

**INFLUENCE OF Fe_3O_4 /OLEIC ACID - CORE/SHELL NANOPARTICLES ON THE
CELLULAR CYCLE AND ADHERENCE OF DIFFERENT EUKARYOTIC CELL LINES**

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Abstract

The purpose of the present work was to investigate the influence of Fe_3O_4 /oleic acid - core/shell nanoparticles on the cellular cycle, adherence and monolayer development of different eukaryotic cell lines. Core/shell nanoparticles have been synthesized by Massart adapted method, under microwave conditions. The dimensions of Fe_3O_4 /oleic acid nanoparticles were in the 5-20 nm range and they were previously characterized by High Resolution Transmission Electron Microscopy. The nanoparticles were deposited on glass slides in one, two and respectively three layers, and thereafter introduced in 6-multi well plastic plates and sterilized by ethanol 70%, for 10 min, followed by UV treatment. The Human epithelial carcinoma (HeLa) and the Human colonic epithelial cancer (HCT) cell lines were cultivated on the pelliculised glass slides and incubated for 24 hours at 37°C in 5% CO_2 atmosphere. Thereafter, the slides were fixed by ethanol, Giemsa stained and examined in reversed microscopy. For the assessment of the cellular cycle, 3×10^5 eukaryotic cells were put in Petri dishes and treated for 24 hours with nanoparticles. The cells were further fixed by ethanol, stained with propidium iodide and quantified by flow cytometry (FacsCalibur). Our results showed that the magnetic nanoparticles of Fe_3O_4 / oleic acid - core/shell exhibit specific influences on the ability of different cell lines to colonize the glass substrata.

Key words: nanoparticles, core/shell, HCT, HeLa, HR-TEM, cell line

1. INTRODUCTION

Adhesion of eukaryotic cells is a dynamic interaction between cells and their microenvironment, affected by many factors, including some properties of the cell itself (hydrophobicity, surface charge, specific adhesins), the target material (chemical composition, roughness, wettability) and the environmental factors (temperature, time period of exposure, cell number, presence of antibiotics, chemicals etc.) [1]. The most recent biotechnological applications in fields like biocompatible surfaces, bioelectronics, biosensors etc. require the production of hybrid systems based on the controlled adhesion of biological systems. Biomaterials are requested with both good mechanical properties and biocompatibility.

Biomaterials is a term used to indicate materials, synthetic or natural in origin, which can be used as a whole or as a part of a system which treats, augments or replaces any tissue, organ or function of the body [26]. Synthetic materials currently used for biomedical applications include metals and alloys, composites and polymers, ceramics and bioglass. Because the structures of these materials differ, they

have different properties and, therefore, different uses. At the nanometer scale, at least two different aspects of controlled biomolecule adsorption/organization must be considered, i.e., the interest in achieving nanosized patterns of biological molecules in a general perspective of bioelectronics devices, and the specificity of nanostructured surfaces in stimulating specific response of large systems, as cells or protein aggregates, opening new, very interesting views on the way the cells can *sense* nanometric surface features [1]. Nanoparticles have been increasingly used in biological and medical applications, including clinical diagnostics, therapeutics development and drug delivery, as well as advanced imaging. However, how nanoparticles could affect cell adhesion has not yet been defined [2]. In view of the above sketched areas of application, the purpose of the present work was to investigate the influence of Fe₃O₄/oleic acid - core/shell nanoparticles on the cellular cycle, adherence and monolayer development of different eukaryotic cell lines.

2. MATERIAL AND METHODS

2.1. Synthesis of core/shell

All the materials were reagent grade and used without further purification. Double distilled, de-ionized water was used. Fe₃O₄ nanoparticles has been synthesized by Massart adapted method using Fe³⁺, Fe²⁺ salts and HO⁻, under microwave conditions. Core/shell[1-4] (figure 1) was also synthesized under microwave conditions using oleic acid as surfactant (10 minutes).

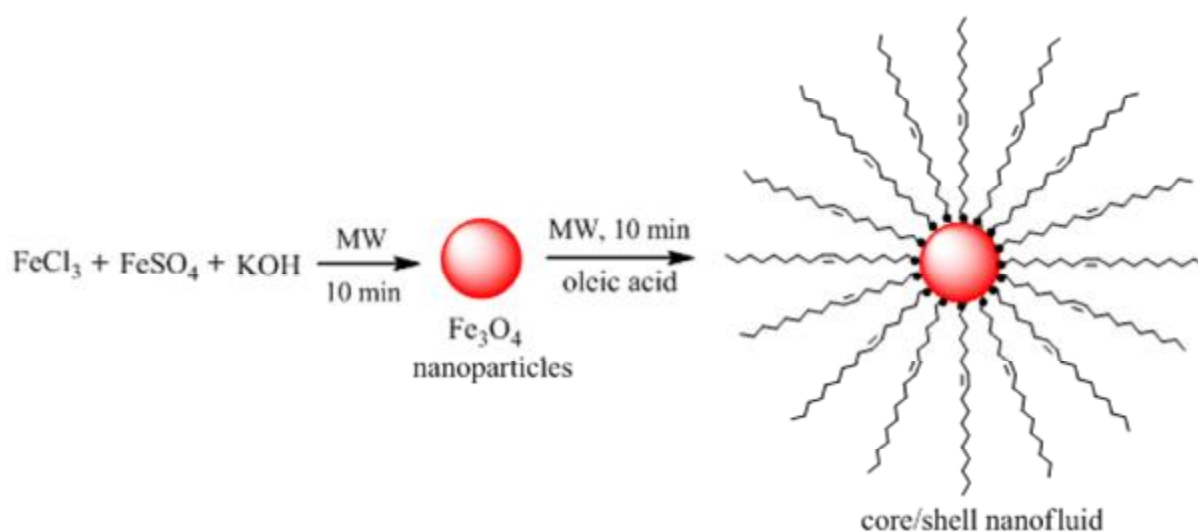


Figure 1: Core/shell nanofluid synthesis by microwave

2.2. Characterization of core/shell

HR-TEM characterization was used as a primary characterization method. Electron microscopy confirmed the formation of magnetic nanoparticles not exceeding 20 nm [3-6].

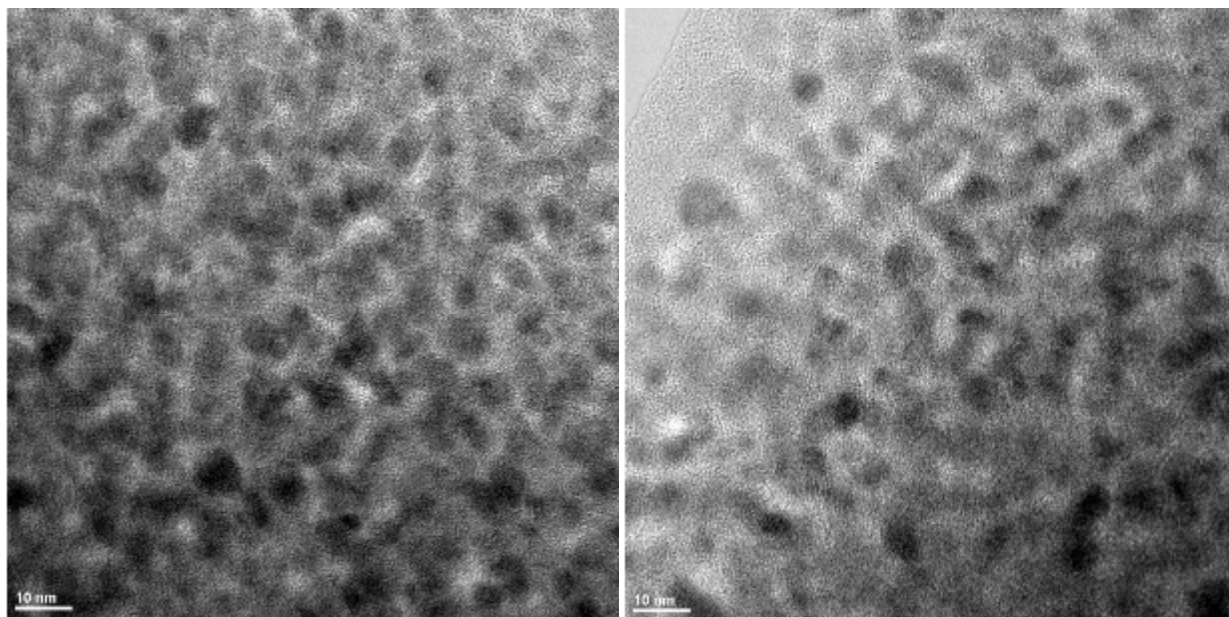


Figure 2: HR-TEM images of magnetic nanofluid

2.3. Preparation of inert substrata

The inert substrata for influence of Fe_3O_4 /oleic acid - core/shell nanoparticles on the cellular cycle, adherence and monolayer development of different eukaryotic cell lines were obtained by coating the glass coverslips with suspended core/shell nanoparticles (Fe_3O_4 /oleic acid: CHCl_3 33%(w/v)). Depositing a layer of functionalized nanoparticles on coverslips was achieved by applying a magnetic field on nanofluid [7]. Subsequently, the coverslips were soft dried and sterilized by ultraviolet irradiation [8].

2.4. Eukariotic cells adhesion to the pelliculised coverslips

The Human epithelial carcinoma (HeLa) and the Human colonic epithelial cancer (HCT) cell lines were cultivated on the pelliculised glass slides and incubated for 24 hours at 37°C in 5% CO_2 atmosphere. Thereafter, the slides were fixed by ethanol, Giemsa stained and examined in reversed microscopy.

2.5. Cell cycle analysis

For the assessment of the cellular cycle, 3×10^5 eukarotic cells were seeded in Petri dishes and treated for 24 hours with nanoparticles. Cell cycle analysis was performed using flow cytometry. The cells were resuspended in 70% ethanol and kept at -20°C . Cells were then washed and incubated with RNase and propidium iodide (50 $\mu\text{g}/\text{ml}$, Sigma) in phosphate buffer solution for 30 minutes and immediately analyzed. Flow cytometric analysis was performed within 1 hour and G_0 , G_1 , S/ G_2 /M cell count determined.

3. RESULTS

Our results showed that the HeLa and HCT cell lines developed a confluent monolayer on the glass slides after 24 hours incubation. The core/shell nanoparticles influenced the adherence pattern of the

two tested cell line, the effect being more evident on the HCT line, consisting in the inhibition of the cells multiplication and of their ability to develop a confluent monolayer.

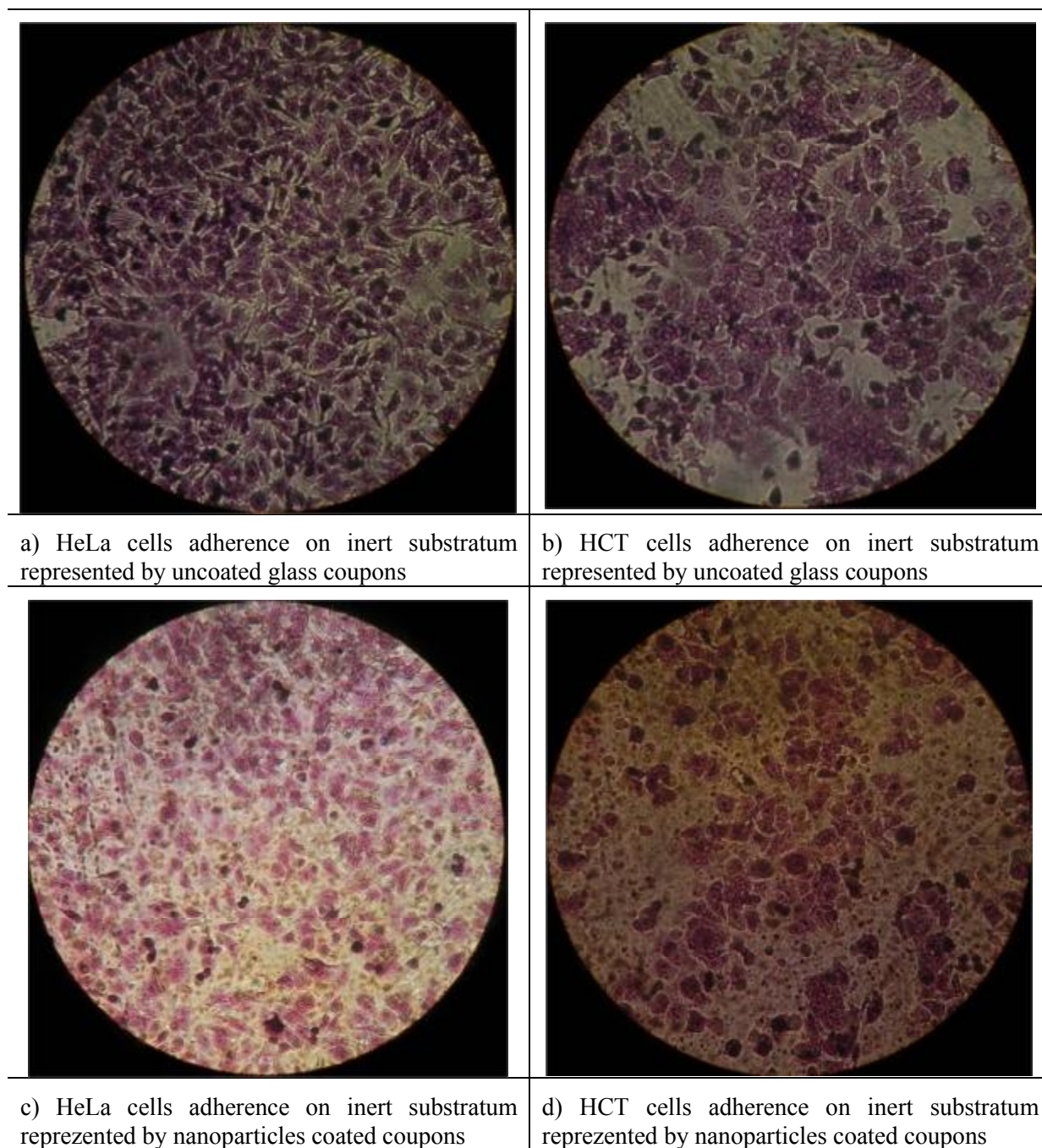


Figure 3: HCT and HeLa cells adherence on inert substratum (un)coated glass coupons (inverted microscope images of the Giesa colores monolayer)

The inhibitory effect was not dependent on the number of nanoparticles' layers. The tested nanoparticles exhibited no cytotoxic effect on the two tested cell lines. The nanoparticles induced a slight increase in G1 phase of both tested cell lines.

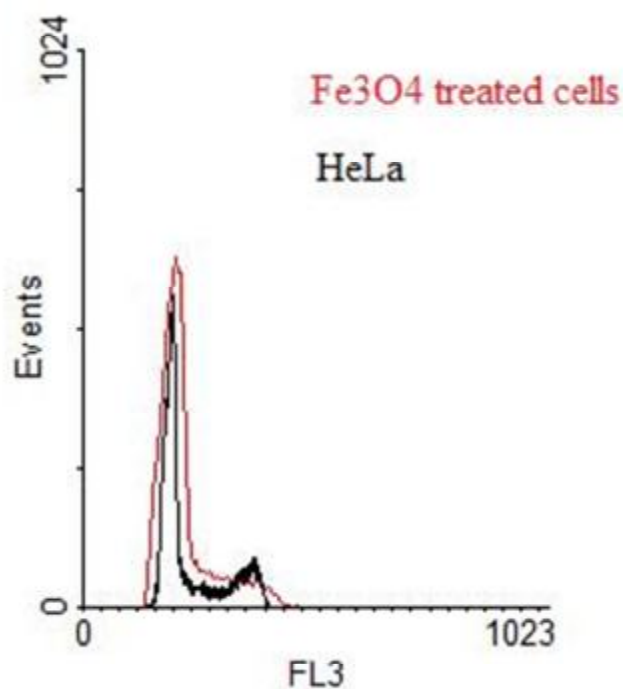


Figure 4: The overlaid histogram (black: HeLa cells control; red: Fe₃O₄ treated cells)

4. CONCLUSIONS

Our results showed that the magnetic nanoparticles of Fe₃O₄ / oleic acid - core/shell exhibit specific influences on the ability of different cell lines to colonize the glass substrata, by impeding their multiplication cycle and by inhibiting their ability to form confluent monolayers at 24 hours, the effect being dependent on the tested cell line, but independent on the nanoparticles layer's thickness.

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