

Effect of nanostructured titanium dioxide on photoinduced cancer treatment

National Technical University of Athens

National and Kapodistrian University of Athens

OpenTox

Dr. Nefeli Lagopati (MSc, PhD)
Prof. Evangelia A. Pavlatou

OPENTOX EURO 2018, 9-11 OCTOBER 2018, ATHENS, GREECE

This research is co-financed by Greece and the European Union (European Social Fund - ESF) through the Operational Programme «Human Resources Development, Education and Lifelong Learning» in the context of the project «Reinforcement of Postdoctoral Researchers» (MIS-282102), implemented by the State Scholarship Foundation (S.F.S.F.).

1

«This research is implemented through IKY scholarships program and co-financed by the European Union (European Social Fund - ESF) and Greek national funds through the action entitled «Reinforcement of Postdoctoral Researchers», in the framework of the Operational Program «Human Resources Development Program, Education and Lifelong Learning» of the National Strategic Reference Framework (NSRF) 2014 – 2020».

OpenTox

Postdoc-Research Scholarships IKY

Operational Programme Human Resources Development Education and Lifelong Learning

European Union Co-financed by Greece and the European Union

NSRF 2014-2020

This research is co-financed by Greece and the European Union (European Social Fund - ESF) through the Operational Programme «Human Resources Development, Education and Lifelong Learning» in the context of the project «Reinforcement of Postdoctoral Researchers» (MIS-282102), implemented by the State Scholarship Foundation (S.F.S.F.).

2

Nanomedicine Cancer Research
Biology Cell Death Laboratory
Medicine Cell Toxicity Drug
Physics Delivery
Titanium Dioxide Systems
Richard Feynman Characterization
Nanoparticles Smart CELLS
Engineering Pharmaceuticals
Nanotechnology Biomaterials Therapy

This research is co-financed by Greece and the European Union (European Social Fund - ESF) through the Operational Programme «Human Resources Development, Education and Lifelong Learning» in the context of the project «Reinforcement of Postdoctoral Researchers» (MIS-282102), implemented by the State Scholarship Foundation (S.F.S.F.).


3

Introduction

This research is co-financed by Greece and the European Union (European Social Fund - ESF) through the Operational Programme «Human Resources Development, Education and Lifelong Learning» in the context of the project «Reinforcement of Postdoctoral Researchers» (MIS-282102), implemented by the State Scholarship Foundation (S.F.S.F.).

4

Titanium Dioxide



TiO₂

This research is co-financed by Greece and the European Union (European Social Fund - ESF) through the Operational Programme «Human Resources Development, Education and Lifelong Learning» in the context of the project «Reinforcement of Postdoctoral Researchers» (MIS-282102), implemented by the State Scholarship Foundation (S.F.S.F.).

5

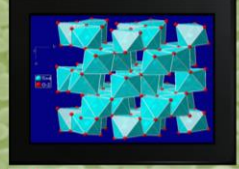
Titanium Dioxide (characteristics)

Color	Usually White
Form	Crystalline Solid
Chemical Formula	TiO ₂
Density	4.23 g/cm ³

This research is co-financed by Greece and the European Union (European Social Fund - ESF) through the Operational Programme «Human Resources Development, Education and Lifelong Learning» in the context of the project «Reinforcement of Postdoctoral Researchers» (MIS-282102), implemented by the State Scholarship Foundation (S.F.S.F.).

6

Titanium Dioxide Crystal Systems



Brookite
Orthorhombic Crystal System

Orthorhombic Crystal System
Bravais Lattice is rectangular parallelepiped with a parallelepiped base

This research is co-financed by Greece and the European Union (European Social Fund - ESF) through the Operational Programme «Human Resources Development, Education and Lifelong Learning» in the context of the project «Reinforcement of Postdoctoral Researchers» (MIS-282102), implemented by the State Scholarship Foundation (S.F.S.F.).

7

Titanium Dioxide (applications)

- dielectric mirrors
- precious stones
- colors, pigments, plastics, papers, inks
- food, cosmetics, sunscreens, medicines
- solar cells (Graetzel cell)
- aeronautics

This research is co-financed by Greece and the European Union (European Social Fund - ESF) through the Operational Programme «Human Resources Development, Education and Lifelong Learning» in the context of the project «Reinforcement of Postdoctoral Researchers» (MIS-282102), implemented by the State Scholarship Foundation (S.F.S.F.).

8

Titanium Dioxide (biomedical applications)

- artificial bone implants
- artificial limbs
- dentistry
- arterial stents, valves

This research is co-financed by Greece and the European Union (European Social Fund - ESF) through the Operational Programme «Human Resources Development, Education and Lifelong Learning» in the context of the project "Reinforcement of Productive Researchers" (MIS-5002322), implemented by the State Scholarship Foundation (SFS).

9

Insulators - Conductors – Semi-conductors

Semi-conductors

endogenous extraneous

TiO_2 n - type semi-conductor (energy gap: 3 – 3.2 eV) p - type n - type

10

Photocatalysis

«*photocatalysis is the acceleration of a photo-reaction in the presence of a catalyst*»
Mills and Hunte, 1997
N. Seprone and A.V. Emeline, 2002

«*process of ROS production by aquatic medium in the presence of a solid heterogenous catalyst and irradiation with light of specific and appropriate wavelength*»
K.T. Pickering 1997
R.J. Bull 2001

This research is co-financed by Greece and the European Union (European Social Fund - ESF) through the Operational Programme «Human Resources Development, Education and Lifelong Learning» in the context of the project "Reinforcement of Productive Researchers" (MIS-5002322), implemented by the State Scholarship Foundation (SFS).

11

Photocatalysis Process

$h\nu \geq 3.2 \text{ eV}$

O_2^-
 O_2
 $\cdot OH$
Pollutants / Cancer Cells
 H_2O
Degradation / Apoptosis

12

Characteristics of an ideal photocatalyst

- ☉ photo-active
- ☉ able to be used in a wide range of electromagnetic spectrum (UV and visible light)
- ☉ photo-stable
- ☉ low cost
- ☉ non-toxic

This research is co-financed by Greece and the European Union (European Social Fund - ESF) through the Operational Programme «Human Resources Development, Education and Lifelong Learning» in the context of the project "Reinforcement of Productive Researchers" (MIS-5002322), implemented by the State Scholarship Foundation (SFS).

13

Titanium Dioxide Photocatalytic Applications

Radiation Radiation Radiation

pathogenic micro-organisms bacteria odors fungi cancer

TiO_2
photocatalysis

air and water cleaning self-cleaning odor control sterilization anticancer effect

This research is co-financed by Greece and the European Union (European Social Fund - ESF) through the Operational Programme «Human Resources Development, Education and Lifelong Learning» in the context of the project "Reinforcement of Productive Researchers" (MIS-5002322), implemented by the State Scholarship Foundation (SFS).

14

Cancer Statistics

American Cancer Society 2011

MEN		WOMEN	
(% incidence)	(% mortality)	(% incidence)	(% mortality)
Prostate Ca (33%)	Lung Ca (31%)	Breast Ca (32%)	Lung Ca (27%)
Lung Ca (13%)	Bladder Ca (10%)	Lung Ca (12%)	Breast Ca (15%)
Colorectum Ca (10%)	Colorectum Ca (10%)	Colorectum Ca (11%)	Colorectum Ca (10%)
Bladder Ca (7%)	Pancreas Ca (5%)	Endometrial Ca (6%)	Ovarian Ca (6%)
Skin Melanoma	Leukaemia (4%)	Non - Hodgkin	Pancreas Ca

This research is co-financed by Greece and the European Union (European Social Fund - ESF) through the Operational Programme «Human Resources Development, Education and Lifelong Learning» in the context of the project "Reinforcement of Productive Researchers" (MIS-5002322), implemented by the State Scholarship Foundation (SFS).

15

Cancer Statistics

Men all ages Women all ages

This research is co-financed by Greece and the European Union (European Social Fund - ESF) through the Operational Programme «Human Resources Development, Education and Lifelong Learning» in the context of the project "Reinforcement of Productive Researchers" (MIS-5002322), implemented by the State Scholarship Foundation (SFS).

16

Conventional Treatment

- control of the proliferation of cancer cells
- induce apoptotic cell death

Warning!!!
The aim is the increase of the apoptotic effect of tumor cells and/or decrease of resistance of cancer cells to treatment.

17

AIM

18

Aim of this study

Development of Ag-doped TiO₂ nanoparticles with the potential to photo-induce anticancer effect via the mechanism of oxidative stress upon irradiation with visible light.

19

Schematic Representation of the study

Step 1

TiO₂ Synthesis – Chemical Doping with Ag – Characterization

20

Schematic Representation of this study

Step 2

Ag-doped TiO₂ photocatalytic effect on breast cancer epithelial cells

21

Schematic Representation of the study

Step 3

Biological effect of Ag-doped TiO₂ Cytotoxicity tests / Apoptosis tests

22

Methods & Results - Part A

23

TiO₂ Preparation

Ag-doped TiO₂ preparation

- heating (100°C) for 12 h
- annealing (400°C) for 6 h

24

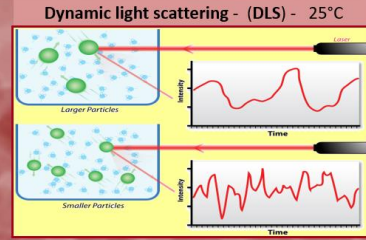
The need of doping!

- Doping with metal ions improves TiO₂ photocatalytic activity
- Reduction of electron-hole recombination
- More effective separation and stronger photocatalytic reactions
- Silver up-regulates TiO₂ biological activity
- Antibacterial properties
- Photo-excitement in visible light (also in UV)

This research is co-financed by Greece and the European Union (European Social Fund - ESF) through the Operational Programme «Human Resources Development, Education and Lifelong Learning» in the context of the project "Reinforcement of Postdoctoral Researchers" (MIS-5002322), implemented by the State Scholarships Foundation (SFS).

25

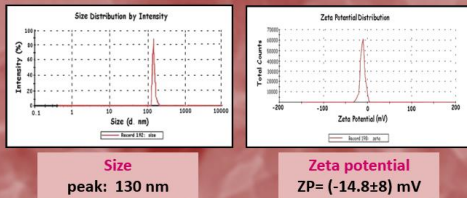
Characterization of Ag-doped TiO₂ (size estimation)



This research is co-financed by Greece and the European Union (European Social Fund - ESF) through the Operational Programme «Human Resources Development, Education and Lifelong Learning» in the context of the project "Reinforcement of Postdoctoral Researchers" (MIS-5002322), implemented by the State Scholarships Foundation (SFS).

26

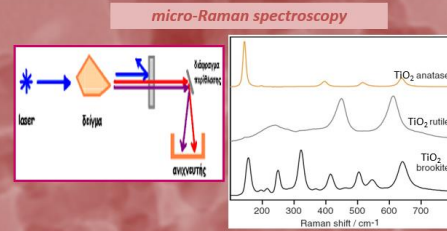
Characterization of Ag-doped TiO₂ (size estimation – zeta potential)



This research is co-financed by Greece and the European Union (European Social Fund - ESF) through the Operational Programme «Human Resources Development, Education and Lifelong Learning» in the context of the project "Reinforcement of Postdoctoral Researchers" (MIS-5002322), implemented by the State Scholarships Foundation (SFS).

27

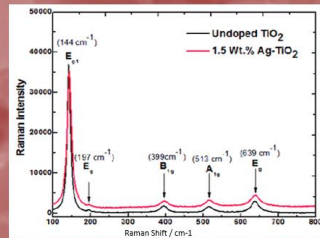
Characterization of Ag-doped TiO₂ (crystal phase estimation - Raman)



This research is co-financed by Greece and the European Union (European Social Fund - ESF) through the Operational Programme «Human Resources Development, Education and Lifelong Learning» in the context of the project "Reinforcement of Postdoctoral Researchers" (MIS-5002322), implemented by the State Scholarships Foundation (SFS).

28

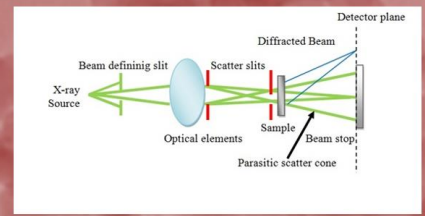
Characterization of Ag-doped TiO₂ (crystal phase estimation - Raman)



This research is co-financed by Greece and the European Union (European Social Fund - ESF) through the Operational Programme «Human Resources Development, Education and Lifelong Learning» in the context of the project "Reinforcement of Postdoctoral Researchers" (MIS-5002322), implemented by the State Scholarships Foundation (SFS).

29

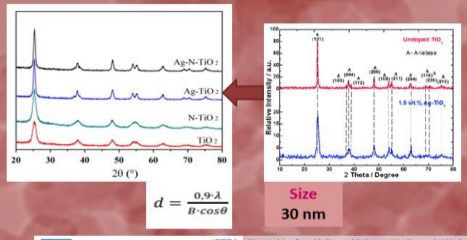
Characterization of Ag-doped TiO₂ (molecular structure estimation - XRD)



This research is co-financed by Greece and the European Union (European Social Fund - ESF) through the Operational Programme «Human Resources Development, Education and Lifelong Learning» in the context of the project "Reinforcement of Postdoctoral Researchers" (MIS-5002322), implemented by the State Scholarships Foundation (SFS).

30

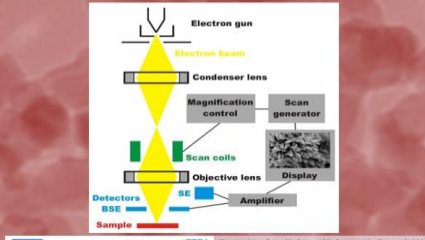
Characterization of Ag-doped TiO₂ (molecular structure estimation - XRD)



This research is co-financed by Greece and the European Union (European Social Fund - ESF) through the Operational Programme «Human Resources Development, Education and Lifelong Learning» in the context of the project "Reinforcement of Postdoctoral Researchers" (MIS-5002322), implemented by the State Scholarships Foundation (SFS).

31

Characterization of Ag-doped TiO₂ SEM (Scanning electron microscopy)



This research is co-financed by Greece and the European Union (European Social Fund - ESF) through the Operational Programme «Human Resources Development, Education and Lifelong Learning» in the context of the project "Reinforcement of Postdoctoral Researchers" (MIS-5002322), implemented by the State Scholarships Foundation (SFS).

32

Characterization of Ag-doped TiO₂

SEM

This research is co-financed by Greece and the European Union (European Social Fund - ESF) through the Operational Programme «Human Resources Development, Education and Lifelong Learning» in the context of the project "Reinforcement of Postgraduate Education" (MIS-1050202), implemented by the State Scholarship Foundation (S.F.).

33

Methods & Results - Part B

This research is co-financed by Greece and the European Union (European Social Fund - ESF) through the Operational Programme «Human Resources Development, Education and Lifelong Learning» in the context of the project "Reinforcement of Postgraduate Education" (MIS-1050202), implemented by the State Scholarship Foundation (S.F.).

34

Cell Cultures

Breast Cancer Epithelial Cells

highly malignant MDA-MB-468

MCF-7 non-metastatic

This research is co-financed by Greece and the European Union (European Social Fund - ESF) through the Operational Programme «Human Resources Development, Education and Lifelong Learning» in the context of the project "Reinforcement of Postgraduate Education" (MIS-1050202), implemented by the State Scholarship Foundation (S.F.).

35

Biological Effect

Visible or UV light

This research is co-financed by Greece and the European Union (European Social Fund - ESF) through the Operational Programme «Human Resources Development, Education and Lifelong Learning» in the context of the project "Reinforcement of Postgraduate Education" (MIS-1050202), implemented by the State Scholarship Foundation (S.F.).

36

Biotoxicity Tests

This research is co-financed by Greece and the European Union (European Social Fund - ESF) through the Operational Programme «Human Resources Development, Education and Lifelong Learning» in the context of the project "Reinforcement of Postgraduate Education" (MIS-1050202), implemented by the State Scholarship Foundation (S.F.).

37

MTT Colorimetric Assay

This research is co-financed by Greece and the European Union (European Social Fund - ESF) through the Operational Programme «Human Resources Development, Education and Lifelong Learning» in the context of the project "Reinforcement of Postgraduate Education" (MIS-1050202), implemented by the State Scholarship Foundation (S.F.).

38

MTT Colorimetric Assay

Tetrazolium salt MTT
 [(3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide)]
 (yellow)

↓ Viable cells

Formazan crystals (purple)

spectrophotometry
 ELISA reader (570 nm, 650nm)

Positive control sample (staurosporin or cis-platin)

Negative control sample TiO₂

$$\text{Cell Viability} = \frac{\text{O.D.treated}}{\text{O.D.untreated}} \cdot 100\%$$

This research is co-financed by Greece and the European Union (European Social Fund - ESF) through the Operational Programme «Human Resources Development, Education and Lifelong Learning» in the context of the project "Reinforcement of Postgraduate Education" (MIS-1050202), implemented by the State Scholarship Foundation (S.F.).

39

MTT Colorimetric Assay

MCF-7
MDA-MB-468

Effect of Ag-doped TiO₂ on cell viability

This research is co-financed by Greece and the European Union (European Social Fund - ESF) through the Operational Programme «Human Resources Development, Education and Lifelong Learning» in the context of the project "Reinforcement of Postgraduate Education" (MIS-1050202), implemented by the State Scholarship Foundation (S.F.).

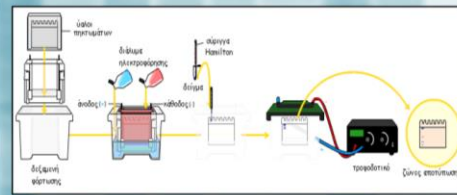
40

Necrosis or Apoptosis...?



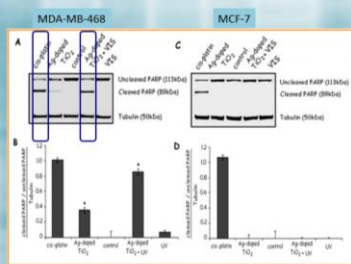
41

Western Blotting



42

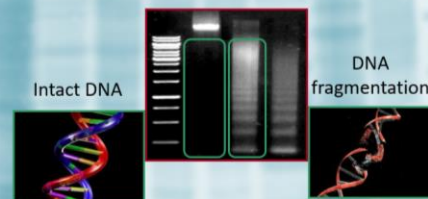
Apoptotic Effect



Ag-doped TiO_2 (15 μM) induces apoptosis on MDA-MB-468 cells, which is visible through PARP fragmentation, while MCF-7 are unaffected

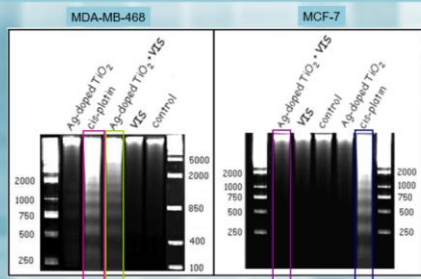
43

DNA laddering



44

DNA laddering



45

Conclusion

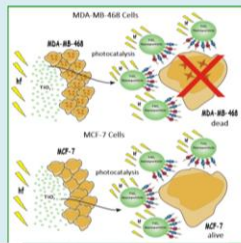
Ag-doped TiO_2 nanoparticles induced cell death specifically in the highly malignant MDA-MB-468 cancer cells, while MCF-7 cells were still unaffected, under the same circumstances.

The molecular mechanism of Ag-doped TiO_2 nanoparticles cytotoxicity was associated with PARP activation thus resulting in DNA fragmentation and programmed cell death.

46

Conclusion

This selective toxicity of Ag-doped TiO_2 nanoparticles is related to the different constitution of cellular membrane and to different interactions between the membrane proteins and Ag-doped TiO_2

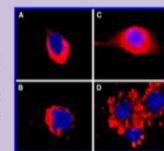


47

Conclusion

- Ag-doped TiO_2 nanoparticles generate pairs of electrons and holes.
- These sub-atomic particles react with water and oxygen, yielding reactive oxygen species (ROS) which can damage cancer cells.

- TiO_2 induces cell death in two separate steps:
- Binding of TiO_2 on the cellular membrane. ROS Production. Oxidative Stress.
 - Destruction of cellular organelles via signaling or entrance of TiO_2 inside the cell with toxic effect.



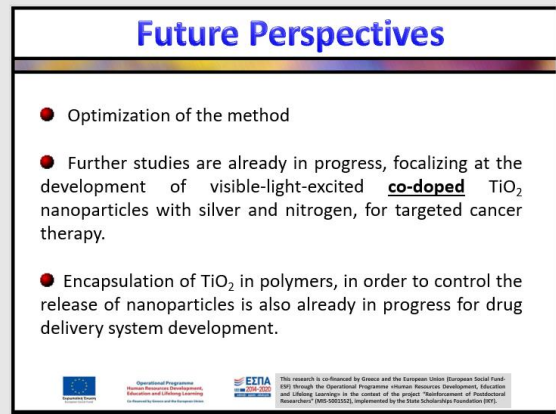
48



Future Perspectives

This research is co-financed by Greece and the European Union (European Social Fund - ESF) through the Operational Programme «Human Resources Development, Education and Lifelong Learning» in the context of the project «Reinforcement of Postdoctoral Researchers» (MIS-501352), implemented by the State Scholarship Foundation (RF).

49




Future Perspectives

- Optimization of the method
- Further studies are already in progress, focalizing at the development of visible-light-excited **co-doped** TiO₂ nanoparticles with silver and nitrogen, for targeted cancer therapy.
- Encapsulation of TiO₂ in polymers, in order to control the release of nanoparticles is also already in progress for drug delivery system development.

This research is co-financed by Greece and the European Union (European Social Fund - ESF) through the Operational Programme «Human Resources Development, Education and Lifelong Learning» in the context of the project «Reinforcement of Postdoctoral Researchers» (MIS-501352), implemented by the State Scholarship Foundation (RF).

50



Thank you!

This research is co-financed by Greece and the European Union (European Social Fund - ESF) through the Operational Programme «Human Resources Development, Education and Lifelong Learning» in the context of the project «Reinforcement of Postdoctoral Researchers» (MIS-501352), implemented by the State Scholarship Foundation (RF).

51