BIOTRANSFORMATION OF SILVER NANOPARTICLES *IN VIVO*

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INTRODUCTION

Our exposure to silver nanoparticles (AgNPs) has rapidly increased in recent years, since they became present in medical equipment, cosmetics, textiles and food as microbicidal agents. Still, their fate in biological systems has so far been a mystery. [1] Because of their small size, NPs interact with biomolecules and biological structures in unique ways. [2]

The aim of this study is to explore possible *in vivo* biotransformation patterns of AgNPs. Agglomeration and dissolution of polyvinylpyrrolidone (PVP) - coated AgNPs were investigated in ultrapure water, simulated biological environment (cell culture medium (DMEM), artificial lysosomal fluid and artificial gastric fluid), and rat liver. Then, AgNP and Ag+ interactions with glutathione (endogenous thiol-containing peptide) were studied, in order to elucidate adsorption, complexation and *in vivo* synthesis of AgNPs.

Table 1. Hydrodynamic diameters (d_H) and corresponding volume percentages, zeta potential (ζ) and percentage of released Ag+ for PVP AgNPs after 1h exposure in different media.

Medium	d_H (nm)	% Volume	ζ (mV)	% Ag+
MQ water	4,99 ± 0,56 31,16 ± 3,54	98,36 1,64	-18,02 ± 3,71	0,74
DMEM	20,7 ± 0,01 68,7 ± 11,7 506,6 ± 141,2	13,3 18,1 68,6	-8,2 ± 0,4	0,85
ALF	7,4 ± 1,2	100,0	-8,6 ± 1,0	0,73
AGF	9,2 ± 2,1 48,0 ± 2,7	98,9 1,1	0,1 ± 0,9	1,30

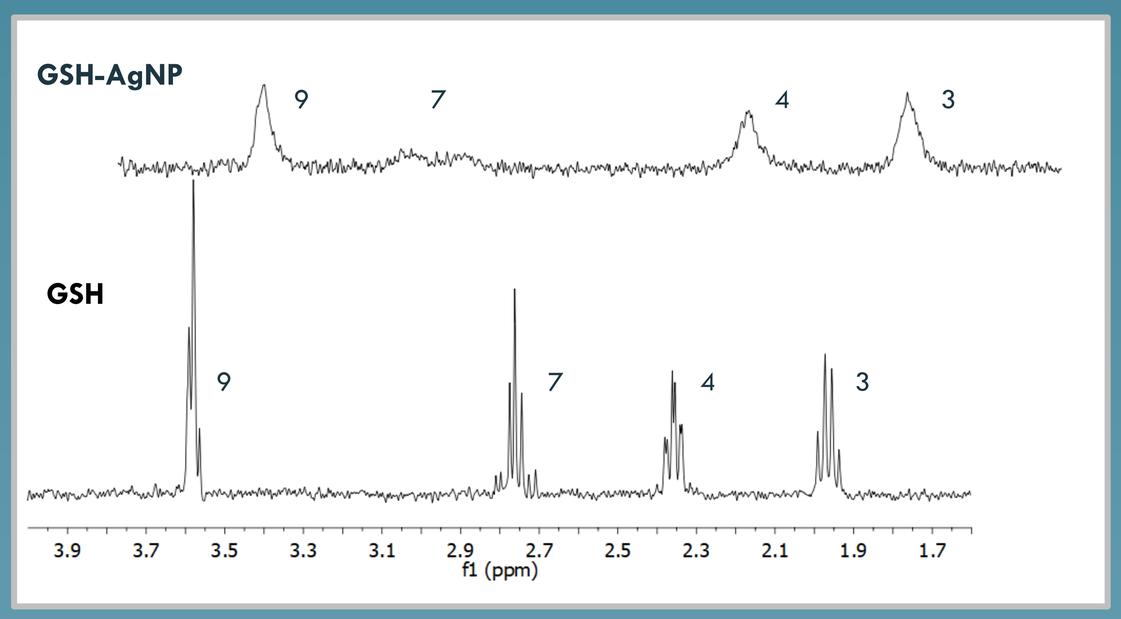

Figure 3. 1H NMR spectra of GSH and GSH-AgNP in phosphate buffer (pH 7). GSH-AgNP spectrum was recorded after 24 h of AgNP synthesis with GSH as a reducing agent.

EXPERIMENTAL DESIGN

AgNPs were synthesised by two different methods: (a) reduction of $AgNO_3$ with $NaBH_4$ in the presence of glutathione (GSH); (b) reduction of $AgNO_3$ using GSH as reducing agent in 25mM phosphate buffer (pH 7).

Size distribution and surface charge were obtained by dynamic and electrophoretic light scattering, respectively. AgNPs were visualized by transmission electron microscopy. Ag+ release was studied using ultrafiltration and atomic absorption spectroscopy, after 1h of exposure to different media. Glutathione binding to AgNPs and NP formation from ionic silver were tracked by proton nuclear magnetic resonance spectroscopy (1H NMR).

The 1H spectra in D2O were recorded with a Varian INOVA 400 spectrometer, at 399.6 MHz. Chemical shifts in the 1H NMR spectra are expressed in parts per million (ppm) vs. TMS as the external standard.

Investigation of stability and behaviour of AgNPs were pereformed in ultrapure water and in simulated biological fluids including cell culture medium, artificial lysosomal fluid, artificial gastric fluid, and liver homogenate obtained from Wistar rats.

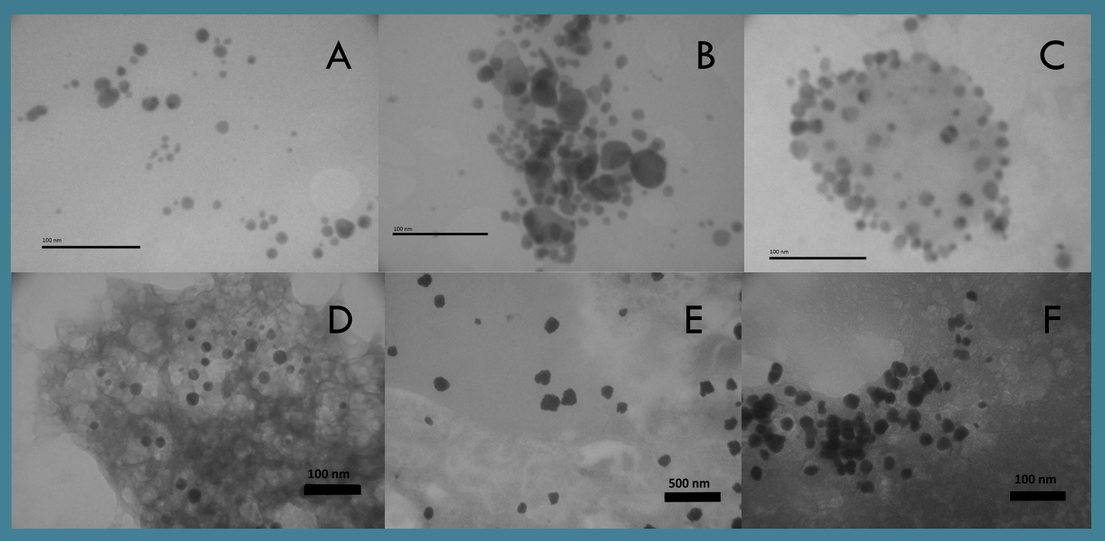

Figure 1. Transmission electron micrographs of PVP-AgNPs in MQ water (A), phosphate buffer (B), cell culture medium DMEM (C), rat liver homogenate (D). Image (E) shows section of the liver of a rat treated with PVPAgNP for 28 days, while (F) demonstrates transformation of ionic Ag in AgNPs in rat liver homogenate. and liver of rat treated with PVPAgNP.

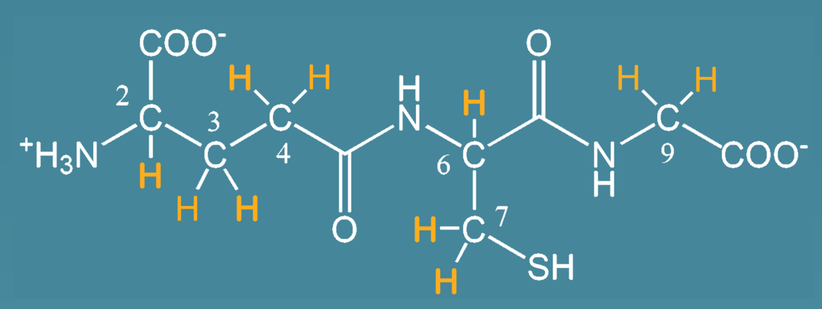

Figure 2. The structure of GSH.

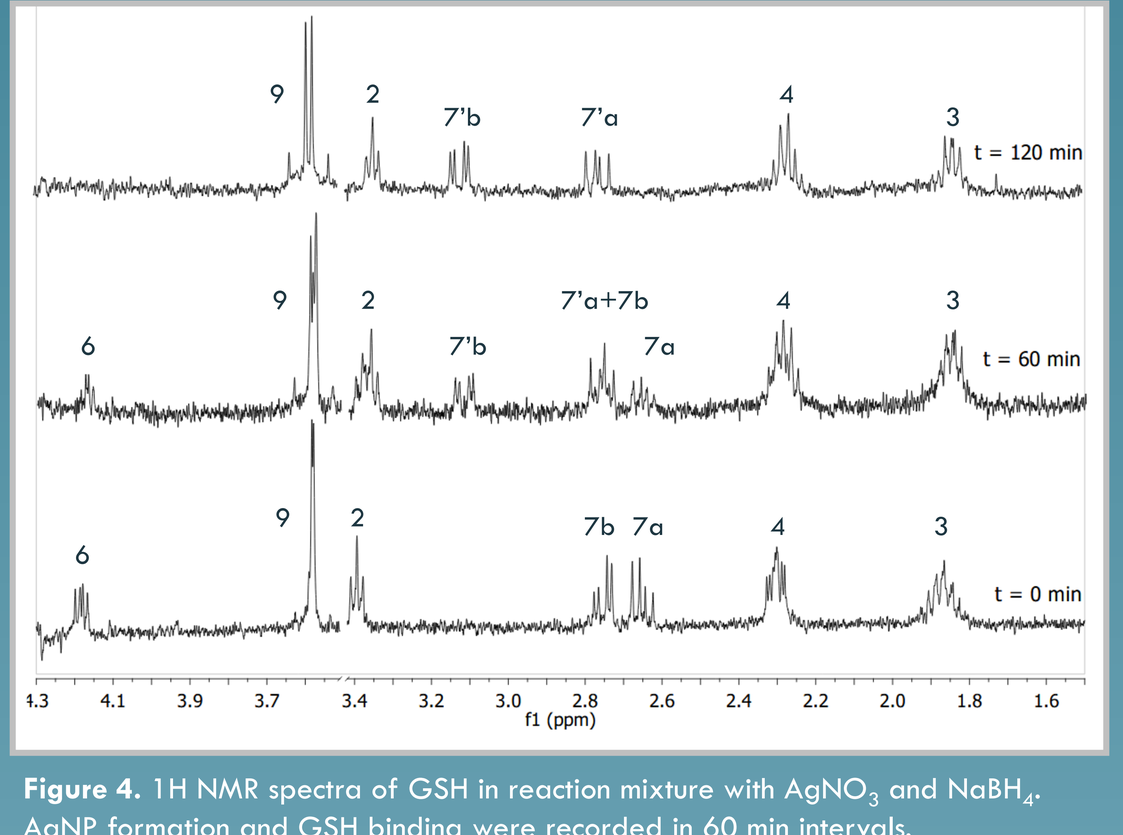

Figure 4. 1H NMR spectra of GSH in reaction mixture with $AgNO_3$ and $NaBH_4$. AgNP formation and GSH binding were recorded in 60 min intervals.

RESULTS

For the first time, this study demonstrates the possible mechanism of *in vivo* synthesis of AgNPs in tissues resulting from the interaction of Ag^+ with glutathione. DLS and dissolution studies confirmed AgNP aggregation in biological media and dissolution at low pH values. 1H NMR binding studies show the adsorption of GSH to the NP surface through the thiol group, evidenced by chemical shifts and separation of the peak 7. Later, AgNPs were synthesized with GSH as the only reducing agent in a pH-controlled system. The process was again monitored with 1H NMR, which displayed significant peak broadening, loss of resolution and the downfield shift of peak 7. AgNP formation was confirmed by transmission electron microscopy.

REFERENCES

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[2] R. Podila, R. Chen, P. C. Ke et al., *Appl Phys Lett* **2012**, *101*, 263701.

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