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Biologic Impact of an Iron Oxide Nanoparticle System on 2D Human Lung Carcinoma

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1. Background

The impact of iron oxide nanoparticles (IONPs) on human lung carcinoma, particularly in the A549 cell line, embodies a complex interplay between therapeutic efficacy and biological responses. These nanoparticles have been demonstrated to induce various cellular alterations indicative of toxicity and stress, which can lead to apoptosis in cancer cells.

2. Study's aim and objectives

The current research reports the fabrication, characterization, and biological evaluation of a mixture of IONPs on the two-dimensional (2D) human lung carcinoma (A549 cells). The IONPs were synthesized through the combustion method, a versatile and energy-efficient approach. This method offers the advantages of rapid reaction kinetics and the formation of crystalline products in a single, self-sustaining step. The fuels used to obtain IONPs were glycine ($C_2H_5NO_2$) and urea (CH_4N_2O), and the $Fe(NO_3)_3 \cdot 9H_2O$ was the oxidizing agent. Four samples were obtained, labeled as G1 and G2 (derived from glycine fuel) and U1 and U2 (derived from urea fuel), in the presence of air (1) and in the absence of air (2).

3. Results and Discussion

3.1. Phase composition through X-ray diffraction

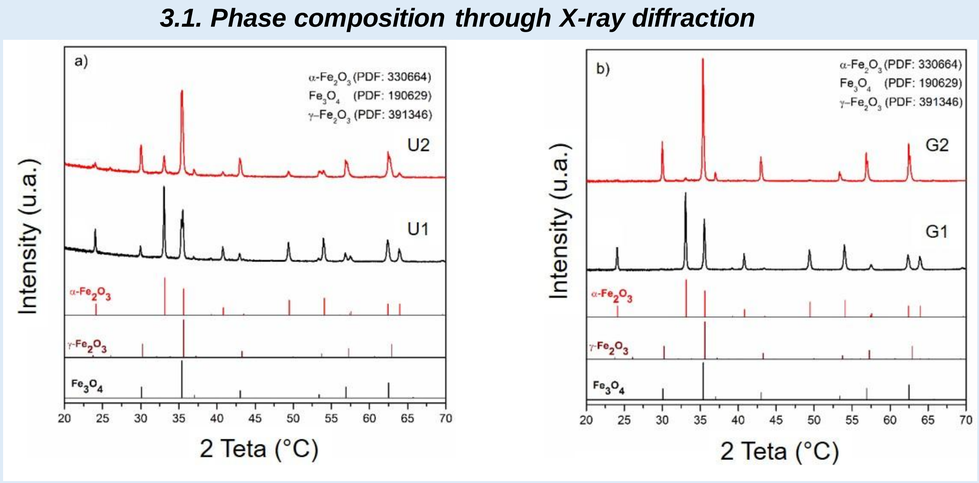


Figure 1. The XRD patterns of IONPs obtained from urea fuel (U1 and U2) (a) and from glycine fuel (G1 and G2) (b) compared to the XRD patterns of hematite - α-Fe_2O_3 (red, PDF file: 330664), maghemite - γ-Fe_2O_3 (brick, PDF file: 391346), and magnetite - Fe_3O_4 (black, PDF file: 190629) from the International Centre for Diffraction Data Powder Diffraction File (ICDD PDF) 4+ 2019.

Table 1. Characteristics of the IONPs obtained by the combustion method

Sample	Fuel used	Reaction conditions, time (minutes)		Phase composition (XRD-RIR)
U1	Urea	Porcelain capsule, in the presence of air	60	74% Hematite + 24% Maghemite + 2% Magnetite
G1	Glycine			96% Hematite + 2% Maghemite + 2% Magnetite
U2	Urea	Flask in a heating mantle, in the absence of air	60	25% Hematite + 32% Maghemite + 43% Magnetite
G2	Glycine			4% Hematite + 2% Maghemite + 94% Magnetite

The phase composition (Figure 1 and Table 1) showed that all the samples contain a mixture of iron oxides (hematite, maghemite, and magnetite), regardless of the working conditions and reaction parameters. The samples obtained in the presence of air (U1 and G1) contained hematite (α-Fe_2O_3) as the major phase, while the samples obtained in the absence of air (U2 and G2) contained magnetite (Fe_3O_4) as the major phase.

3.2. The metabolic activity of A549 cells by MTT assay

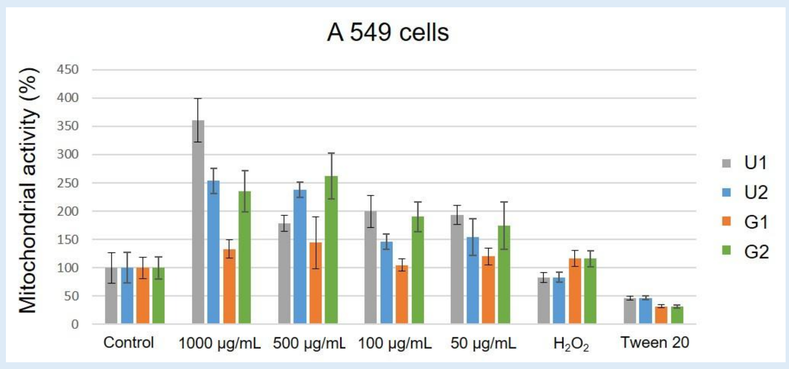


Figure 2. The mitochondrial activity of A549 cells after treatment with IONPs at different concentrations (50 – 1000 µg/mL).

3.3. Plasma membrane integrity of A549 cells by LDH release

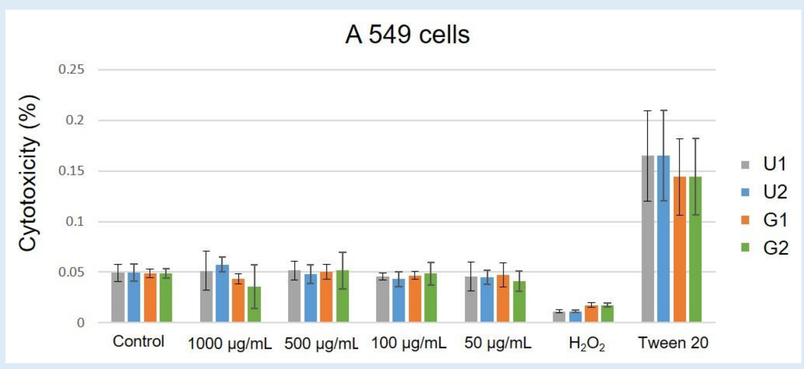


Figure 3. Cytotoxicity percentage of A549 cells after treatment with IONPs at different concentrations (50 – 1000 µg/mL).

3.4. Oxidative stress through NO production

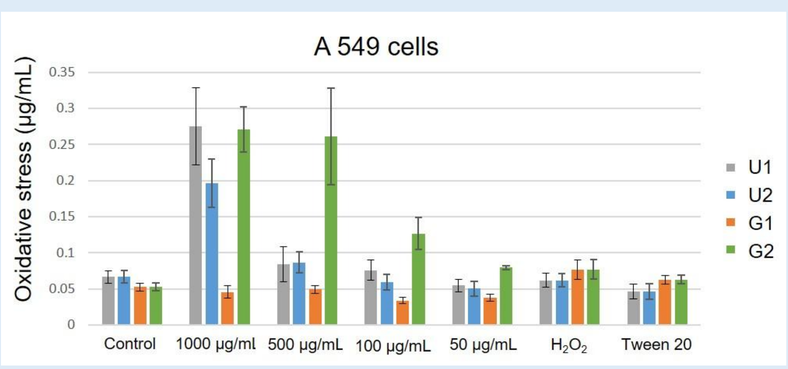
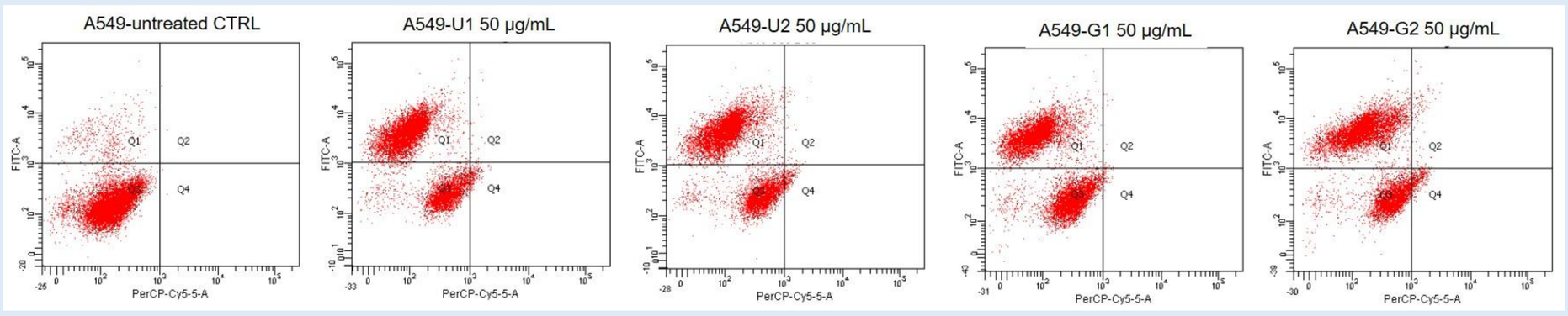


Figure 4. Oxidative stress of A549 cells after treatment with IONPs at different concentrations (50 – 1000 µg/mL).

A549 cells presented an increased mitochondrial activity (Figure 2) for the U1 sample at all tested concentrations, with a 50-100% increase at 50-500 µg/mL and a 250% increase for the 1000 µg/mL concentration. U2 sample produced a dose-dependent mitochondrial reaction, increasing their activity to 150% (at 50 µg/mL) and up to 250% at 1000 µg/mL. G1 sample also induced an increase in mitochondrial activity of up to 130% for the highest concentration, but the obtained absorbance measurements had a large variability and did not seem to depend on particle concentration. G2 sample determined increased mitochondrial activity for all tested concentrations, to 160-260%, again with high variability, but in a dose-dependent way. Tween 20 (2%) reduced their mitochondrial activity to 50% but H_2O_2 did not affect the mitochondrial integrity of A549 cells. The A549 cells did not release LDH enzyme in the cell media at statistically relevant differences compared to untreated control for any of the tested samples and at any of the tested concentrations. Tween 20 (2%) determined a statistically significant increase of LDH (Figure 3). A549 cells suffered increased NO production after treatment with U1 sample, with high values (0.255 µg/mL) at 1000 µg/mL compared to untreated control (0.06 µg/mL nitrite/nitrates) and close to control for the other concentrations. U2 sample also induced NO at 1000 µg/mL (0.19 µg/mL) and at 500 µg/mL (0.08 µg/mL). A549 cells were not affected by G1 sample, as all concentrations gave similar or slightly smaller (0.035 µg/mL) than untreated control values. But G2 sample gave high NO adducts at larger concentrations and in a dose dependent way (0.255 µg/mL down to 0.07 µg/mL for 1000 to 50 µg/mL sample concentration) (Figure 4).

3.5. Viability evaluation by flow cytometry



Q1 – apoptosis
Q2 – late apoptosis
Q3 – viable cells
Q4 – necrosis

Figure 5. A549 cell viability after treatment with IONPs at 50 µg/mL concentration.

A549 cells were clearly separated into two populations: living and apoptotic, with approximately 60% of cells in early apoptosis at 50 µg/mL for all nanoparticle types. Less than 1% were in late apoptosis (for all nanoparticle types) and 2% of cells were necrotic when treated with 50 µg/mL U1 and U2 samples and 3.9% when treated with G2 sample. All other concentrations had smaller values than the 50 µg/mL for apoptosis and necrosis.

4. Conclusions

The biological impact of IONPs on 2D human lung carcinoma showed selective toxicity which may be influenced by the physicochemical characteristics and surface properties of IONPs. However, by manipulating the synthesis parameters one can tailor the obtained nanoparticle characteristics. Their selective cytotoxicity against A549 cells supports the validity of IONPs as a treatment strategy in lung cancer therapy.

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