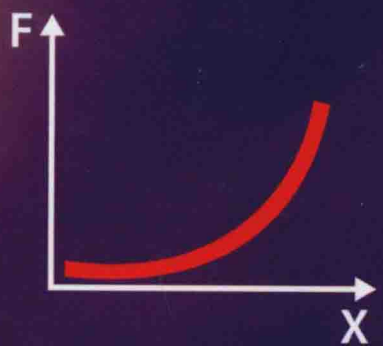
Cellular Analysis by Atomic Force Microscopy

Malgorzata Lekka



In the past two decades, cell biomechanics has become an emerging field owing to a strong link between mechanical properties and alterations observed in various pathologies. Despite substantial evidence showing the feasibility of atomic force microscopy (AFM) for identifying cells with distinct elastic and adhesive properties, the use of this technique as a complementary diagnostic method still remains controversial. This is primarily due to the relatively high complexity of the apparatus, experiment, and data analysis and the lack of a basis for the rapid verification of results.

The measurement of living cells by AFM is not an easy task, but this book facilitates AFM measurements and data analysis. It is a practical textbook that teaches in an easy-to-understand manner how to assess the mechanical characteristics of living, individual cells by AFM. Following a step-by-step approach, it introduces the methodology of measurements in both determination of elastic properties and quantification of adhesive properties.

Malgorzata Lekka is a full professor and head of the Department of Biophysical Microstructures at the Henryk Niewodniczański Institute of Nuclear Physics, Polish Academy of Sciences, Poland. She received her MS in physics in 1993 from the Faculty of Mathematics and Physics, Jagiellonian University, Poland, and doctorate (summa cum laude) and habilitation in physics from the Henryk Niewodniczański Institute of Nuclear Physics in 1998 and 2008, respectively. In 1999, Dr. Lekka presented the first AFM results that proved different mechanical properties of normal and cancerous cells. Her principal areas of interest are AFM and its application in cell biomechanics, structure of the cytoskeleton and its functions, reactive oxygen species and their influence on cells, and mechanical and structural differences between normal and cancerous cells.

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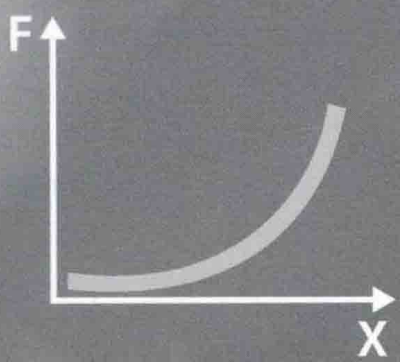

Preface

In the recent two decades, there has been ample evidence that shows the capability of detecting pathologically changed cells basing on their mechanical properties. Such studies commenced to be attractive for biology and medicine with the development of techniques enabling the measurements at a single-cell level. One of such techniques is atomic force microscopy (AFM). Despite evidence showing the feasibility of AFM to identify cells with altered elastic and adhesive properties, the use of this technique as a complementary diagnostic method, for example, in cancer treatment, raises a lot of controversy. This is primarily due to the still high complexity of the apparatus, the complexity of the experiment and data analysis, and the lack of basis for the rapid verification of the results. In addition, there are also doubts whether a high sensitivity of the method (measuring the properties of a single cell) will be sufficient to validate the cancer cell identification at the same level as it is carried out in the analysis of aspirate composed of millions of cells at different stages of progression.

The monograph *Cellular Analysis by Atomic Force Microscopy* presents the use of the AFM as a tool for the characterization of cancerous cells by studies of cellular deformability and cells' adhesive properties. The text is organized as follows: after a brief introduction pointing out the significance of cellular deformability and adhesive properties, Chapter 2 shortly discusses the basic elements of the cell structure, on the knowledge level that facilitates understanding of the role and significance of mechanical properties in cancer. Chapter 3 is devoted to the AFM technique itself. It covers the description of the idea and the construction details of the atomic force microscope, introduces the force spectroscopy mode of its operation, and discusses the calibration issues. The next two chapters are focused on specific applications: Chapter 4 is devoted to cellular deformability measurements by the AFM and Chapter 5 introduces the technique for quantification of cellular adhesive properties. The monograph concludes with

Final Conclusions, briefly summarizing the use of atomic force microscopy in cancer research.

This monograph* is designed to be a kind of a practical textbook, presenting in a concise manner the methodology of assessing the mechanical characteristics of individual cells by AFM. It is addressed to those researchers who need some hints in cases when strange results came out from the AFM experiment. I hope that the monograph will help eliminate at least some questions.

I would like to dedicate this book to all people who helped me in different ways in my research. I am taking this moment to express my deep gratitude to all of them. In particular, I would like to mention Zbigniew Stachura (IFJ PAN), who was always ready to answer questions and give invaluable advice, and whose continuous support enabled to establish and organize the biological activity of the AFM laboratory; Piotr Laidler (CM UJ) for teaching me how to understand biological phenomena, for his help during the realization of my biological experiments, and, most important, for showing me the importance of my studies; Jan Styczeń (IFJ PAN) for his support and for encouraging me in my studies over many years during his heading of the former Department of Nuclear Spectroscopy. I would like to also acknowledge Joanna Wiltowska-Zuber (IFJ PAN) for her invaluable help with managing the AFM laboratory, especially during the time when I was writing this monograph. Finally, I would like to thank my whole family and especially my husband, Janusz, for continuous support.

The topics of this book were also conceived within the networking research activity of the EU COST Action on Applications of Atomic Force Microscopy to NanoMedicine and Life Sciences (AFM4NanoMed&Bio) and the NCN project number NCN DEC-2011/01/M/ST3/00711.

Malgorzata Lekka

*A large portion of the this book has been prepared on the basis of my IFJ PAN Report, 2001/AP entitled "The use of atomic force microscopy as a technique for the identification of cancerous cells."

Contents

Chapter 1

Introduction

Cancer is a very complex disease, involving multiple molecular and cellular processes arising from a gradual accumulation of genetic changes in individual cells. The most apparent morphological change is visible during the transition from a benign tumor to metastatic tumors where cells alter from highly differentiated normal phenotypes to migratory and invasive ones. Around 90% of all cancer deaths are due to metastatic spread of primary tumors. The criteria utilized to detect cancerous cells have been mainly relying on biological and morphological description, additionally complemented by a variety of other techniques, including genetic, chemical, and immunological methods, applied in order to fine-tune diagnosis or therapy. Despite enormous efforts to develop better treatment protocols, our ability to cure solid tumors, such as those of the breast, prostate, cervix or colon, is still lacking sufficient detection methods [1].

The cells transformed oncogenically differ from normal ones in many ways, encompassing variations in any cellular aspects such as growth, differentiation, interactions between neighboring cells and/or with the extracellular matrix (ECM), cytoskeleton organization, and several others. Poor differentiation of the cytoskeleton can result in the larger deformability of cancerous cells. Low stiffness of cancer cells is related to a partial loss of actin filaments and/or microtubules, and therefore by lower density of

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the cellular scaffold [2, 3]. Moreover, one of the key phenomena in metastasis includes adhesive interactions, maintained by distinct type of adhesion molecules present on a cell surface. Cancerous cell aptitude for invasion and migration (clinically interpreted as tumor aggressiveness) has been associated with poor differentiation of the cell and the altered adhesive interactions that characterize a vast majority of cancer cells.

It is obvious that novel techniques are in the limelight if they are able to bring more precise, local information about cancerous changes as early as possible. There are rather few methods capable to assess cell mechanical properties. Historically, the first technique was the micropipette aspiration [4, 5]. Other researchers have employed the magnetic bead rheology [6], microneedle probes [7], acoustic microscopes [8], and the manipulation of beads attached to cells with optical tweezers [9, 10]. Among these techniques, the atomic force microscopy (AFM) can detect malignant changes with a very high resolution, being applied either in imaging mode or as the technique providing information about the mechanical properties of living cells (i.e., their ability to deform and to adhere) in a quantitative manner. Its main advantage is the possibility to measure biological objects directly in their natural environment, such as buffer solutions or culture media.

Many publications in this area were devoted to the characterization of single cells' deformability and adhesiveness, presented in a broad context of biological targets, starting from cell motility, would healing, muscle contraction or differentiation and ending up in characterization of various pathologies such as muscular dystrophies, blood diseases or cancers. Therefore, in this Chapter, the importance of cellular ability to deform and to adhere is presented with the focus on the AFM-related aspects in cancer studies.

1.1 Cell Ability to Deform

Within the past two decades, the cellular ability to deform has attracted great interest in the field of biology. This is because in human body, various cell types are continuously exposed to passive (stretch, compression) and/or active (contraction) deformations. The technological development of techniques, that