Interaction between veterinary medicine and nanotechnology; the present and the near future: A Review

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***Abstract*—** *Judging from the developments already recorded, veterinary medicine can benefit considerably by nanotechnology. Administration of drug treatment or vaccines to free ranging farm, or wild animals can be simplified by nano-scale devices or material that will release the active ingredient, without immediate human intervention. In addition to facilitating administration, nanotechnology has already provided new drug and vaccine candidates with improved characteristics and performance. Furthermore, direct and inexpensive detection of microbial pathogens or of specific disease indicators, using functionalized nanoparticles conjugated to DNA and/or peptide probes, seems ideal for veterinary applications that in most cases must conform within a very strict context defined by cost and the availability of resources. Considering the strong potential of the interaction between the two fields of science, the aim of this article is to provide a concise description of the advances already recorded in nanotechnology, in terms of their potential application in veterinary medicine, in connection specifically to drug and vaccine delivery, and diagnosis of infectious diseases.*

Keywords*— *Diagnostics, Drug-delivery, Nanoparticles, Nanotechnology, Quantum dots.

I. INTRODUCTION

Science is divided by boundaries that are undoubtedly artificial and although useful in terms for example of classification or education, they often prove obsolete. If one would seek an example of the latter, nanotechnology would constitute by all means one of the most ideal candidates: a scientific field with clear intra-disciplinary orientation, the extensions of which seems already to be limited only by fiction. In this respect, the use of applied nanotechnology in veterinary medicine cannot be viewed separately than that of human medicine, biology, bio-physics, bio-chemistry, analytical chemistry or bio-engineering, which demonstrates exactly the validity of the introducing sentences of this paragraph. However, one is obliged to consider the defining characteristics of each of the fields of science mentioned above in order to establish were and exactly how, nanotechnology would or could be used to further the existing state-of-the-art.

As opposed to human medicine in which the value of life usually renders high-cost applications consistent even with their routine use, in veterinary medicine this notion is defined largely by an unavoidable compromise between benefit and cost. Considering the strong potential of the interaction between the two fields of science, the aim of this article is to provide a concise description of the advances already recorded in nanotechnology, in terms of their potential application in veterinary medicine, in connection specifically to drug and vaccine delivery, and diagnosis of infectious diseases.

II. DRUG DELIVERY

The idea of selective targeting of specific types of cells using nanoparticles functionalized with specific biologically active substances, such as cell surface proteins or cytokine receptors, immerged when biological applications of nanotechnology were first being considered. Expectedly this property was put to use in connection to imaging and treatment of cancer in humans. The use of nanoparticles conjugated to peptides expressed by cancerous cells allows selective binding to these cells and the release of the therapeutic agent exclusively to them, minimizing its impact on healthy tissue and effectively, side-effects. The same approach for drug delivery is applicable in veterinary medicine.

Different drug delivery systems incorporating nanotechnology products such as nanotubes, dendrimers or gold coated nanoshells can be constructed to respond to light (infrared and laser) or heat, and could thus be turned on and off. Selective drug release activated by specific stimuli is also possible through the use of automated bio-nano-electrochemical systems, referred to as BioNEMS. These can be implanted near a blood vessel, intramuscularly or subcutaneously, and release their content periodically for administration of therapeutic drugs [1] or post-operative animal monitoring [2]. The use of BioNEMS minimizes human intervention, since they can be activated automatically by external stimuli such as radiation. The same reaction can be evoked by internal cellular signaling associated with tissue damage, such as increase of local

temperature and/or release of cytokines. After stimulation, BioNEMS can respond by releasing antimicrobial agents or hormones and cytokines, leading to tissue regeneration and accelerated healing.

2.1 Nanotechnology and drug delivery to animals

Nanotechnology has been exploited for drug delivery to laboratory animals, especially mice, in practically numerous cases. However, the relevance of most of these applications to veterinary medicine is small, since they constitute part of studies conducted for diseases of humans.

One of the first applications of nanoparticles targeting an animal disease was that which was developed by Kroubi et al., in 2010 [3]. In this case, diminazene, a recognized tryponocidal drug, was incorporated onto a delivery system consisting of colloidal formulations based on porous cationic nanoparticles with oily cores. The aim was to create a more effective method of drug delivery and improve the treatment of African trypanosomiasis. It is interesting to note that the final result was greatly influenced in connection to drug stability, by their content being loaded onto the nanoparticle delivery system during the synthesis stage of the latter, or afterwards. The latter, i.e. the post-loading technique, resulted to over 80% of drug entrapment onto the delivery system, preventing drug oxidation for a period of up to 6 months.

More recently (2012), immune-regulating cytosine-phosphate-guanine-oligonucleotides were administered to allergic horses by Klier et al. [4], using an aerosol of biodegradable and non-toxic nebulized gelatin nanoparticles. Again the aim was to maximize the efficacy of immunotherapy decreasing at the same time, potential side-effect. The specific approach resulted to a "remarkable" increase of anti-inflammatory and anti-allergic IL-10 expression, which according to the team that reported the specific work, can set the pace for similar applications in the treatment of allergy in humans.

A similar approach was followed by Imperiale et al. in 2014 [5], in their attempt to resolve the problem of low bio-availability and stability that results to failure of anti-retroviral treatment administered to AIDS patients. In this case, which is considered of particular interest to veterinary medicine because the trial was conducted on mongrel dogs, the aim was to test the efficacy of a nanoparticle-in-microparticle delivery system, comprised of pure drug nano-crystals of a specific anti-retroviral compound (protease inhibitor indinavir free base). Based on the results of this study, their content's half-life was increased 95-fold whereas that of its oral bioavailability, 47-fold, showing great potential for treating both animals and of course humans. Notably the specific anti-retroviral compound was used as a model of poorly water soluble drugs, the increase of the bioavailability of which, constitutes a challenge for the pharmaceutical industry.

The same problem, i.e. the improvement of pharmacokinetics of poorly water soluble compounds, is often addressed with the use of lipid-based drug delivery systems. These that have already become available commercially, rely on encapsulating or solubilizing their content in lipid excipients, which improves solubilisation, absorption and effectively bioavailability [6].

Equally encouraging results were recorded in connection to oral bio-availability of another water insoluble compound i.e. simvastatin that was administered to beagle dogs through a drug delivery system consisted of glyceryl monooleate poloxamer 407 cubic nanoparticles [7]. Pharmacokinetic profiles showed sustained plasma levels of simvastatin for over 12 h whereas relative oral bioavailability was 241% higher compared to that of simvastatin crystal powder.

In addition to nanotechnology-based drug delivery systems, it is also worth to make reference to nano-technology constructed drugs. One of the most promising compounds of this category is probably soybean oil emulsified with detergents to form nano-drops. These are used as non-selective microbio-cidal agents, effect of their integration into the viral envelope or the microbial cell membrane. The specific nano-therapeutic excerpts no physical effect on nucleated cells, which is currently restricting its use to surface wounds, because it can cause rapture of host non-nucleated cells, such as sperm and erythrocytes [8, 9]. This however maybe a "window of opportunity" for veterinary medicine, in connection to fowl or fish that could constitute an ideal model for an application that would bypass the specific limitation, since their erythrocytes are nucleated.

2.2 Nanotechnology and vaccine delivery to animals

Nanoparticles such as liposomes, PLG (poly-lactide- glycolide), PEG (poly-ethylene glycol), silica, carbon in the form of nanotubes or mesoporous spheres, magnetic beads, cadmium selenide or gold, can be easily functionalised to attach selectively to specific cellular receptors. This property has been used for the preparation of nono-structured vaccine delivery systems in a way similar to that of microbial vaccine carriers. After attachment, nanoparticles enter living cells through picocytosis. Notably virus-like particles, i.e. nanoparticles structured to mimic viruses, have already been used as vaccination vehicles [10]. These particles that are formed by self-assembly of viral capsid proteins induce strong immune response even

without an adjuvant because of their tunable size and repetitive structural order [11]. The specific nanoparticles will interact with the cells but not in a responsive manner, i.e. they may induce biological changes but they will not be structurally or chemically altered by them. The stability of the nanoparticles makes their preservation easier whereas the fact that their use can be combined with BioNEMS facilitates their bio-availability for much longer periods of time.

Greenwood et al. in 2008 [12], have tested the approach mentioned above in connection to vaccination against foot-and-mouth (FMD) disease. The specific team used inert nano-beads carrying a viral synthetic antigen in order to induce an immune response that would be protective against FMDV in sheep. The approach relied on the multi-labelling capacity of the nano-beads and resulted to an interesting outcome in terms of the potential use of nanoparticles as vaccine delivery systems: though single peptides induce an immune response in most sheep, the combination of multiple peptides conjugated either separately or as a mixture to individual nano-beads, induced a significantly stronger cell-mediated and humoral immune response. Chen et al. in 2010 [13], followed a similar approach incorporating a synthetic FMDV peptide conjugated this time with gold nanoparticles, the delivering efficacy of which was assessed comparatively using various sizes. Notably, in this case their content delivery system is inorganic and does not bio-degrade, which implies a more rigid and stable route for the delivery of the active ingredient of the vaccine.

In more detail, the antigen of inorganic nanoparticles, if encapsulated, is released as the nanoparticle is being decomposed either outside or inside the cell. If the antigen is conjugated onto the nanoparticles surface (loosely by absorption due to hydrophobic interaction or strongly, by chemical conjugation), it is presented in a way that mimics antigen presentation of macrophages or dendritic cells and at the conclusion of this process, the nanoparticle carrier is excreted in the urine. One of the most interesting candidates that can be used within the context described above is the mesoporous silica nanoparticle-vaccine delivery system. These can be used both as adjuvants and delivery vehicles, they are stable, their biological interaction is size-dependent and can be thus selectively modulated allowing interaction with different categories of cells, they can be multi-labelled and their delivery capacity can be controlled due to their tunable hollow and mesoporous structure [14, 15].

In addition to the use of nanoparticles as vaccine delivery systems, polysaccharide polymer based nanoparticles such as pullulan, alginate, inulin and chitosan have been incorporated into attenuated vaccines as adjuvants or as both delivery systems and adjuvants. In most cases these applications refer to pathogens of humans, such as HBV [16] or influenza virus [17], though a veterinary application has also been tested in connection to a live vaccine strain for Newcastle disease (ND) that was encapsulated into chitosan nanoparticles. Immunostimulating complexes (ISCOMs) i.e. cage-like particles made of saponin, cholesterol, phospholipids and proteins, have been used as adjuvant/delivery systems for the same disease [18]. In this case, the ISCOM was used to cage a whole virus strain treated with Triton X-100 showing a level of protection upon challenge to virulent NDV that exceeded 80%. A similar combination of delivery/adjuvant system relies on the use of nano-sized emulsions that can be composed as oil-in-water or water-in-oil forms and can carry the vaccine ingredient into their core or they can be mixed with it. Commercially available products of nano-emulsions are available and have been used for a vaccine against FMD proving more efficient in cattle compared to other adjuvants in terms of early immune stimulation [19].

III. DIAGNOSIS OF INFECTIOUS DISEASES

The last two decades of the 20th century was a period during which, at start the emergence, and later on the broad application of molecular biology and more specifically, of the polymerase chain reaction (PCR), revolutionized the field of diagnostics. Expectedly the intense use of PCR revealed some of its "weaknesses". Decreasing the minimum limit of detection (MLD) to minute amounts of target-DNA imposed, especially in connection to routine applications, vigorous precautions to avoid the carry-over effect (successive passage of amplicons from one reaction to the next) and to eliminate every source of potential contamination that could be the cause of false-positive results. Additional quality control measures were required to minimize and detect false-negative results generated by the fragmentation of the target-DNA or the presence PCR inhibitors. Effectively it became apparent that reliable application of PCR is not possible without expert personnel, accredited equipment and dedicated space, which inevitably increase cost.

During mainly the last decade, an increasing number of reports have been published describing an alternative approach, incorporating nanotechnology for a variety of diagnostic applications including pathogen detection. This was most probably associated with the fact that the chemical bio-compatibility of nanoparticles, which is a fundamental property for their use in biology, combined greatly in terms of their potential use in diagnostics, with the fact that they can be constructed in a variety of sizes. Being by definition smaller than 100nm, nanoparticles can be adjusted in size from that of the width of the double helix of DNA i.e. approximately 1-2 nm, to that of proteins, viruses or the smallest of bacteria, i.e. around 100 nm.

Depending on the properties of the material one is interested to make use of, there are a number of metal or polymer nanoparticles from which to choose. In the last years, nano-diagnostics have focused more on colloidal gold nanoparticles (AuNPs) and cadmium selenide (CdSe) quantum dots (QDs) that are being produced commercially in a robust manner. Hence, the specific nanoparticles exhibit characteristics of stability and performance that is consistent with their use even in routine diagnostic applications, targeting different pathogens and/or their immunogenic footprint.

3.1 AuNPs and CdSe QDs

Colloidal AuNPs range in size from 3 to 100 nm, and exhibit strong size-dependent optical resonance. Photo-activation results to a huge enhancement of their electromagnetic field that causes scattering. The negatively charged AuNPs can be chemically (using in most cases salt) or electrically (using micro-circuits) directed to selective deposition, detectable by change of colour (visual detection) or energy (construction of biosensors). Linkage of two or more, properly modified for conjugation AuNPs that will hybridize to adjacent regions of an analyte, such as a nucleotide target, will also result to aggregation due to the decrease of intra-particle distance. Notably the use of AuNPs for optical detection of analytes offers some unique advantages compared to fluorescent dyes: AuNPs are not prone to photodecomposition, they are not toxic, and most significantly they demonstrate a very precise correlation of their chemical/physical properties to their optical characteristics, which if accurately measured can confer improved MLD and sensitivity.

QDs are semiconductor crystals with physical dimensions not larger than a few nanometers. CdSe nanoparticles are already becoming increasingly popular, especially around the size range of 2-6 nm, which makes them dimensionally more compatible with nucleic acid and proteins. When a photon of visible light hits such a semiconductor some of their electrons are excited into higher energy states. A photon with a frequency that is characteristic of the semiconductor is emitted when the electrons return to their ground state. The ability of QDs to exhibit size-dependent fluoresce-emission wavelengths is the foundation of their use as biodetectors, which can be greatly improved by the incorporation in their outer surface of a "shell" such as ZnS or silica. Notably the latter can be easily linked to bioconjugators such as avidin, which allows incorporation of these nanaparticles into several already established diagnostic assays. In summary the QD shells can protect the core from oxidation, they minimize or even eliminate core-derived toxicity and they improve water solubility. CdSe may be size-tuned very accurately and thus it can be used for the simultaneous detection of multiple targets with a single excitation wavelength.

3.2 Nanotechnology and diagnosis of infectious diseases of animals

AuNPs and CdSe QDs have been used for the detection of various microbial pathogens even when present together in the same material (*biofilms*).

Storhoff et al. in 2004 [20], developed a spot-and-read colorimetric detection method for identifying nucleic acid sequences based on the distance-dependent optical properties of AuNPs. The method's low MLD enabled selective targeting of zeptomole quantities of nucleic acid without amplification allowing direct detection and differentiation of methicillin-resistant *Staphylococcus aureus*. QDs have been used to provide in addition to differential detection of pathogens, an assessment of their viability [21, 22]. Assays incorporating bio-conjugated nanoparticles have been used for *in situ* pathogen quantification in less than 20 minutes, with a MLD reaching no more than a single cell. Multi-focal binding of QDs was also used successfully for the simultaneous quantifiable and differential detection of two food-borne pathogens i.e. *Escherichia coli* O157:H7 and *Salmonella typhimurium* [23]. Similarly a rapid (1 hour), simple, high sensitive bacterial detection system was developed using biotin-tagged bacteriophage and QD-nanocomplexes [24]. This method, which was found to be particularly useful for slow growing bacteria such as mycobacteria is already being developed for military applications for fast, accurate and portable detection system applicable directly on clinical samples (*NCI and NIST, USA*).

What is especially intriguing about the diagnostic approach described above in terms of its potential practical use, is in addition to low MLD and small dependency on dedicated equipment, that it can developed for all-in-one assays, i.e. the same principles of detection applied with a variety of nanoprobes designed to detect various peptides or molecules of nucleic acid. This notion was exploited by the author's group in connection to specific pathogens of *Mycobacterium* spp., and *Leishmania* spp., using the same set of assays that rely on AuNP colorimetric, and CdSe-fluorescence detection of microbial DNA and proteins. The methodology consisting of a liquid [25, 26, 27, 28, 29] and a lateral flow [30] sample analysis, was designed for resource-poor setting and was found efficient compared to real time PCR, even for testing heavily contaminated material, as was the case with the detection of *Mycobacterium avium* subspecies *paratuberculosis* in samples of faeces collected from small ruminants with paratuberculosis. The fact that this type of technology relies on the attachment of the functionalized nanoparticles onto several probing sites, sets the basis for the development of assays with increased sensitivity and

simultaneous confirmation of the result. Furthermore, dispensing with the need for pre-amplification of the target region using PCR, makes the proposed approach less prone to false results generated by the carry-over effect or the presence of amplification inhibitors. In addition to this, given that even in the cases of multi-labeling the probing sites are much smaller in length than PCR amplicons, the chances of false negative results caused by fragmentation of the target region is also decreased.

A different approach has been incorporated into the detection of volatile organic or inorganic substances used for the diagnosis of specific diseases. In this case nanoparticles are not used for the identification of the pathogen but for the detection of combinations of volatile substances that have been previously documented as disease indicators. For instance, the air that is exhaled from patients with pulmonary tuberculosis or lung cancer differs in its composition of volatile substances from that of healthy individuals, which can be easily and very accurately detected by an array of gold nanoparticles [31, 32]. These can be readily integrated into micro-devices that respond with an electrical signal, a color or a sound indication, giving a very simple read out that in some cases has been adjusted for screening and/or processing by smartphones. This technology has already been tried successfully in connection to the detection of *Brucella abortus* in bison using a biosensor that detects with high discriminative efficiency specific alternations in the composition of organic volatile substances in exhaled air [33]. One can only imagine the potential of this technology in veterinary medicine not just in connection to exhaled air, but perhaps more significantly to gases released from the intestine as early indicators of gastrointestinal diseases, especially among newborn animals e.g parvoviral enteritis of dogs or newborn diarrhea of ruminants.

IV. CONCLUSION

Judging from the developments already recorded, veterinary medicine can benefit considerably by nanotechnology. Administration of drug treatment or vaccines to free ranging farm, or wild animals can be simplified by nano-scale devices or material that will release the active ingredient, without immediate human intervention. In addition to facilitating administration, nanotechnology has already provided new drug and vaccine candidates with improved characteristics and performance. Furthermore, direct and inexpensive detection of microbial pathogens or of specific disease indicators, using functionalized nanoparticles conjugated to DNA and/or peptide probes, seems ideal for veterinary applications that in most cases must conform within a very strict context defined by cost and the availability of resources. In this respect, it is justified to anticipate that nanotechnology may facilitate the application in practice of many of the research advances already made in various fields of veterinary medicine, to the benefit of animal and public health.

REFERENCES

[1] R. R. Jivani, G. J. Lakhtaria, D. D. Patadiya, L. D. Patel, N. P. Jivani, and B. P. Jhala, "Biomedical microelectromechanical systems (BioMEMS): Revolution in drug delivery and analytical techniques," Saudi Pharm. J., vol. 24, no. 1, pp. 1–20, 2016.

[2] K. C. McGilvray, E. Unal, K. L. Troyer, B. G. Santoni, R. H. Palmer, J. T. Easley, H. V. Demir, and C. M. Puttlitz, "Implantable microelectromechanical sensors for diagnostic monitoring and post-surgical prediction of bone fracture healing," J. Orthop. Res., vol. 33, no. 10, pp. 1439–1446, 2015.

[3] M. Kroubi, S. Daulouede, H. Karembe, Y. Jallouli, M. Howsam, D. Mossalayi, P. Vincendeau and D. Betbeder, "Development of a nanoparticulate formulation of diminazene to treat African trypanosomiasis," Nanotechnology, vol. 21, no. 50, p. 505102, Dec. 2010.

[4] J. Klier, S. Fuchs, A. May, U. Schillinger, C. Plank, G. Winter, H. Gehlen, and C. Coester, "A Nebulized Gelatin Nanoparticle-Based CpG Formulation is Effective in Immunotherapy of Allergic Horses," Pharm. Res., vol. 29, no. 6, pp. 1650–1657, Jun. 2012.

[5] J. C. Imperiale, P. Nejamkin, M. J. del Sole, C. E. Lanusse, and A. Sosnik, "Novel protease inhibitor-loaded Nanoparticle-in-Microparticle Delivery System leads to a dramatic improvement of the oral pharmacokinetics in dogs," Biomaterials, vol. 37, pp. 383–394, 2015.

[6] K. Sandeep, R. Deepika, and S. Anupama, "Design , optimization and characterization of PH sensitive nanoparticles of duloxetine hydrochloride for enhancement of oral," vol. 4, no. 11, pp. 1213–1230, 2015.

[7] J. Lai, J. Chen, Y. Lu, J. Sun, F. Hu, Z. Yin, and W. Wu, "Glyceryl monooleate/poloxamer 407 cubic nanoparticles as oral drug delivery systems: I. In vitro evaluation and enhanced oral bioavailability of the poorly water-soluble drug simvastatin.," AAPS PharmSciTech, vol. 10, no. 3, pp. 960–6, 2009.

[8] U. Buranasuksombat, Y. J. Kwon, M. Turner, and B. Bhandari, "Influence of Emulsion Droplet Size on Antimicrobial Properties," Food Sci. Biotechnol, vol. 20, no. 3, pp. 793–800, 2011.

[9] Y. Y. Hwang, K. Ramalingam, D. R. Bienek, V. Lee, T. You, and R. Alvarez, "Antimicrobial Activity of Nanoemulsion in Combination with Cetylpyridinium Chloride in Multidrug-Resistant Acinetobacter baumannii," Antimicrob. Agents Chemother., vol. 57, no. 8, pp. 3568–3575, Aug. 2013.

[10] Q. Zhao, S. Li, H. Yu, N. Xia, and Y. Modis, "Virus-like particle-based human vaccines: quality assessment based on structural and functional properties," Trends Biotechnol., vol. 31, no. 11, pp. 654–663, 2013.

[11] S. Zhang, "Emerging biological materials through molecular self-assembly," Biotechnol. Adv., vol. 20, no. 5, pp. 321–339, 2002.

[12] D. L. V. Greenwood, K. Dynon, M. Kalkanidis, S. Xiang, M. Plebanski, and J.-P. Y. Scheerlinck, "Vaccination against foot-and-mouth disease virus using peptides conjugated to nano-beads," Vaccine, vol. 26, no. 22, pp. 2706–2713, 2008.

[13] Y.-S. Chen, Y.-C. Hung, W.-H. Lin and G. S. Huang, "Assessment of gold nanoparticles as a size-dependent vaccine carrier for enhancing the antibody response against synthetic foot-and-mouth disease virus peptide," Nanotechnology, vol. 21, no. 19, p. 195101, May 2010.

[14] D. C. de P. Oliveira, A. L. B. de Barros, R. M. Belardi, A. M. de Goes, B. K. de Oliveira Souza, and D. C. F. Soares, "Mesoporous silica nanoparticles as a potential vaccine adjuvant against Schistosoma mansoni," J. Drug Deliv. Sci. Technol., vol. 35, pp. 234–240, 2016.

[15] K. T. Mody, A. Popat, D. Mahony, A. S. Cavallaro, C. Yu, and N. Mitter, "Mesoporous silica nanoparticles as antigen carriers and adjuvants for vaccine delivery.," Nanoscale, vol. 5, no. 12, pp. 5167–79, 2013.

[16] O. Borges, A. Cordeiro-da-Silva, J. Tavares, N. Santarém, A. de Sousa, G. Borchard, and H. E. Junginger, "Immune response by nasal delivery of hepatitis B surface antigen and codelivery of a CpG ODN in alginate coated chitosan nanoparticles," Eur. J. Pharm. Biopharm., vol. 69, no. 2, pp. 405–416, Jun. 2008.

[17] D. L. Gordon, D. Sajkov, R. J. Woodman, Y. Honda-Okubo, M. M. J. Cox, S. Heinzel, and N. Petrovsky, "Randomized clinical trial of immunogenicity and safety of a recombinant H1N1/2009 pandemic influenza vaccine containing Advax™ polysaccharide adjuvant," Vaccine, vol. 30, no. 36, pp. 5407–5416, Aug. 2012.

[18] A. Homhuan, S. Prakongpan, P. Poomvises, R. A. Maas, D. J. Crommelin, G. F. . Kersten, and W. Jiskoot, "Virosome and ISCOM vaccines against Newcastle disease: preparation, characterization and immunogenicity," Eur. J. Pharm. Sci., vol. 22, no. 5, pp. 459–468, 2004.

[19] P. Dar, R. Kalaivanan, N. Sied, B. Mamo, S. Kishore, V. V. S. Suryanarayana, and G. Kondabattula, "Montanide ISA™ 201 adjuvanted FMD vaccine induces improved immune responses and protection in cattle," Vaccine, vol. 31, no. 33, pp. 3327–3332, 2013.

[20] J. J. Storhoff, A. D. Lucas, V. Garimella, Y. P. Bao, and U. R. Müller, "Homogeneous detection of unamplified genomic DNA sequences based on colorimetric scatter of gold nanoparticle probes," Nat. Biotechnol., vol. 22, no. 7, pp. 883–887, Jul. 2004.

[21] M. A. Hahn, J. S. Tabb, and T. D. Krauss, "Detection of Single Bacterial Pathogens with Semiconductor Quantum Dots," Anal. Chem., vol. 77, no. 15, pp. 4861–4869, 2005.

[22] N. I. Chalmers, R. J. Palmer, L. Du-Thumm, R. Sullivan, W. Shi, and P. E. Kolenbrander, "Use of quantum dot luminescent probes to achieve single-cell resolution of human oral bacteria in biofilms.," Appl. Environ. Microbiol., vol. 73, no. 2, pp. 630–6, Jan. 2007.

[23] L. Yang and Y. Li, "Simultaneous detection of Escherichia coli O157:H7 and Salmonella Typhimurium using quantum dots as fluorescence labels," Analyst, vol. 131, no. 3, pp. 394–401, Mar. 2006.

[24] R. Edgar, M. McKinstry, J. Hwang, A. B. Oppenheim, R. A. Fekete, G. Giulian, C. Merril, K. Nagashima, and S. Adhya, "High-sensitivity bacterial detection using biotin-tagged phage and quantum-dot nanocomplexes," Proc. Natl. Acad. Sci., vol. 103, no. 13, pp. 4841–4845, Mar. 2006.

[25] E. Liandris, M. Gazouli, M. Andreadou, M. Comor, N. Abazovic, L. A. Sechi, and J. Ikonomopoulos, "Direct detection of unamplified DNA from pathogenic mycobacteria using DNA-derivatized gold nanoparticles.," J. Microbiol. Methods, vol. 78, no. 3, pp. 260–4, Sep. 2009.

[26] M. Gazouli, E. Liandris, M. Andreadou, L. A. Sechi, S. Masala, D. Paccagnini, and J. Ikonomopoulos, "Specific detection of unamplified mycobacterial DNA by use of fluorescent semiconductor quantum dots and magnetic beads.," J. Clin. Microbiol., vol. 48, no. 8, pp. 2830–5, Aug. 2010.

[27] E. Liandris, M. Gazouli, M. Andreadou, L. A. Sechi, V. Rosu, and J. Ikonomopoulos, "Detection of pathogenic mycobacteria based on functionalized quantum dots coupled with immunomagnetic separation," PLoS One, vol. 6, no. 5, 2011.

[28] M. Andreadou, E. Liandris, M. Gazouli, S. Taka, M. Antoniou, G. Theodoropoulos, I. Tachtsidis, N. Goutas, D. Vlachodimitropoulos, I. Kasampalidis, and J. Ikonomopoulos, "A novel non-amplification assay for the detection of Leishmania spp. in clinical samples using gold nanoparticles," J. Microbiol. Methods, vol. 96, pp. 56–61, 2014.

[29] M. Andreadou, E. Liandris, M. Gazouli, A. Mataragka, I. Tachtsidis, N. Goutas, D. Vlachodimitropoulos, and J. Ikonomopoulos, "Detection of Leishmania-specific DNA and surface antigens using a combination of functionalized magnetic beads and cadmium selenite quantum dots," J. Microbiol. Methods, vol. 123, pp. 62–67, 2016.

[30] F. Cimaglia, E. Liandris, M. Gazouli, L. Sechi, M. Chiesa, E. De Lorenzis, M. Andreadou, S. Taka, A. Mataragka, and J. Ikonomopoulos, "Detection of mycobacterial DNA by a specific and simple lateral flow assay incorporating cadmium selenide quantum dots," Mol. Cell. Probes, vol. 29, no. 6, pp. 534–536, 2015.

[31] G. Peng, U. Tisch, O. Adams, M. Hakim, N. Shehada, Y. Y. Broza, S. Billan, R. Abdah-Bortnyak, A. Kuten, and H. Haick, "Diagnosing lung cancer in exhaled breath using gold nanoparticles," Nat. Nanotechnol., vol. 4, no. 10, pp. 669–673, Nov. 2009.

[32] M. K. Nakhleh, Y. Y. Broza, and H. Haick, "Monolayer-capped gold nanoparticles for disease detection from breath," Nanomedicine, vol. 9, no. 13, pp. 1991–2002, Sep. 2014.

[33] A. Bayn, P. Nol, U. Tisch, J. Rhyan, C. K. Ellis, and H. Haick, "Detection of Volatile Organic Compounds in Brucella abortus - Seropositive Bison," Anal. Chem., vol. 85, no. 22, pp. 11146–11152, Nov. 2013.