Nanotechnology is an innovative tool in the treatment of diseases that affect animals

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ABSTRACT
Nanotechnology is defined as the art of manipulating matter atom by atom. Its materials have a size between 1 and 100 nanometers. In the veterinary field, nanometric-scale devices and systems have been applied for the diagnosis, treatment, monitoring and traceability of agricultural inputs. Some notable applications include sensors used to detect specific substances in biological samples, dispensers that allow the controlled release of medications or nutrients, immunogens that help develop immune responses, and chemotherapy drugs that are used to treat diseases, among other applications. This work describes the application of nanotechnology in different areas of veterinary medicine.

Key words: nanotecnology, essential oils, antiviral, cell morphology, additives.

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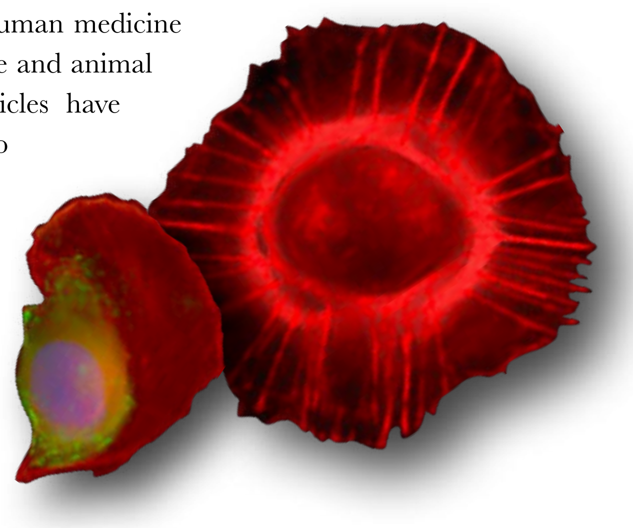
INTRODUCTION

Nanotechnology in veterinary medicine is an emerging field that offers innovative solutions for the diagnosis, treatment, and prevention of animal diseases. It uses nanoscale materials (between 1 and 100 nanometers) to create devices, substances, and systems with unique properties (Arjamend *et al.*, 2023; Coppo, 2009). These may include sensors, dispensers, immunogens, and chemotherapeutics that work at the molecular level to improve the effectiveness and reduce side effects of treatments.

Nanotechnology has proven to be an effective tool contributing to the area of diagnosis and therapy in human medicine and recently its use in veterinary medicine and animal production (Ali *et al.*, 2021). Nanoparticles have been shown to have great potential to revolutionize the veterinary sector in terms of drug and vaccine delivery, diagnostics, animal husbandry and reproduction, and even in the field of animal nutrition.



Natural alternatives are currently being investigated as adjuvants in animal treatments. Nanotechnology allows the creation of controlled release systems that can improve the delivery of natural compounds, such as essential oils and plant extracts, that have antimicrobial and therapeutic properties. These systems can ensure that the active ingredients reach the site of action more effectively, increasing their efficiency and reducing the amount needed, which is beneficial for both animal health and the environment.

Use of Nanotechnology and natural products in the treatment of bovine mastitis

One of the great challenges in the pharmaceutical and food industry is to be able to use the benefits of natural components, avoiding the evaporation of some essential oils and directing the active components more efficiently to the site of action, which is why nanotechnology is a current tool that is managing to innovate the way drugs are delivered (Corona-Gómez *et al.*, 2022). They have conducted significant studies in this field, particularly about bovine mastitis, defined as mammary gland inflammation commonly caused by bacterial infections (Corona-Gómez *et al.*, 2022). One of their studies analyzed the in vitro microbicidal activity of tea tree essential oil, thymol, and carvacrol (compounds of oregano and thyme essential oils) on microorganisms isolated from cases of bovine clinical mastitis. The results showed that these natural substances have potential as alternatives to traditional antibiotics, which is crucial at a time when antibiotic resistance is a growing concern.

Ionic gelation is a method used to manufacture chitosan nanoparticles; a natural polysaccharide derived from chitin. This process involves the interaction of charges between chitosan, which is positively charged, and a negatively charged gelling agent, such as tripolyphosphate (TPP) or sodium alginate (Herrera Barros *et al.*, 2016).

Chitosan nanoparticles manufactured by ionic gelation (Figure 1) have several applications in veterinary medicine, such as the controlled release of drugs, and improving the bioavailability and stability of active compounds (Herrera Barros *et al.*,

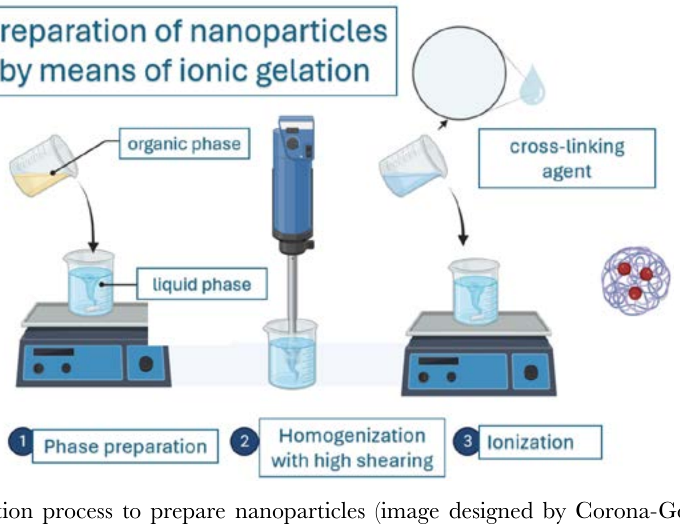


Figure 1. Ionic gelation process to prepare nanoparticles (image designed by Corona-Gómez Lysett with BioRender).

2016). Additionally, they can help mask undesirable odors and flavors and offer better physicochemical stability during processing and storage (Ramírez *et al.*, 2021).

This method is particularly useful for the encapsulation of essential oils due to its ability to protect sensitive compounds and allow a targeted and controlled release at the site of action, which may be beneficial for treating infections or inflammations in animals.

Nanoparticles containing glycyrrhizic acid and the evaluation of their antiviral activity against PRRS virus

Another interesting approach of nanomedicine in the veterinary field is the preparation of nanoparticles with the antiviral agent glycyrrhizinic acid (GA), the most important component from licorice extract (Obolentseva *et al.*, 1999). Urbán-Morlán *et al.* (2018) proposed to study GA due to its different therapeutic properties including its antiviral activity against human and animal viruses (Urbán-Morlán *et al.*, 2018).

In this research, they tested GA water solutions and solid lipid nanoparticles (SLN) containing GA on PRRS-infected cell culture. The Porcine Reproductive and Respiratory Syndrome (PRRS) is caused by a virus and elicits enormous economic losses in hog-producing countries worldwide (Darwich *et al.*, 2010) because currently there is no efficient treatment against this virus. The clinical presentation of the syndrome involves severe reproductive failure mainly in sows and respiratory signs in pigs of all ages; specifically, there is an increase in the number of stillbirths, weak-born piglets, mummified, and an increase in cases of severe pneumonia; sometimes the disease is presented with dyspnea, cyanosis of ears and extremities, pyrexia, lethargy, and anorexia at first stages (Corzo *et al.*, 2010; Huang & Meng, 2010).

The first step of the research was to evaluate the effect of GA water solutions on uninfected and PRRS virus-infected MARC-145 cell cultures. Cytotoxicity and antiviral activity were calculated using the trypan blue dye exclusion test, MTT, and virus titer reduction.

In the second stage, the authors developed SLN with GA. Since the '90s, SLN has been used as carriers of a wide variety of different active molecules because of their advantages, such as high drug payload, low toxicity, increased bioavailability, and drug targeting (Scioli Montoto *et al.*, 2020; Nguyen & Duong, 2022). These colloidal carriers are composed of a lipid matrix stabilized by a surfactant with a size under 1 μm. SLN containing GA (0.54 mg/ml) was obtained by the microemulsion method and tested on cells previously infected with PRRS. After exposure to the SLN, MTT assay, and trypan blue staining were performed.

The results showed that the cytotoxic concentration of GA that reduced cell viability to 50% (CC50) was 4.2 mg/ml and the effective concentration of GA required to inhibit the cytopathic effect to 50% (EC50) was 0.48 mg/ml. Virus titer decreased two logarithms compared to the final titer of a control assay without GA treatment (Figure 2). At this point, these results represent a potential alternative to treat PRRS infection.

Moreover, the effect of GA-loaded SLN on cell culture demonstrated that cell viability by MTT assay was comparable to that exerted by the virus-infected control cells, and the formation of needle-like structures was observed at 48 h, probably due to the presence

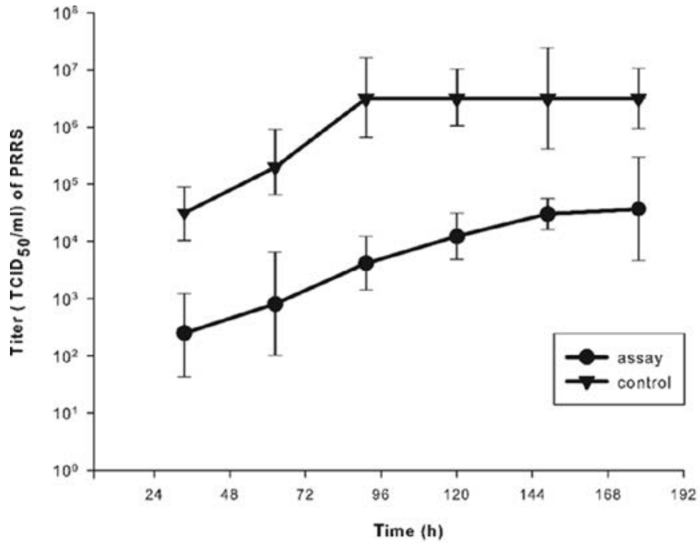


Figure 2. Virus titer was calculated by the Reed and Muench method, where increased concentrations of GA were added after 1 h of incubation of the cells infected with PRRs. n=3. (figure from Urbán-Morlán *et al.* 2018).

of the lipid forming the nanocarrier. The authors concluded that cell culture exposed to SLN interferes with the reliability of MTT assay and it is necessary to combine methods to assess viability. Figure 3 shows the SLN obtained by the microemulsion method.

Drug transporting nanoparticles and their effect on cell morphology

Nanotechnology allows for the development of new drug delivery methods using carriers to transport active ingredients. Nanoparticles can traverse biological barriers and target specific cells, potentially reducing side effects and improving treatment effectiveness. New systems need to be developed with high safety standards, allowing cells to monitor the environment and respond to external signals to survive (Ramos *et al.*, 2018; Kou *et al.*, 2018; Irache, 2008; Jabr-Milane *et al.*, 2008; Tsuji *et al.*, 2006).

The safety of nanosystems can be evaluated by creating cellular models to assess their impact on structure and internal cell function.

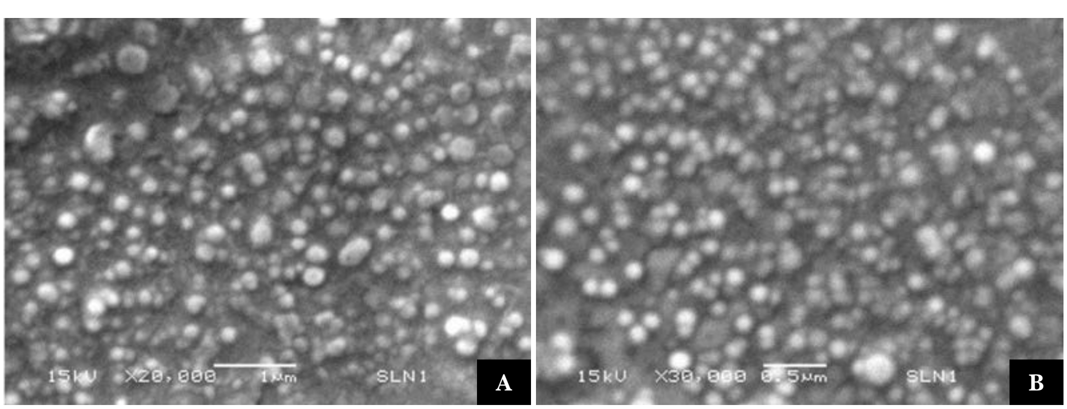


Figure 3. SEM images of SLN were obtained by the microemulsion method. SLN at t=0 (A), and at t=15 days (B). Bars represent 1 μm or 0.5 μm, as specified (figure from Urbán-Morlán *et al.* 2018).

The assessment of cell structure involves studying the rearrangements of the cytoskeleton, a dynamic network composed of different proteins. The most predominant protein is actin, which is responsible for maintaining the cells architecture and structural integrity. These functions are primarily associated with changes in shape and movement through filament rearrangements (Liu *et al.*, 2013).

The actin cytoskeleton induces changes in cell shape and migration in response to various extracellular signals, which can take on multiple arrangements such as bundles (parallel and contractile) and networks (mesh-like and dendritic). Parallel bundles consist of closely associated actin filaments that support structures such as filopodia and microvilli (Liu *et al.*, 2013).

The internal cellular activity of cell lines exposed to nanovehicles is assessed using cytotoxicity tests, which evaluate mitochondrial and lysosomal activity and plasma membrane integrity. Additionally, drug nanocarrier systems can be equipped with fluorophores, which enable their observation using fluorescence microscopy and confocal microscopy to determine the internalization and uptake potential of the nanovehicles.

Effects of additives in nanometric size in veterinary

For a long time, nanotechnology has focused only on humans in areas of health, food, and medicine, among others. Recently, nanotechnology has been applied in veterinary medicine, where several types of nanostructures and nanoparticles have been developed to revolutionize animal science. This is to study disease diagnosis and treatment, nano-vaccines and nano-adjuvants, animal health and nutrition, animal reproduction, and pet care (El-Sayed & Kamel, 2020).

Applications of drug delivery can be applicable in animal nutrition. Drug delivery refers to transporting a pharmaceutical substance to specific areas of the body, such as organs and cellular and subcellular levels of specific tissue, to achieve the desired therapeutic effect (Tewabe *et al.*, 2021). The same strategy used in nanomedicine to reach a specific body area is also applicable in veterinary nutrition.

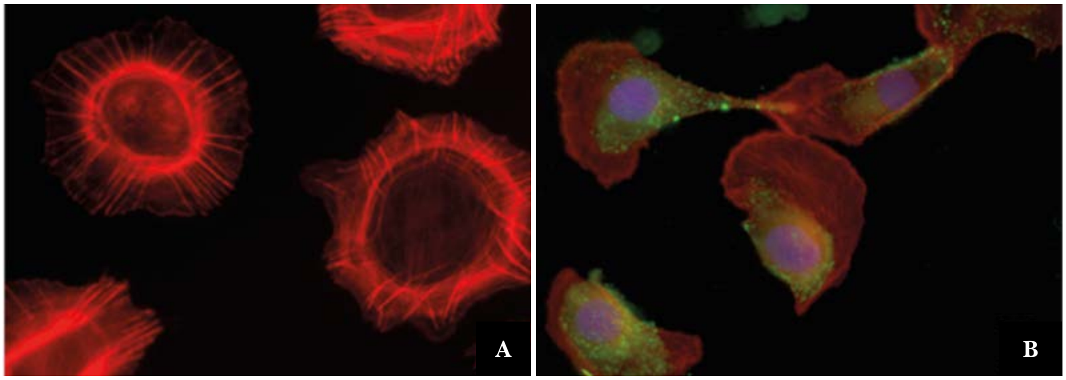


Figure 4. Morphological evaluation of nanoparticle interaction in cell culture. [A] Actin cytoskeleton in MARC-145 cells Direct fluorescence assay, actin filaments stained with TRITC-labeled phalloidin. [B] Internalization of nanoparticles into the cells at 4 h of exposure based on coumarin C6 staining fluorescence microscopy at x40 magnification.

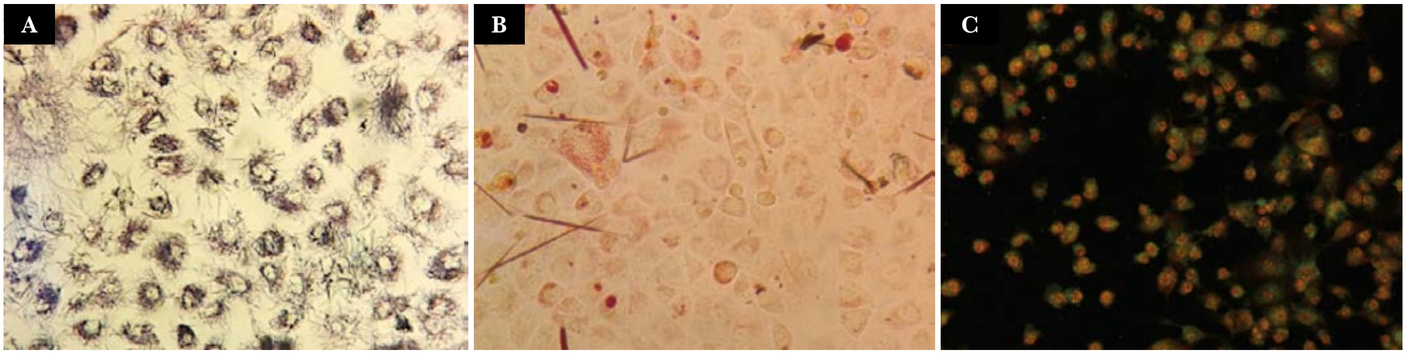


Figure 5. Cytotoxicity after exposure to nanovehicles evaluated by internal cellular activity. [A] Measurement of mitochondrial activity with the MTT assay; [B] determination of lysosomal activity with the neutral red assay; [C] acridine orange staining to evaluate the effect on nuclear integrity.

Administering nutrients to livestock presents inherent challenges in designing carrier nanoparticles, as each compartment of the gastrointestinal tract has its physiological characteristics, such as enzymes and pH level (Hill & Li, 2017). Therefore, nanoparticles must be able to cross many barriers to the delivery of nutritional cargo, particularly to the small intestine.

The delivery of nutrients in the form of carrier nanoparticles has a higher bioavailability than their conventional counterparts. Nanoencapsulation of vitamins allows them to pass through the gastrointestinal tract, deliver the vitamins into the bloodstream, and increase their bioavailability (Thulasi & Sellappan, 2013).

Eggs are consumed for their low price and high nutritional value. To enrich food at the farm level, micro and macro elements are added. Using nano-selenium in laying hens' nutrition decreases total lipids and total cholesterol levels. Additionally, chromium picolinate nanoparticles improve egg yield and increase the accumulation of chromium, calcium, and phosphorous (Konkol D. & Wojnarowsk K., 2018). Similarly, chickens are the most consumed meat in the world due to their easy care and growth. To improve their production, silver nanoparticles were administered to broiler chickens, which improved body weight gain compared to the control group (Reddy *et al.*, 2022).

A study was conducted to evaluate the effects of nanoparticles on growth, carcass characteristics, pork quality, and lipid metabolism in finishing pigs. Nanoparticles of chromium-loaded chitosan were added to the diet of finished pigs destined for market. The results indicated that dietary supplementation with chromium improves growth, carcass characteristics, and pork quality. The chromium-loaded chitosan nanoparticles elevated the activity of hormone-sensitive lipase in adipose tissue while decreasing fatty acid synthase activity (Wang *et al.*, 2014). Additionally, silver and Cu- montmorillonite nanoparticles were used as feed additives to increase the weight gain of pigs. Selenium is an essential element for animal health. Administration of nano-Se and selenomethionate as feed additives for crucian carp showed improvements in final weight in this aquaculture species (Zhou *et al.*, 2009).

Nanotechnology may represent a new and specific product for animal nutrition. Minerals, supplements, and vitamins are important. Through food delivery, many essential nutrients that are not appropriately assimilated could reach a specific body area in the shape of carrier nutrients. Nevertheless, there are several limitations that require solutions. For example, design nanoparticles that must be resilient against enzymatic in multiple environments, and these nanoparticles must be able to either degradation or excretion of the body (Ali *et al.*, 2021).

The inclusion of nanoparticle supplements in livestock, poultry, and aquaculture will be beneficial in increasing the quality of meat for consumers; another advantage is that they are cheaper and needed in lower concentrations.

Nanosensors in Disease Diagnosis

Nanosensors represent an advance in the diagnosis of infectious diseases, particularly those with zoonotic risk. Different strategies using nanoparticles have been examined for both the diagnosis and treatment of these diseases, as well as for prevention (Dhakal, 2023).

Nanoparticles in Vaccine Development

The use of nanoparticles for the development of veterinary vaccines has had a great boom since they have demonstrated greater benefits and are safer than conventional formulations. Self-assembling nanoparticle vaccines (SAPNs) can produce robust cellular and humoral immune responses and have been shown to protect against various animal infectious diseases (Sun *et al.*, 2024).

Improvement of digestive efficiency and quality of products of animal origin

Feeding with nanoparticles has been shown to improve digestive efficiency, immunity, and the quality of milk, meat, and eggs. Nano-minerals offer low-dose use and improved bioavailability, making them an effective alternative to antibiotics, and can also be incorporated into natural feed ingredients (Gelaye, 2024).

CONCLUSIONS

Nanotechnology offers significant potential to improve animal health and welfare, and its continued research will continue to transform veterinary practice. It is essential that scientists, veterinarians and industry work together to make the most of these innovations and ensure their safety and effectiveness in clinical care.

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