27

How to Build Up Biosensors With the Cantilever of the Atomic Force Microscope

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1. Introduction

With the advent of the atomic force microscopy (AFM), the study of biological samples has become more realistic because, in most cases, samples are not covered or fixed and this makes it possible to observe them while alive ***(1,2)***. This advantage of the AFM prompted a new invention: nanobiosensors using the cantilever (probe) of the AFM, which made possible the observation of specific molecules (including medications) as they enter or exit living cells ***(3,4)***.

The nanobiosensor is the smallest biosensor in the world and measures about 100-µm long (about the width of a hair). Beyond sensing the area of interest, this biosensor also makes possible a real-time image of exactly what is occuring on the cell ***(3,4)***.

1.1. Use of the AFM as a Heat Detector

An apparatus for measuring variation of temperature is the thermooptical detector. A reaction cell, whose bottom is a thin gold film (with immobilized enzyme), is immersed into a CCl_4 phase where a probe beam is passed. The heat (from reaction between the enzyme and its substrate) of the water phase is transferred to the gold film and the CCl_4 phase, and thus a temperature gradient is generated in the CCl_4 phase. This temperature gradient induces deflection of the probe beam and the result is registered as a graph ***(3–9)***.

Because AFM has almost the same parts as the thermooptical detector (laser beam, lens, and photodiode; **refs. *3*** and ***4***), if an enzyme was immobilized on the cantilever, one can hope that the temperature gradient generated by reaction heat (between the enzyme and its substrate) could induce a deflection of the cantilever, transforming the AFM into an apparatus that could reveal the presence of specific molecules.

From: *Methods in Molecular Biology, vol. 242: Atomic Force Microscopy: Biomedical Methods and Applications*
Edited by: P. C. Braga and D. Ricci © Humana Press Inc., Totowa, NJ

As mentioned before, an AFM equipped with an enzyme-coated cantilever (nanobiosensor) can visualize living cells, so it should be possible to detect specific molecules being absorbed by living cells and to see this happening. These assumptions were shown to be valid by experiments made with the first nanobiosensor, which had been made to detect the absorption of glucose molecules by *Saccharomyces cerevisiae* cells ***(3)***.

The deflection of the cantilever is explained by the physical phenomenon occurring when two different metal blades (each with a different coefficient of dilation) are bonded together. This is called a bimetallic system, and the presence of heat makes the blades bend. Another illustration of the phenomenon is the working of the taillights of an automobile: a bimetallic blade is heated until it bends and produces a braking light ***(4)***. The cantilever of AFM is not a bimetallic blade but can work as one because it consists of two different materials (a metal and semimetal) with different dilation coefficients: gold and silicon. If a biochemical (or chemical) reaction takes place near this so-called bimetallic blade, it is possible to produce a deflection in the cantilever. This latter produces an enormous deviation in the direction of the laser beam, which is recorded in real time by the computer (via a photodiode; **ref. *4***).

Beyond observing the absorption of glucose molecules by living cells, it was possible to visualize the inverted phenomenon: the release of specific molecules from living cells immobilized on the cantilever surface, in the first instance of this technique, the enzyme alcohol dehydrogenase type II, which transforms ethyl alcohol into glutaraldehyde and releases heat in the process ***(4)***.

Other biosensors were made using the enzymes superoxide dismutase and catalase ***(4)***, proving that this methodology is valid for all enzymes or molecules that release heat from their reactions.

1.2. Identifying Defective Genes

Tiny variations in the genetic code are what make us unique. Changes in one chemical unit in a gene's sequence, called single nucleotide polymorphisms, can influence an individual's risk for disease. Comparing variations will help identify defective genes and may lead to better diagnosis and treatments ***(4)***.

If a cantilever is coated with DNA strands, it is possible to detect genetic variations or single nucleotide polymorphisms when an individual's DNA is tested against them ***(4)***.

In a recent report, scientists from IBM demonstrated that when DNA strands are immobilized on a cantilever surface, it is possible to identify the complementary strand of the DNA in solution ***(10)***. When the immobilized and complementary strands find a match, the heat evolved from the process makes the cantilever bend. This nanomechanical property transforms the cantilever into a DNA chip, making it very useful in identifying mutations ***(10)***.

1.3. Clinical Analysis

The immobilization of para-nitrophenylphosphate on the cantilever can be used to identify cancer in the bones, prostate gland, and ovary. Para-nitrophenylphosphate is the substrate of the acid phosphatase enzyme and it is currently used in clinical analysis ***(11–14)***. The enzyme is released by tumor cells into the extracellular medium.

2. Materials

Enzymes (such as glucose oxidase, catalase, superoxide dismutase, or alcohol dehydrogenase type II) are obtained from Sigma Chemical Co. All reagents are of analytical grade. Water is double distilled and deionized.

The industrial strain of *S. cerevisiae* is commercial. The stock suspensions of living cells are prepared by adding 1.0 g of dry baker's yeast to 10 mL of water (double distilled and deionized) while stirring at room temperature. A drop of the suspension is placed on the surface of glass coverslips and allowed to dry for 15–20 min at room temperature to remove the excess of water. Therefore, the experiments are performed under a thin layer of water.

For AFM, a BioScope (Thermomicroscopes) or a BioProbe (NanoScope IIIa AFM, Digital Instruments) operating in contact mode is used in the experiments along with Si_3N_4 Nanotips (Digital Instruments) with 0.06-N/m spring constants. In some cases, the images are low-pass filtered to remove stray scan lines. All images are collected on the AFM using a scan speed of 2.5 Hz, and all imaging is performed in air at room temperature as described before ***(4)***.

3. Method

1. Stock solution of enzyme: 100 mg of enzyme is mixed in 1 mL doubly distilled and deionized water. Five microliters of this enzyme solution is spread on a 2-cm^2 area on a cover slips.
2. Building the nanobiosensor: a brand new cantilever is put in the cantilever holder of the AFM. An engage is given on the cover slips that contains the spread solution of the enzyme. After 30 s, the cantilever is taken off the cover slip surface and dried for 3 h at room temperature (*see* **Note 1**). The cantilever (with enzyme) is kept in the AFM during drying period, after which, this nanobiosensor is put in a vacuum desiccator for conservation under 5°C. To test the effectiveness of this methodology, an enzyme substrate solution is directly dropped on the nanobiosensor while it is scanning a glass surface; the reaction catalyzed by the immobilized enzyme is monitored by the deflections of the cantilever on the AFM monitor.
3. The nanobiosensors should be kept in a vacuum and under refrigeration. Under these conditions they can be used up to 2 or 3 mo after their preparation, and experiments with them are very reliable and reproducible ***(3,4)***.

4. Notes

1. If the enzyme solution invades the gold mirror area, the biosensor does not work.

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