic of interest. Recently, mechanical phenotyping technologies capable of analyzing large numbers of cells in real time have become available. This has important implications for biology and medicine, especially haemato-oncology and immunology, as immune cell mechanical phenotyping, immunologic function, and malignant cell transformation are closely linked and potentially exploitable to develop new diagnostics and therapeutics. In this review, we introduce the technologies used to analyze cellular mechanical properties and review emerging findings following the advent of high throughput deformability cytometry. We largely focus on cells from the myeloid lineage, which are derived from the bone marrow and include macrophages, granulocytes and erythrocytes. We highlight advances in mechanical phenotyping of cells in suspension that are revealing novel signatures of human blood diseases and providing new insights into pathogenesis of these diseases. The contributions of mechanical phenotyping of cells in suspension to our understanding of drug mechanisms, identification of novel therapeutics and monitoring of treatment efficacy particularly in instances of hematologic diseases are reviewed, and we suggest emerging topics of study to explore as high throughput deformability cytometers become prevalent in laboratories across the globe.

Introduction: Past & Present Tools to Study Cellular Mechanics

The study of cellular mechanical properties stretches back to the advent of microbiology. Leeuwenhoek's earliest observations concerned the shape and size of his "animalcules" as viewed through a light microscope (Egerton, 2006). Early scientists discussed the concepts of cellular viscosity (Heilbrunn, 1927) and elasticity (Treitel, 1944). Viscosity was measured by centrifuging cells and analyzing the time required for granules to return to their original positions (Heilbrunn, 1927). Micropipette aspiration allowed for measurement of cellular and nuclear deformation in response to suction (Evans and Hochmuth, 1976; Hochmuth, 2000). While historical mechanical biology research has been adeptly summarized elsewhere (Pelling and Horton, 2008) powerful new techniques have since been developed.

In recent years, development of advanced technology has allowed for the quantitative assessment of cellular mechanical properties and an increase in research output on this topic [Figure 1]. First developed in 1986, atomic force microscopy (AFM) measures cellular elasticity by quantifying the force on the tip of a probe as it pushes into a sample, generating a topographic map of the cell's surface (Binnig et al., 1986). AFM has highlighted the mechanical nature of the cell and the interplay between structure and function of molecular motors (Taniguchi et al., 2003; Kodera et al., 2003), the cell surface (Pelling et al., 2004; Roduit et al., 2015; Roca-Cusachs et al., 2006), membrane proteins (Horton et al.,

2002), and other cellular components (Sattin and Goh, 2004; Kasas et al., 2005). Although extremely detailed and extensive information is collected, cells must be immobilized, which has implications for accurate analyses of cells in suspension (as in blood). Furthermore, AFM does not allow for the analysis of large numbers of individual cells (Müller and Dufrêne, 2011). Other tools to measure cellular mechanics include contact-based magnetic bead rheology (Wang et al., 1993), contact-free optical tweezers (Sleep et al., 1999) and the optical stretcher (Guck et al., 2001). Optical tweezers are an example of a laser trap; a single beam of light carries momentum, which is transferred to a sample as force. Sample forces can then be quantified; for example, the force of a swimming sperm (Tadir et al., 1990) or the force of a molecular motor (Block et al., 1990). The optical stretcher is based on a double beam laser trap and was developed to fill the gap between optical tweezers and AFM (Guck et al., 2001; Faigle et al., 2014). This tool allows for quantification of contact free cell deformation at speeds of one cell every few seconds, when coupled to an automatic flow chamber (Guck et al., 2005). For a good comparison of these standard techniques to measure cell mechanical properties, the reader is referred to Wu et al., 2018.

Recently, the development of high throughput, microfluidics-based deformability cytometry (DC) techniques has allowed for mechanical analysis of virtually limitless cell numbers in real time. For these analyses, cells must be in suspension (*e.g.* as in blood or purified cells that have been resuspended). One method of high-throughput DC measures the deformation of cells by deceleration as they encounter an opposing flow [Figure 2A] (Gossett et al., 2012; Tse et al., 2013). Real-time deformability cytometry (RT-DC) as introduced by Otto *et al*, similarly deforms individual cells by shear stresses and pressure gradients as they flow through a microfluidic channel [Figure 2B] (Otto et al., 2015). Multi-sample deformability cytometry utilizes a microfluidic chamber resembling that of Otto et al, which allows for analysis of up to ten clinical samples of cells in suspension within a single experiment (Ahmmed et al., 2018). There have been several other, related techniques published in recent years (Rosendahl et al., 2018; Guck, 2019).

DC compliments the strengths and weaknesses of AFM. AFM achieves nanoscale resolution levels, but the cells must be immobilized and the time for analysis of a single cell can reach minutes. Even recent advances with scanning probe AFM require time scales in the milliseconds (Müller and Dufrêne, 2011). In contrast, DC measures over 1000 individual cells per second and also allows for analysis of heterogenous samples in suspension, including whole blood (Tse et al., 2013; Ahmmed et al., 2018; Otto et al., 2015). This suggests opportunities for clinical adaptations (Guck, 2019). However, because DC analyzes a single mechanical signature per cell, resolution is lower and the mechanical information is less detailed. Furthermore, DC relies on a model of a relatively uniform cell to calculate stiffness and volume. Thus, mechanical analysis capabilities of non-uniform cells by DC are currently limited (Otto et al., 2015). As such, AFM will remain a vitally important tool in the study of cell mechanics, however the capability of DC to achieve analysis of millions of cells per minutes, in line with flow cytometers, is opening the door to some fascinating research and clinical opportunities (Guck, 2019).

Cellular mechanical properties alter during processes including differentiation, motility, cell cycle and cell activation, and there is an increasing appreciation of the biomechanical changes in cells during states of disease (Preira et al., 2013; Toepfner et al., 2018). Thus, high throughput mechanical phenotyping has enabled new insights into basic immunology (*e.g.* cell cycle biology and cell differentiation) as well as clinical medicine (*e.g.* malignant transformation in hematology and oncology). In this review we will cover recent advances in our understanding of cell mechanical properties, with a focus on disease in the context of high throughput techniques. Cells from the lymphoid lineage, including lymphocytes and NK cells, have been mechanically phenotyped (Bufi et al., 2015, Toepfner et al., 2018), but this review largely focuses on myeloid cells and specifically on monocytes and granulocytes.

Mechanical Evaluation of Myeloid Cell Differentiation

The differentiation of progenitor cells is coupled to major changes in their mechanical properties. This is partially due to differences in intracellular organization and composition (Darling et al., 2008; Yourek et al., 2007; Lin et al., 2017). In some instances, progenitor mechanical properties can serve as predictive biomarkers for differentiation outcomes (González-Cruz et al., 2012). Neutrophils, monocytes and macrophages develop from a common myeloid progenitor (Weiskopf et al., 2016), and mature monocytes, macrophages, and neutrophils have been successfully distinguished from progenitors and from each other based solely on mechanical properties analyzed by an optical stretcher (Ekpenyong et al., 2012).

Although canonically cells stiffen as they differentiate, due to an increase in cellular cytoskeletal content (Darling et al., 2008; Yu et al., 2010), high throughput mechanical phenotyping has increasingly demonstrated the opposite phenomenon in neural cells (Urbanska et al., 2017). Similarly, neutrophils (Lautenschläger et al., 2009; Tsai et al., 1996) have been reported to soften as they age. Immature myeloid precursor cells are unable to migrate throughout the body and are relatively resistant to deformation (Lichtman and Kearney, 1970). In contrast, mature neutrophils are responsible for actively monitoring distant sites of the body for infection and other danger signals, and need the capacity to move swiftly through large blood vessels, squeeze between epithelial cells, and migrate through narrow microcapillary networks (Schmid and Varner, 2007). Although classical descriptions of myeloid cell trafficking depend largely on biochemical mediators, specifically selectins, chemokines and cell adhesion molecules and their respective receptors (Schmid and Varner, 2007, 2012), it is becoming more appreciated that cellular remodeling and mechanical changes are also essential for motility (Suresh, 2007). Thus, the mature neutrophil becoming progressively more deformable than its progenitors allows the cell to take on its functional duties.(Lichtman and Kearney, 1970). Pharmacological stabilization of microtubules in differentiated neutrophils interferes with deformation and remodeling, and thus slows motility through a 3D system (Lautenschläger et al., 2009). Other characteristics facilitating the deformability of mature neutrophils include the distinctive multi-lobulated nucleus (Olins

et al., 2008; Lammerding, 2011) and the down-regulation of cytoskeletal proteins including vimentin (Olins et al., 2009).

Mechanics in Immunologic Function of Myeloid Cells

In addition to trafficking through microcapillaries, myeloid cells must be capable of a diverse range of carefully modulated effector functions. Exposure of neutrophils to pro-inflammatory cytokines or chemokines (*e.g.* tumor necrosis factor, N-formylmethionine leucyl-phenylalanine, or granulocyte macrophage colony-stimulating factor) induces transition to a 'primed' state. Priming is characterized by increased responsiveness to agonist stimulations and a 'polarized' or 'shape-changed' morphology (Condliffe et al., 1998; Hallett and Lloyds, 1995; Sapey and Stockley, 2014). Priming can also be mediated by mechanical stimulation (Ekpenyong et al., 2017). A second stimulation of the primed neutrophil enhances its functional response, promoting degranulation, NADPH oxidase activation, phagocytosis, neutrophil extracellular trap formation, and other key immunologic defense mechanisms (van der Linden and Meyaard 2016). Priming induces neutrophil mechanical changes, though studies conflict, with reports of neutrophils becoming both smaller and stiffer (Pai et al., 2008; Roca-Cusachs et al., 2006; Worthen et al., 1989; Hiramatsu et al., 2005) or larger and softer (Denk et al., 2017). RT-DC revealed that neutrophil priming via several different stimuli induces a common series of phenotypic changes, with an initial phase of contraction and stiffening lasting one to five minutes, followed by a phase of expansion and increased deformability achieved within fifteen to thirty minutes post-priming. Neutrophils simultaneously became shape changed in a matter of seconds but returned to their original morphologic phenotype within 2-5 hours (Bashant et al., 2019). This finding aligns with previous reports of neutrophil "de-priming" (Ekpenyong et al., 2017; Kitchen et al., 1996). Thus, high-throughput DC demonstrated primed neutrophils could be stiffer or softer depending on the time frame at which cells were analyzed (Bashant et al., 2019). Similarly, in the context of macrophages and dendritic cells, some inflammatory mediators have been reported to stiffen cells while others have the opposite effect (Bufi et al., 2015). It is important to consider kinetics as well as differences in immune cell types and in inflammatory pathways when assessing immunologically-driven mechanical changes.

The mechanics of monocyte activation has been similarly debated. A study in which monocytes were activated with lipopolysaccharide and probed with a cell poker suggested that activated monocytes are stiffer and increasingly retained in the microcapillary bed (E Doherty et al., 1994). A second study employing micropipette aspiration demonstrated that stimulation of monocytes with pro-inflammatory linoleic acid increases monocyte deformability, and the subsequent retention of these cells in the microcapillary bed may drive deformability-driven increases in the surface area available for adherence to the endothelium (Rinker et al., 2004). Interestingly, and like the mechanokinetics of activated neutrophils (Bashant et al., 2019), it was reported that immortalized monocytes stiffen and then soften

when intracellular free calcium levels are increased (Richelme et al., 2000). Other studies showed that monocytes stimulated with inflammatory cytokines are more deformable but less compressible, potentially explaining the above contrasting studies, which did not use techniques capable of analyzing compressive and shear deformation separately (Ravetto et al., 2014).

Myeloid cell deformability has a marked impact on trafficking through the human body. Following neutrophil adherence to the endothelium, cells deform in a matter of seconds, appearing to expand in area by unfurling plasma membrane. This process is necessary for extravasation (Dewitt et al., 2013). Mechanical changes are largely driven by cytoskeletal reorganization and actin polymerization (Renkawitz and Sixt, 2010). The complement activation product and chemoattractant C5a reorganizes the actin cytoskeleton, increases neutrophil deformability within five minutes, and induces migration of neutrophils into inflammatory sites (Denk et al., 2017). Recently, RT-DC demonstrated that chemoattractants chemokine (C-X-C motif) ligand 16 and interleukin-8 enhance neutrophil deformation similarly to C5a, thereby facilitating transmigration through vessel walls (Steffen et al., 2018).

Each cardiac cycle, the entire cardiac output flows through the lung microvasculature. This microvasculature is a complex network of particularly narrow capillaries (Hogg and Doerschuk, 1995). Cytoskeletal stiffening, as it occurs during the first phase of neutrophil priming (Bashant et al., 2019), promotes the sequestration of neutrophils in the lung pulmonary microvasculature (Yoshida et al., 2006). There is both *in vitro* and *in vivo* evidence to indicate that if the priming stimulus is removed, primed neutrophils gradually de-prime, reverting to a quiescent state resembling unprimed neutrophils. With regards to immunologic function, this entails decreased responsiveness to agonists and with respect to morphology, the smoother, "non-polarized" shape of de-primed neutrophils leads to escape from entrapment in the microvasculature (Kitchen et al., 1996). When primed neutrophils are mechanically perturbed by an optical stretcher, active depolarization occurs two orders of magnitude faster than spontaneous de-priming (Ekpenyong et al., 2017).

To further explore this phenomenon *in vitro,* microcirculation mimetics have been employed. These mimetics are physical conduits intended to imitate the pulmonary microcirculation. When neutrophils were trafficked through the microcirculation mimetic, polarized primed neutrophils were hindered more than the smooth, unprimed cells. However, within ten minutes of their repeated compression and release by the mimetic's constrictions, primed neutrophils reverted to a round phenotype, and molecular evidence indicated they had become de-primed. These results confirm the data obtained from the optical stretcher (Ekpenyong et al., 2017). This process, in which a physical force elicits a functional response, is an example of mechanotransduction (Ekpenyong et al., 2015; Mennens et al., 2017; Novikova et al., 2018).

In vivo, radiolabeling studies have demonstrated that primed neutrophils are retained in the lungs, while de-primed neutrophils traffic similarly to their un-primed counterparts (Summers et al., 2014). These studies contribute to the hypothesis that repeated

advection of primed neutrophils through the pulmonary microvasculature may serve to mechanically de-prime neutrophils, thus allowing for regulation of the immune system (Summers et al., 2014, 2014b). This is predicted to have implications for respiratory diseases including acute lung injury and chronic obstructive pulmonary disease (COPD) (Singh et al., 2012).

The Mechanical Signature of Myeloid Cells Associated with Different Diseases

High throughput mechanical phenotyping has begun to identify myeloid cell mechanical signatures present in specific disease states (Toepfner et al., 2018). Neutrophil size increases in trauma patients prior to organ dysfunction (Hesselink et al., 2019) and neutrophil deformability decreases in patients with chronic renal failure (Skoutelis et al., 2000). Treatment of neutrophils and monocytes with serum from patients with acute respiratory distress syndrome induces rapid cellular stiffening (Preira et al., 2016). In line with this observation, RT-DC showed that patients with acute lung injury (ALI) and patients hospitalized with viral respiratory tract infections (RTI) have larger and more deformable neutrophils in their blood than healthy volunteers (Toepfner et al., 2018). This may be due to sequestration and mechanical de-priming of stiff, shape-changed neutrophils in the pulmonary vasculature, and thus a population of larger, softer neutrophils in the circulation (Summers et al., 2014, 2014b; Singh et al., 2012).

Mechanical phenotyping has further demonstrated that monocytes are larger in the blood of both ALI and RTI patients but more deformable only in the blood of patients with viral RTI. Patients infected with Epstein Barr virus exhibited more deformable circulating monocytes, but circulating neutrophils showed less of a response (Toepfner et al., 2018). Eosinophils isolated from patients with asthma are rougher than those from healthy volunteers, but do not differ in size or deformation (Porter et al., 2018). Such phenotypic analysis, in which blood cells including both erythrocytes and immune cells are mechanically profiled, has been termed morpho-rheologic phenotyping, or MORE phenotyping. By analyzing multiple mechanical parameters of multiple blood cell types, researchers hope to develop MORE phenotyping as a diagnostic strategy (Toepfner et al., 2018).

MORE phenotyping also has obvious implications in the field of cancer biology. Somatic cancer cells are more deformable than their healthy counterparts, which may facilitate their metastases to other sites in the body (Cross et al., 2007; Park et al., 2016). By analysis of mechanical properties alone, DC allows for identification of malignant cell subpopulations in mixed blood samples (Tse et al., 2013; Remmerbach et al., 2009). Recently, MORE analysis of blood from patients with acute myeloid (AML) and lymphatic leukemia (ALL) identified characteristic immature blast cells (Zheng et al., 2015; Berger et al., 1994) and characterized them mechanically. In contrast to the general trend in cancer cell mechanics (Guck and Chilvers, 2013; Kumar and Weaver, 2009; Guck et al., 2005), RT-DC confirmed AML and ALL blast cells are stiffer than healthy myeloid cells and healthy lymphoid cells respectively (Toepfner et al., 2018; Ekpenyong et al., 2012; Lautenschläger et al., 2009; Lichtman and Kearney, 1970). Emerging research in this area could have

implications for how we advance initial diagnostics as well as monitor and treat critically ill patients to improve their prognosis.

Pharmacological Effects on Myeloid Cell Mechanics

High-throughput mechanical phenotyping is increasing understanding of the cellular effects of therapeutic strategies. For example, treatment with the cancer therapeutic paclitaxel stabilizes cell microtubule polymers and increases cytoplasmic complex shear modulus (*i.e.* strain response to shear stress, a measure of stiffness) (El Kaffas et al., 2013). Treatment with chemotherapy increases AML blast cell differential stiffness (Toepfner et al., 2018), which is important because increased stiffness has been implicated in development of hyper-leukocytosis, or leukostasis (Lam et al., 2007, 2008) and cell transit through a microvasculature mimetic (Rosenbluth et al., 2008). Especially in patients with AML, leukostasis is a life-threatening complication in which cells aggregate in the vasculature, leading to respiratory failure (Porcu et al., 2000). The possibility of employing high-throughput mechanical phenotyping to assess leukocytosis risk and guide treatment of these critically ill patients has been discussed (Toepfner et al., 2018). Treatment of AML cells with pentoxifylline, a phosphodiesterase inhibitor, attenuates cell stiffness and thereby decreases cellular transit time through the mimetic (Craciun et al., 2013). In this respect, changes in cellular mechanical properties could also be used to anticipate or monitor patients' response to chemotherapy.

Beyond cancer therapies, it has long been known that glucocorticoids induce de-margination of leukocytes from the microcirculation and vascular walls (Fauci, 1978). This elevates the white blood cell count within the clinical blood count (Nakagawa et al., 1998). Historically, this phenomenon has been attributed to down-regulation of selectins and other adhesion molecules (Weber et al., 2004), though some evidence refutes this (Jilma and Stohlawetz, 1998; Doyle et al., 1997). Recently, it was demonstrated that glucocorticoids and catecholamines mediate granulocyte cytoskeleton remodeling and softening. As a result, granulocytes move more swiftly through capillary beds and re-enter the circulation from marginated intravascular pools. These softer cells also demarginate to a position of equilibrium closer to the vessel center than stiffer counterparts, perhaps mitigating extravasation (Fay et al., 2016). Thus, a potential mechanical solution sheds new insight into the mechanism of action of glucocorticoids.

Studies have already used measures of neutrophil deformability to assess drug efficacy in deactivating neutrophils and increasing cellular movement through the microcirculation (Craciun et al., 2013). Milrinone, piclamilast, urinastatin, ketamine, protein C concentrate, and nitric oxide donor FK 409 are drugs that showed promise in reducing inflammation, and mechanical phenotyping demonstrates that these drugs successfully reduce neutrophil deformability in both neonates and adults (Craciun et al., 2013). Future wide-scale testing of the effect of various drugs on myeloid cell mechanical properties may allow for

discovery of new compounds capable of mitigating inflammation or achieving other desired outcomes.

Future of Mechanical Phenotyping: Towards New Diagnostics and Treatments.

High throughput mechanical phenotyping techniques have only been available in recent years. The capabilities of cellular phenotyping for new diagnostics, bedside monitoring of critical conditions including sepsis and leukostasis, assessing patient response to treatment, and scanning compound libraries for potential novel therapeutic options are in their infancy. In the context of basic and translational science, high throughput mechanical phenotyping will increasingly allow research labs to compare purified cells to their pre-processed counterparts found in whole blood. It will further improve isolation techniques, allowing for experiments on cells in suspension (such as cells in bone marrow aspirates, blood, pleura fluid etc.) that that have not been disrupted from their *in vivo* state and allow thus for the characterization of *in vivo* cell functions in health and diseases. These high-throughput techniques could continue to be used in parallel with AFM to achieve a complete picture of cell mechanical properties.

From a clinical standpoint, although MORE phenotyping is a relatively new phenomenon, the high-throughput capabilities of DC mean that we may, very soon, be capable of distinguishing between bacterial and viral infections, between infection, autoimmune and autoinflammatory disease states, and anticipating critical clinical developments such as septic shock. If these aims are feasible, it will most likely be achieved through analysis of multiple phenotypic traits for each of the cell populations found in human blood. Such analysis could be aided considerably by the addition of machine learning to DC set-ups. There may be other mechanical properties (e.g. density, cytoplasmic viscosity) that are analyzable in the time frames dictated by DC, and machine learning could serve to identify and quantify these characteristics (Ge et al., 2019).

This is an exciting time for mechanical phenotyping. As high throughput deformability cytometers become prevalent in laboratories across the globe, we expect numerous discoveries that will tangibly aid our basic research efforts and how we understand and potentially treat diseases.

Abbreviations:

AFM: atomic force microscopy

ALI: acute lung injury

AML: acute myeloid leukemia

ALL: acute lymphatic leukemia

DC: deformability cytometry

RT-DC: real-time deformability cytometry

RTI: respiratory tract infections

MORE: morpho-rheologic

References:

Landmark/milestone references of interest marked by *

Ahmmed, S.M., Bithi, S.S., Pore, A.A., Mubtasim, N., Schuster, C., Gollahon, L.S. and Vanapalli S.A. (2018) Multi-sample deformability cytometry of cancer cells. APL Bioengineering **2**, 032002

Bashant, K.R., Vassallo, A., Herold, C., Berner, R., Menschner, L., Subburayalu, J., Kaplan, M.J., Summers, C., Guck, J., Chilvers, E.R. and Toepfner N. (2019) Real-time deformability cytometry reveals sequential contraction and expansion during neutrophil priming. J Leukoc Biol. **105**, 1143-53

Berger, M., Motta, C., Boiret, N., Aublet-Cuvelier, B., Bonhomme, J. and Travade P. (1994) Membrane Fluidity and Adherence to Extracellular Matrix Components Are Related to Blast Cell Count in Acute Myeloid Leukemia. Leukemia & Lymphoma **15**, 297-302

Binnig, G., Quate, C.F. and Gerber C. (1986) Atomic Force Microscope. Physical Review Letters **56**, 930-33

Block, S.M., Goldstein, L.S.B. and Schnapp B.J. (1990) Bead movement by single kinesin molecules studied with optical tweezers. Nature **348**, 348-52

Bufi, N., Saitakis, M., Dogniaux, S., Buschinger, O., Bohineust, A., Richert, A., Maurin, M., Hivroz, C. and Asnacios A. (2015) Human Primary Immune Cells Exhibit Distinct Mechanical Properties that Are Modified by Inflammation. Biophys J **108,** 2181-90

* Condliffe, A.M., Kitchen, E. and Chilvers E.R. (1998) Neutrophil Priming: Pathophysiological Consequences and Underlying Mechanisms. Clinical Science **94**, 461

Craciun, E.M., Altfelder, F., Kuss, N., Poeschl, J. and Ruef P. (2013) Anti-inflammatory effects of selected drugs on activated neonatal and adult neutrophils. Scandinavian Journal of Clinical and Laboratory Investigation **73**, 407-13

Cross, S.E., Jin, Y., Rao, J. and Gimzewski J.K. (2007) Nanomechanical analysis of cells from cancer patients. Nature Nanotechnology **2**, 780

Darling, E.M., Topel, M., Zauscher, S., Vail, T.P. and Guilak F. (2008) Viscoelastic properties of human mesenchymally-derived stem cells and primary osteoblasts, chondrocytes, and adipocytes. Journal of Biomechanics **41**, 454-64

Denk, S., Taylor, R.P., Wiegner, R., Cook, E.M., Lindorfer, M.A., Pfeiffer, K., Paschke, S., Eiseler, T., Weiss, M., Barth, E., Lambris, J.D., Kalbitz, M., Martin, T., Barth, H., Messerer, D.A.C., Gebhard F. and Huber-Lang M.S. (2017) Complement C5a-

Induced Changes in Neutrophil Morphology During Inflammation. Scandinavian Journal of Immunology **86**, 143-55

Dewitt, S., Francis, R.J. and Hallett M.B. (2013) Ca2+ and calpain control membrane expansion during the rapid cell spreading of neutrophils. J. Cell Sci. **126**, 4627-35

Doyle, N.A., Bhagwan, S.D., Meek, B.B., Kutkoski, G.J., Steeber, D.A., Tedder, T.F. and Doerschuk C.M. (1997) Neutrophil margination, sequestration, and emigration in the lungs of L-selectin-deficient mice. The Journal of Clinical Investigation **99**, 526-33

E Doherty, D., Downey, G., Schwab, B., Elson, E. and Worthen G.S. (1994) Lipolysaccharide-induced monocyte retention in the lung: Role of monocyte stiffness, actin assembly, and CD18-dependent adherence. Journal of Immunology **153**, 241-55

Egerton, F.N. (2006) A History of the Ecological Sciences, Part 19: Leeuwenhoek's Microscopic Natural History. Bulletin of the Ecological Society of America **87**, 47-58

* Ekpenyong, A.E., Toepfner, N., Chilvers, E.R. and Guck J. 2015. Mechanotransduction in neutrophil activation and deactivation. Biochimica et Biophysica Acta (BBA) - Molecular Cell Research **1853**, 3105-16

* Ekpenyong, A.E., Toepfner, N., Fiddler, C., Herbig, M., Li, W., Cojoc, G., Summers, C., Guck, J. and Chilvers E.R. (2017) Mechanical deformation induces depolarization of neutrophils. Science Advances. **3**

Ekpenyong, A.E., Whyte, G., Chalut, K., Pagliara S., Lautenschläger, F., Fiddler, C., Paschke, S., Keyser, U.F., Chilvers, E.R. and Guck J. (2012) Viscoelastic properties of differentiating blood cells are fate- and function-dependent. PLOS ONE **7**, e45237-e37

El Kaffas, Ahmed, Bekah, D., Rui, M., Kumaradas, J.C. and Kolios M.C. (2013) Investigating longitudinal changes in the mechanical properties of MCF-7 cells exposed to paclitaxol using particle tracking microrheology. Physics in Medicine and Biology. **58**, 923-36

Evans, E.A. and Hochmuth R.M. (1976) Membrane viscoelasticity. Biophysical Journal **16**, 1-11

Faigle, C., Lautenschlaeger, F., Whyte, G., Homewood, P., Martín-Badosa, E. and Guck J. (2014) A monolithic glass chip for active single-cell sorting based on mechanical phenotyping. Lab Chip. **15**

Fauci, A.S. (1978) Mechanisms of the Immunosuppressive and Anti-Inflammatory Effects of Glucorcorticosteroids. Journal of Immunopharmacology **1**, 1-25

Fay, M.E., Myers, D.R, Kumar, A., Turbyfield, C.T, Byler, R., Crawford, K., Mannino, R.G., Laohapant, A., Tyburski, E.A., Sakurai, Y., Rosenbluth, M.J., Switz, N.S., Sulchek, T.A., Graham, M.D. and Lam W.A. (2016) Cellular softening mediates leukocyte

demargination and trafficking, thereby increasing clinical blood counts. Proceedings of the National Academy of Sciences **113**, 1987

Ge, Y., Rosendahl, P., Duran, C., Toepfner, N., Ciucci, S., Guck, J. and Cannistracis C.V. (2019) Cell Mechanics Based Computational Classification of Red Blood Cells Via Machine Intelligence Applied to Morpho-Rhelological Markers. IEEE/ACM Trans Comput Biol Bioinform. doi:10.1109/TCBB.2019.2945762

González-Cruz, R.D., Fonseca, V.C. and Darling E.M. (2012) Cellular mechanical properties reflect the differentiation potential of adipose-derived mesenchymal stem cells Proceedings of the National Academy of Sciences of the United States of America. **109,** E1523-E29

Gossett, D.R., Tse, H.T.K., Lee, S.A., Ying, Y., Lindgren, A.G., Yang, O.O., Rao, J., Clark, A.T. and Carlo D.D. (2012) Hydrodynamic stretching of single cells for large population mechanical phenotyping. Proceedings of the National Academy of Sciences. **109**, 7630

* Guck, J., Ananthakrishnan, R., Mahmood, H., Moon, T.J., Cunningham, C.C. and Käs J. (2001) The Optical Stretcher: A Novel Laser Tool to Micromanipulate Cells. Biophysical Journal **81**, 767-84

Guck, J. and Chilvers E.R. (2013) Mechanics Meets Medicine. Science Translational Medicine **5**, 212fs41

Guck, J., Schinkinger, S., Lincoln, B., Wottawah, F., Ebert, S., Romeyke, M., Lenz, D., Erickson, H.M., Ananthakrishnan, R., Mitchell, D., Käs, J., Ulvick, S. and Bilby C. (2005) Optical Deformability as an Inherent Cell Marker for Testing Malignant Transformation and Metastatic Competence. Biophysical Journal **88**: 3689-98

Guck, J. (2019) Some Thoughts on the Future of Cell Mechanics. Biophysics Reviews 10.1007/s12551-019-00597-0

* Hallett, M.B. and Lloyds D. 1995. Neutrophil priming: the cellular signals that say 'amber' but not 'green'. Immunology Today **16**, 264-68

Heilbrunn, L.V. (1927) The Viscosity of Protoplasm. The Quarterly Review of Biology **2**, 230-48.

Hesselink, L., Heeres, M., Paraschiakos, F., ten Berg, M., Huisman, A., Hoefer, I.E., de Groot, M.C.H., van Solinge, W.W., Dijkgraaf, M., Hellebrekers, P., Van Wessem, K.J.P., Koenderman, L., Leenen, L.P.H and Hietbrink F. (2019) A Rise in Neutrophil Cell Size Precedes Organ Dysfunction After Trauma. Shock **51**, 439-46

Hiramatsu, Y., Homma, S., Sato, Y., Sato, S., Matsuzaki, K., Shigeta, O. and Sakakibara Y. (2005) Nafamostat Preserves Neutrophil Deformability and Reduces Microaggregate Formation During Simulated Extracorporeal Circulation. The Annals of Thoracic Surgery **79**, 1326-32

Hochmuth, R.M. (2000) Micropipette aspiration of living cells. Journal of Biomechanics **33**, 15-22

Hogg, J.C. and Doerschuk C.M. (1995) Leukocyte Traffic in the Lung. Annual Review of Physiology **57**, 97-114

Horton, M., Charras G. and Lehenkari P. (2002) Analysis of Ligand-Receptor Interactions in Cells by Atomic Force Microscopy. Journal of Receptors and Signal Transduction **22**, 169-90

Jilma, B. and Stohlawetz P. (1998) Dexamethasone Downregulates l-Selectin In Vitro and In Vivo. Circulation **97**: 2279a-81

Kasas, S., Wang, X., Hirling, H., Marsault, R., Huni, B., Yersin, A., Regazzi, R., Grenningloh, G., Riederer, B., Forrò, L., Dietler, G. and Catsicas S. (2005) Superficial and deep changes of cellular mechanical properties following cytoskeleton disassembly. Cell Motility **62**, 124-32

Kitchen, E., Rossi, A.G., Condliffe, A.M., Haslett, C., and Chilvers E.R. (1996) Demonstration of reversible priming of human neutrophils using platelet- activating factor. Blood **88**, 4330

Kodera, N., Kinoshita, T., Ito, T. and Ando, T. (2003) High-Resolution Imaging of Myosin Motor in Action by a High-Speed Atomic Force Microscope. In Molecular and Cellular Aspects of Muscle Contraction (Sugi, H. eds.), pp. 119-27, Boston, MA: Springer US.

* Kumar, S. and Weaver V.M. (2009) Mechanics, malignancy, and metastasis: The force journey of a tumor cell. Cancer and Metastasis Reviews **28**, 113-27

Lam, W.A., Rosenbluth M.J. and Fletcher D.A. (2007) Chemotherapy exposure increases leukemia cell stiffness. Blood **109**, 3505-08

Lam, W.A., Rosenbluth, M.J. and Fletcher D.A. (2008) Increased leukaemia cell stiffness is associated with symptoms of leucostasis in paediatric acute lymphoblastic leukaemia. British Journal of Haematology **142,** 497-501

* Lammerding, J. (2011) Mechanics of the nucleus. Comprehensive Physiology **1**, 783-807

Lautenschläger, F., Paschke, S., Schinkinger, S., Bruel, A., Beil, M. and Guck J. (2009) The regulatory role of cell mechanics for migration of differentiating myeloid cells. Proceedings of the National Academy of Sciences of the United States of America **106**, 15696-701

Lichtman, M.A. and Kearney E. (1970) Cellular Deformability during Maturation of the Myeloblast. New England Journal of Medicine **283**, 943-48

Lin, J., Kim, D., Tse, H.T., Tseng, P., Peng, L., Dhar, M., Karumbayaram, S. and Carlo D.D. (2017) High-throughput physical phenotyping of cell differentiation. Microsystems & nanoengineering **3**, 17013-13

Mennens, S.F.B., van den Dries, K. and Cambi A. (2017) Role for Mechanotransduction in Macrophage and Dendritic Cell Immunobiology. In Macrophages: Origin, Functions and Biointervention (Kloc, M. eds.), Vol 62, Springer International Publishing: Cham.

Müller, D.J. and Dufrêne Y.F. (2011) Atomic force microscopy: a nanoscopic window on the cell surface. Trends in Cell Biology **21**, 461-69

Nakagawa, M., Terashima, T., D'yachkova, Y., Gregory, P.B., James, C.H. and van Eeden Stephan F. (1998) Glucocorticoid-Induced Granulocytosis. Circulation **98**, 2307-13

Novikova, O.A., Laktionov, P.P. and Karpenko, A.A. (2018) The roles of mechanotransduction, vascular wall cells, and blood cells in atheroma induction. Vascular **27**, 98-109

Olins, A.L., Hoang, T.V., Zwerger, M., Herrmann, H., Zentgraf, H., Noegel, A.A., Karakesisoglou, I., Hodzic, D. and Olins D.E. (2009) The LINC-less granulocyte nucleus. European Journal of Cell Biology **88**, 203-14

Olins, A.L., Zwerger, M., Herrmann, H., Zentgraf, H., Simon, A.J., Monestier, M. and Olins D.E. (2008) The human granulocyte nucleus: Unusual nuclear envelope and heterochromatin composition. European Journal of Cell Biology **87**, 279-90

* Otto, O., Rosendahl, P., Mietke, A., Golfier, S., Herold, C., Klaue, D., Girardo, S., Pagliara, A., Ekpenyong, A., Jacobi, A., Wobus, M., Topfner, N., Keyser, U.F., Mansfeld, J., Fischer-Friedrich, E. and Guck J. (2015) Real-time deformability cytometry: on-the-fly cell mechanical phenotyping. Nat Meth **12**, 199-202

Pai, A., Sundd, P. and Tees D.F.J. (2008) In situ Microrheological Determination of Neutrophil Stiffening Following Adhesion in a Model Capillary. Annals of Biomedical Engineering **36**, 596-603

* Park, E.S., Jin, C., Guo, Q., Ang, R.R., Duffy, S.P., Matthews, K., Azad, A., Abdi, H., Todenhöfer, T., Bazov, J., Chi, K.N., Black, P.C. and Ma H. (2016) Continuous Flow Deformability-Based Separation of Circulating Tumor Cells Using Microfluidic Ratchets. Small **12**, 1909-19

Pelling, A.E. and Horton M.A. (2008) An historical perspective on cell mechanics. Pflügers Archiv - European Journal of Physiology **456**, 3-12

Pelling, A.E., Sehati, S., Gralla, E.B., Valentine, J.S. and Gimzewski J.K. (2004) Local Nanomechanical Motion of the Cell Wall of *Saccharomyces cerevisiae*. Science **305**, 1147

Preira, P., Forel, J.M., Robert, P., Negre, P., Bairnes-Pelicot, M., Xeridat, F., Bongrand, P., Papazian, L. and Theodoly O. (2016) The Leukocyte-Stiffening Property of Plasma in
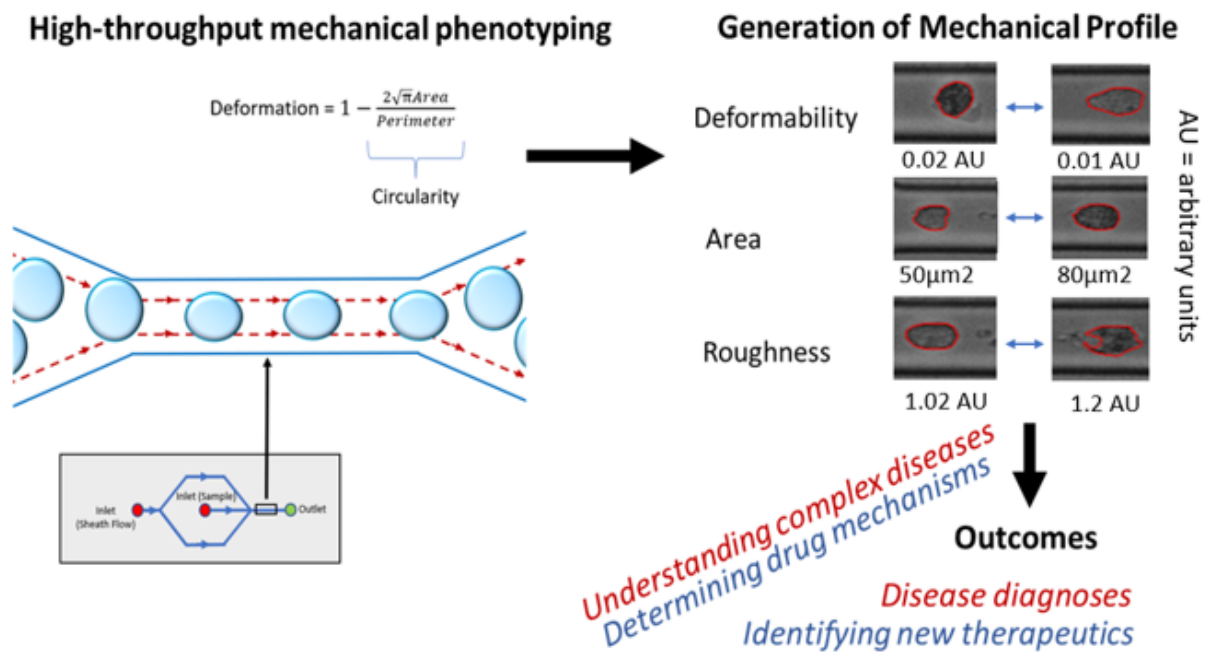
Early Acute Respiratory Distress Syndrome (ARDS) Revealed By a Microfluidic Single-Cell Study: The Rose of Cytokines and Protection With Antibodies. Crit Care. **20**, 8

Porcu, P., Cripe, L.D., Ng, E.W., Bhatia, S., Danielson, C.M., Orazi, A. and McCarthy L.J. (2000) Hyperleukocytic Leukemias and Leukostasis: A Review of Pathophysiology, Clinical Presentation and Management. Leukemia & Lymphoma **39**, 1-18

Porter, L., Toepfner, N., Bashant, K.R., Guck, J., Ashcroft, M., Farahi, N. and Chilvers E.R. (2018) Metabolic Profiling of Human Eosinophils. Frontiers in Immunology **9**

Ravetto, A., Wyss, H.M., Anderson, P.D., den Toonder, J.M.J. and Bouten C.V.C. (2014) Monocytic cells become less compressible but more deformable upon activation. PLOS ONE **9**, e92814-e14

Remmerbach, T.W., Wottawah, F., Dietrich, J., Lincoln, B., Wittekind, C. and Guck J. (2009) Oral Cancer Diagnosis by Mechanical Phenotyping Cancer Research **69**, 1728

* Renkawitz, J. and Sixt M. (2010) Mechanisms of force generation and force transmission during interstitial leukocyte migration. EMBO reports **11**, 744-50

Richelme, F., Benoliel, A.M. and Bongrand P. (2000) Dynamic Study of Cell Mechanical and Structural Responses to Rapid Changes of Calcium Level. Cell Motil Cytoskeleton **2,** 93-105

Rinker, K.D., Kirkpatrick, A.P., Ting-Beall, H.P., Shepherd, R.D., Levin, J.D., Irick, J., Thomas, J.L. and Truskey G.A. (2004) Linoleic acid increases monocyte deformation and adhesion to endothelium. Atherosclerosis 177, 275-85

Roca-Cusachs, P., Almendros, I., Sunyer, R., Gavara, N., Farré, R., and Navajas D. (2006) Rheology of Passive and Adhesion-Activated Neutrophils Probed by Atomic Force Microscopy. Biophysical Journal **91**, 3508-18

Roduit, C., Longo, G., Dietler, G. and Kasas S. (2015) Measuring Cytoskeleton and Cellular Membrane Mechanical Properties by Atomic Force Microscopy. In Methods in Membrane Lipids (Owen, D.M. eds.) Springer New York: New York, NY.

* Rosenbluth, M.J., Lam, W.A. and Fletcher D.A. (2008) Analyzing cell mechanics in hematologic diseases with microfluidic biophysical flow cytometry. Lab on a Chip **8** 1062-70

Rosendahl P, Plak K, Jacobi A, Kraeter M, Toepfner N, Otto O, Herold C, Winzi M, Herbig M, Ge Y, Girardo S, Wagner K, Baum B. and Guck J. (2018) Real-time fluoresence and deformability cytometry. Nat Methods. **15**, 355-358

Sapey, E. and Stockley R.A. (2014) Red, amber and green: the role of the lung in de-priming active systemic neutrophils. Thorax **69**, 606

Sattin, B.D. and Goh M.C. (2004) Direct Observation of the Assembly of RecA/DNA Complexes by Atomic Force Microscopy. Biophysical Journal **87**, 3430-36

Schmid, M.C. and Varner J.A. (2007) Myeloid cell trafficking and tumor angiogenesis. Cancer Letters **250**, 1-8

Schmid, M.C., and Varner J.A. (2012) Myeloid cells in tumor inflammation. Vascular Cell **4**, 14

Singh, N.R.P., Johnson, A., Peters, A.M., Babar, J., Chilvers, E.R. and Summers C. (2012) Acute lung injury results from failure of neutrophil de-priming: a new hypothesis. European Journal of Clinical Investigation **42**, 1342-49

Skoutelis, A.T., Kaleridis, V.E., Goumenos, D.S., Athanassiou, G.M., Missirlis, Y.F., Vlachojannis, J.G. and Bassaris H.P. (2000) Polymorphonuclear leukocyte rigidity is defective in patients with chronic renal failure Nephrology Dialysis Transplantation **15**, 1788-93

Sleep, J., Wilson, D., Simmons, R. and Gratzer W. (1999) Elasticity of the Red Cell Membrane and Its Relation to Hemolytic Disorders: An Optical Tweezers Study. Biophysical Journal **77**, 3085-95

Steffen, S., Abraham, S., Herbig, M., Schmidt, F., Blau, K., Meisterfeld, S., Beissert, S., Guck, J. and Günther C. (2018) Toll-Like Receptor-Mediated Upregulation of CXCL16 in Psoriasis Orchestrates Neutrophil Activation. Journal of Investigative Dermatology **138**, 344-54

* Summers, C., Singh, N.R., White, J.F., Mackenzie, I.M., Johnston, A., Solanki, C., Balan, K.K., Peters, A.M. and Chilvers E.R. (2014) Pulmonary retention of primed neutrophils: a novel protective host response, which is impaired in the acute respiratory distress syndrome. Thorax **69**, 623

Summers, C., Chilvers, E.R. and Peters A.M. (2014) Mathematical modeling supports the presence of neutrophil depriming in vivo. Physiological Reports **2**, e00241

* Suresh, S. (2007) Biomechanics and biophysics of cancer cells. Acta Biomaterialia **3**, 413-38

Tadir, Y., Wright, W.H., Vafa, O., Ord, T., Asch, R.H. and Berns, M.W. (1990) Force generated by human sperm correlated to velocity and determined using a laser generated optical trap. Fertility and Sterility **53**, 944-47

Taniguchi, M., Suzuki, S., Yamane, T., Matsumoto, O., Okuda, A., Taga, T. and Nishino Y. (2003) MgATP-induced conformational changes in a single myosin molecule observed by atomic force microscopy: Periodicity of substructures in myosin rods. Scanning **25**, 223-29

* Toepfner, N., Herold, C., Otto, O., Rosendahl, P., Jacobi, A., Kräter, M., Stächele, J., Menschner, L., Herbig, M., Ciuffreda, L., Ranford-Cartwright, L., Grzybek, M.,

Coskun, U., Reithuber, E., Garriss, G., Mellroth, P., Henriques-Normark, B., Tregay, N., Suttorp, M., Bornhäuser, M., Chilvers, E.R., Berner, R. and Guck J. (2018) Detection of human disease conditions by single-cell morpho-rheological phenotyping of blood. eLife **7**, e29213

Treitel, O. (1944) Elasticity of Plant Tissues. Transactions of the Kansas Academy of Science **47**, 219-39

Tsai, M.A., Waugh, R.E. and Keng P.C. (1996) Changes in HL-60 cell deformability during differentiation induced by DMSO Biorheology **33**, 1-15

* Tse, H.T.K., Gossett, D.R., Moon, Y.S., Masaeli, M., Sohsman, M., Ying, Y., Mislick, K., Adams, R.P., Rao, J. and Di Carlo D. (2013) Quantitative Diagnosis of Malignant Pleural Effusions by Single-Cell Mechanophenotyping. Science Translational Medicine **5**, 212ra163

Urbanska, M., Winzi, M., Neumann, K., Abuhattum, S., Rosendahl, P., Müller, P., Taubenberger, A., Anastassiadis, K. and Guck J. (2017) Single-cell mechanical phenotype is an intrinsic marker of reprogramming and differentiation along the mouse neural lineage. Development **144**, 4313

van der Linden, M. and Meyaard L. (2016) Fine-tuning neutrophil activation: Strategies and consequences. Immunology Letters **178**, 3-9

Wang, N., Butler, J.P. and Ingber D.E. (1993) Mechanotransduction across the cell surface and through the cytoskeleton. Science **260**, 1124

Weber, P.S.D., Toelboell, T., Chang, L., Tirrell, J.D., Saama, P.M., Smith, G.W. and JBurton J.L. (2004) Mechanisms of glucocorticoid-induced down-regulation of neutrophil L-selectin in cattle: evidence for effects at the gene-expression level and primarily on blood neutrophils. J Leukoc Biol. **75**, 815-27

Weiskopf, K., Schnorr, P.J., Pang, W.W., Chao, M.P., Chhabra, A., Seita, J., Feng, M. and Weissman I.L. (2016) Myeloid Cell Origins, Differentiation, and Clinical Implications. Microbiology spectrum **4**, 10.1128/microbiolspec.MCHD-0031-2016

* Worthen, G.S., Schwab, B., Elson, E.L. and Downey G.P. (1989) Mechanics of stimulated neutrophils: cell stiffening induces retention in capillaries. Science **245**, 183

Wu, P., Aroush, D.R., Asnacios, A., Chen, W., Dokukin, M.E., Doss, B.L., Durand-Smet, P., Ekpenyong, A., Guck, J., Guz, N.V., Janmey, P.A., Lee, J.S.H., Moore, N.M., Ott, A., Poh, Y., Ros, R., Sander, M., Sokolov, I., Staunton, J.R., Wang, N., Whyte, G. and Wirtz D. (2018) A comparison of methods to assess cell mechanical properties. Nature Methods **15**, 491-98

Yoshida, K., Kondo, R., Wang, Q. and Doerschuk C.M. (2006) Neutrophil Cytoskeletal Rearrangements during Capillary Sequestration in Bacterial Pneumonia in Rats. American Journal of Respiratory and Critical Care Medicine **174**, 689-98

Yourek, G., Hussain, M.A. and Mao J.J. (2007) Cytoskeletal changes of mesenchymal stem cells during differentiation. American Society for Artificial Internal Organs **53**, 219-28

Yu, H., Tay, C.Y., Leong, W.S., Tan, S.C.W., Liao, K. and Tan L.P. (2010) Mechanical behavior of human mesenchymal stem cells during adipogenic and osteogenic differentiation. Biochemical and Biophysical Research Communications **393**, 150-55

Zheng, Y., Wen, J., Nguyen, J., Cachia, M.A., Wang, C. and Sun Y. (2015) Decreased deformability of lymphocytes in chronic lymphocytic leukemia. Scientific Reports **5**, 7613

High-throughput mechanical phenotyping

$$\text{Deformation} = 1 - \frac{2\sqrt{\pi Area}}{Perimeter}$$

Circularity

Inlet (Sheath Flow)   Inlet (Sample)   Outlet

Generation of Mechanical Profile

Deformability 0.02 AU ↔ 0.01 AU

Area 50µm2 ↔ 80µm2

Roughness 1.02 AU ↔ 1.2 AU

AU = arbitrary units

Understanding complex diseases
Determining drug mechanisms

Outcomes

Disease diagnoses
Identifying new therapeutics

Recently, mechanical phenotyping technologies capable of analyzing the biophysical properties of large numbers of cells in real time have become available. This review introduces mechanical phenotyping techniques and documents the recent discoveries in myeloid cell immunology, human medicine, and drug development afforded by high throughput mechanical phenotyping.
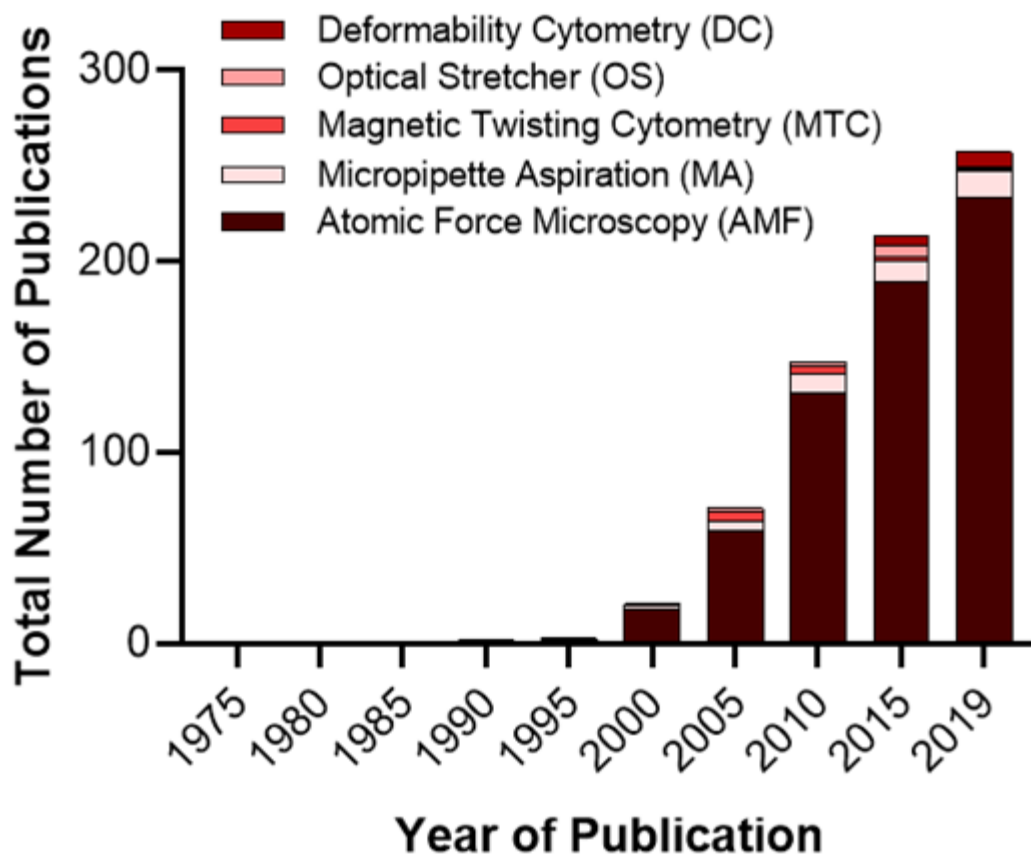
Figure 1: Increase in cell mechanics publications, arranged by analysis technique. These data were obtained from PubMed (www.ncbi.nlm.nih.gov/pubmed) by searching for combinations of "cell deformation" OR "cell deformability" OR "mechanical properties" OR "cell mechanics" OR "mechanical phenotyping " OR "cellular mechanobiology" AND the technique names.
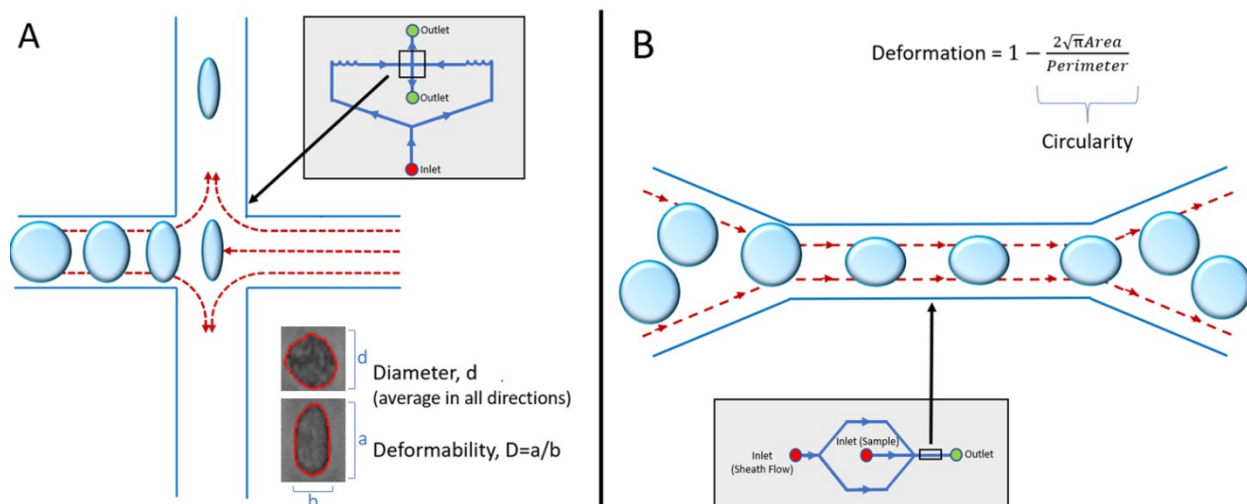
Figure 2: Schematic of deformability cytometry as described by Tse et al. (A) and real time deformability cytometry as described by Otto et al. (B). Red lines demonstrate direction of fluid flow. Microfluidic chips are depicted in grey, with the open black box and black arrow indicating the site and configuration of the deformation channel.