Gold nanoparticle-induced formation of reactive oxygen species in proton therapy is a surface effect

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Proton therapy is an emerging cancer treatment technique characterized by a well-targeted dose delivery to tumors located in or near sensitive organs and fewer adverse side effects in contrast to X-ray therapy [1, 2]. This makes proton therapy particularly useful in sensitive, growing tissues as present in pediatric oncology. Combining proton the apy with noble metallic nanoparticles is an effective strategy to further improve its efficacy [3]. So far, gold nanoparticles (AuNPs) have been at the center of attention as a radiosensitizer for proton therapy. However, nanoparticles of other metals such as platinum, iron, and gadolinium were also tested as sensitive agents to improve proton therapy [3-5]. Even though different mechanisms were proposed to explain the role of nanoparticles in proton beam therapy dose-enhancement, the primary mode of action is believed to be the generation of reactive oxygen species (ROS) leading to higher tumor cell death rates. Nevertheless, a clear idea of the correlation between a) the concentration of nanoparticles as well as b) the particle sizes and c) difference between the mass-driven vs. surface-driven effects on the dose enhancement, is to date not fully understood. In this work, we combined ligand-free noble metal and biocompatible AuNPs obtained via a modern laser ablation in liquids route [6] with proton irradiation. We first investigated the effects of laser-generated AuNPs size, concentration, and irradiation dose on the enhanced ROS production to explore the underlying mechanism by which the nanoparticles act as radiosensitizing agents for proton therapy. Different concentrations and sizes of AuNPs were therefore irradiated at different physical doses of proton fields and the generated ROS was quantified employing fluorescence spectroscopy using the oxidation of the dye coumarin into 7-OH-coumarin upon reacting with the .OH radicals [7]. Our results indicate that smaller particle sizes, higher irradiation doses, and nanoparticle concentration lead to more ROS generation as shown in Fig. 1 and Fig. 2. To further explore whether the observed particle size effects are solely related to the total particle surface area, additional irradiation tests at identical surface area concentrations were conducted for the three different particle sizes (5, 10, and 30 nm). We found that particle size-specific effects were greatly diminished when they are administered at identical surface area concentrations (Fig. 3), which seems to indicate a strong influence of the total available surface area on proton irradiation-induced ROS generation in the presence of gold nanoparticles. However, small but still significantly higher ROS generation was still found for the 5 and 10 nm particles in contrast to those administered at 30 nm, which still points at size effects, probably induced by higher curvature as well as a potentially higher extent of surface defects in the smaller nanoparticles. We further validated the formation of the ROS during proton irradiation of nanoparticles by mixing the AuNPs with sodium citrate, which is a radical scavenger. Here, we observed a significant reduction in the ROS formation (Fig. 4). As citrate is commonly used for the generation of AuNPs by chemical reduction methods, the efficiency of chemically-derived AuNPs in proton therapy is limited, due to the mandatory presence of citrate. The ligand-free AuNPs used in our approach, derived from the laser ablation in liquids

technique, proved to be superior ROS generators and highly suitable sensitizers in proton therapy. In the next steps, these analyses will be extended to *in vitro* and *in vivo* tumor models aiming at a transfer to the clinics.

References

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Figures

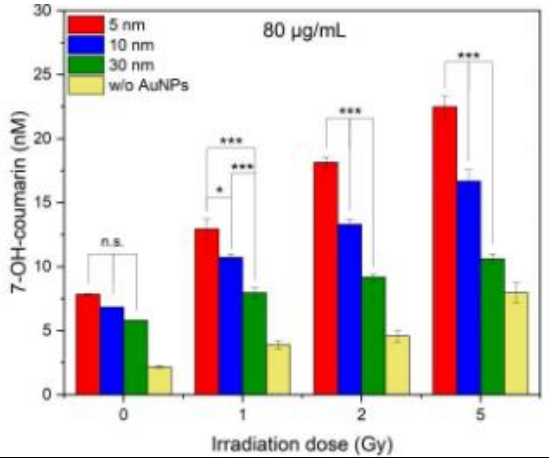

Figure 1: Amount of 7-OH-coumarin formed during the proton irradiation of water phantoms with different sized AuNPs at a constant mass concentration of 80 µg/mL at irradiation doses of 0, 1, 2, and 5 Gy (mean value ± SD of triplicate measurements).

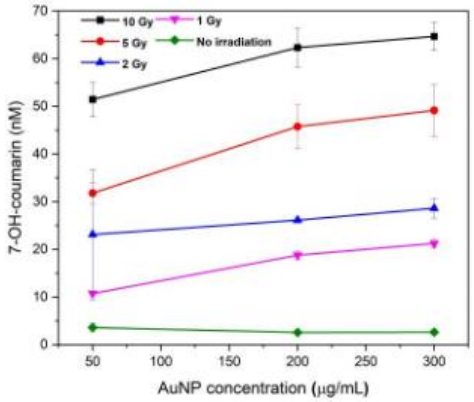

Figure 2: The effect of different AuNPs concentrations on the generation of 7-OH-coumarin (mean value ± SD of triplicate measurements) at different radiation doses. The solid lines connecting the data points are for guiding the eye.

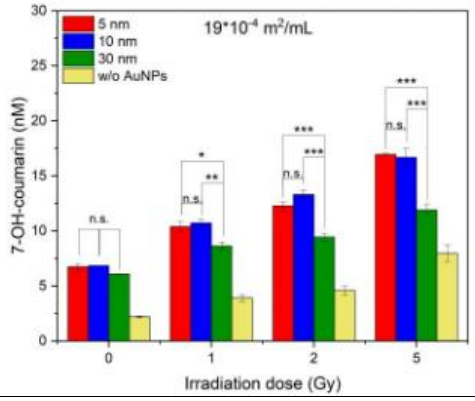

Figure 3: Amount of 7-OH-coumarin formed during the proton irradiation of water phantoms with different sized AuNPs at a constant total surface area of 19*10^{-4} m^2/mL at irradiation doses of 0, 1, 2, and 5 Gy for the evaluation of the size/mass effect (mean value ± SD of triplicate measurements).

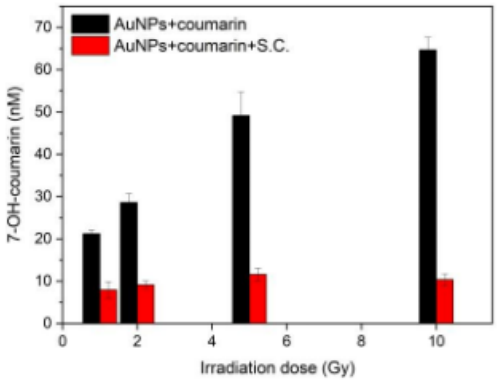

Figure 4: Amount of 7-OH-coumarin formed in the presence and absence of sodium citrate (S.C.) showing that the enhancement effect is due to the radical production which is significantly quenched in presence of the citrate molecules (AuNP+Coumarin+S.C.) (mean value ± SD of triplicate measurements).