1.1

Microfilaments and intermediate filaments contribution to cancer cell migration in physical confinement

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The extra-cellular microenvironment has a fundamental role in tumor growth and progression, and strongly affects the migration strategies adopted by single cancer cells during metastatic invasion. In this study, we focus on the ability of mesenchymal and epithelial breast carcinoma cells to migrate through three-dimensional narrowing microstructures upon chemoattractant stimulation. We address the question of how the dynamical restructuring of the filamentous actin cytoskeleton modulates cell migration through narrowing micro-constrictions. Our findings show that both epithelial and invasive cells have the ability of easily disassemble and reorganize their cytoskeleton, in order to achieve migration through the constriction openings. Additionally, we investigate possible correlations between the migration behavior in the microstructures and the expression of vimentin and keratin intermediate filaments in our cancer cell lines.

1.2

EMT changes actin cortex rheology in a cell-cycle-dependent manner

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The actin cortex is a key structure for cellular mechanics and cellular migration. Accordingly, cancer cells were shown to change their actin cytoskeleton and their mechanical properties in correlation with different degrees of malignancy and metastatic potential. Epithelial-mesenchymal transition (EMT) is a cellular transformation associated with cancer progression and malignancy. To date, a detailed study of the effects of EMT on the frequency-dependent viscoelastic mechanics of the actin cortex is still lacking. In this work, we have used an established atomic force microscope-based method of cell confinement to quantify the rheology of the actin cortex of human breast, lung, and prostate epithelial cells before and after EMT in a frequency range of 0.02-2 Hz. Interestingly, we find for all cell lines opposite EMT-induced changes in interphase and mitosis; whereas the actin cortex softens upon EMT in interphase, the cortex stiffens in mitosis. Our rheological data can be accounted for by a rheological model with a characteristic timescale of slowest relaxation. In conclusion, our study discloses a consistent rheological trend induced by EMT in human cells of diverse tissue origin, reflecting major structural changes of the actin cytoskeleton upon EMT.

1.3

Vimentin intermediate filaments mediate cell shape on viscoelastic substrates

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The ability of cells to take and change shape is a fundamental feature underlying development, wound repair, and tissue maintenance. Central to this process is physical and signaling interactions between the three cytoskeletal polymeric networks: F-actin, microtubules, and intermediate filaments (IFs). Vimentin is an IF protein that is essential to the mechanical resilience of cells and regulates cross-talk amongst the cytoskeleton, but its role in how cells sense and respond to the surrounding extracellular matrix is largely unclear. To investigate vimentin's role in substrate sensing, we designed polyacrylamide hydrogels that mimic the elastic and viscoelastic nature of in vivo tissues. Using wild-type and vimentin-null mouse embryonic fibroblasts, we show that vimentin

enhances cell spreading on viscoelastic substrates, even though it has little effect in the limit of purely elastic substrates. Our results provide compelling evidence that the vimentin cytoskeletal network is a physical modulator of how cells sense and respond to mechanical properties of their extracellular environment.

1.4

Lipid-droplet mediated nuclear deformation occurs independently of cytoskeletal forces in hepatocytes

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Introduction: Hepatocellular carcinoma (HCC) is a deadly primary liver cancer, resulting in ~800,000 deaths globally per year [1]. The mechanisms of HCC development and progression are not well characterized, although ~80% of HCC occurs in stiff cirrhotic livers, and increased tissue stiffness correlates to poorer clinical outcomes [2,3], suggesting that mechanical stress may contribute to the initiation and progression of the disease. However, HCC can occur in soft livers with non-alcoholic fatty liver disease (NAFLD) with minimal amounts of fibrosis. We hypothesized that lipid droplet accumulation in NAFLD acts as an intracellular mechanical stress in hepatocytes, initiating oncogenesis at lower tissue stiffness by disrupting the cell cytoskeleton, and directly deforming the nucleus. Computational models of lipid droplet-mediated nuclear deformation suggested that, due to the relative stiffness of the cell nucleus, active cytoskeletal forces may be necessary to induce large scale nuclear deformations, raising the possibility that lipid-loading and substrate stiffness may work synergistically to induce nuclear deformation.

Materials and Methods: Primary human hepatocytes or isolated rat hepatocytes were cultured on glass and on 500 Pa and 10 kPa polyacrylamide gels, all coated with 1 mg/mL type 1 collagen. After 48 hrs cells were switched to serum-free media supplemented with 400 nM oleate (conjugated to bovine serum albumin) and incubated an additional 48 hrs. To determine whether lipid droplets can directly deform the nucleus, we also inhibited actin polymerization, microtubule polymerization and cell contraction (using 5 uM latrunculin, 10 uM nocodazole, or 5 uM blebbistatin, respectively) for 4 hrs after lipid loading. Cells were fixed and stained using DAPI for nuclei and BODIPY for neutral lipids, and nuclear shape and chromatin organization were imaged by confocal microscopy. HNF-4alpha was stained to determine whether deformation correlates to hepatocyte dedifferentiation. To compare between groups staining intensity was quantified in ImageJ and nuclear deformation was quantified using a specially developed MATLAB program.

Results and Discussion: Primary human hepatocytes readily process lipid droplets, leading to accumulation in the cell cytoplasm. Lipid accumulation is increased on softer substrates with more dense packing of lipid droplets and the nuclei of lipid-loaded cells have lower volume and spread area than controls. Cell volume of lipid loaded cells remains unchanged, however, indicating that hepatocytes are not expanding to accommodate the lipid volume. Lipid droplets visibly deform and indent the nucleus on all stiffness substrates, and when quantified significantly more so in oleate-treated cells than controls. However, the nuclei of oleate-treated hepatocytes on stiff substrates were taller than untreated cells, suggesting that lipid droplets may resist compression in the YZ plane. Experiments inhibiting actin polymerization, microtubule polymerization and myosin-mediated cell contraction showed that lipid droplets indent the nucleus even in the absence of an intact cytoskeletal network, suggesting that the lipid droplets themselves directly exert mechanical stress on the nucleus. The nuclei of oleate-treated hepatocytes were essentially insensitive to cytoskeletal treatment both in radial indentation by droplets and nuclear height, while control cells exhibited altered nuclear morphology as expected. Preliminary experiments indicate that HNF-4alpha may also be reduced in oleate-treated cells, suggesting that nuclear deformation by droplets may lead to hepatocyte dedifferentiation in a similar manner as does increased stiffness.

Conclusions: Lipid-loading of hepatocytes leads to compression and deformation of the nucleus in the XY plane, although lipid droplets appear to resist compression in the YZ plane. Inhibition of the cytoskeleton through various drug treatments has little impact on hepatocyte morphology in oleate-treated cells, suggesting that lipid droplets themselves directly indent the nucleus. Taken together, these results suggest that the intracellular lipid droplets in hepatocytes seen in NAFLD cause nuclear stress and could lead mechanosensitive signaling and differentiation in softer environments.

Acknowledgements: We acknowledge the funding support of the NSF Graduate Research Fellowship, PSOC@Penn (NIH U54 CA193417), the NIDDK Molecular Pathology & Imaging Core (NIH P30 DK050306), and the NSF Center for Engineering MechanoBiology (CMMI:15-48571).

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1.5

3D multicellular spheroids as an in vitro model for bladder cancer: a mechanical and microrheological study by means of atomic force microscopy

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3D cell cultures are known to better mimic the natural environment of cells and some characteristics of the solid tumours, such as the structural organization, cellular layered assembling, physiological responses, gene expression and drug resistance mechanism. Therefore, they have been widely used as new potential in vitro models for finding new biological features of cancer cells and anticancer drug discovery.

In this study, three different cell lines from bladder cancer were used to form the multicellular spheroids: HCV29 (non-malignant cancer), HT1376 (grade III carcinoma), T24 (grade IV transitional cell carcinoma). Firstly, we show that these cell lines have distinct proliferation rate, and they form spheroids in dissimilar ways as they require different amount of initial cell numbers to form spheroids of similar diameter. Secondly, we observe the differences in their morphology by using live/dead staining at different days of growth.

Knowing that cancer progression is associated with changes in the mechanical properties of cells and that Atomic Force Microscopy (AFM) is a versatile tool used to study cell elastic and rheological properties, we decided to investigate biomechanics of the spheroids at these two time points, 3 and 14 days respectively. Three parameters were compared: Young's (compression), storage and loss (shear stress) moduli. We made the mechanical experiments at three different complexity levels: single cells, cell monolayers and 3D spheroids. Our results show that under compression, HCV29 is stiffer than HT1376 and T24. Thus, cancer cells are softer than non-malignant ones. HCV29 and T24 rigidity increases when cells are grown as monolayer. Similar relation is observed for cells undergoing shear stress. The elastic properties of HT1376 cells in monolayer are of the same order as for single HT1376 cells regardless of the type of applied deformation. The mechanical and rheological properties of bladder cancer cells are related to actin filament organisation as stress fibres are present in HCV29 and T24 cells, not in HT1376 ones.

Regarding the spheroids, even though all three cell lines form spheroids diversely and change biomechanics at the 3D level, cancer cells were still softer. These changes seem to be related to their cytoskeleton organization, presence/absence of actin fibers and the amount of extracellular matrix component. These results were confirmed by the histological images of spheroids. Therefore, we conclude that both mechanical (compression) and rheological (shear stress) properties may serve as a biophysical cancer marker.

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2.1

Compressing cell nuclei: From nonlinear mechanics to nontrivial shapes

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Abnormal nuclear shapes are a feature of some human pathologies, such as metastatic cancer, Hutchinson-Gilford progeria syndrome (rapid aging), and mandibulofacial dysplasia disorder. Understanding these nuclear aberrations from a mechanical point of view may suggest new approaches to potential clinical therapies. Recent experimental studies show that the nucleus takes irregular shapes and shows stiffening behavior under compression. We have developed a minimal Brownian dynamics-based model of a nucleus undergoing uniaxial compression. Our model consists of a polymeric protein shell called the lamina, with chromatin, or a chain made up of monomeric subunits, inside the shell. Chromatin binds to itself randomly via crosslinkers and to the lamina via linkages. Extensile and contractile motors remodeling the chromatin represent the ATP-driven activity in the nucleus. We find that for fast compression in the low strain regime, the nucleus demonstrates compression stiffening behavior. We also observe localized bulges on the nuclear surface, indicating possible blebbing initiation. For the same strains, with slow compression, the model nucleus shows a more ellipsoidal shape with fewer localized bulges, as compared to fast compression. Our results suggest that during fast compression cycles, compressive forces do not propagate far into the nucleus. However, with slow compression, force propagation is more efficient, which leads chromatin to rearrange itself to accommodate the high strains. Our model, therefore, captures nuclear compression stiffening and shows how different nuclear morphologies arise in response to applied forces.

2.2

Molecular interactions in semiflexible polymer networks – A science friction story

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The mechanical behavior of soft complex materials composed of semiflexible polymers is determined by the properties of individual filaments and their interplay. In established model theories, developed to capture the emergence of viscoelastic bulk properties, entropic interactions between filaments are assumed to determine the mechanical response[1]. In recent studies the general accepted tube model has been challenged in terms of its basic assumption about filament-filament interactions, but also because of its predictions regarding the frequency dependence of the elastic modulus in the intermediate frequency regime[2,3,4]. The central question is how to theoretically deal with molecular interactions and friction between network constituents. It was shown that friction forces between aligned pairs of actin filaments are not negligible[5]. Here, we systematically investigate the influence of friction forces and attractive interactions on the rheology of entangled networks by means of a targeted surface modification. We show that these forces have a qualitative and quantitative influence on the viscoelastic properties of semiflexible polymer networks..

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2.3

Connective Tissue and Cancer Cross-Talk: Treatment Implications and Biomechanical Signature?

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The most crucial step in cancer progression, often deciding about treatment options, life and death of patients, is the formation of metastases. Unfortunately, in the last decades, progress was slim to treat metastases better. Currently, the best survival strategy is to prevent metastases by detecting the primary tumor as early as possible and to surgical resect it as well as possible.

One step towards a better survival rate is to identify tissues at risk. The second step is elucidating the interaction of tumors and their microenvironment, which often is fatty connective tissue. Here we show that cervical cancers spread from the tissue of origin to tissues of ontogenetic proximity in reverse order of embryogenic development. This “inverse morphogenesis” provides a unique roadmap for tissues at risk of cancer infiltration.

Further, the cross-talk between cancer cells and fatty connective tissue is essential for the mechanical phenotype of cancer cells. We show that stiffness changes of cancer cells are drastic when growing them in proximity to fatty tissue and are one of the largest recorded in optical stretcher measurements. Here, we present the framework for elucidating this emerging field of mechanical phenotyping under tissue cross-talk.

2.4

Fast phenotyping of monocytes mechanics under the cytokine storm

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Monocytes play a key role in the innate immune system during the inflammatory response. Their immune nonspecific response makes monocytes part of the first-line defenses against external agents, like in infections. A current example is the inflammatory response observed in Covid19 patients. Serum analysis of SARS-CoV-2 infected patients presenting hyperinflammatory response revealed high levels of the cytokines IL-6, IL-8, TNF-**α**, and CD14+ CD16+ monocytes. It has been shown that the mechanical properties of monocytes may change in response to inflammatory signals. Thus, mechanical phenotyping may be used as a diagnostic tool for hyperinflammatory syndrome in patients.

The aim of this work was to determine the mechanical properties of monocytes in response to pro-inflammatory proteins in the context of the hyperinflammatory response. We used atomic force microscopy (AFM) as a validation method, and deformation cytometry (DC), a newly developed microfluidics method for high throughput measurements of cell mechanics. Viscoelastic properties were extracted and compared between the methods, showing DC can give a robust and high-throughput approach for mechanical phenotyping of cells.

Furthermore, immunofluorescence imaging of the cytoskeleton proteins revealed how IL-8 downregulates vimentin (intermediate filaments) in monocytes for the first time, which mechanical fingerprint can be detectable with AFM and DC. Therefore, a better understanding of the mechanics of monocytes in the disease may open a window to new therapeutic targets against infectious diseases.

2.5

Morphology and density reveals prognostic relevance of potentially motile breast cancer cells

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We demonstrate that state variables of the cell jamming theory span a space that allows to detect potentially motile cancer cells in static pictures which can be utilized to assess metastatic risk in cancer patients. We observe cancer cells squeezing through dense tissue in vital carcinoma explants, which is accompanied by cell and nucleus deformations that can be measured statically. Building on these observations and hypothesizing that nuclear and cellular deformation are a surrogate for unjammed, motile cancer cells, we measure nuclear shapes and approximate cell shapes in H&E-stained breast cancer tissue slices from 609 patients difficult-to-assess with existing diagnostic methods. A subgroup of patients with more spherical cancer nuclei and cells and higher cancer cell densities – likely representing lower cellular motility – is identified with favorable clinical outcome marked by the absence of metastasis and tumor recurrence.

3.1

Characterization of the mechanical properties of lung adenocarcinoma

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The adverse scenario of lung cancer worldwide encourages research of innovative methods to improve therapeutic arsenals. The advent of immune checkpoint blockade therapy and targeted-drugs to driver mutations has changed the prognosis of lung cancer

patients in the past few years, but the majority of them will not benefit from those treatments. Thus, the development of creative tools and techniques is mandatory to facilitate research and discovery of new treatments. A primary step involved in cancer research is cell culture. To date, most cell culture has long been performed on surfaces that do not reflect the mechanical properties of most tissues in vivo. The use of soft culture environments that have a similar mechano-physiology of in vivo conditions is seen as a promising route for the discovery of new cancer drugs and tissue engineering. Based on this context, it is essential to understand how the mechanical properties are distributed in healthy and tumor tissues to design cell culture substrates that reproduce the in vivo mechanical microenvironment. Here, we present a methodology to characterize the mechanical properties of healthy and adenocarcinoma lung tissues using IT-AFM. Lung tissues are very sticky. In this context, data post-processing algorithms were implemented and optimized using contact mechanics models that consider adhesion effects. Preliminary results from five patients showed that the measured rigidities of both types of tissue obey a log-normal distribution with tumor tissues being slightly stiffer healthy ones. A difference in rigidity texture was observed, showing more heterogeneity for tumor tissues compared to healthy ones. These findings give an indication of the initial parameters necessary to design a mechano-mimetic environment.

3.2

Mechanosensing of primary human breast cells

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Increase in mammographic density and altered expression of extracellular matrix (ECM) proteins correlates with breast cancer progression, but these factors also regulate mammary gland morphogenesis. Nevertheless, it remains unclear how the different cell types in normal human mammary gland respond to changes in biochemical and biophysical adhesion cues. Basal mammary epithelial cells (MECs) form a dynamic barrier between the secretory luminal MECs and the stroma, and harbor the major regenerative and contractile capacity pivotal to mammary gland function. Basal MECs are connected to the basement membrane that envelopes the glandular epithelium, and thus, indirectly sense the mechanical properties of the stroma.

In this study, we isolated primary human breast cells from patients undergoing reduction mammoplasty, plated them on ECM-coated soft hydrogels, and explored a range of mechanobiological readouts in vitro. Our data demonstrate that unpassaged luminal epithelial, basal epithelial, and stromal fibroblastic cells exhibit differential nuclear mechanotransduction, myosin phosphorylation, traction force generation, and growth in soft environment. Importantly, our preliminary data suggest that their adhesion response to increased stiffness is ECM ligand and cell type specific. These data provide previously unknown insight in human breast biology and may even have implications for better understanding breast cancer progression.

3.3

Local structure determines cell rearrangements in epithelial layers

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The transition between fluid-like cell motion in mesenchymal tissue and a jammed or glassy behaviour in epithelial tissue hints at possible underlying general rules for these complex biological processes. The driving forces behind this transition are in discussion at the moment, with different candidates being the cell number density (Angelini et al., PNAS 2011), the cell shape (Bi et al., Nat. Phys. 2015) and protein driven aging (Garcia et al, PNAS 2015).

We observe layers of epithelial-like MCF-10A cells with a stained nucleus, allowing us to track the cells and analyse the individual kinetics. A Voronoi tessellation around these tracks is used to estimate the local structural features and rearrangement events. Thereby, we can describe the local fluidity of a cell layer and look for the onset of cellular jamming. A moderately high density is required for epithelial-like MCF-10A cells to jam. We observed that both round cell shapes and high densities in the local environment indicate a reduced rearrangement dynamic in that local region confirming the role of caging effects. The cell shape influence can be

rescaled by the distance to the critical cell shape parameter p=3.81 of the SPV model. This means that the deviation of this mean cell shape to the critical cell shape index can be understood as a control parameter of the ability of cells to rearrange, similar to critical scaling behaviour near a phase transition. Therefore, this is profound evidence for a shape-dependent jamming transition. This can be reproduced in simulations using the intrinsic velocity of the cells in the system instead of the cell number density, giving a strong indication that the effect of the cell number density on the rearrangement dynamics can be approximated by a slow-down of the intrinsic cell velocity similar to a decrease in temperature of glassy systems.

3.4

Hypoxic conditions alter breast cancer cell mechanics and rheology

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During tumor progression, cells accumulate biochemical and morphological changes, inducing alterations in membrane- and adhesive properties, cell-to-cell contacts, and cytoskeletal arrangement. All these elements contribute to measurable single cell mechanics. Further, solid tumors develop a hypoxic core, in which nutrient availability is low and the microenvironment is inhospitable. Hypoxia is implied in certain hallmarks of cancer progression, such as angiogenesis, invasiveness, and epithelial-to-mesenchymal transition (EMT). Starved cells from the hypoxic core have been shown to migrate out towards the tumor periphery and form part of the metastatic front. Thus, changes in cytoskeletal arrangement and mechanical properties are expected to occur. Here, we examine the influence of hypoxia on the mechanical properties of MCF-7 cells. Indentation and microrheology studies were performed via atomic force microscopy (AFM) force spectroscopy. Mechanical properties like the Young's Modulus, alongside viscoelastic properties following a power law rheology model were determined. Results show that hypoxic conditions induce changes in cell mechanics, such as cell softening, and an increased fluid-like behavior.

4.1

T cell stiffness is enhanced upon formation of immunological synapse

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To exert their effector functions, T cells need to form an intimate contact with their cognate target cells, which is termed the immunological synapse (IS). Mechanobiology has been receiving increasing attention, given its indispensable and previously ignored role in regulating cell functions. In terms of T cells, they can sense the stiffness of targets/substrates and generate force upon IS formation, which are important for their effector functions. However, how the stiffness of T cells per se is regulated upon IS formation still remains elusive. In this work, we determined stiffness of different cell parts in detail during the processes of IS formation in T cells. To this end, we established a method to investigate live T cells on functionalized co-verslips by atomic force microscopy (AFM) based Peak Force Quantitative Na-noscale Mechanical Characterization (Peak Force QNM), which enables simulta-neous determination of the surface profile and stiffness of live T cells. Using primary human CD4+ T cells, we found that upon IS formation, T cells were substantially stiffened at the cell body as well as at the lamellipodia. In general, the stiffness at the lamellipodia is significantly higher than that at the cell body. Furthermore, we identified that calcium is involved in regulation of this IS formation-induced T cell local stiffening at lamellipodia [1].

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4.2

Modulation of viscoelastical properties of MCF-7 cells by substrate stiffness

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Cells sense stiffness of surrounding tissues and adapt their activity, proliferation, motility and mechanical properties based on such interactions. Cells probe the stiffness of the substrate by anchoring and pulling to their surroundings, transmitting force to the extracellular matrix and other cells, and response to the resistance they sense, mainly through changes in their cytoskeleton [1]. Cancer and other diseases alter stiffness of tissues, and the response of cancer cells to this stiffness can also be affected [2]. In the present study we show that MCF-7 breast cancer cells seeded on polyacrylamide gels have the ability to detect the stiffness of the substrate and alter their mechanical properties in response. MCF-7 cells plated on soft substrates display lower stiffness and viscosity when compared to those seeded on stiffer gels or glass. These differences can be associated with differences in the morphology and cytoskeleton organisation, since cells seeded on soft substrates have a round morphology while cells seeded on stiffer substrates acquire a flat and spread morphology with formation of actin filaments, similar to that observed when seeded on glass. These findings show that MCF-7 cells can detect the stiffness of the surrounding microenvironment and thus, modify their mechanical properties.

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4.3

Geometric parameters of entangled and crosslinked polymer networks determined by tracking embedded DNA nanotubes

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In polymer physics, single-filament tracing has proven to be a valuable tool to measure mechanical and geometrical features of entangled polymer networks. Although theory provides a number of predictions concerning the stiffness of filaments on a mesoscopic scale, established polymer systems have not provided the means to examine them experimentally. In our study, we use synthetically polymers hybridized from DNA strands as tracers. They are mechanically programmable and can be embedded both in entangled and crosslinked polymer networks. We present the measurement of structural network properties like tube width and mesh size of F-actin networks with respect to the stiffness of the DNA nanotube tracers [1]. We are able to confirm some predictions from theories based on steric interactions. However, our results strongly indicate that these models may be expanded to account for the behavior of real polymer networks by adding terms describing inter-filament interactions.

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