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Micro- and nanosystems for biology and medicine

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Abstract

The development of new tools and instruments for biomedical applications based on nano- (NEMS) or microelectromechanical systems technology (MEMS) are bridging the gap between the macro- and the nano-world. The well mastered microtechnique allows controlling many parameters of these instruments, which is essential for conducting reproducible and repeatable experiments in the life sciences. Examples are multifunctional scanning probe sensors for cell biology, an arthroscopic scanning force microscope for minimally invasive medical interventions and a nanopore sensor for single molecule experiments in biochemistry. This paper reviews some of the activities conducted in a fruitful interdisciplinary collaboration between physicists, engineers, biologists and physicians.

Keywords: Microsystems; Nanosystems; Cell biology; AFM; Nanopore; Protein; Cartilage; Minimal invasive instrument

1. Nanotools for biology

In cell and structural biology the typical dimensions are spanning the length scales from a few nanometers up to centimeters. The objects of interest are very delicate and often mechanically soft, hence, investigating their functions calls for gentle and precise instruments. Very often several parameters should be simultaneously controlled in such experiments and a more or less complex system of tools is required. An appealing implementation of such systems is to employ micro- and nanofabrication techniques.

Plain atomic force microscope [AFM] probes have proven to provide images of membrane proteins with subnanometer resolution in the past [1]. In order to study structural changes in voltage gated channel proteins at this level of resolution, we have developed a probe, which features a conductive tip [2]. The metal is insulated up to the apex (Fig. 1) such that Faradaic currents in buffer solutions are minimal. Different metals were tested, and PtSi proofed to be the best choice from a fabrication point of view [3]. The basic concept for fabricating such a probe followed that of standard molded silicon nitride tips. We employed anisotropic KOH etching of silicon (100)-wafers through a silicon nitride mask in order to form small pyramidal etch-pits. In one of these pits the tip will be formed. An array of them will be used as contact area. All pits were sharpened by a local low-temperature oxidation process, followed by a deposition of a thin layer of polycrystalline Si. The latter was lithographically patterned such that a thin line connection between the tip mold and the array of pits was formed. Platinum was evaporated on the full wafer and then annealed in order to form the silicide. Residual Pt was removed, and a second silicon nitride layer was deposited by means of low pressure chemical vapor deposition [LPCVD] in order to fully encapsulate the metal structure. The cantilevers were patterned by lithography and reactive ion etching [RIE], a pre-structured Pyrex-glass wafer was bonded to the nitride, and the silicon substrate was dissolved in KOH. The final step was a timed etching in buffered hydrofluoric [BHF] acid in order to break through the thin oxide at the tip apex.

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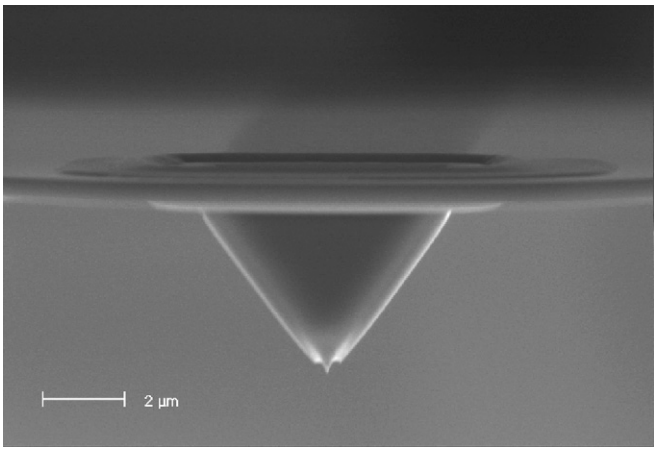

Fig. 1. Conductive cantilever with electrical insulation for operation in physiological buffer solution or for electrochemical experiments. Only the metal tip apex reaches about 100 nm out of the SiO_2 insulation.

It was important to realize that when operating such a probe in a buffer solution one actually conducts an electrochemical experiment [4]. Therefore it was essential, that these probes were first characterized by cyclic voltamograms and electrochemical feedback measurements and second, that the future, routinely performed biological experiments are also to be conducted at the correct potentials under potentiostatic control [5]. Thus, many important parameters and the quality of the tip could be assessed without performing an AFM measurement, which always risks damaging the tip. The Faradaic current, measured when far away from the substrate allowed calculating the tip radius. The cyclic voltamograms of the tips showed a more or less expressed half-wave hysteresis. Transmission electron microscope (TEM) images revealed that tips with such a well pronounce hysteresis showed also an important gap between the electrode and the insulation at the apex. Theoretical modeling confirmed that this gap was responsible for the hysteresis [4]; it acted like a reservoir of ions, which could only be refreshed by slow diffusion through the narrow opening at the tip apex.

The set-up for conducting experiments on cell-membrane patches was to be completed with a planar patch-clamp like sample support [6]. Such a device will allow electronically accessing both sides of the sample: On one side, a global electrode will be used, where as on the other side, the conductive AFM tip will be employed to locally apply a potential. The major design criteria were a low electrical capacity and a smooth surface, which is known to be essential for a good electrical seal between the biological sample and the support. The main structure of the device was a silicon nitride membrane, initially deposited onto a Si wafer, into which a small opening of about 100 nm diameter was machined by means of electron beam lithography and RIE. This membrane was then reinforced by a SiO_2 film and bonded onto a Pyrex-wafer. A plain microfluidic system had been previously etched into the latter, such that the hole could be accessed with different buffer solutions. The Si was removed in KOH exposing the smooth original interface to the silicon nitride. Finally, the SiO_2 which blocked the hole was etched in BHF. The whole set-up could be tested by taking scanning electrochemical microscope images of the hole, using an insulated AFM cantilever described above.

2. Nanopore sensing

The opposite or 'negative' form of a tip is a hole or a nanopore. This plain structure in combination with a fluidic system has a high potential for sensing small particles down to single molecules. The basic layout goes back to an invention made by Coulter in 1953 [7]. The set-up we used is sketched in Fig. 2. Two reservoirs were separated by a membrane into which a small nanometer size hole has been machined, again using electron beam lithography and RIE. If the reservoirs were filled with buffer solution and a potential was applied between them, an ionic current flo-

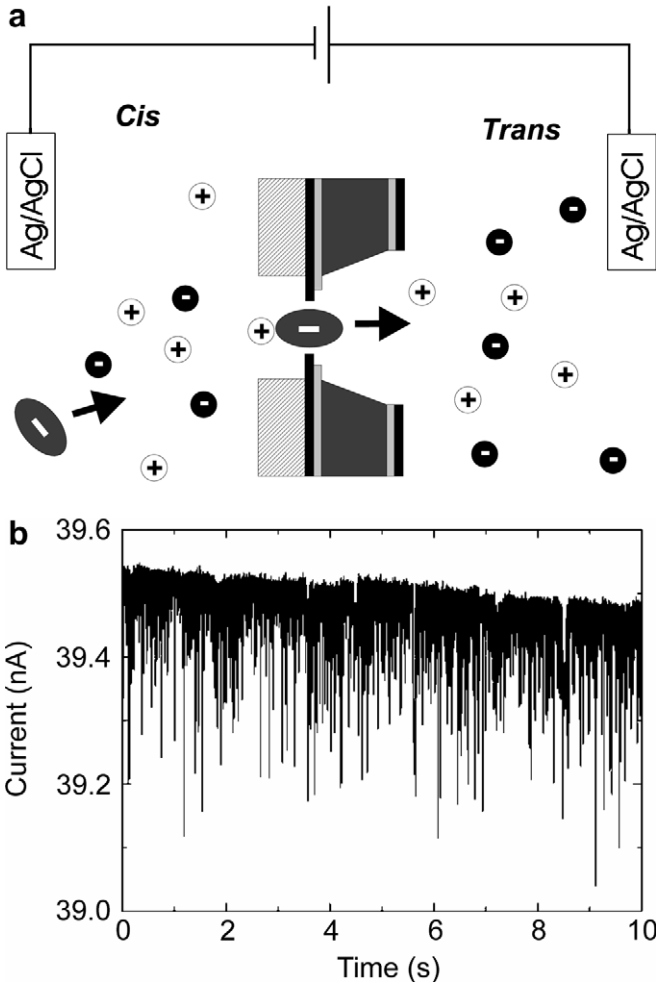

Fig. 2. (a) Concept of nanopore measurements. The ionic current through a small orifice is temporarily reduced when a molecule is translocated, giving rise to the current drops seen on the right hand side (b).

wed through the pore. This current was disturbed by the translocation of large molecules that had been administered into one of the reservoirs. The potential of this technique is large, since no labels are needed for detecting single molecules. So far, it was mostly applied for interrogating DNA [8], and counting and measuring the size of particles [9]. We have successfully applied this technique to detect proteins (bovine serum albumin) in solution [10], which is a first step towards studying protein–protein interactions.

3. Nanotools for medicine

The AFM is very versatile: in addition to imaging matter, it can also be used to measure material properties. By driving an indenter into and withdrawing it from a specimen the material's response can be continuously recorded in form of load–displacement curves. Modeling of such load–displacement curves have been developed for materials science [11] thereby offering the deduction of material properties, such as hardness or the elastic modulus. We have modified this standard model by Oliver & Pharr for measuring highly hydrated, soft biological specimens [12,13]. Most biological samples are limited in size. According to the rule of Bueckle [14], for an accurate measurement of the material properties the indentation depth should be less than 10% of the overall thickness. For example, the thickness of cartilage covering the articulating surfaces of human joints is typically 1–4 mm and consequently the maximal indentation depth is between 0.1 and 0.4 mm. To achieve such small indentation depths, high resolution monitoring of the z-displacement of the indenter is required that is often not provided by classical indentation testing devices [12,13]. Compared to more classical indentation testers, the AFM, which is based on piezoelectric actuators, has far greater dimensional sensitivity compared to conventional clinical indentation testing devices. This allows measuring on much smaller specimens and with higher spatial resolution.

Articular cartilage is a biocomposite material made of hard collagen fibers that are providing the skeletal framework of the material and a soft, gel-like ground substance that is filling up the spaces in between the fibers. The individual fibers of the collagen network are exhibiting a diameter of about 50 nm and are arranged in a random mesh- or sponge-like structure with typical spaces at the order of a hundred nanometers. Therefore, a sharp nanosized AFM tip of typically 10 nm radius can enter the space in between the fibers and, hence, primarily probe the functional properties of the proteoglycan moiety independent from the collagens. In contrast to such small AFM probes, the classically employed millimeter-sized indenters press over a much larger area and, hence, probe the material properties at the level of the overall tissue. Consequently, measurements provided at both scales exhibit two orders of magnitude difference in the elastic moduli [12].

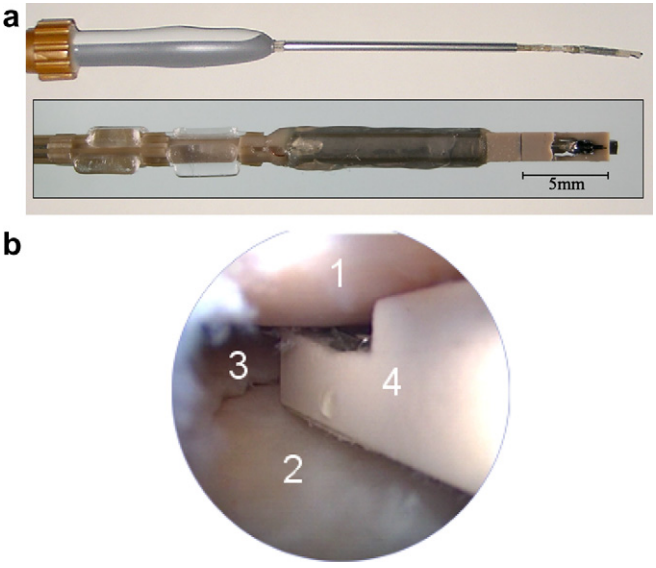

Fig. 3. (a) Arthroscopic AFM for measuring in a minimal invasive approach the elasticity of articular cartilage in the knee joint. (b) Tool tip inside a knee joint as seen through an optical arthroscope. (1) Cartilage of the femoral condyle, (2) cartilage of the tibila plateau, (3) meniscus and (4) AFM-tip measuring the surface of the cartilage of the femoral condyle.

By employing these new capabilities, we have found that early degradation of articular cartilage can be detected by probing its elastic modulus, but only when measured at the nanometer scale [12,13]. In a more recent experiment, we have documented that early changes in osteoarthritis (OA) can be detected at the nanometer scale, but not at the higher levels of tissue organization. It would be highly desirable to develop the AFM into a tool that can be used for diagnosis. In order to also measure the mechanical properties or 'health' of cartilage inside the knee- or hip joint, we have started to develop an arthroscopic version of the AFM that can be inserted into the standard canula that are used in arthroscopic interventions. A bread-board model of such an instrument is shown in Fig. 3a. The inner diameter of a canula is about 3 mm. The main challenges of a scanning force arthroscope (SFA) are the small diameter and the stabilization of the instrument towards the surface to be inspected inside the knee joint. For the latter, we used eight angioplasty balloons to wedge the shaft of the tool tip between the bones and the soft tissue. The handle, which is guided by the orthopedic surgeon during insertion, can then at least partially be decoupled from the sensitive, distal end. Changing the pressure difference between the balloons allowed approaching the probe to the surface. First experiments conducted in an open intervention in a pig's leg, which we got from a slaughter house, showed reasonable good results [15]. The approach curves were qualitatively comparable to load–displacement curves measured on biopsy samples of porcine cartilage. Also a first intervention in knees of human cadavers under the control of an optical arthroscope was very promising. Fig. 3b shows the SFA seen through the optical arthroscope during this first experiment.

4. Conclusion and outlook

This short review demonstrated that microfabricated instruments render new parameters accessible and thus can significantly contribute to the advancement of scientific knowledge. The SFA will hopefully help developing cures for osteoarthritis by e.g. monitoring the efficiency of new drugs. Later, it is conceivable to use a hollow cantilever and tip [16] to inject such a drug right into the affected area, which was detected by the SFA. A further treatment of severally damaged cartilage is to replace it by engineered tissue. The quality of this engineered cartilage is very important. Here, arrays of AFM probes [17] could be used to investigate a large surface at the relevant nanometer scale.

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