Cover story

Scanning probe devices will aid surgeons to diagnose diseases and repair the human body

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Rheumatic diseases – the burden of aging

During the past century the average life expectancy has increased by nearly 20 years. While this definitely represents an unprecedented achievement, this gain is still on the expense of numerous diseases that are becoming more prominent in the elderly. In particular, osteoarthritis develops over many years by typically starting with inoffensive non-specific aches that then often slowly but definitely develop into the full-blown disease. The development of osteoarthritis can be triggered by single events such as a sports injury or a trauma from a traffic accident, as well as by long-term risk factors such as obesity, physical inactivity, stress and smoking. Even somebody that never had a sports injury or trauma from an accident may eventually develop osteoarthritis.

Nanomedicine: the dawn of a new era

It was right at the start of the new millennium that a scientific congress organized by the World Health Organization (WHO) took place in Geneva on "The Burden of Musculoskeletal Conditions". This meeting also marked the beginning of the "Bone and Joint Decade" (BJD), whose mission is to improve the quality of life for people affected by musculoskeletal disorders worldwide. Most importantly, during that congress Kofi Annan, Secretary General of the United Nations (UN), welcomed and strongly supported this collaborative initiative facilitated by the World Bank and the UN. He stated: "Joint diseases, back pain, osteoporosis and limb trauma due to accidents and armed conflict have an enormous impact on the individual, on society and on health care and social systems ... there are effective ways to prevent or treat these disabling conditions. But we must act on them now ... the UN ... welcomes and supports this collaborative initiative."

Annan, K., 1999. The Secretary-General message to launch The Bone and Joint Decade 2000-2010 for the prevention and treatment of musculoskeletal disorders. New York, United Nations Headquarters, Nov. 30,1999. The official letter of support by Kofi Annan is available at:
www.osteofound.org/activities/pdf/annan.pdf

World Health Organization, 2003. "The Burden of Musculoskeletal Conditions at the Start of the New Millennium" is the result of three years of work by an international scientific group of experts. It was undertaken as a collaboration between the WHO and the Bone and Joint Decade. It is published in the WHO Technical Report Series. An electronic version of the report is available at:
www.who.int/ncd/cra/

THE BONE AND JOINT DECADE is an independent global non-profit organization whose mission is to improve the health-related quality of life for people affected by musculoskeletal disorders worldwide. It is the umbrella organization by which National Action Networks, professional medical societies, patient advocacy groups, governments, industry and researchers partner to effect change by: (1) Raising awareness of the growing burden of musculoskeletal disorders on society; (2) Empowering patients to participate in their own care; (3) Promoting cost-effective prevention and treatment; and (4) Advancing understanding of musculoskeletal disorders through research to improve prevention and treatment. For more information, visit the website at:
www.boneandjointdecade.org

New vistas to treat damaged cartilage

The idea of using nanotools for detecting diseases and repairing the human body was put forward in the late fifties by Nobel Laureate Richard Feynman during his famous speech "There's plenty of room at the bottom" (*www.zyvex.com/nanotech/feynman.html*). However, it took another twenty years until Gerd Binnig and Heinrich Rohrer developed the scanning tunneling microscope (STM; (Binnig and Rohrer, 1982), for which they received the Physics Nobel Price in 1986. For the first time, the STM allowed to image and move single atoms (Eigler, D.M. and Schweizer, E.K., 1990). In 1986, the invention of the atomic force microscope (AFM) by Gerd Binnig, Calvin Quate and Christoph Gerber (Binnig et al., 1986) expanded the technology to non-conductive surfaces and, especially, to the world of living matter. The AFM employs a sharp tip at the end of a relatively long cantilever for directly "seeing" (i.e. imaging) and "feeling" (i.e. mechanical testing and manipulating) the surface of a sample at sub-nanometer resolution. This fascinating ability to directly interact with single molecules and atoms in a very controllable manner opened new vistas for the investigation of biological tissues *ex vivo* and *in situ*.

From the bench to the patient

The Swiss National Science Foundation is funding a National Center of Competence in Research Nanoscale Science (NCCR; *www.nccr-nano.org/nccr/*) that started in 2001. The project module "Nanotechnology in Medicine" aims to bring nanotechnology from the bench to the patient by developing a new class of diagnostic and therapeutic tools. In this project module we are working in a truly interdisciplinary team of physicists, engineers, biologists and clinicians. One of the projects is developing a novel approach, called indentation-type atomic force microscopy (IT AFM), for studying the progression of cartilage diseases (Stolz et al., 2004). IT AFM enables us to map, for example, the mechanical properties of healthy and diseased articular cartilage from the millimeter over the micrometer down to the nanometer scale, thus assessing all important scales of cartilage organization. The ultimate goal is to perform IT AFM directly *in situ* by developing an arthroscopic AFM which can be directly introduced into a knee or hip joint. Articular (hyaline) cartilage predominantly consists of a complex network of collagen fibers, glycosaminoglycans (GAGs) and bound water. On the one hand the GAGs act as spacers to keep the collagen fibers apart. On the other hand, by their highly negatively charged nature the GAGs bind a large amount of water (60–80 weight %) into the tissue. Osteoarthritis goes hand in hand with a degradation of the GAGs

which, in turn, leads to a dehydration and therefore a collapse of the cartilage.

As illustrated in *Fig. 1A,* articular cartilage exhibits a random orientation of the collagen fibers, which reveal a characteristic 67-nm axial repeat. For comparison, osteoarthritic cartilage exhibits a preferred orientation and bundling of the collagen fibers, as indicated by the arrows in *Fig. 1B.* Because of the mechanical stress, it is conceivable that upon degradation of the GAGs, the collagen fibers are no longer spaced apart and coalesce on top of each other, thereby slowly but definitely aligning themselves in a direction representing the predominant joint movement. As documented in *Fig. 1C,* when assessed by IT AFM at the nanometer scale the osteoarthritic cartilage appears mechanically significantly stiffer than the healthy cartilage.

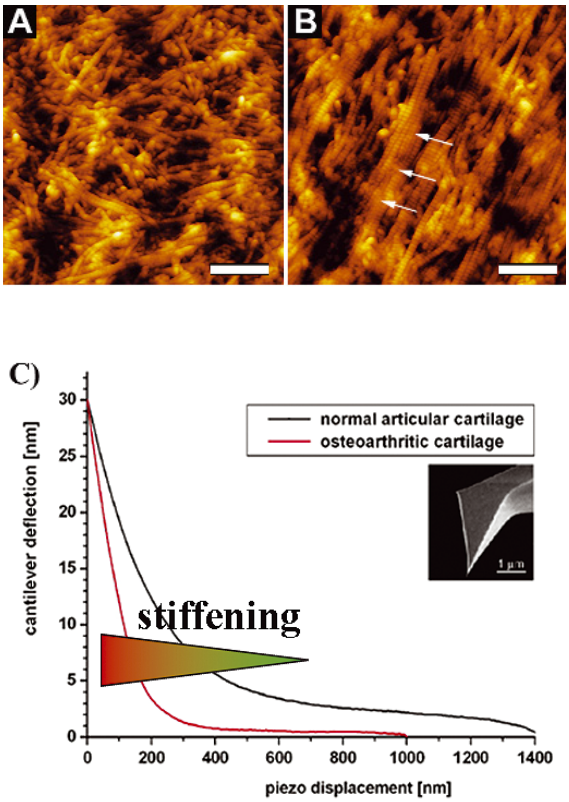

Fig. 1
Surface topography of (A) normal articular cartilage, (B) osteoarthritic articular cartilage, and (C) the corresponding elasticity measurements at the nanometer scale performed with a sharp pyramidal tip. (A) The 67-nm axial repeat distance of individual collagen fibers was clearly resolved by AFM. (B) In contrast to the normal cartilage that exhibited a random orientation of the collagen fiber network, in the diseased cartilage the collagen fibers coalesced on top of each other and exhibited a preferred orientation. This orientation might follow the directed movement within the joint more easily once the GAGs become digested in the course of the disease progression. (C) Comparison of the two slopes of the corresponding force displacement curves indicated stiffening of the osteoarthritic articular relative to the normal cartilage. Scale bars, 1 µm (A and B).

Early detection of cartilage diseases

In further experiments, we were able to mimic osteoarthritic disease progression by enzymatic digestion of the GAGs with cathepsin D. At the nanometer scale, i.e. with a sharp nm-size tip, the same difference in the mechanical properties was found as when comparing normal with osteoarthritic cartilage *(Fig. 2A)*. However, at the micrometer scale, i.e. when assessed with a spherical (µm-size) rather than a sharp (nm-size) tip, no significant hardening was depicted *(Fig. 2B)*. The finding that it is possible to detect alterations at the nanometer scale which are not detectable at larger scales holds a great promise for detecting changes in the cartilage's biomechanical properties as occurring in osteoarthritis at an early stage of disease progression, hopefully at a stage where the disease might be stopped or even reversed. Moreover, IT AFM should be very helpful for a more systematic analysis of different types of normal, diseased, enzymatically modified as well as engineered cartilage. Such analysis, in turn, will allow us to more rationally understand tissue functionality and, ultimately, will enable us to more effectively repair diseased cartilage, stimulate transplanted cartilage, and design and produce tissue engineered cartilage (TEC) exhibiting long-term mechanical stability and biocompatibility (Langer and Vacanti, 1993).

Ultimately, such *ex vivo* measurements should be performed by direct *in vivo* inspection of the articular cartilage

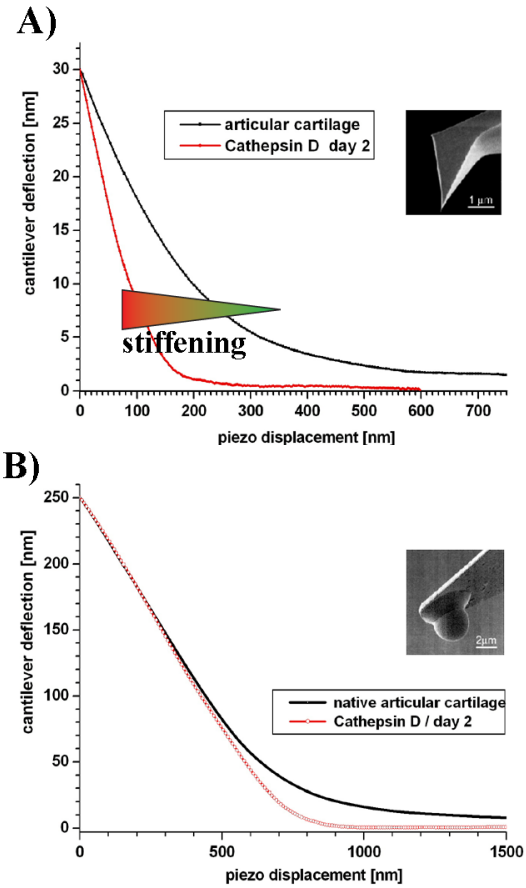

Fig. 2

The elasticity measurements as presented were taken from the same sample by employing two different indenter sizes, i.e. (A) a nanometer-size sharp pyramidal tip (tip radius = 20 nm) and (B) a micrometer-size spherical tip (tip diameter = 5 micrometer). Each set of the two curves was taken on normal articular cartilage and on cathepsin D digested articular cartilage. For digestion the articular cartilage was treated by cathepsin D at 37°C for two days. Comparison of the two slopes recorded at the micrometer scale exhibited no difference between native and cathepsin D treated cartilage. While at the nanometer scale the cathepsin D treated articular cartilage exhibited a clear stiffening (A), at the micrometer scale the cathepsin D treated cartilage appeared indistinguishable from the normal cartilage (B).

in a knee or hip joint in a clinical environment (Stolz et al., 2003; Hunziker et al., 2002). To achieve this ambitious goal, we set out to design and build an AFM which can be directly brought to the disease site in a knee joint by an endoscopic approach (Stolz et al., 2003).

Development of an arthroscopic AFM

As illustrated in Fig. 3, for performing IT AFM within the knee joint the scan head of the AFM is inserted under arthroscopic control into the knee and stabilized by two sets of liquid-inflatable balloons (Fig. 3, inset), similar to those used for heart angioplasty. By filling the balloon with saline solution the head is stabilized against the surfaces within the knee joint. For recording the biomechanical properties (elastic modulus) of the cartilage sample a sharp nm-size tip or a micrometer-size spherical tip is mounted in a holder that is attached at the distal end of a piezoelectric actuator tube (piezo). Next, the coarse approach is achieved by changing the volume of opposite balloon pairs. For recording the force-displacement curves the AFM tip is driven by the piezo into and out of the cartilage surface. After each loading/unloading cycle the AFM tip then is moved to its next position. Because of limited space, the optical readout with a laser system is replaced by a piezo-resistive readout that measures the changes of voltage while deflecting the cantilever.

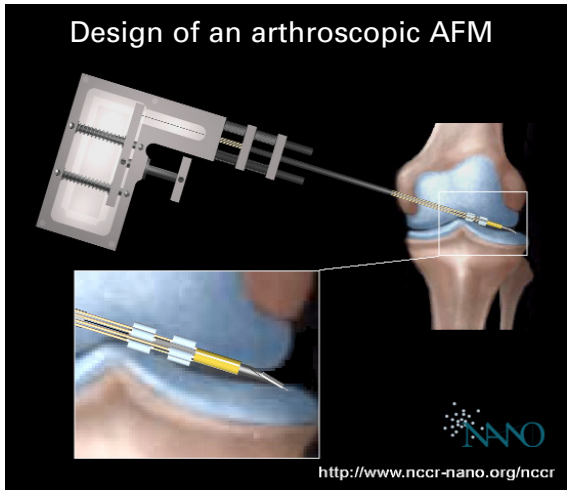

Fig. 3

Illustration of a prototype arthroscopic AFM. The instrument is inserted into the knee cavity under arthroscopic control.

Added value

First, conventional indentation testing devices perform mechanical testing at the mm to cm scale, so they cannot assess tissue properties at the cellular to molecular level, i.e. at the scale were the biochemical processes occur and also where most diseases start. Hence, reliable detection of alterations occurring at all levels of the cartilage architecture is crucial for obtaining a more comprehensive understanding eventually leading towards an early diagnosis of the disease which, in turn, is a prerequisite for developing more effective interventions for stopping or even curing osteoarthritis. Second, from the perspective of orthopedic surgeons, the initial key application of the arthroscopic AFM is in the quality control of transplanted autologous cartilage tissue as well as of tissue engineered constructs (both approaches are already available to the clinician) where it is of major interest to trace the development of the transplanted or tissue engineered cartilage over time in terms of mechanical stability and biocompatibility. Third, unlike any other applied technique used for assessing the morphological and biomechanical state of articular cartilage the AFM enabled us to directly image, measure and manipulate the tissue *in situ* by employing multifunctional tips.

In the context of new applications of nanoscience, the arthroscopic AFM will move from the bench to the patient. The arthroscopic AFM might be just the beginning of a new generation of nanotools for minimally invasive interventions of other parts of the body such as, for example, the detection of vulnerable plaques in the heart coronary arteries by a catheter-based approach. In that perspective, we are still living in the "stone age" of scanning probe-based clinical tools. However, we believe that scanning probe devices will eventually help surgeons to more effectively detect diseases and to repair the human body.

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