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Experiment report MD-1216

X-ray-triggered drug release from liposomes: A proof-of-concept study

Introduction

Drug delivery strategies that can be spatiotemporally controlled by radiotherapy can revolutionize the treatment of cancer. For this purpose, we have developed an oxidation-response lipid-based drug delivery system (liposomes). By embedding high-Z element particles such as gold nanoclusters (AuNC) in these liposomes, we hypothesize that the production of reactive oxygen species upon excitation with X-rays by these particles can facilitate drug release. The aim of this proposal is to obtain proof-of-concept for X-ray-controlled drug release, to determine the optimal X-ray energy, and the minimally effective radiation dose for this application.

Results

Prior to the experiment: Preparation of liposomes

Oxidation-responsive liposomes (OXILs), were composed of 98% unsaturated phospholipids (DOPC) and 2% stabilizing phospholipids (DSPE-PEG). Specific liposomes were prepared containing hydrophobic gold nanoclusters (L-AuDDT, 2nm), or containing AuDDT and benzoporphyrin derivative (L-AuDDT-BPD). With diffuse light spectroscopy, the OXILs were determined to be 130±20nm in size.

Experiment 0: Evaluating the production of reactive oxygen species by gold nanoclusters (AuNC).

This experiment was added to this project to determine the most suitable materials in terms of X-ray-induced ROS production. 96-well sample plates were prepared containing 25µL sample, 25µL of 40µM aminophenyl fluorescein (*OH and $^1O_2$ sensor) and 50uL of PBS. Samples were irradiated at 39, 62, 79 and 82keV, and the ROS production was measured using a fluorescence plate reader. The results in Figure 1A show an X-ray dose-dependent increase in ROS production in PBS, whereas the addition of AuDDT, AuPEG, and BPD did not elevate the ROS production. In contrast, liposomes containing both AuDDT and BPD produced significantly elevated amounts of ROS at 62, 79, and 82 keV. This ROS production was dependent on Au, as there was a 2-fold increase in ROS production at 82keV irradiation compared to 79keV. These findings suggest the existence of an uncharacterized energy transfer between X-ray excited gold and porphyrins, which results in a high ROS production.

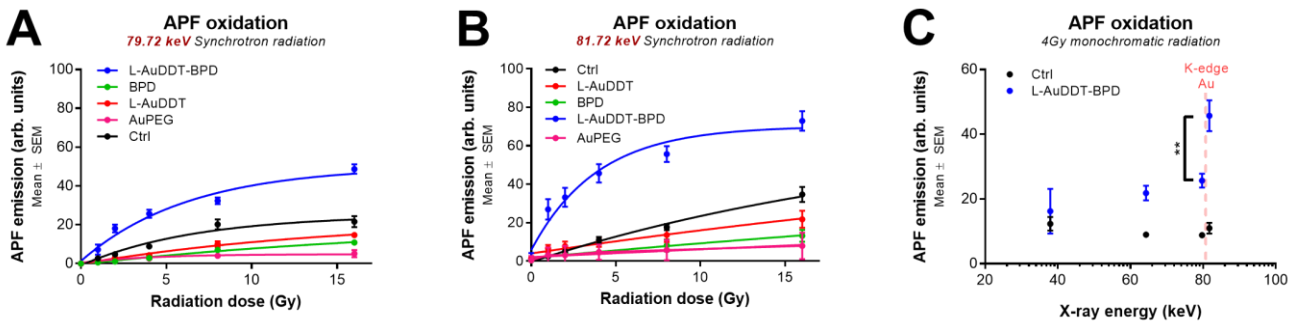

Figure 1: ROS production by various (nano)materials. (A) ROS production upon 79.72 keV (below k-edge of Au). **(B)** ROS production upon 81.72 keV (above k-edge of Au). **(C)** ROS production by L-AuDDT-BPD as a function of X-ray energy.

Experiment 1: Defining the correlation between radiation dose and liposomal drug release *and* Experiment 2: Defining the optimal gold concentrations for radiotriggered liposomal drug release.
A combination of Experiments 1 and 2 was performed to determine whether this ROS production could be used to trigger drug release from liposomes,. OXILs containing a self-quenching calcein solution were prepared, containing either no supplement (Empty), or BPD and AuDDT at different gold concentrations: L-BPD-AuDDT (5µg/mL), L-BPD-AuDDT (10µg/mL), and L-BPD-AuDDT (50µg/mL). The OXILs were irradiated at 82keV (*i.e.*, above the k-edge of Au), at increasing radiation doses. The results in Figure 2 show that empty OXILs released about 1-2% of their total calcein content, whereas L-BPD-AuDDT OXILs released 4-5% of their calcein content. ***We thus obtained proof-of-concept that X-ray triggered drug release from liposomes is possible.*** Regarding the answer for Experiment 1: There was a dose-dependent increase in drug release, and we identified a plateau value at XX gray: at higher X-ray doses, there was no further increase in drug release. Regarding the answer for Experiment 2: There was no clear relation between AuDDT dose, indicating that the liposomes may have been saturated at the lowest concentration. We initially set out to define the ED50 value, *i.e.*, the radiation dose at which 50% drug release has occurred. However, the liposomes need to be substantially optimized to achieve this.

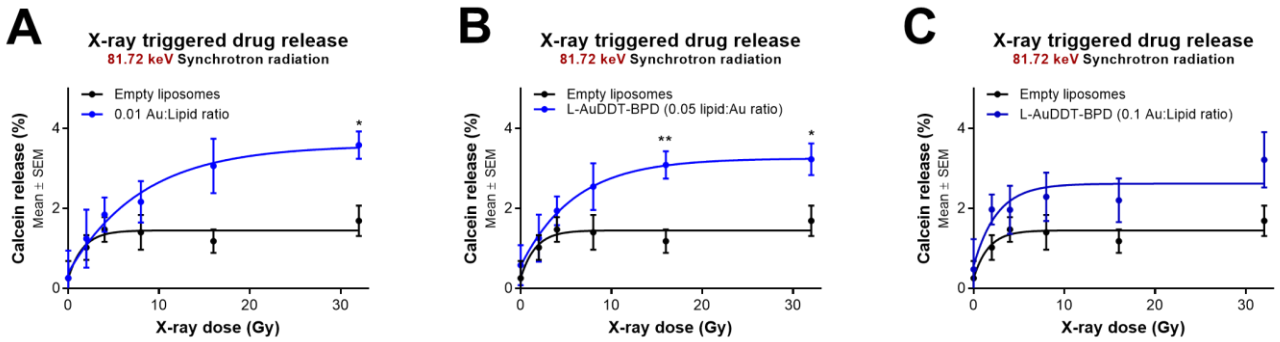

Figure 2: Proof-of-concept for X-ray triggered calcein release from OXILs. (A) L-BPD-AuDDT (5µg/mL), (B) L-BPD-AuDDT (10µg/mL), (C) L-BPD-AuDDT (50µg/mL)

Experiment 3: Defining the optimal X-ray energy for radiation-controlled drug release.
To identify an optimal X-ray energy to achieve liposomal drug release, the results from ***Experiment 0*** were leading. ***Experiment 0 identified 82keV as the most suitable X-ray energy to trigger drug release, as the highest amounts were produced at this energy.*** We thus hypothesize the 82keV is most effective to trigger oxidation-induced drug release. No further experiments could be completed during this beam-time to test this hypothesis.

Conclusion

We were successful in obtaining proof-of-concept for X-ray triggered drug release from oxidation responsive liposomes. We discovered a previously unknown interaction between benzoporphyrin derivative and AuNC, which results in significant ROS production. These ROS could trigger drug release from liposomes that were composed mostly of unsaturated lipids, albeit with a very low (4%) efficiency.

Perspectives

- Substantial optimization of the oxidation-responsive liposomes is necessary to achieve effective X-ray triggered drug release. We have performed, in parallel to this ESRF experiment, light-triggered drug release assays. Such assays are much better characterized and allow us to determine how the liposomes can be optimized. We discovered that DSPE-PEG has a negative influence on the oxidation-triggered drug release, causing a 4-fold reduction in efficacy. We furthermore determined that the inclusion of cholesterol could stabilize the liposomes and also cause a two-fold increase in drug release efficacy.
- A future application for beam-time will be prepared in which an optimized OXIL formulation will be tested.
- The MD1216 experiment was successful, but more shifts would be desired to complete the irradiation at multiple X-ray energies: A single change of energy could consume an entire shift.