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Nanotechnology Applications in Veterinary Science

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Introduction

The rapidly expanding field of nanotechnology holds remarkable promise for transforming veterinary medicine by tackling pressing issues in animal health, welfare, and productivity. This multidisciplinary area utilizes nanoscale materials and systems to create cutting-edge solutions for disease detection, precision drug delivery, and regenerative therapies in various animal species (Catalano, 2021). Owing to their exceptional physicochemical traits—such as enhanced stability, biocompatibility, and precise molecular interactions—nanoparticles are being increasingly applied across diverse areas of veterinary nanomedicine, including diagnostics, therapeutics, and vaccine design (Jafary et al., 2022). Their minute size allows seamless integration into biological environments, improving molecular interaction analyses and enabling advanced high-throughput screening systems (Kher et al., 2016).

Such precision-based drug delivery approaches can also overcome common challenges like low bioavailability and adverse reactions associated with conventional veterinary drugs (Youssef et al., 2019). Beyond therapeutic uses, nanotechnology plays an important role in animal production systems by enhancing nutrient utilization, improving feed efficiency, and acting as biocidal agents against infectious diseases (Hill & Li, 2017).

This review seeks to provide an in-depth overview of the present-day applications of nanotechnology in veterinary diagnostics, therapeutics, and preventive medicine, highlighting the key scientific mechanisms that drive these advancements. It further explores how nanomaterials interact with biological systems, outlines the challenges and future directions in veterinary nanotechnology, and underscores the importance of robust safety evaluations and regulatory frameworks for responsible implementation. Additionally, the review examines economic

feasibility and ethical implications concerning the widespread integration of nanotechnology into animal healthcare.

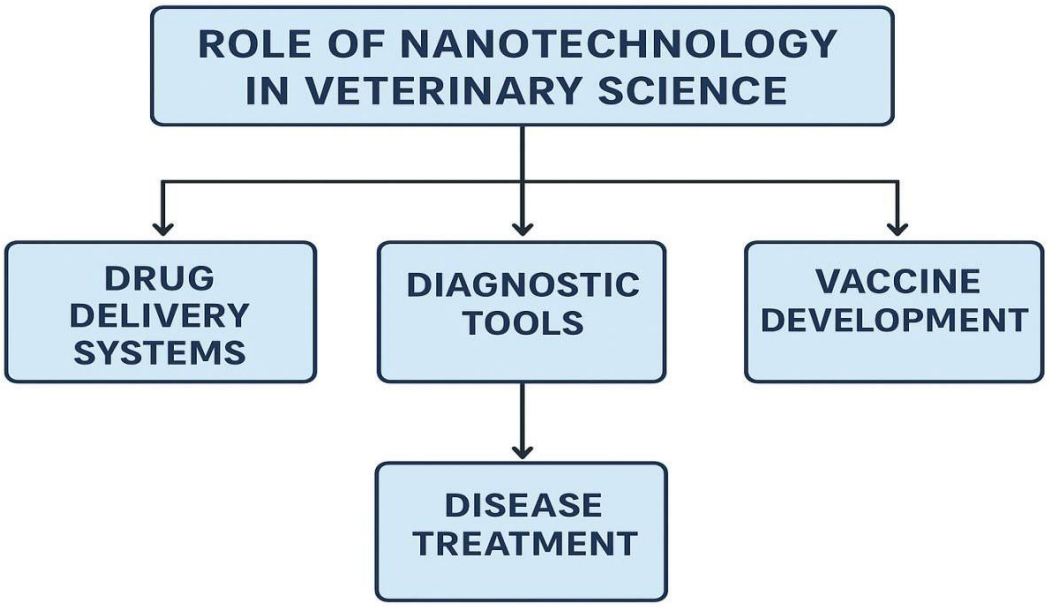
Subsequent discussions will focus on specialized applications, such as the use of nanomaterials in advanced imaging, vaccine adjuvants, and antimicrobial technologies, emphasizing their extensive potential. Nanotechnology is also being explored in veterinary nutrition and dietary supplementation to improve bioavailability and protect sensitive bioactive ingredients (Jampílek et al., 2019). In diagnostic applications, nanoscale materials have demonstrated exceptional specificity and sensitivity, particularly in detecting aquatic pathogens and pollutants, due to their large reactive surface area (Elgendy et al., 2024). In aquaculture, nanomaterials have proven to be effective immunostimulants, boosting disease resistance in fish (Elgendy et al., 2024), and they are instrumental in combating antibiotic resistance through targeted antimicrobial delivery and alternative therapies (Okeke et al., 2022). Nano-based vaccines, such as those developed against *Vibrio anguillarum* and lymphocystis disease virus, show sustained immune responses and promising disease protection in fish (Elgendy et al., 2024).

Furthermore, nanotechnology contributes to reproductive advancements, including improved gamete preservation and enhanced fertility outcomes (Bilgili & Uysal, 2019). Its rapid evolution opens new avenues for addressing major health and productivity issues across species, signaling the advent of precision veterinary medicine. Broader agricultural applications include improving feed conversion, water purification, and next-generation vaccine production (Kumari et al., 2023). Biosensors and nanocarriers are revolutionizing disease surveillance and treatment by enabling earlier diagnosis and more efficient drug delivery (Bhatia, 2017; Sekhon, 2014).

Similarly, cantilever sensors—thin silicon beams coated with DNA—facilitate simultaneous identification of multiple DNA sequences, dramatically enhancing diagnostic precision (Kher et al., 2016). The integration of quantum dots as fluorescent probes has further improved biosensor photostability and multiplexing capability, allowing for rapid multi-pathogen detection (Kumar et al., 2022).

Beyond diagnostics, nanotechnology enables smart packaging for animal products, offering real-time monitoring of freshness and contamination via nanosensors (Raju & Singh, 2014). Innovations such as optical humidity sensors and luminescent CO_2 detectors contribute to food safety and extended shelf life. Incorporating nanomaterials into feed formulations also enhances nutrient uptake and animal health, supporting more sustainable livestock systems (Santos et al., 2023). Moreover, nanostructured materials with unique immunogenic and

structural features hold great potential for developing advanced vaccines and serological assays (Kobayashi et al., 2021).



Literature Review

This section systematically reviews the foundational research and seminal works that underpin the current understanding and application of nanotechnology in veterinary science, critically evaluating the methodologies and findings of key studies. It synthesizes diverse research domains, from materials science and molecular biology to veterinary pathology and pharmacology, illustrating how nanoscale interventions are revolutionizing animal health management. The review also examines the progression of nanotechnology from theoretical concepts to practical applications, highlighting the interdisciplinary collaborations essential for its advancement in veterinary medicine. It delves into the development of novel nanomaterials, their characterization, and their specific utility in addressing prevalent veterinary challenges, such as infectious diseases, reproductive disorders, and drug delivery limitations. Furthermore, it assesses the safety and regulatory considerations associated with the widespread adoption of nanotechnological solutions in animal health, emphasizing the need for robust toxicological evaluations and ethical guidelines. The ethical implications, particularly concerning non-malfeasance, beneficence, autonomy, and justice, are critically examined to ensure responsible integration of these technologies into veterinary practice (Coles & Frewer, 2013). The economic impact and societal acceptance of nanoveterinary products are also considered, providing a comprehensive perspective on the broader implications of these innovative technologies.

This review also explores the molecular mechanisms underlying these effects—ranging from targeted drug delivery and improved bioavailability to heightened diagnostic precision (Malá & Selvaraj, 2018). Nonetheless, the potential cytotoxicity of nanomaterials, particularly at elevated concentrations, must be addressed carefully. For example, while zinc oxide and copper oxide nanoparticles can mitigate mycotoxin-induced toxicity at low levels, excessive doses have been linked to detrimental biological effects (Raza et al., 2023; Onoue et al., 2014). Achieving a balance between efficacy and safety thus necessitates meticulous dose optimization and comprehensive toxicological evaluations in the formulation of nanoveterinary products. Moreover, nanoparticles' adjustable surface chemistry enables precise cellular targeting, reducing off-target interactions and increasing therapeutic efficiency.

The global economic impact of nanotechnology continues to grow, reflecting its expanding adoption across multiple industries, including veterinary science (Dimkpa et al., 2012). For example, integrating nanoparticles—typically less than 100 nm in size—into polymeric matrices has significantly enhanced the mechanical, thermal, and barrier properties of packaging materials, thereby improving food preservation and safety (Raju & Singh, 2014). Furthermore, nanotechnology contributes to improved animal nutrition and feed utilization through nano-encapsulation, which enhances nutrient absorption and targeted gastrointestinal delivery.

Methodology

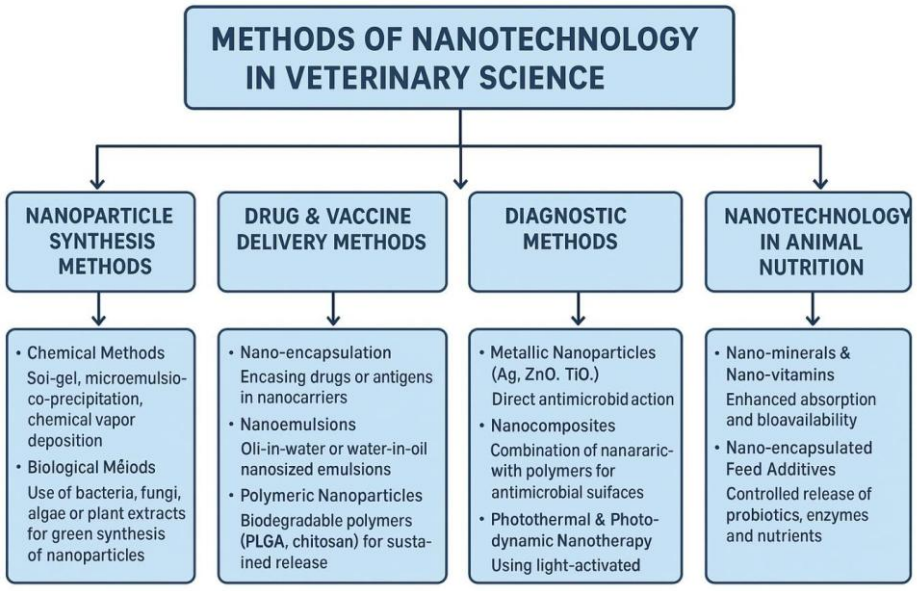
This section describes the systematic methodologies and experimental designs adopted to evaluate the safety and efficacy of nanotechnological applications in veterinary contexts. It outlines detailed protocols for the synthesis and characterization of nanomaterials, along with *in vitro* and *in vivo* experimental studies and comprehensive toxicological assessments to ensure responsible deployment. Special focus is placed on elucidating the pharmacokinetic and pharmacodynamic behaviors of nanomaterials within animal systems using advanced molecular assays and imaging techniques. Computational modeling is also employed to predict nanoparticle interactions and biodistribution at cellular and tissue levels, thereby reducing reliance on animal testing and enhancing translational applicability (Malá & Selvaraj, 2018).

Additionally, the methodology specifies criteria for selecting suitable animal models, accounting for interspecies physiological variations that may affect nanoparticle metabolism, distribution, and excretion. This approach ensures that *in vitro* observations translate effectively into *in vivo* and clinical outcomes, thus reinforcing the reliability of findings (Pandey et al., 2023). Critical investigations include pharmacokinetic profiling of nanoparticle absorption,

distribution, metabolism, and excretion across different species and dosage levels to establish safe and effective therapeutic regimens (Valic & Zheng, 2019).

High-throughput microfluidic systems provide a modern alternative for rapid *in vitro* screening of nanoparticle formulations, allowing early identification of safe, effective candidates while filtering out those with high toxicity risks before proceeding to animal studies (Valencia et al., 2012). This methodology aligns with the 3Rs principles—Replacement, Reduction, and Refinement—by reducing the number of animals used in research while improving predictive accuracy (Ianni et al., 2022; Meng et al., 2018; Lin et al., 2022). Such integrative experimental strategies are vital for generating robust datasets necessary for regulatory approval and widespread implementation of nanotechnology in veterinary practice (Khalil et al., 2023).

Nevertheless, nanoparticle interactions within living systems remain complex and multifactorial, necessitating comprehensive safety evaluations that consider not only conventional toxicological parameters but also molecular and cellular perturbations (Neuer et al., 2023). Integrating *in vitro* and *in vivo* approaches enhances understanding of nanoparticle dosimetry and biological response, ensuring more accurate safety and efficacy predictions (Lin et al., 2022). Although *in vitro* models—such as cell cultures—are valuable for cost-effective, ethical, and high-throughput testing, they cannot fully replicate the intricate physiological conditions of whole organisms (Balakumar & Sugumaran, 2023; Svensson et al., 2023). Consequently, careful interpretation of *in vitro* data is essential, as nanoparticle interference with assay reagents or detection systems can lead to misleading conclusions (Foulkes et al., 2020; Drašler et al., 2017).



Results

In contrast, *in vivo* investigations—though often more resource-intensive and ethically challenging—offer a more realistic depiction of how nanomaterials behave and interact within living organisms. They provide critical insights into nanoparticle biodistribution, accumulation, and long-term toxicological effects within complex physiological environments (Li et al., 2012; Naqvi et al., 2018). Thus, the integration of *in vitro* and *in vivo* methodologies, supported by advanced computational modeling, is essential for a thorough and predictive evaluation of nanomedicine candidates (Charoen et al., 2013; Nikolić et al., 2025). This comprehensive approach expedites the development of innovative nanoveterinary products while ensuring safety and efficacy, thereby facilitating their transition from laboratory research to clinical practice.

The combined framework allows for high-throughput *in vitro* screening of potential nanomaterials, followed by selective *in vivo* testing of promising formulations—optimizing both resources and ethical compliance (Forest et al., 2019; Ianni et al., 2024). Establishing advanced *in vitro* models that closely replicate physiological barriers and cellular microenvironments is vital for improving the predictive accuracy of early-stage testing, particularly regarding nanoparticle uptake, metabolism, and transformation within biological systems (Neuer et al., 2023).

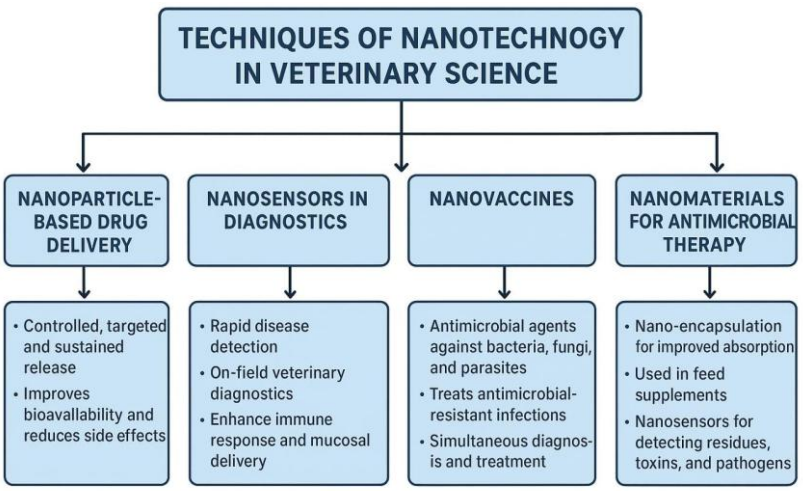
Moreover, computational modeling plays a crucial role in simulating the *in vivo* behavior of nanoparticles based on *in vitro* data, offering a more integrated understanding of their pharmacokinetics, biodistribution, and therapeutic performance (Forest et al., 2019). For example, elucidating the biochemical transformations that inorganic nanomedicines undergo across different biological media—from simple buffers to complex tissue systems—is vital to assessing their stability, bioactivity, and ultimate fate (Neuer et al., 2023). However, correlating *in vitro* bioaccessibility with *in vivo* bioavailability remains a challenge due to the inherent complexity of living systems, which introduces numerous physiological variables absent in controlled laboratory models (Mackie et al., 2020).

To address these discrepancies, more sophisticated *in vitro* platforms are being developed, incorporating dynamic flow systems, co-culture models, and organ-on-a-chip technologies that better mimic physiological conditions (Akbari-Alavijeh et al., 2021). Despite these advancements, *in vitro* and *in silico* methods still face limitations in accurately predicting complex *in vivo* interactions, as they cannot fully reproduce the multifactorial dynamics of biological systems (Burden et al., 2017; Paunovska et al., 2018; Forest et al., 2019).

Consequently, while *in vitro* assays are indispensable for preliminary screening, evaluating nanoparticle absorption, biodistribution, and passage across biological barriers often requires advanced *in vivo* models tailored for these specific endpoints (Pandey et al., 2023).

This is particularly critical for understanding the capacity of nanoconstructs to cross physiological barriers, a phenomenon that remains difficult to model precisely in simplified *in vitro* systems (Carton & Malatesta, 2022). Therefore, continuous refinement of *in vitro* technologies is essential to improve the predictive accuracy of preclinical testing (Brajša, 2016). Integrating artificial intelligence (AI) and machine learning (ML) into these systems represents a promising frontier, enabling large-scale data analysis from multiple experimental platforms to better predict nanoparticle pharmacokinetics and reduce dependency on animal testing (Khakpour et al., 2025).

This shift toward data-driven modeling and simulation is pivotal for accelerating the translation of nanomedicines into viable veterinary applications (Forest et al., 2019). A significant area of ongoing development involves creating *in vitro* models that replicate the complex gastrointestinal environment, improving predictions of nanomaterial dissolution, absorption, and bioavailability—key factors for the design of orally administered nanoveterinary formulations (Cristo et al., 2023). Additionally, advancements in three-dimensional (3D) organoid systems and microfluidic technologies are revolutionizing preclinical testing by providing more physiologically relevant models that bridge the gap between conventional 2D cultures and whole-organism studies (Mazzarella & Curigliano, 2018; Svensson et al., 2023). These 3D models recapitulate native tissue architecture and cellular interactions more accurately, offering deeper insights into nanoparticle behavior under biomimetic conditions (Urzì et al., 2023; Law et al., 2021).



Discussion

Employing such sophisticated 3D systems effectively overcomes the inability of 2D cultures to replicate true tumor microenvironments, improving the precision of anticancer drug screening and biological assessments (Ferreira et al., 2018). These models more accurately

represent cellular organization, drug response, and tissue architecture, capturing the complexity of 3D tumor environments and minimizing off-target effects that are commonly missed in 2D systems (Law et al., 2021; Haykal et al., 2020). The transition toward 3D models marks a paradigm shift in drug discovery, providing more predictive and physiologically relevant insights into drug efficacy and safety profiles by faithfully mimicking *in vivo* conditions (Ye & Eglen, 2017; Taylor et al., 2020). This accuracy is particularly crucial as a large proportion of preclinical candidates fail during clinical trials due to the limitations of conventional 2D models (Henslee et al., 2020; Abbas et al., 2023), underscoring the need for more representative preclinical systems—an unmet requirement effectively addressed by 3D cultures (Lv et al., 2017).

Conclusion

The evolution of 3D cell culture technologies has facilitated the creation of *in vitro* systems that better reproduce physiological and tissue-specific microenvironments, overcoming the shortcomings of previous models and enhancing predictive clinical outcomes (Law et al., 2021). These models more accurately mimic the complex cellular interactions, extracellular matrix composition, and biochemical gradients inherent to native tissues, providing robust platforms for studying disease mechanisms and evaluating therapeutic strategies (Mazrouei et al., 2020; Zoetemelk et al., 2019). Their superior biomimicry greatly improves the translational reliability of *in vitro* studies, contributing to more successful clinical applications and faster therapeutic innovation. Moreover, the use of patient-derived cells in 3D cultures opens avenues for personalized medicine, allowing for pre-screening of drug responses based on individual disease profiles to optimize treatments and reduce adverse reactions (Law et al., 2021).

Despite these advantages, widespread adoption of 3D culture systems remains challenging due to issues in assay standardization, reagent penetration, and analytical compatibility (Urzì et al., 2023; Law et al., 2021). Further investigation is essential to refine assay protocols and ensure consistent reagent delivery across complex 3D constructs to fully exploit their potential in preclinical development and personalized therapy (Svensson et al., 2023). Additionally, the technical complexity and cost of maintaining such models limit their accessibility to many laboratories. Nevertheless, ongoing progress in automation and protocol standardization is expected to facilitate broader implementation, enabling more accurate drug discovery and patient-specific testing (Langhans, 2018). However, challenges persist regarding scalability and reproducibility, particularly in models using patient-derived cells, due to heterogeneity and limited material availability for high-throughput applications (Corallo et al.,

2020). The relatively high costs and specialized infrastructure required also hinder widespread use, necessitating efforts to reduce expenses and simplify workflows for broader adoption (Radajewska et al., 2021). Continued improvements in validation protocols, culture optimization, and analytical imaging will be critical for advancing 3D culture systems as reliable, standardized tools in modern biomedical research (Carragher et al., 2018).

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