
Research Paper

Nanoprobes and Their Applications in Veterinary Medicine and Animal Health

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ABSTRACT

Nanotechnology has the potential to revolutionize veterinary medicine, animal health and other areas of animal production. Second generation nanodevices (e.g., quantum dots, nanotubes, nanoshells, functionalized dendrimers) can potentially target, image, deliver drugs and image cell response to therapy in real time. Nanoemulsions can find application in the delivery of controlled amounts of drugs into the beverage of breeding animals, prevention of bovine tuberculosis, the controlled release of injectable poorly water-soluble drugs and as destroyer of pathogens. Other potential applications of nanotechnology in veterinary medicine and animal health include tissue engineering, treatment of Feline Hyperthyroidism, new tools for molecular and cellular breeding, identity preservation and tracking, animal breeding, multi-colour optical coding for biological assays, the security of animal food products, major impact on animal nutrition scenarios ranging from the diet to nutrient uptake and utilization, modification of animal waste as expelled from the animal, pathogen detection, sensory, surgical aids and many more. Experts are of the opinion that unique developments in nanotechnology will be revolutionising animal health and veterinary medicine and nanotechnology may become the proving ground for untried and more controversial techniques-from nanocapsule vaccines to sex selection in breeding. However, economic and regulatory constraints must still be overcome for nano-based new drugs or therapeutic approaches to become common practice.

Key words: Nanotechnology, animal health, veterinary nanomedicine, nanodevices, pathogen detection, tissue engineering, diagnostic tools, nanoprobes

INTRODUCTION

Veterinary medicine deals with the application of medical, surgical, public health, dental, diagnostic and therapeutic principles to companion, domestic, exotic, wildlife and production animals. In recent years, veterinary medicine has expanded exponentially because of the availability of advanced diagnostic and therapeutic techniques for most species. The application of nanotechnology in veterinary medicine is gaining importance in many areas which include imaging, drug delivery systems and tissue engineering (Narducci, 2007). Applications of nanotechnology in veterinary medicine, animal production and other areas were reported with special emphasis on sensors, dispensers, immunogens and chemotherapeutic available tools, as well as mechanisms of nanometrical scale devices designed for diagnosis, treatment, monitoring and traceability of agropecuary supplies (Coppo, 2009; Scott, 2007; Kumar, 2010; Chakravarthi and Balaji, 2010).

Veterinary medicine will enter a phase of incredible transformations due to changes in our ability to measure, manipulate and organize matter at the nanoscale level. Some reviewers have suggested that nanotechnology has the potential to significantly affect the way veterinarians practice veterinary medicine (Feneque, 2003a; Scott, 2005).

Nanotechnology-a term encompassing the science, engineering and applications of submicron materials-involves the harnessing of unique physical, chemical and biological

properties of nanoscale substances in fundamentally new and useful ways (Sargent and John, 2010). Nanotechnology is considered as the development at atomic, molecular and macromolecular levels and it involves the manipulating of systems at a scale of approximately 1-100 nanometres in at least one dimension (one nanometre (nm) = one billionth of a metre (10^{-9} m)). (Dowling *et al.*, 2004a; Williams *et al.*, 2005). In the context of biological nanostructures, this is the size regime of the molecular machinery that constitutes, for examples, functional viruses 75-100 nm, protein 5-50 nm, thickness of membrane 10 nm, DNA molecule is a double-nanowire, with the strands twisted around each other with a repeat unit every 3.4 nm and a diameter of 2 nm, nucleotides 0.81-0.95 nm, the amino acids 0.42-0.67 nm in size, a water molecule 0.3 nm wide, sodium atom is about 0.2 nm and the length between two carbon atoms bound together is about 0.15 nm. It will be appropriate to define a biological nanostructure as being in the nominal range from 0.5 to 200 nm. Other examples include biological nanostructures that make up the macroscopic tendon: protein 'collagen' (1 nm) which coils into a triple helix (2 nm), a microfibril (3-5 nm), a subfebril (10-20 nm) and a fibril itself (50-500 nm). The most common type of lipid nanoparticle, a liposome, can be thought of as a hollow sphere whose size ranges from 30 nanometers to 10,000 nanometers. For comparison, a red blood cell is about 8,000 nanometers and leukocytes 10,000 nm.

Nanoscale devices may, therefore, be able to penetrate biological barriers such as the blood- brain barrier or the stomach epithelium, barriers that normally make it difficult for therapeutic and imaging agents to reach certain tumours. Nanotechnology is undoubtedly one of the most important technologies of the 21st century and it has the potential to create many new materials and probes/devices with a vast range of applications in medicine (National Science and Technology Council, Committee on Technology, 1999). Nanomedicine refers to programs for application of nanotechnologies for medical treatment, based on molecular processes at the cellular level and nanoparticle fabrication for drug delivery and image enhancement (Office of Portfolio Analysis and Strategic Initiatives, 2006). Medical nanotechnology includes a wide range of nanoscale technologies directly and indirectly applied to medicine. These include areas of biotechnology, bionanotechnology and application of nanotechnology to areas like prosthetics and tissue engineering. Veterinary nanotechnology and medical nanotechnology is sharing most of the things common.

Nanomaterials are among the new type of drug carriers with very promising application. In recent years, great progress was achieved in making drugs own the characteristics of targeted and controlled release via nanotechnologies. Commercially significant types of nanomaterials include carbon nanotubes, nanoclays, fullerenes, quantum dots, metal and metal oxide nanoparticles, silica nanoparticles, dendrimers, nanoemulsions and nanoporous materials (Sekhon and Kamboj, 2010a, b).

Nanoprobes/diagnostic tools: Nanotechnology diagnostic tools may offer a vast number of breakthroughs that will advance the practice of clinical veterinary medicine and animal health. These include: bucky balls and carbon nanotubes, nanoemulsions, quantum dots, dendrimers, metal and metal oxide nanoparticles, nanoclays, nanoemulsions and nanoporous materials (Feneque, 2003b; Singh *et al.*, 2011).

Fullerene and carbon nanotubes: Buckminsterfullerene C60, otherwise known as the 'hucky ball' consists of 60 atoms and is the most spherical molecule in existence. The size of the 60 carbon molecule is just less than a nanometre. They are generally non-toxic and are therefore extremely useful within the body. Shungite (shungit) is a very unique mineral in the world and it contains naturally occurring fullerenes. Besides carbon, this mineral contains a wide range of microelements and biologically active substances which support the biological processes in the body of humans and animals. It is not harmful to living organisms. It has the ability to absorb and expel any harmful agents while restoring anything which is beneficial to life. Skin diseases are treated with Shungite water, the skin is rejuvenated, wrinkles are smoothed down and skin elasticity is restored. It has also

regenerative properties, relieves headaches, backaches, rheumatism, normalizes sleep, stabilizes blood pressure and clears respiratory tracts. Shungite water has antihistaminic effects. Decreased histamine in the blood stream after drinking the Shungite water reduced allergies. The health benefits described above are from accounts over the last 300 years from people in the region and around the world who have used and seen the benefits of Shungite water. Shungite may well prove to be a new tool to provide humanity and nature with good health in the 21st century.

Scientists are also testing fullerenes for drug delivery. Water-soluble groups of peptides or hydrophilic chains are attached to get fullerenes into the blood stream. The medicine loaded fullerene can then be attached to an antibody. Viruses, bacteria and diseases in the body each have unique antigens. Antibodies can recognize and attach to disease antigens in the body. Then the attached loaded fullerene delivers the appropriate medicine in the body.

Application of hydrated fullerenes for efficiency enhancement of existing pharmaceuticals, prevention of animal diseases, including viral ones, to enhance reproductive qualities, viability and quality of life of elite animals and for animals in sports were reported research-in-ukraine-nanomedicine-nanomaterials-on-demand-sports.html). The results on the effects of nanosized hydroxylated fullerenes on fish neutrophil function and immune gene transcription using fathead minnow (*Pimephales promelas*) indicated their potential to interfere with the evolutionary conserved innate immune system responses and encourages the use of fish models in studies of nanoparticle immunotoxicity (Jovanovic *et al.*, 2011).

Nanotubes are a sequence of nanoscale C₆₀ atoms arranged in a long thin cylindrical structure and are also called buckytube. Carbon nanotubes are a natural choice for interfacing with the biological world because of their molecular scale, biocompatibility and stability in aqueous environments. Examples of applications that exploited these features include scanned-probe imaging of biomolecules and biosensing (Balasubramanian and Burghard, 2006). Functionalised carbon nanotubes hold strong promise as novel systems for the delivery of drugs, antigens and genes (Bianco *et al.*, 2005). Externally, derivatives of fullerenes (C₆₀), endohedral metallofullerenes (M@C₆₀) and ultra-short (20 nm long) single-walled carbon nanotube capsules (US-tubes) make them biocompatible and cell-specific through peptide and antibody targeting. On the other hand, internally, the testing of the nanostructures loaded with materials of medical interest for diagnostic and therapeutic medicine is in progress. Further, cancer therapies are also being developed that take advantage of superparamagnetic nanostructures, such as Gd³⁺@US-tubes, that are simultaneously diagnostic (MRI-guided) and therapeutic (magnetic hyperthermia) agents in a single package (Mirakyan and

Wilson, 2002; Mirakyan *et al.*, 2002; Bolskar *et al.*, 2002). The nanotubes are used as a means of tracking oestrus in animals as these have the capacity to bind and detect the estradiol antibody at the time of oestrus by near infrared fluorescence (O'Connell *et al.*, 2002).

Quantum dot particles: Quantum dots are semi-conductor nanocrystals commonly made from binary compounds such as cadmium selenide, cadmium sulphide, indium arsenide and indium phosphide. They absorb white light and then reemits it a couple of nanoseconds later in a specific color. Due to their unique optical and light responsive properties, quantum dots show great promise in veterinary applications; especially in the diagnosis and treatment of cancer, in cell imaging and tracking. Quantum dots are also used to visualize cell pathways which help our understanding about certain drugs behaviour in an animal's body. Moreover, quantum dots promise faster, more flexible and less costly tests for clinical analysis. Quantum dots could become a ground breaking technique in veterinary science, if further research prove these to overcome their toxic nature (MegCoull, 2011). Quantum dots can penetrate skin through minor abrasions penetrate-skin-through-minor-abrasions_102785.shtml). Nanoparticles (CdSe@ZnS quantum dots) and hybrid biosensor

(optical and electrochemical) for cell diagnostic and monitoring has been reported for the study of a veterinary parasitic disease, *Babesia* spp which is transmitted by ticks and infect erythrocytes.

Nanoshells: Nanoshells are concentric spherical nanoparticles (about 100 nm diameter) consisting of a dielectric (typically gold sulfide or silica) core and a metal (gold) shell (Averitt *et al.*, 1997). Nanoshells are being tested as a noninvasive way to detect and destroy tumors (Hirsch *et al.*, 2003).

Nanorods: The application of near-infrared-absorbing gold nanorods (GNRs) for *in vivo* laser closure of a rabbit carotid artery represented a step forward toward the introduction of nanotechnology-based therapies in minimally invasive clinical practices (Paolo *et al.*, 2010).

Dendrimers: Dendrimers are synthetic, three-dimensional macromolecules (typically in the range of 2 to 10 nm) formed using a nanoscale fabrication process which consists of an inner core molecule surrounded by a series of branches. A dendrimer is built up from a monomer, with new branches added in steps until a tree-like structure is created (6). Dendrimers are technically polymers and have a lot to offer to the field of veterinary medicine (Senel, 2011). Dendrimers can be designed to aggregate to form cylinders or spheres depending upon the nature of the fundamental building unit. Polyamidoamine dendrimers have shown promise for biomedical applications because they (a) can be easily conjugated with targeting molecules, imaging agents and drugs, (b) have high water solubility and well-defined chemical structures, (c) are biocompatible and (d) are rapidly cleared from the blood through the kidneys, made possible by their small size (<5 nm) which eliminates the need for biodegradability (Peer *et al.*, 2007). *In vivo* delivery of dendrimer- methotrexate conjugates using multivalent targeting resulted in a tenfold reduction in tumour size compared with that achieved with the same molar concentration of free systemic methotrexate (Hong *et al.*, 2007; Kukowska-Latallo *et al.*, 2005). Although promising, dendrimers are more expensive than other nanoparticles and require many repetitive steps for synthesis, posing a challenge for large-scale production.

Nanoparticles: Nanoparticles include ultrafine particles of metals, metal oxides, non-metals and ceramics.

Metal and metal oxide nanoparticles: Metallic silver nanoparticles (up to 100 nm) allow for a higher antimicrobial effect than silver salts, are more resistant to deactivation by gastric acids and have a low absorption rate through the intestinal mucosa, thus minimising its potential risk of toxicity. Further, it has been shown that the doses that promote animal physiological and productive effects were found to be very low (20 to 40 ppm), especially compared to the 10 to 100-fold higher concentration used with other metallic compounds such as copper and zinc, thus precluding a harmful environmental effect (Fondevila *et al.*, 2009). In a study conducted to examine the potential effects of silver/palladium alloy nanoparticles' hydrosols on growth and development of chicken embryo, results indicated that nanoparticles of Ag/Pd alloy, did not influence chicken embryo development. Injection *in ovo* with physiological saline increased activity of asparagine transferase in blood serum, causing hypertrophy of hepatocytes. However, injection of nanoparticles Ag/Pd showed tendency to restore this negative effect (Studnicka *et al.*, 2009). CeO₂ acts as a putative novel and effective hepatoprotective agent against MCT-induced hepatotoxicity (Amin *et al.*, 2011). Silver nanoparticles synthesized from bark extract of *Boswellia ovalifoliolata* showed moderately toxic to the *E. coli* and *Pseudomonas* species and highly toxic to *Proteus* species. Phytosynthesized silver nanoparticles of *Shorea tumbuggaia* bark extract were moderately toxic to *E. coli* and *Bacillus* species and highly toxic to *Klebsiella* species (Savithramma *et al.*, 2011). Researchers have determined the *in vitro* effect of gold and silver nanoparticles for therapeutics and diagnostic on the microsomes containing wild-type cDNA expressed

human CYP450 enzymes CYP1A2, 2C9, 2C19 and 3A4. Results demonstrated that all of the CYP450s activities were down-regulated by gold and silver nanoparticles, thereby, suggesting the inhibition of oxidation based biological process by penetration of metallic nanosized particles across the microsomal membrane (Sereemasun *et al.*, 2008).

Nano-magnets: Doctors can use 'nano magnets' (MNPs) to carry the drug to the tumour area where they wipe out the cancer cells without harming the surrounding tissue. A revolutionary technique that uses injections of iron nanoparticles to seek out and destroy cancer cells has been developed (Derbyshire, 2009). In this technique, tiny particles are designed to roam through the body's blood vessels in search of tumour cells. Then, upon latching on to their targets, the magnets can be heated from outside the body using a magnetic field, thereby, wiping out the cancer cells without harming the surrounding tissue.

Iron oxide-based nanomagnets with multifunctionalities were reported in cancer diagnostics and treatment, focusing on their combined roles in a magnetic resonance contrast agent, hyperthermia and magnetic force assisted drug delivery. Further, iron oxides as magnetic carriers in gene therapy with a focus on the sophisticated design and construction of magnetic vectors were reported. Furthermore, the iron oxide-based nanomagnet represents a very promising tool in particle/cell interfacing in controlling cellular functionalities, such as adhesion, proliferation, differentiation and cell patterning, in stem cell therapy and tissue engineering applications (Lin *et al.*, 2010).

Researchers have demonstrated the feasibility of site-specific drug delivery to implanted magnetizable stents by uniform field-controlled targeting of MNPs with efficacy for in-stent restenosis. In this context, the enhanced retention of MNPs at target sites due to the uniform field-induced magnetization effect resulted in a significant inhibition of in-stent restenosis with a relatively low dose of MNP-encapsulated PTX (7.5 microg PTX/stent) (Chorny *et al.*, 2010).

Magnetic Nanoparticles (MNPs) can fully explore MRI's potential by drastically increasing tissue contrast and serve the dual purpose of contrast agent and drug delivery system (Vasir and Labhasetwar, 2007). Magnetic nanoparticle design, *in vitro* and animal experiments with MNP-based drug and gene delivery and clinical trials of drug targeting was reported (McBain *et al.*, 2008). Clusters of heated, magnetic nanoparticles targeted to cell membranes can remotely control ion channels, neurons and even animal behaviour (Huang *et al.*, 2010).

Results on biocompatible silica-overcoated magnetic nanoparticles containing an organic fluorescence dye, rhodamine B isothiocyanate (RITC), within a silica shell [50 nm size, MNP@SiO₂ (RITC)] demonstrated that MNPs can be internalized into the cells through an energy-dependent endosomal-lysosomal mechanism (Kim *et al.*, 2006).

Intravascularly administered superparamagnetic nanoparticles made of Fe₃O₄ (core), thermoresponsive polymeric hydrogel (shell) and neurotoxic agent (N-isopropylacrylamide monomer [NIPA-M]) can be magnetically targeted to the inferior right GP (IRGP) and reduced GP activity presumably by the subsequent release of NIPA-M. Such novel targeted drug delivery system can be used intravascularly for targeted autonomic denervation (Yu *et al.*, 2010).

Magnetic nanoparticles that possess accurate sizes, shapes, compositions, magnetizations, relaxivities and surface charges can be used to adjust the toxicity and stability of the nanoparticles and further, to load functionalities, via various mechanisms, onto the nanoparticle surfaces (Xie *et al.*, 2011). Tiny magnets made by bacteria could be used to kill tumours and are better than man-made versions because of their uniform size and shape (<http://www.ayeladdy.com/index.php?page=10>). Using a new *in vitro* assay for assessing monocyte extravasation, researchers showed that the ability of transfected human monocytes to migrate across a human endothelial cell layer into a 3D tumour spheroid is markedly increased when cells are pre-loaded with MNPs and a magnetic force is applied close to the spheroid (Muthana *et al.*, 2008).

Non-metal nanoparticles: In the area of intracellular drug delivery, the intracellular controlled drug release using stimuli-responsive mesoporous-silica-based systems were reported (Vivero-Escoto *et al.*, 2010).

Biological nanoparticles: Protein nanoparticles are biodegradable, non-antigenic, metabolizable and can also be easily amenable for surface modification and covalent attachment of drugs and ligands. They hold promise as drug delivery systems for parenteral, peroral and ocular administration as well as adjuvant for vaccines (Jahanshahi and Babaei, 2008). Nowadays, active research is focused on the preparation of nanoparticles using proteins like albumin, gelatin, gliadin and legumin. Bovine serum albumin was used for generation of nanoparticles in a drug delivery system (Rahimnejad *et al.*, 2006). Scientists have made nanoparticles from DNA that could be used to identify the genes being expressed in a single cell (Ke *et al.*, 2008). α -Lactalbumin, the major whey protein found in the milk of all mammals, can alter its biological function depending on the conformational state and actively interact with lipid membranes which leads to its antimicrobial and antitumor activity that has a vital role to induce apoptosis in tumor cells. Researchers reported fabrication of α -Lactalbumin nanoparticles to use in drug and food delivery system in nanomedicine (Velusamy and Palaniappan, 2011). Chitosan which is a biodegradable, non-toxic biopolymer, is currently being used for therapy in veterinary medicine due to its various biological properties (Senel and McClure, 2004). The potential of dapsone loaded characterized chitosan nanoparticles as a targeted delivery was investigated in memory deficits associated with dementia of Alzheimer disease type (Saluja *et al.*, 2011).

Nanointermediates: Nanointermediates are materials into which raw nanoparticles are incorporated or materials synthesized with nanoscale features to be used in, or applied to, a final nanoenabled product. Examples include fabrics that have been impregnated with silver nanoparticles for their antimicrobial activity (Perelshtein *et al.*, 2008). Silver nanoparticles with diverse medical applications ranging from silver based dressings, silver coated medicinal devices, such as nanogels, nanolotions have been reported by Rai *et al.* (2009). Nanocomposites are multiphase solids where one of the phases has a dimension of less than 100 nm (Ajayan *et al.*, 2003), many of which can be classified as nanointermediates.

Nanoporous materials: Nanoporous materials are materials with uniform and ordered pore structures between 1 and 100 nm. They can be made from a wide range of different compounds such as polymers, ceramics, metal oxides and metals and typically have very large surface area to volume ratios and porosities (volume ratio of pore space to total volume of material) greater than 0.4.

Nanoclays: Nanoclay as drug vehicle for controlled release of drug is one of area in medicinal application, Nanoclays have great potential as compared to polymer and carbon nanotubes for drug delivery applications (Suresh *et al.*, 2010). Nanoclays or organically modified layered silicates (organoclays) were reported with emphasis placed on the use of nanoclays as the reinforcement phase in polymer matrices for preparation of polymer/layered silicates nanocomposites and drug delivery vehicle for controlled release of therapeutic agent (Patel *et al.*, 2006; nanoall.blogspot.com/2011/04/nanoclay-development.html).

Organically modified nano-clay caused rupture of cell membrane and inactivation of the bacteria. Such antimicrobial activity of the organo-clay would open a new opportunity to develop polymer nanocomposites with antimicrobial function (Hong and Rhim, 2008). The chitosan/polygalacturonic acid ChiPgA composite scaffolds containing montmorillonite MMT clay modified with 5-aminovaleric acid were found biocompatible and also appear to satisfy some of the basic requirements of scaffolds for tissue engineering applications (Ambre *et al.*, 2010).

Nanoemulsions: Nanoemulsion involves using nanodrops of oil to physically destroy pathogens. When the nanodrops come into contact with the cell membranes of bacteria, surface tension explodes the cell membrane, thereby, killing the pathogen. The nanodroplets do not affect the cell structures of the higher animal's body, thereby, making them ideal for use in veterinary medicine. Bovine tuberculosis poses a significant risk to human and herd health. The use of a nanoemulsion vaccine to provoke a mucosal immunity against bovine tuberculosis would help cattle farmers to overcome this increasingly problematic disease. Further investigation would be required to ensure that the nanodroplets could not enter the blood stream and cause damage (Whitfield, 2007). Low energy nanoemulsions application in the delivery of controlled amounts of drugs into the beverage of breeding animals (such as poultry, cattle, pigs) and for the controlled release of injectable poorly water-soluble drugs was reported (Vandamme and Anton, 2010). Nanoemulsions are effective against a variety of food pathogens, including Gram-negative bacteria. They can be used for surface decontamination of food processing plants and for reduction of surface contamination of chicken skin (Sekhon, 2010).

Nanoanalysis: Nanoanalysis refers to techniques for determining the atomic structures of materials, especially crystals on a nanometer scale. Nanoanalysis is conducted using any number of technologies that can resolve images at the nanoscale-scanning tunneling microscopes, atomic force microscopes, scanning probe microscopes, transmission electron microscopes, field emission microscopes. Also useful in nanoanalysis are X-ray and UV diffraction, IR microscopy, mass spectrometry, ion-beam machines and precision optical microprobes.

In the nanoanalysis, SEM was useful in observing seed surface and inside structural anatomy of the anise seed and fennel seed (Brooke, 2008). Oxford Instruments AZtec nanoanalysis system was launched in APRIL 2011. Nanoanalysis support a diverse range of science areas and industries including biodiagnostics, pharmaceuticals and support for work on the human and environmental impact of nanoparticles.

Microfluidics: Microfluidics is being used in livestock breeding to physically sort sperm and eggs. Leader in this field is XY, Inc. of Colorado (USA) which is using a microfluidic technique called flow cytometry to segregate male and female sperm for sex selection. XY has successfully bred sex- selected horses, cattle, sheep and pigs and now provides its technology to commercial breeders. Nanotech startup Arryx which has developed a new microfluidic system called MatRyx, uses a nanotechnique in which tiny laser tractor beams trap individual sperm and then sort them by weight. MatRyx can sort around 3,000 sperm per second and aims for commercialization in cattle breeding. Thus, utilizing this technology, dairy farmer can have cows and beef farmers can have bulls that have more meat (ETC Group Report, 2004).

Animals also are likely to be used as the testing ground for less savory or more risky applications that could later be extended to human beings. Using microfluidics for breeding is likely to accelerate genetic uniformity within livestock species and also opens the possibility of applying new nano- eugenic technologies to humans in the future. The ability to remotely regulate animals may have adverse affects as livestock go longer periods without direct human care (<http://www.medlink-uk.org/Site/documents/NanotechnologyVetMedlinkPart2.pdf>).

Nanopharmaceuticals: The areas of veterinary medicine that would benefit most from the nanotechnology research include the field of pharmacology (Feneque, 2000) and the use of nanopharmaceuticals (Feneque, 2003a). The creation and manipulation of new synthetic molecules can provide us with new therapeutical compounds to treat diseases in our pet population. Such new compounds-for example-would protect our patients from viral or bacterial infections and accelerate wound healing. Also these new compounds could carry drugs and genes into cells, making treatment of diseases more efficacious. The current

techniques and future applications in nanopharmaceuticals for veterinary medicine, *in vivo* targeted nanodispensers and nanoactuators were reported (Narducci, 2007).

Feline Hyperthyroidism is recognised as the most common endocrine disorder of the cat. Nanotechnology can be used to manipulate the level of T3 and T4 in the blood and secrete the required levels of thiamazole drug at a level that would be most beneficial to the body, as opposed to the 5 mg (in the form of two tablets every day) that current treatment offers (Longson, 2007). Examples of potential applications in animal agriculture and veterinary medicine include new tools for molecular and cellular breeding, the security of animal food products, modification of animal waste, pathogen detection and many more. Researchers have provided a glimpse at potential important targets for nanotechnology in the field of veterinary medicine (Chakravarthi and Balaji, 2010).

Animal breeding: Management of animal breeding is an expensive and time consuming problem for dairy and swine farmers. One solution that is currently being studied is a nanotube implanted under the skin to provide real time measurement of changes in the level of estradiol in the blood. The nanotubes are used as a means of tracking oestrus in animals because these tubes have the capacity to bind and detect the estradiol antibody at the time of oestrus by near infrared fluorescence. The signal from this sensor will be incorporated as a part of a central monitoring and control system to actuate breeding (O'Connell *et al.*, 2002). With the mapping of the human genome behind them, geneticists are now rapidly sequencing the genomes of cattle, sheep, poultry, pig and other livestock hoping to identify gene sequences that relate to commercially valuable traits such as disease resistance and leanness of meat. By including such probes for these traits on biochips, breeders will be able to speedily identify champion breeders and screen out genetic diseases (Patil *et al.*, 2009). Further, nanotechnology tools like microfluidics, nanomaterials, bioanalytical nanosensors, etc. has the potential to solve many more puzzles related to animal health, production, reproduction and prevention and treatment of diseases (Patil *et al.*, 2009).

Researchers investigated the feasibility of using inert nanobeads that target antigen to Equine osteoarthritis is a degenerative joint disease and one of the most common causes of lameness in horses, particularly in the more athletic e.g., race and event horses. In this direction, nanotechnology could provide great help to thousands of horse owners whose horses are currently forced into early retirement due to tendon injuries and could stop significant economic losses in the racing and eventing industries (Benn and Clarke, 2007; Harris, 2007).

Dendritic cells to induce immune responses against foot and mouth disease virus-specific synthetic peptides in sheep. Their results demonstrated that while single peptides induce responses in most sheep, the combination of multiple peptides either conjugated separately to individual nanobeads or conjugated as a mixture induce significant cell-mediated and humoral immune responses (Greenwood *et al.*, 2008).

Regarding nanomedicine novel approaches in human and veterinary therapeutics, scientists reported various aspects of nanoparticle formulation, characterization, behaviour in the body and some of their potential applications with more particular emphasis on some approaches for the treatment of cancer, treatment of infectious diseases and the potential of these nanoparticles as adjuvants for vaccination purposes (Irache *et al.*, 2011). Nanotechnology tools hold the promise of miniaturizing diagnostic devices which could dramatically reduce costs and increase throughput and sensitivity of a wide range of diagnostic tests for veterinary applications (Bollo, 2007).

Chips can be used for early disease detection in animals. The USD A envisions the rise of 'smart herds'-cows, sheep and pigs fitted with sensors and locators relaying data about their health and geographical location to a central computer. The USDA is completing trials on a system for mass vaccination of fish using ultrasound. Nanocapsules containing short strands of DNA are added to a fishpond where they are absorbed into the cells of the fish. Ultrasound is then used to rupture the capsules, releasing the DNA and eliciting an immune response from the fish. This technology has so far been tested on rainbow trout by

Clear Springs Foods (Idaho, US) - a major aquaculture company that produces about one third of all US farmed trout (Dilnawaz and Sahoo, 2008). Biosensors can monitor food or feed safety and the health of crops, forest areas, fish ponds and livestock (Mongillo, 2007).

Nanovaccines: Nanovaccine is emerging as a novel approach to the methodology of vaccination and nanovaccines are more efficient than conventional vaccines in that they induce both humoral and cell-mediated immune response. Nanovaccines have the promise to harness the body's immune system to kill infections and to prevent infections and diseases from spreading (Sekhon and Saluja, 2011). The progress being made in DNA vaccine technology relevant to a number of diseases and their comparison with the conventional treatment options available was reported (Redding and Weiner, 2009). VGX Animal Health, Inc., a developer of DNA-based vaccines and therapies for companion and food animals, has achieved strong T cell responses from VAH-5000D, its DNA vaccine encoding telomerase reverse transcriptase for the treatment of dogs with cancer.

Environmental and health concern: Nanotechnology is a major innovative scientific and economic growth area which may present a variety of hazards for environmental and human health. The possible hazards associated with nanomaterials and harmful effects that may result from exposure of aquatic animals to nanoparticles has been reported. A precautionary approach has been recommended with individual evaluation of new nanomaterials for risk to the health of the environment (Moore, 2006). As nanotechnologies grow and the number of nanomaterial types and applications increase, so does the likelihood that they will be released into the environment and moreover in significant quantities. This fact has been met with increasing concern over their safety in terms of human health and the environment (Dowling *et al.*, 2004b; Friedrichs and Schulte, 2007). Research on hazards of nanoparticles is still limited and to use the potential of nanotechnology in nanomedicine, full attention is needed to safety and toxicological issues (De Jong and Born, 2008).

Experts are of opinion that the complex and critical issues surrounding the environmental transformation and toxicity of engineered nanomaterials must be accompanied by the creation of new approaches or further developments of existing instrumentation (Sadik *et al.*, 2009). The toxicity of different metal and metal oxide nanoparticles by using zebrafish embryos as a toxicological target was evaluated. Because of their similarities with other vertebrates, rapid development and low cost, zebrafish embryos are ideal animal models for probing toxicological effects of engineered nanomaterials. Among the nanomaterials tested, nickel nanoparticles were characterized by high toxicity and induced delayed development and morphological malformations, while metal oxides nanoparticles (i.e., ceria nanoparticles) had no toxic effects (Ispas, 2010). A series of integrative biological and physicochemical studies on the uptake of unmodified commercial nanoscale metal oxides, zinc oxide (ZnO), cerium dioxide (CeO₂) and titanium dioxide (TiO₂), from the water and diet to determine their potential ecotoxicological impacts on fish as a function of concentration were carried out. Significant uptake of nanomaterials was found only for cerium in the liver of zebrafish exposed via the water and ionic titanium in the gut of trout exposed via the diet. For the aqueous exposures undertaken, formation of large NP aggregates (up to 3 µm) occurred and it is likely that this resulted in limited bio availability of the unmodified metal oxide NPs in fish (Johnston *et al.*, 2010).

The fate and effects of silver NPs in fish, rainbow trout (*Oncorhynchus mykiss*) exposed via the water to commercial silver particles of three nominal sizes: 10 nm (N₁₀), 35 nm (N₃₅) and 600-1600 nm (N_{Bulk}) and to silver nitrate for 10 days was reported. Uptake of silver particles from the water into the tissues of exposed fish was low but nevertheless occurred for current estimated environmental exposures. Of the silver particles tested, N₁₀ were found to be the most highly concentrated within gill tissues and N₁₀ and N_{Bulk} were the most highly concentrated in liver. There were no effects on lipid peroxidation in any of the tissues analyzed for any of the silver particles tested and this is likely due to the low uptake rates. However, exposure to N₁₀ particles was found to induce expression of *cyp1a2* in the

gills, suggesting a possible increase in oxidative metabolism in this tissue (Scown *et al.*, 2010).

Scientists provided a new perspective and method for determining the toxic effects of nanoAg on biological macromolecules (Liu *et al.*, 2009). Silymarin (Sm) nanoparticles from *Silybum marianum* were strongly protective against hepatic damage when tested in a paracetamol overdose hepatotoxicity model (Das *et al.*, 2011).

Upstream oversight assessment has been proposed as a targeted activity that focuses on assessing the technical features of research and development projects, examining potential risks and benefits, exploring data needs for addressing the them and suggesting societal oversight challenges and opportunities. An upstream oversight assessment approach case studies of nanotechnology applied to animal production was examined (Kuzma, 2010).

Nanomedicine and intellectual property rights: Nanomedicine patenting presents unique opportunities and poses numerous challenges. The analysis of the legal implications of nanotechnologies is just at the beginning and the entire patent system is under greater scrutiny and strain, with the Patent and Trademark Office continuing to struggle with evaluating nanomedicine-related patent (Schummer and Pariotti, 2008; Bawa *et al.*, 2005; Bastani and Fernandez, 2005; Koepsell, 2011).

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