Chapter 8
Nanotechnology in Advanced Medical Devices

Sabeeh Habib-Ullah, Dan Fei, and Yi Ge

Abbreviations

AFM	Atomic Force Microscopy
AMD	Advanced Medical Device
CCMV	Cowpea Chlorotic Mottle Virus
CT	Computed tomography
EBID	Electron Beam Induced Deposition
ECM	Extracellular Matrix
ESF	European Science Foundation
HSE	Health and Safety Executive
LOC	Lab on a Chip
MD	Medical Device
MEMs	Microelectromechnical System
MNP	Magnetic Nanoparticles
MPA	Mercaptopropionic Acid (MPA)
MRI	Magnetic Resonance Imaging
NEMs	Nanoelectromechnical Systems
NMs	Nanomaterials
NP	Nanoparticles
OSHA	Occupational Safety and Health Act

S. Habib-Ullah • Y. Ge (✉)
Centre for Biomedical Engineering, Cranfield University,
Cranfield, Bedfordshire MK43 0AL, UK
e-mail: y.ge@cranfield.ac.uk

D. Fei
Leicester School of Pharmacy, De Montfort University,
The Gateway, Leicester LE1 9BH, UK

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PAMAM	Poly(amidoamine)
PB	Prussian Blue
PEG	Polyethylene Glycol
PMMA	Polymethyl Methacrylate
POC	Point of Care
QD	Quantum Dots
REACH	Registration Evaluation, Authorisation and restriction of Chemicals
SPION	Superparamagnetic Iron Oxide Nanoparticles
SPR	Surface Plasmon Resonance
SWCNT	Single Walled Carbon Nanotube
USPION	Ultra-small Superparamagnetic Iron Oxide Nanoparticles
VNP	Viral Nanoparticles
WHO	World Health Organisation

8.1 Introduction

Nano refers to materials and systems being in the proportion of a billionth of a metre, i.e. 10^{-9} m and it originates from the Greek word 'nano' meaning dwarf. The term of 'nano' was first formally introduced by Tokyo Science University Professor Norio Taniguchi in a paper on ionsputter machining 1974 [5]. Generally these systems are agreed to be between 1–100 nm.

The potential of nanoscience was first expounded by Richard P. Feynman in 1959 [4], in his famous speech "Plenty of Room at the Bottom". Nanotechnology is now one of the world's leading industrial, academic, and political drivers. In a short time its emergence as an innovative and multidisciplinary platform has secured funding and investment on an unheard of scale [1]. Nanotechnology is projected to be worth $2.6 trillion in manufactured goods by 2014 [2]. Moreover the market impact of nano-based applications is estimated to be $300 billion within the next 12 years for the United States alone [3].

Nanomaterials (NMs) and nanotechnology hold the potential to initiate a new industrial revolution. The characteristics that make these materials commercially suitable are their size, aspect ratio and possibility to functionalise their surfaces. Most experts agree that this new phase of technology could have an impact on every aspect of life. Their uses range from drug delivery, food hygiene, to astrobiology and ocular implants.

Recently NMs have caused a flurry of activity in the medical field. A large effort has been made to use the technology itself as an analytical tool, as well as using nano-sized material to enhance the current paradigm. This was codified in 2004 [6], the definition for nanomedicine that the Medical Standing Committee of the European Science Foundation (ESF) compiled is:

> the science and technology of diagnosing, treating, and preventing disease and traumatic injury, of relieving pain, and of preserving and improving human health, using molecular tools and molecular knowledge of the human body

Further, the ESF demarcated five main disciplines of nanomedicine:

1. Analytical tools
2. Nanoimaging
3. Nanomaterials and nanodevices
4. Novel therapeutics and drug delivery systems
5. Clinical, regulatory, and toxicological issues

These five categories are generating a considerable amount of research [7]. However, it is difficult to interpret which of these fields are classed under medical devices, because they can be a range of articles. The World Health Organisation (WHO) has developed its own definition for medical devices [8]:

"Medical device" means any instrument, apparatus, implement, machine, appliance, implant, *in vitro* reagent or calibrator, software, material or other similar or related article, intended by the manufacturer to be used, alone or in combination, for human beings for one or more of the specific purposes of:

- diagnosis, prevention, monitoring, treatment or alleviation of disease
- diagnosis, monitoring, treatment, alleviation of or compensation for an injury

This clearly illustrates the breadth of products that could be considered as medical devices (MDs). Tools such as tongue depressors, injections and even spoons can be considered as MDs. Advanced MDs (AMDs) would clearly be a step above these mundane tools, clearly the use of NMs could potentially cause a device to be labelled in that bracket [9]. Instead of looking at a broad range of devices, it is beneficial to focus on the exceptional that are making a noticeable impact in the current climate of AMDs.

Nanoimaging and diagnostics are showing potential to revolutionise the gold standard techniques of today. Imaging systems can be grouped by the energy used to construct pictorial information, the type of information that is acquired or the spatial resolution that is obtained. Macroscopic imaging equipment that provide data for anatomical and physiological systems are regularly used in clinics across the world, the most common being computed tomography (CT), magnetic resonance imaging (MRI) and ultrasound. Whereas the emergence of molecular imaging techniques, that can be attributed to nanotechnology, are beginning to be used in clinical and pre-clinical settings [10]. This advancement has opened the possibilities of actually imaging biological functions [11].

Figure 8.1 shows the possibilities of nanoimaging and diagnostics; its potential to measure biological processes [12] is similar to a biopsy, but better. This can be achieved without surgery and possible long term monitoring of specific conditions. Applications comprise early detection and diagnosis of state and stage of disease, assessing response to treatment, and studying biological processes in real time [13]. Compared to CT scans, MRI scans and others of this ilk, they are advantageous in providing information for evidence based medicine.

Similar to this are biosensors and nanobiosensors [14, 15], which are comprised of two elements, a detection device and a transducer. The first provides sensitivity and specificity, while the latter gives an indication of the presence of the desired substrate [16]. These devices are being primed to make point of care (POC)

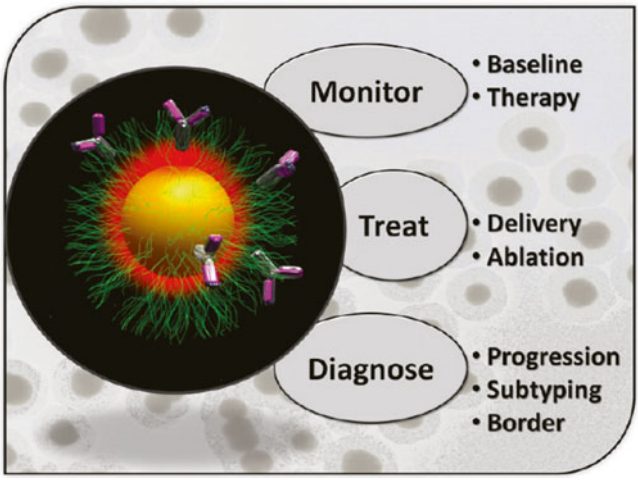

Fig. 8.1 The potential and consequences of nanoimaging and diagnostics [12]

technology feasible, the purpose of which is to deliver real time information on the state of the body. Hence barriers to overcome are to produce something easily fabricated and inexpensive with recognition capabilities, focusing on increasing sensitivity, specificity and enhanced response time [17].

There is already evidence of this on the market with glucose sensors for diabetics. However with nanotechnology in the ascendency, a fast paced diagnostic tool that can run hundreds of tests in less than a minute is conceivable. This lab on a chip (LOC) will utilise the miniaturisation capabilities of nanotechnology to revolutionise patient care. The idea is to produce a small chip that has multiple detection points and transducers to produce a signal, which can then be converted into meaningful information [18]. A key example is the work of Sung et al., in 2010 they demonstrated a microfluidic approach to analyse the pharmacokinetic-pharmacodynamic profile of an anti-cancer drug [19]. The results indicated that their approach was a better alternative to assess drug profiles on cell cultures. Although the work was completed on a micro level, the idea is to exemplify the direction the field is heading towards.

The impact of technology on surgery is often discussed but not truly examined Surgery is also being advanced through the rise of nanotechnology. It can range from the simplicity of the removal of a mole to the complexity of an open heart quadruple bypass. However, surgeons can now use an array of nano-driven materials and devices to aid their work. This extends from advances in intraoperative imaging, tissue healing and wound care to the procedures of nanosurgery involving nanoneedles, nanotweezers and precision lasers [20]. This is leading to more accurate and effective clinical practice combined with surgical precision to produce better invasive procedures.

For completeness, it would also be beneficial to examine the risk to reward ratio. There is scope within nanotechnology for misuse and exploitation. Therefore any regulatory and ethical quandaries should come under consideration in the subsequent discussion.

8.1.1 Nanoimaging

Imaging is a very important aspect within industry. Its uses vary from the x-rays in airports, X-raycrystallography, and MRI to determine chemical compositions. It also plays a critical part in medicine and can be classed as a MD. The WHO clearly states that devices can be used to investigate, diagnose, prevent and alleviate injuries, diseases or a physiological process [8]. The "advanced" can clearly be attributed to the introduction of nanotechnology. This can range from quantum dots, to high-tech processes such as Atomic Force Microscopy (AFM), or a combination of current machinery with a nanotechnology component; such as contrast agents combined with MRI. These tools can be used in prevention, monitoring and treatment of diseases. There is a lag time of information received and can hinder clinician decisions. However, the arrival of new technologies provides real time data along with a unheard of depth of information leading to a more complete and informed diagnosis.

8.1.1.1 Quantum Dots

Quantum dots (QDs) are nanoparticles (NPs) of a few hundred atoms. They absorb light at a wide range of wavelengths, but radiate near monochromatic light of a wavelength that depends on the size of the crystals. Of particular usefulness is the amount of control exerted over QD characteristics; temperature, duration and ligand molecules used during production of QDs can govern size and shape [21]. Hence, the radiation absorbed and light emitted can also be controlled [22]. Furthermore because the materials employed are inorganic, they are more durable in the body than their organic counterparts, they are highly observable using electron microscopy [23].

There are some toxicity issues with QDs, but functionalising them with multiple moieties has led to some favourable outcomes. In 2002, Akerman et al. used polyethylene glycol (PEG) as a surface cap for cadmium selenide QDs [24]. They examined their penetration of tumours in immune-deficient mice and compared it to peptide tagging (Fig. 8.2). Their results demonstrated that QD uptake potential and fluorescent characteristics were enhanced by PEGylation.

Furthermore endocytosis uptake of QDs and labelling of cell surface proteins with QDs conjugated to antibodies were investigated and demonstrated [25]. This procedure has since been improved to show that cells can be imaged and observed in real time. This technology has progressed towards a pilot study of QDs in non-human primates. The use of phospholipid micelle-encapsulated CdSe/CdS/ZnS QDs were observed over a 90 day period. Our results show that acute toxicity of these quantum dots in vivo can be minimal. Blood and biochemical markers remained within normal ranges, but chemical analysis revealed that majority of the initial dose of cadmium remained in the liver, spleen and kidneys. This suggests that the breakdown and clearance warrants further investigation [26]. Furthermore, this technology has been used to investigate specific processes that range from binding of QDs conjugated to the nerve growth factor to membrane specific receptors and intracellular uptake, tracking of membrane protein at the single molecule level, and recognition of ligand bound QDs by T cell receptors [27].

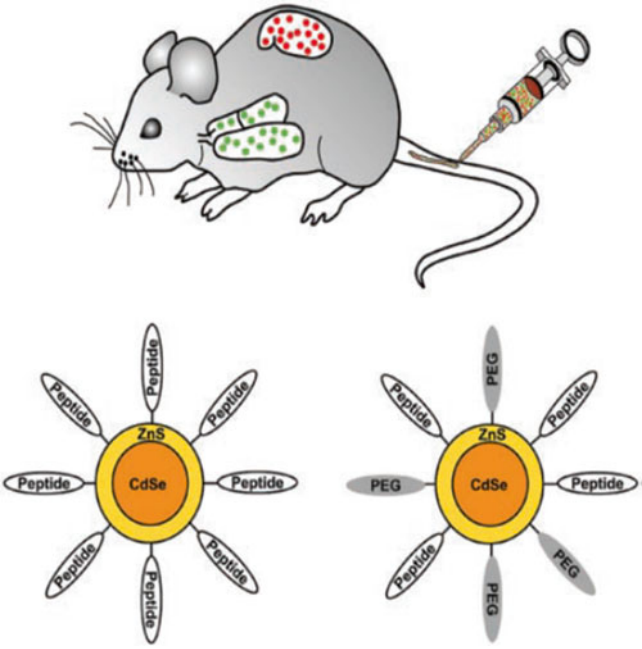

Fig. 8.2 Delivery of QDs into specific tissues of mice. (*Upper*) Design of peptide-coated QDs. (*Lower*) QDs was coated with either peptide only or with peptides and PEG, increasing solubility and bioavailability [24]

8.1.1.2 Magnetic Resonance Imaging

Magnetic resonance imagings (MRIs) are the most widely used diagnostic tools across the world. It is the instrument of choice due to its range, non-invasiveness and minimal side effects. It can be used to give high resolution images of soft tissues; specifically for tumours, brain, central nervous system and cardiac function [28]. MRIs exploit the difference in relaxation times of protons. When protons are exposed to a magnetic field, they become excited and then return to their equilibrium state at different rates T_1 and T_2. It is this information that is used to construct images to help diagnosis and treatment of patients.

Viral nanoparticles (VNPs) are virus-based NP preparations that can be used as a scaffold with a variety of properties. VNPs can be found in the form of bacteriophages and plant or animal viruses. Additionally they are biocompatible and biodegradable; there are organic and non-organic forms. However there is potential to combine these formulations to produce a more functional NP. Yet there have been no explorations of their toxicity, either as singular (organic or non-organic) or in a conjugated format [29].

In 2005, Allen et al. investigated Cowpea chlorotic mottle virus (CCMV) as MRI contrast agents [30]. It has 180 metal binding sites and *in vivo* CCMV binds calcium ions at specific sites; however gadolinium ions (Gd^{3+}) can also do this. Gadolinium is more useful because it increases proton relaxation during MRI, which gives sharper signals. The unusually high proton relaxation values of the Gd^{3+}-CCMV complex can be attributed to the VNP size and number of ions bound. These findings are encouraging, but at the moment VNPs remain proof of concepts until further investigations are completed.

Like VNPs magnetic nanoparticles (MNPs) have gained attention due to their properties. Their magnetic characteristics enable tracking through MRI. MNPs include metallic, bimetallic, Ultra-small and super-paramagnetic iron oxide nanoparticles (USPIONs and SPIONs) [31]. The USPIONs and SPIONs are preferable because of their lack of cytotoxicity, and surface being amenable to functionalization [32]. Hence, there is greater penetration in biological systems, and increased proton relaxation times present sharper signals for better imaging.

New vehicles are being developed that encapsulate multiple imaging molecules onto the MNP for use in integrated imaging systems. These agents can assist investigators to produce images across varied techniques. This signifies that one contrast agent could be used for MRI as well as CT and positron emission tomography scans, offering clinicians the ability to gain a selection of information efficiently [10]. It has been reported that gold NPs coated with gadolinium chelates produces MR contrast [33]. Such MRI active inorganic can be used for imaging of the vasculature, liver and other organs, as well as molecular imaging and cell tracking.

Of course these are not the only particles used for MRI contrast agents. Nanotechnology is developing so quickly that multimodal NPs are being developed not only for MRIs but other imaging techniques. Table 8.1 clearly shows that NPs of different types and sizes can target different organs, and all of them are in the preclinical stages of trials.

8.1.1.3 Atomic Force Microscopy

AFMs are a multipurpose instrument, they can be employed for imaging, determining and manipulating materials at the nanoscale. It can be used to work out surface structure, using forces and interactions such hydrogen bonding, Van der waal, and electrostatic forces. It consists of a small cantilever (of nano-dimensions) that produces a signal when deflected. This is extremely useful in biological imaging, mapping interactions at the cell surface, using high resolution images.

AFM has been used for a multitude of purposes. It can be classed as an AMD due to the fact that it is an analytical tool that is used for monitoring/imaging. Most recently it has proved an effective tool in diagnosis the root causes of protein folding diseases such as Parkinson's, Alzheimer's, and Huntington's diseases. The use of AFM and other nanoimaging techniques has been instrumental in understanding the structure and aggregation of key proteins [35]. Its ability to work on

Table 8.1 A comparison of different nanoparticles, their functions and the cancers they effect [34]

Cancer site	Nanoparticle	Clinical application	Current status
Breast	Iron oxide nanoparticles + Herceptin	Detection of small tumors on MRI	Preclinical
	Iron oxide nanoparticles + uMUC- tumor antigen	MRI and monitoring tumor response to chemotherapy via antigen expression and change in size	Preclinical
	Dendrimer	Contrast agent for micro-MR lymphangiography	Preclinical
	Iron oxide nanoparticle	Detection of sentinel lymph node	Clinical
Colon	Iron oxide particles	MRI of CRC and métastasés	N/A
	QDs	Visualization of cancer using fiber optics	Preclinical
Prostate	Iron oxide nanoparticles	Detection of metastasis with high resolution MRI	Preclinical
	Dendrimer + Prostate specific antibody	Targeting of antigen expressing cells	Preclinical
Brain	Iron oxide nanoparticles	Dual function particles to help define tumor margins accurately intra-operatively	Preclinical
Pancreas	Iron oxide particles	Enhances normal pancreatic tissue on MRI enabling easy visualization of PDAC	Preclinical

singular molecules and isolating its interactions has led to progress in developing understanding and therapies for such diseases. Neurodegeneration in Alzheimer's disease has been linked to β-amyloid (Aβ) peptide build up. AFM has shown that Aβ interacts with the plasma membrane by changing the structure, which leads to the disruption of ionic homeostasis [36]. This was further investigated to conclude that amyloid proteins disrupt distinct regions of the bilayer membranes, which may represent a common mechanism of membrane disruption for protein folding diseases [37].

Nanotechnology has provided the means to make breakthrough machinery more efficient. AFM imaging is generally slow; it takes between 1 and 2 min per frame, making it difficult to record biological processes that generally occur in seconds. Collagen (it is one of the main components of the extracellular matrix) imaging was improved by an inversions feedback/feedforward process. Furthermore it reduced positioning errors as well as increased scan time at high frequencies [38].

In 2006, Voitchovsky and colleagues were able to examine Purple membranes formed by bacteriorhodopsin, it is a combination of crystals and lipids [39]. Using AFM the group were able to observe stiffness and lipid mobility. This work was extended by using AFM to actually study the dynamics of the purple membrane. Yamashita and co-workers were able to film the dynamics of the membrane using high speed AFM, determining how the crystals within it were assembled [40].

8.1.1.4 Optical Tweezers

Optical tweezers are another method of imaging, they can control objects with light. They offer a spatial resolution of 1 nm, a force resolution of a piconewton, and a time resolution of a millisecond. This makes them ideally suited to examine biological processes ranging from the size of a single cell to the minuteness of isolating a single molecule [41].

They were first introduced by AT&T Bell Laboratories. An optical tweezer uses force exerted by the electric field of a focused beam of light. In response to this, a small object develops a dipole, which allows it to be focused at a particular point. The focal point is formed on the optical axis, and the radiation pressure interacts with the electric force, allowing the isolation of a nanosized object. The object of interest can then be examined.

Ermilov and colleagues used optical tweezers to study the interaction of plasma membranes with their own cytoskeletons [42]. Their results showed that combining this technique with fluorescence imaging allowed them to create a profile of forces and stresses acting on the structures of interest. Pine and Chow went further by not only immobilising a neuron but isolating it as well [43]. They found they could move these neurons at a speed of 200 μm/s without actually causing damage to it. The duo also managed to cage these neurons for cell culture. The implication of this study is vast, this technique could be potentially used to grow neurons and graft them for victims of spinal injuries.

Combining this technique with confocal microscopy has allowed the multi-planar imaging of intercellular immune synapses [44]. This collective technology enabled the characterization of complex behaviour of highly dynamic clusters of T cell receptors at the T cell/antigen-presenting cell synapse. This work also identified the presence of receptor rich molecules.

The ability of such techniques to manipulate and measure forces has found several applications such as understanding the dynamics of biological molecules, cell-cell interactions and the micro-rheology of both cells and fluids [45]. A prime example is using optical tweezers to measure the interaction between NPs and prostate cancer cells [46]. The study revealed that the functionalised NP and cell binding was improved by the presence of folic acid. The works presented here show how nano-technology has made a positive impact on medical devices and their capabilities.

8.1.2 Nanobiosensors

The biosensors market has become very lucrative, it is estimated that annual global investment in this technology for research and development was thought to be $300 million. The introduction and fruition of nanotechnology has produced a huge amount of investigation and innovation; over 6,000 articles and 1,100 patents were issued and pending between 1998 and 2004 [16]. This indicates that effort within this field is commercially worthwhile and is rapidly progressing. It is important to note that the addition of NMs is what changes a biosensor to a nanobiosensor.

The uses of biosensors have increased rapidly and have become applicable across a range of fields. Most commonly they are used for clinical, environmental and food purposes, to name but a few [47]. As mentioned before there are two main parts to a biosensor, a recognition element that combines with a transducer (Fig. 8.3). The signal produced is then amplified against some baseline to produce a significant reading. The most dominants types of biosensors can be categorised into three classes, electrochemical, optical and followed by piezoelectric, the latter being the least common. Each of these different categories is differentiated by their transducer technology, as opposed to the molecular recognition element, which can be the same for each of them.

The structure of biosensors has an obvious effect on their functions. The bio-recognition element is based on the high affinity of receptor and analyte, such as an enzyme, a strand of nucleic acid or even an antibody [48].

8.1.2.1 Electrochemical Biosensors

This particular type of biosensor is the most common. It has been used for a variety of reasons. Of interest is the fact that they have been used to detect emerging infectious diseases, however despite the abundance of technology this aspect is still underdeveloped. Environmental detection has been based on monitoring toxic effects in cells, genes and possible endocrine disruptions. This technology is also being heavily mooted to make an impact in POC diagnosis potentially providing information for early detection of disease [17]. Where electrochemical sensors differ is in how signalling is achieved in the transducer element. It can be based on a measured voltage (potentiometric), a current (amperometric), or the transport of charge (conductometric) [16].

8.1.2.2 Optical Biosensors

These particular sensors have been developing rapidly, even more so considering the impact of nanotechnology. Optical sensors employ optical fibres orplanar waveguides to direct light. This is then directed to the sample and interactions produce a signal which can then be compared to its baseline. The signals produced include absorbance, fluorescence, chemiluminescence, surface plasmon resonance to probe refractive index, or changes in light reflectivity and can be read at the sensing film [16, 49].

The advantages of optical biosensors are their rapidity, the insusceptibility of the signal to interference and the potential for greater data, as a result of the changes in the electromagnetic spectrum. Optical methods can be employed to use multiple wavelengths on a sample without interfering with one another. This arrangement can lead to direct or indirect detection [49]. Deposition techniques can be used to produce optical biosensors. Screen printing and ink-jet printing are extremely fast and precise, producing great quantities of low-cost and reproducible biosensors [50].

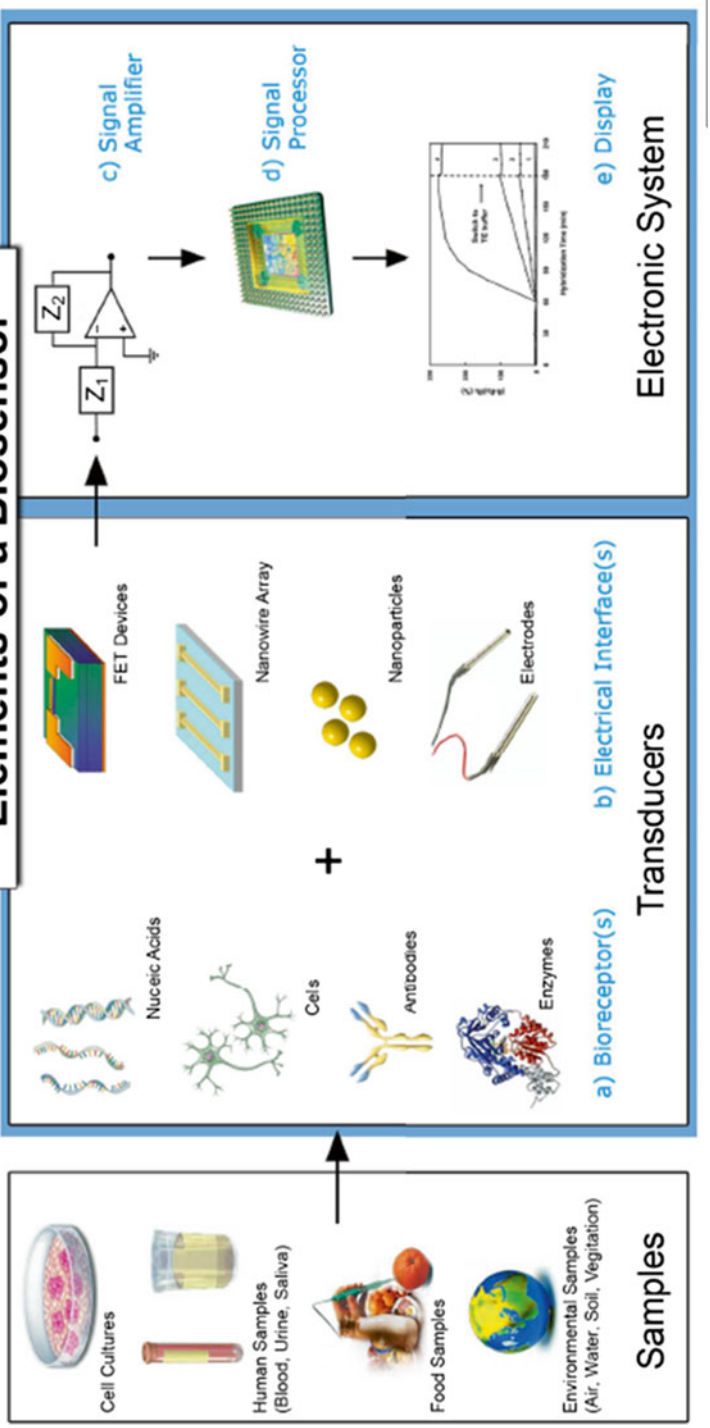

Fig. 8.3 Elements and components of biosensors [48]

8.1.2.3 Piezoelectric Biosensors

As the name suggests this particular biosensor is based on the piezoelectric phenomenon. This was first investigated in 1880 by Jacques and Pierre Curie, who observed that mechanical stress applied to the surfaces of various crystals, caused a correspondingly proportional electrical potential across the crystal, the converse was also true [51]. A multitude of crystalline materials (tourmaline, lithium niobate, aluminium nitride to name but a few) exhibit the piezoelectric effect, however the characteristics of quartz make it the most common crystal employed [52]. These crystals have been used as microbalances owing to their small size, high sensitivity, simplicity of construction and operation, light weight, and the low power required [92]. Similar to the other classes of biosensor, this particular methodology has been employed across a wide range of settings, including environmental and clinical.

8.1.2.4 Nanotechnological Impact

Due to the ubiquitous properties of NMs their use to construct these AMDs is no surprise. In 2003, Davis and co-workers reported on the investigations carried out in their laboratory, which culminated in the construction of a glucose biosensor [53]. The novelty of this sensor was that its construction took place on a single-walled carbon nanotube (SWCNT). The results showed that SWCNTs are highly biocompatible, and their unique structure has added benefits, including having a large surface area that makes it ideal for biological loading. This functionalised surface can exchange electrons and this mechanism can be employed in the fabrication of SWCNT-based biosensing devices.

QDs have also been used in biosensors. Cadmium Telluride was employed because its surface area was conducive to functionalization [54]. Furthermore its conductive properties allowed facilitated the oxidation of the thiocholine-acetylcholinesterase complex, thereby increasing sensitivity. Considering that QDs are so widely used and investigated as biological probes and contrast agents, this is a novel way of using them.

In relation to this green and orange Cadmium Telluride QDs were used as part of a fluorescent biosensor [55]. They were employed to observe proton flux driven by ATP synthesis in viruses. The key findings showed that different fluorescence occurred simultaneously and independently, further they were pH-dependant and showed no interference. An added benefit was that the fabrication of these QDs was inexpensive and convenient, making this commercially viable.

Gold NPs have many uses, in biosensors they are employed because their functions are heavily linked to their structural conformation. Depending on their shape, the surface plasmon resonance (SPR) is affected [56]. This is the oscillation of conduction electrons resonating with the wavelength of light used for excitation. By changing of the gold NPs shape from spheres to rods, the aspect ratio changes, thereby affecting the altering the SPR from the visible region to that of near infrared.

Gold NPs provide better biocompatibility, it also significantly increases the receptor area, thus improving sensitivity. In 2008, Li et al. conjugated gold NPs with

3-mercaptopropionic acid (MPA), poly(amidoamine) (PAMAM) dendrimer to obtain films on which Prussian blue (PB) was electrochemically deposited [57]. The purpose of this was to examine the response to the reduction of hydrogen peroxide. The investigation found that the sensitivity and limit of detection was enhanced, pH range and electrochemical stability and response were also improved. Furthermore, gold NP assemblies were used to trap proteins [58]. Using localised surface plasmon resonance nanotransducers delivers new leverages in hot spot-based nanosensing. The intensity of this electromagnetic hot-spot can be fine-tuned to gain picomolar sensitivity.

Recently, silicon nanowires were found to be able to detect microRNA, at a limit of <100 1 fM [59]. It was found that optimising the surface functionalisation and fabrication protocol, a theoretical limit of detection of 1 fM could be achieved.

It is clear that NMs have enhanced the field of biosensors to new heights. Never before have academics been able to explore biological and cellular processes at such close quarter. Moreover, the addition of NMs can actually enhance specificity, sensitivity and reproducibility. The miniaturisation of this technology is leading to POC and LOC diagnostics.

8.1.2.5 Progress to Lab On a Chip (LOC)

It is imperative to demonstrate the impact that nanotechnology has on the progression from a biosensor to a LOC. In 2009 Lakshmi et al. presented N-phenylethylenediamine methacrylamide (NPEDMA), which contains both aniline and a methacrylamide group, capable of independent polymerisation via free radical or electrochemical methods [60]. This work was then furthered by the group by developing an electrochemical sensor for catechol and dopamine using NPEDMA and a molecularly imprinted polymer (MIP) [61]. The MIP employed in this case is a tyronisase mimicking polymer. It has two copper binding sites and is more stable than its natural counterpart.

MIPs can be considered as polymeric NPs that are able to recognize biological and chemical molecules. The synthesis is based on the formation of a moulding complex between an analyte (template) and a functional monomer. The template is removed leaving impressions of precise recognition sites matching in characteristics to the analyte, intermolecular interactions determine the molecular recognition [62]. Hence it is particularly desirable to use a MIP in conjunction with NPEDMA.

Electropolymerisation of the NPEDMA resulted in the formation of a polyaniline (PANI) layer, a conducting polymer, on a gold electrode surface. The catechol specific MIP was then photochemically grafted on to this layer, in effect creating a network of molecular wires, to conduct a signal for molecule capture. This was a novel method of producing a biosensor with recognition and transducer capabilities.

This approach was then further modified again to examine what impact PANI nanostructures had on the catechol sensor. Berti et al. sputtered gold on an alumina membrane and used it as a mould for poly-NPEDMA [63]. The tryonisase-mimicking MIP was then attached and resulted in the improvement of catechol detection, the lower limit was determined to be 29 nm, a thousand fold increase.

This emphasises the capability of nanostructures to improve diagnostic performances of sensors.

This approach was expanded upon by Akbulut and colleagues. In 2011 they used polychemical grafting to coat NPEDMA to polystyrene microtitre plates [64]. The purpose of this was to create a library of different polymers that could be attached to the plates. The idea of was to assess the feasibility of this to identify a small catalogue of biological and organic compounds. The analytes were a herbicide-atrazine, organic dyes (eosin, bernthsen and meldola), and bovine serum albumin. The results showed that this method of screening is suitable for a myriad of applications and is cost effective, reproducible and easily manufactured.

This work was furthered improved upon by synthesising a polymer to improve and enhance the characteristics shown by NPEDMA [65]. The new polymer is called N-(N_0,N_0-diethyldithiocarbamoylethylamidoethyl) aniline (NDDEAEA). This structure is similar to NPEDMA in that it is based on PANI, but has the ability to be functionalised twice.

This could have far reaching implications over some future applications. In effect, this case study has developed two new polymers with a myriad of different characteristics, the first of which has already been explored to some extent. NPEDMA and NDDEAEA seem to offer potential not only in analytical technology but in other fields. The impact of nanotechnology is vast on AMDs, as demonstrated by this series of work. A polymer and MIP were constructed to form a biosensor, this was essentially modified and optimised to form precursor to a lab on a chip with enhanced limits of detection. This work is only one step short of forming an LOC, consider the key components, there is a molecular circuit linked to a possibility of a library of polymers that can be manipulated to detect any analyte.

The possibilities are endless; furthermore the construct is reproducible and inexpensive. An example of this, is use of nanolithography to create nanoarrays of gold [66]. To demonstrate its multiplex analyses, horseradish peroxidase and anti-horseradish peroxidase antibody was used as a model for a recognition system. The enzyme-linked immunosorbent assay performed had a detection limit 100 pg/mL. It was found that these chips could be stored for 50 days when stored at 4 °C without any significant loss of activity.

8.1.3 Surgery and Clinical Applications

Surgery and clinical applications have been on an upward curve since the exploration of nanotechnology. This was also predicted by Richard P. Feynmann in ‘Plenty of room at the bottom’ talks about the impact of nanotechnology on surgery and its applications:

> It would be very interesting in surgery … if you could swallow the surgeon. You could put the little mechanical surgeon inside the blood vessel and he goes into the heart and looks around (of course the information has to be fed out). He finds out which valve is the faulty one and slices it out. Other small machines may be permanently incorporated into the body to assist some inadequately functioning organ.

It is clear that some of the advances in AMDs are leading to the fulfilment of this vision. This involves the applications of nanotechnology in the surgery. The idea of molecular machines, high precision tools, and nanoimaging are leading to the beginnings of a new field, nanosurgery.

Moreover, giant strides are being made in clinical applications of nanotechnology. This consists of tissue engineering, regenerative medicine and implantation of these AMDs.

8.1.3.1 Nanosurgery

Surgery has always been a macro-scale project, involving cutting, controlling and splicing of organs, muscles and bones. However, as technology has progressed, so have the requirements of this particular field in medicine, leading to more precision and less invasive measures. In the latter twentieth century, the emphasis was on miniaturisation, small incisions, laparoscopic procedures by fibre-optic visualisation, vascular surgery by catheters and microsurgery under microscopes to refine protocols and diminish trauma [67].

One of the major tools that advocate nanosurgery is the use of high precision lasers. A landmark work by Shen and colleagues demonstrated the selectivity and suitability of a femtosecond laser for the ablation of subcellular structures. They focused laser pulses beneath the cell membrane to ablate cellular material. Shen and colleagues were able to selectively remove regions of the cytoskeleton and individual mitochondria without affecting nearby structures or compromising cell viability. Using this approach demonstrated that mitochondria are structurally independent functional units [68].

This work has led to a plethora of development in the use of this 'nanoscissors' technique. Most recently, experiments conducted on human metaphase chromosomes and fixed cell nuclei show it is possible to induce sub-100 nm effects with near-infrared femtosecond laser pulses [69]. Another study used a multimodal imaging system with nanosurgery capabilities, for the selective ablation of sub-cellular components in cancer cells [70]. The work resulted in precise destruction of structures in the cancer cells while leaving them fully functional.

As mentioned before AFM is an instrument that is used in nanomedicine and is highly precise. A study was done to modify the tips of AFM for using electron beam induced deposition (EBIM). This technique was used to make a 'nanoscalpel', 'nanotome' and 'nanoneedle' [71].

Figure 8.4a–c shows the structures of the nanoscalpel being constructing. This was fabricated using an electron beam of low velocity depositing carbon atoms along the tip. This extended along the tip in a self-supporting structure that can then form a blade in the nano scale.

Nanotomes use a similar technique. The process that follows is identical to nanoscalpels, but there are two blades deposited and extended. The difference is that a single filament is extended between the blades, giving it a similar functionality to a vegetable peeler.

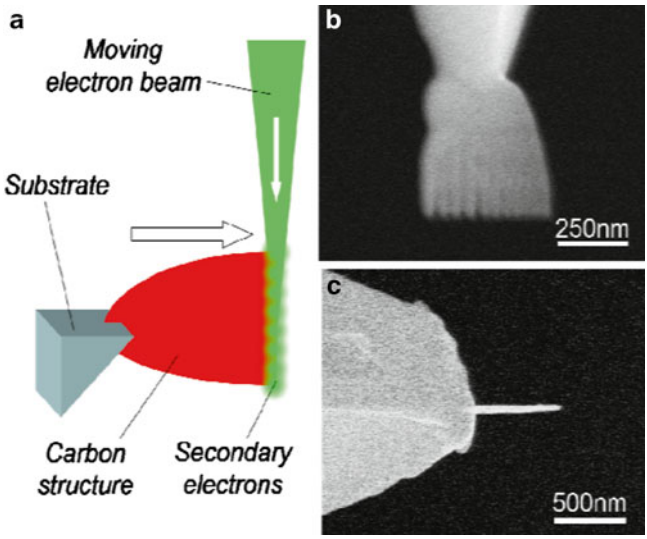

Fig. 8.4 (**a**) Fabrication of nanotool structures using electron beam induced deposition. (**b**, **c**) Nanoscalpel blades imaged from the side (**b**) and from the top (**c**) using SEM [71]

Nanoneedle is processed in a similar way to a scalpel, but the deposition is lateral as well. This allows the needle to be thickened and provide more support, as the thin structure limits the force that can be applied to the nanoneedle. The nanoneedle has several uses, it can be used for profiling structures, and if the needle is thickened then indentations can be made and studied. Beard and colleagues showed that this particular tip could also be used to remove a cluster of proteins from a cell [71]. While the nanoscalpel function can be used for cellular dissections, making controlled incisions. The nanotome can be used to peel back layers of a cell, which can then be imaged.

These nano-surgical tools provide a new route to the manipulation and dissection of cellular structures. They can be used to ablate and manipulate subcellular structures. Although in their infancy, these methods could be developed and used to complete complex surgeries at the nanoscale. Cellular level surgery has been proposed using a nano robot based on AFM technology [72]. It has multiple functions including imaging, characterizing mechanical properties, and tracking. Furthermore, the technique of tip functionalisation facilitates the robot ability for precisely delivering a drug. Therefore, the nano robot can be used for conducting complicated nano surgery on samples such as live cells and bacteria. Additionally, the software in this nano robot provides a "videolized" visual feedback for simultaneously monitoring the operation of nano surgery and observation of the surgery results.

8.1.3.2 Implantable Devices

Nanotechnology is in the process of modernising implantable devices. AMDs that were of macro proportions are now being miniaturised and made more efficient. This is partly due to increased knowledge in the field of biomimetics, the process of

using the way in which nature successfully produces something to create a material or device [20].

Implantable devices can cover many aspects and can deal with sensory issues as well as regulatory issues such as glucose sensors, retinal implants, prostheses with nerve control to name but a few. The ideal scenario is to reach a stage of miniaturisation and biocompatibility that can then produce devices capable of autonomous power, self-diagnosis, remote control and external transmission of data [73].

The auditory nerve contains near to 30,000 axons which cochlear implants stimulate [74]. From 1972 near a 100,000 people have been fitted with the device, however the work being developed is focusing on the nanoscale as the ear contains such structures [75]. The research that produced in this field is focused on developing smaller implants that can aid the work of axons to produce and relay an audible signal for the brain to process.

The optical nerve contains near a million fibres, and must deal with complex data and its processing [74]. In 2007, Alteheld and colleagues compiled a review to report on the developments of visual implants [76]. Electrical stimulation of the retina of blind subjects resulted in ambulatory vision (allowed movements without collisions and stumbling) and some character recognition. This was done by developing a wireless intraocular prosthesis, and having external feedback. Most recently a trial was carried out on 20 patients with varying degrees of blindness, to determine the electric charges needed to stimulate visual perception [77]. In 15 patients this was concluded to be in the range of 20–768 nC. This work illustrates the progress being made within this field, from exacting some visualisation to working out the exact range of charge needed to induce a visual event.

Another key AMD that is being developed for clinical use, is an implantable drug reservoir. This can be used in many scenarios but primarily for diabetics. This concept is to have a system in the body that has sensor that monitors a particular metabolite and releases the needed drug. Microelectromechnical systems (MEMs) and nanoelectromechanical system (NEMs) are ideal for this purpose. Some of these systems include microneedle-based transdermal devices, and micropump-based devices. Most notably the latter has been used for insulin delivery, glucose injection for diabetes, and administration of neurotransmitters to neurons [74].

It is apparent that progress within implantable devices is being made rapidly. This can be attributed to the technological advances and ease of access to information. The range of work being done is broad, focusing on producing an applicable product or technique, as well as optimising it.

8.1.3.3 Tissue Regeneration and Prosthetics

The goal of tissue engineering and prosthetics are very similar. The idea is to provide a platform for the body to either encourage repair or an opportunity to assimilate a new material (the surface of a joint replacement) and possibly to graft new material to a damaged organ. With an ageing population, these options are becoming necessary, especially when you consider the damage done by lifestyles and everyday use of organs and joints.

Considering the lifetime associated with orthopaedic implants and the rigours it goes through, nanomaterials are a viable alternative for current materials. The lifetime of implants are severely hampered by the eventual loosening between the joint and prosthesis [78]. This implies the bonding cement, polymethyl methacrylate (PMMA) that is widely used, is inefficient. Using nano-manipulated titanium showed an improvement in adhesion and promotion of cell growth. By introducing sub-micron features on titanium compared to flat surfaces, led an increase of endothelial cell adhesion density by 200 %. Whereas nanometre surface features promoted a 50 % increase in endothelial cell adhesion density compared to flat titanium surfaces. Using aligned patterns of such features on titanium, results highlighted that both endothelial and bone cells preferentially adhered onto sub-micron and nanometre enhanced surface features when compared to flat regions [79]. The use of titanium oxide nanotubes in dental implants were shown to be arrayed like collagen fibres. Furthermore the nanotubes increased roughness and surface area providing superior performances in multiple areas. It had greater hydrophilicity, greater cell adhesion and growth for osteoblasts and a better bioactivity and compatibility than current materials [80].

Tissue engineering is similar to this aspect in that it wants to promote the body to repair regenerate new cells. This can be done by introducing an extracellular matrix (ECM) that the body recognises as 'self'. Signals are transmitted between the cell and the ECM would facilitate communication for "cell adhesion, migration, growth, differentiation, programmed cell death, modulation of cytokine and growth factor activity, and activations of intracellular signalling" [74].

For example, hybrid biomaterials are being employed to better emulate natural ECM. These materials can be used to encourage healing while reducing the formation of scarring [93]. RGD peptides (R: arginine; G: glycine; D: aspartic acid) have been found to promote cell adhesion, RGD have also been amalgamated into man made ECMs to promote cell proliferation [81].

Some of the topics mentioned in this section are varied; however they do fall under the category of medical devices under the definition of the WHO. The connection may not be obvious but the "replacement, modification, or support of the anatomy or of a physiological process" combined with the monitoring and diagnosis of using a technology, renders that a medical device. The added caveat of the device being of nano-dimensions or functionality not only drives this branch of medicine but provides the epithet of 'advanced'.

8.1.4 Hindrances and Effects to be Considered

So far the focus has been on the positive impacts of nanotechnology and AMDs. However there are some minor and/or serious hindrances and effects that pertain to the success, ethical considerations, regulatory issues and the inherent toxicity of some NMs need to be considered before making an informed decisions.

8.1.4.1 Scientific Hindrances

The work discussed here are some of the more important breakthroughs within AMDs. However this does not even begin to compare to the volume and standard of work begun. In 2007, the amount of papers being published that were nano-related numbered near 15,000, this influx of publications is also reflected within the movements in intellectual property. In 2003, 90,000 patents were submitted for consideration. This illustrates the sheer amount of activity this field has generated.

However, the issues surrounding the use of this technology are manifold. A prime example of this is the use of NPs as imaging agents that are introduced within the body. Usually only 5 % of NPs administered to the body remain after 12 h, while 80 % of the initial dose are eliminated from the body. Further to this it is very difficult to ascertain how swiftly these particles would go to the intended target, NPs would be in systemic circulation for an extended period of time once they are released in a subject [82]. QDs are especially perilous as their outer shells can be worn away and can cause the production of free radicals and DNA nicking [83]

Another issue within progression and realisation of AMDs are the kind of studies that are being done. There is a distinct lack of comparability between investigations which can be attributed to one cause: the drive of getting published. Researchers and their funding are heavily linked to their work being heralded in journals of repute. Consequently, the techniques they use are always new, protocols and models are novel and inventive. Therefore, promising work is sometimes not explored and improved upon by other scientists, which leaves avenues incomplete.

Another issue with publications is that the lack of "no effect" studies is hindering the body of knowledge accrued [84]. This would provide researcher with protocols and methods of investigations that are a waste of resources. Moreover, having access to such data would provide a more complete picture, this would be crucial in designing possible resolutions for projects. In light of this the editors of three scientific journals have agreed to also publish the results of "no-effect studies" [85]. This would be instrumental in the use of resources of academia, governments and industries to translate scientific research into consumable products such as AMDs.

8.1.4.2 Regulatory Hindrances

As has already been discussed NMs size heavily contributes to their unique properties. However, this same feature that allow NMs to interact with biological entities but can also have deleterious effects on cellular mechanisms, and cell viability itself. It must also be considered that as of 2010, NMs have been used or present within over 800 consumer products [86]. There is a growing concern among cynics and advocates of NMs about their toxic potential, and there are calls from some quarters to have a global moratorium on all research and release of products, until regulations are in place to ensure protection from hazards [87].

Toxicity issues range from the points of entry to the range of effects themselves as shown in Table 8.1. Size and biopersistence play a large role in the extent of damage incurred. NMs can enter the body upon contact of the skin, inhalation and ingestion. This can cause a variety of issues ranging from free radical causing DNA damage, cell death and the formations of cancer [85].

In 2007, the European health and safety executive (HSE) initiated the Registration, Evaluation, Authorisation and restriction of Chemicals (REACH). Previous to this regulation, it was supposed that NMs act identically to their bulk equivalents, whereas studies show this is far from the truth. The necessity to weigh the impact of NMs, and also set some guidelines in how they should be approached commercially is key to the propagation of nanotechnology in AMDs. This however is not enough, and to remedy this, a motion has been passed by the European parliament to reassess the regulation of handling of NMs. Guidance has also been set for companies and they are recommended to adopt the precautionary principle [88].

In the US the Occupational Safety and Health Act (OSHA) of 1970 protects employees from illness at work. The slow response of OSHA standards-setting compared to the swiftly developing nanotechnology sector has left many staffs unprotected [89].

However in March 2008, the FDA and nano-health Alliance organised a workshop to identify the most pressing barriers to the success of nanotechnology. They classified the followings as the most important hurdles:

- Determination of the distribution of nanoparticulate carriers in the body following systemic administration through any route;
- Development of imaging modalities for visualizing the biodistribution over time;
- Understanding mass transport across compartmental boundaries in the body;
- Development of new mathematical and computer models that will lead to predicting risk and benefit parameters;
- Establishment of standards or reference materials and consensus testing protocols that can provide benchmarks for the development of novel classes of materials; and
- Realization of an analytical toolkit for nanopharmaceutical manufacturing, accompanied by a specification sheet of toxicological, safety, and biodistribution properties obtained through standardized, validated methods" [74].

These initiatives that are being initialised by various taskforces are an indication of intent. The progress of nanotechnology is held in high regard by industry and academia and its success must be ensured.

8.2 Summary and Outlook

AMDs are part of a wave of health related technology that has been pervading the medical field for at least three decades. However, this aspect of medicine is being driven to new heights by the dawn of nanotechnology. The research presented here is not definitive; the purpose of this work was to highlight the present climate of AMDs and the impact of NMs, NPs and novel techniques.

AMDs range from imaging agents, to techniques such as optical tweezers. The purpose of which is to reach a resolution within cellular activity that actually identifies the structure, function and even movement of specific cell mechanisms. This is typified by the capabilities of AFM and optical tweezers. The issue with the research examined, is that none of these techniques are making it to the clinical setting.

However, the potential within the diagnostic techniques covered is vast. The most common future is the combination of QDs and MRI contrast agents. They have the potential to be combined into theranostics, devices/particles that not only give information about disease but deliver drugs to them as well. Ideally these particles would be loaded with therapeutic agents and functionalised with target and imaging ligands, thereby providing an intermediary that has bio-penetration, medication and surveillance capabilities.

Similarly biosensors have been revolutionised by the introduction of nanotechnology. Generally they all have the same type of technology; a recognition element and the transducer technology. They can be defined by their transducer element, and the three most common were discussed. The issue between them of course lies between the interface between the recognition and transducer, the more integrated it is, the better the sensor. The dynamic range, specificity and sensitivity have been improved beyond compare by NMs. It is yet to be seen whether a particular type of architecture will translate on to the commercial market, what is certain is the possibilities on the horizon [90]. Of course the discovery of NMs and conducting polymers has made this easier and is bringing POC and LOC technologies within grasp, as the case study of MIPs has conveyed.

Surgical applications of AMDs have also been accelerated by the nano-revolution. For example, as shown in Fig. 4, the tips of AFM could be modified using EBIM to create ‘nano scalpels’ and ‘nano needles’. This technique has allowed surgery at the subcellular level. This could be combined with implantable devices and tissue grafts to make near flawless additions to the human body with minimal damage. However most of the literature only deals with in vivo and in vitro. This technology will only move forward if more robust studies can push this into the clinical arena.

The future looks quite interesting in the way of coupling nanosurgery with wireless robotic surgery such as the da vinci robot. In 2010, over 300,000 surgeries were completed using robotic techniques. It is motivating that miniaturisation is being heralded as one of key implications to drive this technology forward [91]. Using this with nanotools and combining them with gold standard clinical practice would drive this even further.

It has been demonstrated that nanotechnology has impacted the development and evolution of AMDs. The use of NMs can promote an ordinary MD to the advanced category. There are some barriers to overcome, but it is globally understood that this technology needs to be nurtured correctly for it to come to fruition. It has provided platforms for interdisciplinary collaborations and is commercially lucrative for all parties involved. Most importantly this nanotechnology and AMDs will change the face of global healthcare.

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