Take a fascinating peek into how nanotechnology may soon help doctors diagnose and target all kinds of diseases with greater accuracy and speed.

Coming Soon
Nanotechnology

to a Clinic Near You in Medicine

by Casey K CHAN

The innumerable connections and intersections of nanotechnology and other disciplines present considerable potential for application in diverse fields. Owing to the nascent stage of development of nanotechnology and the discordant development in the associated disciplines, many of these applications are in the exploratory stage, years away from practical use. It appears, however, that microelectronics and biotechnology represent two areas in which research results from nanotechnology can be immediately relevant.

Nanotechnology applied to therapeutic medicine remains distant. Some people even argue that the idea of nanorobots coursing through the bloodstream to repair damage resulting from blood clots and cancer belongs in the realm of science fiction and will remain so. In reality, nanobiotechnology, the convergence of nanotechnology and biotechnology, has already given rise to real practical application in the form of research and diagnostic tools. Companies in the United States such as Quantum Dot Corp, Nanosphere Inc and Molecular Nanosystems Inc are shipping or are close to shipping real products for research and diagnostic use.

Nanobiotechnology offers the potential of obtaining the most information from the smallest number of test samples in the shortest time at the lowest possible cost. Nanomaterials are so small that when they interact with biomolecules they generate detectable signals in the form of light emissions, a deflection of a nanoscale cantilever beam or magnetic field.

Currently over 200 molecular diagnostic tests for biomolecules have become associated with various disease states. Detection of these biomarkers offers the potential for therapeutic decision, monitoring the progression of disease, early diagnosis, risk assessment of predispositions to certain diseases and consequent preventive care.

For example, doctors of the future, relying on a panel of tests, will be able to tell from a molecular fingerprint whether a tumour is fast- or slow-growing, whether it will be likely to spread to other parts of the body, and then predict which treatment the tumour is likely to respond to. These physicians should be able to get the required molecular biomarkers and gene-based diagnostic tests performed in their offices, interpret the results immediately, and make swift therapeutic decisions.

With advances in nanotechnology, such point-of-care diagnostic tools will take the form of commonplace hand-held or desktop devices in doctors' offices. For example, in 2001 Charles Lieber of Harvard University made boron-doped silicon nanowires with a diameter of just 10 nanometres for use as a biosensor. Adhesion of biomolecules on the surface of the nanowires alters the conductance, allowing the detection of the binding of biomolecules to the surface of these nanowires to be monitored in real time. The laboratory at Harvard has demonstrated that it is possible to detect minute amounts of a biomarker called the prostate-specific antigen (PSA). Elevated levels of PSA in the blood are often associated with prostate cancer.

Arun Majumdar's group from the University of California, Berkeley, demonstrated another sensitive technique for detecting biomolecules such as PSA using microcantilevers. The microcantilevers have also been shown to work with several classes of biomolecular interactions such as DNA–DNA hybridisation (joining of two complementary strands of DNA to form a double-stranded molecule), antibody–antigen binding and protein–protein interaction.

In this technique, one side of the microcantilevers gets coated with a self-assembled monolayer of probe molecules. When the target biomolecules bind to the probe molecules, the intermolecular nanomechanical interactions on one side of the cantilever beam generate a sufficiently large force to bend that beam (Figure 1). The laser beam reflecting off the cantilever measures the amount of deflection.

How an AFM works

The microcantilever was first used in the atomic force microscope (AFM) invented in the mid-1980s. The AFM works in a fashion analogous to the stylus of a vinyl record player. As the nanoscale tip of the cantilever moves across the surface of an object, the interaction of the nanoscale tip with the surface's undulation creates vertical motion in the free end of the microcantilever beam. A laser beam reflecting off the microcantilever greatly magnifies the motion signal, enabling recording of the minute vertical motion. By systematically traversing the surface, the measured signal of the reflected laser beam can be reconstructed in an image that represents the undulation of the surface. The AFM's acute sensitivity permits it to adapt to image various biomolecules.

By controlling the vertical movement of an ink-coated AFM tip as it traverses a surface, a desired number of molecules of organic and inorganic material can be deposited on that surface (Figure 2). This technique, also known as direct-write dip-pen nanolithography (DPN), has been used to deposit DNA molecules in an array format with spots as small as 50–100nm – nearly 100,000 times smaller than the spots on a conventional microarray gene chip. A conventional microarray can routinely accommodate 40,000 spots of DNA material. With the DPN technique, the density can be increased by several orders of magnitude, greatly increasing multiplexing capability in high throughput applications such as in drug discovery, gene expression studies and single nucleotide polymorphism (SNP) detections.

Exciting new methods of rapid sequencing of the DNA molecule based on nanotechnology are on the horizon. These techniques rely on extracting sequence information by direct nanoscale manipulation of a single DNA molecule.

US Genomics, a start-up company with a bold and ambitious plan for developing a GeneEngine that would read the gene sequence information letter by letter directly from a single DNA molecule, was formed by Eugene Chan in 1999 while he was still

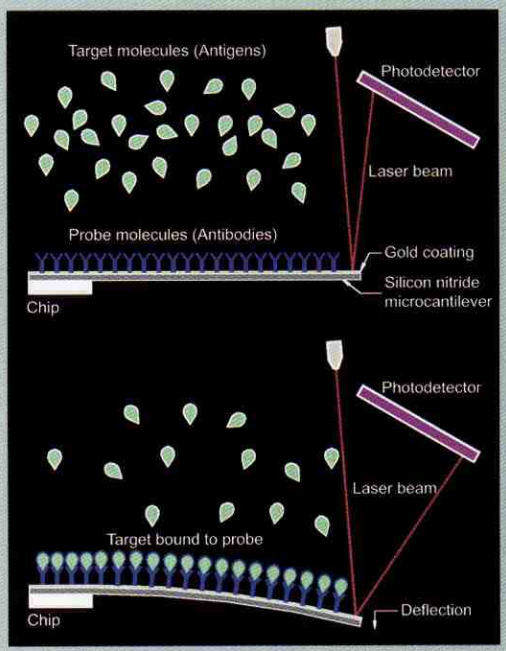

Figure 1: When the target molecule binds to the monolayer of probe molecules on one side of the microcantilevers, the altered intermolecular nanomechanical interactions cause the microcantilevers to deflect.

Adapted from "Bioassay of prostate-specific antigen (PSA) using microcantilevers," *Nature Biotechnology* **19**, 856–860, September 2001.

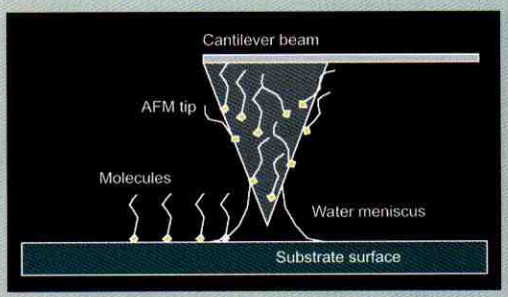

Figure 2: The diagram illustrates one mechanism for the deposition of a monolayer of molecules on a desired surface. Condensation of moisture forms a water meniscus under the atomic force microscope (AFM) tip. As the AFM traverses the surface, molecules attached to the tip get drawn down by the water meniscus and are deposited on the substrate surface.

a medical undergraduate. Eventually he dropped out of medical school to devote himself full-time to the company as CEO.

At the heart of this technology is an array of nanoscale posts about 100nm in diameter. A single-stranded DNA molecule in its natural state has a tendency to coil into a clump. As the DNA molecule flows past the nanochannels formed by the array of nanoscale posts, it unfurls. A funnel then captures the unfurled DNA strand, which flows along a straight 600nm channel to maintain the uncoiled state. Lasers excite the DNA molecule, pre-labelled with fluorescent tags, and photodetectors detect the fluorescence stationed along the narrowed channels (Figure 3).

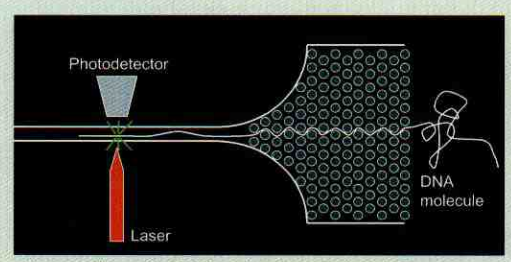

Figure 3: The coiled DNA molecule unfurls as it flows around the nanoscale posts. The laser illuminates the straightened portion of the molecule, and the photodetector senses the fluorescent tags.

This technology can presently handle only short sequences of up to 2,000,000 base pairs. Chan hopes the technology will be able to handle the 3 billion base pairs of one human genome in one go in about four years' time. The ultimate goal is still to sequence one human genome every ten minutes, making personalised genome sequencing within reach of the general public.

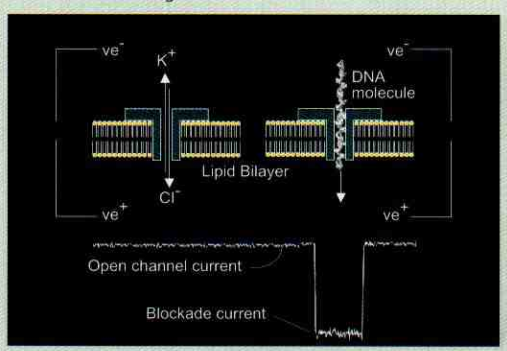

Figure 4: An ionic current of potassium chloride (KCl) is driven through the nanopore by the applied voltage across the membrane. An ionic polymer such as a strand of DNA molecule is driven through the nanopore by the same applied voltage, causing a transient reduction of the KCl ionic current.

Adapted from *Accounts of Chemical Research* **35** (2002) 817–825, Figure 1.

A group at Harvard University led by Daniel Branton has arrived at an even more direct approach than US Genomics in its use of nanochannels and fluorescent tagging of the DNA molecule. Branton's team has been investigating for the past decade the phenomenon of translocation of DNA molecules through nanopores. Applying electric potential across a membrane translocates the electrically charged DNA molecule through a nanopore. The membrane containing nanopores separates two ion-containing solutions. For each unobstructed nanopore, the applied electric potential generates an ionic current.

During the translocation of each nucleic acid polymer through the nanopore, the bulkier nucleic acid polymer causes a transient blockage of ionic flow. DNA lengths and base-pair compositions can be inferred from the recordings of the ionic-current modulation during the transient blockade of ionic flow whenever a nucleic acid polymer translocates across the nanopore (Figure 4). By selecting the appropriate pore size, hybridised DNA can be distinguished from single-stranded DNA. This process provides an alternative to the current microarray technique using fluorescent tags as a reporter for hybridisation events.

Nanowires, microcantilever beams, AFM imaging and nanopores are called label-free techniques. These techniques do not require the addition of labelling molecules, such as a fluorescent organic dye. Nanoparticles in the form of quantum dots, gold nanoparticles

and magnetic nanoparticles can work as identifier tags (an encoder) for a particular species of molecule or as a reporter to indicate that a certain molecular interaction has occurred. Techniques using nanoparticles have many advantages, such as speed, sensitivity and flexibility, when compared to conventional fluorescent or calorimetric techniques.

Chad Mirkin of Northwestern University in the US has developed a technique using 12nm-diameter gold nanospheres for the detection of DNA molecules. This process works on the basis of the phenomenon of gold nanospheres appearing red when the particles are dispersed and separated from one another by a distance greater than their diameter. The particles appear blue when clumped together.

Each gold nanosphere is studded with short strands of DNA complementary to one end of the target DNA molecule. Target DNA molecules are added to a suspension of gold nanospheres coated with DNA strands. When nanospheres are coated with DNA strands complementary in sequence to the ends of the target DNA molecules, the hybridisation of the target DNA molecules to the complementary strands of DNA on the nanospheres will cause the nanospheres to bind to one another. The presence of a target DNA molecule reveals itself as a colour change from red to blue (Figure 5) associated with the clumping of the gold nanospheres. An advantage of this technique is that the read-out of the colour change can be done quickly without sophisticated instrumentation.

Quantum dots are semiconductor nanocrystals with a diameter in the order of 2–10nm. Because of their minute size, they exhibit properties attributed to the quantum effect, such as the ability to emit light with a very narrow spectrum when excited by

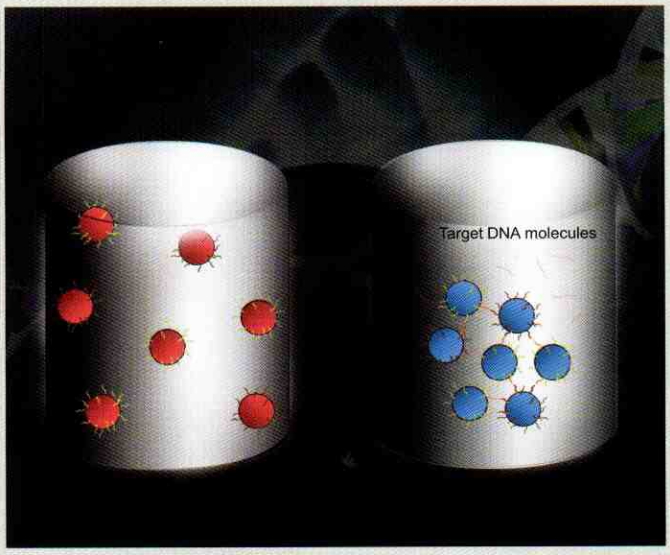

Figure 5: The gold nanospheres are coated with strands of DNA complementary to the ends of the target DNA molecule. The dispersed nanospheres suspended in solution appear red as depicted on the left. In the presence of target DNA molecules, the nanospheres clump together owing to the hybridisation of the target DNA molecules to the complementary strands on the nanospheres. The aggregation of the nanospheres causes a colour change from red to blue, as depicted on the right.

Adapted from *Scientific American* September 2001, and *Science* **277**, 22, 1078, August 1997.

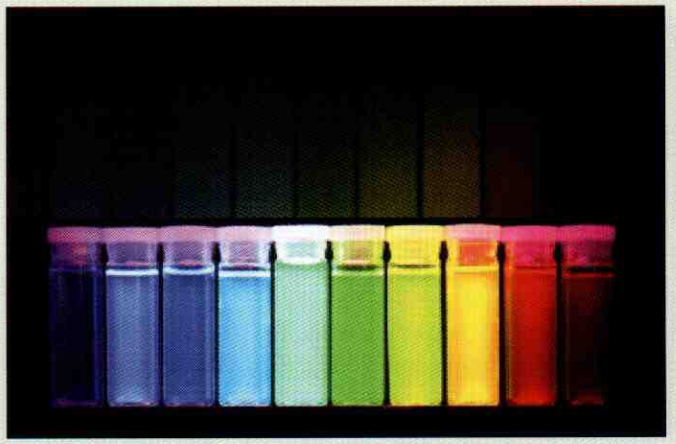

Figure 6: These ten distinct emission colours are a result of the excitation of quantum dots with near-ultraviolet lamplight.

Source: *Nature Biotechnology* **19**, 631, July 2001.

broad-spectrum light. The fluorescent glow from a conventional organic dye can only be elicited by incident light of a specific wavelength.

Unlike organic dye, which has the disadvantage of a bleaching effect, quantum dots continue to emit a non-decaying signal even with repeated cycles of light excitation. Thus, when quantum dots are coupled to biomolecules, intracellular processes can be monitored over a prolonged period. Quantum dots can be coupled to monoclonal antibodies to be used in the standard immunoassay technique. Recent demonstrations showed that this technique can identify specific markers for breast cancer.

The colour of the quantum dots can be adjusted by varying the size of the nanocrystals. Because quantum dots, when excited, emit light with very narrow spectrums, a wide range of colours easily distinguished from one another can be used (Figure 6). The small size and wide range of colours make quantum dots ideally suitable as identifier tags to distinguish various species of biomolecules.

Shuming Nie pioneered the use of quantum dots for tagging biomolecules. Mingyong Han, who has a joint appointment at the National University of Singapore and the Institute of Materials Research and Engineering, Singapore, was one of the original members of Nie's group. By embedding quantum dots in microbeads to tag biomolecules, it is theoretical possible to tag one million species of biomolecules by a combination of ten intensities and six colours. This technology has the potential of making "lab-on-a-bead" a reality. Han is currently working on improving the quality and versatility of quantum dots. (See "Lab-on-a-Bead Deciphers Biomolecules" in this issue.)

The field of nanotechnology is experiencing rapid growth. It appears that one of its first applications will take the form of doctors' office diagnostic tools, clearly demonstrating that cutting-edge technology will be brought to ordinary people thanks to interdisciplinary research.

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