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# *In Vitro* Behaviour and Effects on Cells Induced by Functionalized Nanogold and Nanosilver Particles II. *In Vitro* Comparative Studies for NanoGold Conjugated with Glutathion in RPE D407 Cell Line and *Saccharomyces Cerevisiae*

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**Abstract.** We aimed to demonstrate that the functionalization of nanocolloids of Au *in vitro*, induce the formation of conjugated forms (with glutathion) which can affect the cellular activity, as tested on *Saccharomyces cerevisiae* and RPE cells. The cell line relevant for macular degeneration, type RPE (line D407) proved to be more sensitive to nanogold and conjugated forms. By microscopy we demonstrated the cell capacity to form a monolayer, as a prove of their proliferation and viability, as well in the presence of free AuC, glutathione, but also in the presence of conjugated forms AuC-glutathion. AuC-glutathion conjugate, at pH 5.6 (optimum for cell growth) is stable and can have effect on cellular activity, with impact on proliferation and viability. Obviously, the interaction of AuC and AuC-glutation conjugate with cell components may have effects on cell proliferation and rapid metabolization in presence of appropriate enzymes. (e.g. Glutation peroxidase). Alternative and complementary studies are needed to show the localization of AuC and AuC-glutathion conjugate at cellular level, by microscopy and spectroscopy. It is possible that, inside cell, AuC to conjugate other biomolecules, with a higher stability and affinity, comparing to glutathion.

**Key words:** NanoGold, NanoSilver, conjugation, glutathion, RPE cells, *Saccharomyces cerevisiae*

## INTRODUCTION

We assist, during the last 2 decades, to an exponential development of nano- and micro- biotechnologies, not only with applications in the material sciences, but, more and more, in food science, nutrition and nanomedicine (Albrecht, 1988; Hayat, 1991, 2003). The materials' chemistry is of biological inspiration, the nanostructures from ceramics to DNA structures belonging to natural supramolecular science. Nowadays, scientists mimic in laboratory the "intelligent" materials creating biosensors, good contrast agents for microscopy (Beesley, 1989), detection agents at cellular or molecular level, self-assembling of molecules by biocatalysis, applied in biomedicine and bionanotehnology.

A large interest for nanogold and nanosilver is shown recently, their functionalization, spontaneous in biological systems being beneficial for the organism defense against bacteria or against toxins. Such metals can link proteins, enzymes, even DNA, by specific coordination of nitrogen, sulfur or other non-metals from biomolecules (Bhattacharya, 2007; Kouassi, 2006; Wagner, 2004). Since Roman times, is was known that silver has antimicrobial action, so they preserved food and beverages in silver cups, avoiding fermentations. Gold-based complexes proved to have beneficial effects in rheumatic diseases

and against infections (Hainfeld, 1989; Hongwei, 2006). Interestingly, medicinal plants can store silver and/or gold, being provided with antimicrobial and immune-stimulative or even antitumor properties (Du, 2005; Kouassi, 2006). The preparation of nanogold or nanosilver colloids is possible in laboratory, their covalent functionalization as well, using especially their affinity for amino or thiol groups. These groups, by conjugation, reduce the oxidative status of Au or Ag. This can be a reason of their antioxidant action on cells (Beatty, 2000; Hainfeld, 1996, 2000; Lehmann, 2001). Different cell types were used to demonstrate the effects of nanostructured Au or Ag, such as RPE cells (Davis, 1995) or tumor cells (Du, 2005)

We are interested to demonstrate that the functionalization of Au and Ag colloids in vitro, to study the stability of functionalized forms (by conjugation with cystein, glutathione, insulin) (as presented in the first part of this article) as well and to study their action on cells. Such investigations can prove that natural ways of Au and Ag functionalization, by formation of stable conjugates, can be active also at cellular level and act synergistically as protective, antioxidant agents.

## MATERIALS AND METHODS

### 1. *In vitro* interactions of colloid gold AuC incubated with RPE cells

We used cell RPE (stabilized line type D407, kindly offered by Biophysics laboratory Bremen University, Germany) at a density of $10^5$ cells/ml MEM media, in order to measure the incorporation rate of AuC-glutathion and to test its functionality on cells. We made microscopic investigations (optical, reversed microscopy) for the identification of AuC nanoparticle and AuC-glutathion conjugates localization.

Initially, we made a calibration curve, measuring the cell density (counted by microscopy) and its relation to Vis absorption at 520 and 620 nm, at different dilutions (1:10, 1: 5, 1:2 and 1:1) with UDW. Based on calibration, we could quantify the effect of AuC and AuC-glutathion conjugates on cell proliferation and viability (determined by MTT assay, during 24 hrs). The control was represented by free cells in medium (SA), SG -cells incubated with glutathion, NS-Cells incubated with AuC at 0,1% , NGS- Cells incubated with 10% mixture AuC-glutathion where the ratios AuC: glutathion were 1:1, 1:5 and 1:10

### 2. *In vitro* interactions of conjugated AuC-glutathion with *Sacharomyces cerevisiae* cells

We used cellular suspensions of *Sacharomyces cerevisiae*, as indicator of intracellular interactions with free AuC and AuC-glutathion conjugates.

Initially, we made a calibration curve, measuring the cell density (counted by microscopy) and its relation to Vis absorption at 520 and 620 nm, at different dilutions (1:10, 1: 5, 1:2 and 1:1) with UDW. The yeast cell suspension of *Saccharomyces cerevisiae* (SC) ($10^5$ cells/ml) was incubated with or without AuC, or AuC-glutathion conjugate. Similarly to RPE cells, we could quantify the effect of AuC and AuC-glutathion conjugates on cell proliferation and viability (determined by MTT assay, during 24 hrs). The control was represented by free cells in medium (SA), SG -cells incubated with glutathion, NS- Cells incubated with AuC at 0,1% , NGS- Cells incubated with 10% mixtures of AuC-glutathion where the ratios AuC: glutathion were 1:1, 1:5 and 1:10. N was represented by AuC in UDW, G- glutathione in UDW, GN- glutathion and AuC.

## RESULTS AND DISCUSSION

### 1. *In vitro* Interactions of nanoGold with RPE cells

Retinal RPE cells (of fibroblastic type) are a very interesting experimental model, applied in physiology and pathology. Incubations of these cells can prove oxidative stress under chemical or photochemical stress, one can identify macular degenerescence, tumor induction, anti-tumor effects.

RPE cells in culture can maintain their normal morphology and metabolism as *in vivo*, representing a good experimental model to study the incorporation of nanoparticles, bioactive molecules, including drugs (Davis, 1995).

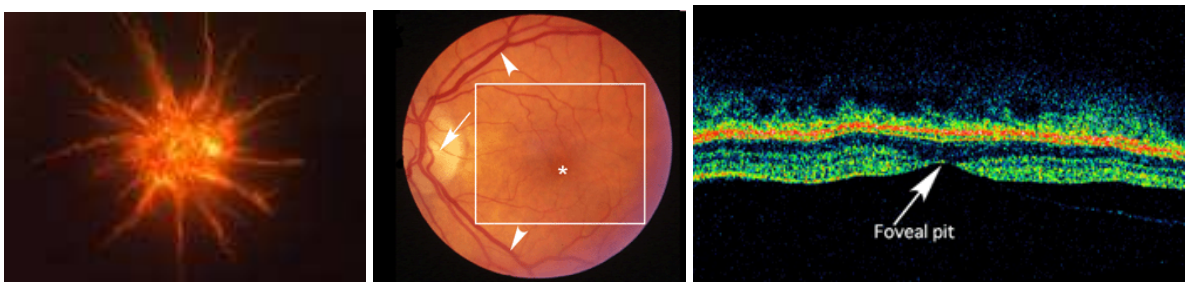
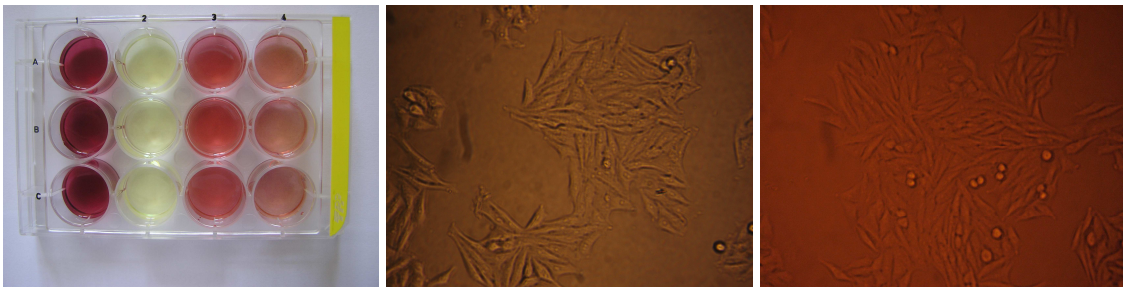



Fig.1. RPE type I cells and their localization, as determined by tomography (unpublished data)

Our aim was to test the effect of a gold nanoparticle layer (AuC) on RPE cell proliferation and viability using a monolayer system where the AuC was immobilized on plates and treated with cells , in order to see if detailed investigations can be made by SERS (Surface enhanced Raman spectroscopy), as suggested by Gulati (2006) or Boca (2010).

### 2. *In vitro* interactions of conjugated nanoGold with *D407* cells

Fig. 2 shows (A) the colors of suspensions after one our of incubation of cells with AuC (line 1), glutathion (line 2), AuC-glutathion (1:1) (line 3), AuC-glutathion (1:5) (line 4). We could not reveal in this case the shift of colour due to the buffer pH pH=6,5, which avoid the formation of the conjugate. On line 1 one can observe the agglutination of cells, as an indication of AuC toxicity, correlated with their reduced viability. In spite of this, the cells keep their ability to form networks in the presence of AuC-glutathion (1:1) (line 3), AuC-glutathion (1:5) (line 4). The microscopic images demonstrate the network formation and keeping the confluence capacity of cells, before or after treatment (B and C)



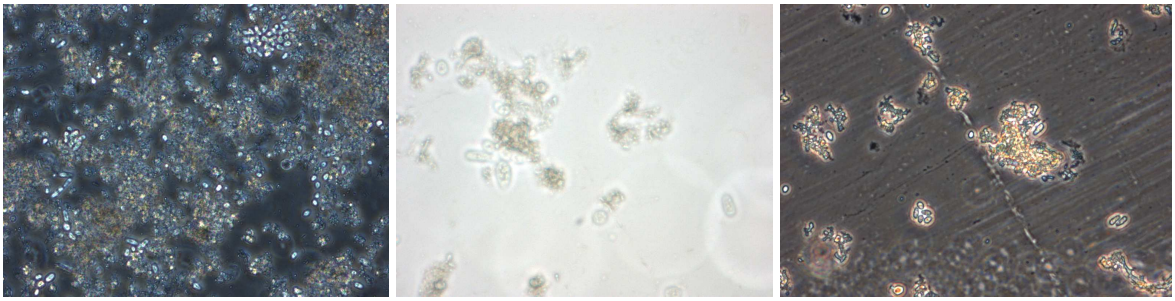
A B C

Fig. 2. A. Cell culture D406 incubated with AuC (line 1), glutathion (line 2), AuC-glutation (1:1) (line 3), AuC-glutathion (1:5) (line 4).
B-Microscopic image of cells Before (B) and after incubation with AuC-glutathion (1:5) (C).

By MTT assay (data not shown) we proved that the viability is maintained in normal limits (above 75%), the presence of AuC and glutathion does not induce toxicity, which may affect the cell viability.

### *3. In vitro* interactions of conjugated nanoGold with *Sacharomyces cerevisiae* cells

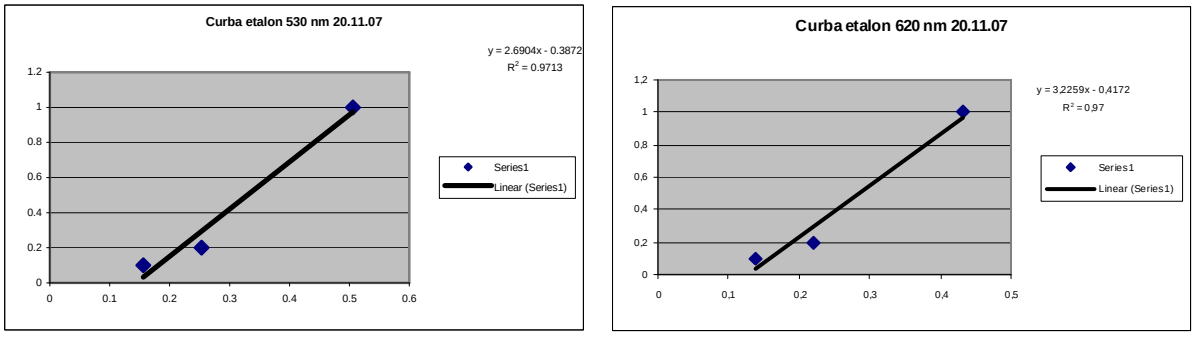
Fig.3 shows the microscopic images of *Sacharomyces cerevisiae* (SC) cells (10x), initially (A) and after incubation with AuC (B) or with AuC-glutathion conjugate (C)



A. B. C.

Fig. 3. Microscopic images of SC cells (10x), initially (A) and after incubation with AuC ( B) or with AuC-glutathion conjugate (C)

To evaluate quantitatively the effect of AuC on cell proliferation, we realized the calibration curves (cell density versus Vis absorption at 530 and 620 nm respectively) (Fig. 4)




A. B.

Fig.4. Calibration curves for SC cells, correlating the cell density (determined by microscopy) and the absorption units at 530 and 620 nm, respectively) (A and B)

Good correlation indexes were established for each case. After 24 hrs incubation, measuring the absorptions at the same wavelengths, we noticed that, at low cell densities, the absorptions increased from 0.15 to 0.250), while for high cell densities, the absorptions remained at a value of 0.500. For SC cells incubated with SA, SG, GN,NS, N, G, NGS we noticed different variations of absorptions in 2-3 and 5-6 (lines) In the first set of cuvettes (2 and 3 in Fig. 5), la initially (line 2) and after 24 hrs (line 3) it was observed a decrease of absorptions for incubations with NS and GN and concomitantly an indication of conjugates AuC - glutation formation (as indicated by GN) or with other cell components (NS). Lines 5 and 6 indicate also decreases of absorptions (from 0 to 24 hrs) when NGS was used, but not for GN, suggesting an inhibition of cell proliferation when incubated with NS and NGS.

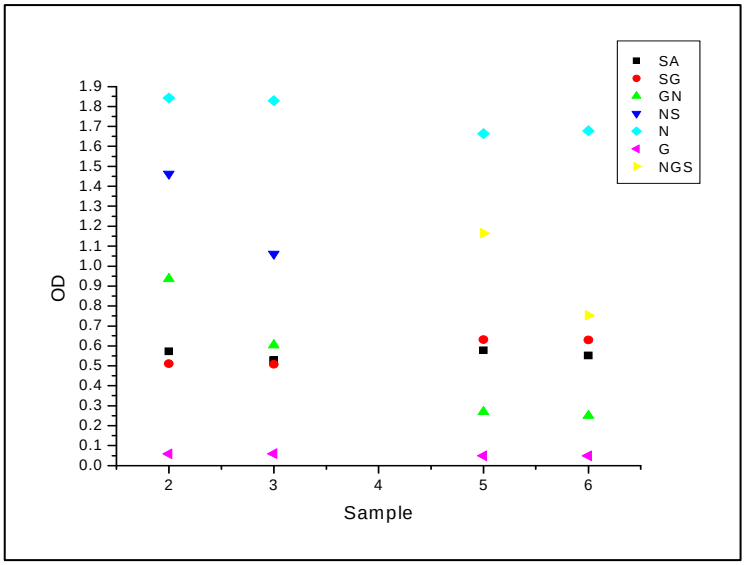



Fig.5. Evolution of Vis absorptions (registered at 530 nm) when SC cells were incubated with or without AuC or AuC-glutathion conjugates. Abbreviations: free cells in medium – SA; SG - cells incubated with glutathion, NS- Cells incubated with AuC; G- glutathion; GN- glutathion and AuC, added separately; NGS -Cells incubated with mixtures of AuC-glutathion Line 2 and 5 – initial time. Lines 3 and 6 – after 24 hrs.

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## CONCLUSIONS

1. Our research demonstrated that colloid Au contains particles which can enter and have effect on cell proliferation, as demonstrated in *Saccharomyces cerevisiae* and RPE cells. The cell line relevant for macular degeneration, type RPE (line D407) which proved to be more sensitive to nanogold and conjugated forms.

2. By microscopy we demonstrated the cell capacity to form a monolayer, as a prove of their proliferation and viability, as well in the presence of free AuC, glutathione, but also in the presence of conjugated forms AuC-glutathion.

3. We showed that AuC-glutathion conjugate, at pH 5.6 (optimum for cell growth) is stable and can have effect on cellular activity, with impact on proliferation and viability. Obviously, the interaction of AuC and AuC-glutation conjugate with cell components may have positive or negative effects on cell proliferation.

4. By UV-Vis spectrometry we observed that conjugation of AuC with glutathione does not induce modifications of media colors, due to its low concentration (1% in culture media) and also, possibly, due to its rapid metabolization in presence of appropriate enzymes. (e.g. Glutation peroxidase).

5. Alternative and complementary studies are needed to show the localization of AuC and AuC-glutathion conjugate at cellular level, by microscopy and spectroscopy. It is possible that, inside cell, AuC to conjugate other biomolecules, with a higher stability and affinity, comparing to glutathion.

## REFERENCES


1. Albrecht, R. M. and G. M. Hodges. (1988). Biotechnology and bioapplications of colloidal gold. Scanning Microscopy International.

2. Beatty, S., H. H. Koh, M. Phil, D. Henson and M. Boulton. (2000). The role of oxidative stress in the pathogenesis of age-related macular degeneration. Survey of Ophtalmology. 45:115-134.

3. Beesley, J. (1989). Colloidal Gold. A new perspective for cytochemical marking, Royal Microscopical Society Handbook No 17.Oxford Science Publications, Oxford University Press.

4. Bhattacharya, R. *et al.* (2007). Attaching folic acid on gold nanoparticles using noncovalent interaction via different polyethylene glycol backbones and targeting of cancer cells, Nanomedicine: Nanotechnology, Biology and Medicine 3:224-238.

5. Davis, A. A, P. S. Bernstein, D. Bok, J. Turner, M. Nachtigal and R. C. Hunt. (1995). A human retinal pigment epithelial cell line that retains epithelial characteristics after prolonged culture, Invest. Ophtalmol. Vis. Sci. 36(5). 955-964

6. Du, D. *et al.* (2005). Colloidal gold nanoparticle modified carbon paste interface for studies of tumor cell adhesion and viability. Biomaterials. 26: 6487-6495

7. Gulati, A., H. Liao and J. Hafner. (2006). Monitoring Gold Nanorod Synthesis by Localized Surface Plasmon Resonance. Spectrochim Acta A Mol Biomol Spectrosc. 3200-3212

8. Hainfeld, J. F. and R. D. Powell. (2000). New frontiers in gold labeling. J. of Histochem. and Cytochem. 48: 471-5.

9. Hainfeld, J. F. (1996). Gold Liposomes. In: Bailey GW, Corbett JM, Dimlich RVW, Michael JR, Zaluzec, NJ, eds. Proc 54th Annu Mtg Microsc Soc Am. San Francisco. San Francisco Press. 898–899.

10. Hainfeld, J. F. (1989). Undecagold-antibody method. In Hayat MA, ed. Colloidal Gold: Principles, Methods, and Applications. Vol 2. San Diego, CA, Academic Press, 413–429.

11. Hayat, M. A. (1991). Colloidal gold: principles, methods, and applications Academic Press. San Diego.

12. Hayat, M. A. (2003). Colloidal Gold: principles, methods and applications. Wiley Publ.

13. Hongwei, Gu, Keming Xu, Chenjie Xu and Bing Xu. (2006). Biofunctional magnetic nanoparticles for protein separation and pathogen detection Chem. Commun. 941 – 949.

14. Kouassi, G. K. and J. Irudayaraj. (006). A nanoparticle-based immobolization assay for prion-kinetics study. J. Nanobiotechnology. 4:8-15.

15. Kouassi, G. K. and J. Irudayaraj. (2006). Magnetic and gold-coated magnetic nanoparticles as a DNA sensor. Anal. Chem. 78:4234-3241.

16. Lehmann, C., U. Wollenberger, R. Brigelius-Flohé and F. W. Scheller. (2001). Modified gold electrodes for electrochemical studies of the reaction phospholipid hydroperoxide glutathione peroxidase with glutathione and glutathione disulfide. Electroanalysis. 13:364-369.

17. Wagner, J. (2004). in: Proc. DNA-Based Molecular electronics. IPHT. Jena.

18. Zhou, Y, W. Chen, H. Itoh, K. Naka, Q. Ni, H. Yamane and Y. Chujo. (2001). Preparation of a novel core-shell nanostructured gold colloid-silk fibroin bioconjugate by the protein in situ redox technique at room temperature. Chem Commun (Camb). (23):2518-9.

19. Boca, S. and S. Astilean. (2010). Detoxification of gold nanorods by conjugation with thiolated poly (ethylene glycol) and their assessment as SERS-active carriers of Raman tags. Nanorechnology. 21:123-129.