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Optimization and Synthesis of Perfluorocarbon Nanoemulsion with Fluorous Photosensitizer for Photodynamic Therapy

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pharmaceuticals MDPI

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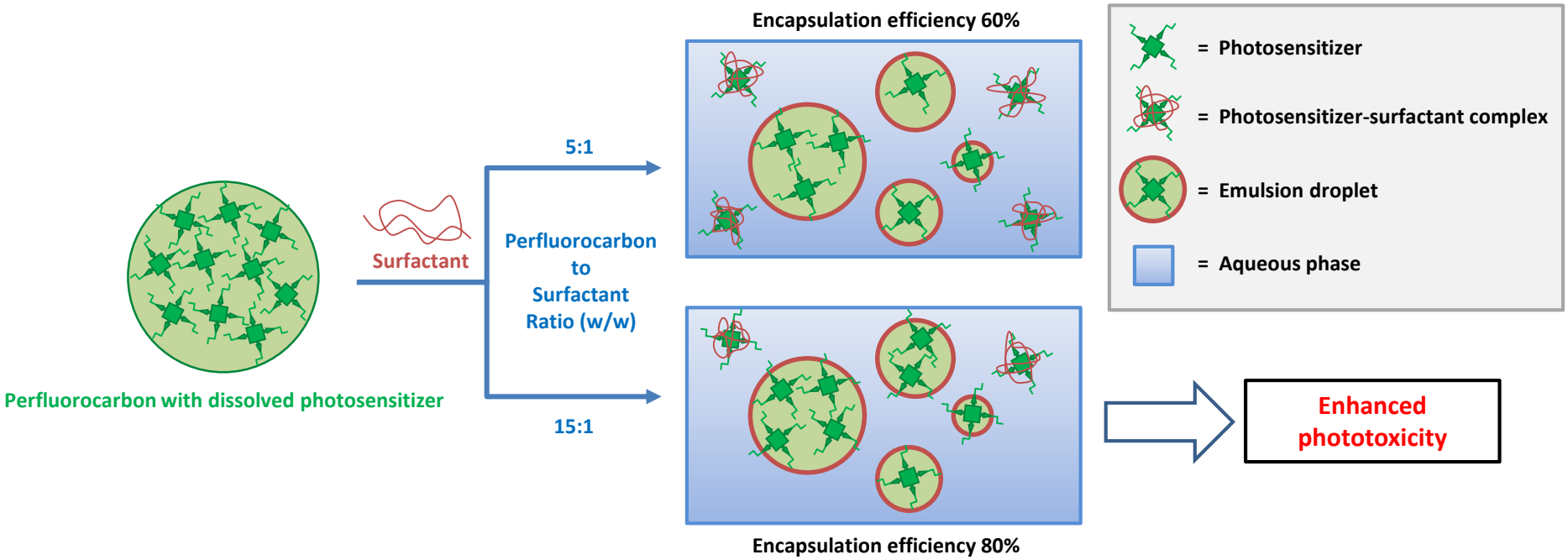
Optimization and Synthesis of Perfluorocarbon Nanoemulsion with Fluorous Photosensitizer for Photodynamic Therapy

Graphical Abstract



Abstract: Perfluorocarbon-based nanoemulsions (PFC-NEs) have been shown to be an effective tool for oxygen delivery in various therapeutic modalities, including photodynamic therapy (PDT) in cancer treatment. PFC droplets are able to transport and supply oxygen to hypoxic cells as well as enhance the singlet oxygen generation by the photosensitizer (PS), functions which make them a promising platform for PDT. To further enhance PDT efficacy, we synthesized PFC-NEs with fluorous PS that is soluble in the perfluorocarbon phase, creating a closer proximity of the PS to the dissolved oxygen in PFC. However, the fluorous PS used in this work had a high tendency to leak into the aqueous phase with a surfactant, leading to less than desirable encapsulation efficiency (EE%). Therefore, the main goal of this study is to develop a formulation to ensure high EE% of fluorous PS in PFC droplets. PFC-NEs were prepared by ultrasonic emulsification and were characterized using dynamic light scattering, UV-Vis and fluorescence spectroscopy. The optimization of the PFC-NE formulation did not significantly affect the nanoemulsion properties, such as hydrodynamic diameter, polydispersity index and colloidal stability, and successfully increased EE%, as well as improved dark cytotoxicity profile and enhanced photoinduced cytotoxicity of PFC-NE.

Keywords: drug formulation; encapsulation efficiency; nanoemulsion; perfluorocarbon; photodynamic therapy.

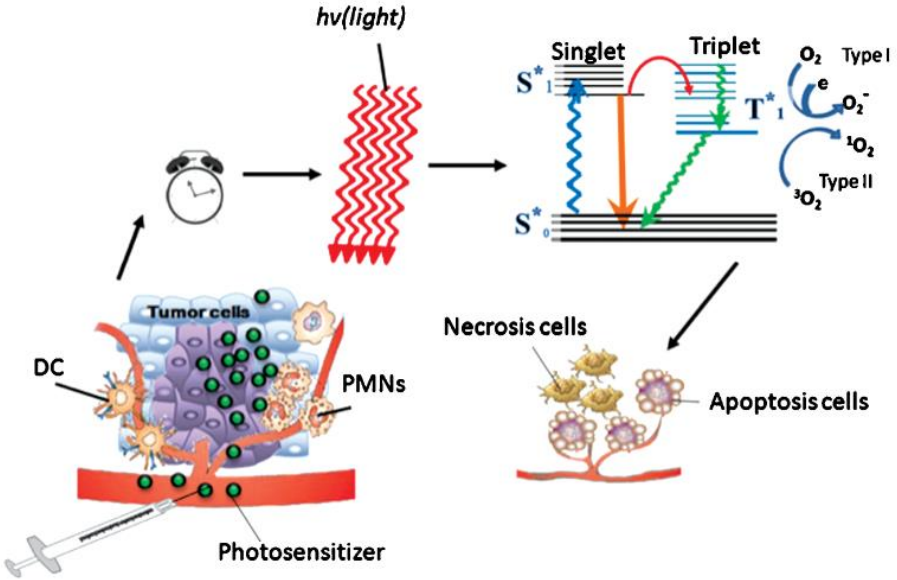
Introduction

Photodynamic therapy (PDT) is a minimally invasive method of cancer treatment

Photodynamic processes are mostly **oxygen**-dependent

Hypoxia leads to tumor progression and resistance to oxygen-dependent therapies (radiotherapy, PDT)

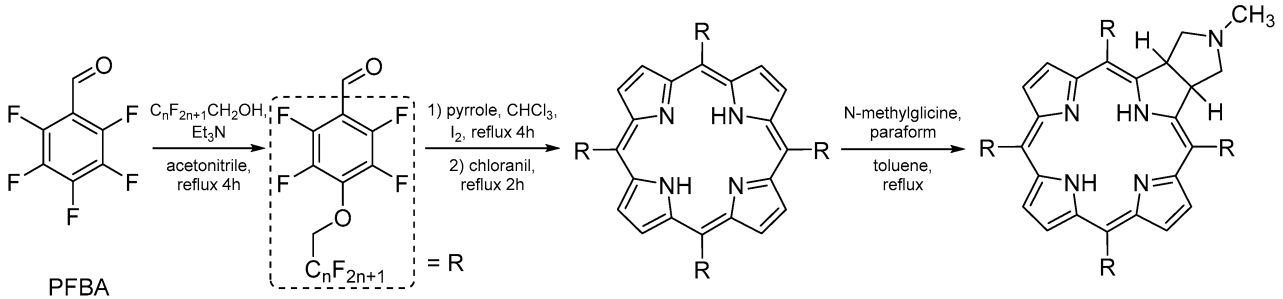
Perfluorocarbons are able to dissolve 20- to 40-fold larger amounts of oxygen compared to water and temporarily alleviate hypoxia



The mechanisms of PDT

*Source: Denis, T. G.; Aziz, K.; Waheed, A. A.; Huang, Y.-Y.; Sharma, S. K.; Mroz, P.; Hamblin, M. R. Combination approaches to potentiate immune response after photodynamic therapy for cancer. Photochemical & Photobiological Sciences **2011**, 10, 792–801. https://doi.org/10.1039/c0pp00326c*

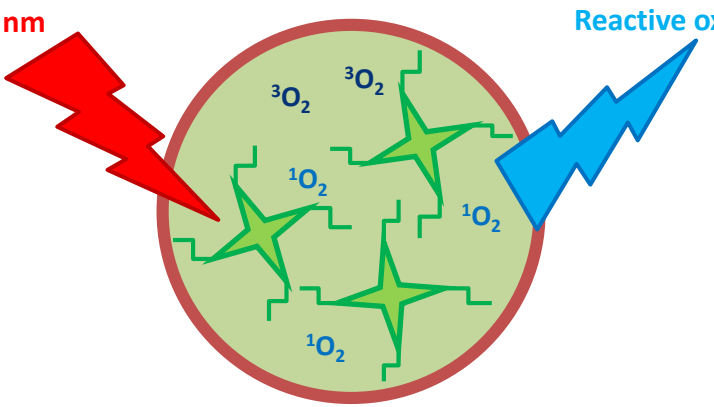
Previous work: Synthesis of Fluorous Photosensitizers and Preparation of Perfluorocarbon Nanoemulsions



1a n = 2
1b n = 4
1c n = 6

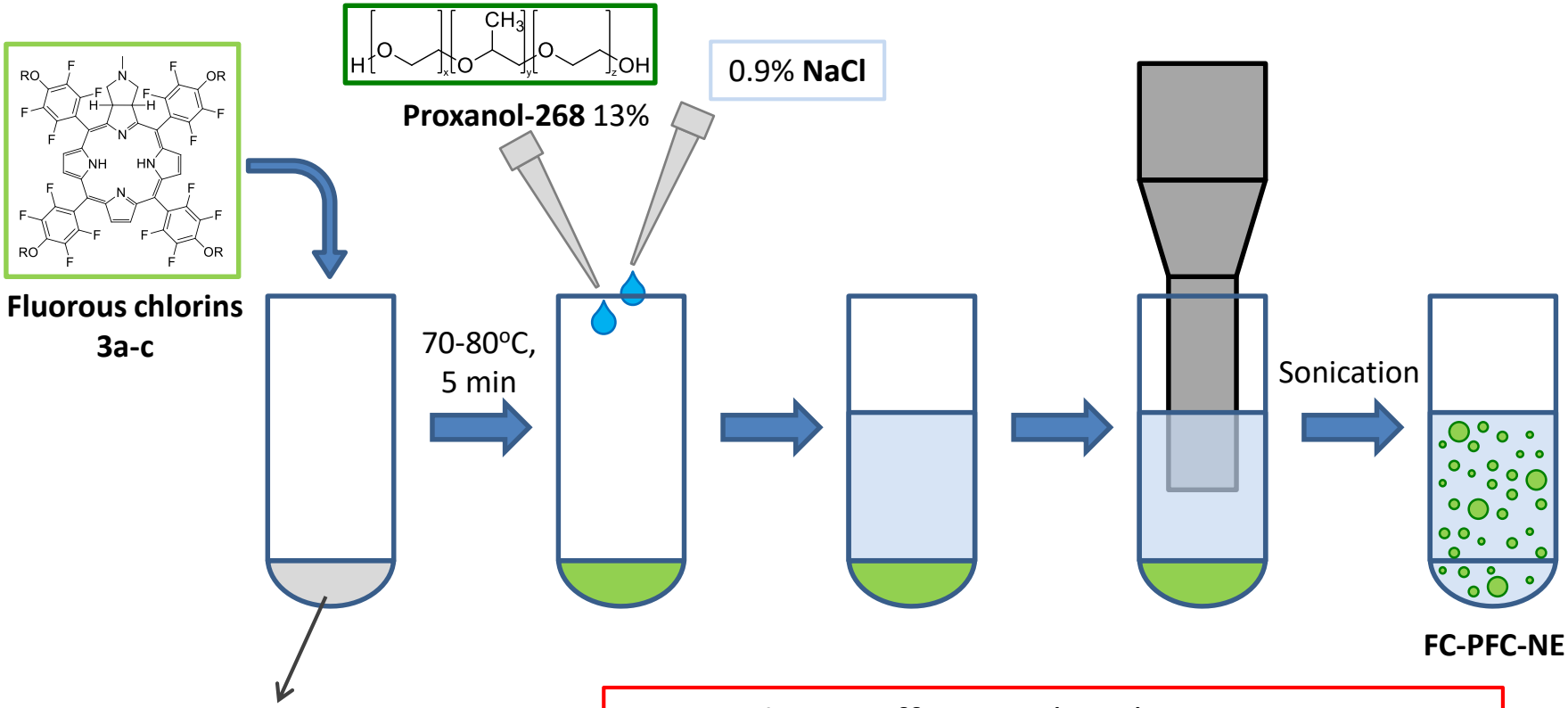
2a n = 2
2b n = 4
2c n = 6

3a n = 2
3b n = 4
3c n = 6



Chlorin-based PSs absorb red light (600-700 nm)
=
Better and deeper tissue penetration

Proximity of fluorous PS to molecular oxygen in PFC
=
Optimized conditions for singlet oxygen generation



Encapsulation efficiency (EE%) is on average 60%

Source: Nguyen, M.T.; Guseva, E.V.; Ataeva, A.N.; Sigan, A.L.; Shibaeva, A.V.; Dmitrieva, M.V.; Burtsev, I.D.; Volodina, Y.L.; Radchenko, A.S.; Egorov, A.E.; et al. Perfluorocarbon Nanoemulsions with Fluorous Chlorin-Type Photosensitizers for Antitumor Photodynamic Therapy in Hypoxia. Int. J. Mol. Sci. ***2023****, 24, 7995. https://doi.org/10.3390/ijms24097995*

Previous work:

Components	Functions
Fluorous chlorin 3b (FC-3b) *0.6507 mg*	Photosensitizer
PFD *0.867 g*	Oxygen carrier, PS solvent
PFMCP *0.433 g*	Oxygen carrier, PS solvent, droplet stabilizer
Proxanol-268 *0.2665 g*	Surfactant
0.9% NaCl solution *Up to 10 ml*	Dispersion medium, buffer solution

PFC/Proxanol-268 5:1

This work:

Components
Fluorous chlorin 3b *1.952 mg*
PFD *2.6 g*
PFMCP *1.3 g*
Proxanol-268 *0.2665 g*
Phosphate Buffer Saline (PBS) *Up to 10 ml*

PFC/Proxanol-268 15:1

Results and discussion

PFC/Proxanol-268 5:1

Translucent, slightly opalescent
Grayish green o/w nanoemulsion

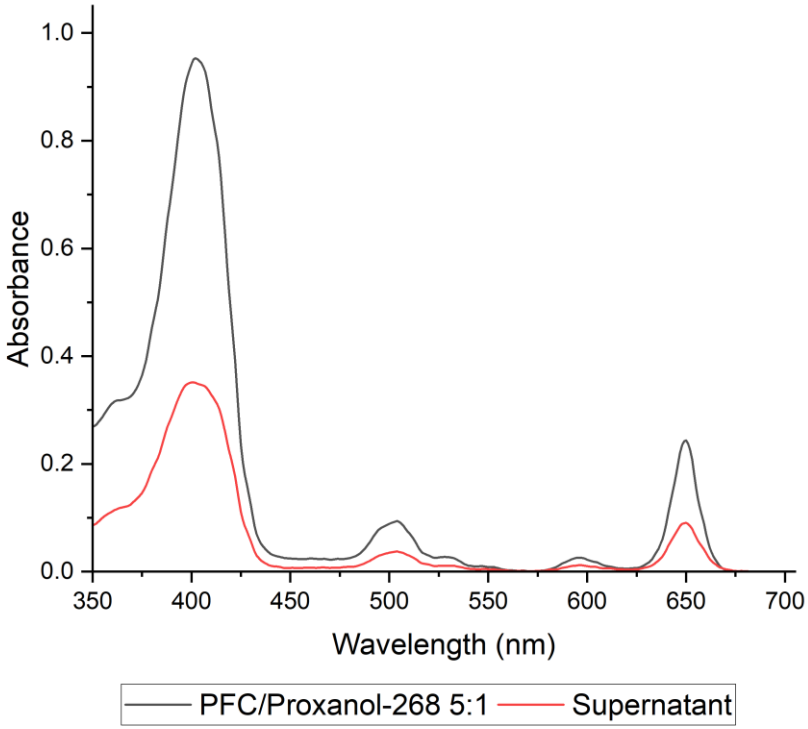
Sonication settings:

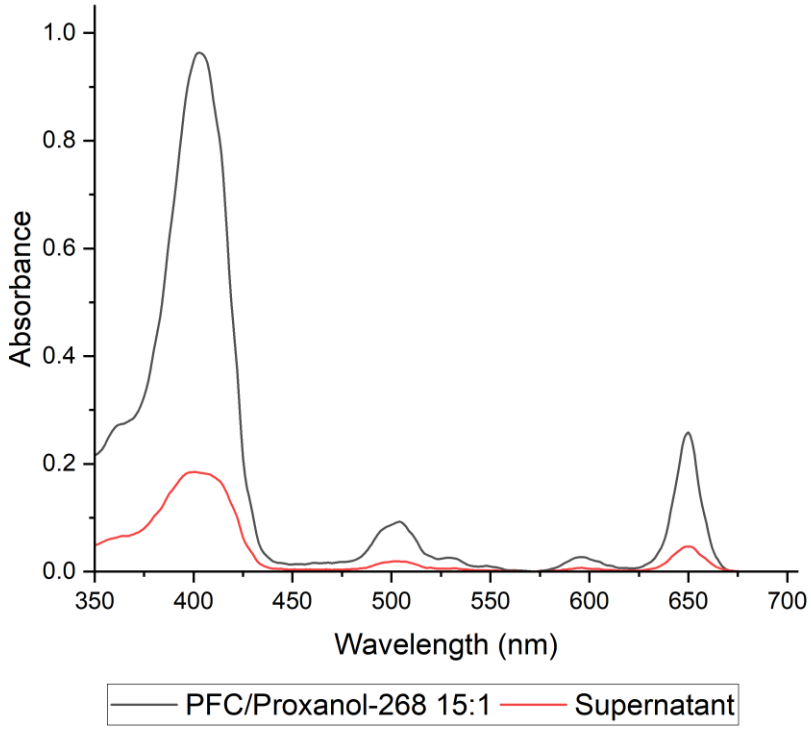
Mode: pulsed
Duty cycle: 50%
Power: 5 (50%)
Duration: 4x2 min
Rest: 1-2 min
Ice bath

PFC/Proxanol-268 15:1

Opaque
Green o/w nanoemulsion

UV-Vis spectra of FC-3b in PFC-NEs and their supernatant





Less absorbance in the supernatant = higher EE%

EE% = (AUC (emulsion) – AUC (supernatant))/AUC (emulsion) × 100%

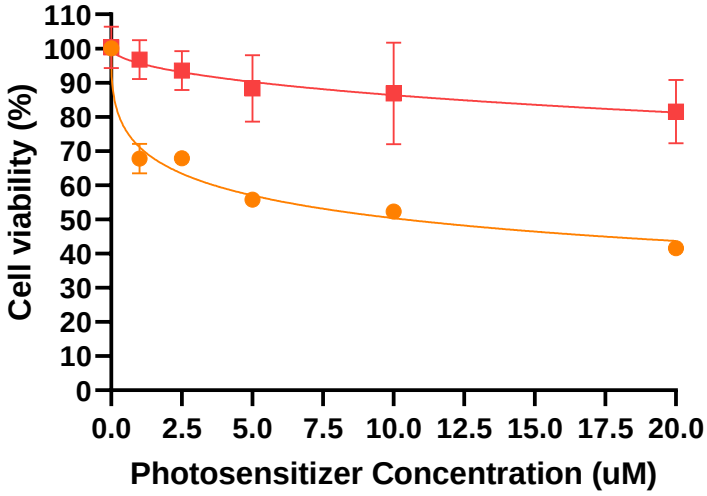
Characterization of PFC-NEs

Emulsion	Storage Temp., °C	z-average hydrodynamic diameter, nm			Polydispersity index (PDI)			EE%	
		Day 1	Day 7	Day 30	Day 1	Day 7	Day 30	Day 1	Day 30
PFC/Proxanol-268 5:1	+4	200.6 ± 6.1	208.7 ± 5.6	221.1 ± 1.4	0.096 ± 0.01	0.027 ± 0.02	0.060 ± 0.04	63.8	44.1
	-20		214.9 ± 4.8	192.9 ± 2.5		0.120 ± 0.03	0.089 ± 0.04		69.6
PFC/Proxanol-268 15:1	+4	214.5 ± 8.5	221.5 ± 4.9	236.7 ± 0.3	0.070 ± 0.02	0.068 ± 0.02	0.025 ± 0.02	81.9	70.5
	-20		217.4 ± 5.9	206.7 ± 0.7		0.083 ± 0.02	0.091 ± 0.02		83.2

- Resulting **PFC/Proxanol-268 15:1** formulation is a **nanoemulsion**;
- Changes in **droplet size** and **PDI** are **insignificant**;
- Both formulations are stable during storage;
- **Encapsulation efficiency** has **improved.**

Improved dark and photoinduced cytotoxicity profile

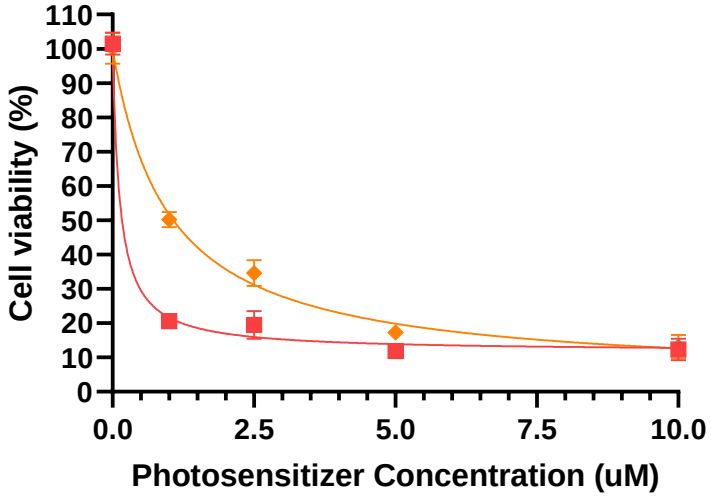
Dark cytotoxicity



↓ Decreased dark cytotoxicity

HCT116 cells, 48h incubation with PFC-NEs

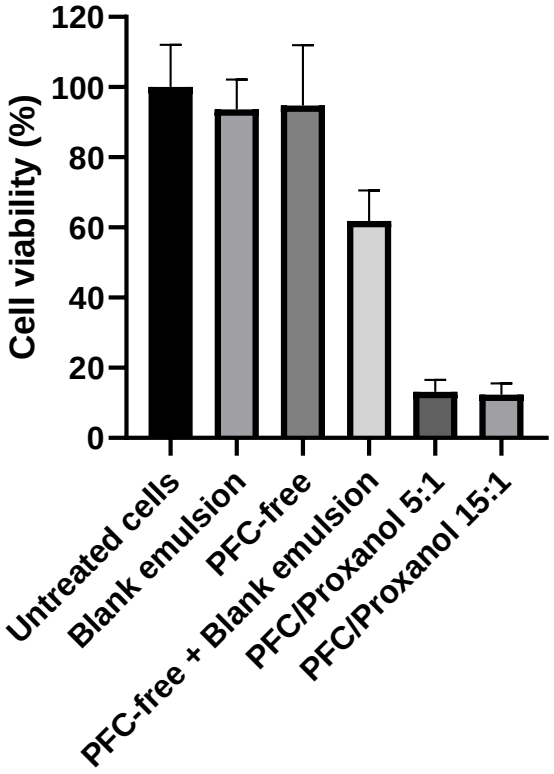
Photoinduced cytotoxicity



↑ Increased phototoxicity

HCT116 cells, 24h incubation + 24h after irradiation
Irradiation: 660 nm, 8.3 mW/cm^2, 45 J/cm^2

Control experiment



HCT116 cells, 24h incubation + 24h after irradiation
Irradiation: 660 nm, 8.3 mW/cm^2, 45 J/cm^2
FC-3b concentration: 10 µM

Treatment groups:

1) Blank emulsion (PFC/Proxanol-268 15:1 without FC-3b) – no cell death
2) PFC-free (PFC/Proxanol-268 15:1 without PFC) – no cell death
3) PFC-free + Blank emulsion – 40% cell death
4) PFC/Proxanol-268 5:1 – 85% cell death
5) PFC/Proxanol-268 15:1 – 85% cell death

Encapsulated FC-3b is the main **photodynamically active** component of PFC-NEs

Conclusions

Optimization of PFC-NE formulation resulted in:
- Higher encapsulation efficiency;
- Improved dark cytotoxicity profile;
- Enhanced photoinduced cytotoxicity.

Optimization process did not affect:
- Particle size and polydispersity index – resulting o/w emulsion remained nanoscale;
- Colloidal stability – optimized formulation is stable during storage.

Further research should and will be focused on:
- Optimizing PS concentration in PFC;
- Studying difference in physical and chemical properties of encapsulated and non-encapsulated PS in PFC-NEs;
- Effect of surfactant on photochemistry of PFC-NEs.

Acknowledgments

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