行政院國家科學委員會專題研究計畫成果報告

奈米金粒子藥物傳送系統之生物界面研究

Investigation for the bio-surface interaction of gold nanoparticles-based drug delivery system

計畫編號：NSC 96-2320-B-009 -001

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一、中文摘要

生物與表面的作用控制微粒子傳輸和交換進入生物系統。許多證據顯示生物表面於奈米尺度下，物理性質的影響變得重要。單純的生物表面物理性質變化可以引起生物反應，因此，對表面物理有基本理解，將有助於發展新世代藥物傳遞系統。我們利用金奈米粒子研究出免疫球蛋白的可撓性。由此可知，金奈米粒子是研究生物表面之最佳選擇。具體的目的是：(1)研究奈米金粒子進入細胞的表面生物物理學 (2)在細胞層級研究金奈米粒子進入細胞之分子機制 (3)研究金奈米粒子傳遞至組織之表面生物物理學 (4)發展具有組織專一性之金奈米粒子藥物傳遞系統

關鍵詞：奈米科技，奈米粒子，自組裝，藥物傳遞，生物界面

Abstract

Bio-surface interaction dominates transporting and trafficking of particles into biosystem. Increasing evidence indicates that at nanoscale, surface biophysics becomes significant and in many cases surface biophysics alone induces major cellular response. A fundamental understanding of surface biophysics will help to facilitate drug delivery system for the next generation. We have applied gold nanoparticles (GNPs) to investigate the flexibility of immunoglobulin. GNPs are proved to be a toll of choice to investigate surface biophysics. The specific aims are : (1) To study surface biophysics of GNP trafficking into cells, (2) To study molecular mechanism governing GNP trafficking at cellular levels, (3) To study surface biophysics of the delivery of GNP to tissue, and (4) To develop tissue-specific GNP-based drug delivery system carrying therapeutic reagents.

Keywords: nanotechnology, melanin, self-assembly, macrophage, dioxins

二、緣由與目的

Nanomedincines Nanodevices and nanoparticles enable study of a wide range of biological phenomena extending from protein-protein interaction mapping to cancer detection in intact animals and man (Huang, Chen et al. 2006;Abrams, Murphy et al.2000;Brown et al.2000). Recent advances in materials sciences; in particular the development of functionalized nanoparticles, united with advances in drug delivery, provided the impetus for the present explosion in nanomedicine (Suki, Ingenito et al.2005; Terray, Marr et al. 2002;Tolic-Norrelykke and N.Wang 2005). Bio-surface interaction dominates transporting and trafficking of particles into biosystem. Biosurface interaction consists of surface biochemistry and surface biophysics. Surface biochemistry deals with the chemistry commonly known as ligand-receptor binding, antibody-antigen binding, etc. which are well known to biologists. Surface biophysics on the other

hand is less familiar to science community (Chan, Chithrani et al. 2006; Roure, Ladoux et al. 2005; Harris 1984). The physics deals with the dimension, size, and structure at the interface. In general, surface biophysics explains how the structure and size induces bio-response (Chan, Chithrani et al. 2006; Ingber, Chicurel et al.1998; Natan, Brown et al. 2000). In a bulk system, such as chemical reaction in a beaker, surface biochemistry dominates the bio-surface interaction. However, increasing evidence indicates that at nanoscale, surface biophysics becomes significant and in many cases surface biophysics alone induces major cellular response.

The nanoparticle-based dug delivery system or nanomedicine is a major focus in the pharmacological study. There has been a variety of nanoparticles applied for drug delivery and targeting (Liu, Dai et al. 2003; Jiang, Sheetz et al. 2003; Lin, Jeon et al. 2005; Suki, Ingenito et al. 2005). For example, dendrimers carrying fluorescence probe have been successfully targeted to cancer cells
through folate-receptor binding. Liposomes have been studied for decades with numerous applications. Recently, polymer-based nanoparticles, metal oxides nanoparticles, and carbon-based nanostructure have joined the taskforce. However, most of these studies take advantage of well-defined surface biochemistry
and utilize ligand-receptor binding for targeting. Surface biophysics is largely ignored and rarely studied.

Gold nanoparticles (GNPs) have been introduced to science community as labeling probes for detection of protein and nucleic acids for decades. The chemistry of GNP synthesis has been improved over years. Size and shape of the GNP products is highly homogeneous. Most important of all, GNPs are well dispersed in solution. GNPs with their unique biocompatibility and convenience are methods of choice to attack fundamental doctrines that govern the surface biophysics (James ,Walter et al.2000; Wang , Regev, et al. 1998).

三、結果與討論

(1) Nanodot array modulates cell adhesion and induces apoptosis-like abnormality in NIH-3T3 cells:

Microstructure that mimics extracellular substratum promotes cell growth and differentiation while cellular reaction to nanostructure is poorly defined. To evaluate cellular response to a nano-scaled surface, NIH 3T3 cells were grown onto nanodot arrays with dot diameters ranging from 10 to 200 nm. The nanodot arrays were fabricated by AAO processing on TaN-coated wafers. A thin layer of platinum, 5 nm in thickness, was sputtered onto the structure to improve biocompatibility. Cells grew normally on the 10-nm array and on flat surfaces. However, 50-nm, 100-nm, and 200-nm nanodot arrays induced apoptosis-like events. Abnormality was triggered as early as 24 hours of incubation on a 200-nm dot array. For cells grown on a 50-nm array, the abnormality started after 72 hours of incubation. The number of filopodia extended from cell bodies decreased for abnormal cells. Immuno-staining using antibodies against vinculin and actin filament was performed. Both the number of focal adhesion and amount of cytoskeleton decreased for cell grown on 100-nm and 200-nm arrays. Pre-coating of fibronectin (FN) or type I collagen enforced cellular anchorage and prevented the nanotopography-induced programmed cell death. Nanotopography in the form of nanodot arrays induced apoptosis-like abnormality for cultured NIH 3T3 cells. The occurrence of abnormality is mediated by the formation of focal adhesions. **(manuscript submitted)**

(2) Assessment of the in vivo toxicity of gold nanoparticles: *Aims :* To investigate the in vivo toxicity of gold nanoparticles (GNP) in mice and to address the size-dependent biological response of nanoparticles.

Main methods: Naked GNPs ranging from 3 nm to 100 nm were synthesized, purified, and injected intraperitoneally into BALB/C mice at a dose of 8 mg/kg/week. Lethality was measured. Pathological examination was performed to major organs. The presence of gold particles at the diseased sites was verified by ex vivo Coherent anti-Stoke Raman scattering (CARS) microscopy. Antibody response of GNP was altered by surface modification through conjugating with synthetic peptides or proteins.

Key findings: GNPs ranging from 8 nm to 37 nm induced severe sickness and finally death in mice. GNPs of 3 nm, 5 nm, 50 nm, and 100 nm did not show harmful effects. Pathological examination of the major organs indicated an increase of Kupffer cells in the liver, loss of structural

integrity in the lungs, and diffusion of white pulp in the spleen. The pathological abnormality was associated with the presence of GNPs at the diseased sites, which was verified by CARS. Modifying the surface of the GNPs by incorporating immunogenic peptides ameliorated their toxicity. This reduction in the toxicity is associated with an increase in the ability to induce antibody response.
Significance: The in vivo toxicity of GNPs is size-dependent. This size-dependent property may be an important parameter when employing gold nanoparticles as a vehicle for drug delivery. It may also elicit the general toxicity of nanoparticles when exposed to the environment.
(manuscript submitted)

(3) Detection of gold nanoparticles using immunoglobulin-coated piezoelectric sensor: Since the existence of nanoparticles in our environment has already caught substantial attention due to their possible toxic impact on biological systems, field detection of nanoparticles is becoming a technology that will be greatly in need. We have constructed a piezoelectric sensor with antibody-coated electrode. The antiserum can bind gold nanoparticles with a high degree of selectivity and sensitivity. The biosensor thus constructed can detect 4 nm, 5 nm, or 6 nm gold nanoparticles (GNP) depending on the coated antiserum. Sensitivity for the detection of 5 nm GNP was 10.3 ± 0.9 ng/Hz, with the low limit of detection at 5.5 ng. The Quartz Crystal Microbalance (QCM) sensor was capable of detecting gold nanoparticles, even in the presence of other types of nanoparticles, such as TiO2, ZnO, or Fe3O4. The current study provides, for the first time, a platform for detecting nanoparticles in a convenient, economical, and most important of all, highly selective manner.
(Accepted by Nanotechnology)

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附件：封面格式

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共同主持人：

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中 華 民 國 年 月 日