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Review

Atomic Force Microscopy and pharmacology: From microbiology to cancerology

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ABSTRACT

Background: Atomic Force Microscopy (AFM) has been extensively used to study biological samples. Researchers take advantage of its ability to image living samples to increase our fundamental knowledge (biophysical properties/biochemical behavior) on living cell surface properties, at the nano-scale.
Scope of review: AFM, in the imaging modes, can probe cells morphological modifications induced by drugs. In the force spectroscopy mode, it is possible to follow the nanomechanical properties of a cell and to probe the mechanical modifications induced by drugs. AFM can be used to map single molecule distribution at the cell surface. We will focus on a collection of results aiming at evaluating the nano-scale effects of drugs, by AFM. Studies on yeast, bacteria and mammal cells will illustrate our discussion. Especially, we will show how AFM can help in getting a better understanding of drug mechanism of action.
Major conclusions: This review demonstrates that AFM is a versatile tool, useful in pharmacology. In microbiology, it has been used to study the drugs fighting *Candida albicans* or *Pseudomonas aeruginosa*. The major conclusions are a better understanding of the microbes' cell wall and of the drugs mechanism of action. In cancerology, AFM has been used to explore the effects of cytotoxic drugs or as an innovative diagnostic technology. AFM has provided original results on cultured cells, cells extracted from patient and directly on patient biopsies.
General significance: This review enhances the interest of AFM technologies for pharmacology. The applications reviewed range from microbiology to cancerology.

1. Introduction

Historically, imaging at high resolution is based on the optical microscope. However this technique suffers from the limitation of the photons wavelength, roughly 200 nm. To overcome this limitation the electron microscopes were developed by Ruska and Knoll. Here the resolution is limited by the electrons wavelength, which is much lower than for visible light (100 000 times shorter). Both technologies are based on lenses that focalize a photon or an electron beam on a sample. Scanning probe microscopes work in a completely different way. The principle relies on the measure of a parameter (*e.g.* the tunneling current [1] or the force [2]) between a sharp tip and a surface and to keep this parameter constant while scanning in order to get a three dimensional image of the sample. As stated by C. Gerber, one of the Atomic Force Microscope (AFM) pioneer, and P. Lang in a 2006 paper in Nature Nanotechnology [3]: the scanning probe microscopes (SPM) have opened the door to the nanoworld. SPM made it possible to explore and to manipulate it. Feynman had dreamed of "the room at the bottom" [4]; SPM had opened the doors (for example, Eigler and Schweizer wrote the acronym IBM with Xe atoms [5,6]). Particularly, AFM has contributed to major advances in very different fields from fundamental physic and chemistry to information technologies, molecular electronic and spintronic. Since 25 years [7–9] AFM has emerged as a first interest characterization technology in life science. The number of research articles, published each year, in which AFM is used has increased exponentially since 1981. Fig. 1A presents this evolution. It must be noticed that the increase of studies on living cells is slow. This is probably due to difficulties inherent to biology and living cells.

AFM can be used in imaging modes like contact mode or oscillation mode as described in Fig. 1B. In these modes a sharp tip mounted on a cantilever is scanned over the sample surface. In contact mode, the cantilever deflection is kept constant in order to apply a constant force and to generate isoforce images of the surface. In oscillation mode, the cantilever is oscillating near to its resonance frequency and the amplitude

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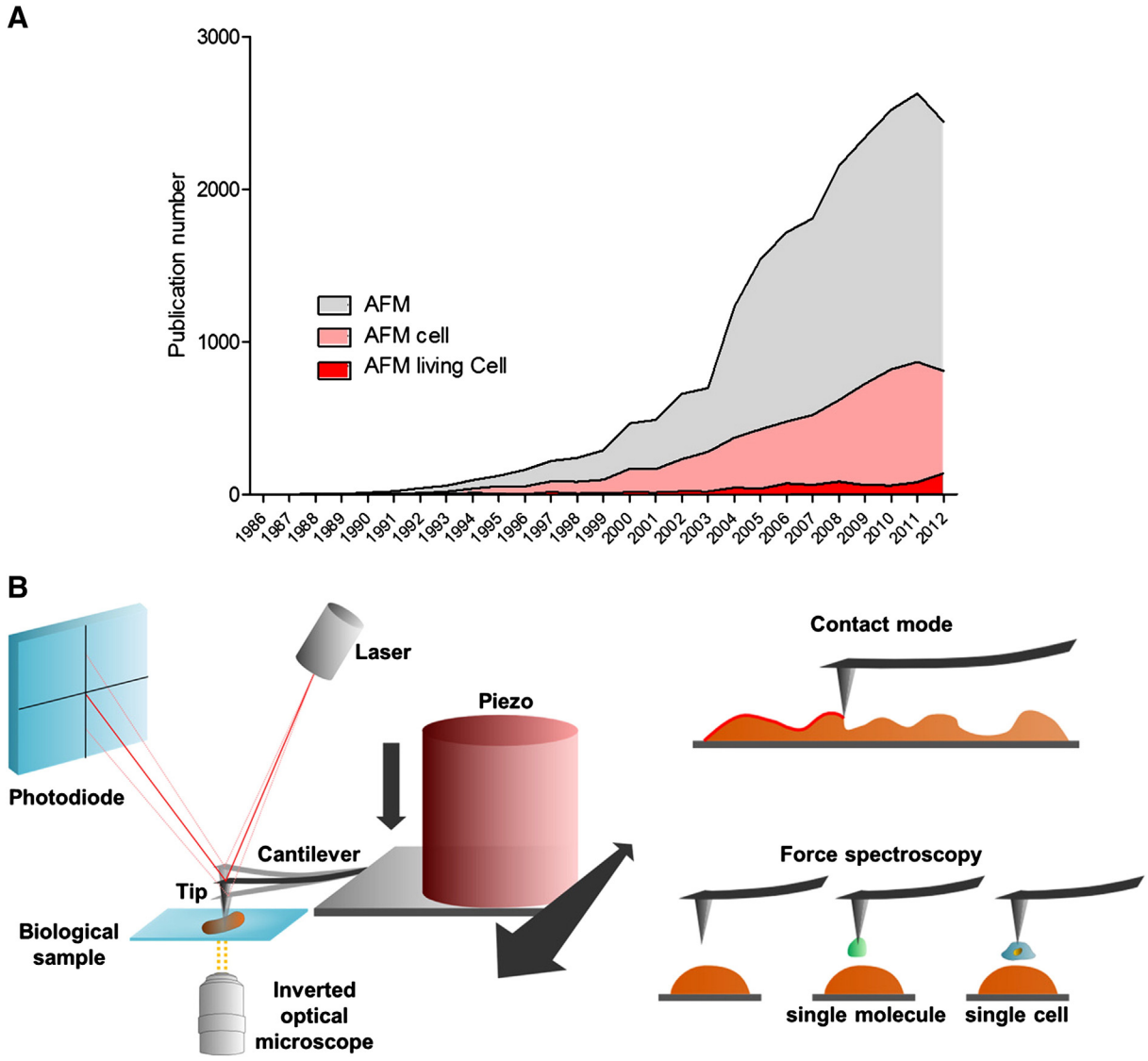

Fig. 1. (A) Evolution of the number of paper published each year, pubmed search using AFM or AFM and cell, or AFM and living and cell. (B) Schema introducing the AFM technology. A sharp tip is mounted on a cantilever that can be moved in the x, y, and z direction thanks to a piezo electric ceramic. The deflection of the cantilever is monitored on a 4 squares photodiode thanks to the reflection of a laser beam, aligned at the end of the, usually gold coated, cantielever. The AFM can be used to produce topographical images (like in contact mode) or to measure forces (in the force spectroscopy mode) between a bare or a functionnalized tip (with a biomolecule or a single cell) and the sample.

of the oscillation is kept constant while scanning, which creates isoamplitude images. However, AFM is not only an imaging technology. It is a highly sensitive force machine, able to measure forces as small as 10 to 20 pN. An AFM is therefore able to record force distance curves, which give measures and properties of the living material (this is sketched in Fig. 1). Nanomechanical properties and nano-adhesive properties of the samples can be measured using the AFM as a force machine. To make a link between the adhesive properties and a cell function it is possible to functionalize the AFM tip with a living cell. The results of such experiments create new paradigms in life science, and the interpretations in term of structure–function relationships are promising for pharmacologists. More and more articles are indeed dealing with the study of the effects of drugs on cells, studied by AFM.

The aim of our review is to give an overview of the AFM applications in biology (fungal cells, prokaryotic cells, mammal cells), with a special focus on the relevance in pharmacology. The first part is dedicated to fungal cells especially *Saccharomyces cerevisiae*, *Candida albicans* and *Aspergillus fumigatus*. The second part treats of bacteria. It gives an insight on the fundamental knowledge that AFM has provided on bacteria and then emphasizes on studies dedicated to the study of antimicrobial (antibiotics, antimicrobial peptides, innovative molecules) effects. Finally, the third part addresses mammal cells, exposed to external stress, like drugs, but also diseases and cancer.

2. AFM for fungal cell wall analysis, from fundamental knowledge to pharmacology

Atomic Force Microscopy is a polyvalent tool that allows biological and mechanical studies of entire living microorganisms, and therefore the comprehension of molecular mechanisms. This first section introduces the AFM modes, with yeast cells as a eukaryotic model to illustrate its potentialities, and their implications in pharmacology. We will first explore morphological and mechanical studies on various yeast cells. Then, we will present molecular mapping principle on cell-wall surface and the applications of this technique for biology. Finally, we will investigate the yeast pathogenicity in cellular invasion and we will give an overview of AFM pharmacology's studies on yeast.

2.1. Morphological and mechanical studies

Since its first development in 1986 by Binnig et al. [10], there have been an increasing number of AFM biological applications (Fig. 1). An