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國立中興大學  
National Chung Hsing University



NSP for Life !

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High-Surface NSP Supported AgNP  
For Pets and Medical Uses

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antimicrobial agent with high safety and efficacy

- ■ ■ ■ ■
- ■ ■ ■ ■

## "NSP-supported AgNP" – (NSP-Ag)

Antibacterial/skin wound dressing and healing/burn treatment,  
mosquito bite caused skin Irritation and itching,

Hemostasis and De-odorant

**anti-MRSA and anti-biofilm**

unique feature: (compared with the conventional AgNP in the market)

1. **Antibacterial Efficiency** (use less dose of Ag and effective for anti-MRSA)
2. **Low Toxicity/Safety** (Safety: (TAg) > (AgNP) > (silver sulfadiazine or trade name, Silvadene or SS))
3. **High Stability under heat and oxygen oxidation** (high stability against heat, and oxidation by O<sub>2</sub>/UV)

●Note: **MRSA** (Methicillin-resistant Staphylococcus aureus)



# NSP and NSP-Ag—medical and health care uses

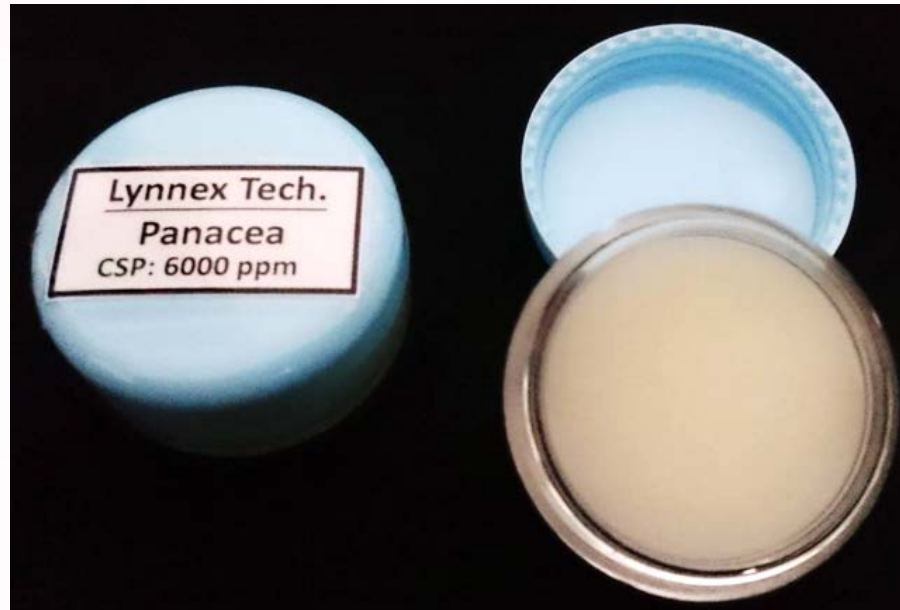
Anti-bacterial for Methicillin-resistance Staphylococcus aureus (MRSA)

mouthwash



- natural and nontoxic
- antimicrobial
- **deodorant**
- anti-virus
- anti gum disease

Skin Cream



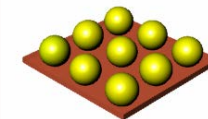
- antimicrobial
- skin-irritation
- **anti-itch**
- **blood-clotting/hemostasis**

Skin Silver-Cream  
(external use only)



- antimicrobial
- **anti-burn**
- **after bites**

# NSP-Ag — applications



**Medical Uses:** wound dressing, Anti-MRSA, surface antimicrobial coating (bone/medical devices, blood clotting/hemostatic spray)

Waste water treatment/  
air filter



Silver/Graphene dispersed  
paste Nano-Ag powder for  
conductivity

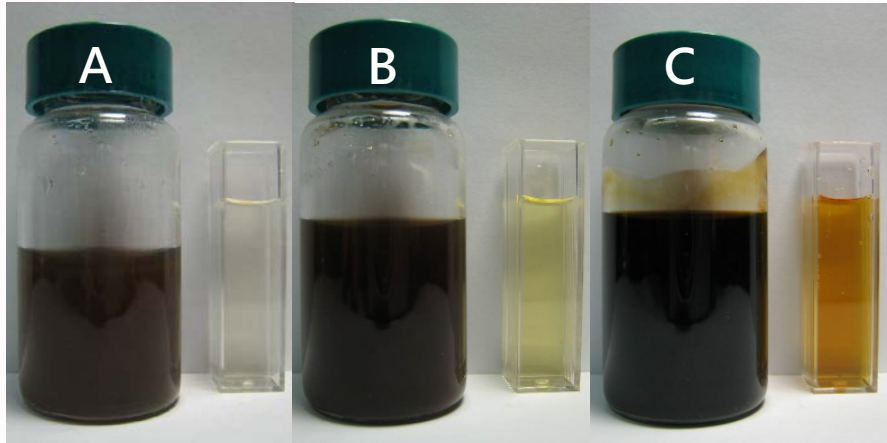
## Colloid, Paste and Powder forms

disperse and thermally stable:  
silver paste, silver powder

Environmental uses (Ag sprays)

Antimicrobial agents for fabrics,  
package films, 3C equipment...

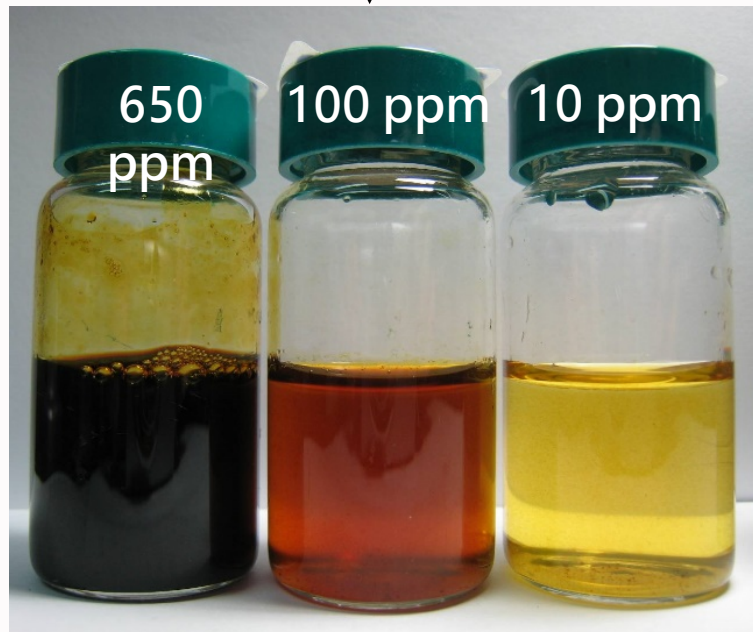
**Safety:** NSP-Ag > nano copper =  
nano zinc >> traditional silver  
nanoparticles (high cytotoxicity)



(golden or ink-colored solution and powder)

1. Highly effective for anti-microbial and anti-MRSA uses
2. low toxicity (cytotoxicity and genotoxicity)

water dilution  
↓



highly stable against air/oxygen

dried  
→



paste or powder forms and thermally stable  
over process temperature



# AgNP/NSP or NSP-Ag rendering the mitigation of the inherent toxicity of Ag-NP

Professor Zhang, NTUH Department of Orthopedic Surgery

## PLOS ONE

### RESEARCH ARTICLE

#### Composition of nanoclay supported silver nanoparticles in furtherance of mitigating cytotoxicity and genotoxicity

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### Abstract

Silver nanoparticle (Ag-NP) is well known for its high antibacterial efficacy. However, its toxicity toward mammalian cells is still a concern in clinical applications. The aim of our study was to evaluate the composition effects of Ag-NP supported by silicate nanospinelite (NSP) with respect to the cytotoxicity and genotoxicity, and was in reference to the poly (styrene-co-maleic anhydride)-supported Ag-NP (Ag-NP/SMA). The NSP at the geometric dimension of averaged 80 x 80 x 1 nm<sup>3</sup> was prepared from the exfoliation of natural clays and used to support different weight ratio of Ag-NP. The supporting limitation of NSP on Ag-NP was below the weight ratio of 15/85 (Ag-NP to NSP), and the detached Ag-NP from the Ag-NP/NSP (30/70) and Ag-NP/SMA hybrids exhibited mutagenicity when the concentration was 1.09 ppm or higher. In viewing of cytotoxicity using MTT tests toward HaCaT cells, the IC50 of Ag-NP/NSP (1/99, 7/93 and 15/85) were 1416.7, 243.6, and 148.9 ppm respectively, while Ag-NP/SMA was 64.8 ppm. The IC50 of Ag-NP/NSP (1/99, 7/93 and 15/85) were at least 833, 78 and 7 folds higher than their corresponding minimum inhibitory concentrations (MIC) respectively, and whereas Ag-NP/SMA was 6.4 folds. The Ag-NP/NSP and Ag-NP/SMA hybrids had been further investigated for genotoxicity by chromosomal aberrations and *in vivo* micronucleus assay within the concentration at IC10 and IC50, only Ag-NP/SMA showed a higher frequency of chromosomal aberrations. Our findings indicated that the viability of utilizing the NSP to maintain Ag-NP for antimicrobial activity, and the high-surface area of NSP served as an excellent support for associating Ag-NP and consequently rendering the mitigation of the inherent toxicity of Ag-NP in clinical uses.



### OPEN ACCESS

**Citation:** Chang CH, Lee YH, Liao ZH, Chen MH, Peng FC, Lin JJ (2021) Composition of nanoclay supported silver nanoparticles in furtherance of mitigating cytotoxicity and genotoxicity. PLOS ONE 16(2): e0247521. <https://doi.org/10.1371/journal.pone.0247521>

**Editor:** Teckjittai Kowatt, Addis Ababa Science and Technology University, ETHIOPIA

**Received:** August 25, 2020

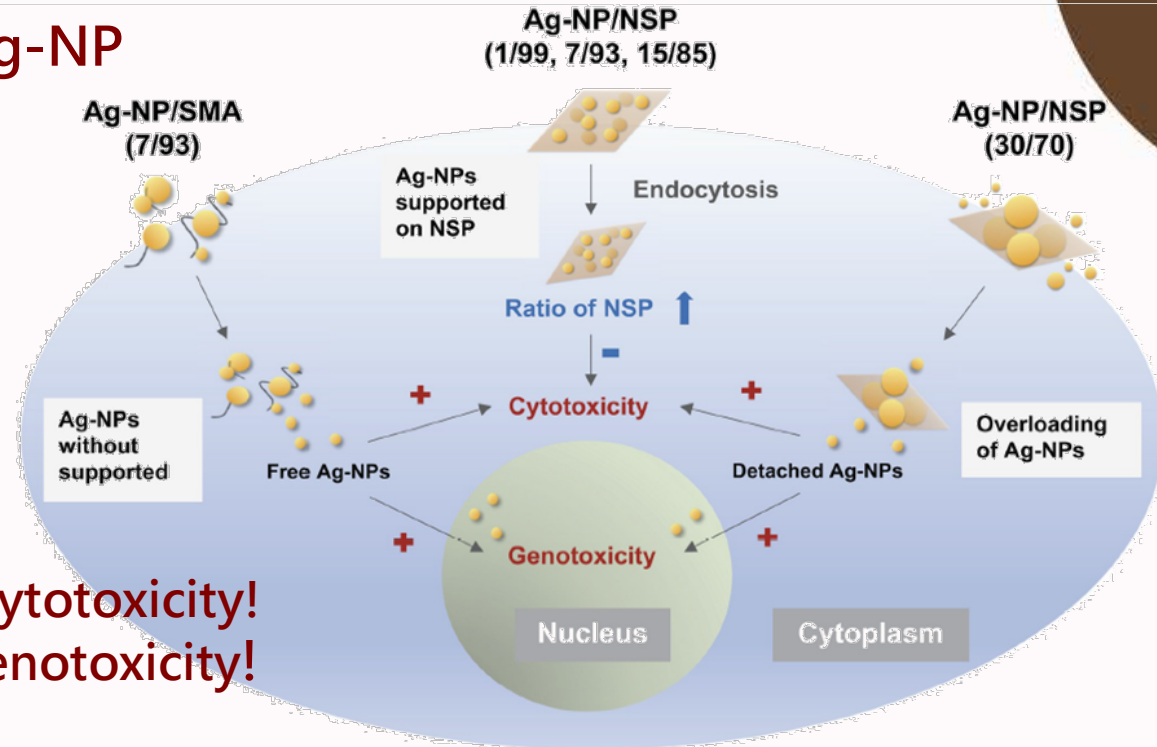
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**Data Availability Statement:** All relevant data are within the manuscript and its Supporting Information files.

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Low cytotoxicity!  
No genotoxicity!

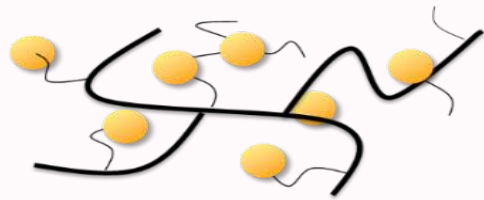
Fig4. Conceptual illustration of the interactions between the hybrids, Ag-NP/SMA (7/93) or Ag-NP/NSP (ranging from 1/99–30/70) with cells.

- The supporting effect of NSP on Ag-NP : *dependent on the weight ratio of 15/85 (Ag to NSP), for controlling adherence of AgNP-on-NSP, revealed by TEM direct observation.*
- NSP may support Ag to mitigate the inherent toxicity of AgNP in cell toxicity and no genotoxicity.

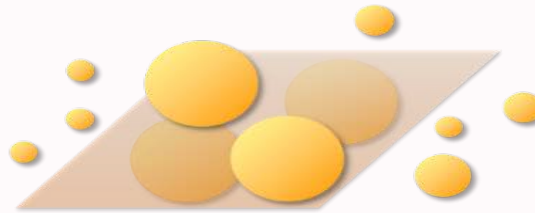
(comparing SMA-polymer stabilized Ag and conventional nanoparticles usually entering cells through various forms of endocytosis)

Professor Zhang, NTUH Department of Orthopedic Surgery

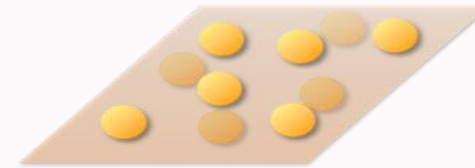
**NSP carrier--Particles do not enter nuclear cells**  
**TAg--low cytotoxicity/no genotoxicity**



Ag-NP/SMA  
(7/93)

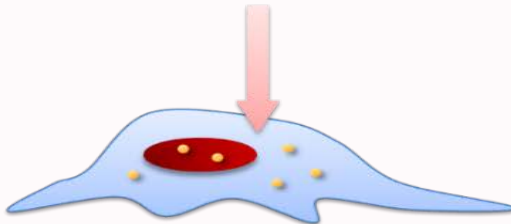


Ag-NP/NSP  
(30/70) (50/50)

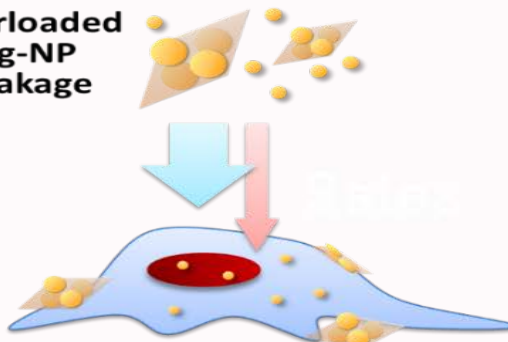


Ag-NP/NSP  
(1/99) (7/93) (15/85)

Ag-NP  
cellular  
uptake

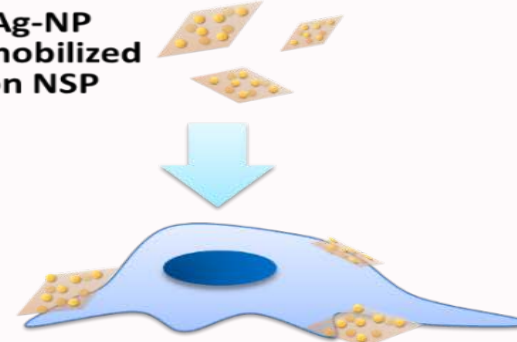


Overloaded  
Ag-NP  
Leakage



Genotoxicity & Cytotoxicity

Ag-NP  
immobilized  
on NSP



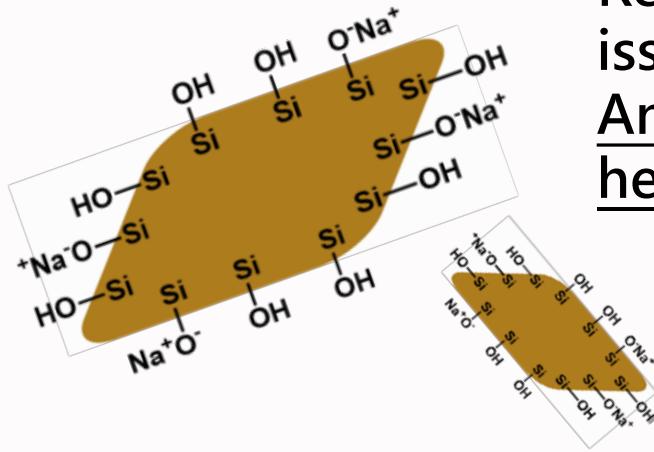
Non-toxic

Mitigating Cytotoxicity and Genotoxicity of Silver Nanoparticles 31 Chang/Lin, Composition of nanoclay supported silver nanoparticles in furtherance of mitigating cytotoxicity and genotoxicity, PLOS ONE , 2021,16, e0247531

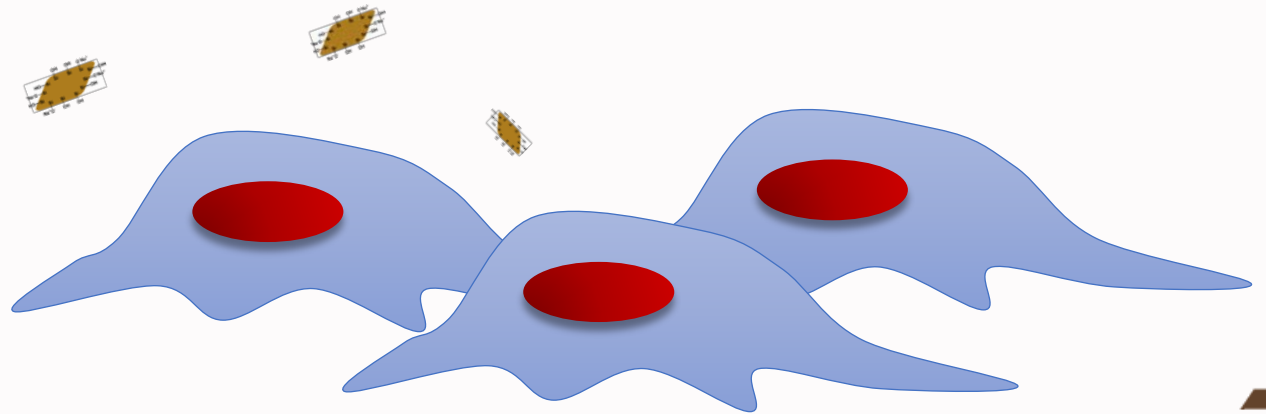
# NSP interactions with virus, microorganism and cell --- NSP physical interaction

What will it happen when NSP contact with living cell?  
Regarding to the toxicity of cytotoxicity and genotoxicity issue:

Answer: NSP-supported AgNP is not entering the cell nuclei, hence causing none of genotoxicity.



231 Chang/Lin, Composition of nanoclay supported silver nanoparticles in furtherance of **mitigating cytotoxicity and genotoxicity**, PLOS ONE, 2021,16, e0247531



cell nuclei

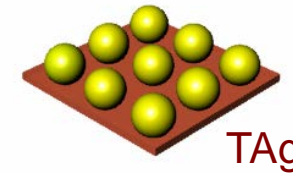


# Antimicrobial Spray (commercial product in Taiwan since 2009)



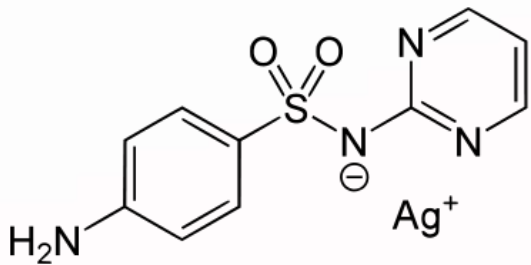
# [NSP-Ag] for wound healing and against infection

154. Chia-Yu Chu\*, Fu-Chuo Peng, Ying-Fang Chiu, Hsing-Chuan Lee, Chien-Wen Chen, Jiun-Chiou Wei and Jiang-Jen Lin\*, 2012.  
Nanohybrids of Silver Particles Immobilized on Silicate Platelet for Infected Wound Healing, PLoS One, 7, e38360



Silver sulfadiazine (Silvadene)

Taiwanese Patent I 462754 to A.T.P. CO., LTD.)



**Adverse effects: hypersensitivity, discoloration of the skin and silver accumulation in organs**

## Regarding NSP-Ag for inhibiting Ag (ion)-resistant bacteria (anti-MRSA)

144.Hong-Lin Su\*, Siou-Hong Lin, Jiun-Chiou Wei, I-Chuan Pao, Shu-Her Chiao, Chieh-Chen Huang, Shinn-Zong Lin and Jiang-Jen Lin\*, 2011. Novel Nanohybrids of Silver Particles on Clay Platelets for Inhibiting Silver-Resistant Bacteria, PLoS One, 6 · e21125

OPEN ACCESS Freely available online




# Novel Nanohybrids of Silver Particles on Clay Platelets for Inhibiting Silver-Resistant Bacteria

**Hong-Lin Su<sup>1,3\*</sup>, Siou-Hong Lin<sup>1</sup>, Jiun-Chiou Wei<sup>2</sup>, I-Chuan Pao<sup>1</sup>, Shu-Her Chiao<sup>1</sup>, Chieh-Chen Huang<sup>1</sup>, Shinn-Zong Lin<sup>4,5,6</sup>, Jiang-Jen Lin<sup>2,7\*</sup>**


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