GOLD AND SILVER NANOPARTICLES IN POLYMERIC NANOCARRIERS: APPLICATIONS IN DRUG DELIVERY

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Gold nanorods and silver nanoparticles have been synthesized and embedded into a biocompatible polymeric matrix. The obtained nanosystems showed promising efficacy as theranostic agents against glioblastoma multiforme

Nanoparticelle di oro e argento in nanovettori polimerici: applicazioni nel rilascio di farmaci
Con metodiche ben riproducibili è stato possibile sintetizzare nanoparticelle di oro e argento intrappolate in matrici polimeriche biocompatibili: esse si sono rivelate promettenti agenti terpeutici e/o diagnostici per la lotta contro il cancro.

Glioblastoma multiforme (GBM) is a solid primary brain tumor with a dramatic scenario due to a median survival of less than one year after diagnosis. Despite surgery, radiotherapy and chemotherapy, GBM remains almost fatal within two years. The necessity to develop tools for the treatment as well as early diagnosis of GBM has assumed enormous importance due to the always-increasing number of patients affected by this pathology. Metallic nanoparticles have been recognized as one of the most powerful tool in nanomedicine field. Particularly gold nanorods (GNRs) and silver nanoparticles (AgNPs), due to their special properties, could find many applications in fields such as diagnostic imaging, hyperthermia and cancer therapy. AgNPs are well known as antibacterial but they seem able also to induce cells death and they could find applications as potential antitumor drugs. GNRs may find applications in hyperthermia and cancerous tissues ablation, but also in the field of diagnostic imaging, thanks to the specific light radiation absorption at wavelength around 800 nm, where the absorption by tissues is negligible. Moreover, the exploitation of these two properties leads to applications in the newly field of *theranostic*, the combination between therapy and diagnosis[1].

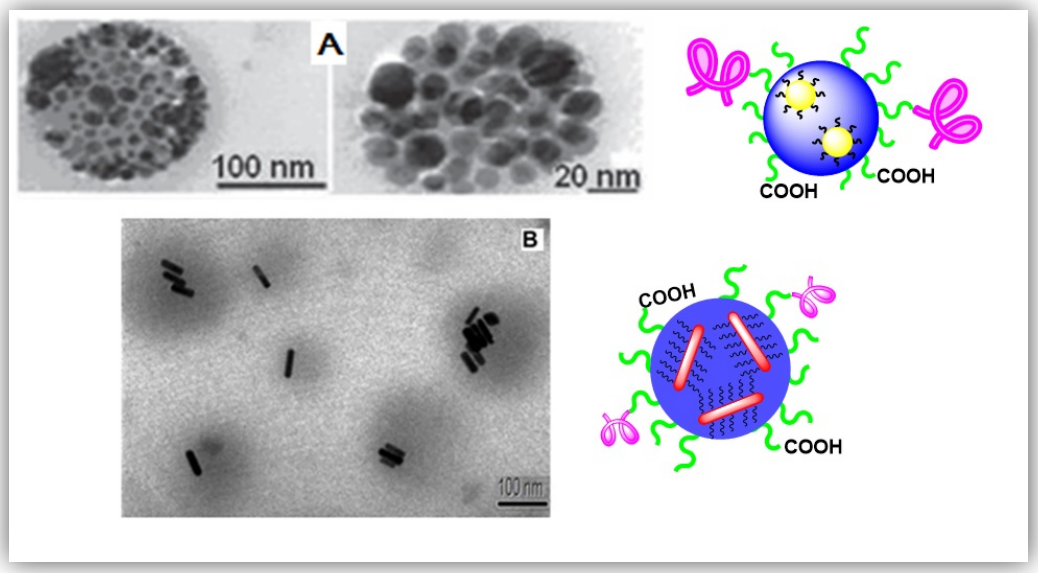

Fig. 1
TEM images and schematic representation of AgNPs@PNPs-Cltx (above) and GNRs@PNPs-Cltx (below)

Either gold and silver nanostructures have been synthesized with the "salt reduction" method: GNRs coated with the surfactant agent hexadecyl-trimethyl-ammonium-bromide (CTAB) were prepared in presence of ascorbic acid and silver nitrate, using a seed mediated growth process[2]. AgNPs were obtained by reduction of silver nitrate with glucose in presence of polyvinylpyrrolidone as stabilizing agent. All the so-obtained nanoparticles are water dispersible; in order to allow the following steps it is important to render them lipophilic, instead of hydrophilic, thus they were coated with the specifically designed organic ligand ethyl 11-(4-mercaptobenzamido)undecanoate (1). This process also removed unwanted cytotoxic ligand (especially CTAB) from the surface of nanoparticles. For a drug delivery aim the so-obtained GNRs-1 and AgNPs-1 were then entrapped into biodegradable and biocompatible polymeric nanoparticles (PNPs). The polymer chosen for this purpose has been the well-known PLGA-*b*-PEG-COOH: it is particularly suitable because it is Food and Drug Administration (FDA) approved and able to self-assemble forming water-dispersible micelles with a nanometric size, which can host in their core lipophilic moieties, such as drugs or nanoparticles, and spread them within the body[3]. By using the nanoprecipitation or the oil-in-water technique, GNRs@PNPs and AgNPs@PNPs were prepared. Moreover, the newly discovered drug Alisertib (Ali), currently in preclinical trials as antitumor drug, was entrapped into the same PNPs. In addition, both AgNPs and Alisertib were entrapped simultaneously in the core of the same PNPs in order to evaluate their synergistic effect. All the so-obtained nanosystems present on their external surface a huge amount of carboxylic acids, deriving from the used copolymer. These carboxylic acids have been exploited for the surface decoration with active targeting agents: indeed, it is of crucial importance to have specific recognizable agents exposed on the outer shell of nanoparticles for an active and more efficient delivery of the entire nanosystems within the body. As targeting agent chlorotoxin (Cltx), a 36-amino acids peptide that specifically binds to metalloproteinase-2 (MMP-2), enzyme over-expressed in brain cancer, was selected. By using the classical 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) chemistry an amide bond between the micelles' carboxylic acids and the free terminal amino group of Cltx was created. When necessary to better visualize the nanosystems with common imaging techniques also a cyanine (Cy5.5) was conjugated on the micelles' outer shell, following the same procedure, or the radioisotope ^{99m}Tc[4,5].

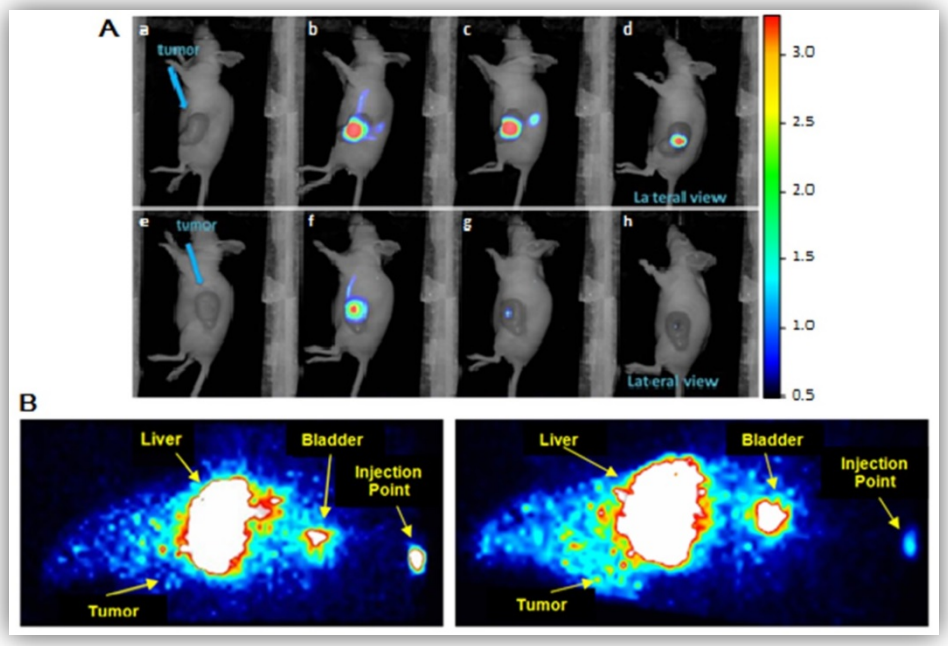

Fig. 2
A. permanence of GNRs@PNPs-Cltx (a-d) and GNRs@PNPs (e-h) in tumor bearing mice after intratumoral injection. B. biodistribution of Ag@PNPs (left) and Ag@PNPs-Cltx (right) in tumor bearing mice

As a general result nanocarriers with a diameter around 100-150 nm were obtained, with low polidispersity index (PDI<0.25) and highly negative ζ-potential values (-40/-20 mV); the dry matters were generally found around 4-6 mg/ml with significant metal contents (200-400 ppm). All nanosystems underwent both *in vitro* and *in vivo* effectiveness tests. GNRs-based nanosystems were tested on healthy (Balb/3T3) cell line at different concentrations for 72 h, where they showed good biocompatibility even at quite high concentrations (20-40 µM).

Indeed, the same nanosystems were tested as contrast agent for optoacoustic imaging: GNRs@PNPs-Cltx was found to be promising, with good enhancement of signal even at low concentrations, both in alginate spheres model or when incubated with glioblastoma cell line (U87MG). In addition to imaging capabilities, also therapeutic possibilities of these nanosystems were tested, exploiting the local hyperthermia developed by GNRs when exposed to laser radiation; cell line U87MG incubated with GNRs-1@PNPs-Cltx and irradiated with appropriate laser radiation showed almost complete cells death while nothing happened to not irradiated cells, thus these can be considered incredibly promising results. Finally tumor retention after intratumoral administration of the nanosystems in glioblastoma bearing mice was evaluated: the results clearly confirm the importance of the active targeting because only the nanosystem with Cltx remains in tumor for days after injection, while in the case of the nanosystem without Cltx the elimination from the tumor results much more faster[6].

Concerning AgNPs, we decided to evaluate the cytotoxicity of the nanosystems in order to select a good candidate as antitumor drug. All nanosystems were tested at different concentrations and for different exposure times onto U87MG cell line. The results showed that AgNPs@PNPs-Cltx is quite cytotoxic (IC_{50}=45 μM). Interestingly a high synergistic effect has been found for the nanosystem containing AgNPs and Alisertib, which showed an IC_{50} of only 0.01 μM, lower also than the one obtained for Alisertib alone (0.02 μM). The same nanosystems were tested *in vivo:* first of all, biodistribution of the nanosystems in tumor bearing mice was observed, exploiting the radioisotope ^{99m}Tc incorporated on the micelles' surface. The results showed a significant accumulation in tumor (more that 5%) compared to healthy tissues (less than 2%) for the nanocarrier with Cltx. Finally, a real therapeutic efficacy test was performed: glioblastoma bearing mice were injected with the nanosystems once at day 24 after tumor inoculation and the tumor mass was monitored until day 45. The results obtained were extremely promising because for the nanocarrier containing both AgNPs and Alisertib with Cltx a tumor reduction of 22% was observed, compared to the non-treated control mice[7]. This confirms the possibility to exploit silver and its synergistic effect with Alisertib as a real antitumor drug.

In conclusions, new and promising multifunctional nanosystems based on noble metal nanoparticles of gold and silver, entrapped in a protective and biodegradable polymer shell have been developed: these nanosystems showed potential capacity for cancer treatment as early diagnostic and therapeutic tools. These properties may open the doors for a real theranostic approach against glioblastoma multiforme and cancer in general.

REFERENCES

[1]P.K. Jain, *Accounts Chem. Res.,* 2008, 1578.
[2]B. Nikoobakht, M.A. El-Sayed, *Chem. Mat.*, 2003, **15**, 1957.
[3]E. Locatelli, M. Comes Franchini, *J. Nanop. Res.,* 2012, **14**, 1316.
[4]E. Locatelli *et al.*, *Adv. Healthcare Mat.,* 2012, **1**, 342.
[5]M. Comes Franchini *et al.*, *J. Mat.. Chem.*, 2010, **20**, 10908.
[6]E. Locatelli *et al.*, *J. Nanop. Res.*, 2014, **16**, 2304.
[7]E. Locatelli *et al.*, *Nanomedicine*, 2014, **9**, 839.