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Microfluidic Synthesis of Protein-Gold Nanoparticle Hybrids: Potential for X-Ray triggered drug release

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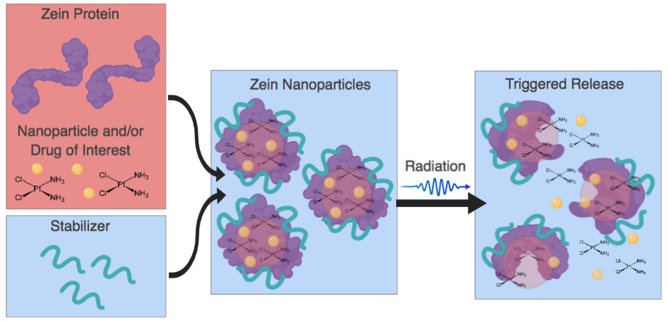
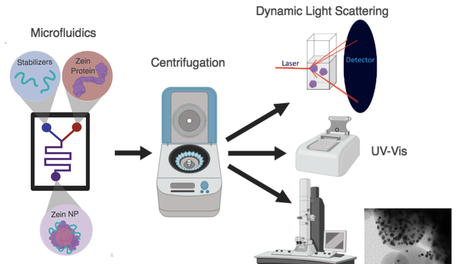
Introduction

Cancer treatments using combined modality approaches, such as high dose cisplatin with radiotherapy (RT), often result in patients experiencing significant toxicities during treatment.[1] In order to better control the side effects, we are examining the use of X- rays as an external trigger to release drugs from Zein nanoparticles (NPs). The radiolysis of water by X-rays generates reactive oxygen species (ROS) which, under certain conditions, is enhanced by the presence of gold (AuNPs). ROS are known to react with surrounding materials, such as DNA and proteins, to degrade and destabilize their structure.[2] The destabilization of Zein NPs could potentially be used to release drug on-demand with the application of X-rays. Herein, we present the preliminary design of a Zein AuNP hybrid system that is destabilised by exposure to X-rays.

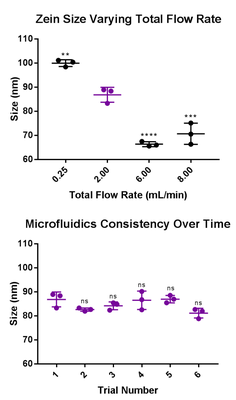
Graphical Abstract



Microfluidic Synthesis of Zein Nanoparticles



Standard Characteristics
Total Flow Rate: **2 mL/min**
Relative Flow Rate: **1 to 3**
% Zein Present: **1% (w/v)**
Solvent: **60% EtOH**

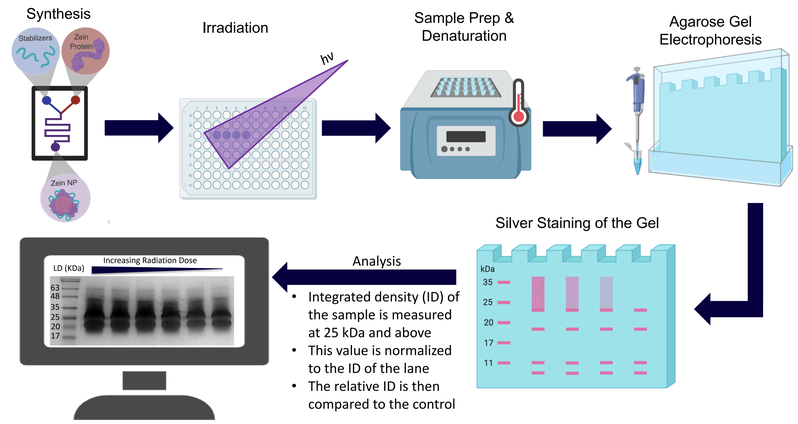


Zein NPs and Zein AuNPs were generated using ”standard characteristics” in our microfluidic system. Aggregates were removed via centrifugation and samples were characterized using DLS (above graphs demonstrate how NP size can be controlled by changing the total flow rate as well as the consistency of the synthesis), UV-Vis (data not shown), and Transmission Electron Microscopy (above image showing Zein AuNP hybrids).

All other variables are held constant for each experiment other than the one being investigated * p<0.05; ** p<0.01; ***p<0.001; ****p<0.0001

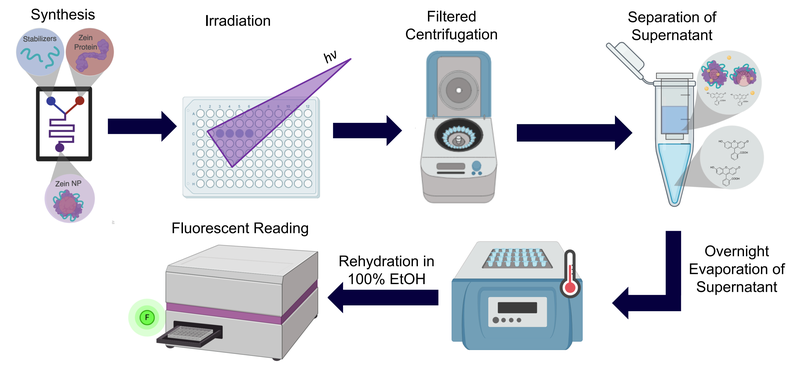
X-Ray Zein Interactions: Preliminary Methods

Measuring Primary Structure Changes of Zein NPs and Zein AuNPs Post Irradiation:

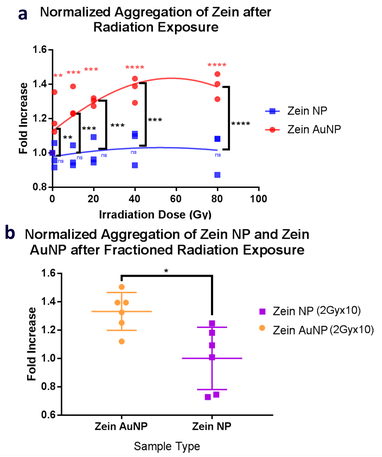


- Integrated density (ID) of the sample is measured at 25 kDa and above
- This value is normalized to the ID of the lane
- The relative ID is then compared to the control

Measuring Triggered Drug Release of Zein NPs and Zein AuNPs Post Irradiation:

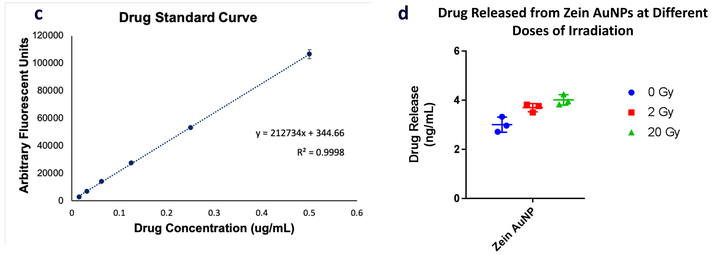


Preliminary Protein Structure Changes



- In Figure a, Zein AuNPs demonstrated a significant increase (red*) in modification (aggregation) at every level of RT dose (2, 10, 20, 40, & 80 Gy) relative to its 0 Gy control.
- This observation was not seen in Zein NP samples (ns). When comparing the two samples, there was a significant difference (black*) between the sample’s fold increase at every level of irradiation other than the 0 Gy control. Zein AuNPs reached a max of a 1.4 fold increase.
- In Figure b, RT doses were administered as fractioned doses to better mimic clinical RT. The significant difference between the Zein NP and Zein AuNP fold increase was maintained when using a fractioned administration method.

Preliminary Drug Release



- In Figure c, a calibration curve of the “drug” (DiI) was generated to determine the concentration of the drug relative to its fluorescence
- Figure d, shows a trend where drug release increases with increasing irradiation dose administered to the Zein AuNP sample.

Conclusion and Future Work

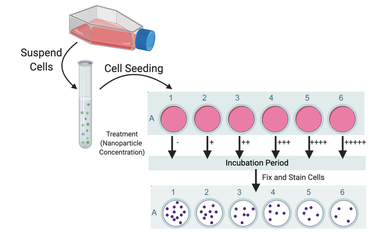
While X-Ray triggered Zein AuNPs has shown to be a promising method for on-demand drug delivery, the following experiments should be performed to further validate the results:

- Additional repeats of the experiments should be performed;
- Structural changes should also be confirmed using another technique, such as circular dichroism.

Future work will include:

- Cisplatin release studies with Zein NPs and Zein AuNPs
- Cell toxicity studies of the Zein NP and Zein AuNP systems
- Triggered drug release studies in the presence of cells

Example Set Up of a Cell Toxicity Study



References

1. Brockstein, B., Vokes, E., and Eisbruch, A. Locally Advanced Squamous Cell Carcinoma of the Head and Neck: Approaches Combining Chemotherapy and Radiation Therapy. UpToDate, 2019.
2. van Ballegooie, C., Man, A., Win, M., Yapp, D. Spatially Specific Liposomal Cancer Therapy Triggered by Clinical External Sources of Energy . Pharmaceutics, 2019. 11(125).
3. Images generated using BioRender

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