THE SYNTHESIS OF BIMETALLIC NANOPARTICLES USING HUMAN CANCER CELLS FOR BIOMEDICAL APPLICATIONS

Miguel Angel Alvarez Sanchez

Advisor: Prof. Thomas J. Webster
Master Thesis Defense

July 31st 2018
Department of Chemical Engineering
Northeastern University, Boston, MA
Universitat Rovira i Virgili, Tarragona, Spain
Overview

1. The problem of cancer
2. Current treatments
3. Nanotechnology in cancer treatments
4. Objectives
5. Experimental part
6. Results and discussion
7. Conclusion
8. Future prospects
The problem of cancer

WHAT IS CANCER?

• Abnormal growth of tissue.
• Increase in the number of cell divisions.
• More than 200 types of cancer.

GLIOBLASTOMA

• Most common tumor in the central nervous system.

STATISTICS

<table>
<thead>
<tr>
<th>Cancer Type</th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung &amp; bronchus</td>
<td>85,920</td>
<td>72,160</td>
</tr>
<tr>
<td>Prostate</td>
<td>26,120</td>
<td>40,450</td>
</tr>
<tr>
<td>Colon &amp; rectum</td>
<td>26,020</td>
<td>23,170</td>
</tr>
<tr>
<td>Pancreas</td>
<td>21,450</td>
<td>20,330</td>
</tr>
<tr>
<td>Liver &amp; intrahepatic bile duct</td>
<td>15,280</td>
<td>14,240</td>
</tr>
<tr>
<td>Leukemia</td>
<td>14,130</td>
<td>10,470</td>
</tr>
<tr>
<td>Eosinophil</td>
<td>12,720</td>
<td>10,270</td>
</tr>
<tr>
<td>Uterine corpus</td>
<td>11,820</td>
<td>8,890</td>
</tr>
<tr>
<td>Lymphoma</td>
<td>11,520</td>
<td>8,630</td>
</tr>
<tr>
<td>Brain &amp; other nervous system</td>
<td>9,440</td>
<td>6,610</td>
</tr>
</tbody>
</table>

RISK FACTORS

• Strong radiation.
• Toxic chemicals.
• Air pollution.

Current treatments

New emerging treatments

**Immune therapy**
- Natural defense stimulation.
- Production of antibodies.
- Combination with chemotherapy and radiation therapy.

**Gene therapy**
- Mutated genes are replaced from the nucleus.
- Very expensive technique.

**Hyperthermia**
- Based on increasing the temperature of tumor cell.
- Use of irradiation or electromagnetic field exposure.
- Need of a sensitizer.

---

Nanotechnology in cancer treatments

**ADVANTAGES OF NANOTECHNOLOGY**

- High surface to volume ratio.
- Ability to cross the cell membrane.
- Uses in cancer for imaging diagnosis and therapeutics.

Metallic nanoparticles: Anticancer properties

**PHOTOTHERMAL THERAPY**

Scheme of photothermal therapy

- High surface to volume ratio.
- Ability to cross the cell membrane.
- Uses in cancer for imaging diagnosis and therapeutics.

Drawback: synthesis of nanoparticles

(A) Cell samples treated with gold NPs and irradiated at 820 nm. (B) Cell samples only irradiated at 820 nm in the absence of nanoshells

---

Synthesis of metal nanoparticles

<table>
<thead>
<tr>
<th>PHYSICO-CHEMICAL METHODS</th>
<th>GREEN METHODS</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Toxic waste production.</td>
<td>• Eco-friendly synthesis processes (room conditions and use of non-toxic reagents).</td>
</tr>
<tr>
<td>• Drastic reaction conditions. (Temperature, pressure, pH...).</td>
<td>• Use of natural sources.</td>
</tr>
<tr>
<td>• Cytotoxicity problems.</td>
<td>• Natural bio-coating surrounding the nanoparticles.</td>
</tr>
<tr>
<td></td>
<td>• Less cytotoxic.</td>
</tr>
</tbody>
</table>

Synthesis by green methods

By plant extracts

Characterization of green synthesized nanoparticles by TEM.

By mammalian cells

TEM image of HEK 293 cells with gold nanoparticles (Black spots) within the cytoplasm.

*Bio-coating*

*Intracellular synthesis within the cytoplasm*

Interacion between cells and nanoparticles

Metal Nanoparticles + Enzymes and organelles

Generation of reactive oxygen species (ROS)

Comparison between the standard activity of ATP production in mitochondria (left) and the generation of ROS through a combination of irradiation and metal nanoparticle targeting (right).

Objectives

1- Synthesis of bimetallic nanoparticles

Glioblastoma cells.

Metal ion salts
Au/Pt Au/Pd.

Bimetallic Nanoparticles.

Au/Pt

Au/Pd
Objectives

2- Nanoparticle treatment with ethanol

- Characterization.
- Cytotoxicity studies.
Objectives

3- Application of nanoparticles in photothermal treatments

Au/Pt

Glioblastoma cells.

Irradiation (785 nm).
Synthesis of nanoparticles

Synthesis of G-Au/Pt nanoparticles.

1. Sonication for 20 minutes
2. Centrifuge at 11000 rpm, 4°C for 30 minutes

Lyophilization

G-Au/Pt

Synthesis of G-Au/Pd nanoparticles.

1. Sonication for 20 minutes
2. Centrifuge at 11000 rpm, 4°C for 30 minutes

Lyophilization

G-Au/Pd
Ethanol treatment of nanoparticles

Synthesis of G-Au/Pt-Tr nanoparticles

Synthesis of G-Au/Pd-Tr nanoparticles
Synthesis of bimetallic nanoparticles

Phase contrast microscopy

Phase contrast microscopy images of the synthesis of G-Au/Pt and G-Au/Pd.

Change of color $\rightarrow$ reduction of metal ions into nanoparticles.
Synthesis of bimetallic nanoparticles

UV-Vis Spectroscopy

G-Au/Pt

G-Au/Pd

Intracellular synthesis

UV-Visible absorption spectra for the synthesis of G-Au/Pt nanoparticles and G-Au/Pd nanoparticles by glioblastoma cells.
Energy dispersive X-ray spectroscopy (EDS)

EDS spectra representing elemental analysis of (A) G-Au/Pt and (B) G-Au/Pd nanoparticles.
Synthesis of bimetallic nanoparticles

Transmission electron microscopy (TEM)

G-Au/Pt

Size mean: 21 ± 7 nm.

G-Au/Pt-Tr

No differences after ethanol treatment.
Synthesis of bimetallic nanoparticles

Transmission electron microscopy (TEM)

G-Au/Pd

Size mean: 21 ± 7 nm.

G-Au/Pd-Tr

Size mean 9 ± 4 nm.

Decrease in particle size.
Cytotoxicity assays

Anticancer Properties

Addition of nanoparticles
Incubation for 1 day, 3 days and 5 days
MTS assay
Measure 490 nm

Biocompatibility

Addition of nanoparticles
Incubation for 1 day, 3 days and 5 days
MTS assay
Measure 490 nm
Cytotoxicity studies

Gioblastoma cells treated with G-Au/Pt and G-Au/Pt-Tr.

Human dermal fibroblasts (HDF) treated with G-Au/Pt and G-Au/Pt-Tr.

Viability percentage of Gioblastoma (left) and Human Dermal Fibroblasts (right) at different concentrations of G-Au/Pt and G-Au/Pt-Tr incubated during 1, 3 and 5 days. * means statistically different to the control.

Anticancer activity.

Decrease in live cells.
Cytotoxicity studies

Gioblastoma cells treated with G-Au/Pd and G-Au/Pd-Tr.

Human dermal fibroblasts (HDF) treated with G-Au/Pd and G-Au/Pd-Tr.

Viability percentage of Glioblastoma (left) and Human Dermal Fibroblasts (right) at different concentrations of G-Au/Pd and G-Au/Pd-Tr incubated during 1, 3 and 5 days.

Anticancer activity.

Higher biocompatibility.
Measure of Reactive Oxygen Species (ROS)

Glioblastoma cells

Addition of nanoparticles 20 µg/mL

Washing

Addition of 2,7-dichlorofluorescein diacetae (DCFH-DA)

Increase in fluorescence

Increase in ROS production

Interaction between cells and nanoparticles

Measure of Fluorescence

$\lambda_{em} = 485$ nm

$\lambda_{ex} = 520$ nm
Measure of reactive oxygen species (ROS)

Reactive Oxygen Species (ROS) generation by glioblastoma cells incubated with G-Au/Pt and G-Au/Pd nanoparticles for 24 hours.

<table>
<thead>
<tr>
<th>Nanoparticle</th>
<th>ROS increase (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G-Au/Pt-Tr</td>
<td>40</td>
</tr>
<tr>
<td>G-Au/Pd-Tr</td>
<td>3</td>
</tr>
<tr>
<td>Zno NPs [20]</td>
<td>400</td>
</tr>
</tbody>
</table>

No significant differences with the control (p > 0.1).

Photothermal Therapy

G-Au/Pt

Incubation
4 hours
37 °C 5% CO₂

Laser irradiation
785 nm (NIR)
10-15 min
1 W
Photothermal studies

Control Glioblastoma cells.

Irradiation for 15 min with 15 µg mL\(^{-1}\) of G-Au/Pt.

Irradiation for 15 min with 20 µg mL\(^{-1}\) of G-Au/Pt.

785 nm laser treatment of glioblastoma cells for 15 minutes with (A) absence of nanoparticles, (B) 15 µg mL\(^{-1}\) and (C) 20 µg mL\(^{-1}\).

Green: live
Red: dead
Photothermal studies

Viability percentage of glioblastoma cells incubated with nanoparticles and irradiated. * means statistically different to the control.

High anticancer activity at 4 hours of incubation.

Irradiation does not increase the number of dead cells.

Viability percentage of cell at different periods of irradiation.
Conclusions

Synthesis of Bimetallic Nanoparticles

- **Synthesized by human cells.** Contribution to the Green Chemistry.
- Reduced size around 20 nm.
- **Anticancer activity** of G-Au/Pt and G-Au/Pd at 15 and 25 µg/mL during the first 24 hours.
- Cytocompatibility.
- Moderated ROS production.
- G-Au/Pt nanoparticles are **not suitable for photothermal therapy irradiating at 785 nm.**
Future prospects

• Improve the release of bimetallic nanoparticles from the cellular matrix by ultrasonication technique.

• Change the irradiation wavelength to one within the absorption range of the nanoparticles.

• Develop drug carrier systems that target glioblastoma cells and with the ability to cross the blood brain barrier (BBB).
Acknowledgments

Special thanks to:

Members of the committee:
Prof. Gallaway, Prof. Pfluger and Prof Webster

Chemical Engineering Department at Northeastern University

Professor, Department Chair and mentor, Thomas J. Webster

Lab members of Webster’s Nanomedicine Lab

Northeastern University, Boston (MA)

Universitat Rovira I Virgili, Tarragona (Spain)
THANKS A LOT FOR YOUR ATTENTION