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The Effects of Gold Nano Sensitizer Photodynamic Therapy on the Proliferation, Invasion, and Migration of Lung Cancer Stem Cells

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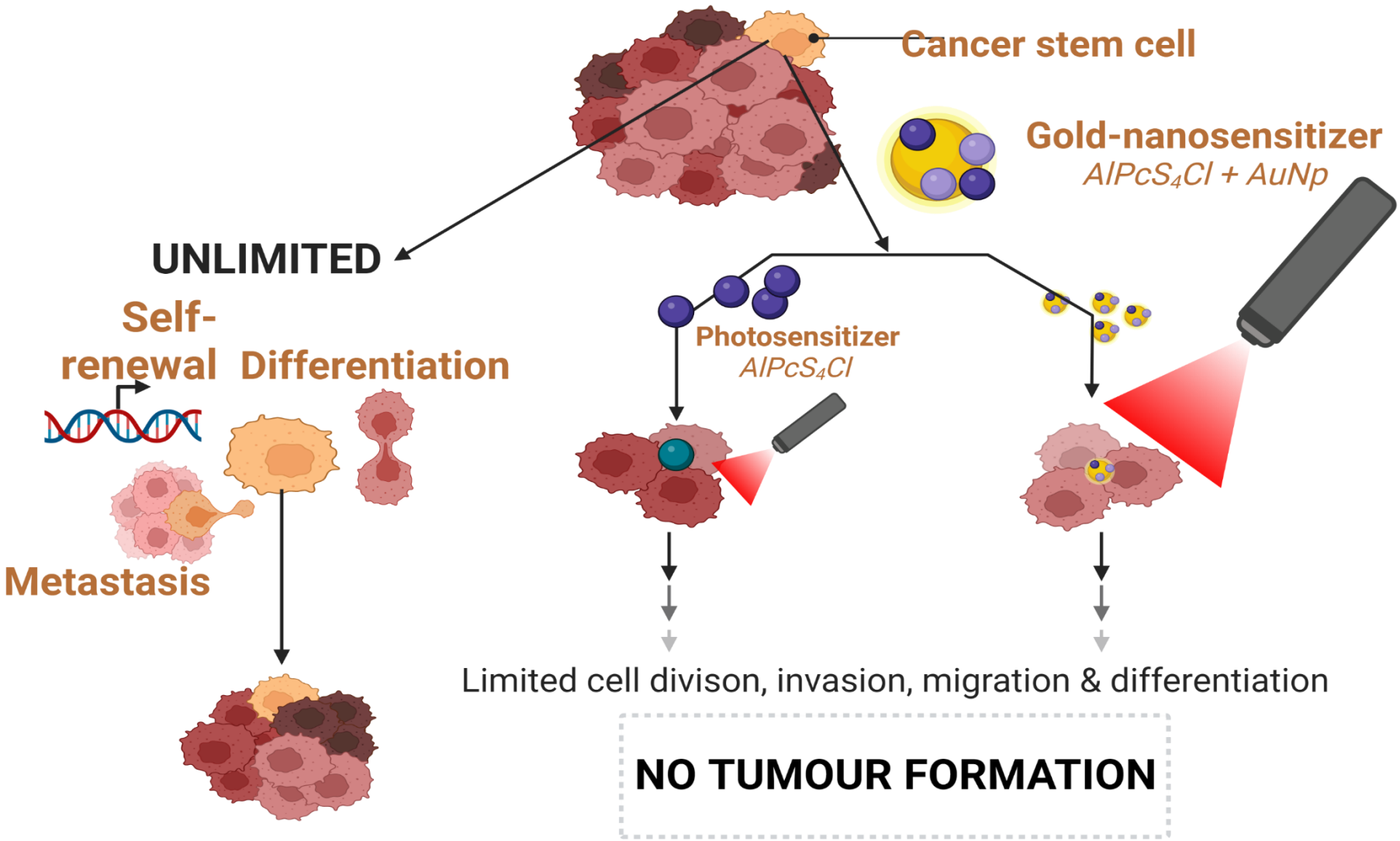

ABSTRACT

Lung cancer relapse and post-treatment dissemination suggest the presence of drug resistant populations of cells called cancer stem cells (CSCs). Cancer metastases and the risk of secondary tumors are the most frequent causes of mortality in many cases. One important feature of lung cancer prognosis is metastases and the invasive ability of the cells, which is driven by CSCs. Considering CSC proliferation and migration associated with metastases, therapeutic strategies targeting these CSCs are considered to improve long-term clinical outcome. A minimally invasive, clinically approved cancer treatment, Photodynamic therapy (PDT), along with the use of a nano drug carrier was used in this study. PDT is based on the principle of light stimulation of a photosensitizing drug that induces tumor cell death. Nano mediated PDT using gold nanoparticles have been seen to induce cell death in lung CSCs. In this study morphological examination and various physiological experiments including, migration, proliferation, cytotoxicity, population doubling time, and cell cycle analysis assay were conducted to determine whether PDT using a gold nano sensitizer prevents CSC migration and invasion. Results show that the use of nanoPDT using a AlPcS4Cl and AuNPs conjugate can inhibit CSC migration and invasion, induce cell cycle arrest, and decrease CSC proliferative abilities. The use of a drug nano carrier in the form of AuNPs can improve the effectivity of PDT cancer treatment and specifically facilitate the inhibition of metastasis seen in lung cancer caused by CSCs, which can clinically relate to an improved prognosis.

Keywords: **G**old nanoparticles; **L**ung cancer stem cells; **M**etastasis; **P**hotosensitizer; **P**hotodynamic therapy

INTRODUCTION
Lung Cancer Stem Cells

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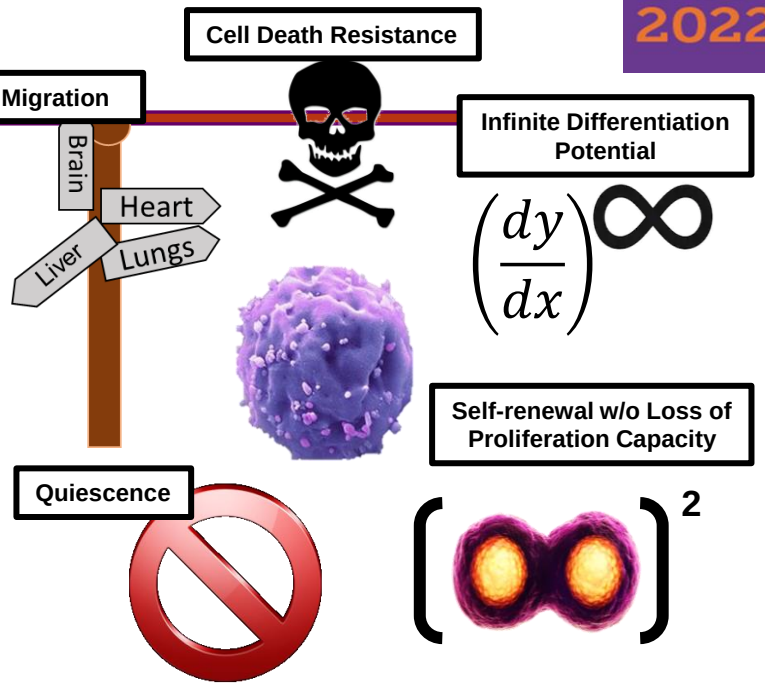
- Tumour cells are **heterogeneous** comprising of rare tumour initiating / **cancer stem cells (CSCs)** and abundant non-stem like cells.
- CSCs share **characteristics** with **normal stem cells** of self-renewal, proliferate, and express **typical stem cell markers** related to drug efflux, recovery, and motility, they are also resistant to cell death.
- CSCs are responsible for tumour growth and **cancer relapse** through metastasis and inhibition of drug-induced cell death, decreasing the effect of traditional cancer therapy and photodynamic therapy (PDT).



Marker	Name	Cellular Function	Role in Cancer	Lung Cancer Marker	Stem Cell/ Other
CD 44	Pgp-1	Hyaluronic acid receptor	Exerts control over cell growth, migration, and tumour progression	NSCLC	Haematopoietic breast
CD 133	Promonin-1	Intracellular accumulation of exogenous compounds, cell metabolism Neurotrophic receptor RET, tyrosine kinase expression	Maintaining stem cell-like properties	NSCLC	Brain, colon, pancreas
CD 56	Neural cell adhesion molecule (NCAM)	**Homophilic binding glycoprotein, cell-cell adhesion or cell-matrix adhesion during embryonic development**	Reduced anti-tumour response	SC/ NSCLC	**Neural, haematopoietic,**

Ebben, J. D., Treisman, D. M., Zorniak, M., Kutty, R. G., Clark, P. A., & Kuo, J. S. (2010). The cancer stem cell paradigm: a new understanding of tumor development and treatment. Expert opinion on therapeutic targets, 14(6), 621–632. https://doi.org/10.1517/14712598.2010.485186
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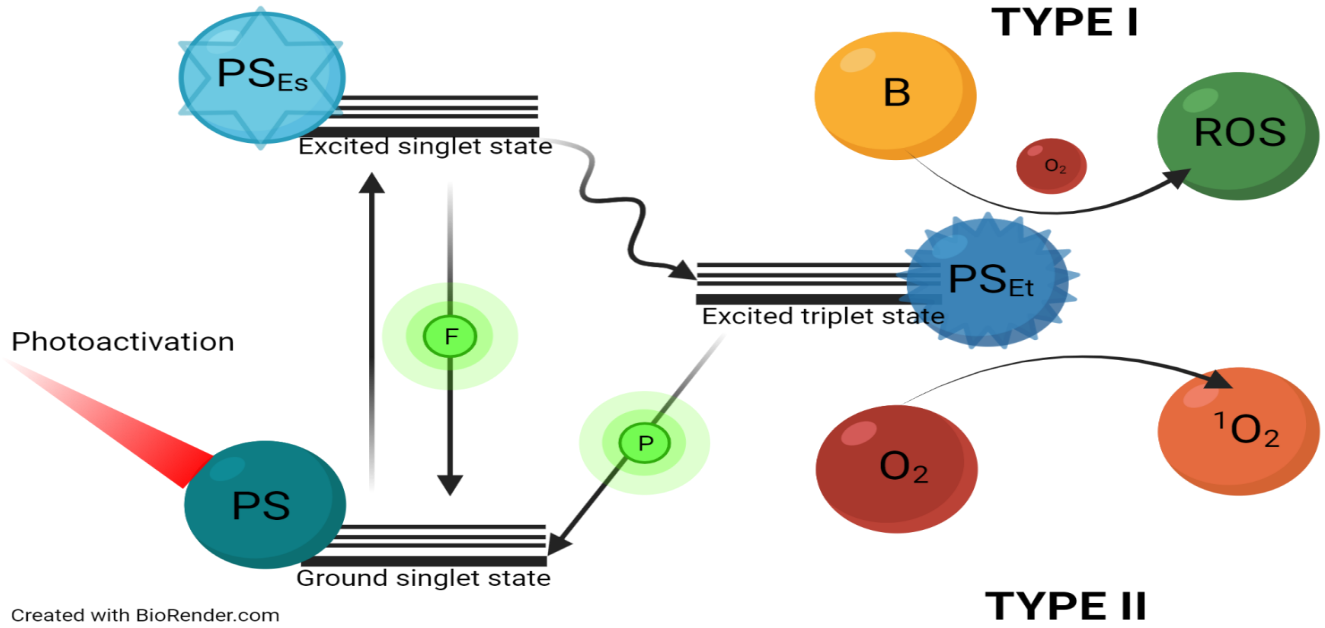
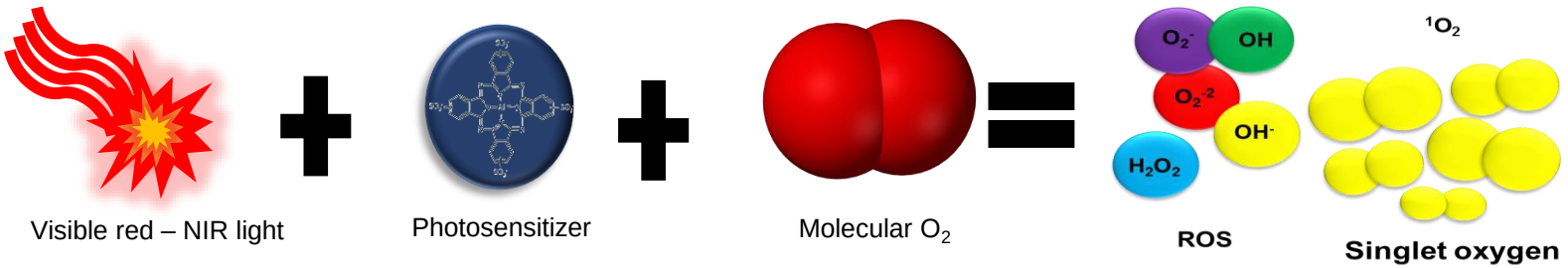
Crous A, Abrahamse H. Effective Gold Nanoparticle-Antibody-Mediated Drug Delivery for Photodynamic Therapy of Lung Cancer Stem Cells. International Journal of Molecular Sciences. 2020; 21(11):3742. https://doi.org/10.3390/ijms21113742

Photodynamic Therapy (PDT)



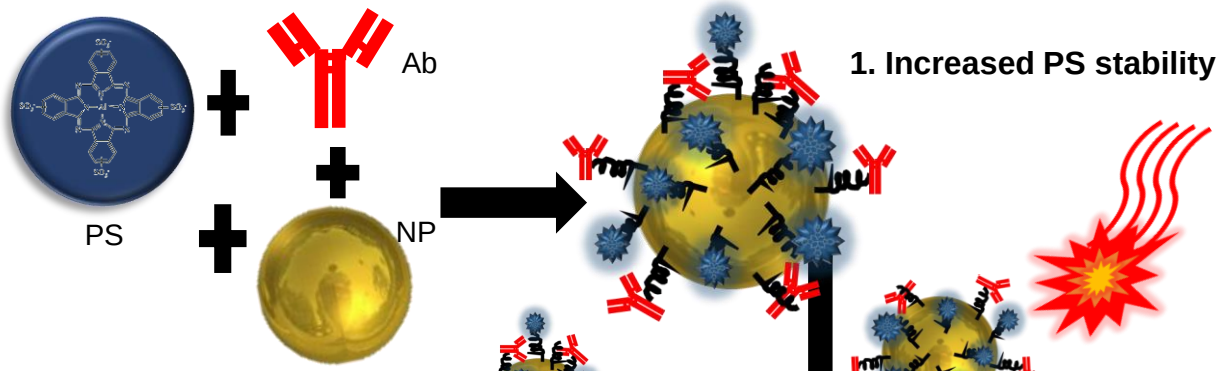


Castano, A. P., Demidova, T. N., & Hamblin, M. R. (2004). Mechanisms in photodynamic therapy: part one-photosensitizers, photochemistry and cellular localization. Photodiagnosis and photodynamic therapy, 1(4), 279–293. https://doi.org/10.1016/S1572-1000(05)00007-4

Crous, A., & Abrahamse, H. (2020). Effective Gold Nanoparticle-Antibody-Mediated Drug Delivery for Photodynamic Therapy of Lung Cancer Stem Cells. International journal of molecular sciences, 21(11), 3742. https://doi.org/10.3390/ijms21113742

Kruger CA, Abrahamse H. Utilisation of Targeted Nanoparticle Photosensitiser Drug Delivery Systems for the Enhancement of Photodynamic Therapy. Molecules. 2018 Oct 13;23(10):2628. doi: 10.3390/molecules23102628

Antibody Nano bioconjugate (NBC) – PDT



1. Increased PS stability and selectivity

2. Specific binding to surface antigens

3. Enhanced endothelial membrane transfer

4. Increased accumulation of PS inside the cell

5. Increased cytotoxic effect upon irradiation, PDT

METHODS

Cell culture
Normal lung epithelial cells (HBEC3-KT (ATCC® CRL-4051™))

Photodynamic Effects of the NBC 24 h post treatment

Morphology
ILM

Biochemical Analysis

Cytotoxicity
LDH
Cytotoxicity

Cell Viability
Trypan Blue/

Proliferation
ATP
Luminescence

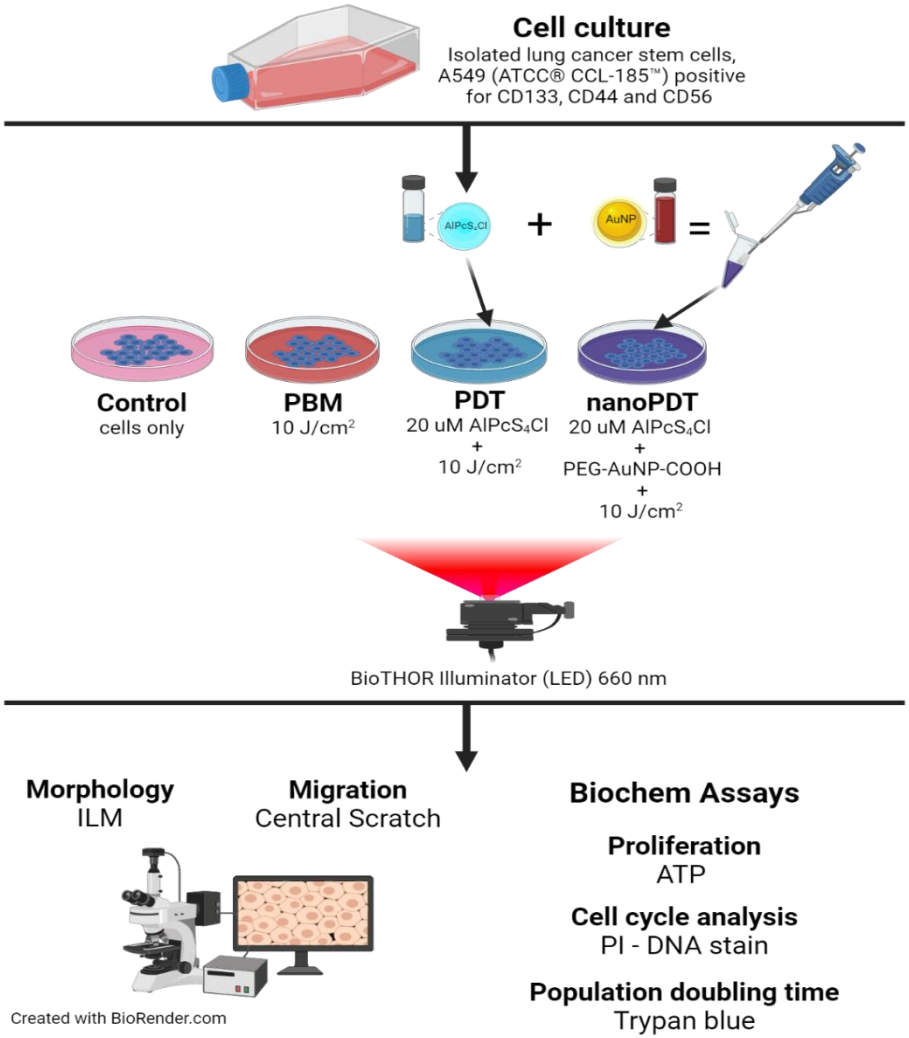


Table 1: LED well plate illuminator parameters.

λ nm	V (volts)	I (amps)	ILX (mW)	Power (mW/cm2)	Fluence (J/cm²)
659,4	22,913	3,02	33,130	117,17	10

RESULTS
Morphology

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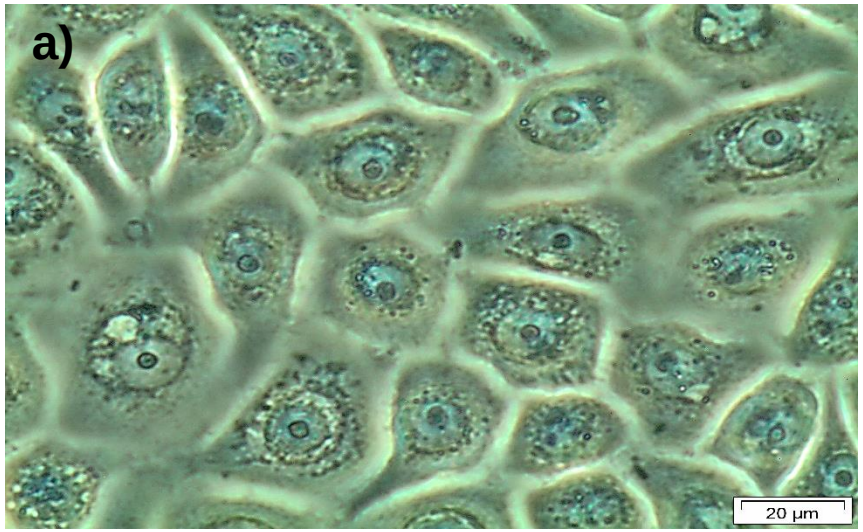
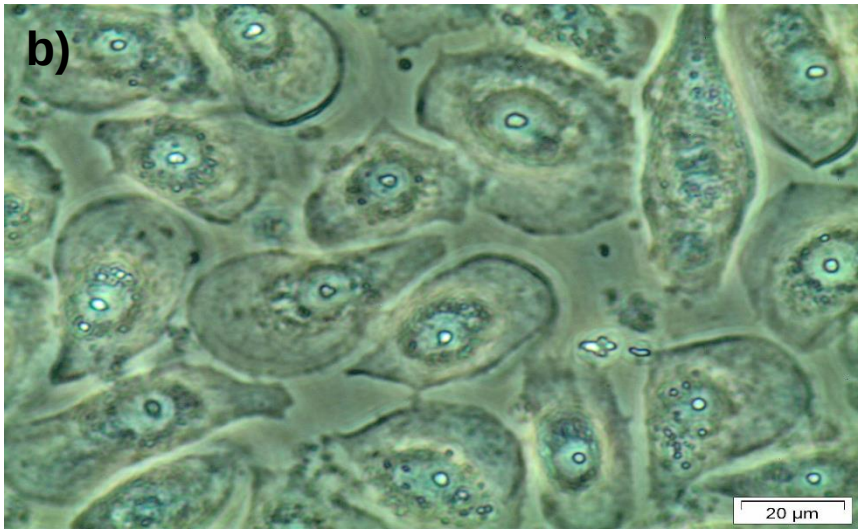
Normal lung epithelial cells (HBEC3-KT (ATCC® CRL-4051™))

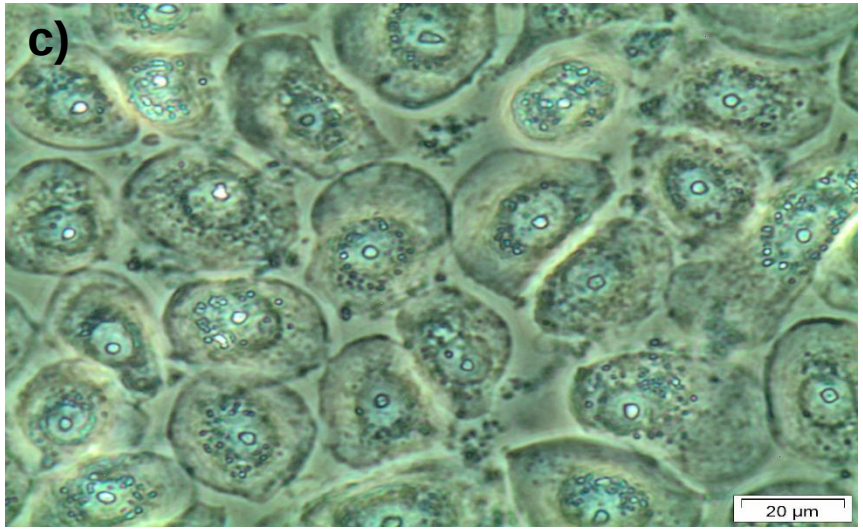
Control

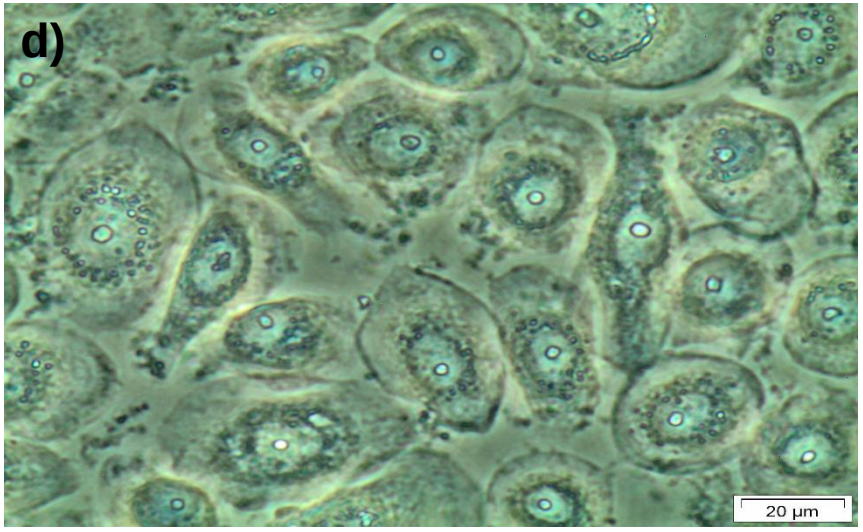




PDT

Nano-PDT



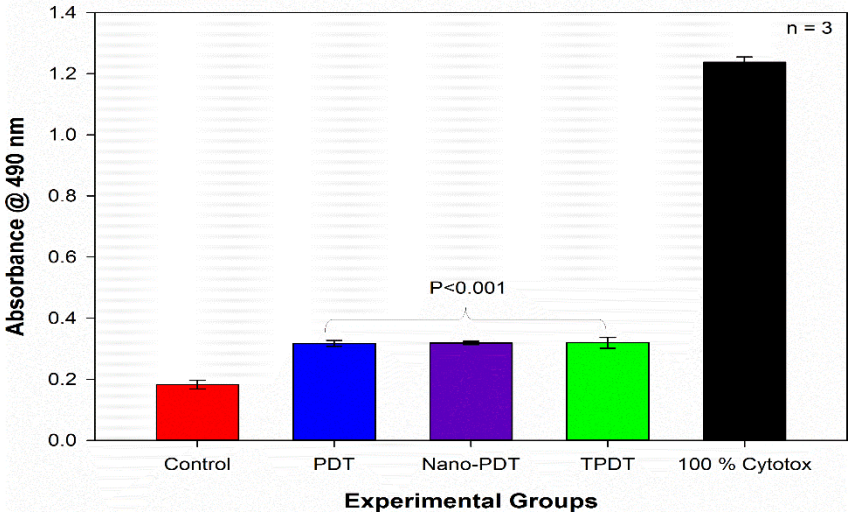


TPDT

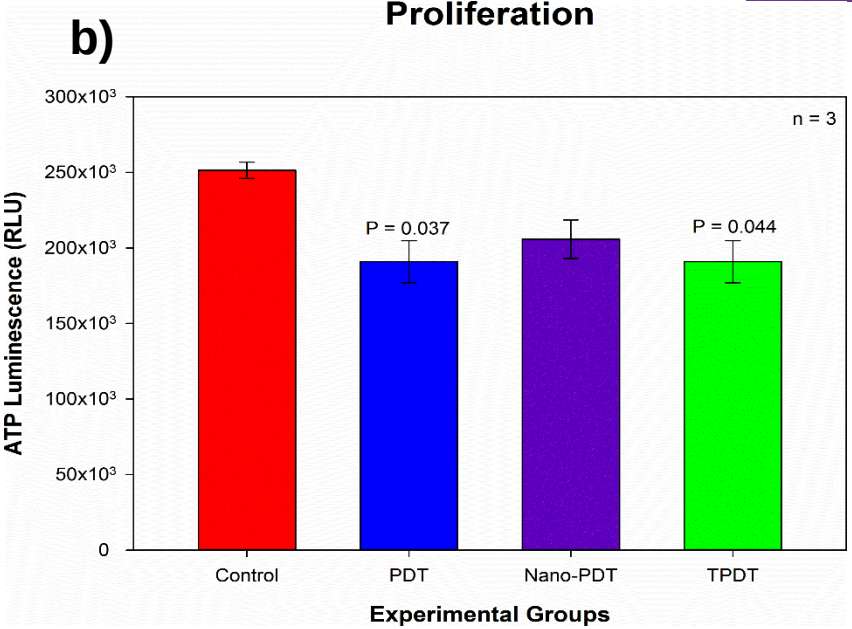
Biochemical assays

Normal lung epithelial cells (HBEC3-KT (ATCC® CRL-4051™))

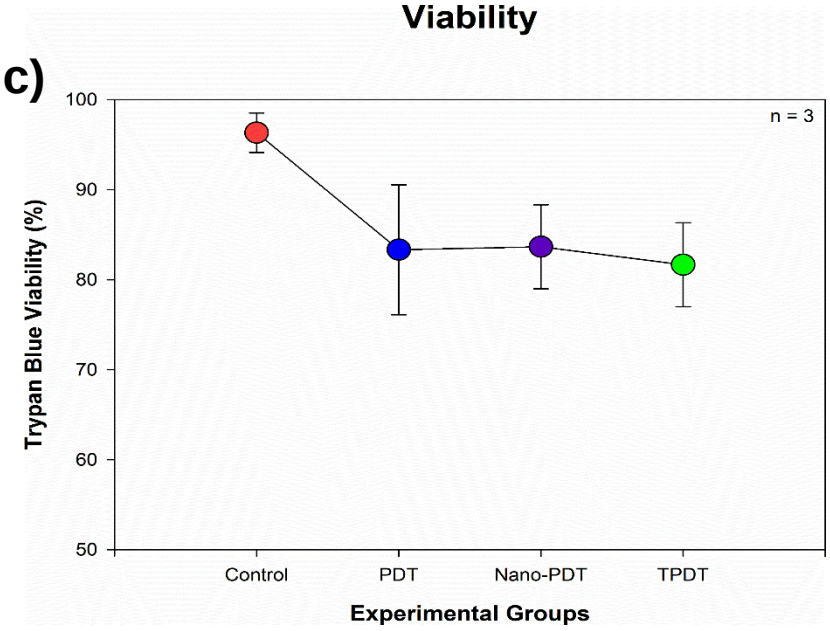
a)



b)



c)



Migration – Scratch Assay

A549 Lung CSCs (ATCC® CCL-185™)

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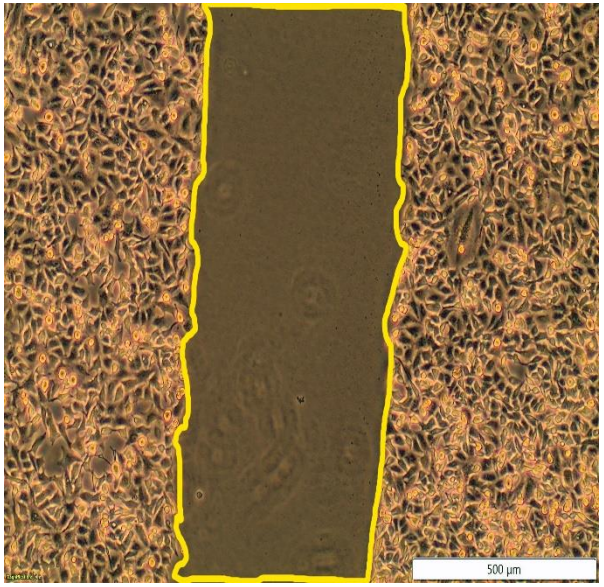
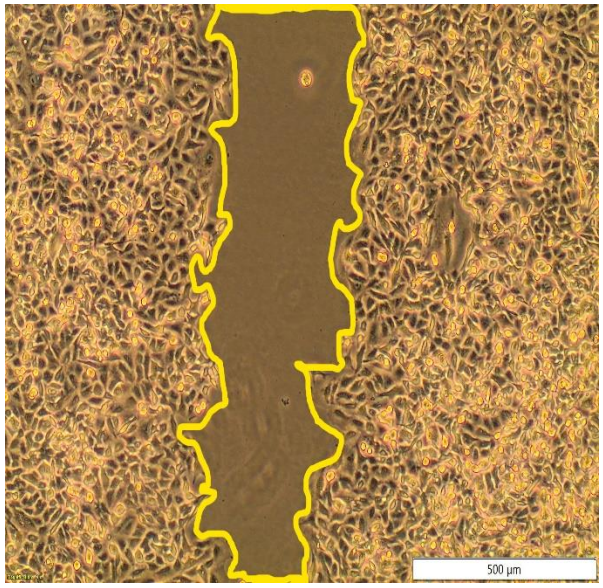
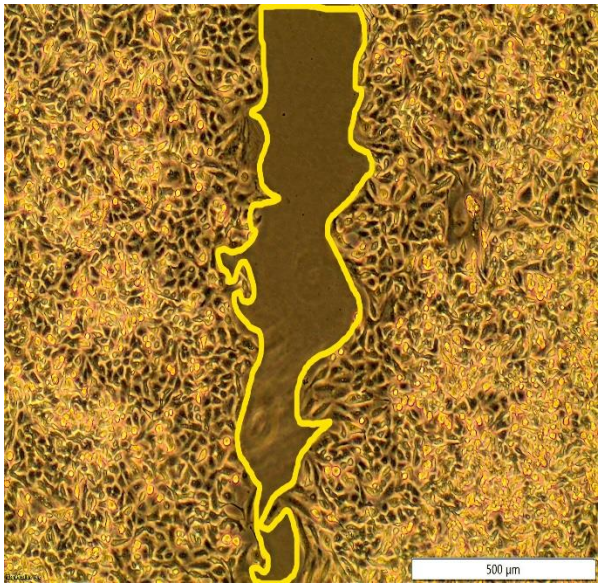
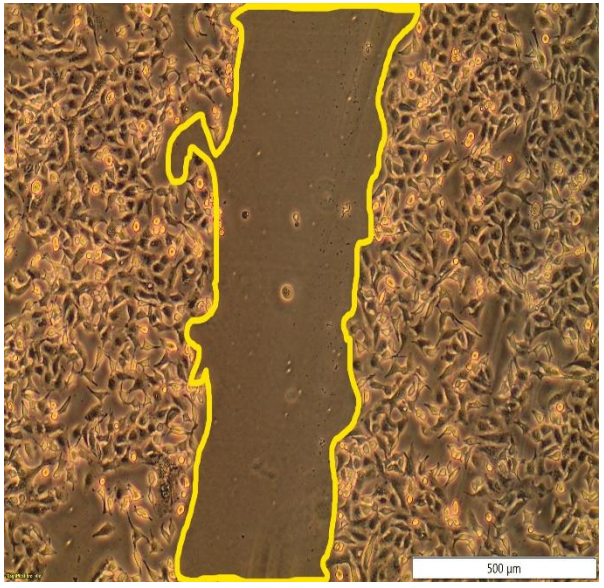
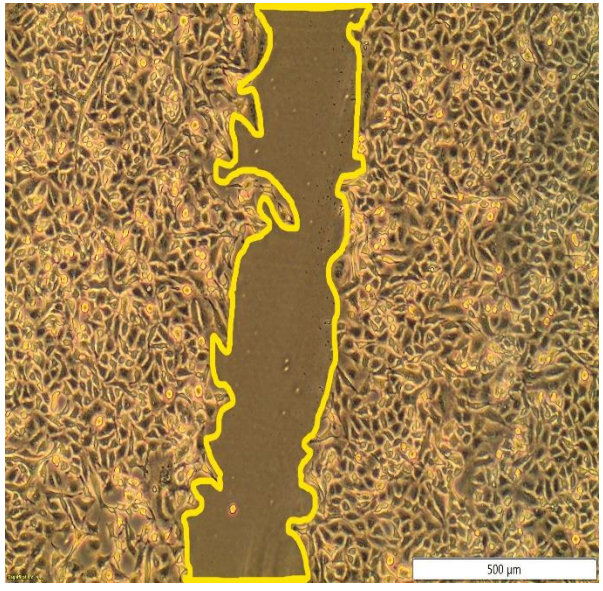
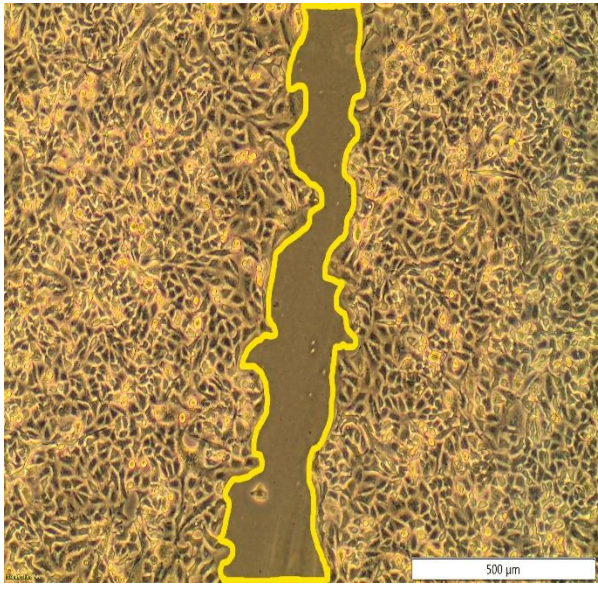
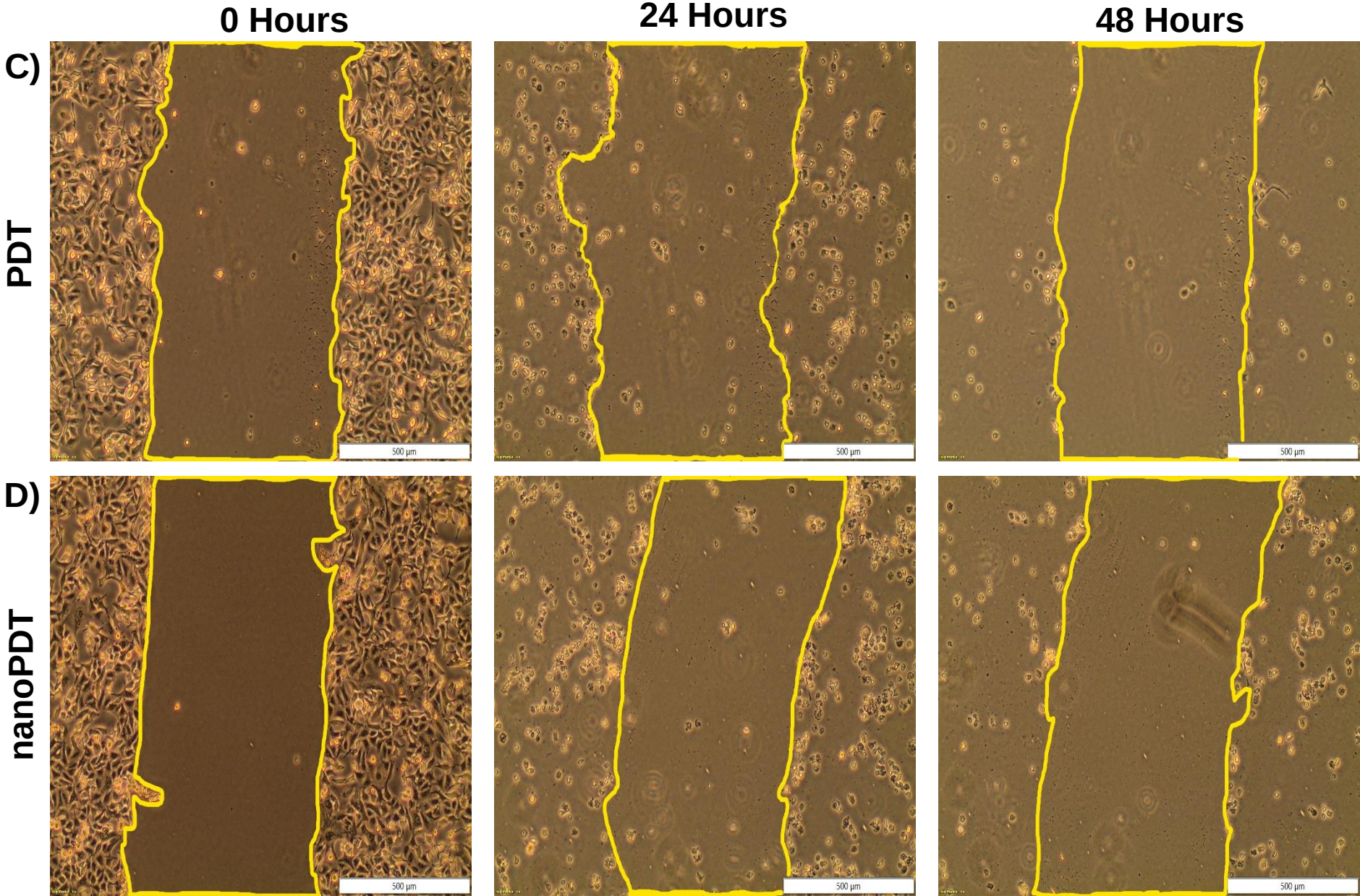
	0 Hours	24 Hours	48 Hours
A) Control			
B) PBM			

Migration

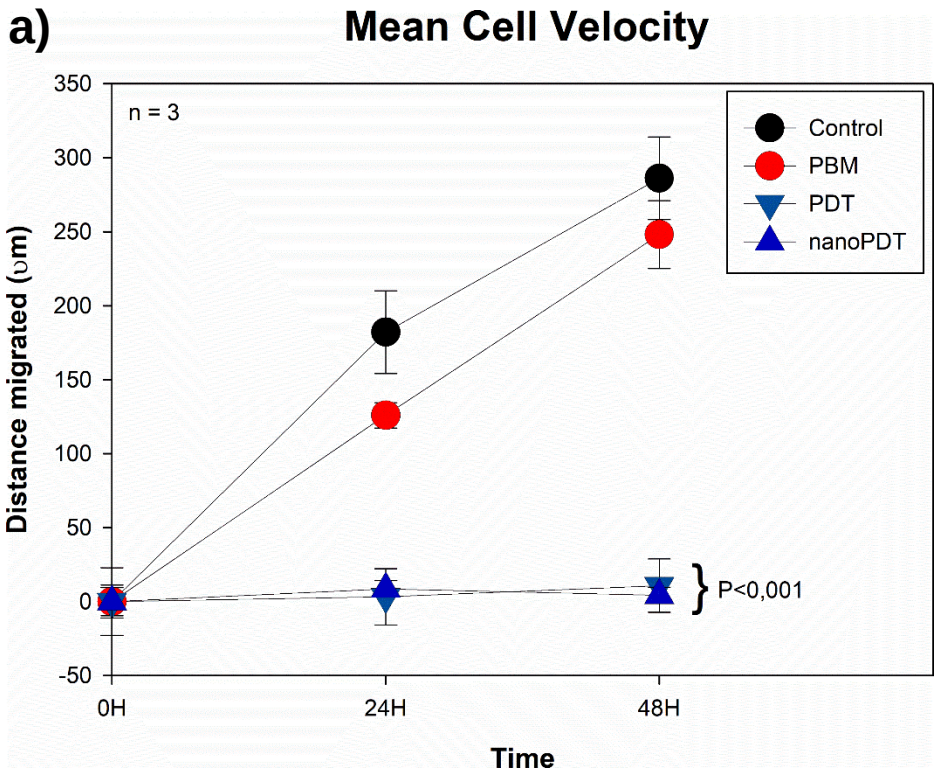
A549 Lung CSCs (ATCC® CCL-185™)

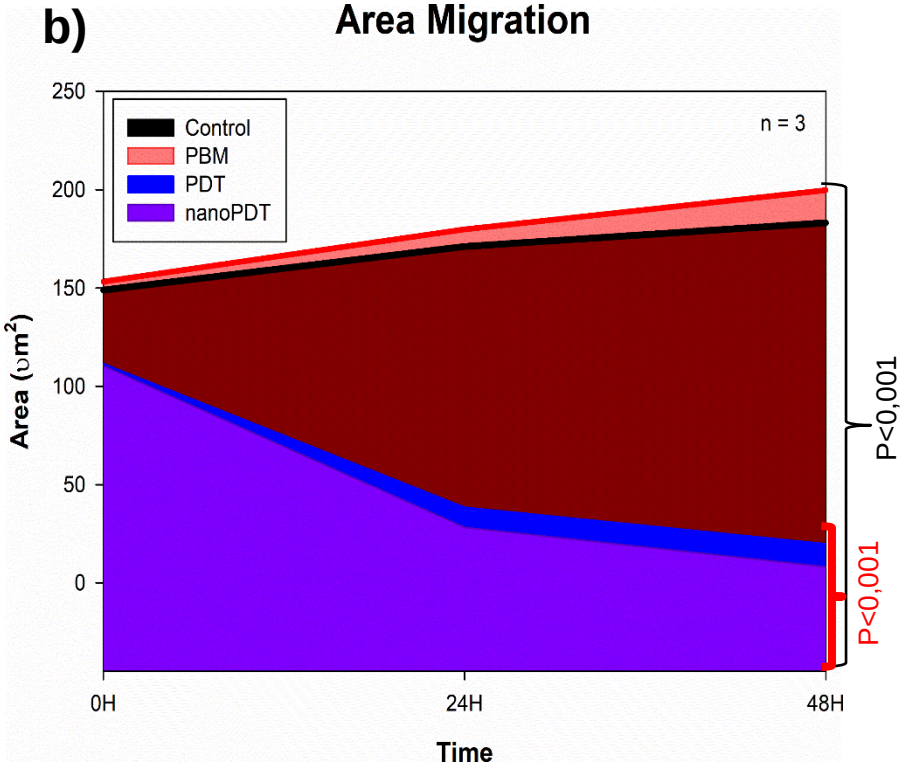
ECMC 2022



Migration - Analysis

A549 Lung CSCs (ATCC® CCL-185™)





	Mean cell velocity (µm/h)
Control	5,96 ±0,5
PBM	5,16 ±0,47
PDT	**0,22** ±0,37
nanoPDT	**0,08** ±0,11

Table: Mean cell velocity of lung CSCs post PDT treatment over 48 hours.

$$\bar{v} = \frac{\Delta x}{\Delta t}$$

Cell Cycle Analysis – PI

A549 Lung CSCs (ATCC® CCL-185™)

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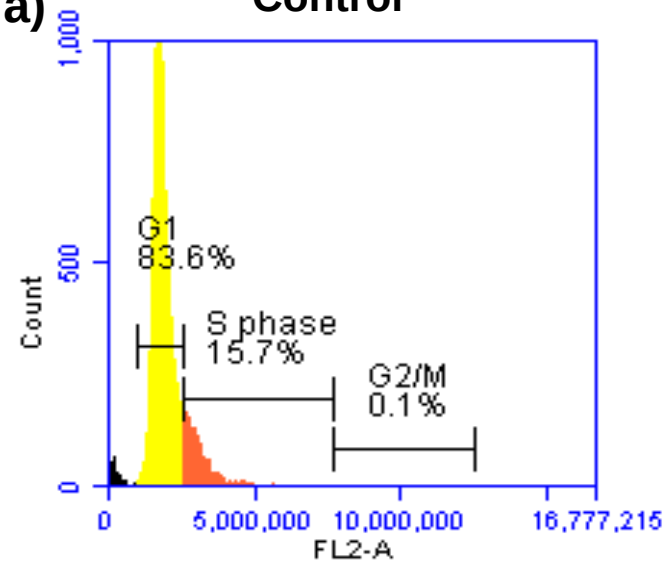
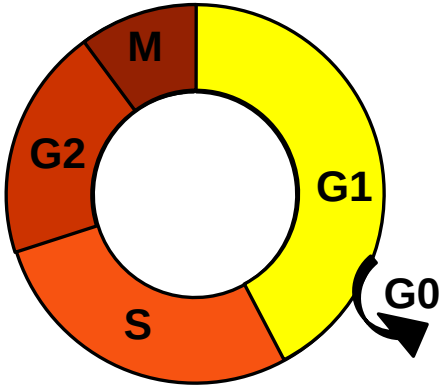
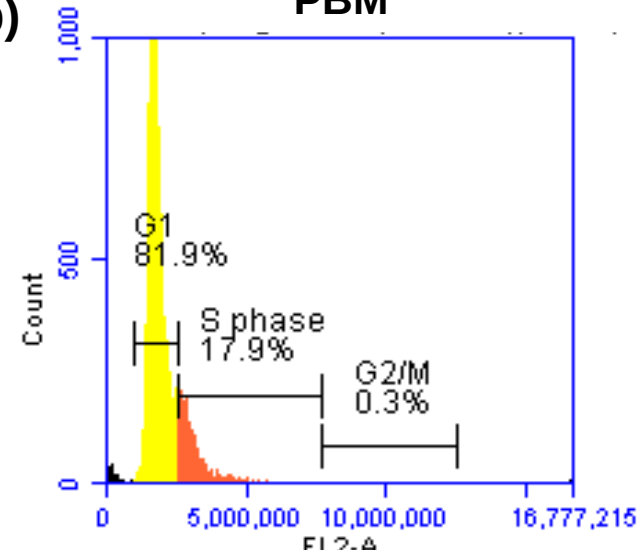
a) Control

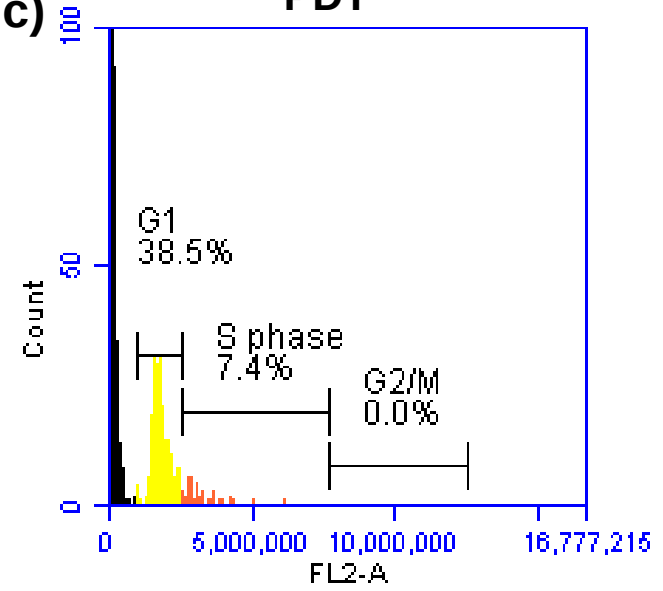




b) PBM

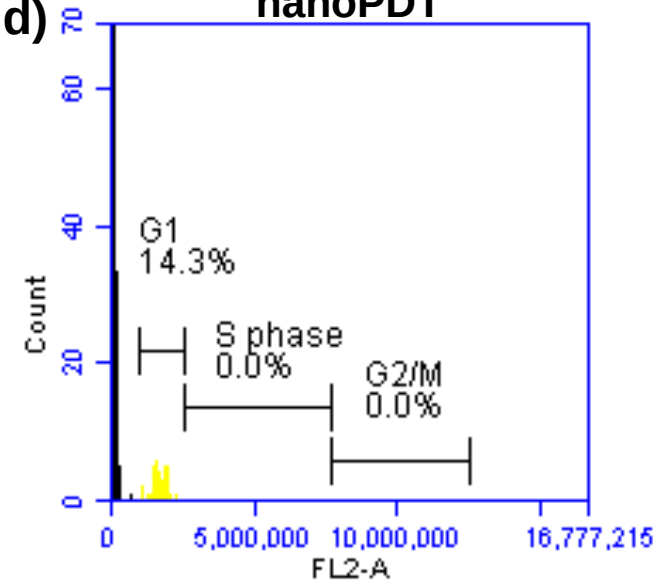


c) PDT



G1 – Growth, cells mature
S – DNA synthesis/copied
G2 – DNA checks
M – Mitosis, cell division
G0 – Cell cycle arrest

d) nanoPDT



Population Doubling Time

A549 Lung CSCs (ATCC® CCL-185™)

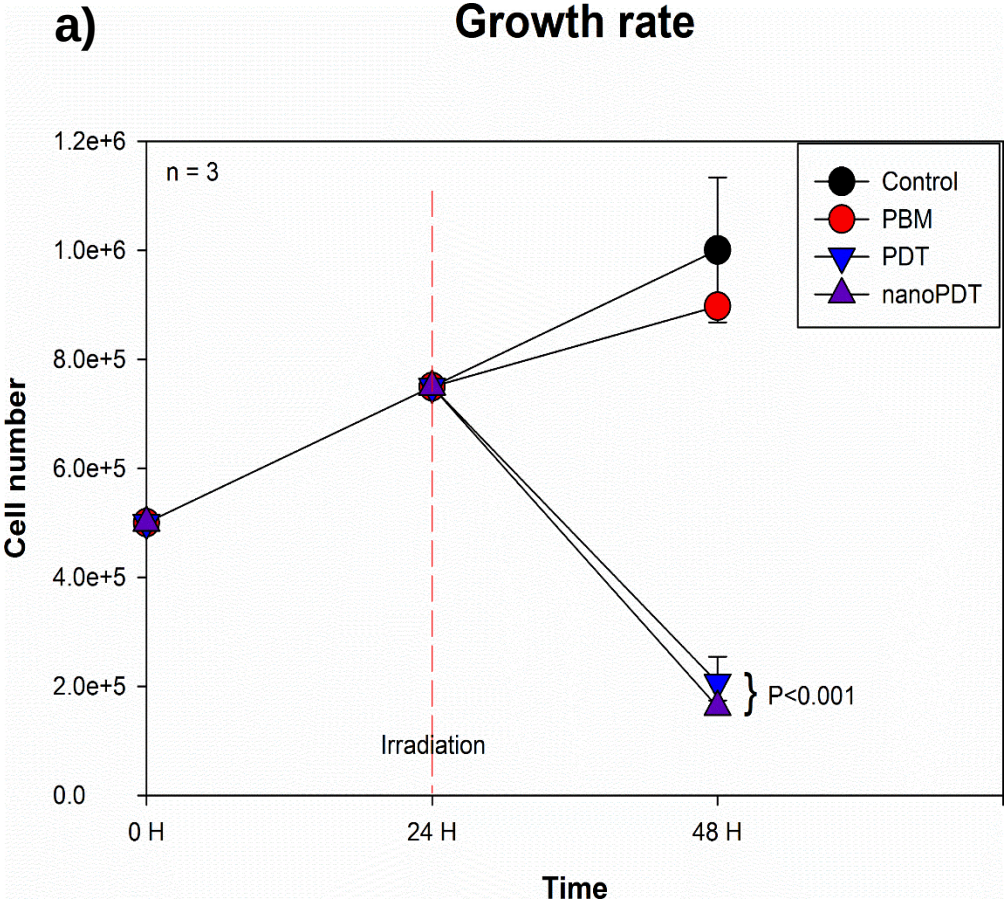


Table: Population doubling time of lung CSCs upon and after PDT treatment.

	Ave cell number @ 48 hours	Doubling Time (Hours)
Control	1^10^6	47.9 ± 2.82
PBM	8.97^10^5	56.89 ± 3.44
PDT	2.07^10^5	- 37.72 ± 3.08
nanoPDT	1.62^10^5	- 29.57 ± 3.54

$$gr = \frac{\ln\left(N(t)/N(0)\right)}{t}$$

$$dt = \frac{\ln(2)}{gr}$$

CONCLUSIONS

- Photodynamic treatment aims at **killing cancerous cells alone**, leaving normal healthy cells in tact. Results from this study shows that PDT on **normal lung** cells show **significant toxicity and decreased proliferation.** However these results are not as significant as PDT treatment of the cancerous cells to the point of eradication. Normal lung cells still indicated **good morphological features and a high viability**.
- Considering CSCs ability to cause cancer relapse and metastasise, cancer treatments needs to be effective at reducing these abilities. The effects of PDT and nanoPDT on CSC Proliferation & Migration showed **significant decreases** in cell **migration**, **velocity, population doubling time** and **cell cycle arrest**. With nanoPDT showing enhanced effects. Signifying a halt in CSC motility.
- A complimentary study of using an ECM transwell assay evaluating cell invasiveness will be repeated to give a clear indication of the photodynamic effects.
- Furthermore the **exact mechanism of cell death** induced by nanoPDT on the CSCs will still be evaluated. This will complete *in vitro* studies and give a comprehensive view on the **effectivity** of using **$AlPcS_4Cl$ nanoPDT** for the **treatment of lung cancer.**

AKNOWLEDGEMENTS