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## Study on the Immune Activity of Mice *In vitro* and *In vivo* with Nano-Material Adjuvant

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**Abstract:** This paper investigates the effects of graphene quantum dots and mesoporous silica as nanomaterial adjuvants on immune activity in mice *in vitro* and *in vivo*. The two materials have distinct properties; graphene quantum dots possess unique optical and electrical characteristics, while mesoporous silica features a regular pore structure. *In vitro* experiments show differences in their effects on immune cell activation and cytokine secretion; *in vivo* experiments reveal varying performances in antibody production and immune cell function regulation. Their mechanisms of action and safety profiles also differ, offering distinct advantages in application prospects. These two nanomaterial adjuvants provide new directions for the development of immunology, warranting further exploration.

**Keywords:** Graphene quantum dots; Mesoporous silica; Nanomaterial adjuvant; Immune activity; Immune regulation

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### 1. Introduction

In modern biomedical research, the regulation of immune activity is of great significance for disease prevention and treatment. Adjuvants, as key components to enhance immune responses, have always been a hot topic in immunology. Although traditional adjuvants have some effect, they come with drawbacks such as significant side effects and limited immune enhancement. In recent years, with the rapid development of materials science, nanomaterials have shown great potential in the field of adjuvants due to their unique nanoscale effects, high specific surface area, and good modifiability. Graphene quantum dots and mesoporous silica are research hotspots in recent years. Graphene quantum dots exhibit unique optical and electrical properties due to their distinctive physical and chemical characteristics, which are related to immune function. Mesoporous silica features regular pore structures, appropriate pore sizes, large specific surface areas, excellent surface chemical properties, and easy modification,

making it a promising new method for immune regulation. This article provides an in-depth analysis of the effects of nanomaterial adjuvants on immune activity both inside and outside mice, as follows.

## **2. Research progress on graphene quantum dots as adjuvants**

### **2.1. Characteristics of graphene quantum dots**

Graphene quantum dots range in size from 1 to 100 nanometers. Their extremely small particle size endows them with a large specific surface area, facilitating extensive interactions with the external environment. Common functional groups on their surfaces include carboxyl ( $-\text{COOH}$ ) and hydroxyl ( $-\text{OH}$ ) groups. These functional groups alter their charge characteristics in aqueous solutions, ensuring dispersion stability while serving as key sites for chemical modification. For example, carboxyl groups can be linked to amino-containing biomolecules through condensation reactions, enabling bio-functional modifications [1].

In terms of optics, graphene quantum dots exhibit excellent fluorescence performance. Their fluorescence emission wavelength can be altered through size control and surface modification, facilitating biological imaging and providing a visual approach to study their immune-regulating effects. In electrical properties, they have good conductivity, and their unique electronic structure features quantum confinement and edge effects. These electrical characteristics may interfere with the ion channels of immune cells, affecting intracellular signal transduction and thus regulating immune cell function.

### **2.2. Effects on in vitro immune activity of mice**

Macrophages, as the forefront of immunity, can take up graphene quantum dots through phagocytosis. Their surface scavenger receptors and Fc receptors play a crucial role in recognition and binding [2]. In terms of lymphocytes, T cell receptors (TCRs) of T lymphocytes interact with antigen-quantum dot complexes processed by antigen-presenting cells, triggering immune responses; B lymphocytes can directly recognize and bind to antigen-modified graphene quantum dots via their surface antigen receptors.

After macrophages take up graphene quantum dots, their volume increases, pseudopodia become more numerous and elongated, significantly enhancing phagocytic capacity. For lymphocytes, graphene quantum dots can promote T lymphocyte proliferation, accelerate the cell cycle, and allow more cells to enter the S phase and G2/M phase; they also induce B lymphocytes to differentiate into plasma cells, enhancing antibody secretion capacity [3].

As an important T-cell growth factor, the secretion of IL-2 is significantly increased under the influence of graphene quantum dots. This is due to its activation of the JAK-STAT signaling pathway within T lymphocytes, promoting the transcription and expression of the IL-2 gene. TNF- $\alpha$  has multiple biological activities; graphene quantum dots can stimulate macrophages and T lymphocytes by activating the NF- $\kappa$ B signaling pathway, enhancing the expression of the TNF- $\alpha$  gene and increasing its concentration in the cell culture supernatant [4].

Chemokines are crucial for the migration and recruitment of immune cells. Studies have shown that graphene quantum dots can induce immune cells to secrete various chemokines, such as CXCL8 (i.e., IL-8), which attract neutrophils and T lymphocytes to migrate towards inflammatory sites, enhancing immune defense. Additionally, graphene quantum dots influence the secretion of immune regulatory molecules like interferons (IFNs). Changes in IFN secretion can have profound effects on the body's immune state.

### **2.3. Effects on immune activity in mice**

In animal models, combining graphene quantum dots with antigens can significantly enhance the production of specific antibodies. The primary mechanism involves activating B cell antibodies by improving antigen-presenting cells' uptake, processing, and presentation of antigens. Enzyme-linked immunosorbent assays show that, compared to using only the antigen group, the antibody titer in the experimental group is notably increased, and it also exhibits a certain affinity for the produced antibodies [5].

Research shows that graphene quantum dots can regulate the distribution and function of T lymphocyte subsets. Immune adjuvants can increase the number of CD4+ T cells, which secrete large amounts of cytokines such as interleukin-4 and interleukin-10. The function of CD8+ T cells is also enhanced, improving their ability to kill target cells, playing a crucial role in antiviral and anti-tumor activities. Fluorescence labeling tracking revealed that during the initial administration, graphene quantum dots primarily accumulate in the liver and spleen [6], where macrophages are abundant. Over time, some gradually spread to other organs such as the lungs and kidneys. By imaging and quantitatively analyzing fluorescence at different time points in various organs, the dynamic distribution process within the body was clarified.

Currently, the metabolic pathways of graphene quantum dots in mice have not been fully elucidated. It is hypothesized that they may be phagocytosed by macrophages and enter lysosomes, where lysosomal enzymes degrade them. However, due to their high chemical stability, degradation is slow. Studies show that the clearance time in mice is relatively long, potentially taking several weeks or even months, which is crucial for evaluating their safety as an adjuvant and assessing long-term effects.

## **3. The research progress of mesoporous silica as an adjuvant**

### **3.1. Characteristics of mesoporous silica**

Mesoporous silica has a highly regular pore structure, with pore sizes mainly ranging from 2 to 50 nm. This precisely tunable pore structure makes it suitable for loading various types of biomacromolecules. The material boasts an excellent specific surface area (ranging from hundreds to thousands of square meters per gram), which not only effectively loads biomolecules but also enhances their interaction with the external environment. When loaded with antigens, a large number of antigen molecules can be adsorbed on the surface and inside the pores, forming stable complexes that provide a favorable environment for subsequent immune responses [7].

Mesoporous silicene is rich in silanol groups (Si-OH), which endow it with excellent hydrophilicity, facilitating its dispersion within biological systems. Silanol is a crucial site for surface modification; through chemical methods such as silanization, different functional groups can be introduced into the material. These functional groups can be targeting ligands, such as antibody fragments or transmembrane peptides, enabling specific binding to the surface of immune cells; they can also be cytokines or immunomodulators that regulate immune responses, achieving precise modulation of immune activity.

### **3.2. Effects on the in vitro immune activity of mice**

The process and mechanism of mesoporous silica being taken up by immune cells: Immune cells primarily rely on endocytosis to take up mesoporous silica. Phagocytes, such as macrophages, engulf larger mesoporous silica particles

through their phagocytic activity; whereas non-phagocytic cells, like lymphocytes, mostly complete the uptake via endocytosis mediated by gridiron proteins or caveolae proteins. In this process, the functional groups on the surface of mesoporous silica play a crucial role. For example, positively charged groups can interact electrostatically with the negatively charged cell membranes of immune cells, enhancing uptake efficiency [8].

At appropriate concentrations, mesoporous silica has a minimal effect on the survival of immune cells but can promote their proliferation. Taking lymphocytes as an example, it can facilitate their entry into the S phase and G2/M phases of the cell cycle, accelerating DNA synthesis and cell division. This promoting effect is closely related to the surface chemical properties of mesoporous silica and the immune modulatory molecules it carries. When loaded with proliferative cytokines, it significantly enhances the proliferation capacity of lymphocytes, thereby intensifying the immune response.

Mesoporous silica can influence multiple signaling pathways within immune cells. In macrophages, it can activate the NF- $\kappa$ B signaling pathway. After being taken up by macrophages, its surface components interact with pattern recognition receptors within the cell, thereby activating NF- $\kappa$ B and promoting the expression of inflammation-related genes such as TNF- $\alpha$  and IL-1 $\beta$ . In T lymphocytes, mesoporous silica can modulate the TCR signaling pathway, affecting the activation and function of T cells. Its surface-modified molecules can interact with TCR complexes, regulating the phosphorylation levels of downstream signaling molecules, thus controlling the immune activity of T cells [9].

As immune signaling pathways are activated or inhibited, the expression and activity of a series of key signaling molecules change. In the NF- $\kappa$ B signaling pathway, I $\kappa$ B kinase (IKK) is phosphorylated and activated, which then phosphorylates and degrades I $\kappa$ B $\alpha$ , releasing the NF- $\kappa$ B dimer to enter the nucleus and regulate the transcription of related genes. In the TCR signaling pathway, the phosphorylation levels of key signaling molecules such as Src family kinases (Lck, Fyn) change, affecting the activity of downstream adapter proteins and kinases, ultimately impacting T-cell functions like cytokine secretion and cell proliferation.

### 3.3. Effects on immune activity in mice

Animal experiments have shown that combining mesoporous silica with antigens can significantly increase their immunogenicity. Its function is to assist antigen-presenting cells in the uptake, processing, and presentation of antigens, as well as to activate B cells to produce antibodies. Studies have indicated that compared to the control group, the treatment group showed a significant increase in serum antibody titers [10]. Additionally, mesoporous silica also plays a role in modulating the immune microenvironment, regulating antibody type conversion, inducing the generation of specific antibody subtypes, and enhancing the body's immune protection.

The study found that mesoporous silica gel had the effect of improving the activity of immune cells. It could improve the killing ability of CTL and the killing effect of NK cells, promote the proliferation of T and B lymphocytes, increase the total number of immune cells, and enhance the degree of cellular immune response of the body.

Research findings indicate that at conventional doses, silica has almost no significant inhibitory effect on vital organs such as the liver, kidneys, and heart. Experimental results show that the tissue structure of various organs in the model rats is normal, with no obvious pathological changes observed. This suggests that the material has good biocompatibility and does not cause severe tissue damage to animals.

Toxicity tests showed that for mice, long-term use of mesoporous silica had no significant weight abnormalities,

no significant changes in hematological indicators, and no significant changes in organ coefficients. However, if exposed to high doses for a long time, there was a tendency for mild inflammatory response and tissue fibrosis <sup>[11]</sup>.

#### 4. The performance comparison of graphene quantum dots and mesoporous silica adjuvants

In terms of immune cell activation, graphene quantum dots, with their unique optical and electrical properties, can more efficiently stimulate T lymphocyte proliferation, accelerating their cell cycle into the S phase and G2/M phases at a higher ratio. Mesoporous silica, through surface modification, significantly enhances macrophage phagocytic activity, promoting the increase and elongation of pseudopodia. In cytokine secretion, graphene quantum dots significantly increase IL-2 production, while mesoporous silica more prominently promotes TNF- $\alpha$  secretion, stimulating macrophages and T lymphocytes to produce large amounts of <sup>[12]</sup>.

In terms of antibody production, graphene quantum dots can effectively enhance the affinity of specific antibodies, while mesoporous silica is more effective in increasing antibody titers. Regarding immune cell function regulation, graphene quantum dots significantly increase the proportion of CD4<sup>+</sup> T cells, whereas mesoporous silica performs better in enhancing the cytotoxic activity of CD8<sup>+</sup> T cells.

The binding of graphene quantum dots to immune cells mainly depends on the physical and chemical interaction between their unique electronic structure and cell surface molecules; mesoporous silica mainly binds to immune cells through chemical reaction and electrostatic interaction between the surface silanol groups and the receptors or membrane components on the surface of immune cells.

Using the unique optical and electrical properties of graphene quantum dots can more effectively stimulate the proliferation of T lymphocytes, promoting their entry into the S phase and G2/M phases. However, after surface modification, mesoporous silica significantly enhances its phagocytic activity against macrophages, promoting the increase and extension of pseudopodia. Studies show that graphene QDs can increase the secretion rate of IL-2, while mesoporous silica can enhance the secretion of TNF- $\alpha$ , stimulating the synthesis by macrophages and T lymphocytes.

Graphene quantum dots can effectively enhance the affinity of antibodies, and mesoporous silica has a better effect on the increase of antibody valence. Based on this, some studies have proposed using graphene quantum dots to improve the ratio of CD4<sup>+</sup> T cells and to improve the killing ability of CD8<sup>+</sup> T cells by using mesoporous silica <sup>[13]</sup>.

Graphene quantum dots primarily achieve the killing of immune cells through their unique electronic structure, interacting physically and chemically with substances on the cell surface. Mesoporous silica is a new type of nanomaterial that can adhere to immune cells via interactions between its silanol groups on the surface and receptors or membrane components on the immune cell surface, as well as electrostatic interactions.

Graphene quantum dots tend to interfere with ion channels within immune cells, affecting electrophysiological activities and thus modulating immune signaling pathways; mesoporous silica, on the other hand, regulates immune responses by activating or inhibiting specific immune signaling pathways such as NF- $\kappa$ B and TCR. The mechanism differences stem from the electrical properties of graphene quantum dots and the pore structure and surface chemical properties of mesoporous silica.

In terms of biological distribution, graphene quantum dots initially concentrate more in the liver, while mesoporous silica tends to cluster more in the spleen. Metabolically, graphene quantum dots degrade slowly and have

a long clearance time; although mesoporous silica also has some stability, it metabolizes relatively faster <sup>[14]</sup>. At high doses, graphene quantum dots may cause mild inflammatory responses, whereas mesoporous silica may exhibit a slight tendency towards tissue fibrosis.

In vaccine development, graphene quantum dots are suitable for enhancing antigen-specific immune responses, while mesoporous silica facilitates the overall immunogenicity of vaccines. In the field of immunotherapy, graphene quantum dots can precisely modulate immune cell functions due to their unique properties, whereas mesoporous silica can exert its effects by loading immune-regulating molecules <sup>[15]</sup>.

## 5. Summary

In the field of nanomaterial adjuvants, graphene quantum dots and mesoporous silica exhibit distinct properties. In terms of enhanced immune activity, *in vitro*, the former promotes T cell proliferation and increases IL-2, while the latter stimulates macrophages and promotes TNF- $\alpha$ ; *in vivo*, the former enhances antibody affinity and increases CD4<sup>+</sup> T cells, whereas the latter boosts antibody titers and strengthens CD8<sup>+</sup> T cell killing. In terms of mechanisms of action, their binding modes and signaling pathways differ. Regarding safety, they vary in distribution, metabolism, and toxicity. In terms of application prospects, vaccine development and immunotherapy each have their advantages. Both offer diverse possibilities for the advancement of immunology and warrant further research.

## Disclosure statement

The author declares no conflict of interest.

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