Hong Kong Student Science Project Competition 2022

Template of Extended Abstract (Investigation)
(Word Limit: 1,000 words, Pages: 2 pages only)

Team Number: SBBC268

Project Title: Green Synthesis of Silver and Copper Nanoparticles and Their Potential Application in Killing Bacteria and Fungi for Burn Wound Dressing

Project Type: Investigation

To our best knowledge and after thorough literature research, as on _3__/_7__/_2022__ , there are / are ~~no~~* similar works. If there are, the reference links are as below:

Balavandy, Sepideh Keshan et al. "Rapid and green synthesis of silver nanoparticles via sodium alginate media." *International Journal of Electrochemical Science* 10 (2015): 486-497.
https://doi.org/10.3390/molecules16097237
https://doi.org/10.1038/s41598-017-07989-w

The enhancement our project has made for the existing related products or research is summarized as below:

We are the first to separate okra pods into mucilage, central axis and seeds and the first to report that mucilage can act as both capping and reducing agents in the synthesis of AgNPs, enabling one-pot AgNPs synthesis, and providing a greener synthesis pathway.
The synthesised AgNPs can self-crosslink with PVA without adding crosslinking agents such as tetraborate, which enhances the potential of applying AgNPs to burn wounds in the form of hydrogels.

***Please delete if not applicable. HKSSPC values the originality of works. Students must conduct literature research thoroughly to ensure that their works are unique, and to list relevant reference materials to complement the research or invention.**

I. Background

Among the various capping agents of AgNPs, natural polymers, such as chitin, alginate and starch, are desired because of their high biocompatibility [1]. AgNPs are also capable of being functionalized with hydrogels. A hydrogel is a three-dimensional, hydrophilic, crosslinked insoluble polymeric matrix with the ability to extend and retain a significant fraction of water within its structure. Hydrogels are usually synthesized by the crosslinking of hydrophilic polymers by the reaction between functional groups and external chemicals. The AgNPs-hydrogel composite often features properties like high porosity, hydrophilicity, swelling capacity and lack of toxicity, which is desired in an ideal wound dressing [2]. K. K. Y. Wong *et al.* [3]also reported that the healing process of second-degree burn wounds was accelerated in BALB/C mice when they are treated with AgNPs. It has been shown that AgNPs can modulate the cytokines involved in the wound-healing process and give a better cosmetic appearance. In this report, AgNPs were synthesized in a green way with different natural capping agents, and their antibacterial and antifungal properties were investigated. The approach to forming nanoparticle-hydrogel composite was brought by the gelation of the synthesized nanoparticles in hydrogel-forming polymers by crosslinking long polymer chains of PVA with mainly nanoparticles and a significantly low amount of borax.

II. Objectives

Our project aims at investigating and optimizing the methodologies of the synthesis of natural-product-capped metal nanoparticles, which exhibit the best antibacterial and antifungal effect, which also can form hydrogel composites with a high potential in the application for burn and chronic wounds treatment.

III. Hypothesis

The natural polymers and carbohydrates in the natural products could be used to chelate and reduce the metal ions into metal nanoparticles.
All synthesized nanoparticles could be able to exhibit considerable antibacterial and antifungal properties.
Well-known crosslinkers such as sodium tetraborate could be used as the only crosslinker in the synthesis of hydrogel.

IV. Methodology

Synthesis of nanoparticles:
Materials: $CuCl_2$, $AgNO_3$, $NaBH_4$, KI, starch, sodium alginate, chitosan, cysteine, acetic acid, L-ascorbic acid, natural products such as: okra, aloe vera, avocado seeds, green tea.
Natural products were extracted(if necessary), metal precursors were added into the capping agent for chelation, followed by addition of reducing agent (or self-reduce). UV-Vis absorbance spectroscopy and nanoparticle tracking analysis were conducted (if the synthesis was successful). Experiments were repeated 4 times each. The peak of absorbance would confirm the formation of NPs and the size of the NPs were deduced from the blue-red shif of the UV-vis spectrum and NTA.

Antibacterial test:
Materials: Lysogeny broth (LB), E. coli (cultured in LB), LB agar plate.
Disc-diffusion assay and OD_{600} quantitative analysis were conducted. The antibacterial strengths of NPs were qualitatively tested in disc diffusion assay by comparing the inhibition zones. The OD_{600} analysis was used to quantitatively monitor the growth of bacteria in different [NPs] by measuring its absorbance at $\lambda = 600$ nm, to determine the inhibition ratio and minimum inhibitory concentration (MIC). Both experiments had a control (without NPs) for comparison and were repeated 3 times each.

Antifungal test:
Materials: bread (without preservatives), fungi (Penicillium, collected from a molding fruit)
NPs of different concentrations were brushed on the left-hand side of the bread while the right-hand side was served as a control, and the bread were added with a drop of fungi dispersed in water in the centre. The anti-fungal effect of each type and concentration of the NPs was determined by the number of days taken for the fungi to contaminate the left-hand side of each slice of bread.

Synthesis of hydrogel:
Materials: Polyvinyl alcohol (PVA), sodium tetraborate
PVA and NPs solution were mixed and stirred until the mixture was gel-like (significant higher viscosity). NPs at low concentrations were added with sodium tetraborate since they failed to crosslink with PVA. The experiment was repeated 3 times.

V. Results

Chitosan, alginate, okra, cysteine and starch chelated AgNPs were successfully produced. Chitosan, Starch and alginate chelated nanoparticles were of higher monodispersity, with lower FWHM. In okra chelated AgNPs, 2 particle sizes were observed and the UV-vis spectrum was more polydispersed.

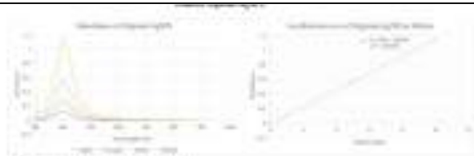


Figure 4: Left: the absorbance of Alginate/AgNPs. Right: the calibration curve of Alginate/AgNPs at 404 nm.

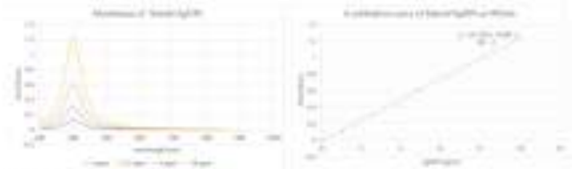
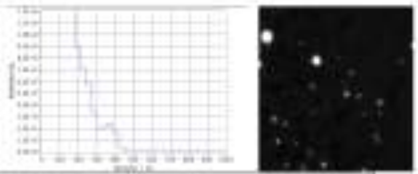


Figure 2: Left: the absorbance of Starch/AgNPs; Right: the calibration curve of Starch/AgNPs at 402 nm

Figure 3

Left: histogram showing the distribution of Starch/AgNPs by particle size;

Right: the NTA image of starch-chelated AgNPs.



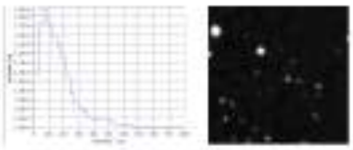
Peak analysis of NTA of Starch/AgNPs			
Diameter / nm	Particles / mL	FWHM / nm	Percentage / %
82.5	2.4E+6	152.5	100.0

Table 1: peak analysis of NTA of Starch/AgNPs

Figure 5

Left: histogram showing the distribution of Alginate/AgNPs by particle size;

Right: the NTA image of Alginate/AgNPs.



Peak analysis of NTA of Alginate/AgNPs			
Diameter / nm	Particles / mL	FWHM / nm	Percentage / %
84.8	3.8E+6	186.1	100.0

Table 2: peak analysis of NTA of Alginate/AgNPs

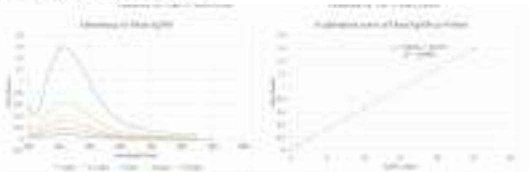
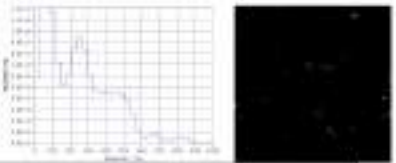


Figure 7: Left: the absorbance of Okra/AgNPs. Right: the calibration curve of Okra/AgNPs at 414 nm.

Figure 8

Left: histogram showing the distribution of Okra/AgNPs by particle size;

Right: the NTA image of Okra/AgNPs.



Peak analysis of NTA of Okra/AgNPs			
Diameter / nm	Particles / mL	FWHM / nm	Percentage / %
234.3	8.1E+5	147.1	55.8
76.6	1.4E+6	82.9	42.8

Table 3: peak analysis of NTA of Okra/AgNPs

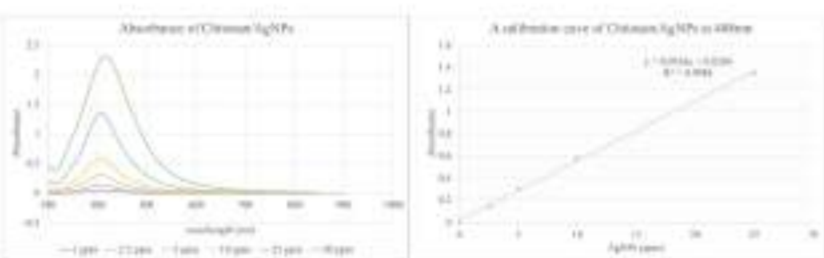
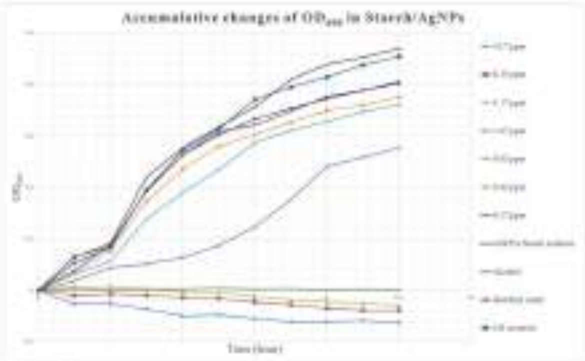


Figure 6: Left: the absorbance of Chitosan/AgNPs.
Right: the calibration curve of Chitosan/AgNPs at 408 nm.

However, the synthesized CuNPs were very unstable and oxidized quickly by reacting with the oxygen dissolved in the solution. Thus, they are unsuitable for the application onto hydrogels.

A significant antibacterial effect was shown by starch, alginate and chitosan-chelated NPs, with MIC of 4.17 ppm, and the growth of fungi was delayed by AgNPs with a concentration of 100 ppm. The resultant MIC, 4.17 ppm, was less than half of that of commercial AgNPs (10 ppm) as reported by Li et al. [4].



Hydrogels were successfully synthesized with the AgNPs. AgNPs at higher concentrations can self-crosslink with PVA without the addition of sodium tetraborate, which increases the potential of applying our AgNPs on burn wounds by means of hydrogel.

VI. Conclusion

Chitosan, alginate, okra, cysteine and starch-chelated AgNPs were successfully produced by one- step synthesis. Among these samples, the okra extract could be used as both the capping and reducing agents, without the addition of $NaBH_4$, providing a green synthesis pathway of AgNPs. However, the synthesized CuNPs were very unstable and oxidized quickly by reacting with the oxygen dissolved in the solution. They were also unsuitable for the application onto hydrogels.

The antibacterial effect of Chitosan/AgNPs was the strongest, followed by Starch/ and Alginate/AgNPs. The antibacterial effect of Okra/AgNPs was not significant, due to their polydispersity of diameters. The MIC of Starch/AgNPs and Alginate/AgNPs are 4.17 ppm, which was less than half of that of the commercial one. However, a relatively low antifungal effect was observed in AgNPs, in which a notable delay in fungal growth was only presented in AgNPs with a concentration of 100 ppm. It is concluded that the synthesized AgNPs can be used to prevent bacterial infection, but cannot prevent fungal infection just as effectively.

Hydrogels were successfully made from our Starch/ and Alginate/AgNPs and multiple possible crosslinking mechanisms were suggested. This enhances the potential of the application of our synthesized AgNPs towards wound treatment.

Our project is developed based on our school's previous project and the enhancement is as below:

(1) Kalantari, K.; Mostafavi, E.; Afifi, A. M.; Izadiyan, Z.; Jahangirian, H.; Rafiee-Moghaddam, R.; Webster, T. J. Wound Dressings Functionalized with Silver Nanoparticles: Promises and Pitfalls.

Nanoscale **2020**, *12* (4), 2268–2291. https://doi.org/10.1039/C9NR08234D.
(2) Boonkaew, B.; Suwanpreuksa, P.; Cuttle, L.; Barber, P. M.; Supaphol, P. Hydrogels Containing Silver Nanoparticles for Burn Wounds Show Antimicrobial Activity without Cytotoxicity. *J. Appl. Polym. Sci.* **2014**, *131* (9), n/a-n/a. https://doi.org/10.1002/app.40215.
(3) Tian, J.; Wong, K. K. Y.; Ho, C.-M.; Lok, C.-N.; Yu, W.-Y.; Che, C.-M.; Chiu, J.-F.; Tam, P. K. H. Topical Delivery of Silver Nanoparticles Promotes Wound Healing. *ChemMedChem* **2007**, *2* (1), 129–136. https://doi.org/10.1002/cmdc.200600171.
(4) Li, W.-R.; Xie, X.-B.; Shi, Q.-S.; Zeng, H.-Y.; OU-Yang, Y.-S.; Chen, Y.-B. Antibacterial Activity and Mechanism of Silver Nanoparticles on Escherichia Coli. *Appl Microbiol Biotechnol* **2010**, *85* (4), 1115–1122. https://doi.org/10.1007/s00253-009-2159-5.