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Gold nanorods functionalized to reach cell nucleus: new tools for theragnostic applications

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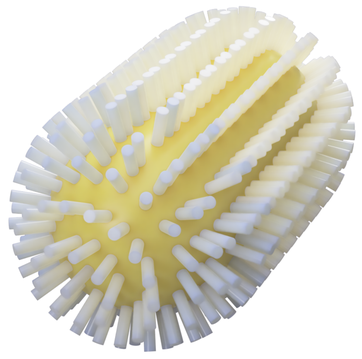
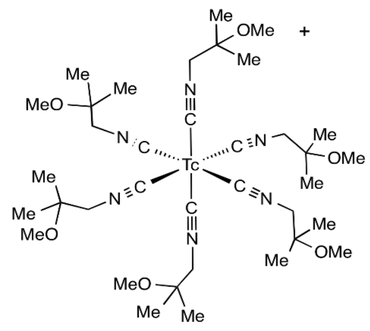
INTRODUCTION

Gold nanords (AuNRs) are a particular kind of nanomaterials that have same specific property that makes them interesting for biological application. The most researched property are the Localized Surface Plasmon Resonance (LSPR) which allows a study of particles using UV spectroscopy and their surface-to-volume ratios, which being high allows particles to be specifically functionalized with different molecules. As far as LSPR is concerned, it allows us to investigate the size of the particles, because the spectrum that is produced has two peaks due to the different resonance of the electrons on the long and short sides, so the greater the distance between the peaks, the greater the size of the rods [1,2] . In recent years, their interest in the field of nuclear medicine has increased as they can be used as radiopharmaceutical carriers to create theragnostic systems. The problem is that once in the cells, AuNRs remain in the cytoplasm and thus their function would not be fulfilled [3] .

AIM

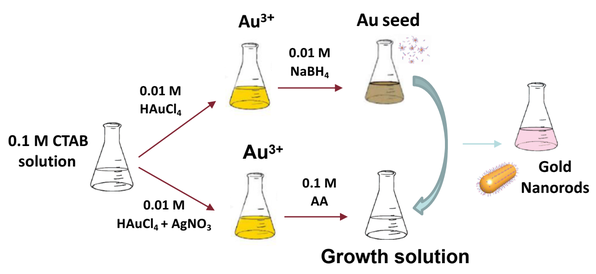
The aim of this work is the functionalization of AuNRs with the radiopharmaceutical base on ^{99m}Tc and a suitable peptide, TAT (nuclear clue sequence) [4]. In the first case, the radiopharmaceutical serves the theragnostic function, while the peptide allows the nanorods to enter the nucleus. In order to perform biological tests using cold rather than hot radiopharmaceutical, a decayed radiopharmaceutical was used and external gamma rays irradiations were delivered in order to mimick the damage at cellular and/or molecular level.





PROCEDURES

SCHEMATIC REPRESENTATION OF THE AuNRs SYNTHESIS PROCESS



Step 1: aqueous solutions of hexadecylcetyltrimethylammonium bromide (**CTAB**), hydrogen tetrachloroaurate(III) hydrate (**$HAuCl_4$**), and sodium borohydride (**$NaBH_4$**) - *Au seeds solution*

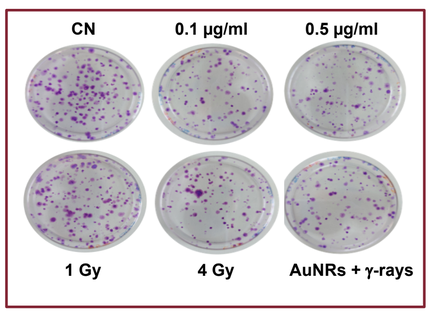
Step 2: aqueous solutions of **CTAB**, **$HAuCl_4$**, silver nitrate, Ascorbic Acid (**AA**) and *Seed solution - Growth solution*

Au seed solution + Growth solution = Gold Nanorods

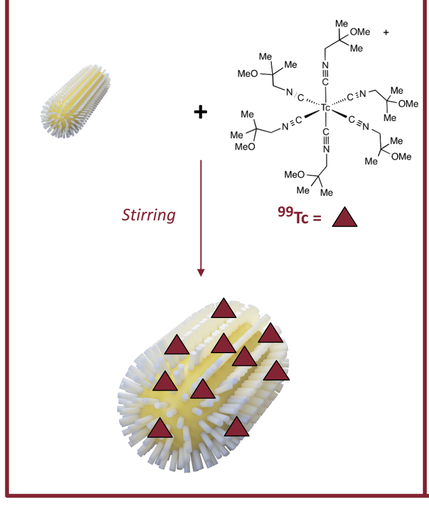
BIOLOGICAL EXPERIMENTS

For the biological tests, a ***gamma irradiator*** was used to simulate the radiation emitted by radioactive technetium 99m.

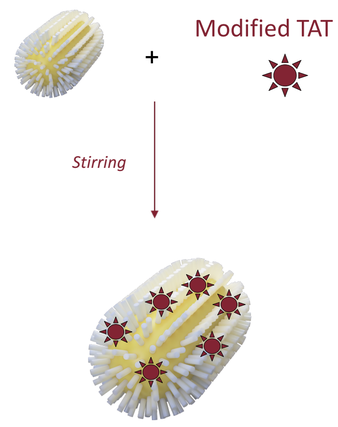
gamma irradiator



^{99}Tc SestaMIBI LABELING

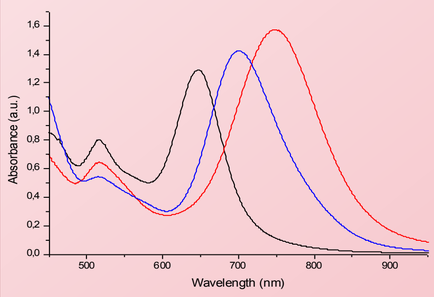


TAT LABELING



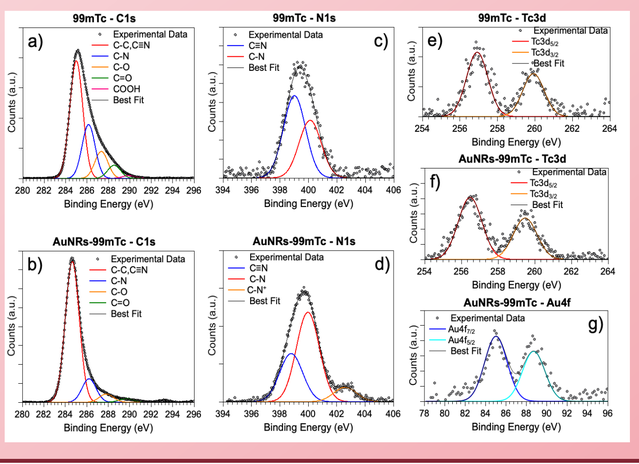
RESULTS

UV-VIS SPECTROSCOPY



Absorption in the Uv-Visible region shows the presence of the characteristic two components (Picks) Localized Surface Plasmon Resonance (LSPR), at 520 nm and from 650 to 900 nm, typical for gold nanorods. The nano-dimensions were verified by FESEM studies.

X-RAY PHOTOEMISSION SPECTROSCOPY

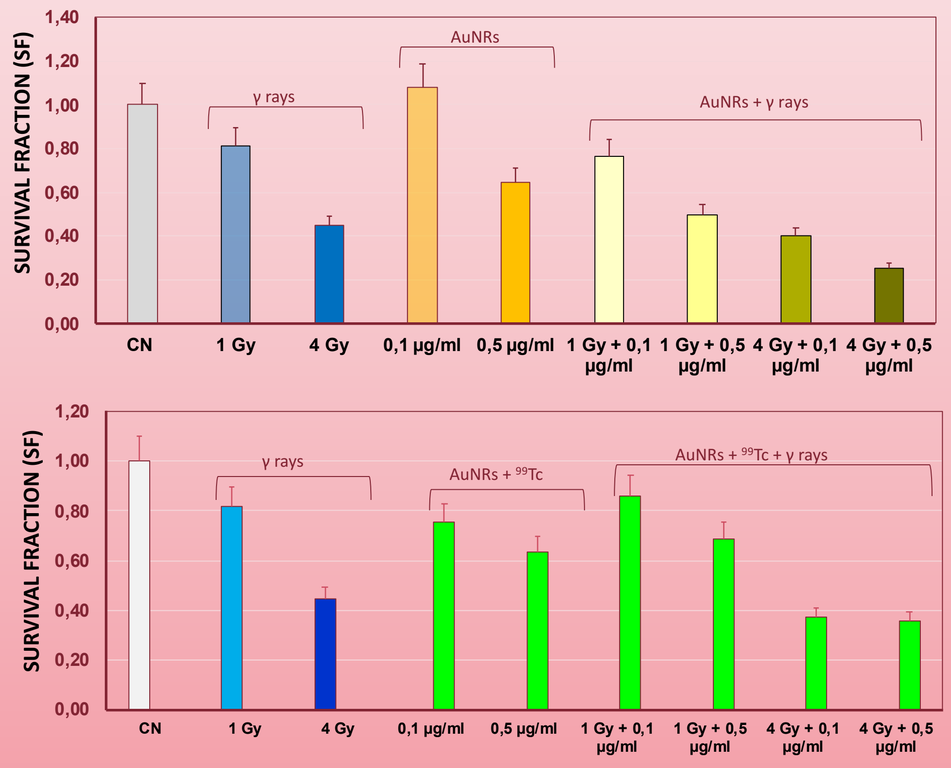


LOADING EFFICIENCY

The loading was performed after AuNRs synthesis and veryfied by XPS studies

η(%) = 5,5 ± 2 %

DATA FROM CLONOGENIC ASSAY EXPERIMENTS IN T98G CELLS



Error bars represent the standard error of the mean (SEM) coming from 3 independent experiments. All data are significantly different (p< 0.01) except for 0.1 µg/ml.

CONCLUSIONS

- **The synthesis and chemical characterization of AuNRs has led to the development of an established and reproducible protocol.** *Further studies will be needed to optimize radiopharmaceutical loading with ^{99m}Tc*. However, **preliminary data show uptake of the radiopharmaceutical within cells**: *further studies are underway to evaluate the mechanism*.
- **Auger electrons emitted increase the detrimental effect in terms of cell survival resulting in a decrease in SF**, in the case of 1 Gy + 0,1 µg/ml, **from 86% to 71%**. *Further studies are in progress.*
- **These preliminary results show that AuNRs conjugated with ^{99m}Tc could be promising as a theranostic system.**

REFERENCES

1 Chen, H et al. Gold nanorods and their plasmonic properties. Chem. Soc. (2013) Rev., 42(7), 2679–2724

2 Maccora, D et al. Gold Nanoparticles and Nanorods in Nuclear Medicine: A Mini Review. Applied sciences 2019; 9 (16): 3232

3 **Binelli, L.et al.** Gold nanorods as a vehicle for Technetium-99m radiopharmaceuticals: preparation and preliminary radiobiological in vitro tests Nanomaterials 2023, 13, 1898.

4 **Santos-Cuevas, CL, et al** 99mTc-N2S2-Tat (49-57)-bombesin internalized in nuclei of prostate and breast cancer cells: kinetics, dosimetry and effect on cellular proliferation. Nucl Med Commun. 2011 Apr;32(4):303-13.