HPPH Liposomes for Phototherapy-Induced Intracellular Release of TNO-155 in Macrophages

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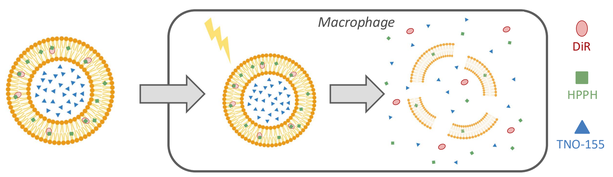
Background

- Neurofibromatosis Type 1 (NF1) affects 0.03% of newborns[1]
- NF1 is associated with malignant peripheral nerve sheath tumors (MPNSTs) which exhibit extensive immune cell infiltrate; up to 40% of tumor volume consists of macrophages in some cases[1]
- Despite immune cell infiltration, the tumor microenvironment (TME) allows for continued tumor growth[1]
- Past studies have shown that TNO-155, a SHP2 inhibitor, is able to target both tumor cells and alter macrophage polarization[2]
- Photochlor (HPPH) is an FDA-approved phototherapy that can be used to degrade liposomes[3]
- Phototriggered release of TNO-155 from Lipid Nanoparticles (LNPs) will activate and repolarize macrophages and will have dual therapeutic action on the TME by enabling direct cancer cell death as well as altering the TME to tumor suppressive rather than tumor supporting.

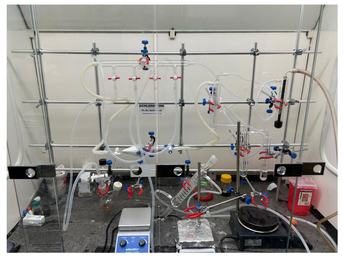
Hypothesis

- HPPH liposomes containing TNO-155 can be phagocytosed when added to a macrophage culture, and the contents will be released as a result of phototherapy.
- A custom cell segmentation algorithm can be designed to more effectively segment macrophages

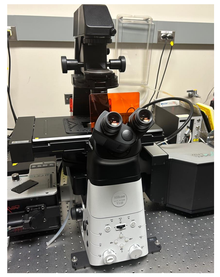


Methods: Liposome Synthesis

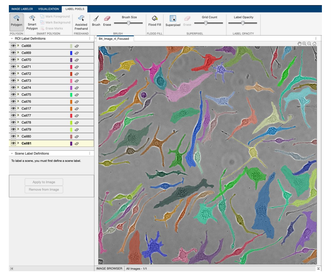
- Liposomes were prepared by thin film organic synthesis
- Lipids used: DPPC, $DC_{8,9}PC$, and DSPE-PEG2000
- DPPC and $DC_{8,9}PC$ were mixed with TNO-155 and stirred in the presence of Argon for 6 hours in $CHCl_3$:MeOH (1:1)
- DSPE-PEG2000, DiR, and HPPH were added and stirred for 6 hours under inert conditions
- A thin film was formed under high vacuum rotation followed by sonication. Purification was done through column chromatography and centrifugation



Methods: Cell Segmentation Algorithm



- A Nikon TI-2E was used to image M2 macrophages
- Matlab was used to train a Cellpose 2.0 model[5,6]
- Once initial model was generated, human-in-the-loop training was used to further refine the model using Matlab's Image Labeler
- Both focused and unfocused images used in model training to enhance model's ability to segment cells out of the plane of focus



Results

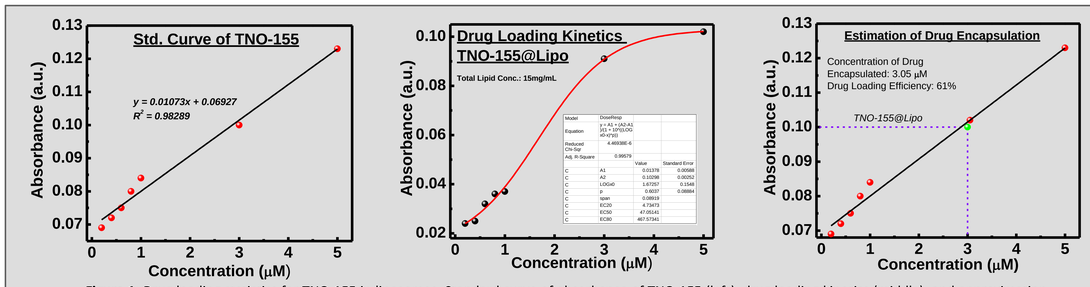


Figure 1: Drug loading statistics for TNO-155 in liposomes: Standard curve of absorbance of TNO-155 (left), drug loading kinetics (middle), and approximation of drug encapsulation (right)

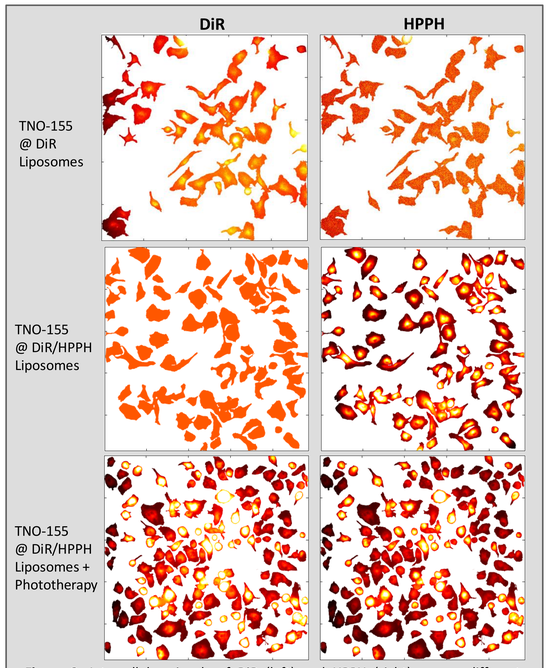


Figure 2: Intracellular signals of DiR (left) and HPPH (right) among different treatment groups: Liposomes with TNO-155 and DiR (top), Liposomes with TNO-155, DiR, and HPPH (middle), and Liposomes with TNO-155, DiR, and HPPH after phototherapy (bottom). Brighter and darker areas indicate stronger and weaker fluorescent signals, respectively.

- The graphs in Figure 1 show a drug loading efficiency of 61% based on a standard curve of TNO-155 absorption and readings of surfactant-induced burst release of TNO-155 from liposomes
- Fluorescent microscopy images in Figure 2 show minimal fluorescent signal from DiR when loaded in a particle with HPPH prior to phototherapy and is revealed post-therapy
- The images in Figure 3 shows the improved M2 macrophage segmentation performance when using a Cellpose model trained on a custom dataset compared to using the same model on the pre-trained counterpart

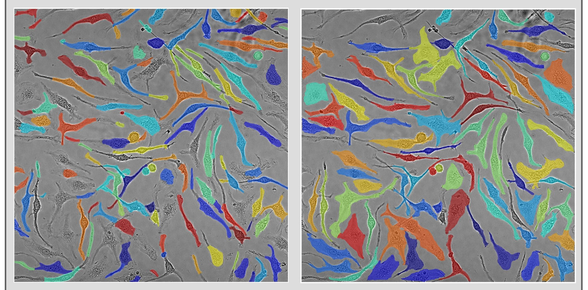


Figure 3: Cell segmentation with default Cellpose Cyto 2.0 model (left), and custom trained Cyto 2.0 model (right)

Discussion

- The results show the ability to load therapeutic compounds into liposomes and have the particles phagocytosed when added to an in vitro culture of M1/M2 macrophages, making it a potential candidate for targeted drug delivery and photodynamic therapy

Nanoparticle Design Concept

- There is an existing challenge to showcase the photodynamic property of LNPs in a multimodal photophysical environment. Here, the synthetic methodology involving organic phase synthesis yielded the electrostatic interaction of aza-crown-HPPH and DiR with excellent lipid layer compartmentalization. This allowed us to explore the multimodality of the through utilization of the photophysical characteristics of both HPPH and DiR as shown in Figure 2. This desired photodynamic mechanistic approach can be beautifully correlated with pre/post treatment photophysics.

Future Work

- M1 and M2 macrophages will be treated with particles containing S-nitroso-N-acetyl-penicillamine +/- phototherapy and their morphology analyzed as M1-like or M2-like using a narrow neural network model (Figure 4)

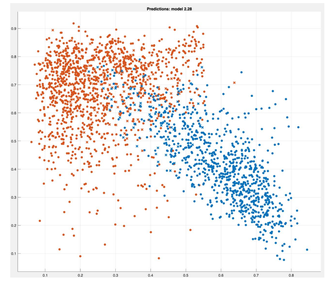


Figure 4: Scatterplot of macrophage classification model with 92.5% accuracy, with dots representing correct classifications. Blue = M1 and Orange = M2

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