Review Article

NANOBIOTECHNOLOGY: APPLICATION OF NANOTECHNOLOGY IN DIAGNOSIS, DRUG DISCOVERY AND DRUG DEVELOPMENT

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ABSTRACT

Nanobiotechnology is a recently coined term describing the junction of the two existing but isolated worlds of engineering and molecular biology. It is a combination of three words: "nano" tiny, "bio" is living things, and "technology" is about tools. It refers to the ability to create and manipulate biological and biochemical materials, devices, and systems at atomic and molecular levels. Thus, it is an integration of physical sciences, molecular engineering, biology, chemistry, and biotechnology, and holds considerable promise of advances in pharmaceuticals and health care. Nanomaterials are at the leading edge of the rapidly developing field of nanotechnology. Their unique size-dependent properties make these materials superior, crucial in many areas of human activity, and above all a tiny tool to learn about living things. Consequently it applies the tools and processes of nanofabrication to build devices for studying biosystems. In this review we discussed the role of nanobiotechnology in molecular diagnosis, drug discovery, and development of nanomedicine and personalized medicine. The FDA approval is essential for clinical applications of nanotechnology and substantial regulatory problems could be encountered in the approval of nanotechnology-based products therefore we also discussed about the limitations of use of nanobiotechnology in drug discovery.

Key words: Nanobiotechnology, Nanoparticles, Nanomedicine, Nanotechnology, Drug discovery.

INTRODUCTION

The word "nano" comes from the Greek for "dwarf". A nanometer is a thousandth of a thousandth of a thousandth of a meter (10-9 m). One nanometer is about 60,000 times smaller than a human hair in diameter or the size of a virus, a typical sheet of paper is about 100,000 nm thick, a red blood cell is about 2,000 to 5,000 nm in size, and the diameter of DNA is in the range of 2.5 nm.

Therefore, nanotechnology deals with matter that ranges from one-half the diameter of DNA up to 1/20 the size of a red blood cell. [1] Further, it is interesting to note that nanomaterials are so small, even bacteria would need a microscope to see them. [2]

Nanoparticles are generally accepted as those with a particle size below 100 nanometers where unique phenomena enable novel applications and benefits. Nanomaterials on which most of the research has been carried out are normally powders composed of nanoparticles which exhibit properties that are different from powders of the same chemical composition, but with much larger particles.

Nanobiotechnology is the branch of nanotechnology that deals with biological and biochemical applications or uses. It often studies existing elements of living organisms and nature to fabricate new nanodevices.

It holds considerable promise of advances in pharmaceuticals and healthcare. It is widely seen as having a great potential to bring benefits to many areas of research and applications. It is attracting increasing investments from governments and private sector business in many parts of the world. Concurrently, the application nanobiotechnology is raising new challenges in the safety, regulatory, and ethical domains that will require extensive debates on all levels.

The combination of nanobiotechnologies plays an important role in drug development and drug discovery as shown in figure 1. This article will provide an integrated overview of application of nanobiotechnology- based molecular diagnostics, drug discovery, and drug delivery in the development of nanomedicine with the relationships.

Nanobiotechnology in molecular diagnostics

Nanomolecular diagnostics is the use of nanobiotechnology in molecular diagnostics and can be termed "nanodiagnostics". [4] Nanobiotechnology extends the limits of molecular diagnostics to the nanoscale. The use of nanotechnologies for diagnostic applications shows great promise to meet the rigorous demands of the clinical laboratory for sensitivity, multiplexing capabilities and cost-effectiveness. [5] Some examples of the use of nanoparticles for molecular diagnosis are given here.
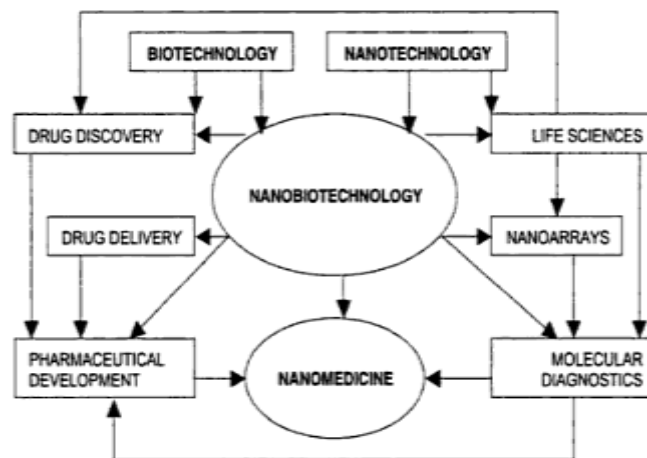

Figure -1: Combination of nanobiotechnologies for the development of nanomedicine. [3]

Nanoparticles

Nanoparticles have emerged as promising nanoplatforms for efficient diagnostics and therapeutics by merging the characteristic properties they possess at the nanometric scale with the feasible immobilization of specific ligands on the surface. Therefore, they have become ideal candidates for molecularly sensitive detection, highly efficient contrast agents for molecular imaging, as well as carriers for targeted drug and gene delivery, and therapeutical reagents for targeted photothermal therapy. [6]

Nanoparticle usually forms the core of nano-biomaterial. It can be used as a convenient surface for molecular assembly, and may be composed of inorganic or polymeric materials. It can also be in the form of nano-vesicle surrounded by a membrane or a layer. The shape is more often spherical but cylindrical, plate-like and other shapes are possible.

The size and size distribution might be important in some cases, for example if penetration through a pore structure of a cellular membrane is required. The size and size distribution are becoming extremely critical when quantum-sized effects are used to control material properties. A tight control of the average particle size and a narrow distribution of sizes allow creating very efficient fluorescent probes that emit narrow light in a very wide range of wavelengths. This helps with creating biomarkers with many and well distinguished colors. The core itself might have several layers and be multifunctional. For example, combining magnetic and luminescent layers one can both detect and manipulate the particles. [7]

Nanoparticles developed on a platform of biotechnology, nanotechnology and information technology can be used to take part in molecular, biochemical and biological processes, e.g. genetics and pharmacogenomics:

- The surface of nanoparticles can be decorated with various molecules in order to avoid being recognized by the immune system, enabling them to reach their target more efficiently.

- Nanoparticles may be designed to overcome physiological barriers like the blood brain barrier and dermal tight junctions.

- Due to the leaky constitution of neovasculature in malignant tumors, nanoparticles may penetrate the lesion.

- Nanoparticles may carry drugs and be designed to release their contents at a site of disease.

- Nanoparticles may consist of an inorganic core of superparamagnetic materials coated with polymer such as dextran. These particles are used as contrast agents in magnetic resonance imaging for diagnostic applications and therapy monitoring.

More specific tissue targeting ("functionalizing") can be achieved by the conjugation of the nanoparticle with a ligand, e.g. monoclonal antibodies. [8]

Nanoparticles for molecular diagnostics

Multifunctional nanoparticles, which incorporate diagnostic (quantum dots, magnetic, metallic, polymeric and silica nanoparticles) and/or therapeutic (magnetic and metallic nanoparticles) properties, are in the process of development. They have been used in vivo to protect the drug entity in the systemic circulation, restrict access of the drug to the chosen sites and to deliver the drug at a controlled and sustained rate to the site of action. [9-13]

Nanoparticles that are commonly used for diagnostics are

- Gold nanoparticles

- Magnetic nanoparticles

- Quantum dot (QD) technology

- DNA-protein and nanoparticle conjugates

Gold nanoparticles

Fascinating features such as ease of synthesis and surface functionalization with thiol-containing molecules, non-cytotoxicity, high biocompatibility, low nonspecific binding in control cells as well as broad-based optical properties, make gold-based nanoparticles still another attractive nanomaterial and one of the most studied in the bioanalytical field. Gold nanoparticles with tunable size (~0.8 - 60 nm in diameter) and narrow size distribution (± 10% in deviation) can be synthesized by physical methods, including photochemistry, sonochemistry and radiolysis, and by chemical methods, including reduction of HAuCl4, microemulsion and seeding growth.

Owing to the quantum size effect, gold nanoparticles possess strong surface plasmon resonance (SPR) bands in the visible wavelength range. Their SPR frequencies are also strongly influenced by the interparticle distance. When the gold nanoparticles in solution are in close proximity with others, their overall change in surface plasmon is translated into absorption spectra shifts resulting in a change in sample color. [14]

Applications of Gold Nanoparticles

Gold particles can be real jewels - at least at the nano size they are in great demand by scientists. Today gold nanoparticles are being used for an ever-growing number of applications. Gold nanoparticles have bioapplications in four areas: labeling, delivery, heating and sensing. For labeling, certain properties of the particles are exploited to generate contrast. For example in transmission electron microscopy, the strong electron absorbing properties of gold nanoparticles make them suitable as a stain for samples with poor contrast, such as tissue samples.

Their small size and the possibility of functionalizing the particles, for instance with antibodies (immunostaining), mean that they also provide extremely high spatial resolution and specificity in many labeling applications. Similarly, the particles' optical properties - strong absorption, scattering and especially plasmon resonance - make them of value for a large variety of light-based techniques including combined schemes such as photothermal or photo-acoustic imaging. Secondly, gold nanoparticles can serve as carriers for drug and gene delivery. Biologically active molecules adsorbed on the particle surfaces can be guided inside cells and released. DNA delivery, for instance, is the basis for gene therapy.

Thirdly, their strong light absorbing properties makes gold nanoparticles suitable as heat-mediating objects; the absorbed light energy is dissipated into the particles' surroundings, generating an elevated temperature in their vicinity. This effect can be used to open polymer microcapsules, for example, for drug delivery purposes. Appropriately functionalized nanoparticles might bind specifically to certain cells, which might one day find a use in cancer targeting and hyperthermal therapy by heating the particle-loaded tissue in order to destruct the malignant cells

Finally, gold nanoparticles can also be used as sensors. Their optical properties can change upon binding to certain molecules, allowing the detection and quantification of analytes. The absorption spectra of gold nanoparticles change drastically when several particles come close to each other. This property can be used for very sensitive DNA detection. [15]

Magnetic nanoparticles

Magnetic nanoparticles are a class of nanoparticles which can be manipulated using magnetic field. Such particles commonly consist of magnetic elements such as iron, nickel and cobalt and their chemical compounds. Magnetic nanoparticles are a powerful and versatile diagnostic tool in biology and medicine. It is possible to incorporate sufficient amounts of superparamagnetic iron oxide nanoparticles into cells, enabling their detection in vivo using magnetic resonance imaging (MRI). [16]

Magnetic nanoparticles offer some attractive possibilities in biomedicine. First, they have controllable sizes ranging from a few nanometres up to tens of nanometres, which places them at dimensions that are smaller than or comparable to those of a cell (10–100μm), a virus (20–450 nm), a protein (5–50 nm) or a gene (2 nm wide and 10–100 nm long).

Second, the nanoparticles are magnetic, which means that they obey Coulomb's law, and can be manipulated by an external magnetic field gradient. This 'action at a distance', combined with the intrinsic penetrability of magnetic fields into human tissue, opens up many applications involving the transport and/or immobilization of magnetic nanoparticles, or of magnetically tagged biological entities.

Third, the magnetic nanoparticles can be made to resonantly respond to a time-varying magnetic field, with advantageous results related to the transfer of energy from the exciting field to the nanoparticle. For example, the particle can be made to heat up, which leads to their use as hyperthermia agents, delivering toxic amounts of thermal energy to targeted bodies such as tumors. [17]

Applications of Magnetic Nanoparticles

Magnetic nano-particle applications are being investigated in multiple disciplines from biomedical sensors, drug delivery, magnetic resonance imaging, data storage, nano-electronics, etc. Magnetic nanoparticles, bound to a suitable antibody, are used to label specific molecules, structures or microorganisms. Magnetic immunoassay techniques have been developed in which the magnetic field generated by the magnetically labeled targets is detected directly with a sensitive magnetometer.

Quantum dots

Quantum Dots (QDs), which are the most promising nanostructures for diagnostic applications, are semiconductor nanocrystals characterized by high photostability, single-wavelength excitation, and size-tunable emission. Quantum dots are of much interest for the properties that they possess. These properties include electrical and nonlinear optical properties. These unique properties of nano sized particles are partly the result of the unusually high surface to volume ratios for their particles, as many as one-third of the atoms are on the surface of the particle. As a result electrons and "holes" (holes result when an electron moves away from a bond, leaving a positively charged particle) are confined in a limited space inside the cluster. Quantum dots can emit light if excited, the smaller the dot, the higher the energy of the emitted light. This ability to create dots that emit a rainbow of colors suggests that they could be used as biosensors. Unlike the dyes currently being used as biosensors, quantum dots do not degrade as rapidly.

Applications of QDs in molecular diagnostics

- Cancer
- Genotyping
- Whole blood assays
- Multiplexed diagnostics
- DNA mapping
- Immunoassays and antibody tagging
- Detection of pathogenic microorganisms
- Used as inorganic fluorophores

DNA protein and nanoparticles conjugates

Semi-synthetic conjugates of nucleic acids and proteins can be generated by either covalent coupling chemistry, or else by non-covalent biomolecular recognition systems, such as receptor-ligands of complementary nucleic acids. These nucleic acid-protein conjugates are versatile molecular tools which can be applied, for instance, in the self-assembly of high-affinity reagents for immunological detection assays, the fabrication of laterally microstructured biochips containing functional biological groups, and the biomimetic 'bottom-up' synthesis of nanostructured supramolecular devices. [18]

Other nanodiagnostic techniques

Various nanodiagnostics that have been reviewed will improve the sensitivity and extend the present limits of molecular diagnostics. Nanodiagnostics: applications of nanotechnology in molecular diagnostics, indicates, researchers within the field of nanodiagnostics are currently studying the ways nanotechnology will be able to extend the limits of current molecular diagnostic techniques. [4]

Nanochips

One of the most common techniques used today to analyze DNA sequences is hybridization, or the pairing of separated strands of DNA with complementary DNA strands of known sequence that act as probes. [19] Currently, DNA chips called DNA micro array assays are used to analyze DNA. Passive (non-electronic) technologies can be slow, tedious, and prone to errors because of nonspecific hybridization of the DNA. [20] A company called Nanogen has developed a product called the "Nanochip" that employs the power of an electronic current that separates DNA probes to specific sites on the array based on charge and size. Once these probes are on specific sites of the nanochip, the test sample (blood) can then be analyzed for target DNA sequences by hybridization with these probes. The DNA molecules that hybridize with target DNA sequences fluoresce, which is detected and relayed back to an onboard system through platinum wiring that is present within the chip. [21]

Microfluidics (Lab-on-a Chip)

The newest technologies within nanodiagnostics involve microfluidic or "lab on a chip" systems, in which the DNA sample is completely unknown. The idea behind this kind of chip is simple: the combination of numerous processes of DNA analysis are combined on a single chip composed of a single glass and silicon substrate. The device itself is composed of microfabricated fluidic channels, heaters, temperature sensors, electrophoretic chambers, and fluorescence detectors to analyze nanoliter-size DNA samples. This device is described as capable of measuring aqueous reagent and DNA-containing solutions, mixing the solutions together, amplifying or digesting the DNA to form discrete products, and then separating and detecting those products. Using a pipette, a sample of DNA-containing solution is placed on one fluid-entry port and a reagent-containing solution on the other port. Capillary action draws both solutions into the device, but hydrophobic patches positioned just beyond the vent line in each injection channel stop the samples. [22]

Microelectromechanical systems (MEMS)

MEMS are related to microfluidic systems. The difference between MEMS and microfluidic systems are that MEMS do not require reagents or a fluidity based substrate to react upon. Although MEMS are primarily used in drug-delivery systems, one primary application of MEMS in diagnostics are the swallowed capsule technology pills that allow doctors to visualize GI bleeding. The patient swallows a capsule containing a light-emitting diode for illumination, a CMOS (complementary metal-oxide semiconductor) video camera and optics for taking images, a battery, and a transmitter". [23] Nanotechnology-on-a-chip is one more dimension of microfluidic/lab-on-a-chip technology. Biological tests measuring the presence or activity of selected substances become quicker, more sensitive and more flexible when certain nanoscale particles are put to work as tags or labels. Nanopore technology for analysis of nucleic acids converts strings of nucleotides directly into electronic signatures. DNA nanomachines can function as biomolecular detectors for homogeneous assays. Nanobarcodes, submicrometer metallic barcodes with striping patterns prepared by sequential electrochemical deposition of metal, show differential reflectivity of adjacent stripes enabling identification of the striping patterns by conventional light microscopy. All this has applications in population diagnostics and in point-of-care hand–held devices. [24]

Nanobiotechnology in drug discovery

The application nanobiotechnology is already having an impact on diagnostics and drug delivery. Now, researchers are starting to use nanotechnology in the field of drug discovery. Future prospects for the application of nanotechnology in healthcare and for the development of personalized medicine appear to be excellent. [25]

Nanoparticles for Drug Discovery

Nanocrystals (QDs) and other nanoparticles (gold colloids, magnetic nanoparticles, nanobarcodes, nanobodies, dendrimers, fullerenes, and nanoshells) have received considerable attention recently with their unique properties for potential use in drug discovery. For example, QDs and magnetic nanoparticles can be used for barcoding of specific analytes. Gold and magnetic nanoparticles are key components of the bio-barcode assay, which has been proposed as a future alternative to the protein chain reaction. [11]

Use of Gold Nanoparticles for Drug Discovery

Gold nanoparticles are the most commonly used nanomaterial in diagnostics; they have many other uses as well. They are also used as a connecting point to build biosensors to detect disease DNA.

Instead of a fluorescent molecule, a gold nanoparticle can be attached to an antibody and other molecules, such as DNA, can be added to the nanoparticle to produce bar codes. Because many copies of the antibodies and DNA can be attached to a single nanoparticle, this approach is much more sensitive and accurate than the fluorescent-molecule tests used currently. Although they can be used for drug discovery, they need to be combined with another technology for visualization. Gold nanoparticles have been used to demonstrate multiphoton absorption-induced luminescence (MAIL), where specific tissues or cells are fluorescently labeled using special stains that enable them to be studied. [25]

Use of Quantum Dots for Drug Discovery

The use of QDs for discovery has been explored extensively. Both advantages and drawbacks have been investigated. [26]

Advantages of the use of QDs for drug discovery are as follows:

- Enhanced optical properties as compared with organic dyes. QDs offer great imaging results that could not be achieved by organic dyes.

- Multiple leads can be tested on cell culture simultaneously. Similarly, the absorption of several drug molecules can be studied simultaneously for a longer period of time.

- Using the surface functionalization properties of QDs, targeting capabilities can be added as well.

- Due to the inorganic nature of QDs, their interaction with their immediate environment at in vivo states can be minimal compared with their organic counterparts.

Limitation of use of nanotechnologies for drug discovery

With a large number of nanotechnologies and nanomaterials, no generalizations can be made about safety and toxicity. In vitro diagnostic use does not pose any safety risks to people but there is a concern over the in vivo use of nanoparticles, particularly those <50 nm in size, which can enter the cells and there are still many unanswered questions about their fate in the living body. Because of the huge diversity of materials used and the wide range in sizes of nanoparticles, these effects will vary a lot. It is conceivable that particular sizes of some materials might turn out to have toxic effects and further investigations will be needed. The FDA approval is essential for clinical applications of nanotechnology and substantial regulatory problems could be encountered in the approval of nanotechnology-based products. [25]

Role of nanobiotechnology based drug delivery in development of nanomedicine

Nanomedicine is now within the realm of reality starting with nanodiagnostics and drug delivery facilitated by nanobiotechnology. Miniature devices such as nanorobots could carry out integrated diagnosis and therapy by refined and minimally invasive procedures, nanosurgery, as an alternative to crude surgery. Nanotechnology will markedly improve the implants and tissue engineering approaches as well. It offers the key to faster and remote diagnostic techniques including new high throughput diagnostics, multi-parameter, tunable diagnostic techniques, and biochips for a variety of assays. It also enables the development of tissue-engineered medical products and artificial organs, such as heart valves, veins and arteries, liver and skin. These can be grown from the individual's own tissues as stem cells on a 3-D scaffold, or by the expansion of other cell types on a suitable substrate. [27]

Nanoparticles hold tremendous potential as an effective drug delivery system. To overcome the problems of gene and drug delivery, nanotechnology has gained interest in recent years. [28, 25] Nanoparticles can be used in targeted drug delivery at the site of disease to improve the uptake of poorly soluble drugs [29] the targeting of drugs to a specific site, and drug bioavailability. Several anti-cancer drugs including paclitaxel, [30-31] doxorubicin, [32] 5-fluorouracil, [33-34] have been successfully formulated using nanomaterials. Quantum dots, chitosan, polylactic/glycolic acid (PLGA) and PLGA-based nanoparticles have also been used for in vitro RNAi delivery. [35] The use of nanomaterials including peptide-based nanotubes to target the vascular endothelial growth factor (VEGF) receptor and cell adhesion molecules like integrins, cadherins and selectins, is a new approach to control disease progression. [36]

Application of nanobiotechnology in various therapeutic areas

Nanobiotechnology has been applied in almost every area of human healthcare. Some examples are given of applications in stem-cell-based therapies and important therapeutic areas: cancer, neurological disorders, cardiovascular diseases, and infections.

Oncology

Nano-engineered particles have been developed to reach specific molecular targets on diseased cells and have been used in various experimental and clinical conditions. For cancer diagnostics nanoparticles have been engineered to optimize magnetic resonance imaging, ultrasound imaging and nuclear medicine imaging. The potential benefits of nanoparticle cancer treatment are highly selective and rapid tumor destruction with minimal damage to surrounding healthy tissue. Additionally, this therapeutic device may be used in combination with and could increase the effectiveness of standard chemotherapy and radiation. [8] Nanoparticles can deliver chemotherapy drugs directly to tumor cells and then give off a signal after the cells are destroyed. Drugs delivered this way are several times more potent than standard therapies. Combination of gold nanoparticles followed by X-ray treatment reduces the size of the tumors, or completely eradicates them in mice. [37] Prior research has indicated the ability to deliver the appropriate levels of infrared light at depths of up to 15 cm, depending upon the tissue. Photothermal tumor ablation in mice has been achieved by using near-infrared-absorbing nanoparticles with success rates over 90%. [38]

Neurological Disorders

The brain represents one of the most complex systems in biomedicine. With an improved understanding of brain function, nanotechnology offers better diagnosis and treatment for neurodegenerative diseases like multiple sclerosis, Alzheimer's disease and Parkinson's disease. Applied nanobiotechnolgy aimed at the regeneration and neuroprotection of the central nervous system (CNS) will significantly benefit from basic nanotechnology research conducted in parallel with advances in cell biology, neurophysiology, and nanopathology. [39] Nanomaterials are delivered as a specific structure, or combination of structures, designed to deliver the therapeutic effect, directly to the site, requiring a much lower dose. These materials use very specific and deliberate molecular structures that can interact with neurons or protein structures inside the cells. A well-planned treatment spaced over time will produce functional return in the CNS. The four parts of CNS regeneration is a new framework for approaching CNS injury and evidence shows that nanotechnology is currently being used for stroke rehabilitation and, in several clinical trials, the treatment of scar formation blockade in the spinal cord. The four components are preserve, permit, promote, and plasticity. [40]

Cardiovascular Diseases

The diagnosis and treatment of unstable plaque is an area in which nanotechnology could have an immediate impact. Nanoprobes can be targeted to plaque components for noninvasive detection of patients at risk. Targeted nanoparticles, multifunctional macromolecules, or nanotechnology- based devices could deliver

therapy to a specific site, localized drug release being achieved either passively (by proximity alone) or actively (through supply of energy as ultrasound, near-infrared, or magnetic field). Restenosis after percutaneous coronary intervention continues to be a serious problem in clinical cardiology. [41]

Infections

An important role of nanotechnology in the management of infections is use of formulations which improve the action of known bacterial agents. The bactericidal properties of some agents are

manifest only in nanoparticulate form. Certain formulations of nanoscale powders possess antimicrobial properties. These formulations are made of simple, nontoxic metal oxides such as magnesium oxide (MgO) and calcium oxide (CaO) in nanocrystalline form, carrying active forms of halogens, for example, $MgO.Cl_2$ and $MgO.Br_2$. [42] A simple molecule from a hydrocarbon and an ammonium compound has been used to produce a unique nanotube structure with antimicrobial capability. [43] Antimicrobial nanoemulsions, containing water, alcohol, soybean oil and detergents emulsified into uniformly sized droplets in the 200 to 400nm range, can destroy microbes effectively without toxicity or harmful residual. [44]

Nanobiotechnology for Developing Stem-Cell-Based Therapies

Stem-cell-based therapies are one of the most promising areas of development in human therapeutics. Nanobiotechnology can be applied to delivery of gene therapy using genetically modified stem cells ad further applied in tracking stem cells introduced into human body. Nanofibrous scaffolds are being developed for stem cells to mimic the nanometer-scale fibres normally found in that matrix. [45] They are being used to grow stem cells derived from adipose tissue. Nanofibrous scaffolds which could potentially mimic the architecture of extracellular matrix (ECM) have been considered a good candidate matrix for cell delivery in tissue engineering applications. [46]

CONCLUSIONS

It has been proved that nanobiotechnology is a promising area of scientific and technological advancement. In nanobiotechnology, big things are expected from really small things. The introduction of biocompatible materials and devices that are engineered on the nanometer scale that interact with biological molecules and cells and provide specified diagnostic, therapeutic, and imaging functions will utterly change the way in which health care is provided in the future. For nanobiotechnology to prosper there needs to be a true unification of sciences, which will require a multidisciplinary approach.

AUTHORS' STATEMENT

The authors declare no divergence of awareness.

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