Recent progress in AFM and Nanomedicine - Applications of Force Spectroscopy and Peak Force Tapping

By Sean Griffiths (Swansea University) and Alex Berquand (Bruker Nano)
Principle of AFM

Optical detection system

Different feedbacks for different AFM modes
Combining AFM with IOM
2 techniques in 1 tool

AFM

Optics
Combining AFM with IOM

Automatic Overlay (MIRO)

1) Capture 3 sample images at 3 different locations.
2) Capture 1 tip image.
3) Capture a background image and overlay it with an AFM image.

1) Import optical image into Nanoscope
2) Target a location for the AFM scan
3) Overlay optical and AFM images
Combining AFM with IOM
Automatic Overlay (MIRO)

Live Hela cells
Combining AFM with IOM

Compatibility with various optical techniques

AFM + ...

Bright Field

DIC

Epifluorescence

Confocal

TIRF

RAMAN

Sample courtesy Nat. Univ. Singapore

Sample courtesy NIH

Sample courtesy SWMC Stanford

Sample courtesy Nat. Univ. Singapore

Sample courtesy INRA Nantes

Sample courtesy Zeiss
Tapping mode and phase signal

Are they quantitative?

- height
- amplitude
- phase $\rightarrow$ $R^*$

---

Topography

- surface pties
- volume pties

$F_{adh}$

$E^*$, $\mu$
Force Spectroscopy

Get access to stiffness and adhesion
Application: pharmacology

Testing drug specificity

Vancomycin

D-Ala-D-Ala

Au support

WT

Mut D-Ala-D-Lac

Mut D-Ala-D-Val

- Proves the very high specificity of the interaction

Dufrene et al. (2005) Nature methods
AFM/IOM Application:
*Correlating specificity of interaction to fluorescence intensity*

Cells transfected with GPI/GFP plasmids

Transfected cells: fluorescence + specific interactions

Non-transfected cells: non-fluorescence + no interactions

Pro-Aerolysin

GPI-anchor protein
AFM/IOM Application: neurology

Stimulating live neurons with a modified SPM probe
**AFM to study infectious diseases**

*Listeria monocytogenes*

- Septins play a role in the interaction between *L. monocytogenes* invasion protein InlB and the Met receptor.

- Septin depletion or overexpression dramatically changes the shape and mechanical properties of cells and the interaction between InlB and Met receptor.

- Proves the role of the septins in the function of surface receptors.

Mostowy et al. (2011) *Biophys J*
Need for a new characterization technique

**Peak Force Tapping and Peak Force QNM**

PFT is based on **ScanAsyst** (fully Automated AFM)

Works with most **standard AFM probes** in the standard AFM cantilever holders.

Z piezo is driven with sinusoidal waveform (not a triangle as in force-distance curves).

**Z drive frequency is 1-2 kHz.** Z drive amplitude is fixed at typical value of 150 nm (300 nm peak-to-peak)

Vertical motion of probe produces **force-distance plots as it taps on the sample.**

Imaging **feedback is based on the Peak Force** of the force-distance curve.

The probe can be calibrated before the experiment so that all the channels are directly quantitative: **PFQNM**
Needed range of Young’s moduli

**Example: Human Body**

How soft/hard are your body tissues?

- **Brain**: 1-4 kPa
- **Spleen**: 4-8 kPa
- **Heart**: 10-15 kPa
- **Ventricles**: 15-20 kPa
- **Tendon**: 1300-1700 kPa
- **Fat**: 0.5-1.0 kPa
- **Kidney**: 5-10 kPa
- **Muscle**: 10-20 kPa
- **Intestine**: 20-40 kPa
- **Bone Marrow**: 0.5-1.5 kPa
- **Lung**: 3-6 kPa
- **Liver**: 8-12 kPa
- **Arteries**: 12-20 kPa
- **Skin**: 3-7 kPa
- **Cartilage**: 1000-1500 kPa
- **Teeth**: 20000-25000 kPa
- **Bone**: 15000-20000 kPa
Overview: PeakForce QNM®

**Basic Principle**

- **PeakForce Tapping** is an oscillating technique that can be used to image a wide range of samples at a high resolution.

- The probe can easily be calibrated prior to the experiment. The technique is referred to as **PeakForce QNM**. In that case, all the recorded data are directly quantitative.

- **Peak Force QNM** can now be operated on live cells and can be used to test changes in morphology and any other property in response to various factors.
Background: Glyphosate
A Harmful Herbicide

- The Human skin is the first physiological barrier against physical and chemical aggressions.

- Glyphosate (N-(phosphonomethyl)Glycine) is a broad spectrum systemic herbicide used to kill weeds. It’s been extensively used in the late 2000’s.

- Its toxicity on lab animals has been clearly demonstrated but its effects on humans remain unclear.
• **Glyphosate-treated** samples show a **higher number of necrotic and apoptotic cells**, and a higher \([\text{H}_2\text{O}_2]\) than controls.

• **Keratinocytes** and **HaCat** cells are **difficult to image** by classical AFM modes. A **more reliable technique** is required.

• **Need for a faster way** to probe the oxidative stress. Changes in morphology and mechanical properties might be detectable by using **PeakForce QNM**.
Drug treatment induces a significant increase in Young’s modulus (x3) and a decrease in deformation (x2).

Changes in mechanical properties can directly be correlated to changes in morphology.
Glyphosate-induced stiffening of HaCaT keratinocytes, a Peak Force Tapping study on living cells

Celine Heu**, Alexandre Berquand*, Celine Elie-Gaill†, Laurence Nicod*†

* Université de Franche-Comté, Laboratoire de Biochimie, CNRS, UMR 6154, 1 rue de l’Université, 25000 Besançon, France
** University Hospital, Clinical and Experimental Pharmacology, Dijon, France (CPPIT, UFR 6154 CNRS, 21001 Dijon cedex, France)
† Bruker - Nano Surface Science Systems, 9, Marsland, Germany

ABSTRACT

The aim is to: study biological functions of living cells, using a complex construct, that provides different responses to multiple physical and chemical aggressions. glyphosate is an extremely used herbicide that has been shown to increase the risk of cancer. Moreover, there is increasing evidence suggesting that the mechanical phenotype plays an important role in malignant transformation. Atomic force microscopy (AFM) has emerged as a novel tool for providing nanometer-scale resolution imaging of biological samples. Peak Force Tapping® (PFT) is a highly advanced AFM-based imaging technique allowing extraction of chemical and mechanical properties from a wide range of samples at a variety of resolutions and high resolution. The present work uses the PFT technology to investigate HaCat keratinocytes, a human epidermal cell line, and offers an original approach to study cellular changes in the cellular mechanical properties under non-physiological conditions. These experiments indicate that phosphorylation levels are the first step towards malignancy, and the agreement of results is a morphological change. The present results confirm that the mechanical behavior depends on different experimental conditions. Therefore, a well-known antioxidant, renolin, and the glyphosate-induced mechanical phenotype.

© 2012 Elsevier Inc. All rights reserved.

1. Introduction

The aim is to: study biological functions of living cells, using a complex construct, that provides different responses to multiple physical and chemical aggressions. glyphosate is an extremely used herbicide that has been shown to increase the risk of cancer. Moreover, there is increasing evidence suggesting that the mechanical phenotype plays an important role in malignant transformation. Atomic force microscopy (AFM) has emerged as a novel tool for providing nanometer-scale resolution imaging of biological samples. Peak Force Tapping® (PFT) is a highly advanced AFM-based imaging technique allowing extraction of chemical and mechanical properties from a wide range of samples at a variety of resolutions and high resolution. The present work uses the PFT technology to investigate HaCat keratinocytes, a human epidermal cell line, and offers an original approach to study cellular changes in the cellular mechanical properties under non-physiological conditions. These experiments indicate that phosphorylation levels are the first step towards malignancy, and the agreement of results is a morphological change. The present results confirm that the mechanical behavior depends on different experimental conditions. Therefore, a well-known antioxidant, renolin, and the glyphosate-induced mechanical phenotype.

*Author for correspondence: Celine Elie-Gaill, Laboratoire de Biochimie, CNRS, UMR 6154, 1 rue de l’Université, 25000 Besançon, France. E-mail address: celine.elie-gaill@univ-fcomte.fr

**Author for correspondence: Alexandre Berquand, University Hospital, Clinical and Experimental Pharmacology, Dijon, France (CPPIT, UFR 6154 CNRS, 21001 Dijon cedex, France). E-mail address: Alexandre.Berquand@univ-fcomte.fr

†Author for correspondence: Laurence Nicod, Université de Franche-Comté, Laboratoire de Biochimie, CNRS, UMR 6154, 1 rue de l’Université, 25000 Besançon, France. E-mail address: laurence.nicod@univ-fcomte.fr

DOI: 10.1016/j.jsb.2012.06.007

Data was published in a paper entitled ‘Glyphosate-induced stiffening of HaCat keratinocytes, a Peak Force Tapping study on living cells’ in Journal of Structural Biology.

The System: Bioscope Catalyst with PeakForce QNM and operated in fluid

Authors are Celine Heu, Celine Elie and Laurence Nicod (FEMTO) and Alexandre Berquand (Bruker Nano GmbH)
- Parasites enter bloodstream and infect RBCs.
- As parasites multiply, the RBCs breaks open and infects more RBCs.
- Infected RBCs are *misshapen* with *knob-like structures* on surface.
Background: Cytoadherence

Erythrocyte (Red Blood Cell) Infection

- *P. falciparum* infected erythrocytes (IE’s) exhibit cytoadherence (stick to blood vessel walls /endothelium).

- Prevents IE elimination by the spleen and causes vascular blockages.

- Interaction with endothelium surface receptors (eg. CD36).

Adapted from http://cmr.asm.org/cgi/content-nw/full/13/3/439/F1.

Adapted from the Plasmodium Genome Resource.
The Biological Question:
Can we map the distribution of cytoadherent molecules to specific cell surface structures?

- AFM probes were functionalized with endothelial surface receptor CD36.
- Used PeakForce QNM with functionalized probe to obtain 2D map of the distribution of CD36 molecular binding sites on IE.

Adapted from Li et al. (2011) PLoS ONE 6: 1-10.
• CD36 binding sites (high adhesion) correlate to knob structures (circles).
• Adhesion is a result of specific interactions between CD36 and knobs and not due to crosstalk between data channels (arrow).
Application Note #135
Quantitative imaging of living biological samples by Peak Force QNM Atomic Force Microscopy

It is now well established that measuring the mechanical properties of living cells in vivo can be a good indicator of the health of the organism from which they were extracted. Atomic force microscopy (AFM) is a powerful investigation and diagnostic tool, especially in force mode. Nevertheless, force spectroscopy suffers from several limitations, including the speed of acquisition, a relative lack of resolution, and the fact that it doesn’t directly provide quantitative information. In order to fill this gap, Bruker has developed a new imaging technique, PeakForce QNM®, which provides much more informative data at a high resolution and with remarkable ease-of-use. Up to now, this very promising technique has been tested successfully on a wide range of samples but not on living organisms. This Application Note gives an overview of the biological samples that can be imaged using this technique and the information that can be extracted from the atomic force microscopy data.

AFM and Cell Mechanics
Since its development, AFM has proven itself to be a tool of choice to image super soft biological samples, especially with the emergence of TappingMode® and force spectroscopy® and the fact that it is one of the few microscopy techniques that allow observation of cells under near-physiological conditions. It is now well documented that the mechanical properties of living cells can be quantified by AFM and used to estimate the effect of drug treatment or different types of pathologies, as well as many natural processes as aging.[7] For instance, it is becoming more and more evident that cancer cells and their normal homologues express clearly different mechanical properties.[8,9] Moreover, AFM is often used to correlate elastic behavior and cell migration or division.[9,10] The vast majority of these studies are based on TappingMode, single-force curves, or force-volume measurements. TappingMode offers the advantage of applying negligible nominal, friction, and shear forces, and phase imaging reflects the energy dissipated between the tip and the sample during each tap on the surface. Nevertheless, the nature of the phase signal remains always unclear since it is a mixed contribution of several surface and volume parameters, including adhesion forces, contact area, elasticity, viscosity, and dissipation. Even if working at specific ratio serpents allows more sensitivity to surface or volume properties, a quantitative extraction is impossible.

Force volume is another powerful technique based on force measurements achieved on a matrix of points defined by the user. Stiffness (and by extrapolation the sample Young’s modulus) and the adhesion between the tip and the sample can be extracted from each force curve. If the tip is functionalized with a molecule of interest, specific unbinding events can also be detected on the retraction.

- A comprehensive review of Peak Force QNM applications on soft biological samples
- Author is Dr. Alex Berquand and Dr. Ben Ohler (Bruker Nano Inc.).
Centre for NanoHealth
Swansea University

Nanotechnology, Medical School and Hospital System serving ~600,000 people in Wales
Centre for NanoHealth
Swansea University

- £22 million centre located between Swansea University campus and Singleton Hospital
- Combines nanotechnology with medical science
Nano-Health

Outline

- Nano-Health is the medical application of nanotechnology

‘Human health has always been determined on the nanometer scale; This is where the structure and properties of the machines of life work in everyone of the cells in every living thing. The practical impact of nanoscience on human health will be huge’

Richard E. Smalley, PhD **1996 Nobel Laureate in Chemistry** for the discovery of fullerenes.

‘Research and development activity in nanotechnology needs to be undertaken to improve the effectiveness of in-vivo and in-vitro diagnostics’

*European mission statement nanomedicine* (2005)
Centre for NanoHealth
Swansea University

**Nano-suite**
- Class 100/1000 clean room
- Bio-clean room
- Nanostructure growth
- SEM & AFM/SNOM
- NMR & Rheology
- Printing and Coating

**Bio-suite**
- NanoToxicology
- Cell Imaging
- Tissue Engineering
- Molecular biology
- Microbiology

**Direct access to**
- Clinical Trials Unit (Bedded first-into-man)
- Medical imaging (research MRI & CT)
Nanohealth

Quantum dots

Molecular imaging & therapy

- Targeted drug delivery systems
- Improved imaging
Nanohealth

Diagnostics – Biosensors / Biomarker Detection

- Novel Materials

ZnO nanowires (AFM)

Ultra-sensitive biosensor for the detection of bio-markers using bio-compatible ZnO nanowires.
Nanohealth

Effect of Se deficiency on cartilage degeneration: Kashin-Beck Disease

Corridor of Se deficiency in China

Mechanical Elasticity

H&E Stained Integration

Subcellular distribution of metal ions
The Menstrual cycle
An outline

Reproductive Cycle

Ovarian Cycle
- Primordial Follicle
- Primary Follicle
- Secondary Follicle
- Mature Follicle
- Ovulation
- Corpus Luteum
- Corpus Albicans
- Primordial Follicle

Hormonal Cycle
- Estrogen
- Progesterone
- LH
- FSH

Menstrual Cycle
- Menstrual Blood
- Spiral Artery
- Gland
- Glandular Pit
- Secretrions
- Thickness of Uterine Endometrium

Day
- Menstruation
- Proliferative
- 4
- 14
- 21
- 28
- Menstruation
Infertile pathologies and Cancer

An outline

• Cancer
  • Ovarian cancer

• Infertile pathologies
  • Polycystic ovary syndrome (PCOS) (ovulatory and non-ovulatory)
  • Endometriosis
  • Unexplained infertility
  • What uterine symptoms are disrupted in these disorders?

• Endometrial cancer
Spectrum of Reproductive Biology

Endometrial Function and Development

* Infertile Pathology
* Cancer

Cell Differentiation and Characterisation

Gene  Protein  Cell  Tissue
Regulation  Function

Co-activators  ERE  PRE  RNA POL II
Co-repressors

ERE  PRE
PR
P
ER  E

MUC1  OPN  $\alpha_{v}\beta_3$  CD44
PHD studentship
A systems approach to understanding adhesion in the endometrium

- High Throughput Quantitative Data

Morphological
Electrical
Elasticity
Adhesion

CLSM
FRET
RAHMAN
TIRF

Tissue Cells Membranes Molecules
Mechanical Strain Plasticity Motility Differentiation Drug Delivery/Effect Fate??

Uterus Cancer Adhesion

• Increases knowledge of cell-cell interactions
• IVF efficiency

Improves ART
Infertility
Affects 1/7 couples

Causes of infertility

- Tubal problems
- Endometriosis
- Unexplained
- Male factor

28% of all cases unexplained

Endometrial factor

Fallopian tubes
Ovary
Cervix
Endometrium
Infertility
At the microscale

Embryo

L-Selectin

Uterine epithelium

MUC1

Implantation site

Implantation markers
NanoHealth applications

Surface roughness changes on epithelial cells after progesterone treatment

Francis LW et al, Biology of the Cell 2010

• Control

• Progesterone
NanoHealth applications

Surface roughness changes on epithelial cells after progesterone treatment

- Progesterone up-regulates MUC1 protein
MUC1 Protein
A biomarker for endometrial receptivity

- Highly glycosylated, high molecular weight glycoproteins

- Present on the surface of human epithelial cells.

- MUC1 is a candidate for selection of implantation site. *One of the first molecules recognised by the egg.*

- MUC1 provides scaffold for attachment of the egg to the endometrium.
MUC1 Protein
Relation to pathology

- Infertile pathologies exhibit altered MUC1 expression

MUC1 Protein
A tale of glycosylation

Regulation of MUC1

An in vitro cellular model

- Endometrial Epithelial cell lines Hec-1-A and Hec-1-B
  - Hec-1-A is a relative low expresser, Hec-1-B is a relative high expresser
  - Provides a basic model system
AFM imaging
Hec-1-A and Hec-1-B EEC cell lines
Peak Force QNM
Mapping the microadhesome

- Hec-1-B has 2x the adhesion of Hec-1-A
- No difference in other nanomechanical properties
Molecular tools
Small interfering RNA (siRNA)

Control

siRNA

SiRNA Knockdown of MUC1
PeakForce QNM

siRNA knockdown of a specific target: MUC1. Direct correlation with adhesion changes
Force Spectroscopy

Functionalised probes

Characterised event

Event mosaic

No event
Force Spectroscopy
Functionalised probes

- Decrease in binding
- Increase in binding
Project summary

- Reduction of MUC1 from the surface of the cell reduces adhesion
- However within the reproductive context MUC1 is anti-adhesive
- AFM and specifically PFQNM has been useful in understanding protein and cell-cell interactions
- These techniques have not been used before in the endometrium
- Understanding the endometrial interface is key to improving ART (assisted reproduction therapies)
Industry and academia
A collaborative effort

With special thanks to:

- Alexandre Berquand
- Drew Murray
- Boumedienne Boudjelida
- Steven Minnie
- Steven Badger
- Bruker Germany staff

- Lewis francis
- Steven Conlan
- Deya Gonzalez
- Paul Lewis
Acknowledgements

Principal Investigators
• Steve Conlan
• Deyarina Gonzalez
• Lewis Francis
• John White (ret)

Postdoctoral Researchers
• Abdulkader Azouz
• Abdullah Alvi
• Helen Whiteland

Clinical
• Lavinia Margarit
• Kerryn Lutchman Singh
• Kinza Younas
• Lisa Joels (Peninsula)
• Kalyan Dhar
• Adnan Bunkheila
• Paul Flynn
• Gian Bertelli

Pathology
• Christine Davies
• Paul Griffiths

Graduate Students
• Natalie DeMello
• Sean Griffiths
• Zoe Coombes
• Julia Davies
• Roberta Paravati
• Hanifa Koguna (Clinician)
• Sarika Nandanan (Clinician)
• Gurpreet Kalra (Clinician)

Collaborators
• Paul Lewis (Swansea)
• Gareth Brenton (Swansea)
• Mauro Ferrari (Methodist Hospital, Houston)
• Jason Sakamoto (Methodist College, Houston)
• Armando Rojas (Talca, Chile)
• Franco DeMeyo (Baylor College, Houston)
• John Lydon (Baylor College, Houston)
• Alexandre Berquand (Bruker)

• Porvair Filtration Ltd
• Bruker Ltd
• Keucept Ltd
• London Womens Clinic Ltd
Contact information

Alexandre Berquand

*Life Science Applications Scientist*

[Alexandre.Berquand@bruker-nano.com](mailto:Alexandre.Berquand@bruker-nano.com)

**Tel**: +49 174 333 94 62

Sean Griffith

*PhD student reading in Nanomedicine*

[366566@swansea.ac.uk](mailto:366566@swansea.ac.uk)

**Tel**: +441792...