National Technical University of Athens

National and Kapodistrian University of Athens

International Conference on Nanomedicine and Nanotechnology 2018

Effect of silver doped nanostructured titanium dioxide (TiO_2) on breast cancer epithelial cells

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«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».

Postdoc-Research Scholarships IKY

European Union

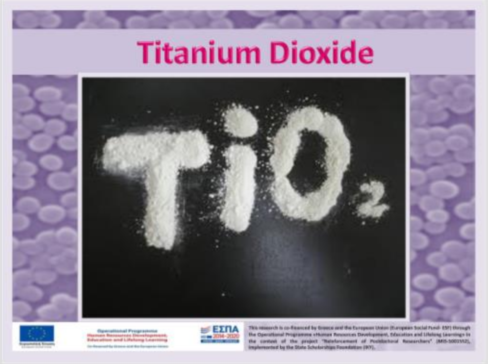
Operational Programme Human Resources Development Education and Lifelong Learning

Co financed by Greece and the European Union

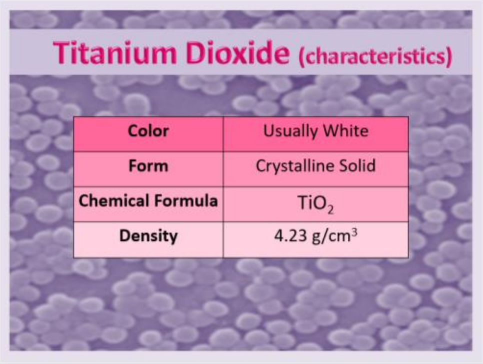
NSRF 2014-2020

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

Introduction



Titanium Dioxide



Titanium Dioxide (characteristics)

Color	Usually White
Form	Crystalline Solid
Chemical Formula	TiO_2
Density	4.23 g/cm^3

Titanium Dioxide Crystal Systems

Brookite

Orthorhombic Crystal System

Orthorhombic Crystal System

Bravais Lattice is rectangular parallelepiped with a parallelepiped base

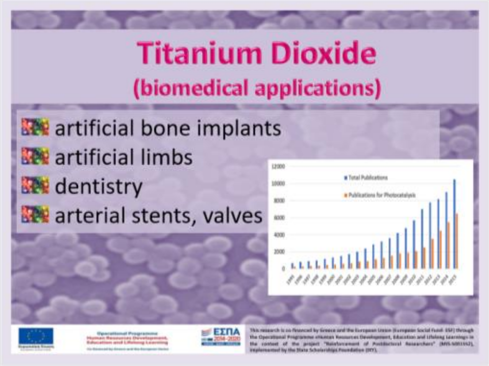
Titanium Dioxide (applications)

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

Ευρωπαϊκή Ένωση
Ευρωπαϊκό Κοινωνικό Ταμείο

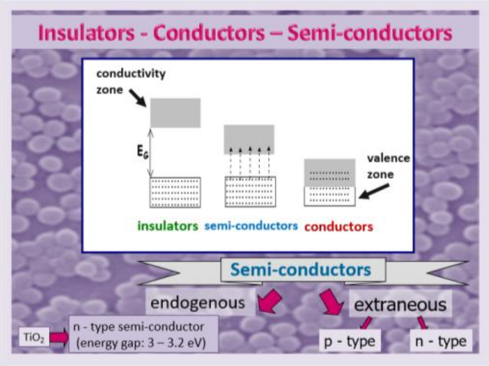
Επιχειρησιακό Πρόγραμμα
Ανάπτυξη Ανθρώπινου Δυναμικού, Εκπαίδευση και Διά Βίου Μάθηση
Με τη συγχρηματοδότηση της Ελλάδας και της Ευρωπαϊκής Ένωσης

ΕΣΠΑ 2014-2020
ανάπτυξη - εργασία - αλληλεγγύη



Titanium Dioxide
(biomedical applications)

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



Insulators - Conductors – Semi-conductors

Semi-conductors

endogenous

extraneous

p - type

n - type

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

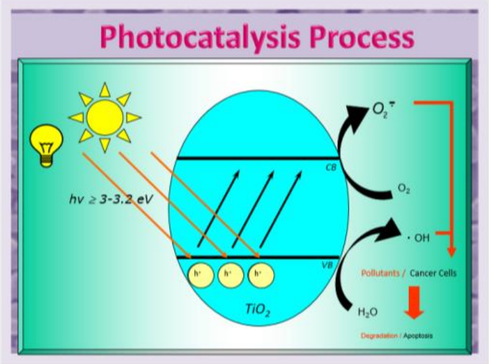
Photocatalysis

«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»

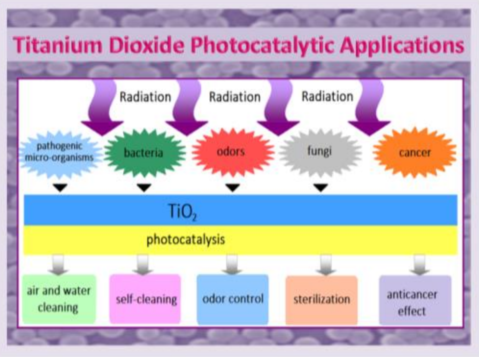
K.T. Pickering 1997
R.J. Bull 2001



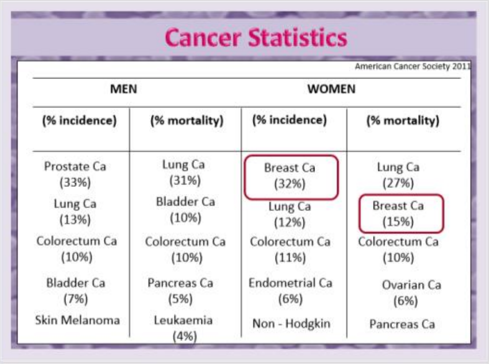
Photocatalysis Process

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



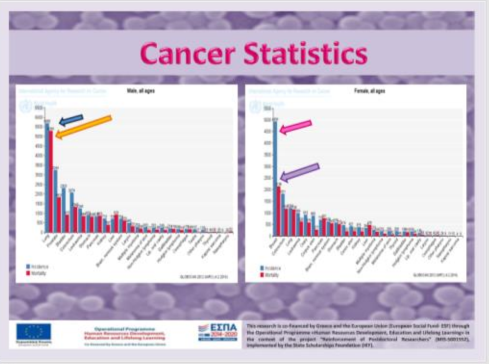
Titanium Dioxide Photocatalytic Applications



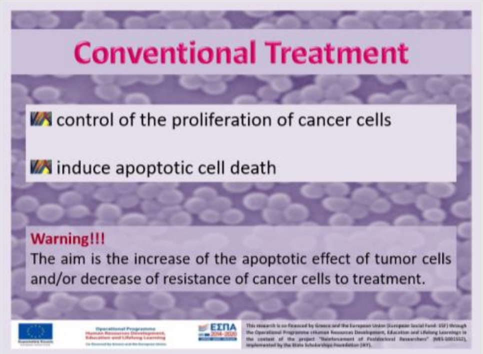
Cancer Statistics

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



Cancer Statistics



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.

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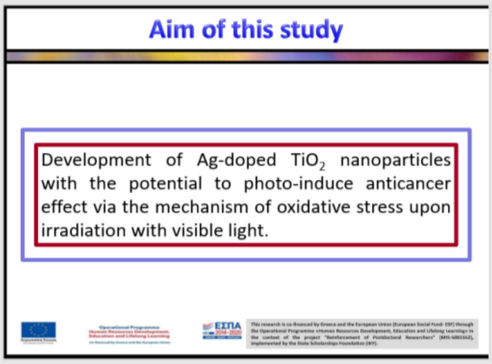
AIM



Aim of this study

Development of Ag-doped TiO_2 nanoparticles with the potential to photo-induce anticancer effect via the mechanism of oxidative stress upon irradiation with visible light.

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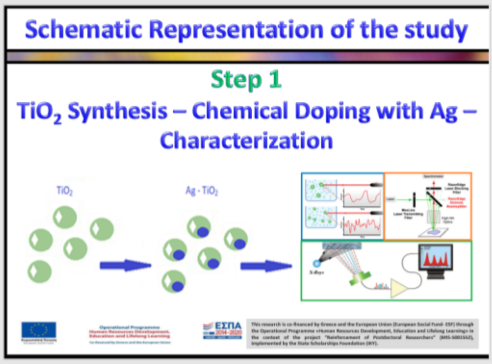

Schematic Representation of the study

Step 1

TiO_2 Synthesis – Chemical Doping with Ag – Characterization

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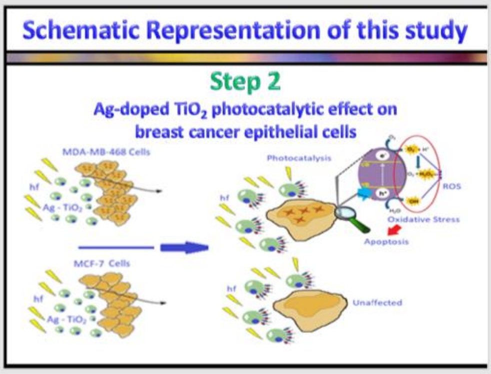
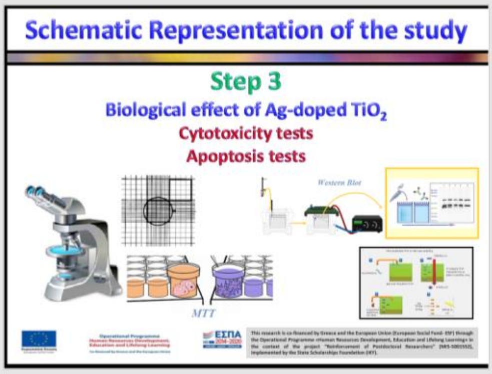


Schematic Representation of this study

Step 2

Ag-doped TiO_2 photocatalytic effect on breast cancer epithelial cells



Schematic Representation of the study

Step 3

Biological effect of Ag-doped TiO_2

Cytotoxicity tests

Apoptosis tests

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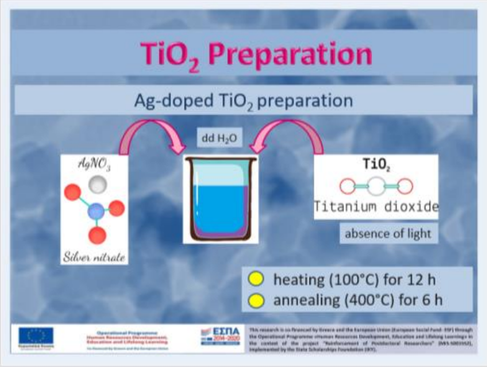
Methods & Results - Part A

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-5001552), implemented by the State Scholarships Foundation (ΙΚΥ).



TiO_2 Preparation

Ag-doped TiO_2 preparation

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

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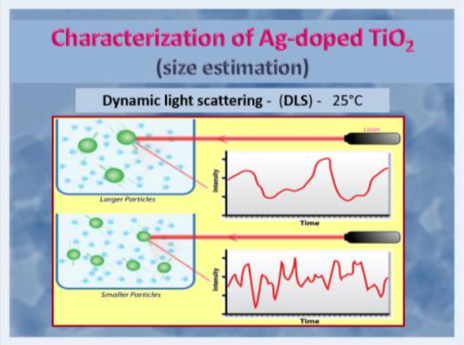
The need of doping!

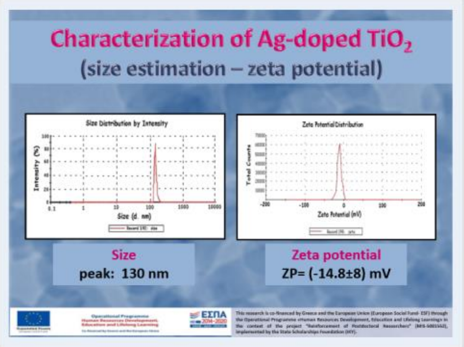
- Doping with metal ions improves TiO_2 photocatalytic activity
- Reduction of electron-hole recombination
- More effective separation and stronger photocatalytic reactions
- Silver up-regulates TiO_2 biological activity
- Antibacterial properties
- Photo-excitement in visible light (also in UV)



Characterization of Ag-doped TiO_2

(size estimation)

Dynamic light scattering - (DLS) - 25°C

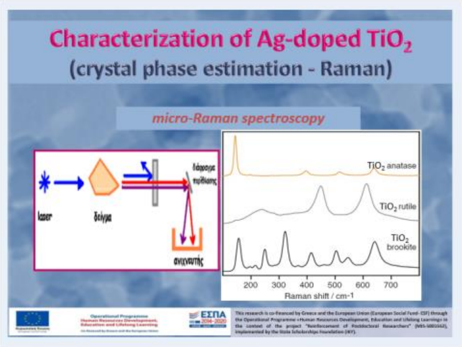


Characterization of Ag-doped TiO_2

(size estimation – zeta potential)

Size
peak: 130 nm

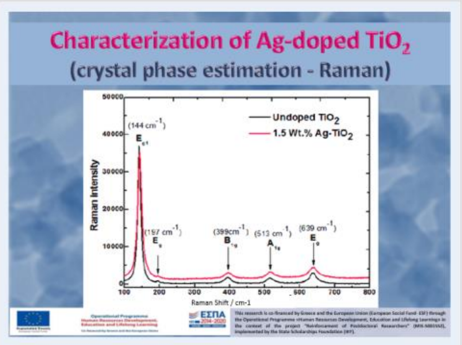
Zeta potential
ZP= (-14.8±8) mV



Characterization of Ag-doped TiO_2

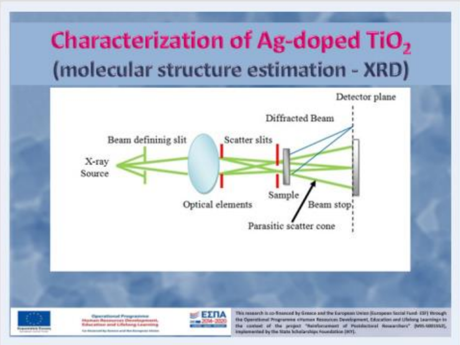
(crystal phase estimation - Raman)

micro-Raman spectroscopy



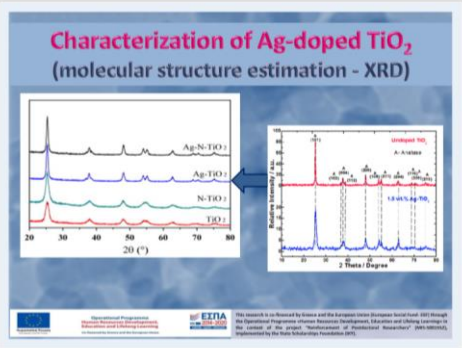
Characterization of Ag-doped TiO_2

(crystal phase estimation - Raman)



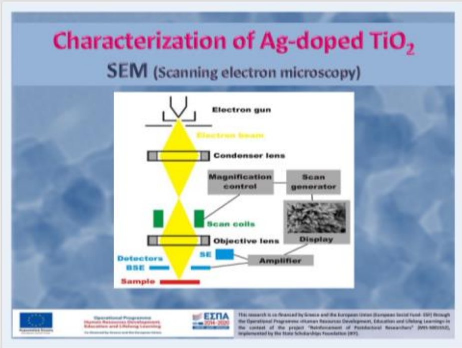
Characterization of Ag-doped TiO_2

(molecular structure estimation - XRD)



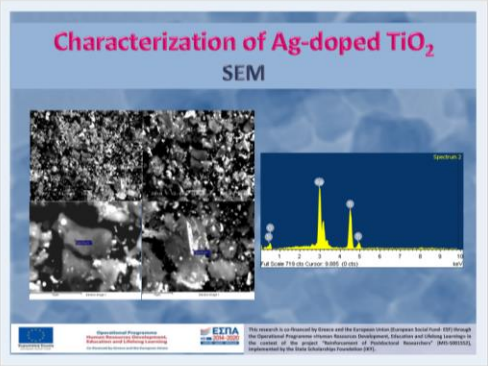
Characterization of Ag-doped TiO_2

(molecular structure estimation - XRD)



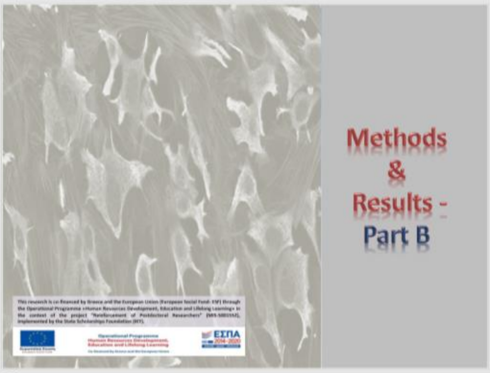
Characterization of Ag-doped TiO_2

SEM (Scanning electron microscopy)

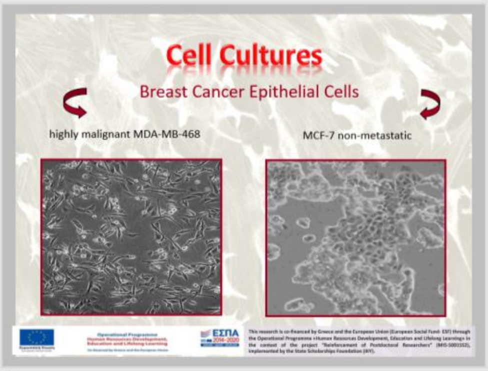


Characterization of Ag-doped TiO_2

SEM



Methods & Results - Part B

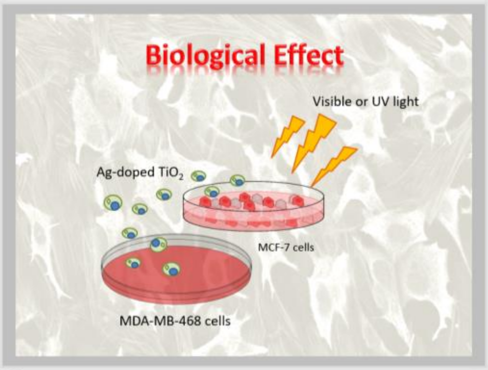


Cell Cultures

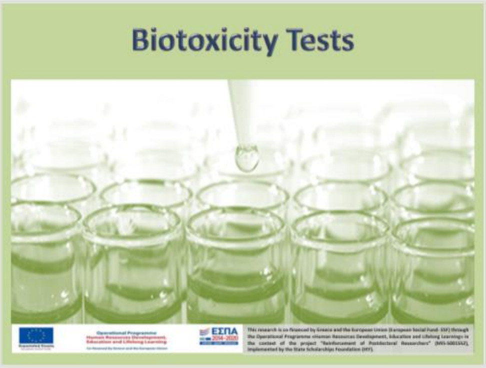
Breast Cancer Epithelial Cells

highly malignant MDA-MB-468

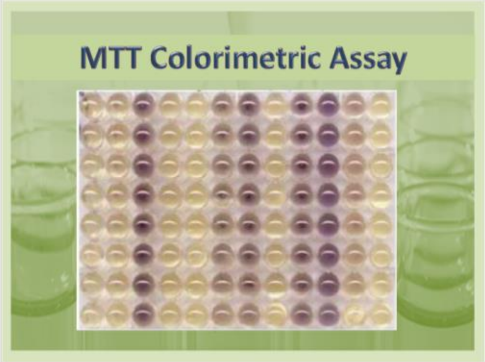
MCF-7 non-metastatic



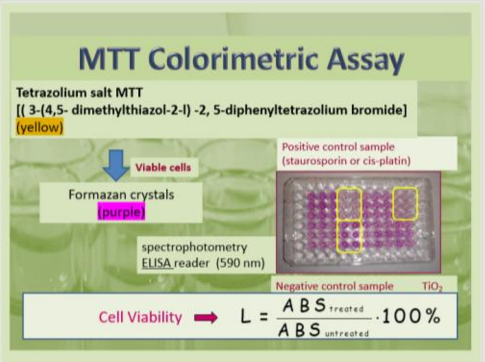
Biological Effect



Biotoxicity Tests



MTT Colorimetric Assay



MTT Colorimetric Assay

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

Viable cells

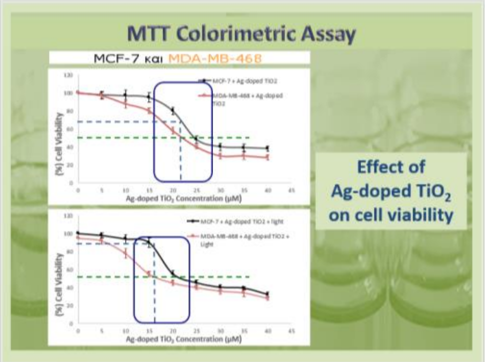
Formazan crystals
(purple)

spectrophotometry
ELISA reader (590 nm)

Positive control sample
(staurosporin or cis-platin)

Negative control sample TiO_2

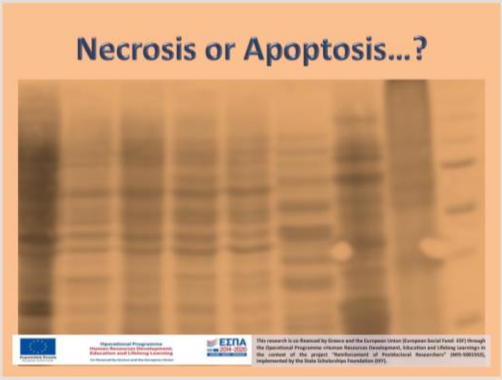
Cell Viability ➡ $L = \frac{ABS_{treated}}{ABS_{untreated}} \cdot 100\%$



MTT Colorimetric Assay

MCF-7 και MDA-MB-468

Effect of Ag-doped TiO_2 on cell viability



Necrosis or Apoptosis…?

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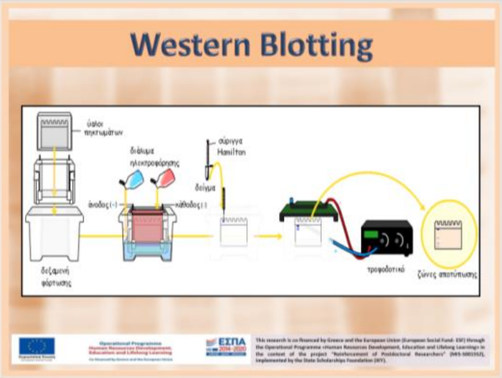

Western Blotting

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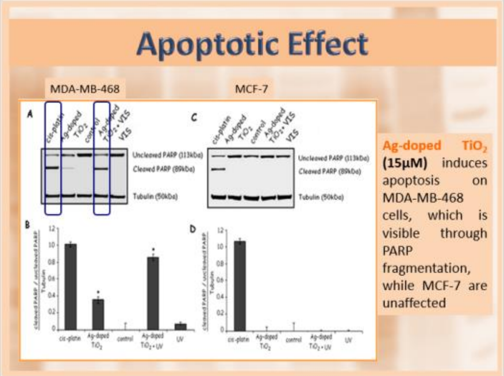
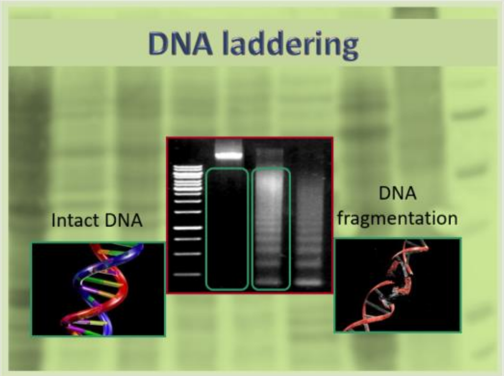


Apoptotic Effect

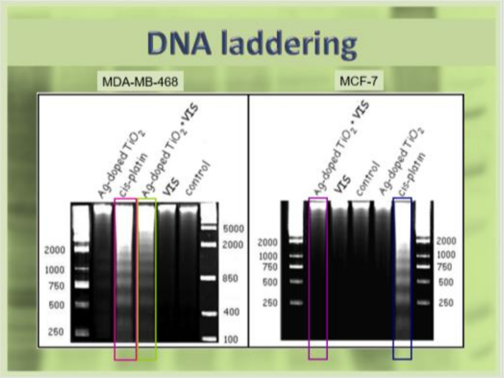
Ag-doped TiO_2 (15μM) induces apoptosis on MDA-MB-468 cells, which is visible through PARP fragmentation, while MCF-7 are unaffected



DNA laddering

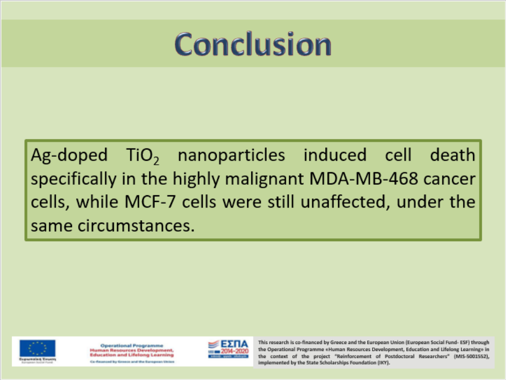
Intact DNA

DNA fragmentation



DNA laddering

Conclusion



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.

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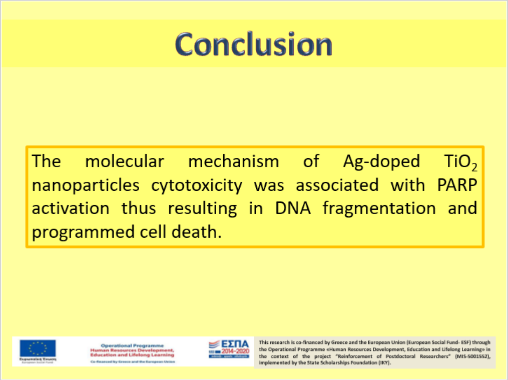

Conclusion

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

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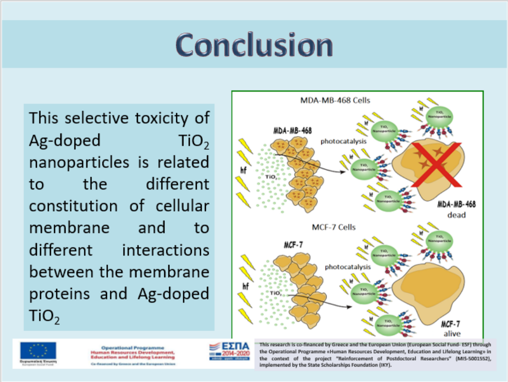

Conclusion

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

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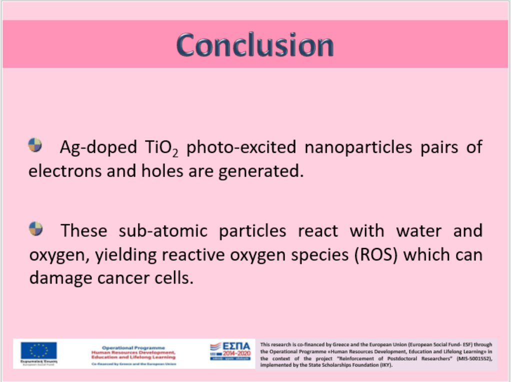

Conclusion

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

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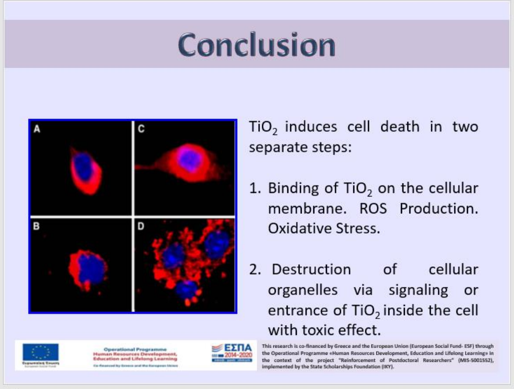

Conclusion

TiO_2 induces cell death in two separate steps:

1. Binding of TiO_2 on the cellular membrane. ROS Production. Oxidative Stress.
2. Destruction of cellular organelles via signaling or entrance of TiO_2 inside the cell with toxic effect.

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Future Perspectives

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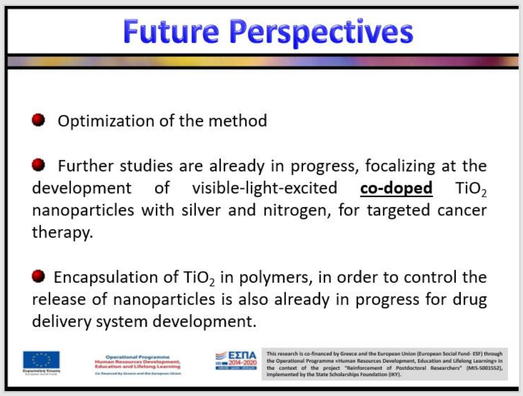

Future Perspectives

- Optimization of the method
- Further studies are already in progress, focalizing at the development of visible-light-excited **co-doped** TiO_2 nanoparticles with silver and nitrogen, for targeted cancer therapy.
- Encapsulation of TiO_2 in polymers, in order to control the release of nanoparticles is also already in progress for drug delivery system development.

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Thank You !!!!!

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