Photo-enhanced ferroptosis achieved by FSP1 inhibition and photodynamic therapy

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Introduction

Disease Burden of Cancer and Therapy Resistance

❖Cancer: 1st and 6th leading cause of death in Hong Kong and worldwide (2019) [1, 2]
❖Therapy resistance: the cause of 90% cancer-related mortality[3, 4]

Emerging promising therapeutic module --- Combination of Ferroptosis Inducer and Photodynamic Therapy (PDT)

PDT:
❖ Mechanism: Under laser irradiation of a specific wavelength, photosensitizers can produce cytotoxic reactive oxygen species (ROS) and trigger anti-tumour response
❖ Efficacy is limited by the typical hypoxia tumour microenvironment, altered intake and efflux rate of photosensitizers, and antioxidant defense mechanisms[5, 7]

Ferroptosis:
❖ Iron- and ROS- dependent programmed cell death which generates ROS via Fenton reaction[8, 9]

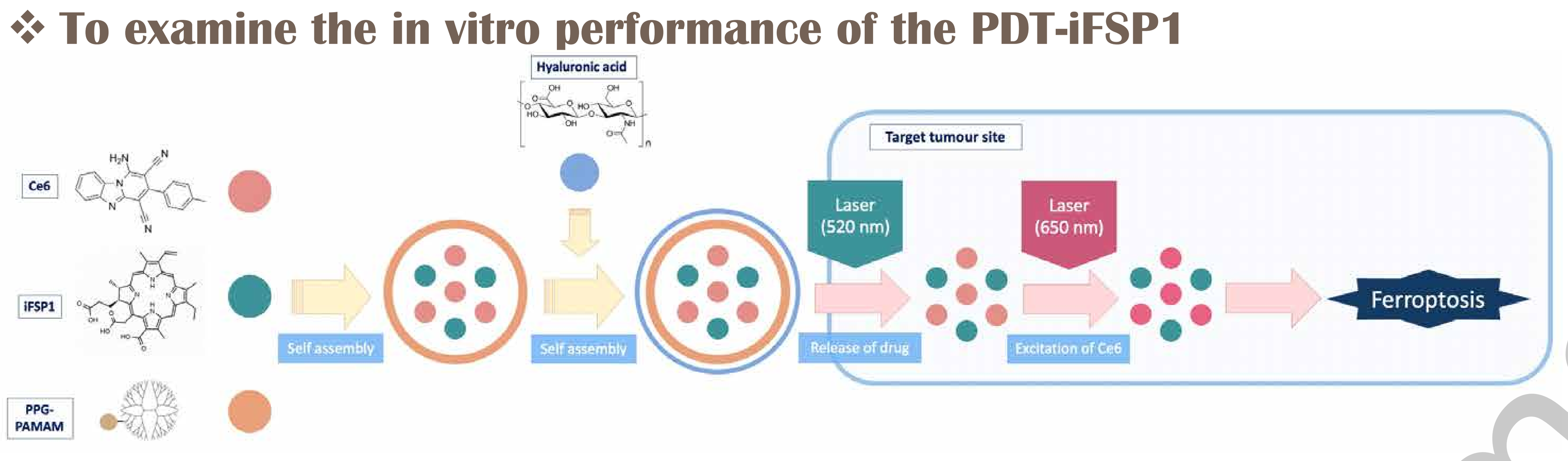
Co-administration of photosensitizer and ferroptosis inducer:
❖ PDT can enhance Fenton reaction either by supplying substrate ROS or depleting glutathione
❖ Oxygen product from Fenton reaction allows maintenance of PDT function[10-16]

Newly identified anti-ferroptosis pathway --- Ferroptosis Suppressor Protein 1 (FSP1)

❖ FSP1: newly recognized ferroptosis inhibitory mediator which activity is parallel to conventional glutathione pathway[17, 18]
❖ Inhibitor of FSP1 (iFSP1): ferroptosis inducer with potentiality to overcome cellular ferroptosis resistance[18]
❖ No research has studied the combination of iFSP1 and PDT as cancer therapeutics yet

Objectives

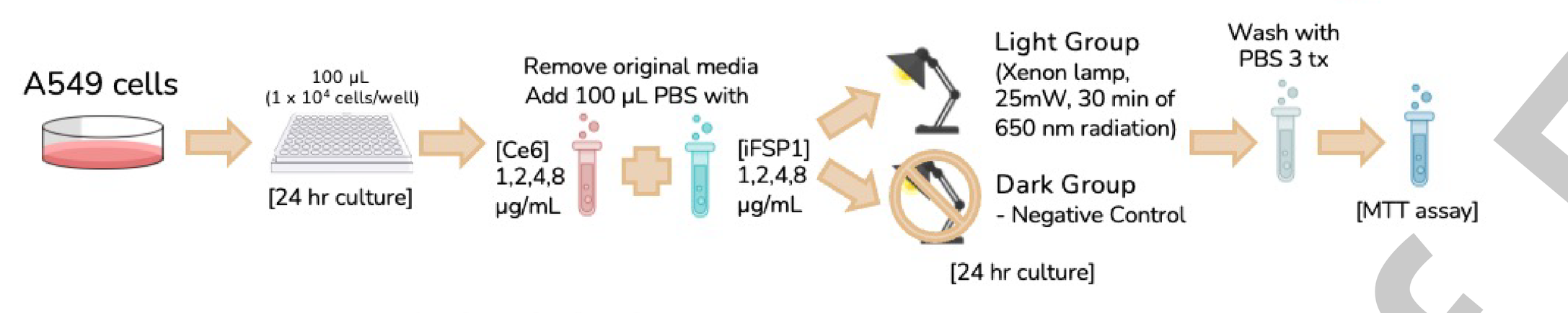
❖ **To examine any synergistic interaction between iFSP1 and PDT**
❖ **To develop a dual light-responsive nanoplatform for co-delivery of photosensitizer and iFSP1**
❖ **To examine the in vitro performance of the PDT-iFSP1**



Scheme 1. Schematic illustration of the construction and cellular pathway of BMP60@Ce6-iFSP1.

Materials and Methodology

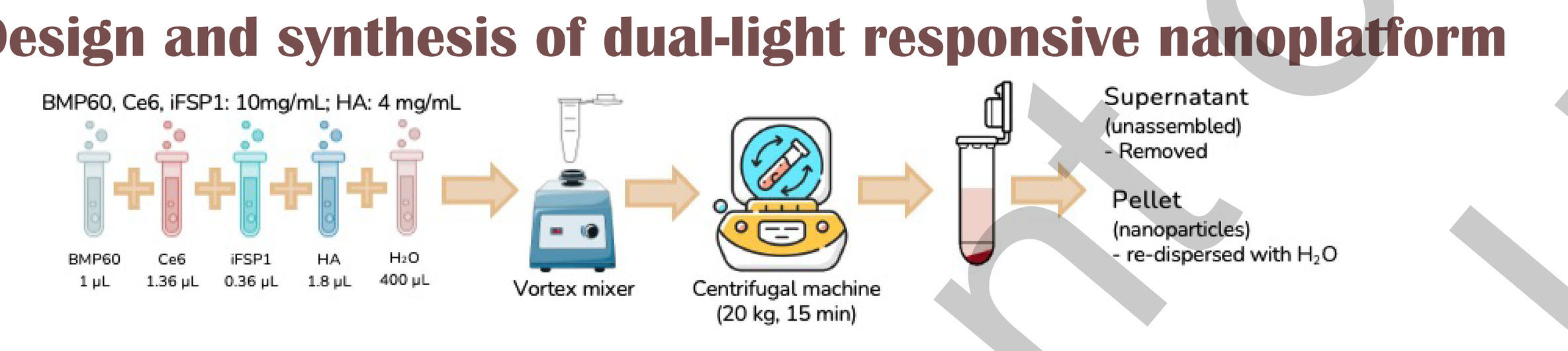
1. Cytotoxicity assay of Chlorin 6(Ce6)-iFSP1 via MTT assay



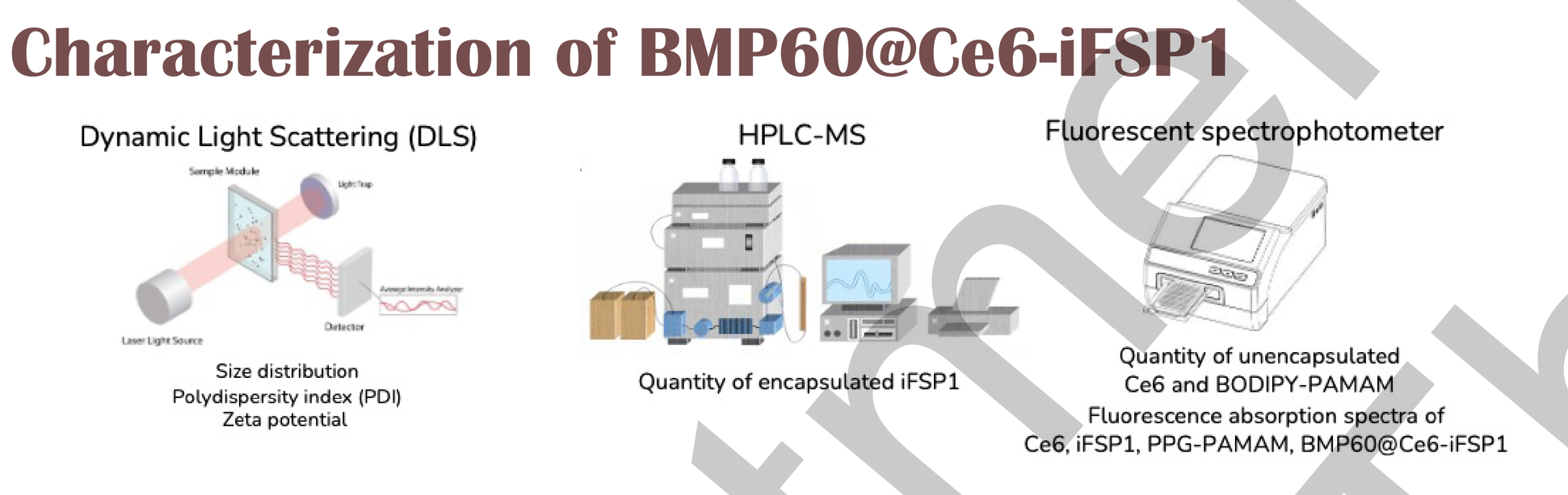
2. Synergy assay of Ce6-iFSP1

Loewe additivity model of Ce6-iFSP1 was constructed by SynergyFinder Plus® Software[19]

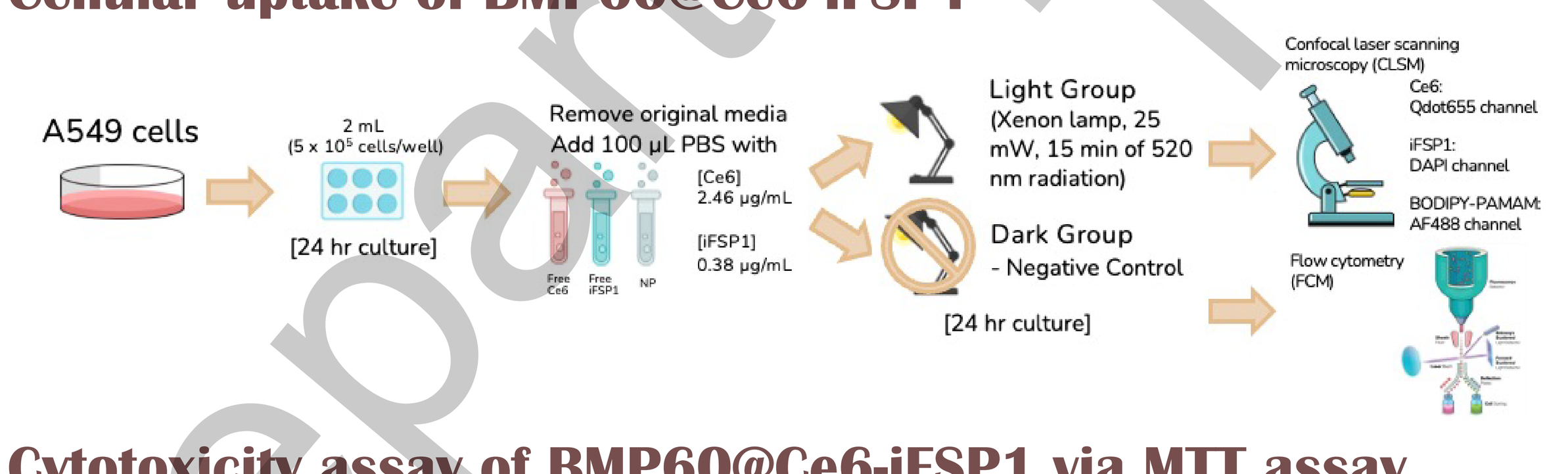
3. Design and synthesis of dual-light responsive nanoplatform



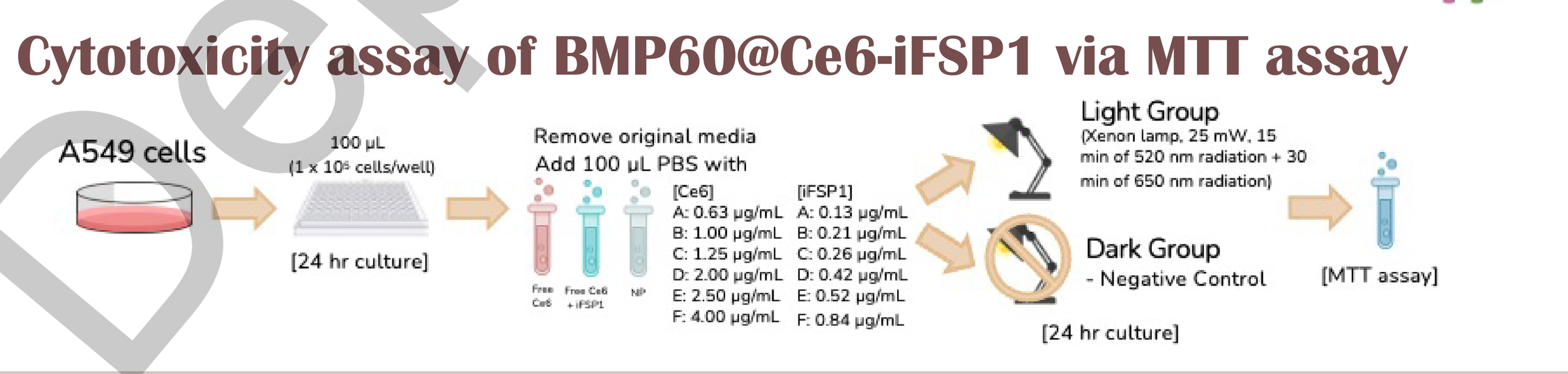
4. Characterization of BMP60@Ce6-iFSP1



5. Cellular uptake of BMP60@Ce6-iFSP1

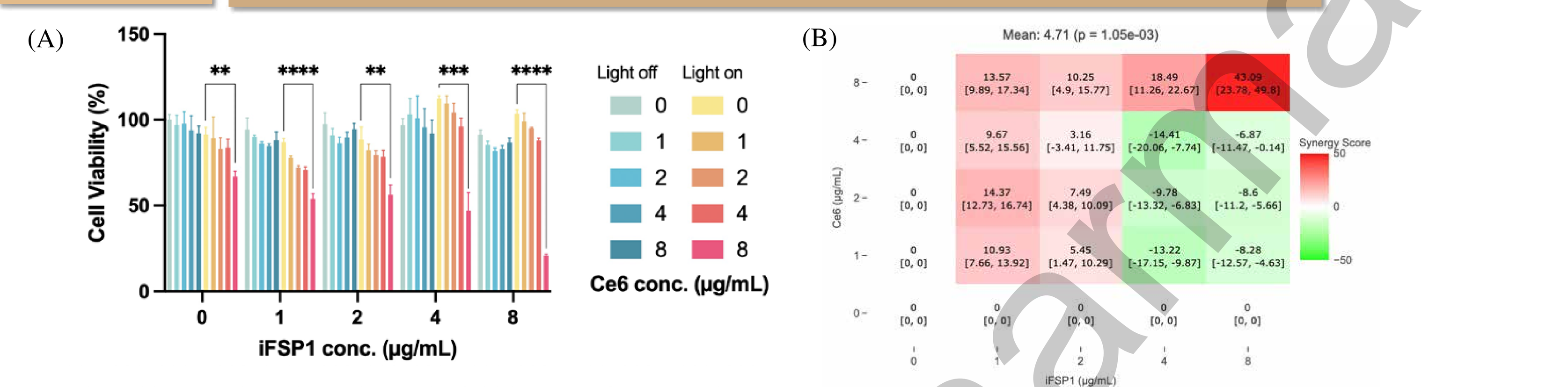


6. Cytotoxicity assay of BMP60@Ce6-iFSP1 via MTT assay



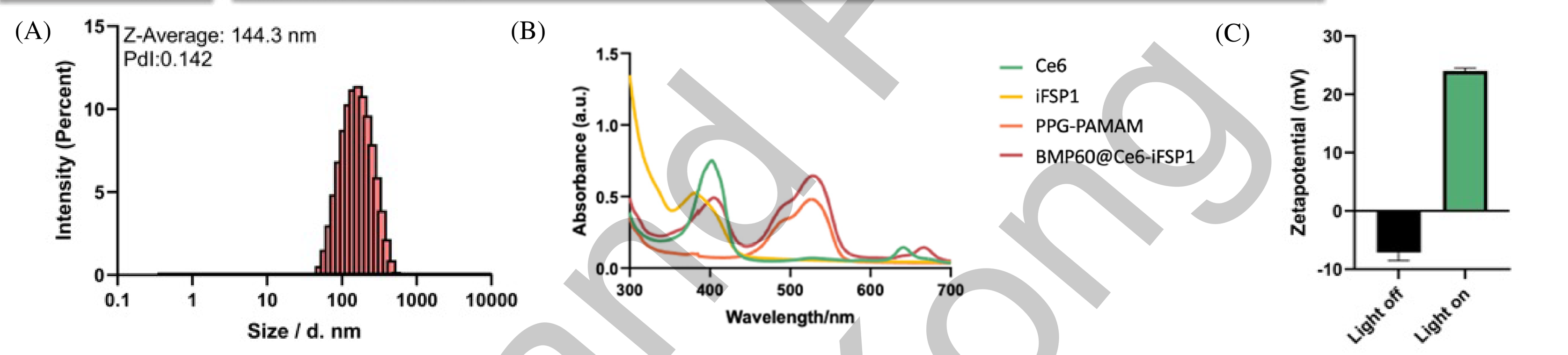
Results

Figure 1. Presence of Synergism in Ce6-iFSP1 combination



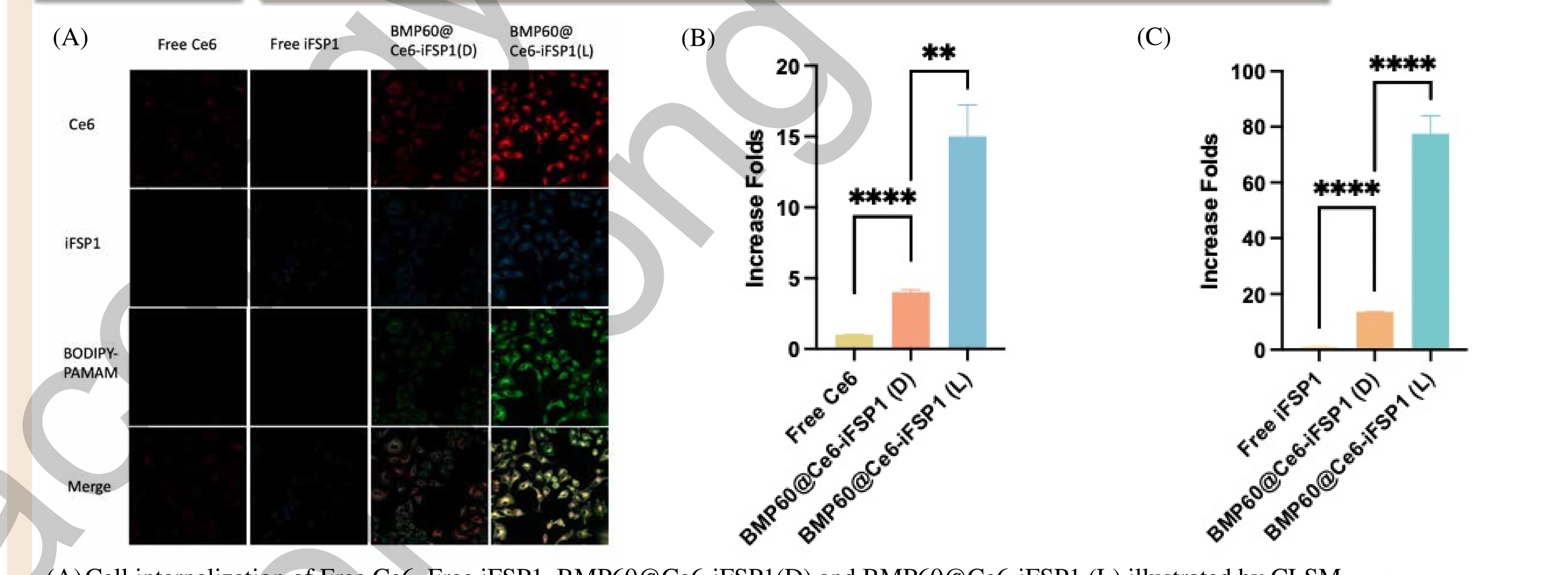
(A) Ce6- and iFSP1- dose dependent cell viability (%) of A549 cells.
(B) Loewe additivity synergy model of Ce6-iFSP1(L) illustrated as heatmap.

Figure 2. Characterization of BMP60@Ce6-iFSP1



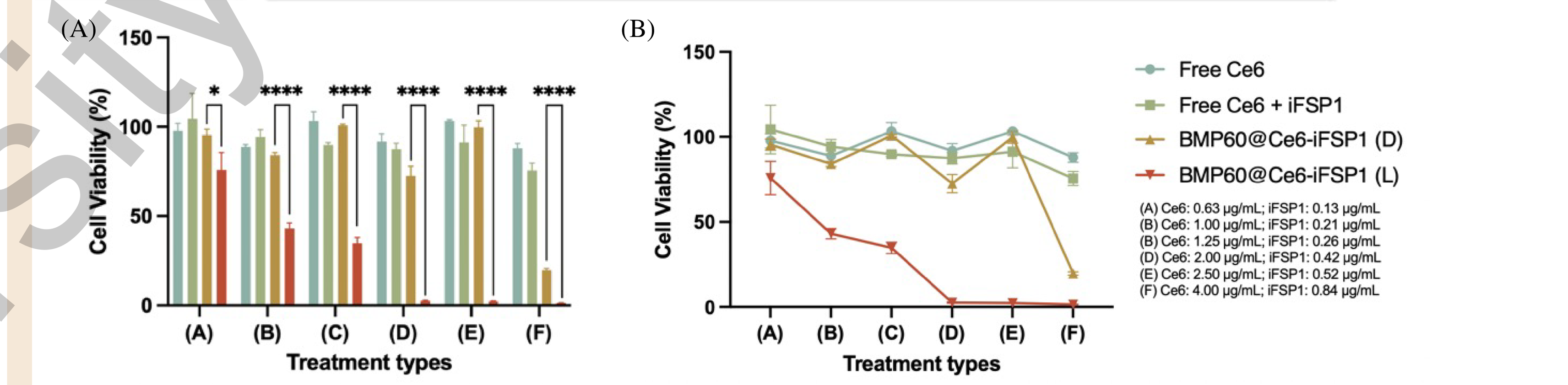
(A) Representative DLS measurement of HA-coated BMP60@Ce6-iFSP1.
(B) Fluorescence absorption spectra of Ce6, iFSP1, PPG-PAMAM, BMP60@Ce6-iFSP1.
(C) Zeta potential profile of BMP60@Ce6-iFSP1 in the absence and presence of light irradiation.

Figure 3. Superior cell internalization of BMP60@Ce6-iFSP1 (L)



(A) Cell internalization of Free Ce6, Free iFSP1, BMP60@Ce6-iFSP1(D) and BMP60@Ce6-iFSP1 (L) illustrated by CLSM.
(B) Increase folds of cellular intake of Free Ce6, BMP60@Ce6-iFSP1(D) and BMP60@Ce6-iFSP1(L) from FCM fluorescence measurement.
(C) Increase folds of cellular intake of Free iFSP1, BMP60@Ce6-iFSP1(D) and BMP60@Ce6-iFSP1(L) from FCM fluorescence measurement.

Figure 4. Potent cellular cytotoxicity of BMP60@Ce6-iFSP1 (L)



(A) Free Ce6, Free Ce6+iFSP1-, BMP60@Ce6-IFSP1(D)- and BMP60@Ce6-IFSP1(L)- dose dependent cellular viability illustrated as bar chart.
(B) Free Ce6, Free Ce6+iFSP1-, BMP60@Ce6-IFSP1(D)- and BMP60@Ce6-IFSP1(L)- dose dependent cellular viability illustrated as line graph.

Discussion

Synergism between iFSP1-Ce6

❖*In the absence of 650 nm light stimulation, synergistic effect was not identified:*
! Suggested iFSP1 exclusive cellular sensitization action towards ferroptosis inducer
❖*In the presence of 650 nm light stimulation, potent synergistic effect was observed:*
! Specifically at high concentrations of Ce6 and iFSP1
❖*A synergy model depicting synergism with a slight shade of negative additive effect:*
! Suggested the involvement and upregulation of other cellular anti-ferroptosis system in the interaction between Ce6 and iFSP1

Characterization of BMP60@Ce6-iFSP1

❖*Nanocarrier's characteristics:*
! Allowed successfully incorporation of Ce6 and iFSP1
! Allowed maximal preservation of Ce6 monomer status and its therapeutic action
❖*Size:*
! Allowed utilization of cancer's enhanced permeability and retention effect
❖*Light-responsiveness characteristic and associated alteration of the outer shell charge:*
! Allowed enhanced cellular internalization of Ce6 and iFSP1 after 520 nm light irradiation was confirmed

Cytotoxicity of BMP60@Ce6-iFSP1

❖In the presence of dual light stimulation, superior cellular cytotoxicity was observed
❖In the absence of dual light stimulation, no dose-response relationship was observed

Conclusions

❖ **Presence of PDT-iFSP1 synergistic action confirmed**
❖ ***In vitro* experiment, BMP60@Ce6-iFSP1 displayed favorable potentiality:**
! **minimal dark toxicity**
! **enhanced uptake and potent cellular cytotoxicity under dual-light stimulation**

Limitations

❖Failure to present that PDT-iFSP1 synergistic cytotoxicity are induced through enhanced ferroptosis action and elevated ROS production
❖Insufficient penetration degree of 520 nm light stimulation in respond to cancer located at deeper lesion

Acknowledgement

This work was supported by the Department of Pharmacology and Pharmacy and Dr Li Dak-Sum Research Centre, The University of Hong Kong. Special thanks to Dr Weiping Wang, Assistant Professor at the Department of Pharmacology and Pharmacy and Dr Li Dak-Sum Research Centre for his professional guidance and generous support as a supervisor throughout the project. Also, credit to his graduate student, Mr. Yang Zhou, who assisted in the formulation of study design and collection of preliminary results.

Reference

[illegible]