Some aspects of Nanodiamond application

I. Medicine aspect

Virus Particle Absorption(or attenuation virulency?)

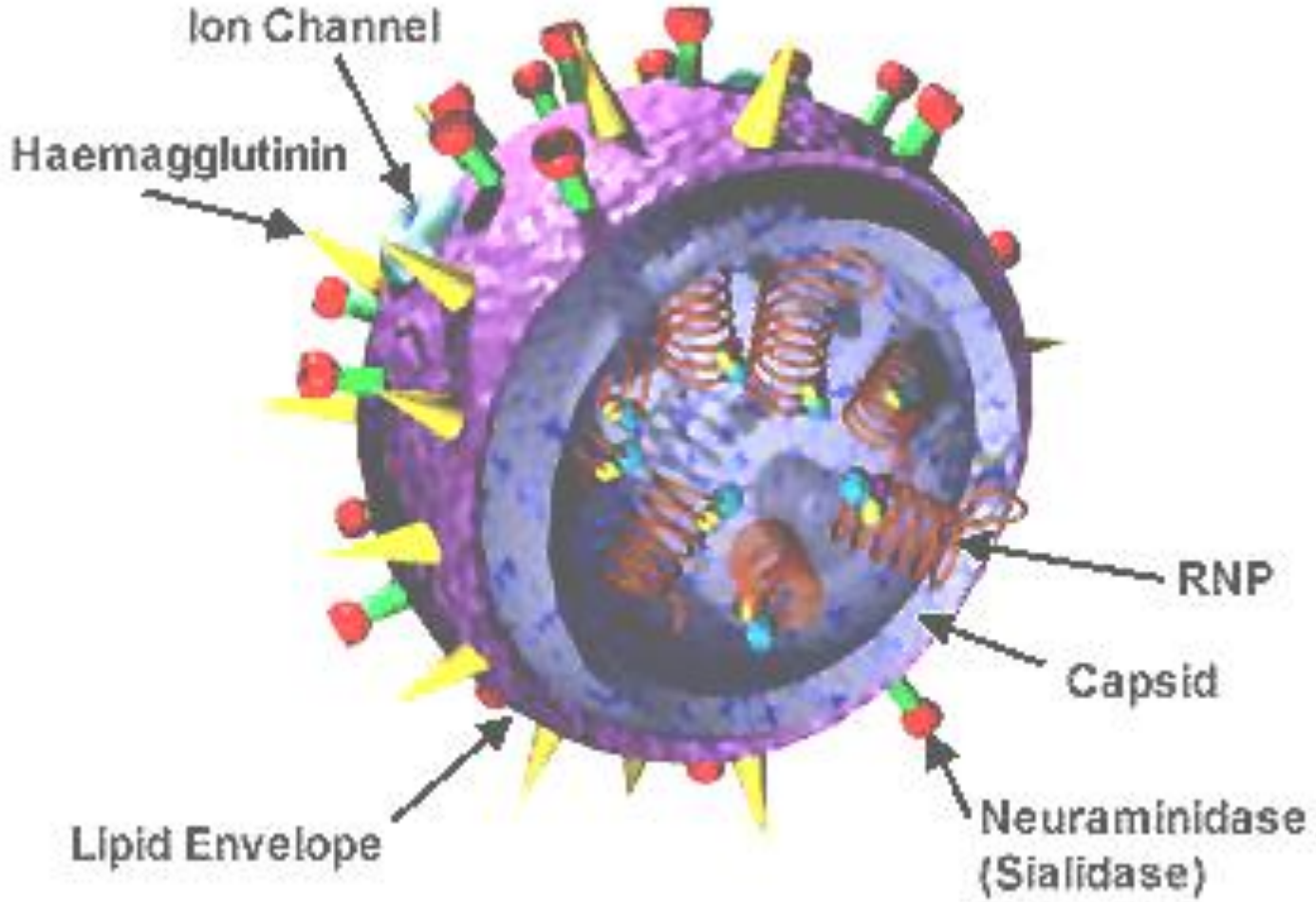
- Medical usage
  - Mask
  - hand scrub agent
  - surgical gloves & operating coat
  - Medical instrument disinfectant (eg.endoscope, surgical or dental instruments)

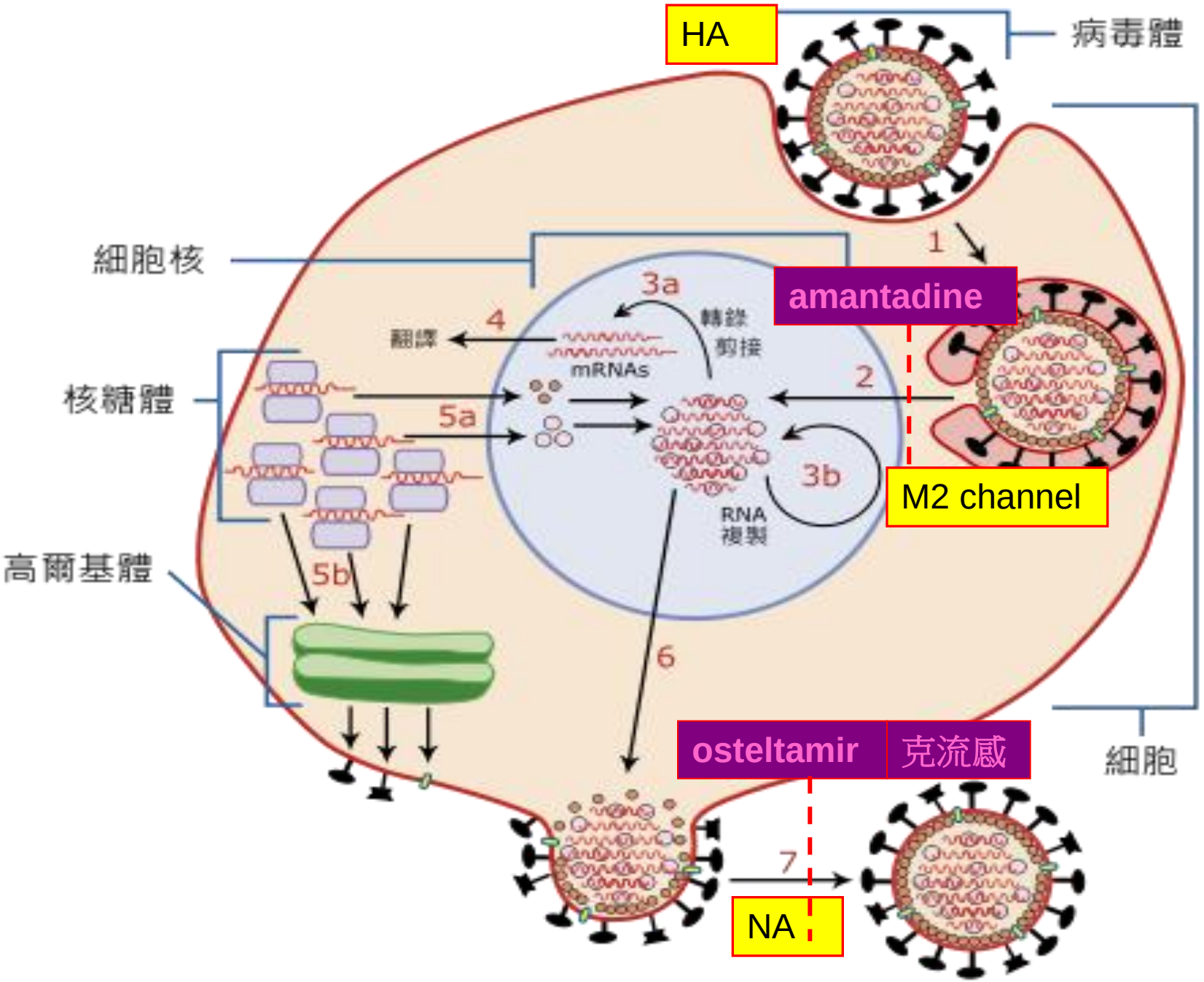
- Commercial products
  - hand cleaning gel
  - Environment sterilization agent
  - Mouth gargle agent & tooth paste
  - Condom for protect HIV, HPV,HSV
  - Water filtration membrane
  - Air conditioner filter (prevant disease outbreak)

About swine flu H1N1:

nanodiamond
&
Influenza Virus
sterilization mechanism?

Influenza virus




HA
病毒體
細胞核
1
amantadine
3a
轉錄
剪接
4
翻譯
mRNAs
2
核糖體
5a
3b
M2 channel
RNA
複製
高爾基體
5b
6
細胞
osteltamir
克流感
7
NA

Possible mechanisms of ND with different functional groups

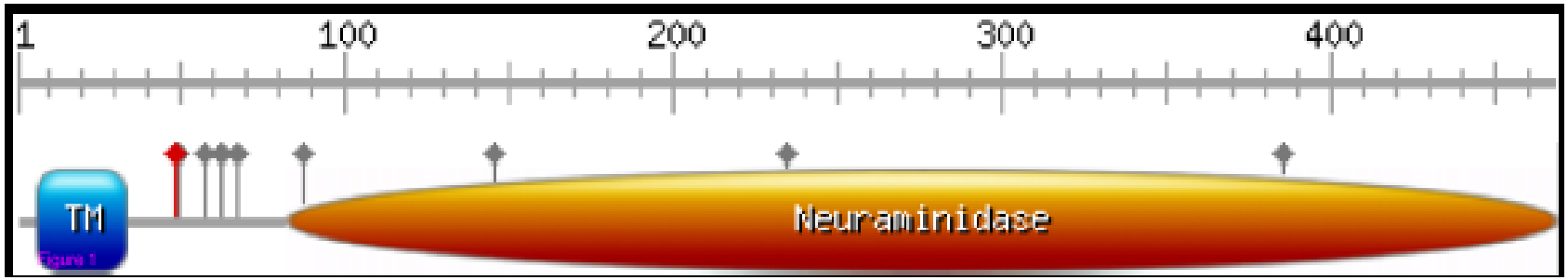
- Protein Capsid destruction

  (as 台大抗煞一號,電性相斥崩解)
- 8 pieces (-)RNA genome:
  - 11 proteins: hemagglutinin (HA), neuraminidase (NA), nucleoprotein (NP), M1, M2, NS1, NS2(NEP), PA, PB1, PB1-F2 and PB2
  - 破壞此8段RNA中重要基因結構(人體無negative RNA?)
  - 攜帶核苷酸類似物插入virus RNA製造複製過程停滯
  - 破壞其RNA-dependence RNA polymerase(人體內無此enzyme)
- 更強之M2 channel或 NA 抑制劑
- Lipid envelope destruction?(親油性?)

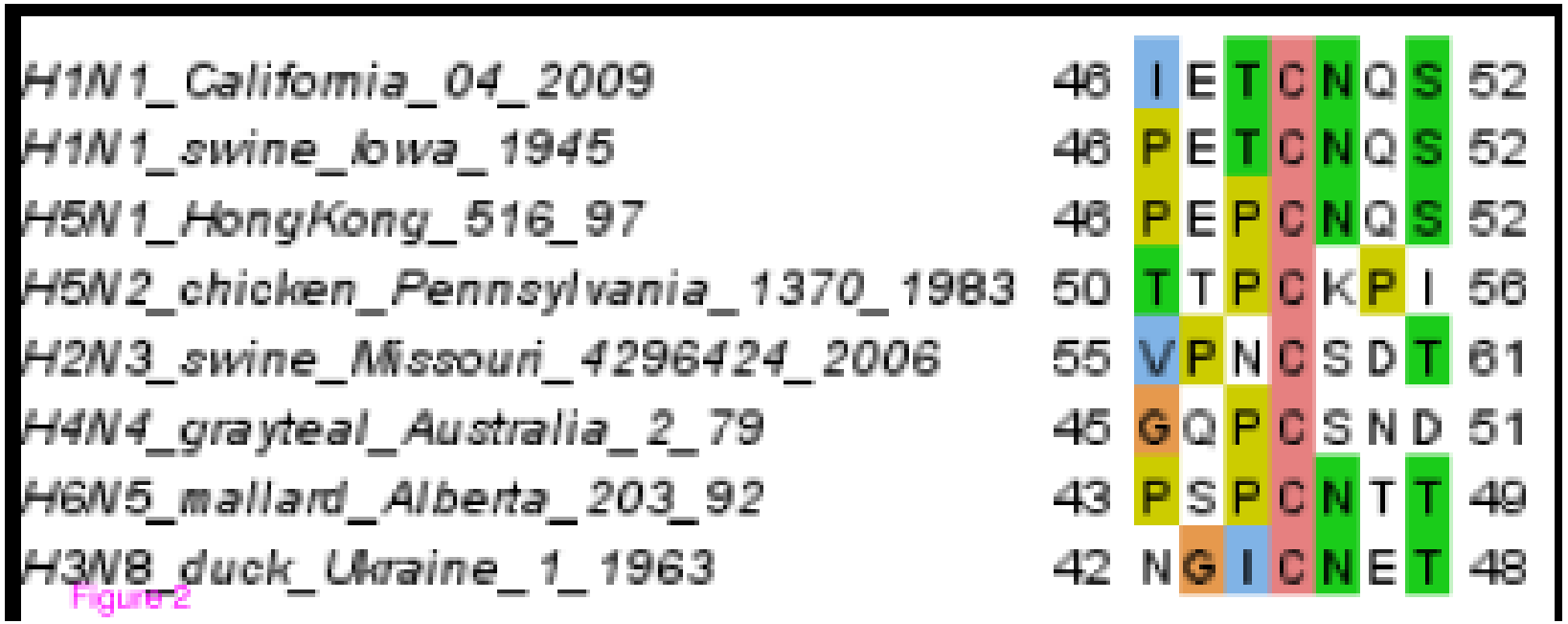
Mapping the sequence mutations of the 2009 H1N1 influenza A virus neuraminidase relative to drug and antibody binding sites

Biology Direct 2009, **4**:18

- sequence variations of the “2009 H1N1” influenza A virus strain neuraminidase
- Homology-based protein 3D structure modeling reveals that the novel mutations
- 3 currently used neuraminidase inhibitors: oseltamivir (Tamiflu®), zanamivir (Relenza®) and peramivir
- the antigenic regions of the neuramidase relevant for vaccine development, serological typing and passive antibody treatment



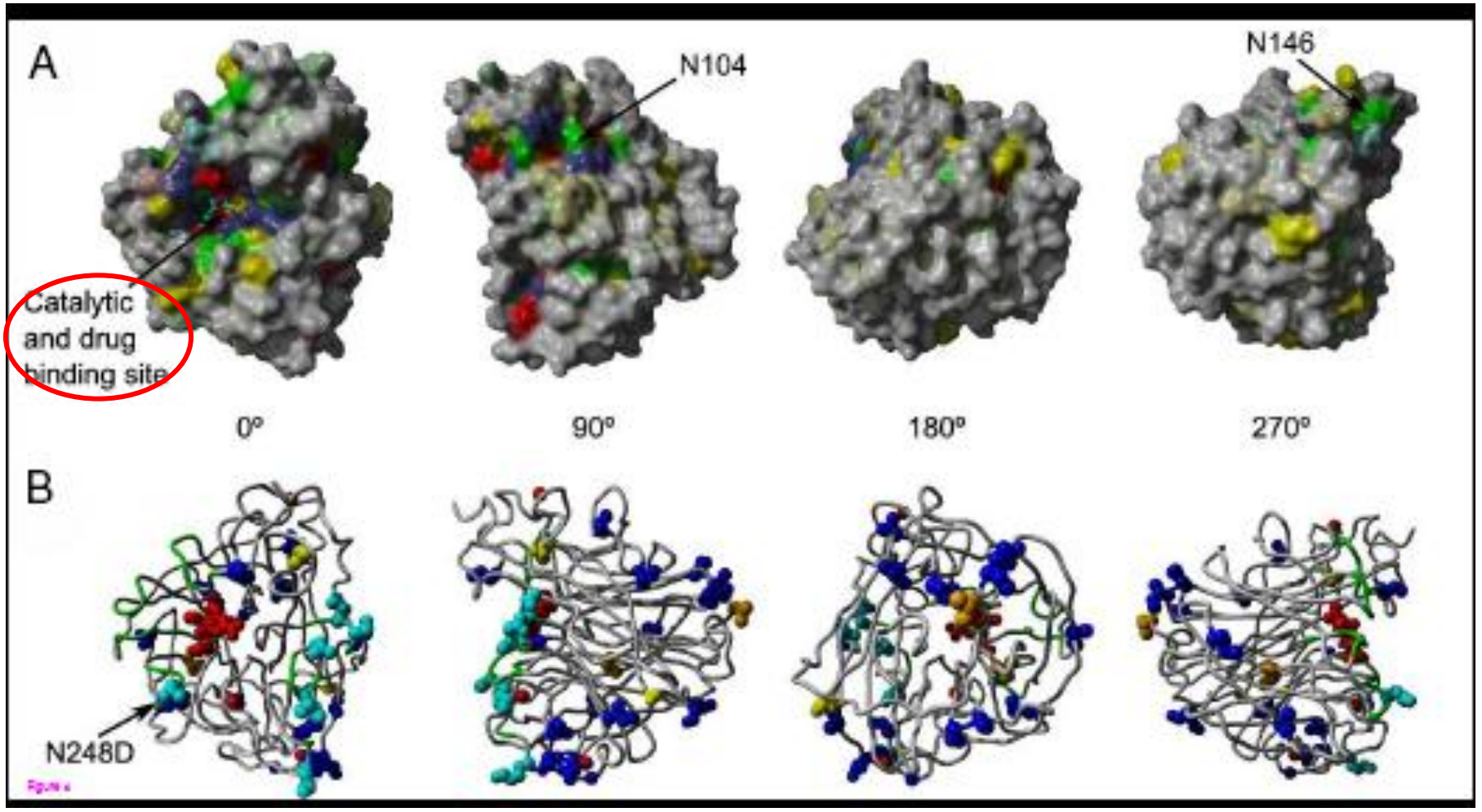
469 amino acid long

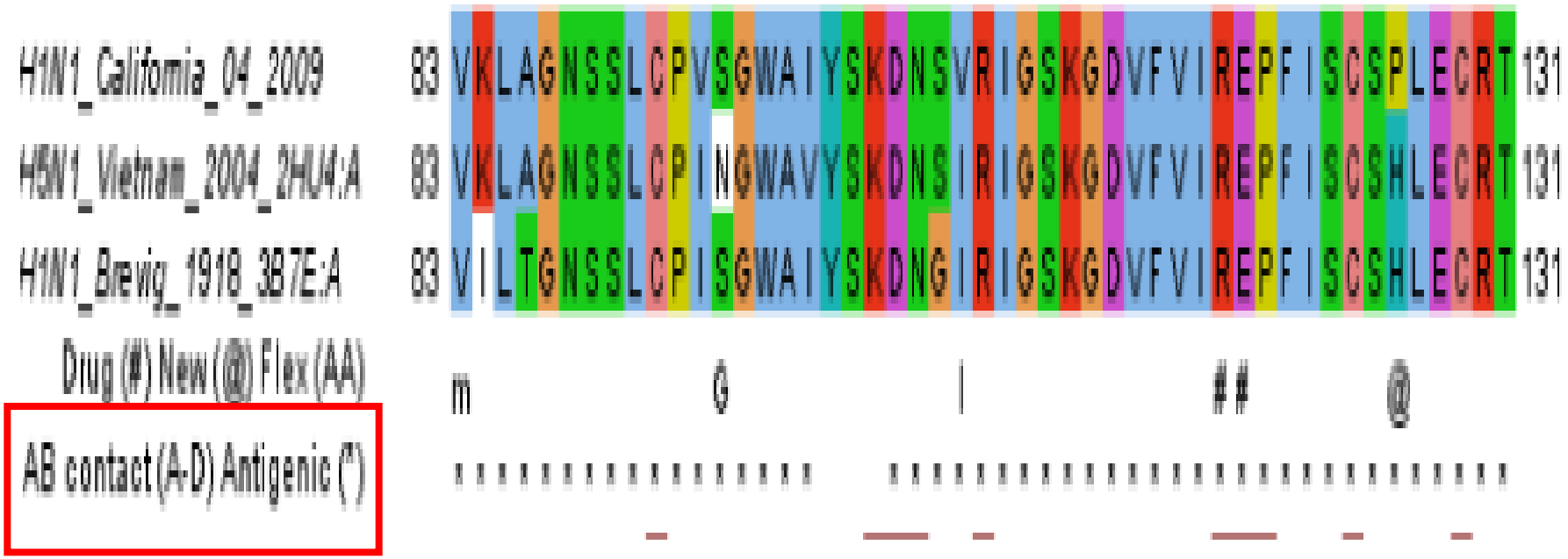


Cysteine C49—anchor?? Intermolecular disulfide bond??

Dimer

tetramer




H1N1_California_04_2009 83 VKLAGNSSLCPVSGWAIYSKDNSVRIGSKGDVFVIREPFISCSPLECRT 131
H5N1_Vietnam_2004_2HU4:A 83 VKLAGNSSLCPINGWAVYSKDNSIRIGSKGDVFVIREPFISCSHLECRT 131
H1N1_Brevig_1918_3B7E:A 83 VILTGNSSLCPISGWAIYSKDNGIRIGSKGDVFVIREPFISCSHLECRT 131
Drug (#) New (@) Flex (AA)
AB contact (A-D) Antigenic (*)

ND application for H1N1

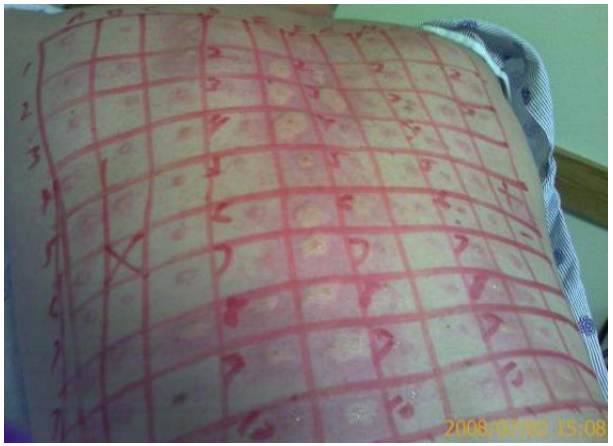
- neuraminidase destruction (protein level)
  - Cysteine 49? Destruct disulfur bond or lipid anchor
  - Destruct N104 & N146 for protein polymerization
- Enhance the NA inhibitor efficiency

ND application for immune

- Vaccine:
  - Traditional: carrier of antigen into human body
  - Gene vaccine: carry the antigenic genes into human genomes
- Artificial antibody:
  - epitope peptide chain synthesis
  - Carry genes into B cells?

ND application for medical examination

- Allergen test
  - 測血清中allergen-specific IgE antibodies,目前僅有skin test & 健保給付35種常見過敏原檢查
  - 未知allergen,具有allergic shock之危險!
  - 以nanodiamond二維矩陣製成千至萬種之過敏原檢驗盤, 再以血清反應觀察IgE結合強度,增強藥物過敏篩檢效率



ND application for medical examination

- Viral serology test
  - 病毒檢測方法僅限數種
  - 需在活細胞中培養,如雞胚
  - 空窗期之考量,無抗體產生,故呈陰性

  - Nanodiamond 可辨認病毒外殼glycoprotein—(ie.病毒靠岸之船錨)

Using Detonation Nanodiamond for the Specific Capture of Glycoproteins

Anal. Chem. **2008,** *80,* 4659–4665

- **the functionalization of detonation nanodiamond (ND) with aminophenylboronic acid (APBA)**

  for the purpose of targeting the selective capture of glycoproteins from unfractionated protein mixtures
- **A loading capacity of ~350 mg of glycoprotein of ND could be attained on ND**
- **functionalized ND as a high-efficiency extraction and analysis platform for proteomics research**

Nanodiamond:

Biocompatiblity
&
non-cytotoxic

For implant coating, local drug delivery and in vivo usage?

Protein-mediated assembly of nanodiamond hydrogels into a biocompatible and biofunctional multilayer nanofilm **ACS Nano. 2008 Feb;2(2):203-12**

- diameter of 2-8 nm

- assembled into a closely packed ND multilayer nanofilm

  with positively charged poly-L-lysine

  via the layer-by-layer deposition technique

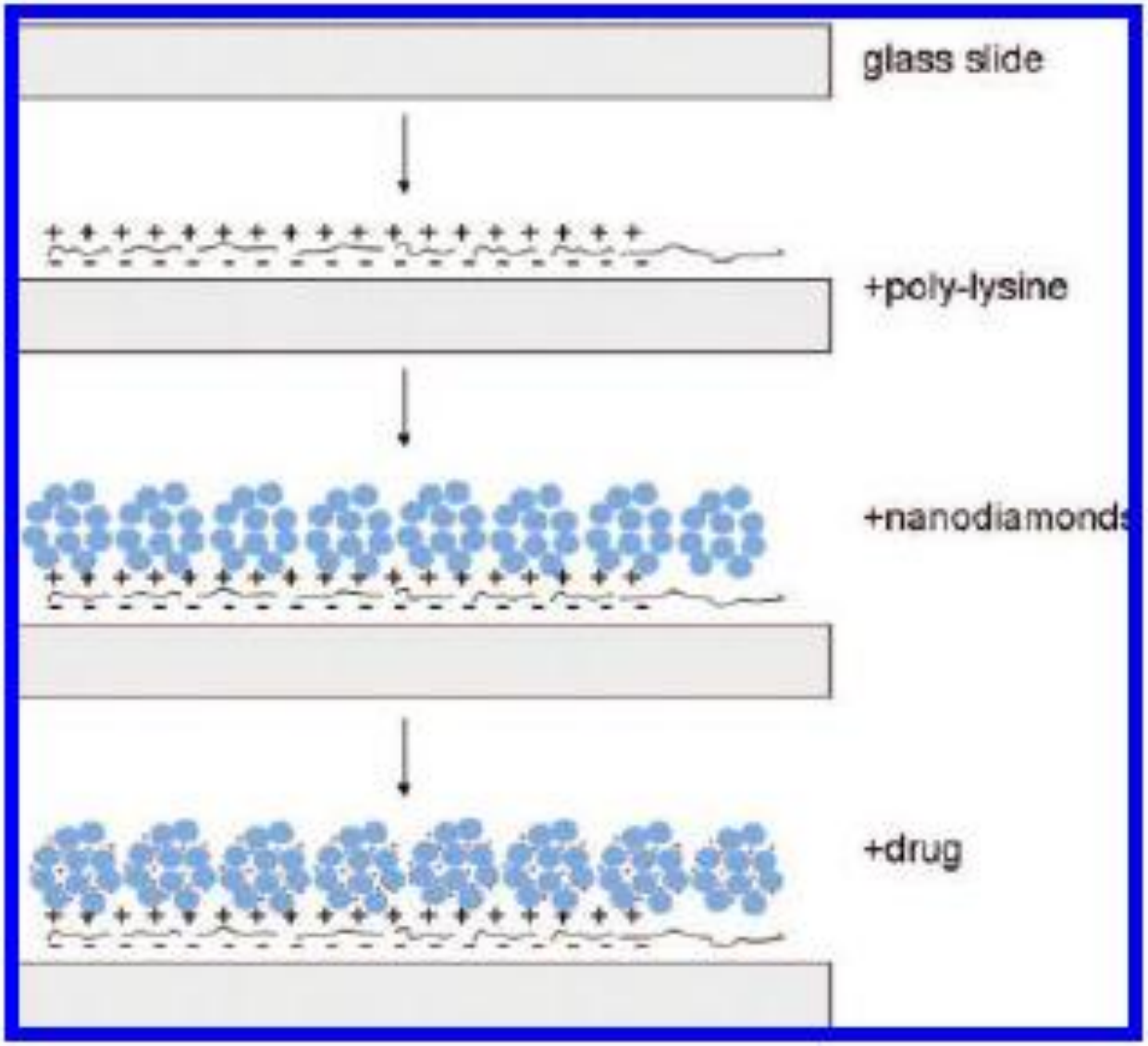


Figure 2. Schematic drawing of nanodiamond nanofilm formation and drug incorporation into the film. Poly-L-lysine is used to attract NDs onto the glass surface.

- The innate biocompatibility of the NDs:

  (free-floating and thin-film forms)

(1)cellular gene expression examination by real-time polymerase chain reaction

(2)MTT and DNA fragmentation assays

- Knockdown of lipopolysaccharide-mediated inflammation

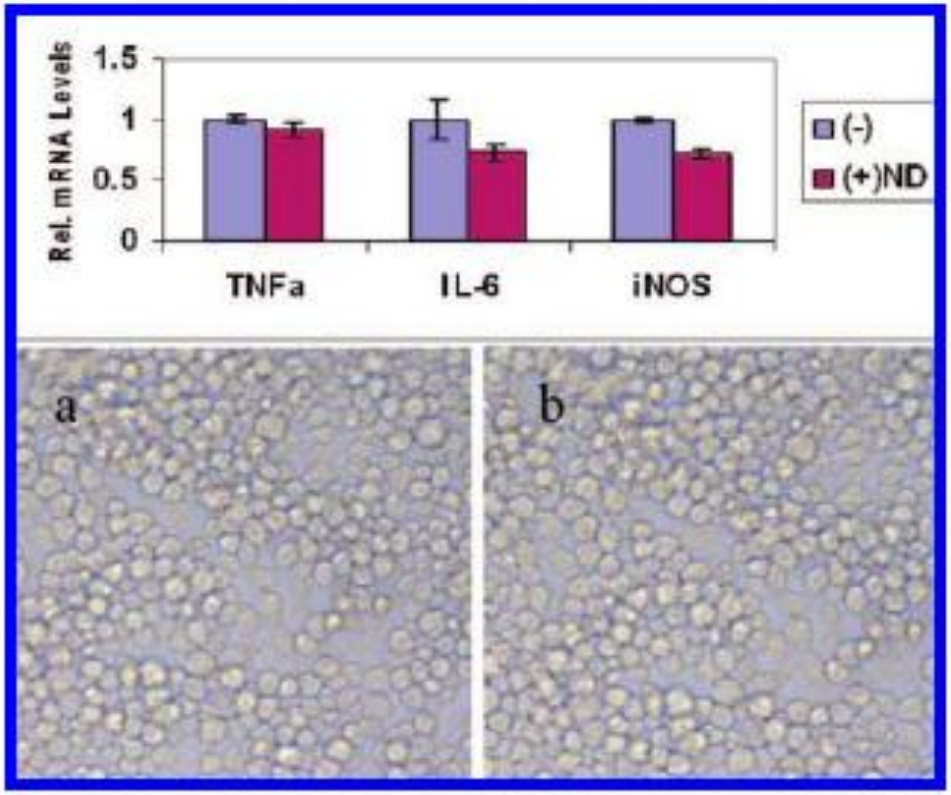


Figure 7. (Top) Genetic analysis (from RT-PCR) of ND innate biocompatibility, performed to monitor inflammation of RAW 264.7 murine macrophage grown for 24 h without and with addition of free-floating nanodiamonds (30 μg/mL).

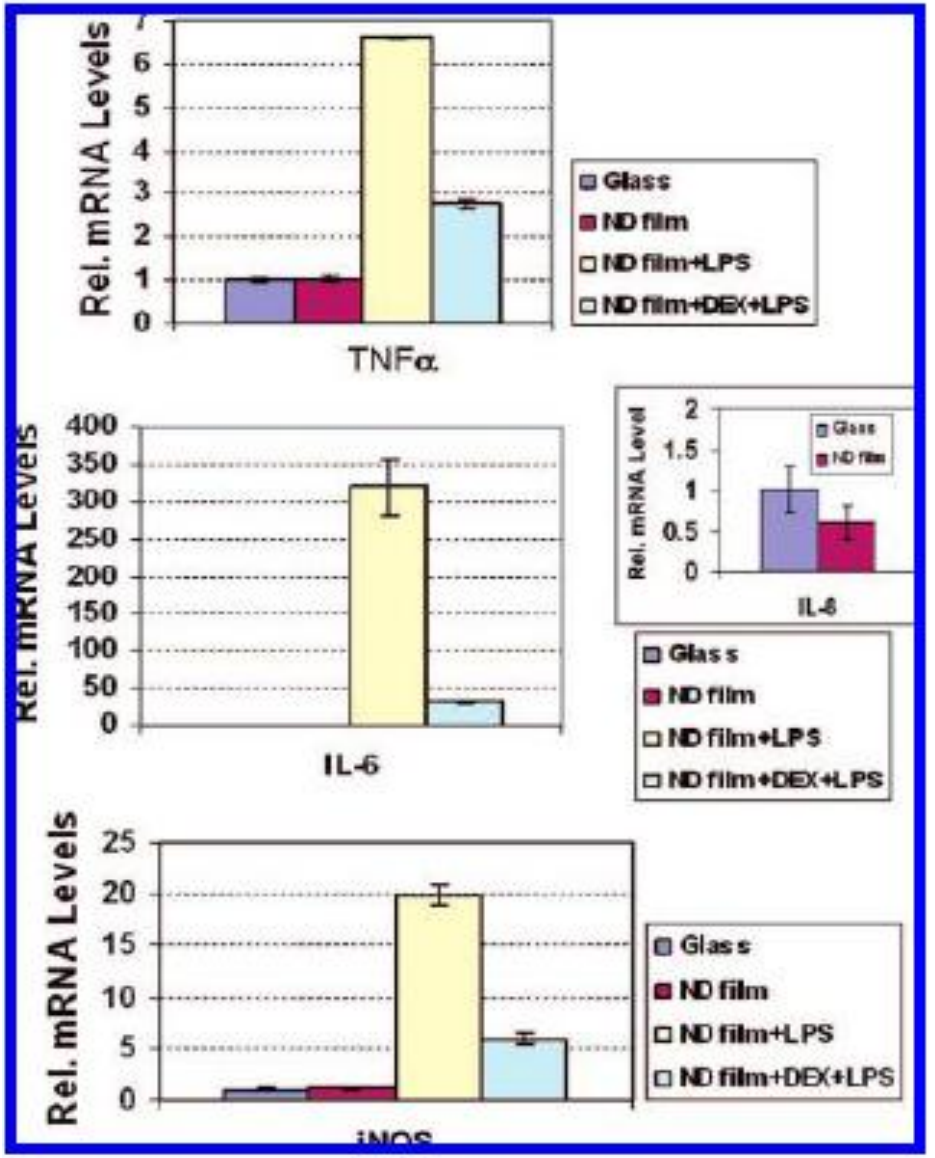


Figure 8. Genetic analysis (from RT-PCR) was performed to monitor the inflammation of RAW 264.7 murine macrophage grown for 24 h on ND films, ND films with lipopolysaccharide (LPS)-mediated inflammation (4 h), and ND–dexamethasone (Dex) films with LPS-mediated imflammation (24 h). Glass slides coated with PLL were used as a control. From top to bottom are gene expression profiles for

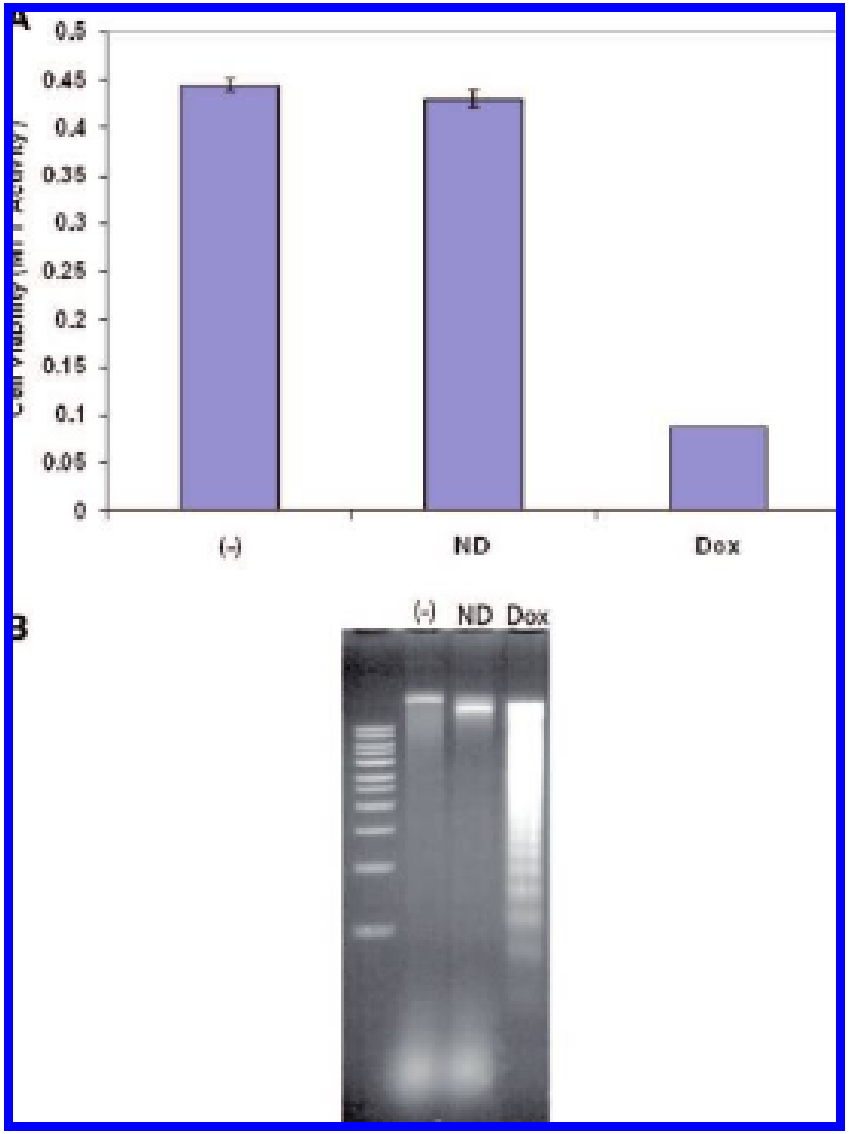


Figure 10. (A) Cell viability assay. Murine macrophage cells (RAW 264.7, ATCC) were grown in the absence (−) or presence of 25 μg/mL nanodiamonds (ND) or 2.5 μg/mL doxorubicin (Dox) for 24 h and were subjected to an MTT-based cell viability assay (Sigma-Aldrich). (B) DNA fragmentation assay. Murine macrophage cells (RAW 264.7, ATCC) were grown in the absence (−) or presence of 25 μg/mL nanodiamonds (ND) or 2.5 μg/mL doxorubicin (Dox) for 24 h and were harvested. DNA was purified and loaded on an agarose gel for analysis.

Are Diamond Nanoparticles Cytotoxic?

J. Phys. Chem. B, Vol. 111, No. 1, 2007

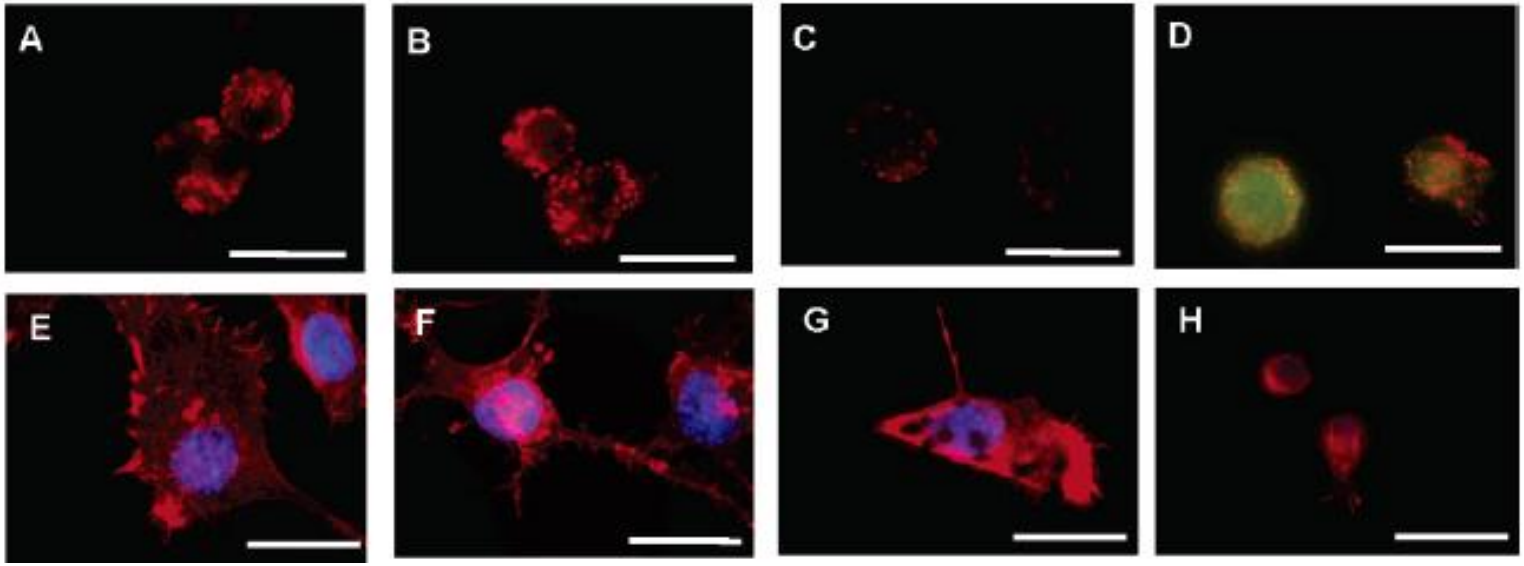
- nanodiamonds size from 2 to 10 nm
- **Assays of cell viability**

  (1)mitochondrial function(MTT)
  (2)luminescent ATP production
- not produce significant reactive oxygen species
- Cells can grow on nanodiamond-coated substrates without morphological changes

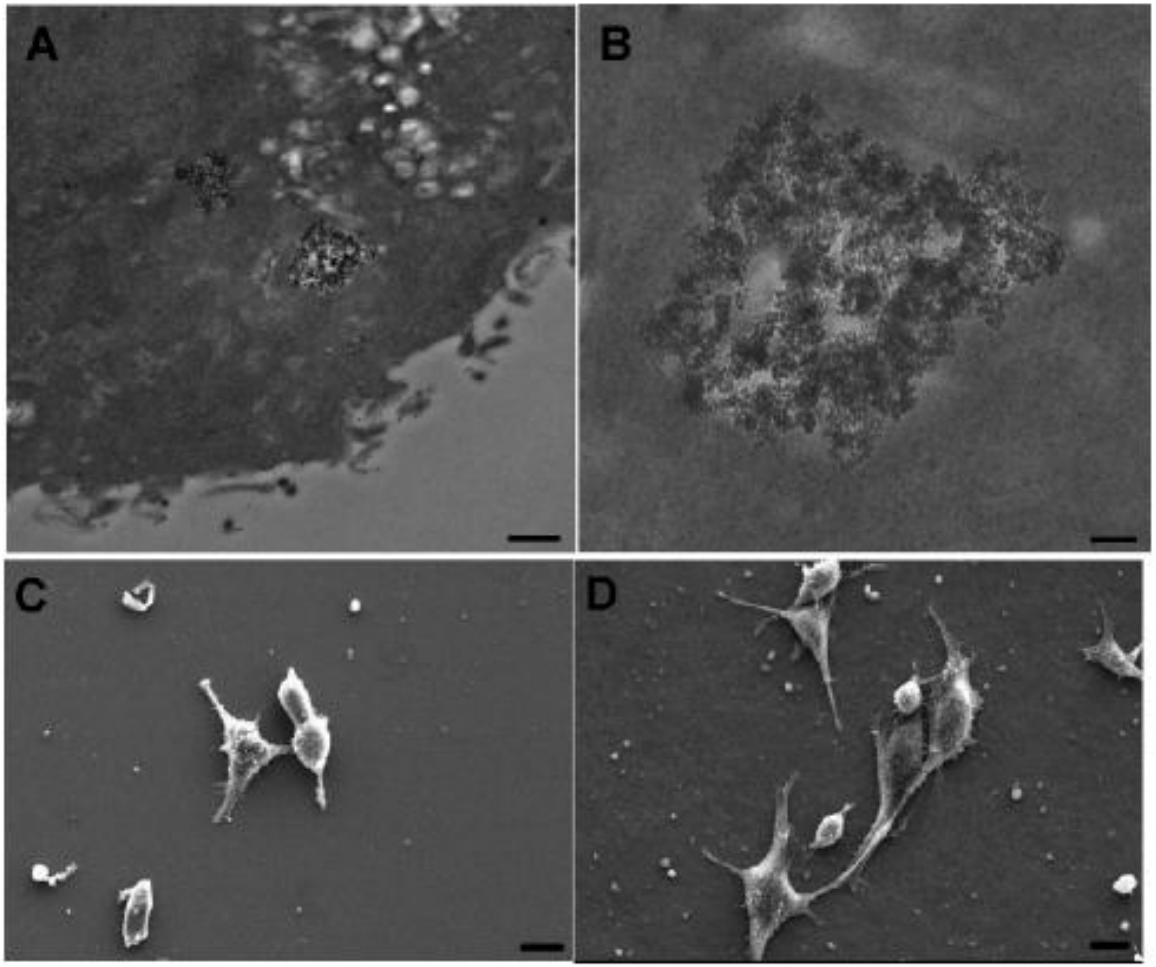
ND-raw ND-COOH CB(-) CdO(+)

Figure 1. Incubation of cells with various nanoparticle concentrations after 24 h viewed with light microscopy: (A) control; (B) 100 μg/mL ND-raw; (C) 100 μg/mL ND−COOH; (D) 100 μg/mL CB; (E) 2.5 μg/mL CdO. Note that nanoparticles are seen surrounding the cell borders and attached to neurite extensions whereas cell morphology is unaffected by the presence of nanodiamonds. Scale bars are 20 μm.

ND-raw CB CdO



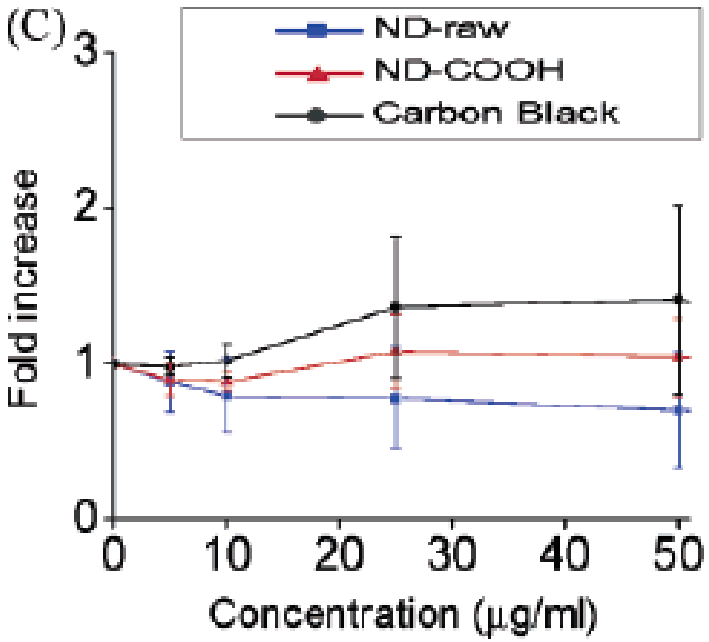
NO Mitochondrial membrane & cytoskeleton change

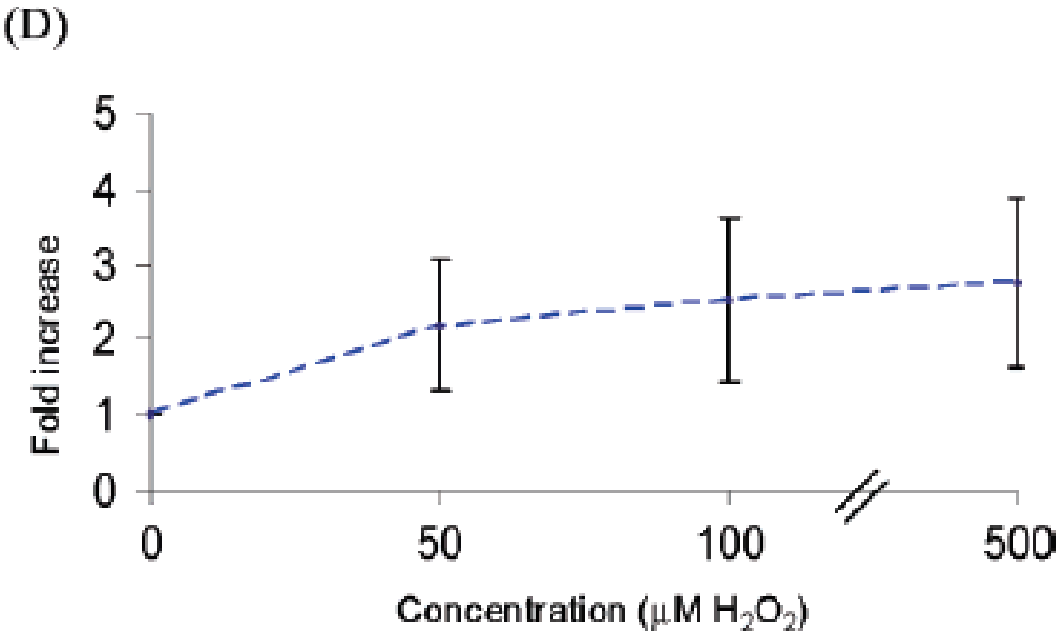


(1)internalized nanodiamonds after exposure to 25 *í*g/mLND-COOH for 24 h.

(2) cells grown on a ND-COOH coated collagen substrate after 96 h

ROS production test





not produce significant reactive oxygen species

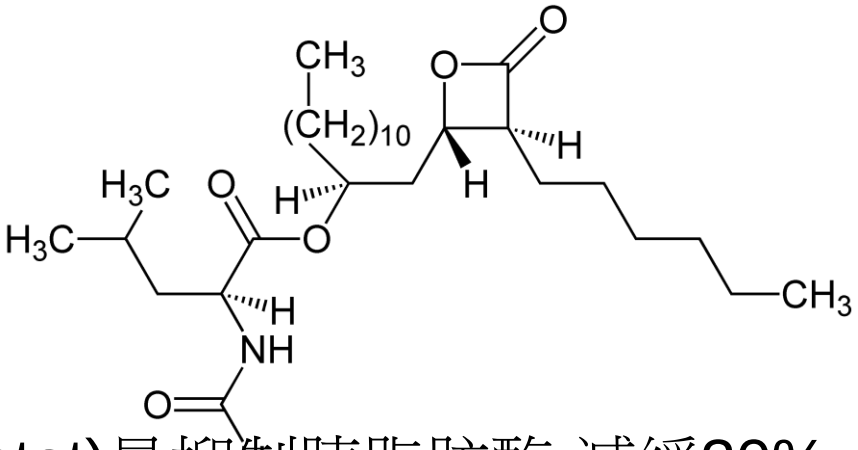
- Implant coating
  - Artificial joint(骨髓炎之預防,加入ND合併微小包覆體抗生素釋出)

- 無排斥性植入式晶片
  - 失智老人,精神分裂患者,寵物
  - Biosensor: 血糖,血脂肪,血壓值
- 長期投與藥物釋出系統
  - 避孕荷爾蒙類藥物
  - 癌末病人止痛如fentanyl

II.Biochemical molecule absorption & excretion?

Health food or pharmacy

- Lipid
  - 減重

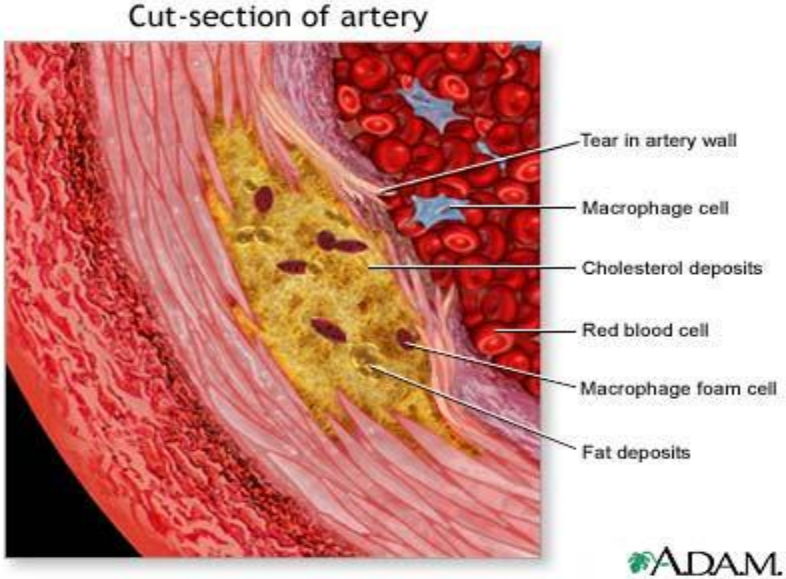


    - 目前僅有Xenical(Orlistat)是抑制胰脂肪酶,減緩30%腸道脂肪吸收其餘皆為抑制食慾或促交感神經藥物

  - ND於腸道中吸附攝食之油脂(如何定量?)
  - 目前有1064nm Nd-YAG Laser於熔解皮下脂肪之應用,但仍需傷口使脂肪抽吸出體外

    - ND 可進入細胞層次,或可藉由ultrasound加速其深入帶出脂肪?

- Lipid
  - Fatty acid
    - 增強攜帶多元unsatuated FA 被細胞利用能力,如魚油
  - Cholesterol
    - 降低血清中低密度脂蛋白(LDL)濃度,預防心血管疾病
    - 已存在之血管璧脂肪條塊或粥狀硬化中,使fat之清除(血管清道夫),延緩冠狀動脈硬化心肌梗塞



- Diabetes
  - Type I : insulin gene therapy carrier
  - Type II:
    - **Enhance blood sugar into cell ,reduce blood sugar conc.**
    - **Absorption and excretion excess insulin**

- **排毒??(諾得膠囊—active charcoal only)**
  - **腎: creatinine,blood urea nitrogen,uric acid**
  - **肝:ammonia, bilirubin**

Keloid or hypertrophic scar

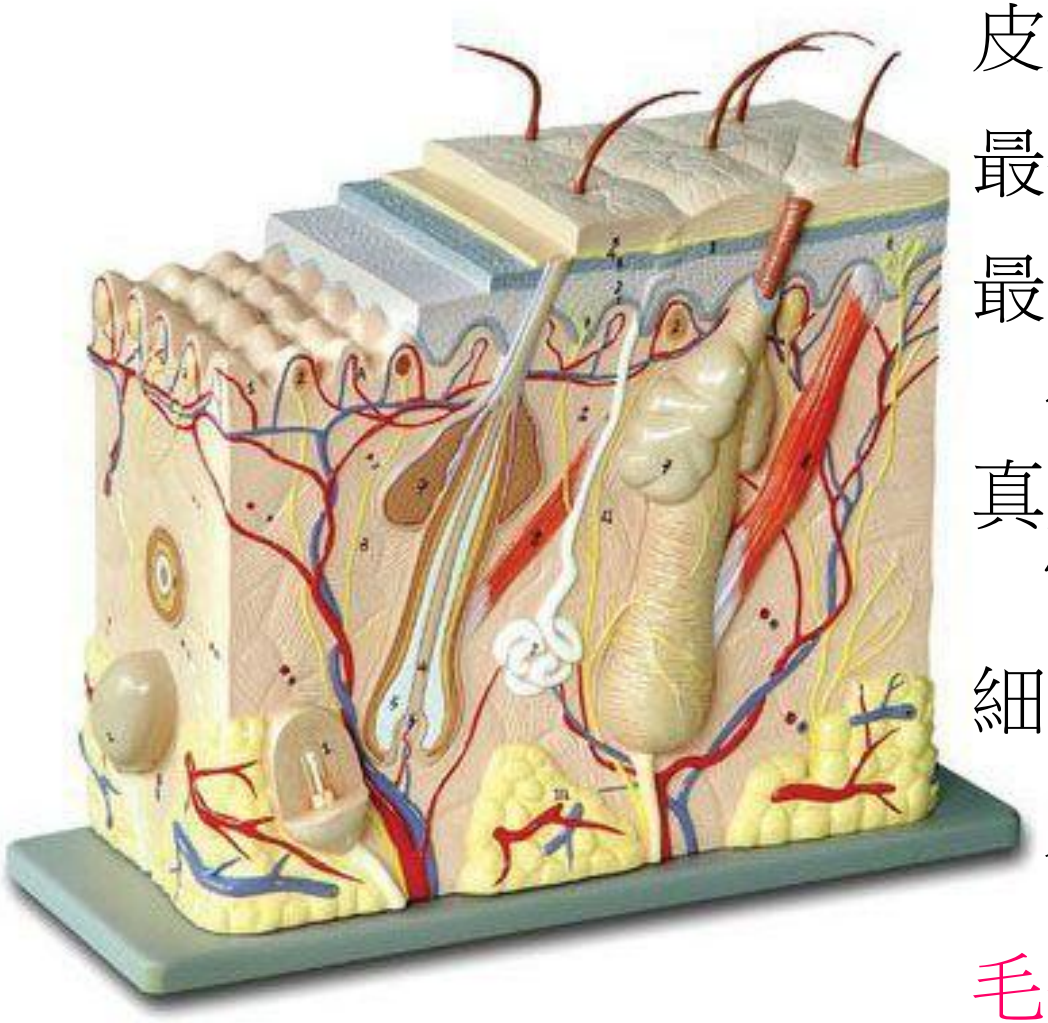
- Type I , III collagen增生
- Surgery: 50%復發
- 注射類固醇:3~6months復發
- Laser, cryotherapy:傷害大

- ND可攜帶collagenase或新研發中之 alfa-interferon, 5-FU, bleomycin進入疤痕組織

Others

- 抽油煙機濾網
- 生命事業
  - 胎毛,結婚,葬儀紀念
- 傳熱性佳應用於鍋具,烹飪爐具烘烤用途

III.Aesthetic & cosmetics



皮膚厚度:平均0.2mm

最外角質層:→黯沉淨白

最內基底層:細胞分裂,決定皮膚外觀→修復

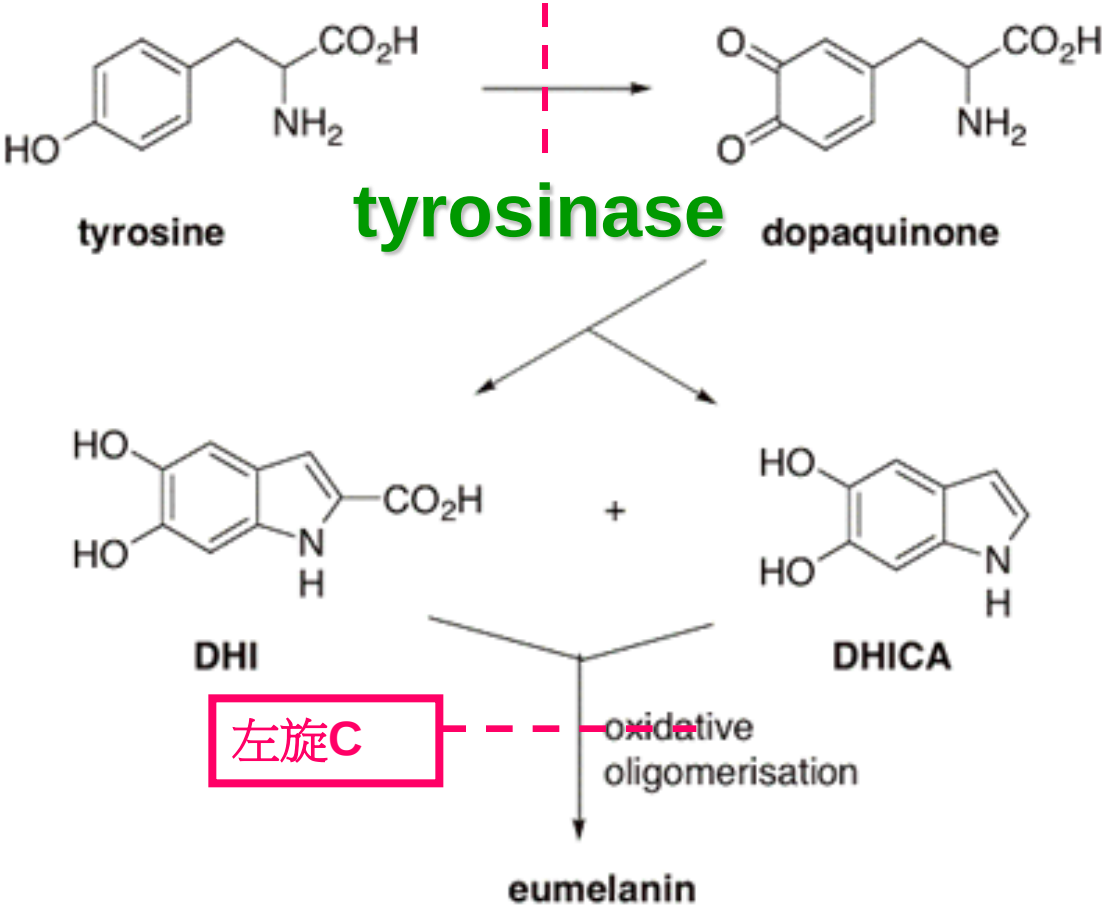
真皮: 合成膠原蛋白&彈性蛋白→回春除皺

細胞間隙:natural moisturing factor (主含玻尿酸)→保濕

毛孔粗大→過度出油 or 保濕不足

黑色素合成

Hydroquinone, 熊果素,鞣花酸, 甘草萃取,傳明酸



去角質排除melanin: 水楊酸,杜鵑花酸

防曬,除皺,美白?

- UVA1,2(98%)320~400nm→PA係數: 曬黑
- UVB(<2%)290~320nm→SPF :曬傷曬紅
- UVC(被O3吸收)
- 物理性:
  - Zinc oxide(收斂抗菌)→UVA&UVB
  - TiO2:UVA&UVB
  - 易阻塞毛孔

- 化學性:
  - Octyl salicylate(UVB)
  - Parsol 1789(UVA1 &UVB)
  - Mexoryl SX(UVA & UVB)

- 防曬+美白:nanodiamond鍵結美白成分深入黑色素細胞抑制tyrosinase,基底加入防曬成分

- 防曬+美白+除皺緊實:

  除上述美白成分,利用nanodiamond可深入毛孔及基底細胞與真皮層處,藉由熱能刺激膠原蛋白及彈性蛋白增生(同1064nm Nd-YAG Laser原理),達到除皺緊實以及縮小毛孔效果
- 熱能之來源:
  - 由UV轉換而來?

美容儀器

- 除皺紋老化
  - 如何達到與Laser同樣功效?
  - 利用放射腫瘤科治療前的simulation原理,做出適合個人臉型的構模,此為可塑性材料,接觸臉部含有nanodiamond
  - 或塗佈一層含有nanodiamond的介質於臉部,以光照或是其他能量使其傳熱於皮膚深部

Bipolar Fractional Radiofrequency Treatment Induces Neoelastogenesis and Neocollagenesis

Lasers in Surgery and Medicine 41:1–9 (2009)

- The FRF system delivered RF energy directly within the dermis via 5 micro-needle electrode pairs
- Tissue temperature was held at 72C for 4 seconds
- marked induction of tropoelastin, fibrillin, procollagens 1 and 3 by 28 days post-treatment

臨床常見皮膚問題

- 出油
  - ND可吸附油脂
- 毛孔粗大
  - ND可深入毛孔,藉由熱能促進collagen增生縮小毛孔
- 粉刺
  - 先以蛋白酵素去角質,ND再包覆吸附阻塞毛孔中的油脂,可利用靜電原理配合剝除面膜帶離

- 青春痘

  同上,但可再測試抗菌能力以及抗發炎附加功效,並加速帶走游離脂肪酸
- 黑眼圈
  - 色素型:同美白原理
  - 血循不良型:同配合導熱性佳原理促進循環
  - 過敏鼻炎型:可作為鼻噴劑抑制局部發炎反應