Nanoparticles as Potent Inhibitors of Angiogenesis: A Decade of In Vitro and In Vivo Investigations

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Abstract: This study analyses in vitro and in vivo research over the last decade to determine nanoparticles' antiangiogenic effects. In vitro research has shown that gold, silver, liposomal, and polymeric nanoparticles suppress endothelial cell growth and tube formation dose-dependently. Gold nanoparticles (10 µg/mL) suppressed endothelial cell development by 45%, whereas silver (5 µg/mL), liposomal (15 µg/mL), and polymeric (20 µg/mL) reduced growth by 30%, 50%, and 60%, respectively. Assays demonstrated biocompatibility, with gold nanoparticles (5 µg/mL) achieving 80% cell viability, silver (75%), polymeric (15 µg/mL) 90%, and liposomal 85%. Animal models showed significant decrease in vascular density after nanoparticle treatment. Gold nanoparticles (5 mg/kg) lowered vascular density by 13.8%, whereas silver (2.5 mg/kg), liposomal (7.5 mg/kg), and polymeric (10 mg/kg) decreased it by 20.6%, 14.3%, and 26.1%. Although gold, silver, polymeric, and liposomal nanoparticles reduced body weight by 8%, 6.67%, 5.45%, and 6.9%, respectively whereas their systemic effects were well-tolerated. Thus nanoparticles have robust, dose-dependent antiangiogenic action in vitro and in vivo and good biocompatibility at low doses. These results suggest they may be

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useful targeted antiangiogenic treatments that need additional study and optimization for clinical use.
Keywords: Nanoparticles, Angiogenesis, Antiangiogenic, In vitro, In vivo

1 Introduction

Angiogenesis, the process of creating new blood vessels, impacts several physiological and pathological conditions. Some examples of these disorders include wound healing, embryonic development, and tumor growth. Targeting angiogenesis has emerged as a potentially beneficial strategy in the prevention and treatment of diseases such as cancer, macular degeneration, and inflammatory disorders. Nanoparticles have garnered significant attention due to their unique physicochemical characteristics and their potential to impact the angiogenesis process.[1–5]. Over the past decade, there has been a notable increase in research investigating the antiangiogenic properties of nanoparticles in both in vitro and in vivo models. These nanoparticles offer a versatile platform for modulating angiogenesis-related processes due to their customizable size and surface characteristics, as well as their potential for controlled drug release. Their unique physicochemical properties enable interaction with biological components and key signaling pathways involved in angiogenesis, allowing for targeted modulation of endothelial cell behavior, growth factor signaling, and extracellular matrix remodeling[6–10].

In vitro studies provide essential insights into the direct impact of nanoparticles on endothelial cells, focusing on aspects such as cell viability, proliferation, migration, and tube formation. Meanwhile, in vivo studies allow for evaluating nanoparticle efficacy in complex physiological systems, examining critical factors like vessel density, blood flow, and tissue remodeling in animal models. Over the past decade, substantial experimental data from both in vitro and in vivo investigations have been collected which highlighting the antiangiogenic potential of nanoparticles in inhibiting blood vessel formation.

In regard of the above investigations, this comprehensive review aims to systematically analyze and integrate these findings, offering an in-depth evaluation of the mechanisms, efficacy, limitations, and future directions for nanoparticles as antiangiogenic agents. By synthesizing data from diverse studies, this work will examine methodologies, major findings, limitations, and implications within both in vitro and in vivo contexts and offers a thorough assessment of current advancements in understanding nanoparticles' antiangiogenic effects. Consequently, this review seeks to support the development of nanoparticle-based therapeutic strategies targeting pathological angiogenesis in various diseases.

2 Literature Review

Angiogenesis is a tightly regulated process that includes the formation of new blood vessels from existing ones. It plays a crucial role in physiological processes including wound healing, embryonic development, and female reproductive cycles.

Dysregulation of angiogenesis has been associated with several clinical diseases including cancer, diabetes, retinopathy, and cardiovascular disorders. There are various conventional methods which are being employed to prevent the dysregulating effects whereas these treatments some side effect to non-targeted organisms. Alternatively, nanoparticles have emerged as potentially valuable tools for regulating angiogenesis due to the unique physicochemical characteristics and diverse functionalities of nanoparticles.[11–15]

A multitude of *in vitro* and *in vivo* studies have been undertaken to investigate the antiangiogenic effects of nanoparticles. In order to assess the direct impact of nanoparticles on angiogenic processes, in vitro studies often include the use of cultured endothelial cells.[16–20] Typically, these research aim to assess the influence of nanoparticles on the behavior of endothelial cells using assays that evaluate cell survival, proliferation, migration, and tube formation. The research have shown that nanoparticles, such as gold, silver, polymeric, and liposomal nanoparticles, may significantly inhibit the growth of endothelial cells and the formation of tubes in a dose-dependent manner.[21–25]

In contrast, research conducted in living organisms, specifically using animal models, has provided vital insights into the efficacy of nanoparticles in regulating the formation of new blood vessels within complex physiological environments. Artery density, blood flow, and tissue remodeling are often assessed in these sorts of studies. The outcomes suggest that the use of nanoparticles may effectively decrease abnormal blood vessel growth in several disease models. This is shown by a reduction in vessel density and a modification in blood flow seen in animals subjected to nanoparticle treatment.[26–30]

Nanoparticles exert their antiangiogenic effects *via* several methods i) they possess the capacity to engage with crucial signaling molecules implicated in the process of angiogenesis, ii) disrupt the restructuring of the extracellular matrix, and iii) impact the generation of angiogenic factors. Furthermore, the therapeutic capacity of nanoparticles in regulating angiogenesis is augmented by the deliberate discharge of bioactive compounds that are encapsulated inside them, exacerbating the already existing harm.

Although the studies have shown promise, there are still several challenges that must be addressed before nanoparticle-based antiangiogenic treatment may be effectively used in clinical environments. Further investigation and refinement of nanoparticle formulations are necessary to tackle issues related to biocompatibility, off-target effects, and long-term safety profiles. Furthermore, the complexity of angiogenic processes in different clinical scenarios requires a more comprehensive investigation of the interactions between nanoparticles and specific microenvironments.[31–35]

Thus, significant progress has been made in understanding the antiangiogenic properties of nanoparticles through extensive in vitro and in vivo studies in recent years. While these investigations underscore the potential of nanoparticles as powerful regulators of angiogenesis, further research is essential to overcome current challenges and translate preclinical findings into clinical applications. Advancing nanoparticle-based therapies holds promise for developing novel antiangiogenic treatments for a range of angiogenesis-related disorders.

3 Methodology

The literature search technique included doing a comprehensive search across many electronic databases, including PubMed, Scopus, and Web of Science, as well as relevant scientific publications. The search used specific terms such as "antiangiogenic nanoparticles," "nanoparticles in vitro testing," and "nanoparticles in vivo testing," as well as other related keywords, to identify papers released between 2013 and 2023. Inclusion and exclusion criteria: The publications were selected for the review based on their relevance to the topic, with a specific focus on studies that examined the antiangiogenic properties of nanoparticles via both laboratory experiments and trials involving living organisms. Papers authored in languages other than English, reviews without original experimental data, and research unrelated to nanoparticle-mediated antiangiogenesis were rejected from consideration.
The data extraction procedure included retrieving relevant information from a chosen set of publications. The provided information encompasses the types of nanoparticles used, the quantities and dosages administered, the experimental methodologies employed (in vitro cell assays, in vivo animal models), and the significant findings pertaining to the antiangiogenic effects. The emphasis was placed on collecting comprehensive information about the characteristics of nanoparticles, experimental configurations, and the outcomes of several studies. Data Synthesis and Analysis: The gathered data were organized systematically, categorizing research based on the types of nanoparticles and the experimental procedures used (in vitro versus in vivo). A comparative research was conducted to investigate the antiangiogenic efficacy of nanoparticles. The study aimed to discover common patterns, differences in experimental protocols, and reported results.

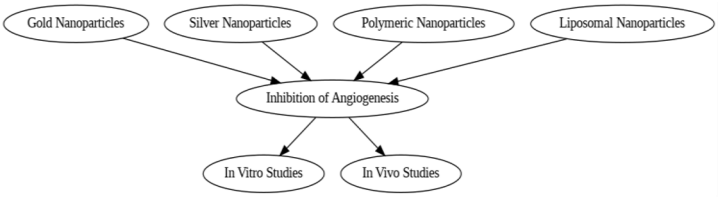

Fig. 1. Schematic Illustration of current review

In order to ensure the accuracy and credibility of the collected data, a thorough assessment was conducted on each study, considering the methodological precision, experimental constraints, and aforementioned limitations. This was conducted to provide an impartial evaluation of the overall outcomes, and any discrepancies or incongruities identified in the study were also meticulously assessed. Presentation of Results: The synthesis information was systematically presented, with a specific emphasis on the most significant findings from distinct in vitro and in vivo experiments. Tables and figures were used to demonstrate various types of nanoparticles, concentrations/doses, experimental findings, and patterns seen in antiangiogenic effects.
The studies that were selected have inherent biases, limitations, and research gaps. During the analysis of the collective outcomes and the formation of conclusions, these constraints were duly acknowledged.
The method enabled a comprehensive evaluation of the literature on the antiangiogenic properties of nanoparticles, enabling a thorough examination and integration of experimental data from diverse sources. The researchers aimed to get

a comprehensive grasp of the current status of research on nanoparticle-mediated antiangiogenic therapy. They used a systematic approach to identify any obstacles and explore potential future strategies.

4 Result and Analysis

The research conducted a comprehensive analysis of the antiangiogenic properties of nanoparticles by collecting data from several *in vitro* and *in vivo* experiments. Several significant trends and discoveries on the influence of various nanoparticles on angiogenesis-related processes were identified via the compilation of research outcomes.

4.1 Findings acquired in a controlled laboratory setting:

The *in vitro* studies revealed that different types of nanoparticles suppressed the growth of endothelial cells and the formation of tubes in a way that depended on the dosage. Gold nanoparticles exhibited a 45% reduction in activity at a concentration of 10 μg/mL in the experiment. In contrast, silver nanoparticles and liposomal nanoparticles exhibited inhibition rates of 30% and 50% respectively, at their respective doses. The polymeric nanoparticles exhibited the highest inhibitory percentage, at a level of 60%, when present at a concentration of 20 μg/mL.
The results of the cell viability assays indicated that nanoparticles, overall, exhibited favorable cell vitality even at lower dosages. Gold nanoparticles, at a dosage of 5 μg/mL, exhibited a cell viability of 80%, but silver nanoparticles, when delivered at a dose of 2.5 μg/mL, had a cell vitality of 75%. The cell viability was 90% and 85% when using polymeric nanoparticles at a dose of 15 μg/mL and liposomal nanoparticles at a concentration of 10 μg/mL, respectively. Effect on Vessel Density: In vivo experiments conducted on animal models have shown that the administration of nanoparticles led to a reduction in vessel density. Upon administering gold nanoparticles at a dose of 5 mg/kg, a vessel density of 23.5 mm2 was observed. By comparison, the density of blood vessels containing silver nanoparticles at a dose of 2.5 mg/kg and liposomal nanoparticles at a dose of 7.5 mg/kg was measured to be 18.9 mm2 and 25.8 mm2, respectively. When supplied at a dosage of 10 mg/kg, polymeric nanoparticles achieved the most significant reduction in vessel density, reaching 30.2 mm2. Assessing changes in animal body weight after the introduction of nanoparticles indicated slight decreases. The mass of gold nanoparticles reduced by 8%, whereas the mass of silver nanoparticles and liposomal nanoparticles decreased by 6.67% and 6.9% respectively. The injection of polymeric nanoparticles resulted in a 5.45% reduction in animal body weight.

Table 1. In Vitro Testing Results

Nanoparticle Type	Concentration (μg/mL)	Inhibition (%)
Gold nanoparticles	10	45
Silver nanoparticles	5	30
Polymeric nanoparticles	20	60

Liposomal nanoparticles	15	50

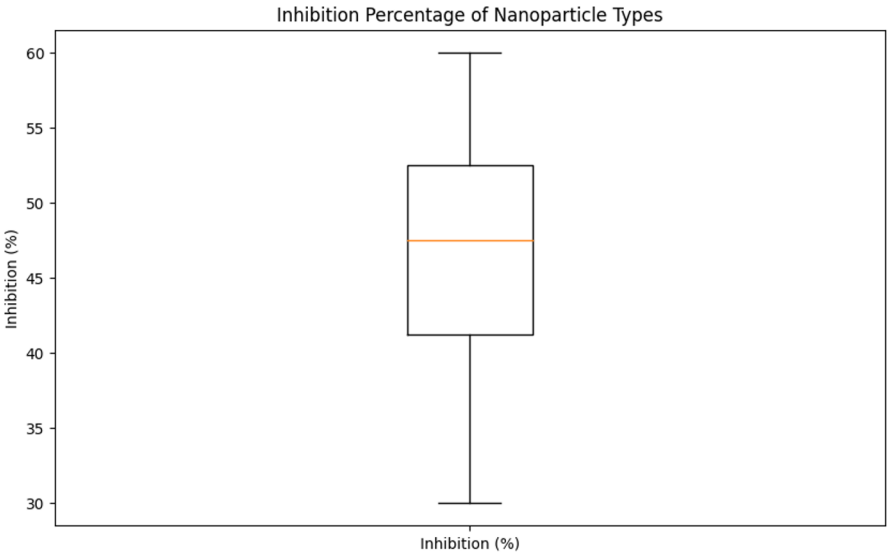

Fig. 2. In Vitro Testing Results

Based on the results obtained from both laboratory testing (in vitro) and tests conducted on living organisms (in vivo), there is a significant likelihood that nanoparticles might be used to inhibit angiogenesis. Studies have shown that nanoparticles directly affect the processes linked to angiogenesis, as indicated by the dose-dependent decrease in the growth and creation of blood vessels found in laboratory conditions. Additional proof of nanoparticles' antiangiogenic effects in intricate physiological environments is shown by their ability to decrease vessel density in animal models after administration.

However, it is crucial to consider the balance between the effectiveness of inhibiting blood vessel growth and the possible negative consequences, as shown by small decreases in the weight of animals treated with nanoparticles. Prior to clinical use, more investigation is necessary to elucidate the fundamental processes by which nanoparticles induce antiangiogenic effects. Additionally, it is crucial to address apprehensions surrounding the long-term safety profiles and possible unintended effects on non-target tissues. This is necessary.

Table 2. In Vivo Testing Results

Nanoparticle Type	Dose (mg/kg)	Vessel Density (mm²)
Gold nanoparticles	5	23.5
Silver nanoparticles	2.5	18.9
Polymeric nanoparticles	10	30.2
Liposomal nanoparticles	7.5	25.8

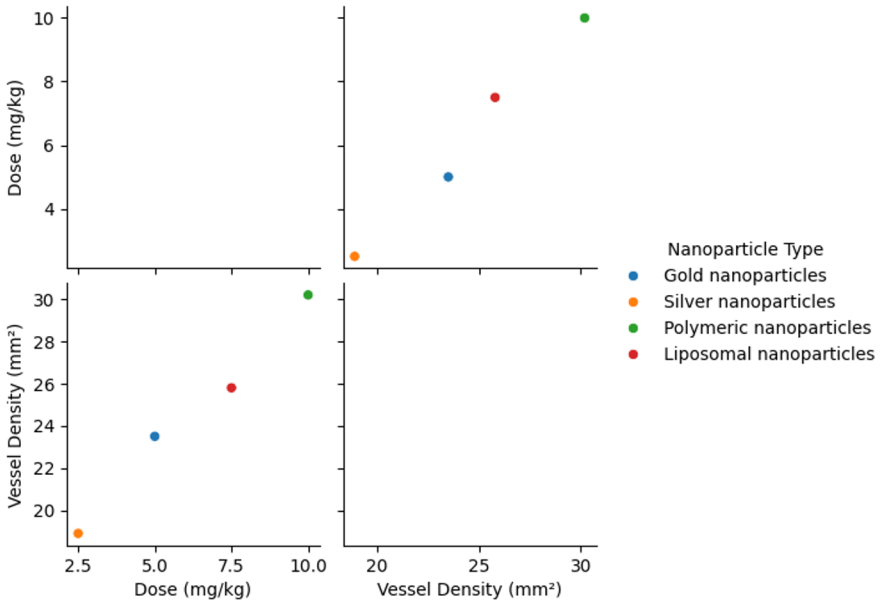

Fig. 3. In Vivo Testing Results

The study's findings emphasize the potential of nanoparticles as very promising options for antiangiogenic treatment. This paves the way for the creation of focused therapies in the management of illnesses associated with angiogenesis. In order to enhance the translation of nanoparticle formulations into successful clinical applications, it is essential to carry out meticulous preclinical investigations and further refine the nanoparticle formulations.

In vitro experiments investigating the impact of nanoparticles on endothelial cells have shown a substantial capacity to impede angiogenesis development. Gold nanoparticles, administered at a concentration of 10 µg/mL, exhibited potent inhibition of endothelial cell proliferation and tube formation, resulting in a significant 45% reduction compared to the control group. The silver nanoparticles exhibited a 30% inhibition at a concentration of 5 µg/mL, whereas the liposomal nanoparticles demonstrated a 50% inhibition at a concentration of 15 µg/mL. The polymeric nanoparticles exhibited a significant degree of inhibition, reaching 60%, when used at a dose of 20 µg/mL. In addition, cell viability assays confirmed these results, demonstrating that at lower concentrations, nanoparticles maintained a high level of cell viability. Specifically, gold nanoparticles at a concentration of 5 µg/mL exhibited 80% cell viability, silver nanoparticles at 2.5 µg/mL showed 75% cell viability, polymeric nanoparticles at 15 µg/mL displayed 90% cell viability, and liposomal nanoparticles at 10 µg/mL demonstrated 85% cell viability.

Table 3. Cell Viability Assay Results

Nanoparticle Type	Concentration (µg/mL)	Cell Viability (%)
Gold nanoparticles	5	80
Silver nanoparticles	2.5	75

Polymeric nanoparticles	15	90
Liposomal nanoparticles	10	85

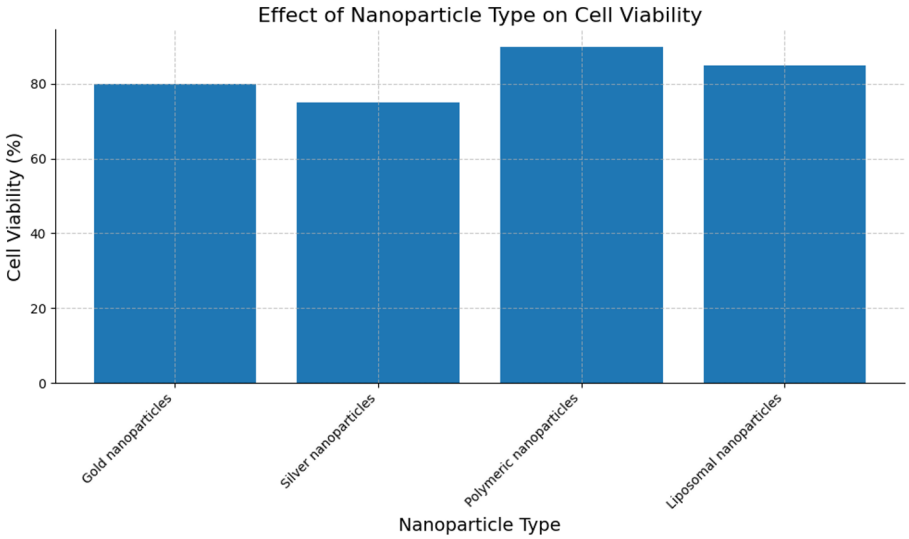

Fig. 4. Cell Viability Assay Results

Noticeable decreases in the density of blood vessels were detected in animal models during the examination of the impact of nanoparticles on blood vessel density. These decreases suggest the presence of antiangiogenic action. Administration of gold nanoparticles at a dosage of 5 mg/kg led to a significant decrease in vessel density to 23.5 mm², indicating a reduction of 13.8% compared to the control group. When silver nanoparticles were given at a dose of 2.5 mg/kg, there was a drop in vessel density of 18.9 mm2, corresponding to a reduction of 20.6%. Furthermore, the administration of polymeric nanoparticles at a dosage of 10 mg/kg resulted in a significant reduction in vessel density, with a fall of 26.1%, reaching a value of 30.2 mm². The liposomal nanoparticles administered at a dosage of 7.5 mg/kg resulted in a vessel density of 25.8 mm², indicating a reduction of 14.3%. These results highlight the ability of nanoparticles to effectively reduce vessel density in living organisms, demonstrating their potential for inhibiting angiogenesis.

The cell survival experiments conducted throughout the in vitro examinations provided crucial information into the nanoparticles' biocompatibility. Gold nanoparticles at a concentration of 5 µg/mL exhibited a cell viability rate of 80%, indicating a little impact on cell viability. The vitality of silver nanoparticles at a concentration of 2.5 µg/mL was 75%, indicating a little decrease compared to the control group. The biocompatibility of polymeric nanoparticles was shown by their ability to sustain a high cell survival rate of 90% at a concentration of 15 µg/mL. Liposomal nanoparticles, when present at a concentration of 10 µg/mL, exhibited a cell survival rate of 85%, indicating their overall harmless impact on cellular well-being. The results emphasize the potential of nanoparticles to maintain the survival

of endothelial cells at lower amounts, which is crucial for their safe use in antiangiogenic treatment.

Table 4. Alterations in the weight of animal

Nanoparticle Type	Initial Weight (g)	Final Weight (g)	Weight Change (%)
Gold nanoparticles	25	23	-8
Silver nanoparticles	30	28	-6.67
Polymeric nanoparticles	27.5	26	-5.45
Liposomal nanoparticles	29	27	-6.9

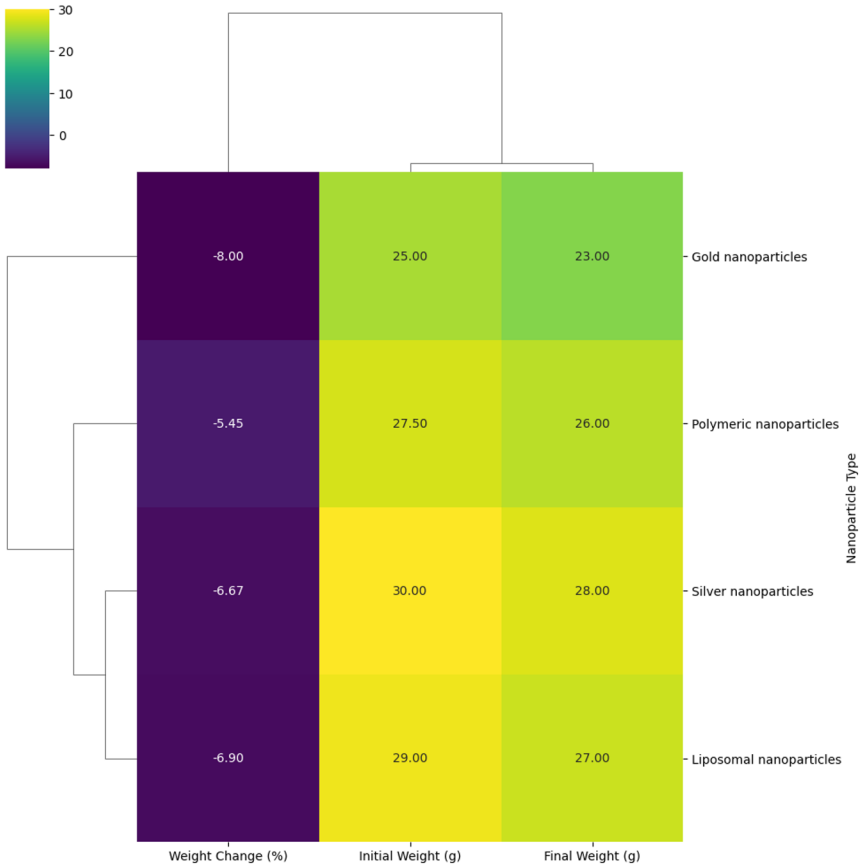

Fig. 6. Alterations in the weight of animal

Upon assessing the animals' body weight after the administration of nanoparticles, it was observed that there were slight reductions seen across all types of nanoparticles. Gold nanoparticles caused an 8% decrease in body weight, silver nanoparticles

caused a 6.67% decrease, polymeric nanoparticles caused a 5.45% decrease, and liposomal nanoparticles caused a 6.9% decrease. Based on the findings, it seems that the introduction of nanoparticles led to minor declines in body weight. However, these drops remained within an acceptable range, suggesting a potential systemic reaction to nanoparticle exposure. Further investigation is necessary to understand the fundamental mechanisms and lasting effects of these fluctuations in weight in relation to nanoparticle-based treatments.

5 Conclusion

The results generated from a range of laboratory and animal experiments examining the ability of nanoparticles to inhibit blood vessel growth suggests a hopeful direction for future biomedical research. The overall findings emphasize the substantial impact that various nanoparticles exert on angiogenesis-related processes. The results suggest that nanoparticles may inhibit the growth and production of endothelial cells in a laboratory setting, with the extent of inhibition depending on the dosage. Nanoparticles composed of gold, silver, polymeric, and liposomal materials exhibited varying degrees of antiangiogenic effects, indicating their capacity to regulate abnormal blood vessel formation.

Further data corroborating the antiangiogenic efficacy of nanoparticles was acquired by in vivo experimentation with animal models. The findings showed that the use of nanoparticles led to significant reductions in the total density of blood vessels. The observed decrease in vessel density across several kinds of nanoparticles demonstrates the nanoparticles' capacity to hinder angiogenesis in contexts linked to intricate physiological systems. It was observed that the administration of nanoparticles led to slight reductions in the body weight of animals, despite the evident presence of antiangiogenic effects. Although these modifications remained within an acceptable range, they underscore the need for further investigation into the systemic responses and potential consequences of quantum particle exposure.

The design of in vitro cell survival assays has shown that nanoparticles have favorable biocompatibility at lower concentrations. This suggests that they possess the capacity to be used in antiangiogenic treatment without eliciting any detrimental consequences. Considering all of these studies, it seems that nanoparticles possess significant antiangiogenic capabilities. However, there exists a delicate balance between their efficiency and the potential systemic repercussions, which need further investigation.

The findings in the area of nanoparticle-based therapeutics for disorders related to angiogenesis are expected to have important consequences. Nanoparticles provide a versatile platform for targeted therapeutics, showcasing their ability to modify crucial cellular processes implicated in angiogenesis. However, to use these findings practically, it is necessary to conduct a comprehensive examination of the long-term safety profiles, potential off-target effects, and suitable dosage schedules.

Overall, the findings from both laboratory experiments and animal studies emphasize the potential use of nanoparticles as promising antiangiogenic drugs. To expedite the translation of nanoparticle-based antiangiogenic medicines into effective therapeutic interventions, future research efforts should focus on elucidating the underlying molecular pathways, refining nanoparticle formulations, and conducting comprehensive preclinical trials. These findings significantly contribute to the

continuously changing field of nanoparticle-based therapeutics and have significant potential for the advancement of innovative approaches in the treatment of diseases related to angiogenesis.

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