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Interactions of Nanomaterials with Emerging Environmental Contaminants

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Interactions of Nanomaterials with Emerging Environmental Contaminants

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Foreword

The ACS Symposium Series was first published in 1974 to provide a mechanism for publishing symposia quickly in book form. The purpose of the series is to publish timely, comprehensive books developed from the ACS sponsored symposia based on current scientific research. Occasionally, books are developed from symposia sponsored by other organizations when the topic is of keen interest to the chemistry audience.

Before agreeing to publish a book, the proposed table of contents is reviewed for appropriate and comprehensive coverage and for interest to the audience. Some papers may be excluded to better focus the book; others may be added to provide comprehensiveness. When appropriate, overview or introductory chapters are added. Drafts of chapters are peer-reviewed prior to final acceptance or rejection, and manuscripts are prepared in camera-ready format.

As a rule, only original research papers and original review papers are included in the volumes. Verbatim reproductions of previous published papers are not accepted.

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Preface

Emerging environmental contaminants are newly identified or previously unrecognized pollutants, which primarily include human and veterinary pharmaceuticals and personal-care products, surfactants, plasticizers, flame retardants, metals and metalloids, various industrial additives, pesticides, and pesticide metabolites. The use of novel nanomaterials with unique characteristics has been demonstrated to increase the removal efficiency of emerging pollutants, which provides a promising strategy to control the distribution of environmental contaminants. In addition, nanomaterials also can serve as ideal platforms for precise and accurate detection and sensing of emerging contaminants in the environment and biological fluids; this is because of their novel characteristics on optical and electrochemical properties. The interactive research of nanomaterials with emerging environmental contaminants will improve our understanding of the implication and application of nanomaterials in the environment.

This book is derived from the symposium "*Interactions of Nanomaterials with Emerging Environmental Contaminants"* at the 244th ACS National Meeting in Philadelphia during the fall of 2012 sponsored by the American Chemical Society (ACS) Division of Environmental Chemistry. Many topics addressing issues of interaction of emerging environmental pollutants with nanomaterials (including physical, photochemical, and biological interactions) were presented in this symposium, and they constitute the main content of this book.

This book contains 12 peer-reviewed chapters that cover various aspects of interaction of various nanomaterials with environmental contaminants. These chapters can be organized into two major sections: (I) interaction of nanomaterials with biomolecules for biosensing and detection of contaminants (Chapter 1-4) and (II) interaction of nanomaterials with contaminants to enhance the removal efficiency and rate of emerging pollutants (Chapter 5-12). Chapter 1 by Ren, Zang, Qie, and Baker provides an overview of the adjuvant effect of emerging nanomaterials. The nanoparticles can serve as drug delivery to deliver antigens to species targets. In contrast, ambient nanoparticles exhibit adverse effect on human and ecological health. To address the accurate and precise detection of environmental contaminants in the environment (as well as in the human body), Chapter 2 and Chapter 3 have developed photoluminescent gold nanomaterials — including gold nanoparticles and gold nanodots — for the monitoring and detection of metal ions, proteins, and bacteria in an aquatic ecosystem. In Chapter 2, Unnikrishnan and Huang have synthesized luminescent core-shell Au nanodots using different kinds of capping ligands (including alkanethiols, proteins, DNA, thiol derived carbohydrates, and aptamers) as the shell layer for detection and determination of mercury ions, proteins, and *E. coli*. Chang and

In Interactions of Nanomaterials with Emerging Environmental Contaminants; Doong, R., et al.;
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his team (Chapter 3) have developed several functional Au nanoparticles and Au nanodot-based sensors that allow the sensitive and selective detection of mercury, lead, and copper ions through analytes induced changes in colors, absorption, and fluorescence. In addition, a biosensing system (AlGaN/GaN high electron mobility transistors immobilized with antibodies) is developed by Wang and his team (Chapter 4) to effectively detect a short peptide only containing 20 amino acids, which opens a door for investigation into nanomaterials with biomolecules.

The applications of nanomaterials for various reactions (including adsorption, photocatalytic degradation, and reductive dechlorination) are also addressed. Huang, Padhye, and Wang (Chapter 5) describe the generation of N-nitrosamines from transformation of amines catalyzed by activated carbons. This interaction is important because activated carbon and amine are often used in water treatment plants. In Chapter 6, Du and Jing explore the dynamic adsorption process of propranolol at the TiO_2/water interface on the molecular level. In addition to adsorption behavior, Hung and his colleagues (Chapter 7) combine TiO_2 and carbon nanotubes to adsorb and look at the photocatalytic decomposition of bisphenol A, which is an endocrine disrupting chemical widely existing in the environment.

Iron-based nanomaterials are effective catalysts for the removal of emerging pollutants in the environment. Ren, Han, Al Anazi, Nadagouda, and Dionysiou (Chapter 8) discuss the application of different iron-based nanomaterials for environmental remediation — including water treatment, groundwater remediation, and soil decontamination. Ferrate, ferrites, and TiO_2-composite magnetic iron oxides are used for the removal of contaminants of emerging pollutants. In addition to iron oxide catalysts, zerovalent metals are also common nanomaterials widely used for reduction of emerging pollutants. Su, Tso, Peng, and Shih (Chapter 9) have used nanoscale zerovalent iron (nZVI) and bimetallic Pd/Fe as the dual functional tools for adsorption and reduction of aromatic contaminants (including decabrominated diphenyl ether, hexachlorobenzene, penrachlorophenol, and Congo red). The combination of biological or sequential Fenton treatment on the mineralization of some emerging contaminants is also evaluated in this chapter. In Chapter 10, McPherson, Goltz, and Agrawal summarize the role of polyelectrolyte stabilization and catalytic metal modification in the enhanced performance of nZVI. The addition of polyelectrolyte stabilizers to nZVI decreases particle agglomeration and reduces particle size — resulting in the increase in reactivity and transport in porous media. The modification of nZVI with Pd and Ni for the enhancement of reactivity is also summarized and discussed. In addition, three field studies using Pd-nZVI for the remediation of chlorinated compounds in groundwater are introduced. Sharma, Siskova, and Zboril (Chapter 11) review the recent development of nanoscale zerovalent iron and magnetic bimetallic Fe/Ag nanoparticles with core-shell structures. They also show the usefulness of these nanomaterials on nutrient removal, transformation of halogenated aromatic contaminants, and antimicrobial activity. In addition to zerovalent iron, Lee and Doong (Chapter 12) have demonstrated the feasibility of using another environmentally friendly metal to remove chlorinated compounds. In this chapter, the authors review and discuss the reductive dechlorination of chlorinated hydrocarbons and emerging pollutants by zerovalent silicon and

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In Interactions of Nanomaterials with Emerging Environmental Contaminants; Doong, R., et al.;
ACS Symposium Series; American Chemical Society: Washington, DC, 2013.

bimetallic Fe/Si and Ni/Si. More importantly, they demonstrate the synergistic effect of nickel ions and polyethylene glycol on the dechlorination rate.

The understanding of interaction of emerging contaminant with various nanomaterials is essential to exploring the applications of nanotechnology in the environment. We hope that this collection will benefit graduate students who are engaged in research and development in the advancement of nanotechnology and environmental science and technology. We wish to thank Anne Brenner and Timothy Marney of the editorial department of ACS for their assistance in preparing this volume and for keeping us on schedule.

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Chapter 1

The Adjuvant Effect of Emerging Nanomaterials: A Double-Edged Sword

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[†]This manuscript is in memory of my advisor and friend, Dr. Gregory L. Baker who passed away unexpectedly while this paper was being written.

Nanoparticles have growing applications in industry, consumer products, biology and medicine. One of those applications involves the interaction between nanoparticles and immune components. The nanoparticles can act as antigen carriers to deliver and release antigens to specific targets, and enhance the immune response against a variety of antigens as adjuvants. The adjuvant effects of nanoparticle size, shape, surface charge, linkage method on the immunological response are also discussed. In contrast, as-prepared nanomaterials and ambient particulate matter (PM) from air pollution exhibit adverse adjuvant effect *in vitro* and *in vivo*, and recent advances to address their potential risk on human health are also included in this review.

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Introduction

Nanomaterials are characterized by their sizes which are in the range of several nanometers to several hundreds of nanometers, well below the micrometer range. Because of their nanoscale dimensions and hence large specific surface area, nanomaterials exhibit remarkable physicochemical properties, such as optical property (*1*), catalytic property (*2*), mechanical property (*3*) and drug delivery property (*4*), which are usually not active for their bulk materials. Due to these specific properties, research and development of new nanomaterials have steadily increased, which can be reflected by the increasing number of publications on nanomaterials research, from about 110 publications in 1990 to 4200 publications in 2000 followed by a burst to more than 77900 publications in 2013 (key word: nano, ISI web of knowledge, 05/2013). As a result, nanomaterials, rapidly introduced into electronic devices, construction and composite materials, are more and more present in workplaces as well as consumer products since large-scale producing, handling and processing facilities of nanomaterials are easily available.

Although properties of nanomaterials are impressive from physicochemical viewpoint, they also raise safety concerns of nanomaterials. One major concern is that nanomaterials may lead to potential toxic effect on environment or human health, because nanomaterials readily penetrate cell membranes, travel throughout the body, and deposit in target organs. Therefore, it may trigger injurious responses (*5*, *6*). In recent years, nanotoxicology has become one of the major research focuses in nanoscience. While the number of publications dealing with nanotoxicity study was about 1350 in 2000, this increased to 5500 in 2010, then it rapidly jumped to more than 8200 in 2013 (key words: particle/toxicity, ISI web of knowledge, 05/2013). These clearly indicate that nano toxicology has been widely recognized and gained more and more attention. These studies would help to determine whether and to what extent these properties may present a threat to environment and human beings, and guide applications of nanomaterials in daily life as well (*7*). Among several mechanisms proposed to explain the adverse effect of nanomaterials, generation of reactive oxygen species and oxidative stress has received the most attention (*8*). Dimension, surface chemistry, surface charge and aggregation of nanomaterials are related to their nanotoxicity (*5*). It is difficult to identify the health risk of each new nanomaterials because all material properties need to be taken into account during the toxicity study.

Despite safety concerns that nanomaterials could present adverse effect on human beings, it has been reported that nanomaterials serving as effective vaccine/drug carriers could improve and/or facilitate the extended release of antigens, and hence enhance the immune response level and quality of antigens (*9*, *10*). This has drawn more and more research focuses worldwide in nanomedicine field, especially in vaccine immunology, and nanomaterial has been referred as nano adjuvant to enhance the immunogenicity of specific vaccine or antigen. From ISI web of knowledge (key words: particle/adjuvant, 05/2013), there were only 380 publications on nano-adjuvant research in 2000 and it increased to more than 1350 in 2010, and then rapidly jumped to more than 1800 publications in 2013.

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ACS Symposium Series; American Chemical Society: Washington, DC, 2013.

Publication Date (Web): October 29, 2013 | doi: 10.1021/bk-2013-1150.ch001

An adjuvant is a substance which is not immunogenic itself, but increases or prolongs immune response if introduced in combination with a vaccine or antigen *in vitro* or *in vivo* (*11*). Ideally, adjuvants should be biodegradable, stable with long shelf life, cheap to produce (*12*). They are introduced to enhance immunogenicity by reducing the administration amount of required vaccine or antigen for protective immunity without inducing immune responses against themselves (*13*). Nano adjuvants take the advantages of nanomaterials to deliver vaccines, drugs, peptides and nucleic acids efficiently to targets, control their release and induce immune response while protecting the integrity of delivered vaccines from enzymatic degradation or from degradation at physiological conditions in body (*9*, *14*, *15*). However, due to the concerns about safety and toxicity, there is only limited vaccine adjuvant approved for human use in the United States, such as aluminum hydroxide, or alum. Despite the wide use of alum, it has comparatively weak immune response and works only with certain diseases. So nanomaterial as adjuvant in vaccine immnunology is one of the major areas currently being studied widely because new, safe and efficient nano adjuvants are highly demanded. Apparently, further efforts in improvement or optimization of nanomaterial-based adjuvants in immunology, such as new nanoparticles, particle sizes, surface properties and loading methods of vaccine etc., are required to help induce, strengthen and prolong the immune response before nano adjuvants are to be used with vaccine in human beings.

Due to their positive adjuvant effect on immunology and their adverse adjuvant effect on human health, nanomaterials could be viewed as a double-edged sword. Therefore, the purpose of this manuscript is to present an overview of the positive adjuvant effect of nanomaterials in immunology where nanomaterials, more specifically, nanoparticles are developed as vaccine or antigen carriers/adjuvants. Two categories of nanoparticles from previous reports (polymeric and inorganic nanoparticles) will be discussed. We also consider the fact that human exposure to nanomaterials in the environment is more and more common, which brings potential adverse effect when allergens/antigens are present in the body. So the adverse adjuvant effect of as-prepared nanomaterials and ambient particulate matter from air pollution will also be discussed at the end of this manuscript.

Adjuvant from Polymeric Nanoparticles

Over the last several decades, many nanoparticles-based delivery systems have been developed and these systems have received extensive interests as potential adjuvants for immunology. Based on the polymer ingredients, the nanoparticles can be divided into biodegradable and non-biodegradable polymeric carriers.

Publication Date (Web): October 29, 2013 | doi: 10.1021/bk-2013-1150.ch001

Nanoparticle Adjuvant from Biodegradable Polymers

Polymeric nanoparticles formulated from different biodegradable polymers and copolymers have been widely explored as controlled delivery vehicles of different agents, such as peptides, proteins and nucleic acid vaccines (*16*, *17*). They are well established delivery systems and the encapsulation of biomolecules in polymeric nanoparticles protects them from extreme conditions while maintaining their integrity and activity, and herein enhances the immune response of the antigens (*18*).

Polyester is a thermoplastic polymer with labile aliphatic ester bonds in the backbones which can degrade hydrolytically under physiological conditions (*19*). It is one of major nanoparticle materials used in vaccine delivery to determine the antigen immunogenicity. Poly(lactide) (PLA) and poly(lactide-*co*-glycolic acids) (PLGA), the most popular materials for formulation of polymeric nanoparticles, have been approved by FDA for the applications in human beings. The application of these PLGA nanoparticles in antigen delivery enhanced cellular and humoral immune response compared to the free antigen dose (*20*, *21*), which confirms their adjuvant effect. Both nano- and micro-particles of PLGA can be used as adjuvants and antigen carriers to facilitate presenting antigens to T cells and increase the immune response (*22*, *23*). In 2012, Bershteyn and coworkers synthesized PEGylated phospholipid-enveloped PLGA (50:50) microparticles (MP) conjugated with thiolated protein antigens (ovalbumin, OVA) and studied the adjuvant effect of MP carriers in mice (*22*). The $CD8^+$ T-cells responses on day 7 from different dose combinations of MP, antigens, monophodphoryl lipid A (MPLA, an immunostimulatory molecule) showed very different levels. The $CD8^+$ T-cells response of mice from antigen-MP was significantly greater than soluble antigen immunization alone at both doses (either no detectable or slightly higher responses compared to control experiment). It showed much higher response than alum-immunized (FDA approved) mice which was not detectable compared to control, even at very high alum dose (Figure 1A). Thus these results clearly identify that lipid-enveloped PLGA MP is one efficient adjuvant in immune response in mice. More recently, Moon and coworkers synthesized similar lipid-enveloped PLGA nanoparticles and then conjugated antigen, *vivax* malaria protein (VMP001) to the lipid membrane of particles with MPLA (*23*). The mice were immunized twice on days 0 and 21, and the sera were collected and analyzed on days 35 and 120 to compare the antibody responses promoted by the immunization of free VMP001 and VMP001-nanoparticles. As shown in Figure 1 B-F, the nanoparticle vaccines generated a more balanced Th1/Th2 antibody response. The level of IgG_1 and IgG_{2c} were persistent while IgG_{2b} and IgG_3 responses were lost on day 120. Compared to free VMP001+MPLA, the immunization with nanoparticles showed much higher antibody responses (Figure 1 B-F), though cytokine production by restimulated spenocytes *ex vivo* did not show greater differences in either nanoparticles loaded or not loaded groups.

Particle size is a critical factor that affects the immune response. Generally, small nanoparticles are known to be more effective targeted-delivery system than large particles because small nanoparticles can easily penetrate the biological barriers. So one would expect stronger immune response from small

nanoparticle-antigen conjugates. However, several reports related to degradable particles from PLA, PLGA or sulfobutylated poly(vinyl alcohol)-*graft*-PLGA have shown that immune response from model antigens entrapped in particles exhibits no such trend (*24–27*) and the relation between immune response and particle size varies with different polymer materials and antigens. But there is evidence that the size of particle adjuvants may be related to different categories of the immune responses with preferred humoral immune response for large particles and preferred cellular immune response for small particles (*25*, *28*). Overall, polyester nanoparticle vaccines elicited enhanced immunogenicity and prolonged antibody response in mice which proved the adjuvant effect of nanoparticles (*29*).

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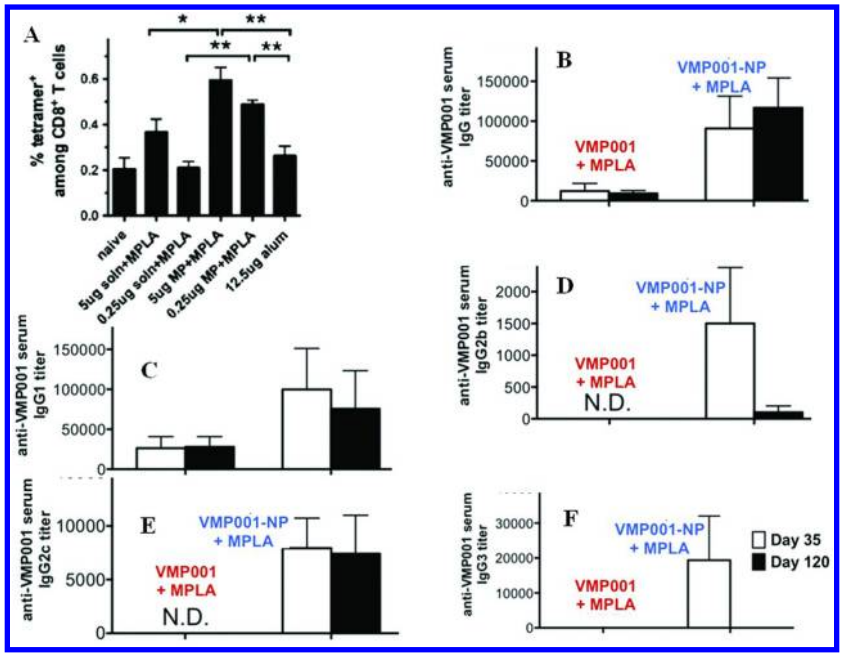

Figure 1. (A) Frequencies of ova-specific CD8⁺ T-cells in spleens were analyzed by peptide-MHC tetramer staining and flow cytometry. (, P<0.05; **, P<0.05). (Reproduced with permission from reference (22). Copyright 2012 Elsevier Ltd.). (B-F) C57Bl/6 mice were immunized s.c. on days 0 and 21 with 25 μg of MPLA and 1 μg of VMP001 in either soluble or VMP001-NP formulations, and anti-VMP001 IgG sera were characterized on days 35 and 120 for (B) IgG, (C) IgG_1, (D) IgG_{2b}, (E) IgG_{2c}, and (F) IgG_3 titers. (Reproduced with permission from reference (23). Copyright 2012 PLoS One)*

Publication Date (Web): October 29, 2013 | doi: 10.1021/bk-2013-1150.ch001

Chitosan (poly(D-glu-cosamine)), the most investigated polysaccharide, can be prepared from natural polymer (chitin) via partial *N*-deacetylation. It is soluble in acidic solutions and prone to chemical or biological functionalization due to the presence of highly reactive amino groups in its structure (*30*). It is widely studied as nano vaccine carriers and adjuvants against a variety of antigens, such as H1N1 hemagglutinin antigen delivered by chitosan-coated poly(ε-caprolactone) (PCL) nanoparticles (*31*), swine influenza DNA vaccine (*32*) and hepatitis B surface antigen (rHBsAg) (*33*, *34*). And the results suggest enhanced immunological properties of these chitosan-based nano adjuvants. Zhao and coworkers reported that spherical chitosan nanoparticles conjugated with swine influenza antigen (plasmid DNA) were prepared by complex coacervation method with high encapsulation efficiency and high antigen stability (*32*), which indicates that the encapsulated DNA was protected from degradation after incorporated with nanoparticles. The presented much higher antibody serum IgG titers (2 to 3 times higher than naked DNA), using ELISA from BALB/c mice which were immunized with antigen-loaded chitosan nanoparticles, naked antigen and blank chitosan nanoparticles, suggest significant adjuvant immune response from chitosan nanoparticles. Even more, the antibody presentation sustained almost same level at prolonged time (up to 8 weeks during the tested time interval) which indicates continuous release profile of the incorporated DNA antigen from chitosan nanoparticles. This may bring the potential to increase DNA vaccination efficiency and is worth of further study for clinical use in the future. A similar adjuvant effect was also reported by Prego and coworkers (*33*, *34*) when ionic gelated chitosan nanoparticles loaded with hepatitis B antigen (HB) were prepared and intramuscularly administrated to mice (two doses). Although the immune response (IgG level) from antigen-loaded nanoparticles existed a slight delay compared to FDA approved alum, the adjuvant immunological effect of nanoparticles was ~9-fold higher than the alum vaccine with extended antigen release profile accounting for the prolonged immune response. The surface charge and composition of the antigen-loaded chitosan nanoparticles also play important roles in modulating the immune response (*34*). When incorporating nanoparticles with less excess or excess of antigen, the resulted antigen-nanoparticle complexes could have positive ζ potential (CSNC+, leaving chitosan as predominant composition on the particle surface when less excess antigen was used) or negative ζ potential (CSNC-, leaving antigen as predominant composition exposure towards the external medium when excess antigen was used). The immunological response of CSNC- (two doses) was much lower than CSNC+, even lower than the control (alum-antigen) during the 27-week study, and it was not able to induce effective immunogenicity against antigen (*33*, *34*). This is consistent with previous reports that exposure to repetitive antigens induced weak immune response (*35*), and cationic surface charge of nano adjuvant enhanced the immune response (*36*). This might be explained by the fact that CSNC+ could facilitate antigen internalization through strong association with outer membrane of dendritic cells (*37*), and therefore, CSNC+ could induce adequate presentation to the immune system and an enhanced activation of APCs with further development of a strong adaptive immune response. Despite that immunization via multiple doses are widely applied in immunology research,

single-dose approach of antigen (HB) on chitosan nanoparticles also show high specific and long-lasting IgG antibody levels against antigen (*34*), which suggest nano adjuvant could achieve the goal of vaccination by reducing the injection frequency while maintaining efficient immune protection.

Other biodegradable polymers have also been studied as adjuvants in immunonology, such as micro/nano-particles from poly(ε-caprolactone) (PCL) (*38*), nanoparticles from poly(anhydrides) (*39*) and poly(γ-glutamic acid) (*40*) etc. The results from those studies all suggest nanoparticles formulated from these polymer materials exhibit adjuvant immune response while co-administrated with specific antigens.

Nanoparticle Adjuvant from Non-Biodegradable Polymers

Unlike biodegradable polymers, non-biodegradable polymers cannot be broken down or degraded *in vivo* by hydrolysis and/or bacteria digestion. Hence the study on non-degradable nano adjuvants in immunology attracted much less attention compared to biodegradable polymer due to slow clearance and the risk of chronic toxicity after administration. However, they are considered to have extended immune response and thus improve the immunogenicity due to the prolonged persistence of nanoparticles in tissues (*21*). Especially, a great number of non-biodegradable nanoparticles have been proved to be biocompatible, though not biodegradable. So there are still considerable reports related to their adjuvant studies on immune response.

Polystyrene or latex nanoparticles have been incorporated with OVA to investigate their adjuvant immunogenicity (*41–44*). When antigen OVA was covalently linked to polystyrene nanoparticles (*41*), it displayed much higher MHC class I-restricted T cell immune response and Ab titers than those currently used adjuvants (Alum, Freund's complete adjuvant (CFA), QuilA, monophodphoryl lipid (MPL)) after one or two immunization. And the conjugates of antigen and nanoparticles via covalent linkage showed higher immune response than soluble antigen alone or a simple mixture of antigen and nanoparticles. Especially, the effect of particle size was addressed in the study and the result suggests that 40-nm sized particles exhibited superior antibody and cellular immune response which indicate they are ideal as immune adjuvants in the model. Moreover, in tumor models EG7-OVA (ovalbumin-expressing EG7 tumors), E7-HPV (HPV 16-expressing tumors) and their established tumor, mice immunized with nanoparticle-antigen conjugates cleared the tumor after certain time challenge, whereas mice treated with antigens only all showed grown tumors. Other literatures (*42*, *43*) using latex nanoparticles also demonstrated 1000-10000 fold more efficient immune response than soluble antigen alone via MHC-class I or II molecules presentation.

Another biocompatible but non-biodegradable polymer, poly(*N*-isopropylacrylamide) (PNiPAAm) with thermo-responsive property, has been studied as nano adjuvant to investigate antigen-specific immune responses (*45*, *46*). Shakya et al. synthesized well-controlled PNiPAAm with lower critical solution temperature (LCST) at 32 °C. PNiPAAm conjugated with collagen type II and formed clear and visible white precipitate around the injection site

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(>32 °C), which confirms the thermo-responsive property while immunization. And this adjuvant enhanced immunogenicity of collagen type II with induced collagen-related arthritis, and PNiPAAm covalently conjugated with antigen induced much weaker arthritis than that with physically adsorbed antigen, which suggests that one of major mechanisms corresponding to the adjuvant effect of polymeric nanoparticles might be depot effect (slow release of the entrapped antigen). Moreover, this polymer adjuvant also showed enhanced immune response to OVA. These results suggest PNiPAAm may be used as one general adjuvant in immunology and vaccination.

However, several factors might need to be considered in order to use non-biodegradable polymer nanoparticles as immunological adjuvants, such as toxicity, particle aggregation and accumulation in the tissue after immunization, which require further studies on *in vivo* clearance and safety administration.

Although several factors of the conjugates of polymeric nanoparticle and antigen (from either biodegradable or non-biodegradable polymer) can affect the adjuvant immunogenicity, such as particle size and covalent linkage or physical adsorption, there are no clear trends which can be used to predict whether nanoparticles would exhibit negative or positive adjuvant effect. However, it is clear that positive surface charge of nanoparticle and antigen conjugates can form complex with DNA easily, facilitate higher transfection efficiency and herein enhance the adjuvant immune response (*47*, *48*).

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Adjuvant from Inorganic Nanoparticles

The development of functional, inorganic nanoparticles has progressed exponentially over the past two decades. As an alternative to polymeric nanoparticle adjuvant, inorganic nano adjuvants have also been widely studied as antigen carriers in vaccine immunology due to the fact that there are a variety of inorganic nanoparticles being prepared (*49*) and utilized in biomedical applications (*50*), such as imaging (*51*), tumor detection (*52*), and drug delivery (*53*). These inorganic particles may also have prolonged adjuvant effect due to the slow clearance from tissues. Particle size and surface functionality or charge can be easily tuned during particle preparation or via simple surface chemical reactions (*50*). This may extend the research varieties of inorganic nano adjuvants. Several inorganic nanoparticles acting as immune adjuvants will be included here in details for understanding their adjuvant activities.

Alum ($Al(OH)_3$) is the first adjuvant and has been approved and used in human beings for long time. Recently, aluminum oxide nanoparticles have been addressed more attention perhaps due to the similar component to alum. Antigen peptomers (head-to-tail linked 18-mer synthetic peptides) were covalently conjugated to surface-derivatized aluminum oxide nanoparticles (355 nm mean diameter) and their systemic immunization study was reported with the peptomer-particle conjugates without co-administration of hydrophilic adjuvant muramyl dipeptide (MDP) resulted in the highest serum antibodies titers (*54*). Both free C4 (the 4th conserved region of HIV-1 gp120) peptides and C4 peptomers with and without MDP exist lower antibody titers. This suggests the

peptomer–particle formulation provided adjuvant activity when administered systemically. More recently, Maquieira and coworkers have first covalently coupled non-immunogenic hapten (one small molecular mass compound) to aluminum oxide nanoparticles as carrier and adjuvant for haptens immunization (*55*). Aluminum oxide particles (amorphous 40 and 3000 nm, crystalline 300 nm) were prepared and coupled with hapten covalently. The immune response is related to particle size (Figure 2) and crystallinity with the highest response from crystalline 300nm particles-hapten conjugates with or without Freund's adjuvant. It is consistent to previous reports with higher response from crystalline materials (*56*), and particle size effect is also consistent with previous results (*54*, *57*). The results also suggest the covalent linkage in aluminum oxide nano adjuvant system is essential to induce the enhanced immune response as compared with no response from free hapten or a simple mixture of particles and hapten.

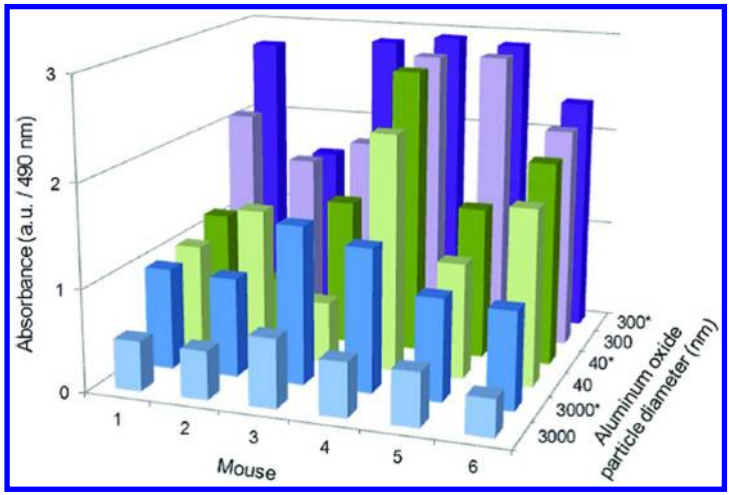

Figure 2. Effect of the size of aluminum oxide particles in the atrazine−hapten conjugates on mouse serum antibody titers, expressed as absorbance values. Asterisk (*) denotes immunization using Freund's adjuvant. (Reproduced with permission from reference (55). Copyright 2012 American Chemical Society.)

Gold nanoparticles have also attracted much attention as antigen carriers and adjuvants. Chen *et al.* conjugated pFMDV (VP1 protein of foot-and-mouth disease) with gold nanoparticle with different sizes (2-50 nm) to form nanoparticle vaccines and immunized mice model to study the size-dependent immunogenicity (*58*). The results showed significant size-dependent immuno-genicity against pFMDV with the highest immune response from 8 and 12 nm particle conjugates. And gold nanoparticle was demonstrated to be an ideal candidate as vaccine carrier due to no detectable antibody-binding activity. The effect of size and shape of gold nanoparticles on immune response was studied by Niikura and coworkers (*59*). Different sized and shaped gold nanoparticles were synthesized (Figure 3 A-D) via seeding growth method and coated with anionic polymer (PSS-MA) to electrostatically attach West Nile virus envelope (WNVE) protein to produce

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20 and 40 nm spherical (Sphere40-E, Sphere20-E), rod (Rod-E, 40 × 10 nm), and cubic (Cube-E, 40 × 40 × 40 nm) particle-antigen conjugates (AuNP-Es) for *in vivo* and *in vitro* immunization study. Sphere40-Es induced the highest level of WNVE specific antibodies and Rod-Es induced only 50% of that from Sphere40-E (Figure 3 E). Results from cell uptake experiments of nanoparticles showed the uptake of Rod-Es is more efficient than others, which suggest that antibody production was not dependent on the uptake efficiency of different AuNP-Es. Although the mechanism of shape-dependent WNVE antibody production needs to be further investigated, this report (*59*) will pave the way for future development of nano vaccines. Other researchers also demonstrated gold nanoparticles along with alum (*60*) or with Fc fragment from human IgG (*61*) can improve the cell uptake of antigen and enhance the immunogenicity against respective antigens.

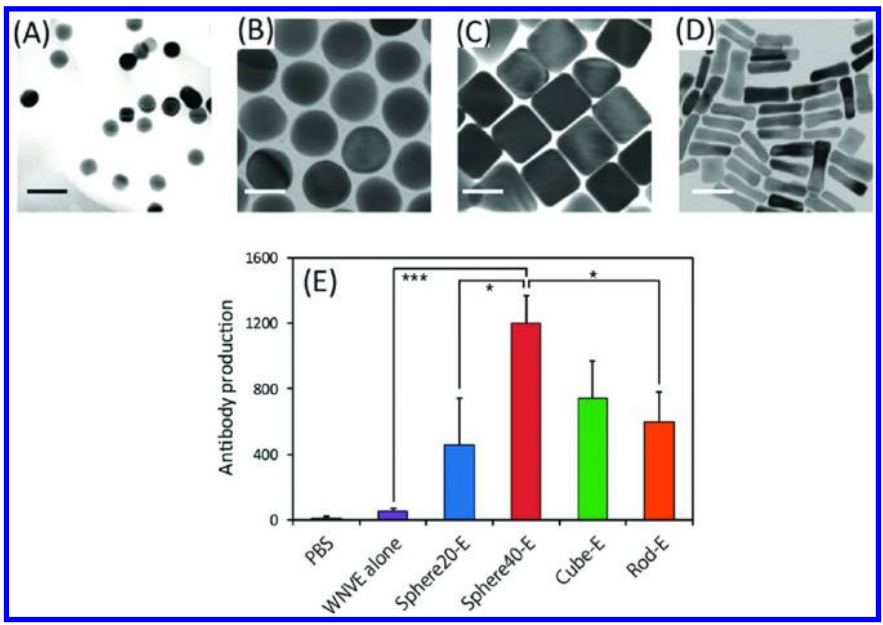

*Figure 3. TEM images of as-prepared (A) Sphere20, (B) Sphere40, (C) Cube, and (D) Rod gold nanoparticles before conjugated with antigen (Scale bar: 40 nm). (E) WNVE-specific IgG ELISA end point titers in mice immunized twice at 3-week intervals with 100 ng WNVE/animal/dose of AuNP-E. Significant differences: *p < 0.05; ***p < 0.001 (mean ± SEM, n = 10). (Reproduced with permission from reference (59). Copyright 2013 American Chemical Society.)*

Mesoporous silica nanoparticle (MSN) is one of the most popular materials used as antigen delivery systems due to its high surface area and porous structure. Wang and coworkers (*62*) prepared three different sized MSNs as vaccine adjuvants and investigated the effect of their pore structure on immune response. After bovine serum albumin (BSA) was entrapped into MSNs, oral

immunization suggests MSN-BSA conjugates (430 nm) displayed the highest response compared to those from 130-nm and 1-2-μm MSNs which might attributed to the releasing rate (depot effect) and pore structure for different sized MSNs. MSN-BSA conjugates (430 nm) also showed much higher immune response than free BSA or BSA-CFA adjuvant, which suggests MSNs are acting as effective adjuvants. Co-presentence of Th1 and Th2 responses was proved by the comparison of IgG_1 and IgG_{2a} titer's change after 4 week vaccination. Most recently, OVA model antigen was efficiently conjugated to amino-functionalized MSN (AM-MSN, 90 nm) at 72 mg OVA/g MSN compared to only 29 mg OVA/g non-aminated MSN (*63*) due to the stronger electrostatic interaction between OVA and AM-MSN. Mice immunization using OVA-AM-MSNs at high (10 μg) and low (2 μg) loading of OVA induced enhanced antibody immune response, but cell-mediated immunity was observed when mice were immunized with high loading of OVA on AM-MSNs, not with low loading of OVA on AM-MSNs. This indicates that there might be a minimal threshold amount of antigen loading on AM-MSNs required to induce both cellular and humoral adaptive immunity. No discernible local or systemic morphology change at the injection site was observed after administration of OVA-AM-MSNs. These results indicate the adjuvant activity with OVA antigen and a new nano vaccine method. Non toxic mesoporous silica cylinders (SBA-15) have also been used to investigate the adjuvant immunogenicity against antigens, BSA (*64*) and *Micrurus* snake toxins and *Int1*β (*65*). The results clearly suggest that SBA-15 increased the immunogenicity compared to respective antigens alone or other common adjuvants, and positively modulated the immune response of low responder individuals into high antibody producers. These cylindrical silica nanomaterials stimulated mutually TH1 and TH2 immune responses.

Single-walled carbon nanotubes (SWNT) were covalently conjugated with Wilm's tumor antigen, WT1Pep427, and were used to immunize mice with or without another immunological adjuvant, Titer max (*66*). The SWNT-WT1Pep427 conjugates with Titer max induced specific serum IgG response against antigen, whereas antigen alone, SWNT-antigen alone or antigen-Titer max alone did not induce such response. This clearly suggests carbon nanotubes could serve as antigen carriers and induce immune response against weak MHC class II antigen. Quantom dots (QD, CdSe/ZnS) and Iron oxide nanoparticles (IO) conjugated with merozoite surface protein 1 (rMSP1), a recombinant malaria vaccine antigen, were also used as adjuvants to immunize mice in literatures (*67–69*). QD-rMSP1 induced significantly higher antibody response and parasite inhibitory antibodies against rMSP1 than those from rMSP1 with other conventional adjuvant together. The enhanced immunogenicity might be due to the uptake of QDs by dendritic cells resulting in the activation of dendritic cells and secretion of key cytokines (*67*).

Similar to polymeric nano adjuvants, there are a variety of inorganic nano adjuvants due to the extensive development of synthesis of inorganic nanoparticles. Their adjuvant effect on immune response is related to their size, shape, linkage method between antigen and nanoparticles, their chemical composition and antigen loading amount on nanoparticles. These inorganic nanoparticles are biocompatible due to the low toxicity examined *in vitro*, which

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suggests they are novel vaccine carriers and adjuvant candidates for the future clinical application.

Both polymeric and inorganic nanoparticles can act as adjuvants, combined with the antigens, usually through a depot effect via slow dissolution after administration. This overcomes the problem of rapid loss of free antigen and allows lower administration dose of antigen while maintaining similar or enhanced immune response to benefit the vaccination development.

Allergic Adjuvant Effect from Nanomaterials and Ambient Particulate Matter

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We have discussed a variety of nanoparticles prepared from different materials as adjuvants to enhance immune responses against specific antigens and prevent human diseases. As mentioned above, adjuvants are intended to lower antigen dosage and improve efficiency of the delivered vaccine (*12*); hence they are beneficial to vaccine immunology and could be used in future novel vaccine development. However, like a double-edged sword, nanoparticles have also been reported to exhibit adverse allergic effect raised from allergen/nanoparticle complexes (*70*). Yang and coworkers (*71*) have reported that silica nanoparticles (nano-SiO_2, 10-20 nm) showed adverse effect on lung function of rats with OVA immunization compared to the saline-treated control rats. The OVA-sensitized and saline-sensitized control rats were treated daily with intratracheal instillation of nano-SiO_2 solutions (0.1 mL of 0, 40 and 80 μg/mL) for 30 days, rather than few immediately high dose exposure, which simulates the real environmental exposure. The results suggest that nano-SiO_2 has adverse effect on large airways of the lung as evidenced by the increasing inspiratory and expiratory resistance while increasing the dose of methacholine (MCH) challenge in airway hyper-responsiveness assessment. The pulmonary histological assay of lung indicates the obvious airway remodeling in rats via exposure to 80 μg/mL nano-SiO_2, and OVA-treated rats exhibit much more aggravated inflammation than that from the saline-treated control ones. This may attribute to the unbalance of Th1/Th2 cytokine accelerated by nano-SiO_2 through the increasing IL-4 production in the tissue. We have studied the enhanced allergic effect of engineered silica nanoparticles (SNP, polyethylene glycol coated particle surface, 90 nm hydrodynamic diameter) on airway diseases by assessing the magnitude of OVA-induced histopathological and immunological responses in the lung of mice (*72*). Female BALB/c mice were intranasally sensitized with allergen OVA along with co-exposure to SNP (0, 10, 100 and 400 μg) and secondary OVA challenge. The significantly greater level of OVA-specific serum IgE and IgG1, airway eosinophil inflammation, mucous cell metaplasia, and Th2 and Th17 cytokine gene and protein expression were observed from the SNP/OVA-mice compared to those from OVA-mice or SNP/saline-mice, and these results indicate that airway exposure to engineered SNP during the sensitization of mice to OVA enhances allergic airway disease with a dose-dependent fashion upon secondary OVA challenge. Besides silica nanoparticles, the adverse adjuvant effect of other