The Mechanics of Nanomedicine

Today’s nano tools are enhancing our understanding of disease and enabling new therapies.

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If you attempt to look inside the tissues of the body and examine the fundamentals of disease, you must enter the nano-world. Viruses such as HIV and the common cold are invisibly small nanoparticles that attack cells and replicate using nano-mechanics. Nanoscale electrical networks enable the brain to process information and the heart to maintain its beat. DNA is wound up in nano structures, and read by nano ‘machines’. Alzheimer’s and Parkinson’s diseases are caused by nanoscale fibres. Tuberculosis and pneumonia are caused by bacteria, which use complex nano motors such as tail-like flagellae and pili to invade the body.

However, when people talk about ‘nanomedicine’, they often mean ‘nanomedicines’; nano-sized drugs for therapy and imaging. This article instead deals with the nano-mechanics and electronics in medicine, from the tools to visualize biological processes, to the engineering of tiny devices. Nanotechnology in this sense is particularly relevant to medicine: the 1-1000 nanometre range, which is larger than a few atoms but smaller than a human blood cell, is the scale at which much of human biology takes place.

The Nano Tool Box

If we want to understand the causes of disease so we can create better cures or even prevent it altogether, we have to be able to visualize the very earliest manifestations of disease within a cell. This activity takes place at the nanoscale, often well beyond the resolution of conventional light microscopes. So new tools and techniques are necessary to explore and understand this hitherto hidden nanoworld. Some of the more important of these tools, and their role in uncovering the secrets of disease, are described below.

Imaging

Scanning Probe Microscopies, including Atomic Force Microscopy (AFM)

Tools for achieving atomic resolution, originally designed for the semiconductor industry, are now being applied to the molecules of life. The precursor of the Atomic Force Microscope (AFM) won Gerd Binnig and Heinrich Rohrer, scientists at IBM Switzerland, the Nobel Prize in 1986. It was famously used to write the letters ‘IBM’ in individual silicon atoms, a breakthrough in the ability to control the building blocks of matter. Twenty five years later, new types of AFM have been developed for studying biology. An AFM works by vibrating a tiny silicon lever with a sharp silicon tip, close to the surface under investigation. Changes in this vibration caused by surface topography are detected. The smaller the tip and the faster the vibration, the smaller the features that can be observed.

It is now possible to image the molecular structure of DNA and its surface charge with nanometre resolution using scanning probe microscopy, which helps us understand how all our DNA fits
into each cell nucleus. The AFM is also currently being used to image nanopores in our cells. Hereditary diseases such as frontal lobe epilepsy and cystic fibrosis are associated with the malfunction of these pores, which have been particularly difficult to study. A high frequency AFM, however, is able to image in liquid and explore these pores in their natural state.

**Focused Ion Beam Scanning Electron Microscopy (FIBSEM)**

FIBSEM is a combination of two techniques. A beam of charged atoms is used to physically slice away nanthin layers of a specimen, as they are imaged using an electron microscope. The structure is peeled away one layer at a time, to reveal a three dimensional image. This is ideal for imaging the network of connections between the cells that make up the brain, the most complex organ of the body. The cells of the brain are connected in a dense network, the study of which has become known as ‘connectomics’. The objective is to map the detailed wiring of the brain’s circuits. It is extremely difficult to follow the pathways of nerve cells in the brain because they are so small and thin. Reconstructing them in three dimensions is a delicate task and images take weeks to acquire. With FIBSEM, it is possible to image neural circuits at a resolution of a few nanometres in all three dimensions. Disentangling the network of neural cells and their connections will enable the link between structure and function in the brain to be explored, which is vital to the quest to understand neurological disorders.

![FIBSEM image of an entire Purkinje neuron in cerebellar cortex](image: Image courtesy of Michael Hausser)

**Coherent X-ray diffraction**

X-ray crystallography has been the workhorse of biological imaging since the time when Rosalind Franklin produced diffraction patterns corresponding to the double helix of DNA. It relies on being able to produce a crystal from the molecule to be imaged, an often tricky and lengthy exercise. A modern adaptation of this technique, the imaging of single molecules or complexes, is coherent X-ray diffraction. The ability to image single complexes with nanometre resolution requires the use of a synchrotron facility, which covers an area of several football pitches in size, where electrons are accelerated to near the speed of light. From the movements of these electrons a bright beam of X-rays is released, sufficiently bright and ‘coherent’ or ordered, to image the molecule under investigation. Already used to image the interactions between biological molecules and metal surfaces, fundamental to the fabrication of biosensors, these bright and uniform X-ray beams could be used to show the make-up of our own chromosomes. This has implications for understanding and treating genetic disease due to chromosome defects, and also for elucidation of the mechanism by which chromosomes are formed and read.

**Electron Paramagnetic Resonance Spectroscopy (EPR)**

EPR is a type of spectroscopy that detects free radicals, that is, molecules with ‘free electrons’. The state of these free radicals can provide information on their surrounding environment. Although not many biological species have free radicals, those that do are fundamentally important, such as the proteins, small molecules and vitamins involved in respiration and metabolism. EPR allows a researcher to observe the processes involved in respiration, for example, by tracking the free radicals. This is especially powerful when combined with static information, such as crystallography, to give a picture of the dynamic process.
Diagnostics

Not only can nano tools be used to ‘visualise’ and thereby understand how a cell functions, other techniques and tools based on nanotechnology are leading to faster diagnosis, more effective screening for new drugs as well as tracking the progress of a therapy. Some of the more important of these include:

Gold nanoparticles and thin films

Most new types of diagnostic sensor for healthcare are made from thin gold layers or small gold spheres, this is due to the ease by which biological molecules can be attached to gold. The smaller these devices, the more sensitive they become. Gold nanoparticles have been studied using the nanoscopic technique of coherent X-ray diffraction. It was found that an individual nanometre-sized grain of gold becomes distorted when a single layer of molecules is attached, such as those that would be used in sensing. As sensors get smaller to increase sensitivity, effects such as these become more significant and understanding nanoscale behaviour become increasingly important when developing new diagnostics.

Surface Plasmon Resonance

As well as using mechanical sensors to analyse blood and other liquids, optical techniques such as Surface Plasmon Resonance (SPR) can also be used as a sensor. Here nano-sized metal features, such as gold drops on glass are irradiated with laser light. This causes a thin, invisible electromagnetic ‘field’ to be created on the gold surface. This delicate field is easily disrupted by the arrival of a protein or an interaction between a protein bound to the surface and a drug, and can therefore be used for diagnosing infection, or screening for novel drugs.

Cantilevers

One approach to detecting nanosized species and studying their interactions is to use tiny ‘cantilevers’. Silicon levers the width of a human hair are arranged like a series of diving boards in a row. These levers are coated with receptor molecules, which can bind to bacterial cells, viruses and proteins. Nanometre bending of these tiny levers is detected using a laser.

Infectious bacteria such as hospital-scare Methicillin-resistant Staphylococcus aureus (MRSA) are becoming increasingly resistant to antibiotics and new drugs must be found to combat them. Critical to finding the correct drug is understanding the mechanism by which it works. To investigate new antibiotics for these so-called ‘superbugs’, cantilevers have been coated with specific capture proteins. An antibiotic interaction with the proteins bends the levers, due to stress. Experiments such as these can tell us which drug binds most effectively, indicating its potential to treat the disease.

Cantilevers are not limited to detecting drugs; the size range is relatively wide, from whole organisms, such as the tuberculosis bacterium, down to tiny HIV virus particles and HIV-specific proteins. Through using cantilevers, it is possible to detect disease and to understand better the mechanism of infection and potential cure.

Virus Particle sorting

Often when diagnosing a viral infection or reacting to an epidemic outbreak, for example avian flu, it is necessary to isolate the virus and find out what it contains as fast as possible. Virus particles themselves are only few nanometers in size, invisible under a microscope and often in such small quantities in the blood that only a few hundred are hidden amongst the billions of much larger cells in a sample. In order to detect these, the viruses have to be found, sorted and concentrated, a task for a nano-engineered device. A forest of nano-pillars can be used as an obstacle course that causes particles flowing through it to be deflected according to size. Thus the hidden viruses can then be separated from the sea of red and white blood cells and collected. This type of sorting is quite remarkable, and furthermore can also be used to make viral vaccines, where the tiny viruses must be delicately sifted from the host in which they are grown.
Implantable medical devices

Many medical procedures involve the implantation of metals, polymers and other non-biological structures and devices within the body. The engineering and design of these implants can be critical to their performance, and their nanoscale properties in particular can have a large impact on their acceptance by, and interaction with cells and molecules in the body.

Super-hydrophobic surfaces

In nanotechnology, inspiration often comes from nature. In order to develop a super-hydrophobic surface – one that is extremely repellent to water - researchers copied the nanoscopic structure of the lotus leaf. The surface of a lotus leaf is rough and waxy, covered in tiny wax pyramids that are invisible to the eye. By mimicking this design, it is possible to engineer a surface coating that causes water to form perfectly spherical droplets, which then roll off at the slightest movement. These superhydrophobic surfaces also exhibit a self-cleaning effect with benefits including the elimination of biofouling in medical devices and in situations where high-precision dosing of medication from say implants is essential.

Nanodiamond micro-electrode arrays

Receiving and transmitting electrical signals is fundamental to so many processes in the body. Our heart beats to an electrical rhythm, our brain computes electronically, our muscles twitch using electrical impulses and we are even able to see due to images being transmitted as a series of electrical pulses. In order to measure and understand these signals, and replace and repair damaged function, we need electrical devices. The smaller and more biocompatible the device, the better it can integrate with the body, this is where nanotechnology plays an important role.

The stimulation of ganglion cells in the retina of the eye causes the brain to form images of the outside world. Micro-electrode arrays are being developed that can be implanted in the eye, replacing damaged cells and stimulating the eye to form an image. Creating an intimate contact between the cells in the eye and the electrodes of the array is a delicate task, as eye cells in particular don’t like to grow on electrodes. By understanding and manipulating the nanotopography of the electrodes, cells can be encouraged to form a natural contact with the foreign device. An engineered material such as nanocrystalline diamond offers both the desired electrical properties, as it can transmit tiny electrical signals to the cells, as well as other characteristics that are cell-friendly.

Cell patterning

Novel materials can be designed that support and help the regrowth of cells. Cells are very fussy about surfaces, and will only grow and thrive when the surface has a specific nano-topography. By controlling the nanoscopic shape and chemical properties of a cells environment, patterns of growth can be stimulated, a technique that has been used with success on the surface of hip implants. Neurons, the cells responsible for the transmission of information in the brain, are notoriously difficult to re-grow after damage. Initial experiments indicate that a carefully engineered surface such as nano-crystalline diamond can be as effective as a protein-treated surface designed specifically for neurons, and will encourage regrowth.

Cardiac stents

A good example of a medical device that can benefit from nano-engineering is the stent. A stent is a small tube made of steel mesh, commonly used to open up arteries in the case of blockage. Such blockages inside the arteries of the heart are often caused by a build up of fatty layers and plaques. The stent can be expanded inside the artery, concertina-like to increase the size of the opening and hold it open. The surface of the stent or the stent material itself can be nano-engineered to provide useful properties.

Implantation of a stent carries a risk of clotting, and the cells of the artery are usually damaged leaving the wall weak. In the future, stents will be manufactured with superhydrophobic coatings (mentioned previously) that may help reduce clogging and minimize the surgeon’s predominant worry of thrombosis. Surface patterning or coating with a nano-engineered material, for example nanodiamond, may also encourage the weakened cells of the artery epithelium to re-grow at the region of the implant.

Conclusion

Many techniques derived from physics and engineering are being applied to the medical field to increase our knowledge of cell behaviour and understanding of the causes of disease at the nanoscale. Tools developed for the semi-conductor industry and nanomaterials research are being successfully applied to the biological world, leading to new therapeutic solutions. These nanotechnologies are enabling a 21st century revolution in the way disease is diagnosed, treated and prevented.