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# ESPS PEER REVIEW REPORT

**Name of journal:** World Journal of Biological Chemistry
**ESPS manuscript NO:** 12213
**Title:** SHORT- AND LONG-TERM EFFECTS OF SILVER NANOPARTICLES ON HUMAN MICROVASCULAR ENDOTHELIAL CELLS
**Reviewer code:** 00631914
**Science editor:** Fang-Fang Ji
**Date sent for review:** 2014-06-28 19:13
**Date reviewed:** 2014-07-09 04:24

| CLASSIFICATION | LANGUAGE EVALUATION | RECOMMENDATION | CONCLUSION |
|---|---|---|---|
| [ ] Grade A: Excellent | [ ] Grade A: Priority publishing | Google Search: | [ ] Accept |
| [ ] Grade B: Very good | [Y] Grade B: Minor language polishing | [ ] Existing | [ ] High priority for publication |
| [ ] Grade C: Good | [ ] Grade C: A great deal of language polishing | [ ] No records | [Y] Rejection |
| [Y] Grade D: Fair | [ ] Grade D: Rejected | BPG Search: | [ ] Minor revision |
| [ ] Grade E: Poor | | [ ] Existing | [ ] Major revision |
| | | [ ] No records | |

## COMMENTS TO AUTHORS

This manuscript investigates in vitro cytoxicity of silver nanoparticles (AgNP) in human vascular endothelial cells with assessment of cellular growth rate, integrity of cell membrane, and DNA injury using MTT, LDH, and comet assay, respectively. However, toxicity of commercially synthesized AgNP has been widely investigated and reported, even in human endothelial cells (Ucciferri N, et.al. Nanotoxicology. 2014 and Reference#5 in this manuscript). Given heterogeneity of vascular endothelial cells, the authors indicated that the significance of this study was to characterize microvascular endothelial cells exposed to AgNP. There are several major concerns with this manuscript that weakens its overall significance. 1. This manuscript lacks clear hypothesis and cohesive experimental design with focusing on characterizing toxicity of AgNP in microvascular endothelial cells per se. As the authors mentioned in the introduction, heterogeneity of vascular endothelial cells is dependent on the origin of cells etc. However, the authors did not state clearly the origin of HMEC in the entire paper. Moreover, some experiments were performed using HUVEC that are not belonged to microvascular EC. The toxicity of AgNP has been reported in the peer-reviewed journals. 2. How is AgNP dissolved in the solution is not indicated. It has been clearly demonstrated that solubility of AgNP can affect cytotoxicity. The manuscript indicated that AgNP had been incubated with cells for days. Whether AgNP is truly dissolved in the solution or only suspended for a certain period of time would significantly influence all parameters measured in this

study. Likewise, what agents and conditions were used to dissolve AgNP also could produce significant influences. 3. The rationale for the selection of anti-oxidant agents and their concentrations used in the study is not indicated. The negative results could be caused by which antioxidant agent and what concentration were used.

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| CLASSIFICATION | LANGUAGE EVALUATION | RECOMMENDATION | CONCLUSION |
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| [ ] Grade D: Fair | [ ] Grade D: Rejected | BPG Search: | [ ] Rejection |
| [ ] Grade E: Poor | | [ ] Existing | [ ] Minor revision |
| | | [ ] No records | [ Y] Major revision |

## COMMENTS TO AUTHORS

The article of Castiglioni et al. evaluated the cytotoxicity and genotoxicity of silver nanoparticles (AgNP) on human microvascular cells (HMEC) and endothelial progenitor cells. They found that AgNP reduce HMEC and ECFC viability (MTT and HDL), induce DNA damage (comet assay) in a time and concentration-dependent manner and that this inhibitory effect was not blocked by antioxidant drugs. The results are original and interesting but major revision should be done before its acceptance. Figure 1 Data in A and B are quite different. While in B 1 and 2.5 ug/ml of AgNP appears to completely suppress cell viability, in panel A, cell viability at similar concentrations is 60 and 40% of control. Please, explain these differences and show more uniform data. In B, there is almost no difference in the effect of AgNP at 3 or 6 days. What happens at 24 or 48 hs? Please, show these data as well as cell count numbers. The authors state that at 0.5 ug/ml of AgNP ther is only a slight increase. However after 6 days appears to be a reduction of 50% compared to control samples. Please explain these differences. Figure 2 The values are more like those from Fig. 1 B but quite different from Fig. 1 A so please, uniform data. Please, also include a positive control showing that antioxidants were active. Figure 3 There is not much information for one figure so Figure 3 can be included in Fig. 1. The effect at 1 and 2.5 ug/ml is quite impressive so it would be important to correlate these data with the MTT values at the same time ( 16 hs) and to confirm that LDH matches the data from the MTT assay as it is stated in the discussion. What is the total LDH content of the cells?

Figure 4 A) It is not clear why the authors state that "2.5 µg/ml Ag NP markedly reduced cell number after 4 days treatment". The other two concentrations also induce a marked reduction comparing with non-treated cells. Please explain. B) It is not evident from the results that removal of AgNP rescues cell growth as it is written in the abstract. More accurate is to state that removal partially rescues cells growth as it is written in the result section. -Page, 7 line 13: Please, indicate the mean ±SE of the IC50 instead of a range. -What is the concentration of the AgNP used in vivo? Please indicate it in the text and compare with that used in this study. Minor -Please as they are in vitro studies; use the word "concentration" instead of "dosis". -Abstract line 18 replace to for in angiogenesis. - In Figure 1C and 4 C please indicate the absence of AgN

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| [ ] Grade E: Poor | | [ ] Existing | [ Y] Minor revision |
| | | [ ] No records | [ ] Major revision |

## COMMENTS TO AUTHORS

The purpose of this study was to evaluate the effects of silver nanoparticles on human microvascular endothelial cells, and found that silver nanoparticles are cytotoxic and genotoxic for endothelial progenitors. This is a very well written and designed manuscript. The purpose of the study is clear. Method, Results and Discussion are well documented. I have only minor concerns, described below.

1. Figures 1C, 4C, 5B: scale bars are missing.