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PhD THESIS

CONTRIBUTIONS REGARDING THE EXPERIMENTAL ASSESSMENT OF DIFFERENT MAGNETIC MATERIALS BASED ON IRON OXIDE NANOPARTICLES, FROM A PHYSICOCHEMICAL AND BIOLOGICAL POINT OF VIEW

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SUMMARY OF THE PhD THESIS

1. INTRODUCTION

Nanobiotechnology is the most promising technology of the 21st century and represents a field with strong interdisciplinary features, as it gathers the physicochemical characteristics of a substance (at molecular levels), the material science and materials engineering regarding the design, development and application of these and also biological aspects, to manipulate molecular, genetic and cellular processes. As concerns medicine, nanobiotechnology is broadly used in drug targeting, imaging, diagnosis, prevention and treatment. In health science, this technology is usefull by appliyng an agent *in vitro* and/or *in vivo*, with biomedical potential, but the greatest potential of this science is still the development of new and effective medical treatments. Nanotechnology is focused on the fabrication, manipulation and utilization of various materials included in the nanometric size scale (1-100 nm).

The inorganic nanoparticles (NPs) are considered to be the most promising nanomaterials in the biomedical field, automotive industry, environmental protection, as well as, in the development of new electronic devices. The most important class of inorganic NPs is represented by the iron oxide nanoparticles (IONPs). Since U.S. Food and Drug Administration (FDA) approved nanomedicines, IONPs are the most studied materials, due to their outstanding and unique properties which make possible their application in catalysis, agriculture, environment and biomedicine. In nature are found many forms of IONPs, but the most technologically used are: magnetite (Fe_3O_4), maghemite (γ-Fe_2O_3) and hematite (α-Fe_2O_3).

In the above context, the PhD thesis is part of the research topic related to the synthesis, characterization and use of some magnetic nanomaterials based on IONPs. The subject addressed is of the utmost topicality, considering the applications of MIONPs in the biomedical field.

2. CURRENT STATE OF KNOWLEDGE

Every day nanoscience registers novel advances in the production of NPs, environmentally-friendly, with a dimension less than 100 nm, with multiple biological properties. The design of magnetic NPs (MNPs) plays an essential role in obtaining NPs with tailored properties, therefore in the past decade, numerous synthesis methods have been developed to obtain magnetic iron oxide nanoparticles (MIONPs) suitable for application in nanomedicine. Many factors should be taken into consideration in the design process that can dramatically change the expected outcomes, i.e. controling the size, shape, morphology, stability, biocompatibility, and magnetic properties of these nanoparticles. These factors continue to be a permanent challenge which can still be optimized in the early design process. In the same time, the synthesis method has to be environmentally-friendly, simple, inexpensive and reproducible. The methods described in the thesis allow to obtain MNPs with narrow size dimensions, desired shape and morphology, by changing the conditions and/or parameters of the synthesis.

In the last decades, many researchers were focused on design strategies of multifunctional IONPs with application in the biomedical domain, by combining them with bioactive constituents aiming to enhance their biomedical potential. The fabrication of nanomaterials via biological entities represent the forefront of the nanobiotechnology field. In addition, the NPs obtained can be applied in a variety of biomedical fields, such as cancer therapy, drug delivery, hyperthermia, bioimaging, biolabeling etc.. In comparison with conventional chemical synthesis, the biosynthesis which involves a green chemistry approach, represents an attractive alternative for the development of biologically compatible and environment benign nanoparticles. Thus, green nanotechnology is based on the use of green nanomaterials, safe for human health or nanoproducts designed to provide solutions for medical system as well as for the environmental problems.

Green nanotechnology is a relatively new field focused on the use of green nanomaterials or NPs without using toxic or hazardous chemicals, which is safe for human health. In the same time, green nanotechnology deals with nanoproducts, designed to provide solutions for advanced biotechnology used in medical system as well as for the environmental problems.

Green synthesis is a simple eco-friendly approach employed to synthesize various NPs, which does not imply the use of harmful substances, is cost-effective, biocompatible and benign for the environment. For the synthesis of MNPs via green approach, bacteria, fungi, yeast, algae, viruses, plant extracts, actinomycetes, plant biomasses etc., are considered biological alternatives which offer a clean, nontoxic and environmentally-friendly route of synthesizing MNPs with a wide range of compositions, surface chemistry and physico-chemical features (size, shape, morphology). Each biological entity listed above has more or less biochemical degree of capabilities that can be used in the synthesis of iron or IONPs. But, with all these, due to their intrinsic metabolic process and enzyme activity, not all biological entities can synthesize IONPs. Therefore, the selection of the most suitable biological entity is required to obtain IONPs with well-defined composition, surface chemistry, size and shape.

From all biological entities, plants or plant based extracts are the most used in green synthesis due to the ease of the obtaining process, reduced costs, large-scale production and environmental innocuity. The whole plant or different parts of plants (leaves, fruits, fruit peels, stems, roots, seeds etc.) can participate in the synthesis of MNPs, due to the notable amount of phytochemicals which can act as reducing and stabilizing agents in the synthesis process and to the potential to accumulate heavy metals. In the reaction medium, the phytochemicals reduce metal ions in a single step, from their mono/divalent oxidation states to zero valent states, i.e. to NPs. The zero valent NPs lead to the nucleation of particles followed by growth, which is more stable thermodynamically and then takes place the reduction of metal ions. The growth process is followed by particles aggregation in a variety of morphologies (spheres, cubes, rods, triangles, pentagons etc.). The biochemical reaction is easily to conduct, at room temperature or not and it’s completed within few minutes.

Nowadays, the development of green MIONPs is more oriented towards biomedical potential, due to the fact that human health is threaten by several issues which can cause plenty of serious diseases, among which, the most severe is cancer. The potential biomedical application of MIONPs includes therapeutic carriers for drug delivery, visualization agents in magnetic resonance imaging (MRI) or heat intermediaries in cancer thermotherapy (so-called hyperthermia).

Cancer still remain a huge health concern worldwide, with an incidence, at the present time, over 4.9 million of cases in Europe, from which over a hundred thousand only in Romania. The incidence from cancer in Romania, are expected to rise with 5.4% by 2030. According to global cancer observatory data, till now, deaths from cancer in Europe are over 2.2 million, from which, over fifty thousand in Romania, being expected to rise with 7.6% by 2030. For this purpose, in the last decade, considerable measures has been developed for the diagnosis, monitoring and therapy of cancer.The diagnostic stage is primordial because the discovery of cancer in an early phase is determined by the choice of the most suitable and effective cancer therapy. Although it presents many disadvantages and side effects, the viable conventional therapies used for cancer treat-ment are radiation therapy, chemotherapy and surgery. In consideration of the limited efficacy of viable conventional therapies, the development of new additional treatment approach is strongly required. One of the antitumor therapy known and used for more than 30 years, is magnetic hyperthermia

For intravenous or oral administrations FDA approved in the last years several MIONPs formulations – as MRI visualization agents, but most of them have been taken off the market. For instance, Ferumoxides (Feridex IV®, Endorem™) which consists in a mixture of Fe_3O_4 and γ-Fe_2O_3 nanoparticles, having the surface functionalized with dextran, were used as biological target by reticuloendothelial system (RES) to liver stem cell labelling application. GastroMARK and Oral-SPION was also used and discontinued, for gastrointestinal bowel marking, the formulation consist in a mixture of Fe_3O_4 and γ-Fe_2O_3 nanoparticles, coated with silicon on the metallic iron surface. However, currently there are some MIONPs with superparamagnetic properties which have been approved and are still on the marked. For instance, Nano Therm which has the magnetic core coated with aminosilane and is employed in hyperthermia of brain tumors, is still used in Europe. Also, Ferucarbotran (Resovist), which possesses Fe_3O_4 as core material and has the surface coated with carboxydextran is used as biological target in blood pool, as stem cell labelling applications.

For magnetic targeting, the magnetic nanoparticles based on iron oxides should possess some key parameters including: i) high surface area to maximize the drug loading amount; ii) nanoparticles surface functionalization, with specific targeting moieties to facilitate drug distribution at the tumor site, avoiding damage to other organs; iii) high saturation field to provide maximum signals; iv) synergistic activity with anti-tumor agents, to improve the efficacy of cancer treatment by decreasing the resistance of cancerous cells.

For drug delivery, it is imperative that the magnetic core of the nanomaterial (usually magnetite, Fe_3O_4, or maghemite, γ-Fe_2O_3) to be nanocrystalline and to possess superparamagnetic behavior only in the presence of an external magnetic field, and out-side of its presence, the nanoparticles should no longer exhibit magnetic interactions. Nowadays, the optimization process of these MIONPs is based both on the reduction of the associated side effects (reducing the amount of systemic distribution of the drug), and also on the reduction of the required dose, by improving the drug target precision.

Therefore, the present thesis describe general concepts about IONPs (various physical, chemical and biological methods for IONPs fabrication as well as their features), the biomedical applications of MNPs especially in cancer therapy, as well as several concerns related to the possible toxicity of these magnetic nanomaterials (the *in vitro* and *in vivo* mechanism of toxicity, including remarks to reduce the toxicity). The second part of the thesis reveal the synthesis and physico-chemical characterization of the three main iron oxides (magnetite, maghemite and hematite) by employing the combustion synthesis and green chemistry approach, and their biological impact in some normal and cancer cells lines alongside with the impact on bronchial 3D respiratory tissues.

3. AIM AND OUTLINE

The aim of this PhD thesis was to synthesise MIONPs with suitable features for biomedical applications, especially for cancer therapy – as tools with great heating capacity used in hyperthermic treatment and drug delivery systems used for targeted cancer therapy. As far as the NPs fabrication is concerned, the idea focuses on the employment of two original synthesis method, as an alternative to the currently conventional used methods – the combustion method and the green method, for synthesising magnetite, maghemite and hematite. Both methods exhibits multiple advantages which are presented by comparison.

The doctoral thesis makes an important contribution in this direction, due to the fact that, MIONPs resulted from green synthesis as well as from combustion were tested in terms of their heating capacity, quantified by intrinsic loss power (ILP) and specific absorption rate (SAR), two mandatory parameters for practical applications in hyperthermia. Through combustion method it was obtained MIONPs with excellent magnetic properties which were further coated with citric acid in order to obtain highly stable, biocompatible magnetic colloidal suspension (MCS), further evaluated in terms of heating capacity quantified by ILP. Through green method, MIONPs were obtained for the first time, starting from two aqueous extracts of *Artemisia absinthium* L., based on leaf and stems. In order to obtain MNPs with hyperthermia inducing features, the synthesis reactions was conducted, both at 25°C and at 80°C, and with two formulations of the precipitating agent. Due to the fact that by green chemistry approach it was obtained MIONPs with higher heating capacity, the rest of the objectives established to fulfill the aim of the thesis refers only to the synthesis of green MIONPs.

As far as the novelty and originality of this PhD thesis is concerned, it was assessed the *in vitro* biological impact of the green MNPs obtained, on healthy (HaCaT-immortalized human keratinocytes) and tumorigenic 2D cell lines (A375 - human melanoma cells; A431 - epidermoid human carcinoma; A 549 - human lung carcinoma and NCI-H460 - large human lung carcinoma), with very good results. Also, the biosafety profile of the synthesized MIONPs was assessed using the EpiAirway^{TM} model–3D functional microtissues, from normal bronchial cells. For targeted drug delivery application, a solid-lipid nanoformulations (SLNs) were obtained by loading the green MNPs with an antitumoral compound – oleanolic acid. It was analyzed the cytotoxicity of the SLNs on tumorigenic 2D cell line (A549) as well as their biocompatibility on 3D functional microtissue EpiAirway^{TM} model, an analyze which has never been done before. This complex investigation allow us to closely mimic the *in vivo* conditions of MIONPs use.

Through this thesis, it was intended to eliminate all dissonance that shows when we pass from 2D to 3D *in vitro* studies, or when we advance from *in vitro* to *in vivo* studies, with the purpose that at the end of each study, we will obtain coherent data. The cells may have a slight different response to antitumoral compounds when 2D and 3D in vitro studies are employed, representing the main reason why the present PhD thesis is essential, as it constitutes a complete, integrating and complex study.

For a better understanding of the information described above, Figure 3.1. summarizes the entire outline of the thesis. Within the scope of this thesis, a brief overview is given to MIONPs, along with two different methods to synthesize these NPs, their physico-chemical properties and their biologic impact as well as hyperthermia inducing features, depicted in Chapters 2 - 6.

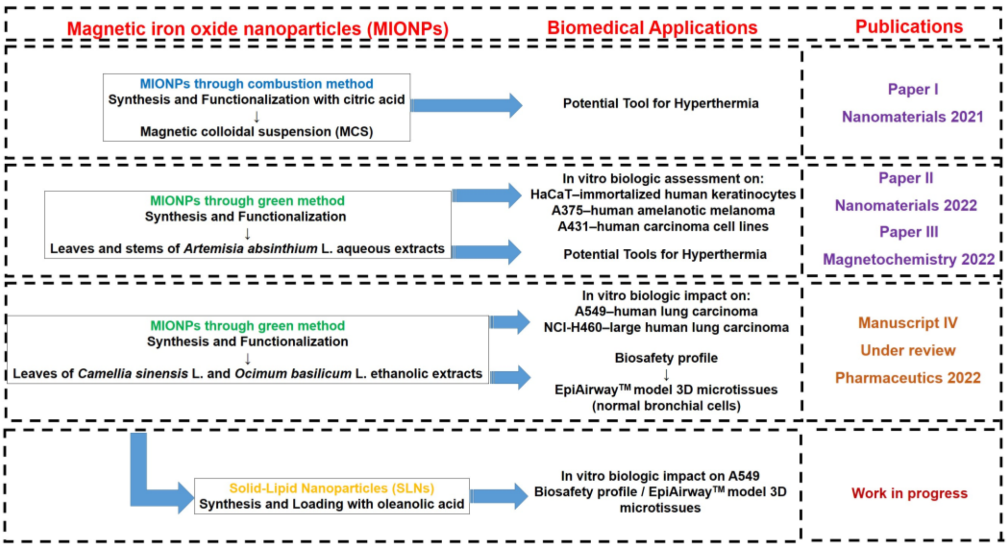

Figure 3.1. The outline of the entire PhD thesis, the intended field of applications and the published/under review or work in progress papers.

4. PERSONAL CONTRIBUTIONS

4.1. SYNTHESIS AND CHARACTERIZATION OF Fe_3O_4 NPs FABRICATED BY COMBUSTION METHOD, AS A TOOL FOR HYPERTHERMIA APPLICATIONS

The results of this study are presented in the PhD thesis in ***Chapter 2*** and refers to the design of MNPs based on iron oxides, using the combustion method. Further, a facile single-step process for the preparation of a highly stable and biocompatible MCS based on citric-acid coated MIONPs used as an effective heating source for the hyperthermia treatment of cancer cells is presented. The coating process of the as-prepared magnetic materials, was performed in order to obtain a MCS suitable for medical purpose, meaning, with hyperthermia inducing features.

Regarding the solid MIONPs obtained, the combustion reaction took place in a controlled atmosphere (in the absence of air), precisely to ensure the formation of magnetite (Fe_3O_4) and prevent its oxidation to maghemite (γ-Fe_2O_3). The structural characterization of solid MIONPs showed broad diffraction peaks consistent with nano-sized crystallites around 9 nm. The peaks observed were assigned to the magnetite and hematite diffraction planes. It was explained that the presence of hematite is not disturbing, du to the fact that this NPs remain in suspension not being attracted by the magnet due to the lack of magnetic moment, thus being able to be removed. The electron microscopy showed that the solid MIONPs obtained had polygonal shapes distributed uniformly. The saturation magnetization of the solid MIONPs, was 46.26 emu/g, quite big considering that the magnetite bulk is around 90 emu/g.

Regarding the physicochemical analyses of MCS, these revealed that the obtained colloidal suspension had a z-average diameter of 72.7 nm at 25°C with a polydispersity index of 0.179 and a zeta potential of -45.0 mV, superparamagnetic features, and a heating capacity that was quantified by an intrinsic loss power analysis. Raman spectroscopy showed the presence of magnetite and confirmed the presence of citric acid on the surfaces of the MIONPs. The heating capacity of MCS revealed a value of 0.055 $nH{*}m^2/kg$, at a magnetic field amplitude of 120 Oe. It was showed that this value increases with the increased of the magnetic amplitude field. Considering this, one can affirme that the value obtained indicates that the MCS could be used in magnetic heat generation.

4.2. SYNTHESIS AND CHARACTERIZATION OF BIOACTIVE MNPs FROM THE PERSPECTIVE OF HYPERTHERMIA APPLICATIONS

As a comparison, in ***Chapter 3*** were developed MIONPs through green synthesis method, with the aim of investigating to what extent these MIONPs possess magnetic hyperthermia features, so that they can be used for the development of new therapeutic strategies that contribute to the improvement of current cancer therapies.

In this chapter, MIONPs via green chemistry approach were obtained for the first time, starting from two aqueous extracts of wormwood, based on leaves and stems. In order to obtained MIONPs with hyperthermia inducing features, the synthesis reactions were conducted, both at room temperature (25°C) and at 80°C, using two formulations of the precipitation agent. In terms of phase composition, the results showed that regardless of the precipitating agent used, all the samples with one exception (WL 25-2 – leaf wormwood extract, using only $NH_{3(aq)}$ as precipitating agent at 25°C), showed the inverse spinel structure of cubic magnetite or maghemite. The electron microscopy exhibit nearly spherical shape particle with size range of 1 to 10 nm. A slighty agglomeration of NPs was observed in the case of samples precipitated only with $NH_{3(aq)}$, especially whn the synthsis occurs at 25°C.

The magnetic investigation of the as-obtained MIONPs revealed that the synthesis at 80°C using a mixture of NaOH and $NH_{3(aq)}$ increases their diameter and implicitly enhances their specific absorption rate (SAR), a mandatory parameter for practical applications in hyperthermia. High SAR values were obtained especially when the stems aqueous extract was used (904 W/kg in the case of the synthesis at 80°C and 880 W/kg in the case of the synthesis at 25°C), as well as high saturation magnetization (~62 emu/g for the synthesis at 80°C and 42 emu/g for the synthesis at 25°C).

In conclusion, considering that, to date, literature reveal no study regarding the synthesis of magnetite and maghemite designed for magnetic hyperthermia applications, through green synthesis, starting from aqueous extracts of wormwood, thus we can affirm that this is the first research study in which is detailed the biosynthesis of MIONPs, using an aqueous extract of wormwood based on leaves and stems, designed for magnetic hyperthermia applications.

In summary, considering both studies, further research is needed in order to optimize the synthesis procedure, with the aim of increasing the nanoparticles' magnetic diameter (with both methods NPs under 10 nm it were obtained) and implicitly the AC magnetic heating efficiency, due to the fact that the practical applications of nanoparticles in hyperthermia are limited by their sizes. Nevertheless, we believe that this research opens new methods regarding the synthesis conditions taken into account for the preparation of biocompatible and highly stable magnetic nanoparticles via both combustion and plant-mediated green chemistry approach.

4.3. BIOSYNTHESIS OF IONPS: CHARACTERIZATION AND THEIR IN VITRO CYTOTOXICITY ON HEALTHY AND TUMORIGENIC CELL LINES

Chapter 4 present the synthesis of hematite through green chemistry approach, since in the previous chapter magnetite and maghemite were obtained. One more time the leaves and stems of Artemisia absinthium L. were employed, but modifying the synthesis conditions with the purpose of obtaining α-Fe_2O_3. For the record, to the best of our knowledge, no studies have been reported on the synthesis of α-Fe_2O_3 NPs starting from aqueous extract of any species of *Artemisia* based on leaves and stems. The NPs were designed with the purpose of assessing the in vitro biological effects of both extracts of *Artemisia absinthium* L. and of the IONPs resulting from them, on three different cell lines: HaCaT–immortalized human keratinocytes, A375–human amelanotic melanoma and A431–human carcinoma cell line.

The results showed that the lack of magnetic properties and the reddish-brown color of all the samples confirms the presence of hematite as majority phase. In addition, the FTIR bands located at 435 cm^{-1} and 590 cm^{-1}, are assigned to Fe-O stretching vibration from hematite, confirming the formation of α-Fe_2O_3 NPs. According to electron microscopy investigation, the as synthetized IONPs are extremely small (4.7 ± 0.8 nm from SEM and 2.8 ±

0.9 nm from TEM), agglomerated and quasi-spherical in shape. The hydrodynamic diameter revealed that the aqueous suspensions with IONPs obtained from leaves are of the microns order (denoting giant clusters) as against aqueous suspensions with IONPs obtained from stems are in the nanometric scale. The in vitro screening of the samples revealed that the healthy cell line (HaCaT) presents a good viability (above 80%) after exposure to IONPs and lack of apoptotic features, while the tumorigenic cell lines manifested a higher sensitivity, especially the melanoma cells (A375) when exposed to concentration of 500 µg/mL IONPs for 72h. Moreover, A375 cells elicited significant apoptotic markers under these parameters (concentration of 500 µg/mL IONPs for a contact time of 72h).

In summary, the newly synthetized IONPs showed promising potential for biomedical applications, as the preliminary in vitro screening revealed that the phyto-mediated α-Fe_2O_3 NPs did not show cytotoxic activity on non-tumorigenic immortalized human keratinocytes (HaCaT), while the tumorigenic cell lines, especially A375 cells are significantly affected, the cells releasing a high amount of extracellular LDH and specific signs of apoptosis when exposed to concentration of 500 µg/mL for an interval of 72h.

4.4. BIOLOGIC IMPACT OF GREEN MIONPs ON TWO DIFFERENT LUNG TUMORIGENIC MONOLAYERS AND A 3D NORMAL BRONCHIAL MODEL-EPIAIRWAY™ MICROTISSUE

In ***Chapter 5*** other plant materials were also tested for the synthesis of IONPs by the green method, namely *Camellia sinensis* L. and *Ocimum basilicum* L. The study was undertaken with the purpose of evaluating the biological impact of the synthesized MIONPs through green method, on two morphologically different lung cancer 2D cell lines: human lung carcinoma - A549 cells and large human lung carcinoma - NCI-H460 cells. In addition, the biosafety profile of the newly synthesized MIONPs was assessed using the EpiAirway™ model three-dimensional (3D) functional microtissues obtained from normal bronchial cells, cultured at the air-liquid interface. Due to the fact that literature present few reports regarding the fabrication of MIONPs through green synthesis approach with anticancer potential against lung cancer, but none study regarding the use of green tea or basil as plant material for

MIONPs biosynthesis and their potential applications in lung cancer, we consider that our research study will be of extremely scientific importance.

The effect of both green raw materials as reducing and capping agents were taken into account for the development of MIONPs, as well as the reaction synthesis temperature (25°C and 80°C). The physico-chemical analysis showed that, regardless of the type of plant extract, the samples prepared at 25°C contain a mixture of Fe_3O_4 and γ-Fe_2O_3 NPs, while the synthesis at 80°C led to the formation of Fe_3O_4 NPs as a unique phase. The electron microscopy exhibited that the formed MIONPs have nearly spherical shape with narrow size under 8 nm. Moreover, the MIONPs diameter statistics, determined from TEM and SEM images, was in accordance with the crystallite size, determined by XRD analysis and with their magnetic diameter. The magnetic measurements demonstrated the strong saturation magnetization, around 60 emu/g in the case of the samples prepared from green tea ethanolic extract, which makes this magnetic nanoparticles being suitable also for hyperthermia applications as well as for drug delivery.

The biological effect of the MNPs obtained from *Camellia sinensis* L. ethanolic extract was compared with that of the MNPs obtained from *Ocimum basilicum* L. ethanolic extract, by using two morphologically different lung cancer cell lines (A549 and NCI-H460); the results showed that the higher cell viability impairment was manifested by A549 cells after exposure to MNPs obtained from *Ocimum basilicum* L. ethanolic extract. Regarding the biosafety profile of the MNPs, it was shown that the EpiAirway^{TM} models did not elicit important viability decrease or significant histopathological changes after treatment with none of the MNPs, at concentration up to 500 µg/mL.

In summary, the data revealing that the MIONPs are more active on A549 cells compared to NCI-H460 cells. Nevertheless, the biosafety level of MIONPs is good, the EpiAirway^{TM} microtissues manifesting viabilities above 80% with no significant histopathological changes.

Taking into account all the promising features presented above, the newly synthesized MIONPs can be considered suitable candidates for development of nanotechnology-enabled formulations for lung cancer treatment.

4.5. BIOLOGIC IMPACT OF SOLID-LIPID NPs (SLNs) ON HUMAN LUNG CARCINOMA (A549) AND ON 3D NORMAL BRONCHIAL MODEL – EPIAIRWAY^{TM} MICROTISSUE

In ***Chapter 6*** are depicted the investigations regarding the biologic impact of the solid-lipid nanoparticles (SLNs) loaded with a pentacyclic triterpene (oleanolic acid – OA), derived from the MIONPs synthesized by green method and highly detailed in the previous chapter. As concerns the biologic impact, it was followed to determine the anticancer potential on lung cancer 2D cell line – human lung carcinoma - A549 cells, as well as the biosafety profile of the newly synthesized SLNs, by using the EpiAirway^{TM} 3D microtissue model, obtained from normal bronchial cells.

The outcomes revealed that the human lung carcinoma cell line (A549) was strongly affected after treatment with both SLNs samples originated from green tea (Cs); the viability rates induced by the SLNs were around 50%. Moreover, after an exposure of 24h at SLN_Ob 25 and SLN_Ob 80, the A549 cells were significantly affected, manifesting a viability of around 28% and 18%, respectively. As concerns the biosafety profile of SLNs samples, the outcomes exhibited a negative impact on the viability of 3D normal bronchial microtissues (below 50%) after exposure to all the test samples (SLN_Cs 25, SLN_Cs 80 and SLN_Ob 25, SLN_Ob 80). This aspects were also highlighted after the histological investigation regarding the morphological changes of SLNs on 3D respiratory tissues model. All the samples induces major changes such as loose of the surface epithelium integrity, loose of epithelial junctions, loose of cilia, hyperkeratosis and the cells death occurs by apoptosis. It is very likely that the culprit for the negative impact on viability and morphology of 3D normal bronchial microtissues, to be the too high dose (500 µg/mL) used. Neverthels, further adjustments in the SLNs synthesis process as well as another complex in vitro evaluation will be taken into consideration.

The PhD thesis is based on the original results obtained from three research articles, in which the undersigned is the first author, published as follows:

- **The data presented in Chapter 2, are the subject of an ISI article, published in Nanomaterials Journal from MDPI Publisher, Journal Rank Q1 (I.F. = 5.719)**.

The most important discoveries from this study, is based on the fact that MNPs were developed by the combustion method with a narrow diameter, which were the basis for obtaining stable, biocompatible, magnetic colloidal suspensions and it was demonstrated that the obtained MCS have a hydrodynamic diameter below 100 nm, a good polydispersivity index and a very negative zeta potential, which denotes the stability and biocompatibility of the obtained colloidal suspension. As regards the heating capacity of the MCS, the obtained value demonstrates that it can be successfully used in magnetic heat generation

- **The data presented in Chapter 3, are the subject of another ISI article, published in Magnetochemistry Journal from MDPI Publisher, Journal Rank Q2, (I.F. = 3.336)**.

As regards this study, for the first time, MIONPs were obtained via green chemistry approach, starting from two aqueous extracts of wormwood, based on leaves and stems. It was demonstrated that depending on the synthesis conditions (temperature, metal precursor used, reducing agent used, precipitation agent, etc.), MIONPs with excellent magnetic properties as well as with high value of specific absorption rate (SAR), can be obtained through green synthesis approach. In addition, it was demonstrated that by optimization of the synthesis procedure, increase the nanoparticles' magnetic diameter and implicitly the AC magnetic heating efficiency, due to the fact that the practical applications of nanoparticles in hyperthermia are limited by their sizes.

- **The data presented in Chapter 4, are the subject of the third ISI article, published in Nanomaterials Journal from MDPI Publisher, Journal Rank Q1, (I.F. = 5.719)**.

As concerns this study, for the first time, it was reported the synthesis of α-Fe_2O_3 NPs starting from aqueous extract of Artemisia species, based on leaves and stems. Considering the biological impact, it was demonstrated that the newly synthetized IONPs showed promising potential for biomedical applications. Moreoveer, it was showed that the phyto-mediated α-Fe_2O_3 NPs did not show cytotoxic activity on non-tumorigenic immortalized human keratinocytes (HaCaT), but the tumorigenic cell lines, especially A375 cells were significantly affected.

- **The data presented in Chapter 5, are submitted for publication in Pharmaceutics Journal from MDPI Publisher, Journal Rank Q1, (I.F. = 6.525)**.

As concerns this study, for the first time, it was reported the fabrication of MIONPs through green synthesis approach with anticancer potential against lung cancer. It was demonstrated the strong saturation magnetization (60 emu/g) obtained for the samples prepared with green tea ethanolic extract, which underline their unique features for hyperthermia applications as well as for drug delivery. In addition, it was revealed the biosafety profile of the MIONPs synthesized by green method, using the EpiAirway^{TM} model. It was not show important viability decrease or significant histopathological changes after treatment with none of the green MIONPs.

- **The data presented in Chapter 6 are still work in progress**.

In the initial phase, it was demonstrated the high antitumoral effect of the SLNs based on green MIONPs loaded with OA. The viability rates were under 50%, which means that the human lung carcinoma cell line (A549) were significantly affected. As concerns the biosafety profile of the SLNs using the Epiairway^{TM} model, all the samples induces major changes such as loose of the surface epithelium integrity, loose of epithelial junctions, loose of cilia, hyperkeratosis and the cells death occurs by apoptosis. But, with all these, further adjustments in the SLNs synthesis process as well as another complex in vitro evaluation will be taken into consideration.