

Selective Entrance of Gold Nanoparticles into Cancer Cells

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Abstract

We report that Au(0) nanoparticles, stabilized by 5-aminovaleric acid, selectively penetrate into K562 cancer cells in a short time. These experiments were carried out in order to verify the specific recognition of gold sol by abnormal cells. The observed selectivity towards gold nanoparticles by K562 makes the metallic system attractive for cancer therapy.

Introduction

Nanotechnology is widely employed in biological systems and there are many examples in literature of studies on gold-bioconjugate systems and magnetic nanoparticles employed in therapies against tumours or other pathologies [1,2]. The work reported here was stimulated by the study of Hainfield et al. [3-6] in which mice were firstly injected with cancer cells and gold nanoparticles, then treated with X-ray radiation at 250 KV. A synergistic effect was observed between gold nanoparticles and the X-ray treatment resulting in tumour reduction or eradication. Moreover it was also observed that cancer cells treated only by X-rays or only by gold continued to grow. The survival after one year of the combined therapy was of 70%. The success of this technique is related to the high ability of gold to accumulate within tumours and absorb X-rays. Other mechanisms previously investigated [7] have demonstrated the susceptibility of adhesive properties of embryonic and cancer cells to HEP (hydroxyethylpyrrolidine); these alterations in endothelial cell-extracellular matrix interactions are central to the process of angiogenesis.

In this field we consider an improvement in the therapy the admittance of the gold particles into the cell by recognition instead of the injection. With the objective of finding new vehicles which can transport nanoparticles into the cells, we have used gold sols stabilized by natural and non-toxic organic molecules [8-13] such as, amino acids, amino sugar or sugars. In particular a 5-aminovaleric acid protected gold sol (Au@AVA) was prepared by reduction of an aqueous solution of the aurate precursor NaAuCl_4 in the presence of the ϵ -amino acid by reduction with NaBH_4 ($\text{AuCl}_4^- : \text{BH}_4^-$ molar ratio equal to 1:3, reaction time = 1h). The mean diameter of the gold nanoparticles, determined by transmission electron microscopy (TEM) measurements, was found close to 7 nm. (Figure 1). The reaction was followed by UV/visible spectroscopy by observing the decrease of the 217 nm UV band, due to the AuCl_4^- anion, and the formation of

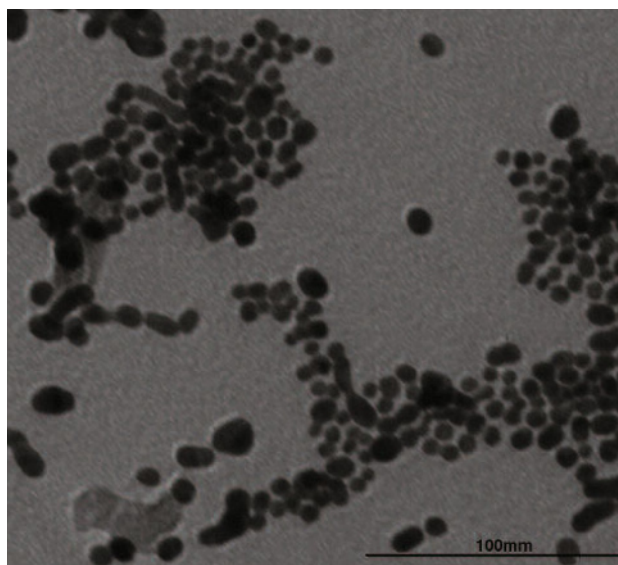


Figure 1

TEM image of nanoparticles of Au@AVA sol

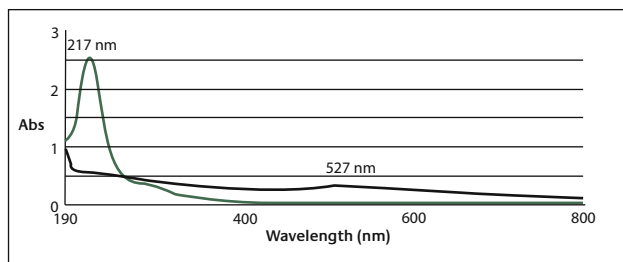


Figure 2
UV/visible spectrum of Au@AVA sol

the plasmon resonance peak at 527 nm consistent with the resulting red-purple colour of the sol (Figure 2). Moreover, the characterization was performed by immobilization of the sol on silica (Aerosil 200, 5%w/w) recording UV/visible/NIR spectra in which we observed the position of the combination bands and the stretching and deformation modes of the AVA ligand, free (1984 and 1438 nm) and coordinated to gold nanoparticles (1936 and 1454 nm) besides the plasmonic band. Although these bands are weak in absorbance, they resulted specific of the electronic interaction between particle and ligand.

Once formed, the AVA-gold colloidal solution was evaporated to dryness and carefully re-dispersed in an aqueous D-MEM cell culture medium obtaining a dark violet sol [λ_{max} = 559nm (plasmon peak) in the UV/visible spectrum using the D-MEM cell culture medium as a blank]. This gold-DMEM system was admixed with a K562 cancer cell suspension (for the preparation see supporting material) and incubated for different periods of time (from 15 minutes to 8 hours). Observations were made using confocal microscopy in reflectance and transmission modes. Figure 3a shows the gold aggregates dispersed in the cell culture medium highlighting the green colour of gold particles in accordance with its self-fluorescence at 488 nm.

It is interesting to note that the gold sol entered into the cancer cell after just 15 minutes of the contact between K562 and Au@AVA nanoparticles (Figures 3b and 3c) and the total uptake of gold into the cancer cell took place after 8 h (Figures 3d and 3f). As confocal microscopy can reconstruct a three-dimensional image by the optical sections of the sample, in Figure 3e four selected sections are presented, in which it can be seen that nanoparticles are located in the cytoplasm and not in the nucleus, suggesting that the cell keeps its metabolism unchanged and there is no carrier for Au@AVA into nucleus.

A negative control was also carried out in order to verify that the selectivity of the synthesized gold sol towards cancer and not towards normal human cells. For these studies human epithelial cells, kindly offered by Dr. Francesca Andretta (Neurological Institute "C. Besta" of Milan) were used. These were supplied as a growing tissue (Fig. 4). After 8 hour of contact between epithelial cells and Au@AVA sol no uptake of the gold particles had taken place.

In order to determine the toxicity of Au@AVA, 0.5 ml of the sol ($[Au]=1.81 \times 10^{-3}$ M) was intraperitoneally injected into

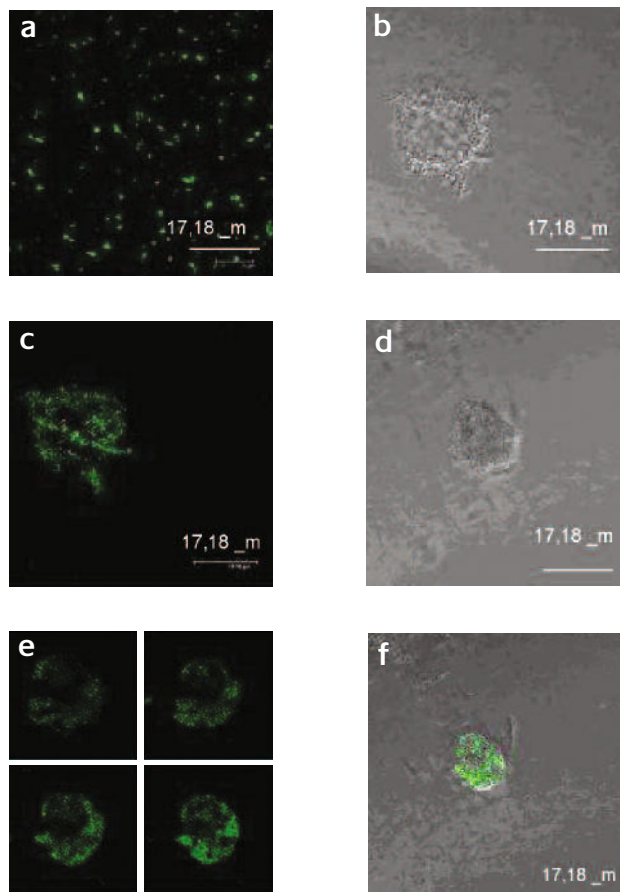


Figure 3
Confocal microscopy images from the interaction between cancer cells and gold nanoparticles
a gold nanoparticles in the D-MEM cell culture
b gold uptake after 15 minutes (image in the reflexion mode)
c gold uptake after 15 minutes (image from the superimposed sections)
d gold uptake after 8 hours (image in the reflexion mode)
e optical sections of the image
f gold uptake after 8 hours (image from the superimposed sections)

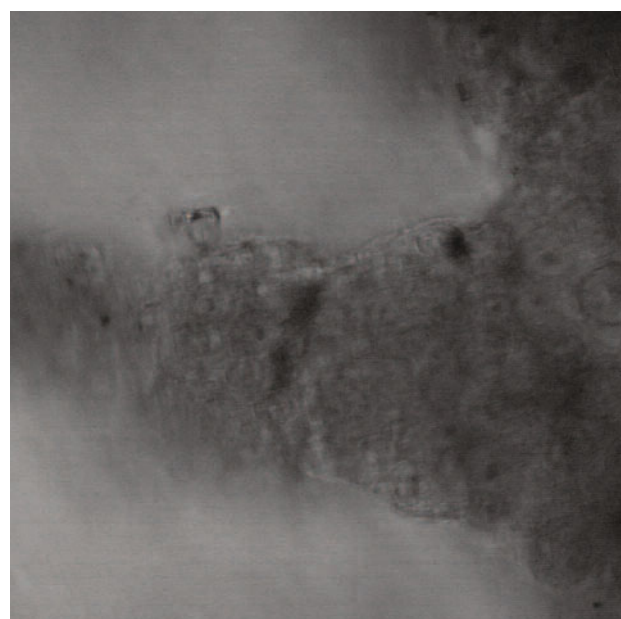


Figure 4
Confocal image of epithelial cells

mice CD1 and no toxic effects were observed at the concentration used. All the mice were born and kept in the animal facility according to the criteria outlined in the "Guide for the Care and Use of Laboratory Animals" prepared by the National Academy of Science and published by the National Institute of Health. [14].

Conclusions

In this brief report we have highlighted the activity of the AVA-gold sol of being recognized by cancer cells of K562. The colloidal system shows a good selectivity and atoxicity. In the future our research will be aimed to investigate other stabilized gold sols in order to correlate behaviour and properties.

Supporting material (materials and methods)

NaAuCl₄·2H₂O 99.0%, NaBH₄ 99.0% and 5-aminovaleic acid (AVA) 97% purity from Aldrich were used. The K562 cells were obtained from American Type Culture Collection (ATCC, Rockville, MD, USA). DMEM (Dulbecco minimal essential medium), FBS (Fetal Bovine serum), glutamine and gentamycine were provided from Sigma-Aldrich.

Preparation of Au@AVA sol. 0.817 ml of 3.5 10²M stock aqueous solution of 5-aminovaleic acid (AVA, aminovaleic acid, C₅H₁₁NO₂) were added to 25 ml of 1.14 10⁻³M NaAuCl₄ stock aqueous solution, obtaining a yellow solution. After 15 min, NaBH₄ (0,858 ml, 0,1M) was added under vigorous stirring. A bordeaux-red sol was reacted for 1h. The gold nanoparticle size (7.5 nm, figure 1) was determined by TEM microscopy (JEOL 100SX EM). The size distribution populations are shown in figure 5.

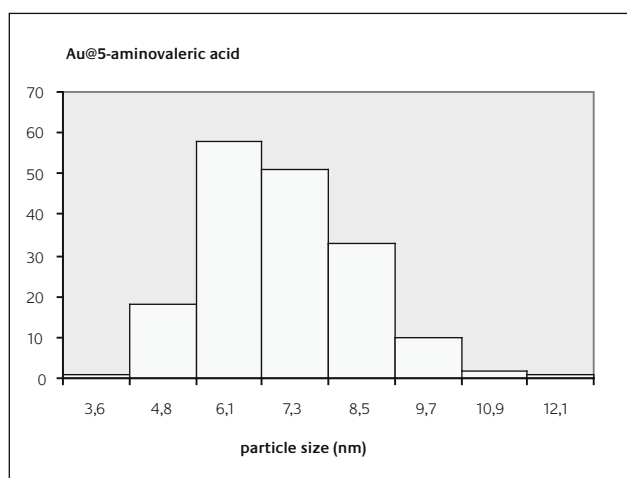


Figure 5

UV-visible spectra of the sols were performed on HP8452 and HP8453 Hewlett-Packard spectrophotometers in Milli-Q water between 190 and 800 nm, in a quartz cuvette.

The reflectance diffuse UV-VIS-NIR spectra were recorded on a JASCO V-750 spectrophotometer.

Cell culture. K562 cancer cells (Leucemia myelogenous cronica caucasica humana) and epithelial cells were deposited on a glass in petri dish were grown by using the following aqueous solutions: a) D-MEM (Dulbecco Minimal essential medium), b) 10% v/v FCS (Fetal Calf serum), c) 1% w/w glutamine, d) 0,1% w/w gentamycine. The cell density, measured by Burcher room, resulted equal to 4,5 10⁶ cells/cm³. Then they were incubated at 37°C in a 5% CO₂ sterile atmosphere.

Immunofluorescence. For confocal microscopy observations the cells were fixed on a microscopy glass and 100 µl of Au@AVA sol was added to the medium. The system was reacted for variable periods of time (from 15 minutes to 8 hours) and at the end of each reaction quenched by using an aqueous solution of *para*-fomaldehyde (3.7% w/w) buffered at pH 7.4 (by 0.2M phosphate solution) mixed with Evans's blue solution (0,01% w/w) for cell self-fluorescence removal. Immediately after GEL/MOUNT™ was added to the prepared samples and the samples covered with cover-glasses.

The samples were observed by Leica TCS NT confocal microscope (Ar/Kr laser, 75 mW) exploiting the self-fluorescence of the gold nanoparticles at 488 nm.

References

- G.F. Paciotti, L. Myer, D. Weinreich, D. Goia, N. Pavel, R.E. McLaughlin L. Tamarkin, *Drug Delivery*, 2004, **11**, 169
- Y.P. Bao, M. Huber, T.F. Wei, S.S. Marla, J.J. Storhoff, U.R. Muller, *Nucleic Acids Research*, 2005, **33**, e151
- J.F. Hainfeld, D.N. Slatkin, H.M. Smilowitz, *Phys. Med. Biol.*, 2004, **49**, N309
- D.M. Herold, I.J. Das, C.C. Strobbe, R.V. Iyer, J.D. Chapman, *Int. J. Radiat. Biol.*, 2000, **76**, 1357
- H. Matsudaira, A.M. Ueno, I. Furuno, *Radiat. Res.*, 1980, **84**, 144
- J.H. Rose, A. Norman, M. Ingram, C. Aoki, T. Solberg, A. Mesa, *Int. J. Radiat. Oncol. Biol. Phys.*, 1999, **45**, 1127
- G. Scari, E. Giavini, *Animal Biology*, 2001, **10**, 27
- M. Daniel, D. Astruc, *Chem. Rev.*, 2004, **104**, 293
- P.R. Selvakannan, S. Mandal, S. Phadtare, R. Pasricha, M. Sastry, *Langmuir*, 2003, **19**, 3545
- T.R. Tshikhudo, Z. Wang, M. Brust, *Materials Science and Technology*, 2004, **20**, 980
- W.J. Parak, D. Gerion, T. Pellegrino, D. Zanchet, C. Micheel, S.C. Williams, R. Boudreau, M.A. Le Gros, C.A. Larabell, A.P. Alivisatos, 2003, **14**, R15
- N.L. Rosi, C.A. Mirkin, *Chem. Rev.*, 2005, **105**, 1547
- A.G. Tachenko, H Xie, D. Coleman, W. Gloman, J. Ryan, M.F. Franzen, D.L. Feldheim, *J. Am. Chem. Soc.*, 2003, **125**, 4700
- Guide for the Care and Use of Laboratory Animals, Institute for Laboratory Animal Research (HYPERLINK "http://dels.nas.edu/ilar" ILAR), Commission on Life Sciences, National Research Council, The National Academic Press, (1996)