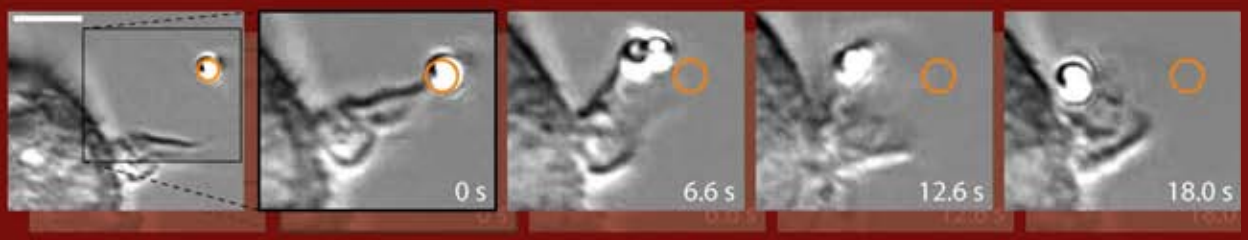
Cell mechanics during phagocytosis studied by optical tweezers-based microscop

Holger Kress



Cuvillier Verlag

Dissertation

submitted to the Combined Faculties
for the Natural Sciences and for Mathematics
of the Ruperto-Carola University of Heidelberg, Germany

for the degree of Doctor of Natural Sciences

---

presented by
Dipl.-Phys. **Holger Kreß**
born in Essen
Oral examination: July 26th 2006

Bibliografische Information Der Deutschen Bibliothek
Die Deutsche Bibliothek verzeichnet diese Publikation in der Deutschen Nationalbibliografie; detaillierte bibliografische Daten sind im Internet über http://dnb.ddb.de abrufbar.
1. Aufl. - Göttingen : Cuvillier, 2006
Zugl.: Heidelberg, Univ., Diss., 2006
ISBN 3-86727-007-4

Nonnenstieg 8, 37075 Göttingen
Telefon: 0551-54724-0
Telefax: 0551-54724-21
www.cuvillier.de

1. Auflage, 2006
Gedruckt auf säurefreiem Papier

ISBN 3-86727-007-4

Cell mechanics during phagocytosis studied by optical tweezers-based microscopy

---

Holger Kreß

Referees:
Prof. Dr. Jörg Schmiedmayer, University of Heidelberg
Prof. Dr. Alexander Rohrbach, University of Freiburg

Zusammenfassung

Phagozytose ist ein zentraler Bestandteil des Immunsystems von Säugetieren und wurde bislang im Rahmen zellbiologischer Studien hauptsächlich mit Hilfe konventioneller Licht- und Elektronenmikroskopie untersucht. Die mechanischen Eigenschaften dieses Prozesses, wie beispielsweise die Kräfte und Energien die hierbei wirken, sind bislang kaum bekannt. In der vorliegenden Arbeit wurde eine auf einer optischen Pinzette basierende Mikroskopie verwendet, um im Zusammenspiel mit beschichteten Beads, die als bakterielles Modellsystem fungierten, die mechanischen Eigenschaften des Phagozytoseprozesses zu untersuchen. Die Anbindung, die Aufnahme in die Zelle und der intrazelluläre Transport von einzelnen, optisch gefangenen Beads wurde durch die optische Pinzette induziert und in 3D mit Nanometer-Präzision mit einer interferometrischen Technik gemessen. Dabei wurde ein neuartiges zelluläres Verhalten entdeckt: Einige Sekunden nach dem Anbinden von Beads an Filopodien und Membran-Ruffles zogen diese die angebundenen Beads zur Zelle hin. Die Beobachtung von diskreten, F-Aktin-abhängigen 33-Nanometer-Schritten während dieses Zurückziehens führte zur Hypothese, dass ein prozessiver Myosin-Motor involviert ist. Kraft-Geschwindigkeits-Messungen konnten die mechanischen Eigenschaften dieses potentiellen Motors aufdecken. Ein hier entwickeltes Modell zur Kraft-abhängigen Motor-Kinetik konnte die Messungen theoretisch untermauern. Um die erwähnten Experimente durchzuführen, wurden die Fang- und Detektions-Eigenschaften des Mikroskops signifikant verbessert und aufgezeigt, dass diese sehr gut mit theoretischen Vorhersagen übereinstimmen, welche auf Fourier-Optik und erweiterter Mie-Streutheorie basieren.

Abstract

Phagocytosis, which is a central cellular mechanism in the mammalian immune system, was so far - in the context of cell-biological studies - mainly investigated by conventional light and electron microscopies. However, the mechanical properties of this process, like the forces and energies involved were barely known up to now. Here, optical tweezers-based microscopy in combination with coated beads as bacterial model system was applied to investigate the mechanical properties of phagocytosis. The binding, the uptake into the cell and the intracellular transport of single optically trapped beads was induced by the optical trap and measured in 3D with nanometer precision by an interferometric tracking technique. Thereby, a novel cellular behaviour was discovered: A few seconds after binding, filopodia and ruffles retracted and pulled the bound beads towards the cell. The observation of discrete F-actin dependent 33-nanometer steps during retraction led to the hypothesis that a processive myosin motor plays an important role in the retraction. Force-velocity measurements revealed the mechanical properties of this putative motor. A model for the force-dependent motor kinetics confirming these results was developed. In order to perform these measurements, the microscope's optical trapping and tracking properties were significantly improved and shown to be in very good agreement with theoretical predictions based on Fourier-optics and extended Mie-scattering theory.

Publications

This thesis is partly based on the following publications:

Articles

Holger Kress, Ernst H.K. Stelzer, Daniela Holzer, Folma Buss, Gareth Griffiths, and Alexander Rohrbach
Phagocytic tentacles - forces, velocities and step sizes of filopodial retraction
(*in preparation*)

E. Anes, P. Peyron, L. Staali, L. Jordao, M.G. Gutierrez, H. Kress, M. Hagedorn, I. Maridonneau-Parini, M.A. Skinner, A.G. Wildeman, S.A. Kalamidas, M. Kuehnel, and G. Griffiths
Dynamic life and death interactions between *Mycobacterium smegmatis* and J774 macrophages
Cellular Microbiology, **8** (6), 939-960 (2006)

Holger Kress, Ernst H.K. Stelzer, Gareth Griffiths, and Alexander Rohrbach
Control of relative radiation pressure in optical traps: Application to phagocytic membrane binding studies
Physical Review E, **71**, 061927 (2005)

Holger Kress, Ernst H.K. Stelzer, and Alexander Rohrbach
Tilt angle dependent three-dimensional position detection of a trapped cylindrical particle in a focused laser beam
Applied Physics Letters, **84** (21), 4271-4273 (2004)

Alexander Rohrbach, Holger Kress, and Ernst H.K. Stelzer
Reply to comment on 'Trapping forces, force constants and potential depths for dielectric spheres in the presence of spherical aberrations'
Applied Optics, **43** (9), 1827-1829 (2004)

James E.N. Jonkman, Jim Swoger, Holger Kress, Alexander Rohrbach, and Ernst H.K. Stelzer
Resolution in Optical Microscopy
Methods in Enzymology, **360** - Biophotonics Part A, 416-446 (2003)

Alexander Rohrbach, Holger Kress, and Ernst H.K. Stelzer
Three-dimensional tracking of small spheres in focused laser beams: influence of the detection angular aperture
Optics Letters, **28** (6), 411-413 (2003)

Conference Proceedings

Holger Kress, Ernst H. K. Stelzer, and Alexander Rohrbach
Measuring and adjusting the trapping position in optical tweezers
DGaO Proceedings 2005, ISSN: 1614-8436 (2005)

Conference Abstracts

Holger Kress, Ernst H. K. Stelzer, Gareth Griffiths, and Alexander Rohrbach
Investigating phagocytosis by optical tweezers-based microscopy
DPG Conference - Biophysics Section, March 27-31 (2006) Dresden, Germany

Alexander Rohrbach, and Holger Kress
Optical trapping and tracking: novel approaches in cell biophysics
DPG Conference - Biophysics Section, March 27-31 (2006) Dresden, Germany

Holger Kress, Peter Seitz, Nils Becker, and Alexander Rohrbach
Interferometric imaging of interactions: From single proteins to whole cells
International Imaging Symposium, November 4-5 (2005) Freiburg, Germany

Holger Kress, Ernst H. K. Stelzer, Gareth Griffiths, and Alexander Rohrbach
Phagocytic cell membrane mechanics probed by photonic force microscopy
Optical Trapping and Optical Micromanipulation II, SPIE's 50th Annual Meeting, July 31 - August 4 (2005) San Diego, USA

Holger Kress, Ernst H.K. Stelzer, Gareth Griffiths, and Alexander Rohrbach
Membrane and particle binding dynamics during the earliest events in phagocytosis
WEH Seminar: Dynamics of Cell and Tissue Structure, May 22-26 (2005) Bad Honnef, Germany

Holger Kress, Ernst H.K. Stelzer, Gareth Griffiths and Alexander Rohrbach
Radiation pressure control in photonic force microscopy and application to biophysical membrane binding studies
106th Conference of the DGaO, May 17-21 (2005) Wroclaw, Poland

Alexander Rohrbach, Holger Kress, Nils B. Becker, and Ernst H.K. Stelzer
Imaging interactions with photonic force microscopy
Focus on Microscopy Conference, March 20-23 (2005) Jena, Germany

Holger Kress, Ernst H.K. Stelzer, Gareth Griffiths, and Alexander Rohrbach
Membrane dynamics and membrane binding events investigated by photonic force microscopy
DPG Conference - Biophysics Section, March 4-9 (2005) Berlin, Germany

Alexander Rohrbach, Ernst H.K. Stelzer, Holger Kress, and Nils B. Becker
Imaging of molecular interactions with photonic force microscopy
DPG Conference - Biophysics Section, March 4-9 (2005) Berlin, Germany

Holger Kress, Ernst H.K. Stelzer, and Alexander Rohrbach
Measuring phagocytic binding and uptake by photonic force microscopy
Optical Trapping and Optical Micromanipulation, SPIE's 49th Annual Meeting, August 2-6 (2004) Denver, USA

Holger Kress, Alexander Rohrbach, and Ernst H.K. Stelzer
Non-spherical probes in photonic force microscopy
Focus on Microscopy Conference, April 13-16 (2003) Genova, Italy

Contents

1. Introduction

About sixty years ago, Erwin Schrödinger raised in his book 'What is Life?'[1] the question whether physics and biology are compatible and whether living organisms can be explained by the laws of physics. In his book, Schrödinger discussed questions about genetic stability and mutations in terms of basic physical principles by assuming that genes have a molecular basis. He did this at a time when the word 'gene' was still used as a conceptional term. Exactly in the publication year of 'What is Life?' it was revealed that genes consist of DNA (deoxyribonucleic acid) molecules. An eighteen year old student was so impressed by Schrödinger's book that he desired to research the gene. A few years later, he revealed - together with Francis Crick - the double-helix structure of DNA.[2]

At the time of Watson's and Crick's discovery, living cells were described as 'chemical reactors', small vessels which contain a complex composition of substances undergoing chemical reactions through diffusion and random collision.[3] Since then, the structural knowledge about the molecular cell components increased strongly by techniques like X-ray crystallography, nuclear magnetic resonance spectroscopy and electron microscopy. Simultaneously, more and more attention was drawn to the question of the structural basis of biomolecular interactions.

Cells were no longer considered as homogeneous reaction vessels but rather as small 'factories' containing molecular 'machines' to carry out highly specialized processes like DNA transcription, RNA translation, organelle transport, chromosomal segregation, cell motility or protein folding. On the scale of individual molecules, many of these processes are mechanical processes and can be described by basic physical concepts like force, torque, energy or power.[3]

The recent advent of experimental tools for the direct mechanical manipulation of single cells or even single molecules enables the investigation of the mechanical cellular machinery. One of the tools that are in particular suitable for this purpose are optical tweezers. Optical tweezers consist of a tightly focussed laser beam which allows the three-dimensional trapping of small objects like beads. A small bead, attached to a single molecule or a cell can be trapped by optical tweezers and used as a handle to measure mechanical properties such as forces, torques, energies or powers.

In the work presented here, optical tweezers with an interferometric fluctuation tracking system were applied to study cellular mechanics during the process of phagocytosis. Phagocytosis is a central cellular mechanism in the innate mammalian immune system. Although many cell types can undergo this process under some conditions, it is a major function of macrophages, which are cells specialised on phagocytosis. When an invading bacterium binds to the membrane of a macrophage, the cell membrane starts to wrap around the invader and internalizes the bacterium. Thereby, the bacterium is enclosed into an intracellular membrane-organelle, the phagosome.

The process of phagocytosis is highly interesting from a biomedical point of view as well as from a biophysical one. Biomedically, phagocytosis is very important because macrophages are able to kill most of the potential infectious agents fast and with only little inflammation. However several pathogens, are able to undermine this process. One of these pathogens is *Mycobacterium tuberculosis*, the causative agent of the disease tuberculosis, which causes about two million deaths per year. The mechanisms used by *M. tuberculosis* to survive inside macrophages are still poorly understood and a deeper knowledge of the phagocytosis process might help in fighting this disease. So far, phagocytosis was mainly investigated by conventional light and electron microscopies, which provide primarily kinematic and structural information (kinematic and structural information at a resolution on the order of hundreds of nanometers in the case of conventional light microscopies and structural information at a resolution in the range of Ångström in the case of electron microscopies). However, the mechanical properties of the molecular machinery driving this process, like the physical forces and energies involved were barely known up to now.

Biophysically, phagocytosis involves various interesting aspects: The first step of phagocytosis is the binding of the bacterium to the macrophage plasma membrane. This process involves the (sequential) bacterial binding to receptors in the cell membrane. The dynamics of this binding process should depend on e.g. the receptor density within the membrane or the strength of the receptor-ligand bond. The next phagocytosis step is then the wrapping of the membrane around the bacterium. This process is thought to be caused by a remodeling of the actin-cortex which is located on the cytosolic side of the plasma membrane. After the bacterium is taken up by the cell, the newly formed phagosome is transported inside the cell by molecular motor proteins along cytoskeletal tracks. Neither the dynamics of the binding process nor the mechanical properties of the uptake and the subsequent three-dimensional intracellular transport were investigated so far on the molecular level of nanometers and picoNewtons.

In order to tackle these tasks, optical trapping and tracking tools were developed and their optical properties were compared to theoretical predictions based on Fourier-optics and extended Mie theory (Chapter 2). These tools were then applied to investigate the mechanical properties of phagocytic binding, uptake

and intracellular transport in single living cells (Chapter 3). Finally, a Brownian dynamics simulation was developed to validate the experimental data and to increase to amount of information that can be gained experimentally about mechanical cellular processes (Chapter 4).