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13. ABSTRACT (Maximum 200 words)

A workshop was held in February, 2004 at the University of California at Irvine to bring together some of the leading experts on advanced computational modeling and simulation of biological systems. The proceedings of the workshop were published in a new journal, "Mechanics and Chemistry of Biosystems". It is expected that the discussions held at the workshop should point to future fruitful avenues for research to incorporate biology into future Army systems.

The attached report includes the title page from selected presentations and abstract pages from the workshop proceedings.

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Enclosure 1

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Re: Army Research Workshop on Mechanics and Chemistry of Biosystems
University of California, Irvine
P.I. S.N. Atluri

Dr. Rajendran:

The material found in this packet is subsequent to the February 2004 meeting on Mechanics and Chemistry of Biosystems, held at the University of California, Irvine. Please find included the agenda, the list of attendees, and a CD with a few presentations. The other presenters chose to publish their full length papers, based on their presentations at the Symposia, in the new journal, "MCB: Mechanics & Chemistry of Biosystems". We have included print outs from the journal for review by those who were in attendance. Dr. Gang Bao and Dr. Satya Atluri are the Editors-in-Chief, and Dr. Y.C. Fung is the Honorary Editor of MCB. Nearly all participants in the MCB Workshop are on the Editorial Board of the MCB journal. Hence, the outcome of the Army Research Workshop on Mechanics and Chemistry of Biosystems was scientifically valuable and successful.

Sincerely,

Satya N. Atluri
Principal Investigator

Army Research Office Conference on Mechanics and Chemistry of Bio-Systems

Monday February 9th, 2004

9:00-9:30	Registration
9:30-9:35	Introductory Comments Michael Gottfredson Executive Vice Chancellor University of California, Irvine
9:35-9:45	Dr. Jim C.I. Chang Director, Army Research Office
9:45-10:00	Dr. A. Rajendran Senior Research Scientist, Army Research Office
10:00-10:40	Professor Gang Bao Department of Biomedical Engineering Georgia Institute of Technology and Emory University "Nanostructured Molecular Probes for Gene detection in Living Cells"
10:40-10:50	Break
10:50-11:30	Professor Donald E. Ingber Departments of Pathology and Surgery Harvard Medical School "The Mechanochemical Basis of Cell & Tissue Regulation"
11:30-12:10	Professor Roger Kamm & Dr. Mohammad R. Kaazempur-Mofrad Department of Mechanical Engineering & Division of Biological Engineering MIT "Mechanical Aspects of Cellular Deformation and Mechano-transduction"
12:10-1:00	Lunch
1:00-1:40	Dr. Meyya Meyyappan Director, Center for Nanotechnology Ames Research Center "Bio-Nano Fusion in Sensor and Device Development"
1:40-2:20	Professor Deborah E. Leckband Department of Chemical and Biomelecular Engineering University of Illinois at Urbana-Champaign "Molecular Mechanisms of Cell Adhesion"
2:20-2:40	Break
2:40-3:20	Dr. Jun Li NASA Ames Research Center "Ultrasentsitive Label-free Electronic Chip for DNA Analysis Using Carbon Nanotube Nanoelectrode Arrays"
3:20-4:00	Professor Arun Majumdar University of California, Berkeley Department of Mechanical Engineering "Mechanics at the Interface of Hard and Soft Matter"

Army Research Office Conference on Mechanics and Chemistry of Bio-Systems

Tuesday, February 10th, 2004

9:00-9:40	Professor Geert W. Schmid-Schoenbein Department of Bioengineering University of California, San Diego "Cardiovascular Cell Activation and Inflammation: Interaction between Fluid Shear Stress and Humoral Mediators"
9:40-10:20	Professor Van C. Mow Stanley Dicker Professor of Biomedical Engineering Columbia University Fixed Electrical Charges and Mobile Ion Affect the Properties of Charged-Hydrated Biological "Tissues: The Articular Cartilage Paradigm"
10:20-10:40	Break
10:40-11:20	Professor Stephen R. Quake Division of Engineering and Applied Science Caltech "Biological Large Scale Integration"
11:20-12:00	Professor Michael P. Sheetz Department of Biomedical Engineering Columbia University "Local Force Transduction Acts Globally through Oncogenes and Sytoskeletal Transport of Signals"
12:00-1:00	Lunch
1:00-1:40	Dr. Wouter Roos Biophysical Chemistry University of Heidelberg "Mico- and Nanolithographic Tools for Designing Biophysical Models of Cell Adhesion and Mechanics"
1:40-2:20	Professor Masasuke Yoshido Chemical Resources Laboratory Tokyo Institute of Technology "The Engine and Brake of Rotary Motor ATP Synthese"
220-2:30	Break
2:30-3:10	Professor Satya N. Atluri Department of Mechanical & Aerospace Engineering Samueli/Von Karman Chair in Aerospace Engineering University of California, Irvine "Multi-scale Modeling in Mechanics and Chemistry of Biosystems"

Army Research Office Conference on
Mechanics and Chemistry of Bio-Systems

Dean Nicolaos G. Alexopoulos

Dr. Satya N. Atluri

Dr. Gang Bao,

Dr. Jim C.I. Chang

Dr. Stephen Davis

Dr. Y.C. Fung

Miss Carly Herrera

Dr. Donald E. Ingber

Dr. Roger D. Kamm

Dr. Ghassan Kaasab

Dr. Mohammad R. Kaazempur-Mofrad

Dr. Bruce LaMattina

Dr. Deborah E. Leckband

Dr. Jun Li

Dr. Arun Majumdar

Dr. Meyya Meyyappan

Dr. Van C. Mow

~~Dr. Stephen R. Quake~~

Heun Jin Lee

Fixed Electrical Charges and Mobile Ions Affect the Measurable Mechano-Electrochemical Properties of Charged-Hydrated Biological Tissues: The Articular Cartilage Paradigm

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Columbia University, New York, NY

UC Irvine Army Research Office Conference of
Mechanics and Chemistry of Bio-Systems

Cardiovascular Cell Activation and Inflammation:

Interaction between Fluid Shear Stress and Humoral Mediators.

Geert W. Schmid-Schönbein
Fariborz Moazzam, Shunichi Fukuda, Peter Marschel,
Mark Coughlin, Masako Sugihara-Seki, Tony Hugli, Erik Kister,
Florian Fitzal, Hiroshi Mitsuoka, Frank A. DeLano

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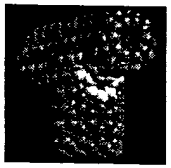
Bio-nano Fusion in Sensor and Device Development



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MCB, vol.1, no.1, pp.23-36, 2004

Engineering Nanostructured Probes for Sensitive Intracellular Gene Detection

Gang Bao[1], Andrew Tsourkas[2] and Philip J. Santangelo[2]

Abstract: The ability to detect, localize, quantify and monitor the expression of specific genes in living cells in real-time will offer unprecedented opportunities for advancement in molecular biology, disease pathophysiology, drug discovery, and medical diagnostics. However, current methods for quantifying gene expression employ either selective amplification (as in PCR) or saturation binding followed by removal of the excess probes (as in microarrays and in situ hybridization) to achieve specificity. Neither approach is applicable when detecting gene transcripts within living cells. Here we review the recent development in engineering nanostructured molecular probes for gene detection *in vivo*, describe probe design approaches and its structure-function relations, and discuss the critical issues and challenges in performing living cell gene detection with high specificity, sensitivity and signal-to-background ratio. The underlying biological and biochemical aspects are illustrated.

1 Introduction

Quantitative methods to measure gene expression such as real-time PCR and microarrays have revolutionized molecular biology and drug development. Such approaches, however, are generally used with purified DNA or RNA obtained from cell lysate. The ability to detect and quantify the expression of specific genes in living cells in real-time will offer tremendous opportunities for biological and disease studies, provide another leap forward in our understanding of cell and developmental biology, disease pathophysiology, and significantly impact medical diagnostics (Tsien, 2003). However, currently available technologies for gene detection in intact cells such as in-situ hybridization (ISH) are not capable of detecting and quantifying gene expression in living cells. Unlike ISH studies and solid-phase *in vitro* assays in which probes not hybridized to their target (i.e., background) can be removed by washing (Femino et al, 1998; Raap et al, 1997), gene detection in living cells requires more sophisticated methods to distinguish signal from background. Probes must be able to recognize the target with high specificity, convert target recognition *directly* into a measurable signal with high signal-to-background ratio, and allow for differentiation between true and false-positive events. To meet these challenges and definitive needs, recently, extensive studies have been conducted to develop novel nanostructured molecular probes with high specificity, sensitivity, and signal-to-background ratio.

Gene expression in a living cell can be quantified through the level of either mRNA expression or protein expression; the former is controlled by transcription, the latter by translation. Although both are dictated by the regulation of the specific gene, their expression levels can be very different, since an mRNA molecule can produce multiple proteins. In this article, we focus on the detection of mRNA molecules based on Watson-Crick base pairing, which is one of the most specific molecular interactions in a living cell. Over the last decade or so, there is increasing evidence to suggest that RNA molecules have a wide range of functions in living cells, from physically conveying and interpreting genetic information, to essential catalytic roles, to providing structural support for molecular machines, and to gene silencing. These functions are realized through control of both the expression level of specific RNAs, and their spatial distribution. Therefore, there is an urgent need to image specific mRNAs in living cells in real-time in order to provide essential information on mRNA synthesis, processing, transport, localization, and on the dynamics of mRNA expression and localization in response to external stimuli. However, existing methods cannot reveal the spatial and temporal variation of RNA within a single cell.

One approach to tagging and tracking endogenous

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 MCB, vol.1, no.1, pp.37-52, 2004

Flaw tolerant bulk and surface nanostructures of biological systems

Huajian Gao[1], Baohua Ji[1], Markus J. Buehler[1], Haimin Yao[1]

Abstract: Bone-like biological materials have achieved superior mechanical properties through hierarchical composite structures of mineral and protein. Gecko and many insects have evolved hierarchical surface structures to achieve extraordinary adhesion capabilities. We show that the nanometer scale plays a key role in allowing these biological systems to achieve their superior properties. We suggest that the principle of flaw tolerance may have had an overarching influence on the evolution of the bulk nanostructure of bone-like materials and the surface nanostructure of gecko-like animal species. We demonstrate that the nanoscale sizes allow the mineral nanoparticles in bone to achieve optimum fracture strength and the spatula nanoprotrusions in Gecko to achieve optimum adhesion strength. In both systems, strength optimization is achieved by restricting the characteristic dimension of the basic structure components to nanometer scale so that crack-like flaws do not propagate to break the desired structural link. Continuum modeling and atomistic simulations have been conducted to verify the concept of flaw tolerance at nanoscale.

A simple tension-shear chain model has been developed to model the stiffness and fracture energy of biocomposites. It is found that, while the problem of low toughness of mineral crystals is alleviated by restricting the crystal size to nanoscale, the problem of low modulus of protein has been solved by adopting a large aspect ratio for the mineral platelets. The fracture energy of biocomposites is found to be proportional to the effective shear strain and the effective shear stress in protein along its path of deformation to fracture. The bioengineered mineral-protein composites are ideally suited for fracture energy dissipation as the winding paths of protein domain unfolding and slipping along protein-mineral interfaces lead to very large effective strain before fracture. The usual entropic elasticity of biopolymers may involve relatively small effective stress and may not be able to ensure simultaneous domain unfolding and interface slipping. Cross-linking mechanisms such as Ca^{++} induced sacrificial bonds in bone can increase the shear stress in protein and along the protein-mineral interface, effectively converting the behavior of entropic elasticity to one that resembles metal plasticity. The sacrificial bond mechanism not only builds up a large effective stress in protein but also allows protein deformation and interface slipping to occur simultaneously under similar stress levels, making it possible to engineer a very long range of deformation under significant stress in order to maximize energy absorption. Optimization of mineral platelets near theoretical strength is found to be crucial for allowing a large effective stress to be built up in protein via cross-linking mechanisms such as Ca^{++} induced sacrificial bonds. Similarly, for gecko adhesion, the strength optimization of individual spatulas is found to play a critical role in enhancing adhesion energy at the higher hierarchical level.

1 Introduction

An important objective of materials science and engineering has been to understand the relationships between microstructure of materials and their macroscopic properties. New challenges to this field in the 21st century will include the development of multi-functional (e.g., strength, transport, self-repair, self-replicate, etc) and hierarchical (properties optimized at all length scales) materials systems. Nanotechnology promises to enable mankind to eventually design materials using a bottom-up approach, i.e. to construct multi-functional and hierarchical material systems by tailor-designing structures from atomic scale and up. However, currently we barely have any theoretical basis on how to design a hierarchical material system to achieve a particular set of functions. One strategy is to look among convergent evolutions in nature for hints on basic principles of multiscale and multifunctional materials design.

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 MCB, vol.1, no.1, pp.53-68, 2004

The Mechanochemical Basis of Cell and Tissue Regulation

D.E. Ingber[1]

Abstract: This article is a summary of a lecture presented at a symposium on "Mechanics and Chemistry of Biosystems" in honor of Professor Y.C. Fung that convened at the University of California, Irvine in February 2004. The article reviews work from our laboratory that focuses on the mechanism by which mechanical and chemical signals interplay to control how individual cells decide whether to grow, differentiate, move, or die, and thereby promote pattern formation during tissue morphogenesis. Pursuit of this challenge has required development and application of new microtechnologies, theoretical formulations, computational models and bioinformatics tools. These approaches have been used to apply controlled mechanical stresses to specific cell surface molecules and to measure mechanical and biochemical responses; to control cell shape independently of chemical factors; and to handle the structural, hierarchical and informational complexity of living cells. Results of these studies have changed our view of how cells and tissues control their shape and mechanical properties, and have led to the discovery that integrins and the cytoskeleton play a central role in cellular mechanotransduction. Recognition of these critical links between mechanics and cellular biochemistry should lead to novel strategies for the development of new drugs and engineered tissues, as well as biomimetic microdevices and nanotechnologies that more effectively function within the context of living tissues.

keyword: Mechanotransduction, integrins, cytoskeleton, tensegrity, cell engineering, morphogenesis

1 Introduction

This paper is based on an invited lecture I presented at a symposium on "Mechanics and Chemistry of Biosystems" in honor of Professor Y.C. Fung that convened at the University of California, Irvine in February 2004. Our work on mechanobiology, and especially on cellular tensegrity, has always been controversial; this became even more so as we crossed disciplines from biology into engineering. Professor Fung was one of the few established scientists and engineers who saw the potential value of our message early on, and who spoke out in our defense in public forums when our work was openly attacked. I still keep a letter Professor Fung wrote me early in my career, in which he wrote: "I am sure you are on the right track to discover the mystery of growth. Your theory is the best so far". I cannot tell you how validating this was for me as a young scientist. To me, it demonstrated the depth of his commitment in support of new ideas, regardless of how heretical they may seem at first glance. The advancement of science and engineering was his only concern. Thus, it is indeed my pleasure, and my honor, to be able to participate in this wonderful symposium and journal issue in appreciation of Professor Fung's seminal contributions to the field of bioengineering.

My laboratory is interested in the general mechanism of cell and developmental regulation: how cells respond to signals and coordinate their behaviors to produce tissues with specialized form and function. Molecular cell biologists have made great advances in terms of uncovering the biochemical signaling pathways that mediate behavioral control. But if we want to fully understand biological regulation, we also have to consider how these chemical interactions function in the physical context of living tissues. This is critical because it has been known for over a century that mechanical forces also impact tissue development [Wolff, (1892); Thompson (1952)]. The effects of large-scale forces on tissue growth, such as of compression on bone, tension on muscle, and hemodynamic forces on blood vessels, are obvious examples.

What is less clear, however, is that microscale forces also impact the development of all living tissues. For example, we work in the area of vascular development and angiogenesis – the growth of blood capillaries. Most biologists tend to think of vascularization in a relatively

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System Optimization for the Development of Ultrasensitive Electronic Biosensors Using Carbon Nanotube Nanoelectrode Arrays

Jessica E. Koehne, Jun Li[1], Alan M. Cassell, Hua Chen, Qi Ye, Jie Han, and M. Meyyappan

Abstract: Vertically aligned multi-walled carbon nanotubes (MWCNTs) have been reported in fabricating nanoelectrode arrays. Further studies on optimizing this system for the development of ultrasensitive DNA sensors are reported here. The mechanical stability of the as-grown MWCNT array can be improved by polymer coating or SiO_2 encapsulation. The latter method provides excellent electronic and ionic insulation to the sidewall of MWCNTs and the underlying metal layer, which is investigated with electrochemical impedance spectroscopy. The insulation ensures well-defined nanoelectrode behavior. A method is developed for selectively functionalizing biomolecules at the open end of MWCNTs while keeping the SiO_2 surface passivated, using the unique graphitic chemistry. An ultrahigh sensitivity approaching the limit of fluorescence techniques is obtained with this system for DNA detection.

1 Introduction

Electrochemical (EC) methods are attractive techniques for molecular analysis of biological samples [Kuhr, 2000; Umek, 2001; Boon, 2000; Popovich, 2002], which can be directly integrated with microelectronics and microfluidics devices to gain advantages in miniaturization, multiplexing, and automation [Sosnowski, 1997]. Recently, many studies have reported the modification of the electrode surface with organic monolayers or nanostructured sensing elements to enhance biosensing ability. In a series of reports [Li, 2003a; Koehne, 2003; Koehne, 2004], we have demonstrated that vertically aligned multiwalled carbon nanotubes (MWCNTs) embedded in a SiO_2 matrix could be employed as well-defined nanoelectrode arrays (NEAs) for ultrasensitive DNA detection. Specific hybridization of PCR amplicons of the BRCA1 gene as well as oligonucleotide targets were measured by the combination of the MWCNT NEAs with $Ru(bpy)_3^{2+}$ mediated guanine oxidization [Li, 2003a; Koehne, 2003]. An ultrahigh sensitivity approaching the limit of fluorescence techniques was achieved [Koehne, 2003]. This makes it possible to realize the advantages of electronic biosensing technologies for applications requiring rapid results. Here we report further studies on optimizing the MWCNT NEA platform for the development of biosensors, with particular emphasis on the following issues: (1) the mechanical stability, (2) electrical characteristics, and (3) chemical selectivity and reliability for surface functionalization/passivation.

There are many reports on using carbon nanotube (CNT) arrays or random ensembles as electrodes for electrochemical sensors [Ng, 2001; Guiseppi-Elie, 2002; Gooding, 2003; Yu, 2003, Sotiropoulou, 2003]. However, such raw CNT arrays or ensembles have poor mechanical stability (particularly apt to bunch due to the capillary force of liquid droplets) [Li, 2002a; Chen, 2002] and high electronic noise due to the large surface area [Koehne, 2004; Li, 2002a; Chen, 2002]. The chemistry is also complicated due to the complex nanostructure. In contrast, a critical step was introduced in our fabrication of NEAs [Li, 2002b, 2003; Koehne, 2003, 2004], which encapsulates the as-grown MWCNT array in a SiO_2 matrix by chemical vapor deposition (CVD) of tetraethylorthosilicate (TEOS). This method improves the mechanical stability of the MWCNT array dramatically so that an aggressive chemical mechanical polishing (CMP) process can be applied to planarize the top surface and expose only a fraction of the MWCNTs [Koehne, 2003]. Together, these processing steps make it possible to fabricate reliable and well-defined MWCNT NEAs for biosensor development. In this report, we further address the properties of the NEAs from an electronic signaling and surface chemistry points of view. Electrochemical impedance spectroscopy (EIS) is employed to illustrate the equivalent circuit of MWCNT

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 MCB, vol.1, no.1, pp.81-99, 2004

Fixed Electrical Charges and Mobile Ions Affect the Measurable Mechano-Electrochemical Properties of Charged-Hydrated Biological Tissues: The Articular Cartilage Paradigm

Leo Q. Wan[1], Chester Miller[1], X. Edward Guo[2], Van C. Mow[1,3]

Abstract: The triphasic constitutive law [Lai, Hou and Mow (1991)] has been shown in some special 1D cases to successfully model the deformational and transport behaviors of charged-hydrated, porous-permeable, soft biological tissues, as typified by articular cartilage. Due to nonlinearities and other mathematical complexities of these equations, few problems for the deformation of such materials have ever been solved analytically. Using a perturbation procedure, we have linearized the triphasic equations with respect to a small imposed axial compressive strain, and obtained an equilibrium solution, as well as a short-time boundary layer solution for the mechano-electrochemical (MEC) fields for such a material under a 2D unconfined compression test. The present results show that the key physical parameter determining the deformational behaviors is the ratio of the perturbation of osmotic pressure to elastic stress, which leads to changes of the measurable elastic coefficients. From the short-time boundary layer solution, both the lateral expansion and the applied load are found to decrease with the square root of time. The predicted deformations, flow fields and stresses are consistent with the analysis of the short time and equilibrium biphasic (i.e., the solid matrix has no attached electric charges) [Armstrong, Lai and Mow (1984)]. These results provide a better understanding of the manner in which fixed electric charges and mobile ions within the tissue contribute to the observed material responses.

keyword: Articular Cartilage, Triphasic Theory, Unconfined Compression, Fixed Charge Density

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1 Introduction

Articular cartilage is a thin layer of white, dense connective tissue covering the moving and load-supporting bony articulating ends in diarthrodial joints [Mow, Ratcliffe and Poole (1992)]. It is composed of an electrically charged and hydrated organic matrix of collagen and proteoglycan, where a sparse population of chondrocytes reside. Normal articular cartilage contains on average approximately 75% water within the pores of the tissue that are estimated to be 50-65 Å in diameter; even though its water content is high, its permeability is extremely low at approximately $10^{-15} m^4/(N \cdot s)$ [ibid]. Figure 1 shows the organizational arrangement of the structural macromolecular components of the extracellular matrix of such tissue as articular cartilage [Heinegard, Bayliss and Lorenzo (2003)]. The most abundant components are collagen at about 10% per wet weight, followed by large aggregating proteoglycans at about 7.5% per wet weight [Mow and Ratcliffe (1997)]. Type II collagen is the dominant component of the collagen that exists in the tissue that also includes a number of quantitatively minor but functionally important collagen types, including V, VI, IX, and XI. Type II collagen fibers have an estimated half-life of 67 years, and they are capable of forming a strong meshwork of fine pores that traps proteoglycans in its intrafibrillar space. The quantitatively minor collagen types, particularly Type IX, are useful in facilitating interactions between the proteoglycans and collagen Type II fibers (Fig. 1). No covalent bonds exist between proteoglycans and the collagen meshwork; electrostatic interactions, frictional interactions and steric exclusion effects are responsible for immobilizing the large proteoglycans within the extracellular compartment of the tissue. Proteoglycans are architecturally complex macromolecules that are capable of forming networks in solution and within the interstitial fluid of the intrafibrillar space. Though labile these proteoglycan net-

 MCB, vol.1, no.2, pp.101-111, 2004

Forced Dissociation of the Strand Dimer Interface between C-Cadherin Ectodomains

M.V. Bayas[1], K.Schulten[2] and D. Leckband[3]

Abstract: The force-induced dissociation of the strand dimer interface in C-cadherin has been studied using steered molecular dynamics simulations. The dissociation occurred, without domain unraveling, after the extraction of the conserved trypthophans (Trp2) from their respective hydrophobic pockets. The simulations revealed two stable positions for the Trp2 side chain inside the pocket. The most internal stable position involved a hydrogen bond between the ring Nε of Trp2 and the backbone carbonyl of Glu90. In the second stable position, the aromatic ring is located at the pocket entrance. After extracting the two tryptophans from their pockets, the complex exists in an intermediate bound state that involves a close packing of the tryptophans with residues Asp1 and Asp27 from both domains. Dissociation occurred after this residue association was broken. Simulations carried out with a complex formed between W2A mutants showed that the mutant complex dissociates more easily than the wild type complex does. These results correlate closely with the role of the conserved tryptophans suggested previously by site directed mutagenesis.

keyword: C-cadherin, Cell Adhesion Molecules, Steered Molecular Dynamics.

1 Introduction

C-Cadherin, also called EP-Cadherin, is a representative classical cadherin expressed in the early embryo of the frog *Xenopus laevis* [Levi et al. (1991); Lee and Gumbiner (1995)]. It plays an important role in the transformation of a ball of undifferentiated cells into a well-organized embryo [Lee and Gumbiner (1995)]. As for all classical cadherins, C-cadherin comprises a highly conserved cytoplasmic domain, a single transmembrane segment and an extracellular segment containing a tandem of five cadherin-like domains [Takeichi (1990)]. The extracellular domains are numbered EC1 through EC5, starting from the N-terminal domain as shown in Fig. 1A. In the presence of Ca^{++} these domains form a rigid structure necessary for adhesion [Nagar et al. (1996); Häussinger et al. (2002)].

The EC1 domain plays a central role in the adhesive activity of classical cadherins. It is thought to be responsible for the specificity of the interaction [Nose et al. (1990)]. Several studies demonstrated the importance of two elements of the EC1 domain in adhesion: namely, the conserved Trp2 and the hydrophobic pocket in the vicinity of the conserved HAV sequence [Blaschuk et al. (1990); Nose et al. (1990); Shapiro et al. (1995); Boggon et al. (2002); Häussinger et al. (2002)]. The recent X-ray structure of C-cadherin suggested that Trp2 is important for the *trans* association of classical cadherins [Boggon et al. (2002)]. According to this study the interacting cadherins form a strand dimer interface This interface involves an exchange of EC1 domain β-strands with the insertion of the conserved Trp2 side chain into the hydrophobic pocket in the opposite molecule (Fig. 1B). The exchanged β-strands form an anti-parallel β conformation involving hydrogen bonds between residues 1 to 3 of strand A and residues 27 to 25 of the partner B strand.

The evidence favoring the role of the *trans* strand dimer interface in adhesion is not completely conclusive. Site directed mutagenesis experiments demonstrated the importance of the Trp2 and the hydrophobic pocket for adhesive function [Tamura et al. (1998)]. However these experiments cannot discriminate between *trans* or *cis* associations. Moreover, electron microscopy studies showed that the mutation W2A abolishes *trans* but not *cis* interactions between chimeric E-cadherins [Pertz et al. (1999)]. To further complicate this scenario, recent ex-

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 MCB, vol.1, no.3, pp.169-180, 2004

Nonlinear Elastic and Viscoelastic Deformation of the Human Red Blood Cell with Optical Tweezers

J. P. Mills[1], L. Qie[2], M. Dao[1], C. T. Lim[2] and S. Suresh[1,3]

Abstract: Studies of the deformation characteristics of single biological cells can offer insights into the connections among mechanical state, biochemical response and the onset and progression of diseases. Deformation imposed by optical tweezers provides a useful means for the study of single cell mechanics under a variety of well-controlled stress-states. In this paper, we first critically review recent advances in the study of single cell mechanics employing the optical tweezers method, and assess its significance and limitations in comparison to other experimental tools. We then present new experimental and computational results on shape evolution, force–extension curves, elastic properties and viscoelastic response of human red blood cells subjected to large elastic deformation using optical tweezers. Potential applications of the methods examined here to study diseased cells are also briefly addressed.

1 Introduction and overview

Optical tweezers are finding increasing application in the study of single biological cells. Ashkin et al (1987) used infrared laser beams to optically trap and manipulate single cells. An optical trap was used by Svoboda et al (1992) to isolate the membrane skeleton from a red blood cell, which was treated with a non-ionic detergent, so as to study the effect of ionic strength on the contraction of the spectrin network. Bronkhorst et al (1995) employed triple traps to deform and to explore shape recovery of red blood cells.

Direct tensile stretching of the human red blood cell using optical tweezers to extract elastic properties was first reported by Hénon et al (1999) who attached two silica beads non-specifically to diametrically opposite ends of the cell, trapped both beads with laser beams, and imposed tensile elastic deformation on the cell by moving the trapped beads in opposite directions. Forces were calibrated by subjecting a trapped bead to counter flow following the procedures outlined by Svoboda and Block (1994) and Simmons et al (1996). Stokes' law was used to estimate the force on the trapped bead from known fluid velocity. With a 1.064 μm Nd:YAG laser beam of 605 mW maximum emission power and silica beads 2.1 μm in diameter, they imposed maximum tensile forces that were estimated to be 56 pN on discocytic and osmotically swollen, nearly spherical cells. By employing simple analytical expressions based on two-dimensional linear elastic, small deformation, idealization of the cell, they examined variations in only the transverse diameter of the cell with applied force, and ignored possible contributions to deformation arising from the bending stiffness of the cell membrane and cytoskeleton. Finite contact between the beads and the cell membrane during stretching by optical tweezers was also not considered. The in-plane shear modulus of the cell membrane was estimated from this approach to be 2.5 ± 0.4 μN/m. This estimate is lower than the range of shear modulus values of 4 to 10 μN/m obtained from a number of independent investigations which employed the more commonly known micropipette aspiration technique (Evans and Skalak, 1980; Hochmuth and Waugh, 1987; Discher et al, 1998; Boal, 2002). Studies by the same group (Lenormand et al, 2001) employing triple bead optical tweezers measurements showed that the area expansion modulus and shear modulus of red blood cells were higher in an isotonic buffer than in a low hypotonic buffer.

Sleep et al (1999) also estimated elastic properties of the human red blood cells by optical tweezers where two polystyrene latex beads of 1 μm diameter were trapped using a 1.047 μm Nd:YLF laser beam. In this experiment, one bead was held fixed and the other moved with a second trap to induce tensile deformation in the cell.

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An Improved Mathematical Approach for Determination of Molecular Kinetics in Living Cells with FRAP

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Abstract: The estimation of binding constants and diffusion coefficients of molecules that associate with insoluble molecular scaffolds inside living cells and nuclei has been facilitated by the use of Fluorescence Recovery after Photobleaching (FRAP) in conjunction with mathematical modeling. A critical feature unique to FRAP experiments that has been overlooked by past mathematical treatments is the existence of an 'equilibrium constraint': local dynamic equilibrium is not disturbed because photobleaching does not functionally destroy molecules, and hence binding-unbinding proceeds at equilibrium rates. Here we describe an improved mathematical formulation under the equilibrium constraint which provides a more accurate estimate of molecular reaction kinetics within FRAP studies carried out in living cells. Due to incorporation of the equilibrium constraint, the original nonlinear kinetic terms become linear allowing for analytical solution of the transport equations and greatly simplifying the estimation process. Based on mathematical modeling and scaling analysis, two experimental measures are identified that can be used to delineate the rate-limiting step. A comprehensive analysis of the interplay between binding-unbinding and diffusion, and its effect on the recovery curve, are presented. This work may help to bring clarity to the study of molecular dynamics within the structural complexity of living cells.

keyword: GFP, cytoskeleton, nuclear matrix, diffusion, mathematical model

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1 Introduction

Our understanding of cell structure and function is largely based on static images, yet virtually all structures and molecular species are in a state of dynamic equilibrium and undergo continual turnover within living cells. Moreover, it is becoming increasingly clear that many molecules also may become immobilized through binding interactions with insoluble molecular scaffolds, such as signaling complexes, cytoskeletal filaments, and nuclear matrix that appear beneath the surface membrane, in the cytoplasm, and within the nucleus, respectively (Ingber, 1993). A powerful approach for measuring the dynamics of structures and molecules is to track fluorescently tagged molecules in living cells (Lippincott-Schwartz et al., 1999; Lippincott-Schwartz J, 2001). The mobility of molecules (characterized by the diffusion coefficient) can be determined using laser photobleaching techniques, such as fluorescence recovery after photobleaching (FRAP)(Jacobson et al., 1976; Salmon et al., 1984; Schindler et al., 1980) in conjunction with mathematical models (Axelrod et al., 1976; Phair and Misteli, 2001; Tardy et al., 1995). In FRAP, fluorescently-labeled molecules within a small region of the surface membrane, cytoplasm, or nucleus are exposed to a brief pulse of radiation from a laser beam. When the appropriate wavelength of light is utilized, this irradiation bleaches all of the molecules within the path of the beam without altering their structure or function (Phair and Misteli, 2001). Repeated fluorescence images of the bleached zone can be used to measure the rate at which fluorescent molecules redistribute and replace photobleached ones.

If the fluorescent molecules were freely diffusing, then the resulting recovery curve could be used to estimate their diffusion coefficient. However macromolecules in cells usually recover much more slowly than would be predicted for diffusion under similar solution conditions.

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On the Molecular Basis for Mechanotransduction

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Abstract: Much is currently known about the signaling pathways that are excited when cells are subjected to a mechanical stimulus, yet we understand little of the process by which the mechanical perturbation is transformed into a biochemical signal. Numerous theories have been proposed, and each has merit. While cells may possess many different ways of responding to stress, the existence of a single unifying principle has much appeal. Here we propose the hypothesis that cells sense mechanical force through changes in protein conformation, leading to altered binding affinities of proteins, ultimately initiating an intracellular signaling cascade or producing changes in the proteins localized to regions of high stress. More generally, this represents an alternative to transmembrane signaling through receptor-ligand interactions providing the cell with a means of reacting to changes in its mechanical, as opposed to biochemical, environment. One example is presented showing how the binding affinity between the focal adhesion targeting domain of focal adhesion kinase and the LD motif of paxillin is influenced by externally applied force.

1 Introduction

Studies during the past two decades have illustrated the wide range of cellular responses to mechanical stimulation. Much of the impetus for this work stems from the discovery that stresses experienced in vivo are instrumental in a wide spectrum of pathologies. One of the first diseases found to be linked to cellular stress was atherosclerosis, where it was demonstrated that hemodynamic shear influences endothelial function [Dewey, Bussolari, Gimbrone, and Davies (1981)], and that conditions of low or oscillatory shear stress are conducive to the formation and growth of atherosclerotic lesions [see reviews (Davies (1995), Lehoux and Tedgui (2003))]. Even before then, the role of mechanical stress on bone growth and healing was widely recognized, and since then, many other stress-influenced cell functions have been identified. Collectively, this work has evolved into a field of its own, sometimes referred to as "mechanobiology".

Many have investigated the signaling cascades that become activated as a consequence of mechanical stress, and these are generally well characterized. The initiating process, however, by which cells convert the applied force into a biochemical signal, termed "mechanotransduction", is much more poorly understood, and only recently have researchers begun to unravel some of these fundamental mechanisms. Several theories exist that might explain the process of mechanotransduction, but most are still in their infancy. For example, it is known that the cell membrane contains stretch-activated ion channels that change conductance as membrane tension is increased. While many such channels have been identified, the atomic structure of only a few is known, and molecular dynamics simulations have proven somewhat inconclusive. It is also widely appreciated that proteins, in addition to transmembrane channels, undergo conformational change when subjected to stress. Such conformational change can alter binding affinities to other proteins, but might also initiate enzymatic activity, which has the attractive potential of leading to signal amplification. It is straightforward to demonstrate that the forces experienced by single proteins at the threshold of mechanotransduction are above the level of thermal noise and of sufficient magnitude to induce such changes in conformation or even bond rupture. Finally, we also know that changes in the intracellular distribution of structural proteins occur rapidly under the action of external force. All this points to the role of force-induced conformational change as a primary mechanism

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