UNIVERSITY OF MEDICINE AND PHARMACY

"CAROL DAVILA", BUCHAREST

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MEDICINE FIELD

Colon cancer – a new approach.

PhD THESIS SUMMARY

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List of published scientific papers

Papers published in specialized journals (selection)

1. **Prodan AM**, Iconaru SI, Predoi MV, Predoi D, Motelica-Heino M, Turculet CS, et al. Silver-Doped Hydroxyapatite Thin Layers Obtained by Sol-Gel Spin Coating Procedure. *Coatings*, 10, 14, 2019. https://doi.org/10.3390/coatings10010014. ***IF = 2.436***
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Patents

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1. Project PN II 131/2014: "Study by immunofluorescence of the effect of functionalized iron oxide nanoparticles on malignant rectal tumor cells (DIAGTHR)"; - **member of the research team**
2. Project PNII 259/2014: "Bioceramic composites with local applications in antibacterial therapy (HAPAGTHR)" - **member of the research team**
3. Project 43 / PCCDI / 2018: "Innovative bionanomaterials for treatment and diagnosis (BIONANOINOV)". Component projects: "Bionanomaterials for tissue regeneration, diagnosis, prevention and treatment of osteoporosis", "Intelligent nanocomposites for

the diagnosis and treatment of bone cancer"; "Nanostructured Probes for Diagnosis" - **member of the research team**

4. Project POCU / 468/4/9/128106: "ROCCAS Development and implementation at national level of the organizational framework necessary to initiate screening in colorectal cancer"; - **member of the research team**
5. Project PN-III-P2-2.1-PED-2019-1375: "Development of new antiseptics based on zinc oxide for the clinical treatment of wounds" (MANAGEWOUND) "- **project director**

GENERAL INTRODUCTION

Recent studies have shown that colon cancer ranks the third most common cause of death, following cardiovascular diseases [1]. We know that many factors are involved in the onset of this type of cancer, but the most important are the "western" lifestyle, especially if we talk about developing countries, obesity, lack of physical activity, sustained consumption of red meat, alcohol and tobacco [1,2]. All of this has led scientists around the world to work hard to improve both diagnostic and treatment methods [3]. In the last century, the treatment of colon cancer has undergone an important evolution, both in terms of surgical treatment and in terms of radio and chemotherapy treatment.The genetic testing has brought a major benefit in terms of diagnostic methods.

The twentieth century brought a number of neoadjuvant treatments for colon cancer, but in addition to the benefits of these treatments, there were a number of side effects, which led researchers to seek solutions to limit these inconveniences. This has led to the emergence of a new branch of medicine called nanomedicine, which has found applicability both in the diagnosis of diseases and especially in their treatment, including neoplasms.

The results of studies on nanoparticles have shown that due to their unique properties, chemical, physical, optical, electronic and magnetic, nanoparticles can be used successfully in biotechnology and biomedicine. There are a number of advantages to using these nanoparticles, such as improved solubility, reduced rate of degradation of administered substances, low systemic toxicity all leading to improved clinical efficacy. The magnetic nanoparticle systems that receive the most attention from the medical sphere are iron oxides (magnetite and maghemite), due to their ease of preparation and stability to oxidative stress. In addition, they have a high biocompatibility.

Due to the large number of colon tumors that occur annually [1], the mortality caused by this disease, and the problems caused by the treatment of these tumors, we decided to take a multidisciplinary approach to this type of cancer, based on innovative therapies. With all current studies in the field, we must specify that the most important existing data on the behavior of nanoparticles in vivo are based on data obtained from animals, and their application to humans is a growing field of research.

In this study, the in vivo toxicity of iron oxide nanoparticles was tested on laboratory animals. Subsequently, in vivo studies were performed with iron oxide nanoparticles (γ-Fe2O3) functionalized with the therapeutic drugs 5-Fluorouracil (5-FU) and Doxorubicin (DOX), in order to study their effects on both different types of tumors. induced in laboratory animals as well as on their organs. This paper is structured in four chapters.

Chapter I is dedicated to the general presentation of data from the literature on colon cancer. We have made a detailed documentation of the current methods of diagnosis and treatment used in the case of this neoplasm. General data on innovative therapies used in the treatment of colon cancer are also presented. The types of nanoparticles currently used in medical applications, their magnetic properties, the general principles of multifunctional nanoparticles, therapeutic and diagnostic strategies are described in detail.

Chapter II presents the preparation methods and techniques used for morphological and structural characterization of iron oxide and iron oxide functionalized with chemotherapeutic drugs. Also, in this chapter are presented the results of physico-chemical studies, haematological analyses as well as the results of in vitro cytotoxicity studies performed on various cell lines. Thus, the in vitro behaviour of iron oxide nanoparticles and iron oxide nanoparticles functionalized with chemotherapeutic drugs is presented. For the study of the size, chemical structure and morphology of the samples we used: transmission electron microscopy (TEM), high resolution transmission electron microscopy (HRTEM), electron diffraction on selected area (SAED) and scanning electron microscopy (SEM).

Fourier transform infrared spectroscopy (FTIR) studies were also used to demonstrate the presence of specific peaks of both iron oxide and chemotherapeutics in the analysed samples. Also, here are presented the influence of maghemite nanoparticles on cell viability depending on the concentration of nanoparticles, as well as the time of exposure of cells to the action of nanoparticles.

Chapter III presents original results obtained by *in vivo* testing of iron oxide nanoparticles and iron oxide particles functionalized with chemotherapeutic agents, 5-FU and DOX, respectively. The influence of the magnetic field was also studied, when it is applied on tumors in which γ-Fe_2O_3 functionalized with DOX and 5-FU were injected. The results obtained in this study highlited the biocompatibility of γ-Fe_2O_3 particles. Studies have shown that, unlike simple chemotherapy, γ-Fe_2O_3 functionalized with chemotherapeutics, together with the

administration of an electromagnetic field, helps to reduce the size of the tumor and prevents the appearance of metastases in laboratory animals.

Chapter IV is dedicated to conclusions and personal contribution. Here, PhD student Alina Mihaela Prodan presents the original results obtained in this study and emphasizes the need to develop further studies on human subjects, in order to understand the interactions between these nanoparticles and the human body and the challenges related to their chemistry.

CHAPTER 2: CONTRIBUTIONS TO THE PHYSICO-CHEMICAL AND BIOLOGICAL STUDIES OF IRON OXIDE WITH POTENTIAL APPLICATIONS IN THE TREATMENT OF COLON CANCER

Recently, special attention has been focused on the study of several types of iron oxide nanoparticles (magnetite (Fe_3O_4) and maghemite (γ-Fe_2O_3)), due to its special properties. Biocompatible, water-based magnetic fluids, have been intensively studied in order to develop new biomedical applications, such as the administration of targeted drugs, magnetic resonance imaging, intracellular hyperthermia therapy, etc. A number of nanoparticles based on iron oxide nanoparticles are now approved by the US Food and Drug Administration (FDA) and are used successfully in the medical field (eg. Feridex I.V).

Iron oxide suspensions (P1) and iron oxide functionalized with 5FU suspensions (P3) were obtained by an adapted co-precipitation method. Under the conditions described above, suspensions based on iron oxide functionalized with doxorubicin were also obtained.

The physico-chemical properties of the obtained samples were analyzed using dynamic light scattering (DLS) and Zeta Potential studies; Transmission electron microscopy (TEM); Scanning Electron Microscopy (SEM) and Fourier Transform Infrared Spectroscopy (FTIR). The biocompatibility of both iron oxide and iron oxide functionalized with a chemotherapeutic agent suspensions has also been studied by *in vitro* studies on several cell lines (Caco-2 (HTB-37, ATCC) - cells human colorectal adenocarcinoma; HeLa cell line; primary human osteoblast cells (obtained from the upper part of the femur). Moreover, the blood of rats injected with P3 solution was studied by haematological and biochemical analyses. The average nanoparticle size of samples P1, P2 and P3 determined by DLS measurements was estimated to be around 47 nm (P1), 58nm (P2) and 67nm (P3). As can be seen, the hydrodynamic diameter of the nanoparticles increased in the case of P3 sample, where we have maghemite nanoparticles functionalized with 5-Fluorouracil. The results of the SEM studies regarding the morphology of P2 and P3 samples are shown in Figures 2.4. Therefore, it can be seen that the particles have nanometric dimensions, with a pronounced tendency to agglomerate.

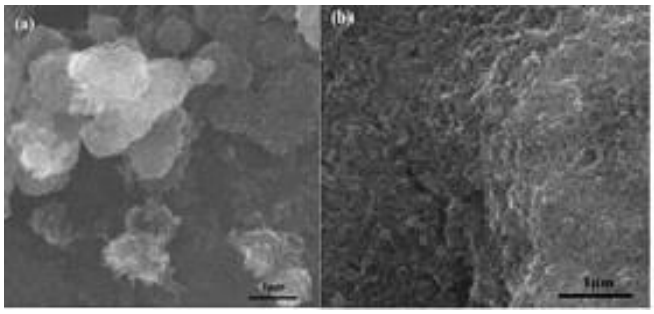

Figure 2.4. SEM micrographs specific to P2 (a) and P3 (b) suspension.

In the EDX spectra obtained on P3 sample (Figure 2.7.) can be observed both the constituent elements of 5-FU (C, F, N, O) and the chemical elements specific to the iron oxide structure (Fe and O). There are also noticed peaks that are assigned to the chemical elements characteristic to the sample-holder on which the sample was placed in order to be analysed.

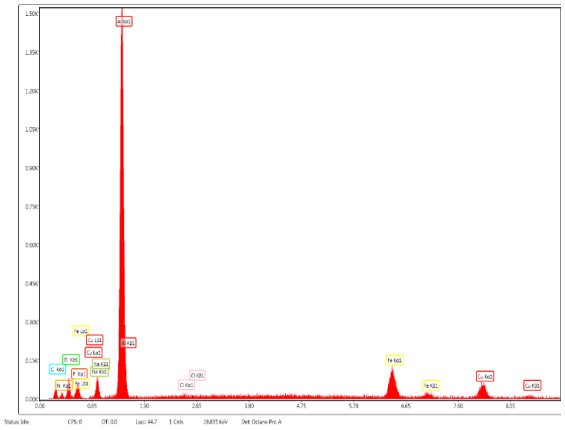

Figure 2.7. EDX spectra characteristic of iron oxide / 5-FU suspension (P3).

The FTIR spectra obtained on the analysed samples highlight the presence of maxima specific to the vibrational groups in the structure of iron oxide / 5-FU. The cell viability was evaluated by the MTT quantitative test. Cellular viability studies have shown that the exposure of colon tumor cells to ɣ-Fe_2O_3 nanoparticles, in concentrations between 0.1 - 100 µg / mL Fe did not induce a decrease in cell viability, thus demonstrating their enhanced biocompatibility.

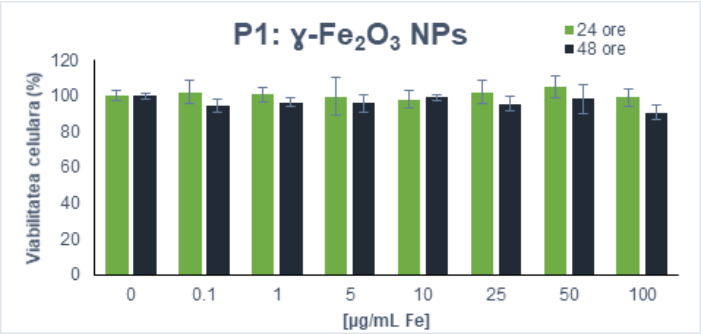

Figure 2.18. Viability of Caco-2 cells after exposure for 24 and 48 hours at different concentrations of NPs (between 0.1 and 100 µg / mL Fe).

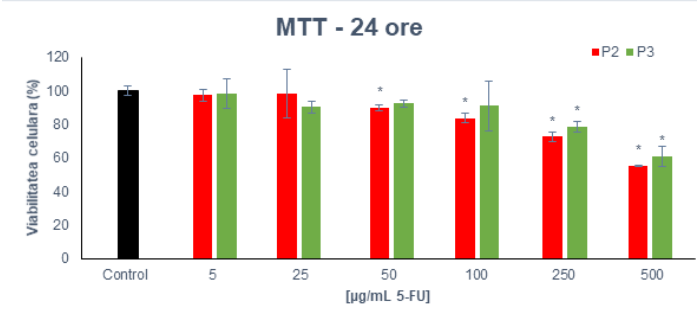

Figure 2.19. Viability of Caco-2 cells after exposure for 24 hours to different concentrations of 5-FU between 5 and 500 µg / mL of P2 and P3 samples. Statistically significant results were considered those with the value of p <0.05 (*).

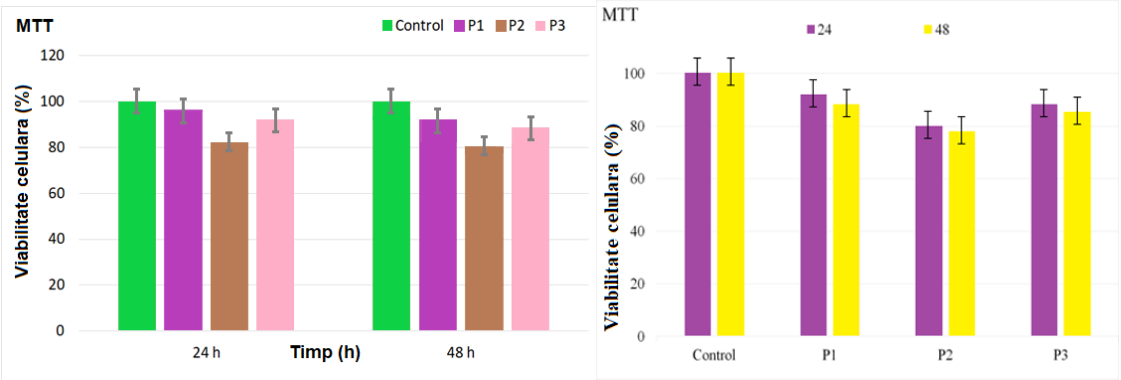

Figure 2.21. Viability of HeLa cells after incubation for 24 and 48 h with samples P1-P3 (left). Viability of osteoblast cells after incubation for 24 and 48 h, with samples P1-P3 (right).

Moreover, the results of the MTT test showed that, after 48 hours of incubation, a slight decrease in the viability of HeLa cells was observed for the three samples. The results also showed that the viability of osteoblast cells was dependent not only on the type of samples but also on the incubation period. Microscopic analysis of untreated (C) and treated Caco-2 cell for 24 and 48 hours with suspensions (250 μg / mL) of NPs and 5-FU revealed significant changes in tumor cell morphology. As can be seen in Figure 2.24. these cells showed a damaged morphology compared to the control cells. The appearance was characteristic of apoptotic cells. Moreover, a decrease in cell density was observed, which confirmed the results obtained by MTT test. On the other hand, cells that were exposed to iron oxide nanoparticles had a normal morphology, with a regular contour and a well-defined edge similar to untreated cells (control).

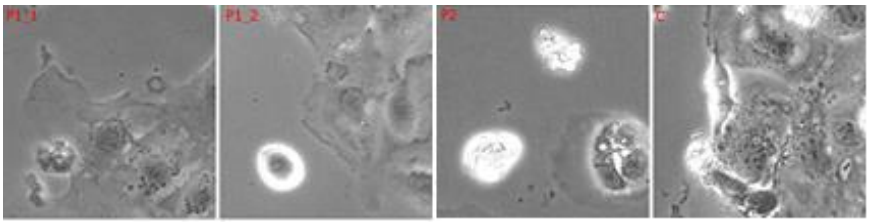

Figure 2.24. Untreated (C) Caco-2 cells and treated Caco-2 cells for 48 hours with suspensions of NPs and 5-FU (250 μg / mL). 20X magnification.

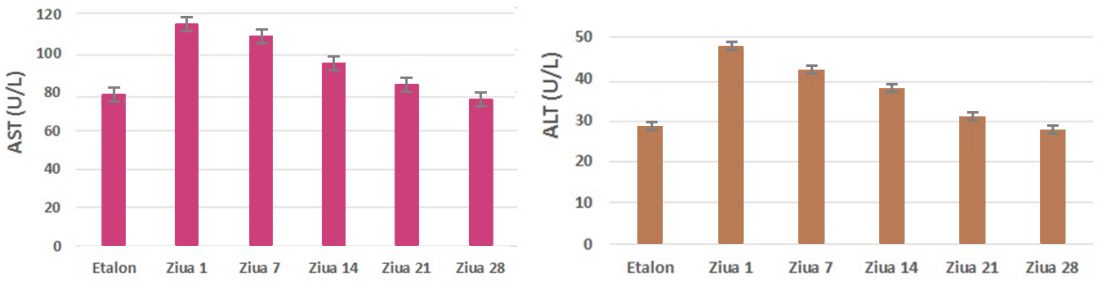

Figure 2.36. Hepatic function of rats, assessed by aspartate aminotransferase (AST) - left and by alanine aminotransferase (ALT) -right.

We can conclude that the haematological and biochemical studies performed in this PhD thesis did not show significant changes in the case of the studied biochemical parameters. The results obtained highlighted the fact that samples P1 and P3 are good candidates to be used in the development of biomedical devices with applicability in the cancer treatment [270-274].

CHAPTER 3: CONTRIBUTIONS TO THE *IN VIVO* STUDY OF THE EFFECT OF CHEMOTHERAPEUTIC FUNCTIONED IRON OXIDE NANOPARTICLES

The purpose of this study was to develop a delivery system for chemotherapeutic drugs that would strictly target tumor tissue. In this sense, we synthesized iron oxide nanoparticles (γ-Fe_2O_3), which we tested both in vivo and in vitro, to determine their biocompatibility in tissues. Subsequently, we functionalized these nanoparticles with two chemotherapeutic drugs often used in the treatment of colorectal cancer, namely 5-Fluorouracil (5-FU) and Doxorubicin (DOX).

3.1. DETERMINATION OF γ-Fe_2O_3 NANOPARTICLES TOXICITY

As previously mentioned, in order to determine the level of toxicity exerted by γ-Fe_2O_3 nanoparticles in the body, we performed in vivo experiments on laboratory animals. Nude, adult, male mice, weighing ~ 30 ± 5 g were used. These mice are immunosuppressed due to the absence of thymus and due to a small number of T cells, which favors the rapid development of tumors. The animals were purchased from the National Research and Development Institute for Microbiology and Immunology "Cantacuzino", Bucharest. In accordance with the NIH Guide for the Care and Use of Laboratory Animals, prior to the experiments, all mice were housed in identical conditions, had a standard diet, were exposed to a cycle of 12 hours of light, 12 hours of darkness, were kept in an environment with controlled temperature and humidity (60 ± 10%). All animals were kept in a pathogen-free environment. To analyze the biodistribution of nanoparticles and iron oxide, respectively, in vivo, nude mice were divided into two groups (n = 4) and a control group. Subsequently, the laboratory animals were injected into the peritoneal cavity with a suspension containing γ- Fe_2O_3, in saline. Concentrations of γ-Fe_2O_3 of 1 and 1.5 ml / kg were used. After injection of these suspensions, toxicity was studied at different time intervals, respectively 24h and 72h. In this sense it was performed histopathological analysis of various tissues and organs, such as liver, kidney and spleen, taken from nude mice injected with these suspensions.

Organs taken from nude mouse were placed in plastic containers, then fixed with 10% formaldehyde. The containers were labeled with the data of each laboratory animal. For each laboratory animal we made a sampling sheet, on which we wrote the following data:

identification data of the laboratory animal, date of tissue sampling, date of receipt in the histopathology laboratory, type of organ taken, solution used for fixation, sending doctor, the type and purpose of the required examination.

Histopathological analysis, respectively the influence of γ-Fe_2O_3 nanoparticles on the sampled tissues was performed on paraffin-coated glass slides, on which hematoxylin and eosin were deposited. The interpretation of the histopathological result was analyzed by an anatomopathologist from the Bucharest Emergency Clinical Hospital, being subsequently recorded in the accompanying sheet of the laboratory animal. We specify that after the administration of γ-Fe_2O_3 all the laboratory animals used in this experiment survived. They also showed no change in behavior, weight loss or other side effects.

The comparison of the two groups, respectively the group in which the γ-Fe_2O_3 suspension was administered, with the control group, which was administered physiological serum, after 24 and 72 h after the intraperitoneal injection, did not show irreversible histopathological changes in the organs taken, for none of the tested concentrations. Considering the results obtained by injecting the γ-Fe_2O_3 suspension in the peritoneal cavity, we decided to continue the studies and test the effect of γ-Fe_2O_3 nanoparticles. by intratracheal instillation, in concentrations similar to those administered intraperitoneally.

In this regard we used a similar number of laboratory animals, as in the previous experiment. We administered these animals intratracheally, γ-Fe_2O_3, in different concentrations, respectively 1 and 1.5 ml / kg. As with intraperitoneal administration of γ-Fe2O3, at the end of the experiment, the animals were sacrificed and the organs were analyzed histopathologically in order to determine the effect of γ-Fe_2O_3 nanoparticles, administered intratracheally on the organs.

We mentioned that all laboratory animals survived the administration of γ-Fe_2O_3, at all concentrations tested and showed no signs of discomfort, such as vomiting or diarrhea, throughout the experiment. We analyzed the changes induced by γ-Fe_2O_3 on lung, liver and splenic tissue. As with intraperitoneal administration, these changes induced by γ-Fe_2O_3 nanoparticles were dependent on the concentrations used and the time elapsed since the nanoparticles were instilled. As demonstrated by histopathological analysis of organs, there is no toxic effect of γ-Fe_2O_3 nanoparticles, exerted on tissues and cells at 24 and 72 hours after administration of 1 and 1.5 mg / kg γ-Fe_2O_3, respectively. Our results demonstrate that the

administration of iron oxide nanoparticles by intratracheal instillation has no toxic effect on the analyzed tissues. Once the biocompatibility of iron oxide particles was established, we decided to test them in the treatment of tumors induced by laboratory animals.

3.4. STUDY OF THE EFFECT OF γ-Fe_2O_3 NANOPARTICLES. FUNCTIONALIZED WITH 5-FLUOROURACIL (5-FU) AND DOXORUBICIN (DOX).

One of the chemotherapeutic drugs used in the treatment of colon cancer is Doxorubicin (DOX) [331]. This drug is used in the treatment of several types of cancer, such as colon cancer, breast cancer, bladder cancer, lung cancer, ovarian cancer [332], having a broad antitumor spectrum [333]. Although this drug is considered an effective chemotherapeutic, its widespread use has led to decreased efficacy, leading to resistance and numerous side effects throughout the body, such as nephrotoxicity, inhibition of medullary hematopoiesis function and cardiotoxicity [333-334]. In order to test the suspension of γ-Fe_2O_3, functionalized with Doxorubicin, in vivo we used nude mice. Tumors were induced in laboratory animals by injecting colorectal tumor cells, HT 29. To obtain these tumors we injected 19 nude mice, at the level of the anterior thigh region, with $7x10^6$ colorectal neoplastic cells, HT 29. They had a weight of 25 ± 5 g. They were purchased from the National Institute of Research-Development and Immunology "Cantacuzino", Bucharest. Throughout the experiment, the nude mice were kept according to the NIH guide for the care and use of laboratory animals. About three to four weeks after the injection of the tumor cells, tumors were obtained, with sizes ranging from 1-2 cm to the anterior region of the thigh.

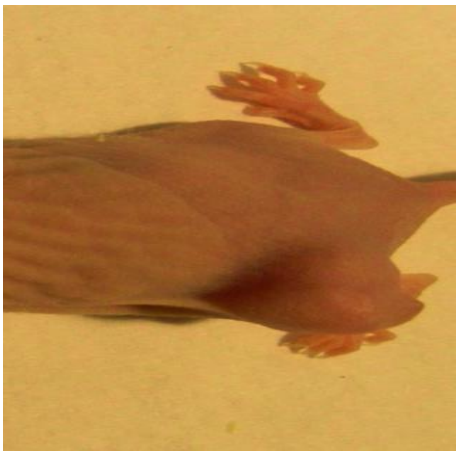

Figure 3.35. Tumor obtained one month after inoculation of 7x106 colorectal neoplastic cells, HT 29, in the anterior region of the thigh..

In this study we wanted to analyze both the effect of γ-Fe_2O_3, functionalized with Doxorubicin, and the effect of the magnetic field on these tumors. The application of a magnetic field on the neoplastic areas was done with the help of an instrument made by the company Nuclear NDT, within the research project 131-PN-II-PT-PCCA-2013-4-0006. The resulting heat can be used for various applications. It induces apoptosis of tumor cells by hyperthermia. It also helps speed up the release of antitumor drugs.

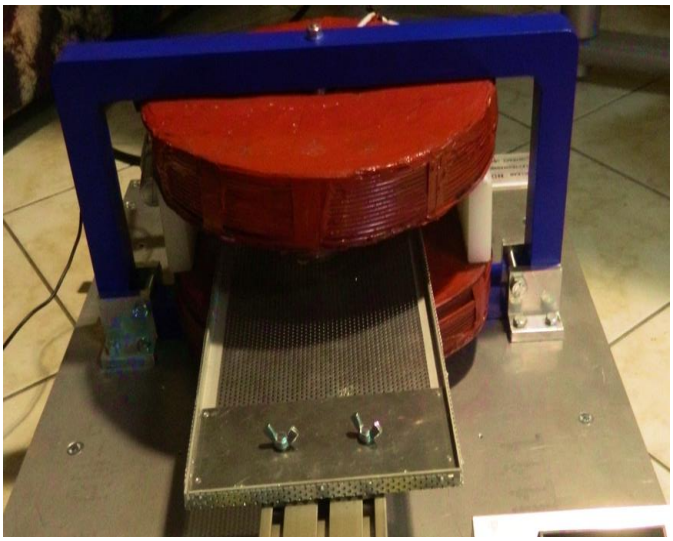

Figure 3.36. Electromagnet made by Nuclear NDT company, within the research project 131- PN-II-PT-PCCA-2013-4-0006 [335].

The tests performed on laboratory animals were performed according to several parameters, namely: the concentration of the solutions used, the time elapsed since the administration of the solution and the magnetic field applied. We divided the laboratory animals into eight groups (n = 2 animals / group). They were injected according to the following protocol: the first two groups were injected with a solution 1, respectively 1.5 mg / kg suspension of γ-Fe_2O_3 functionalized with Doxorubicin (γ-Fe_2O_3 _DOX), those in groups 3 and 4 were injected with the same suspension quantities. In addition, a magnetic field produced by a coil supplied at a voltage of 8 V was applied to these two groups. Groups 5 and 6 were injected with 1 and 1.5 mg / kg suspension of γ- Fe_2O_3 functionalized with Doxorubicin (γ- Fe_2O_3_DOX), respectively. A magnetic field produced by a coil supplied at a voltage of 12 V was applied to

them. Groups 7, 8 were injected with simple Doxorubicin (DOX), 0.5 and 1 mg / kg, respectively. A mouse was considered a standard.

The obtained solutions were administered at 24 h, 72 h, 7 days and 10 days by injection to the tumor 0.5ml γ-Fe_2O_3 and 0.5ml DOX were used to obtain the injectable solutions, respectively 0.5ml γ-Fe_2O_3 and 1ml DOX. At each intratumoral administration of the suspensions, respectively 24h, 72h, 7 days and 10 days, groups 3-6 were exposed to an electromagnet, to which different voltages were applied, respectively 8 V and 12 V. The maximum intensity of the magnetic field was oriented at the level of the tumor region. Exposure time was 7 minutes. At the end of the experiment, 14 days after the start of treatment, the animals were euthanized and their organs removed.

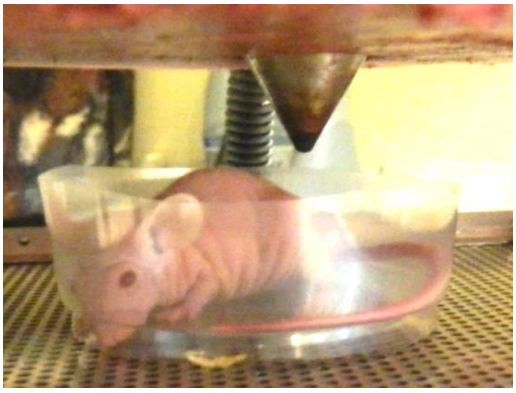

Figure 3.38. Targeted application of the magnetic field on the tumor region [335].

In order to identify the effect of the administered substances, as well as the magnetic field, on the tumor formations and organs of laboratory animals, we performed their histopathological analysis on liver, kidney and splenic tissues. We mention that after the administration of the suspensions, all the laboratory animals used in this experiment survived. No changes in animal behavior, such as weight loss or other side effects, were detected. Both in the administration of the solutions and in the exposure of the laboratory animals to the magnetic field, we used 0.01ml / Kg Xylazine for anesthesia. Comparing the groups in which we administered simple Doxorubicin, as well as the groups in which γ-Fe_2O_3_DOX was administered and subsequently a magnetic field, with the standard, we did not find macroscopic changes in the organs taken.

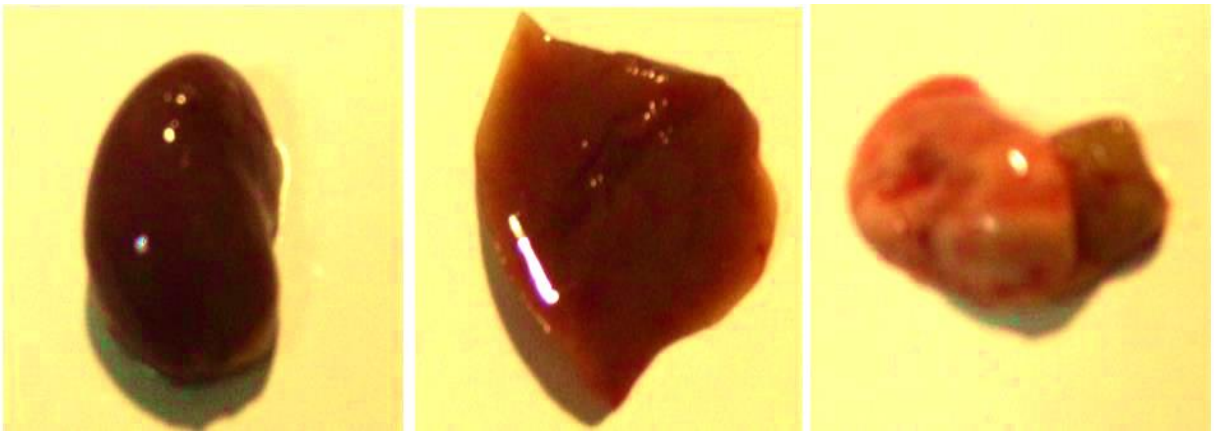

Figure 3.39. Macroscopic appearance of the kidney, liver, and lung 14 days after injection of a solution of γ -Fe_2O_3_DOX and magnetic field supplied at a voltage of 12 V.

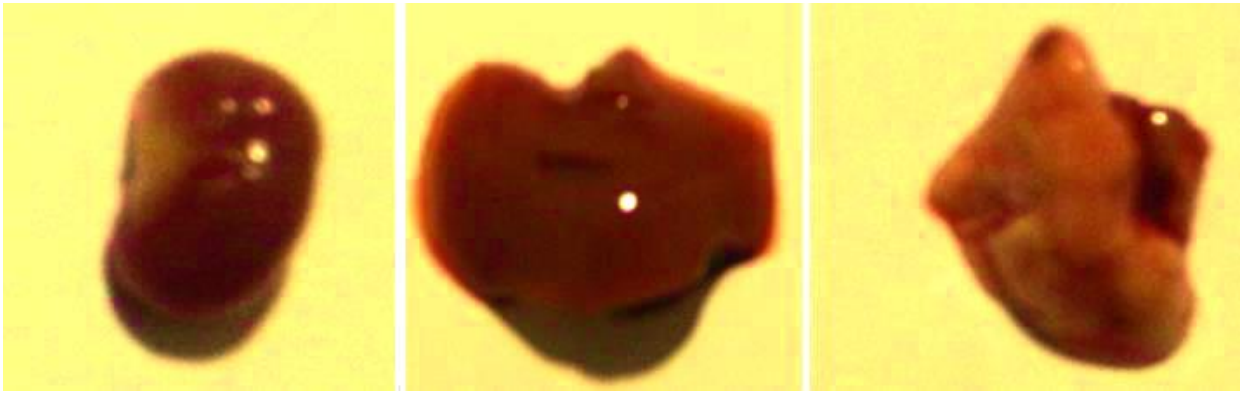

Figure 3.40. Macroscopic appearance of the kidney, liver, and lung 14 days after injection of a DOX solution.

Figure 3.41. Appearance of the tumor 7 days after injection of a solution of γ-Fe_2O_3_DOX and application of a magnetic field obtained after application of a voltage of 12V.

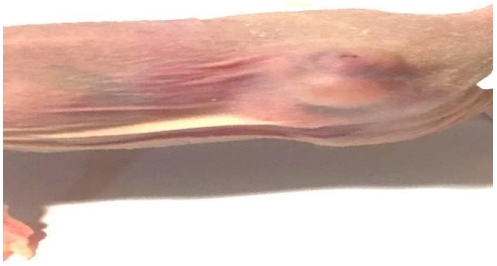

Figure 3.42. Appearance of the tumor 14 days after injection of a solution of γ-Fe_2O_3_DOX and application of a magnetic field obtained after application of a voltage of 12V.

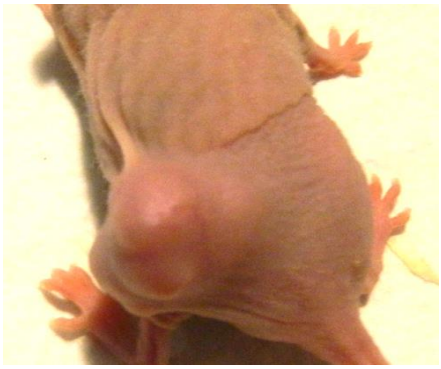

Figure 3.43. Appearance of the tumor 14 days after injection of a simple Doxorubicin solution.

Histopathological analysis of tissues taken from laboratory animals did not identify any changes in renal tissue in the laboratory animals analyzed after administration of γ-Fe_2O_3 _DOX and Doxorubicin simple at any of the concentrations tested. Pathological changes were observed and no secondary determinations appeared in the case of the groups in which γ-Fe_2O_3_DOX and magnetic field were administered, supplied with a voltage of 8V and 12V, respectively.

In the case of laboratory animals given simple Doxorubicin, we found both an increase in the size of the tumor formation, 14 days after the start of treatment, and the appearance of secondary determinations in the lung and liver tissue, in the case of two nude mice.

Following the experiments performed on laboratory animals to which we injected suspensions of γ-Fe_2O_3 _DOX and after applying a magnetic field produced by a coil fed at a voltage of 8 and 12 V, respectively, we found a decrease in tumor size and lack of secondary

determinations to the other organs. To study the effect of γ-Fe_2O_3 functionalized with 5-FU we used an identical number of laboratory animals, as in the case of Doxorubicin. We used the same tumor-obtaining protocol as for Doxorubicin testing. Treatment was started 4 weeks after injection of HT 29 colorectal neoplastic cells into the anterior thigh region of laboratory animals. The suspensions were administered according to the following protocol: the first two groups were injected with a solution 1, respectively 1.5 mg / kg suspension of γ-Fe_2O_3 functionalized with 5-FU (γ-Fe_2O_3 _5-FU), those in groups 3 and 4 were injected with the same amounts of suspension. In addition, a magnetic field supplied with a voltage of 8V was applied to these two groups. Groups 5 and 6 were injected with 1 and 1.5 mg / kg suspension of 5-FU-functionalized γ_Fe_2O_3 (γ_Fe_2O_3 _5-FU), respectively.

A magnetic field produced by a coil supplied at a voltage of 12 V was applied to them. Groups 7, 8 were injected with simple 5-FU, 0.5 and 1 mg / kg, respectively. A mouse was considered a standard. The obtained suspensions were administered at 24 h, 72 h, 7 days and 10 days by injection to the tumor. 0.5ml γ-Fe_2O_3 and 0.5ml 5-FU, respectively 0.5ml γ-Fe_2O_3 and 1ml 5-FU were used to obtain the injectable solutions.

At each application of the treatment, respectively 24h, 72h, 7 days and 10 days, groups 3-6 were exposed to an electromagnet, to which different voltages were applied, 8 V and 12 V. The maximum intensity of the magnetic field was applied to the tumor region. Like in the case of Doxorubicin, the exposure time was 7 minutes. 14 days after the start of treatment, the animals were euthanized and their organs removed.

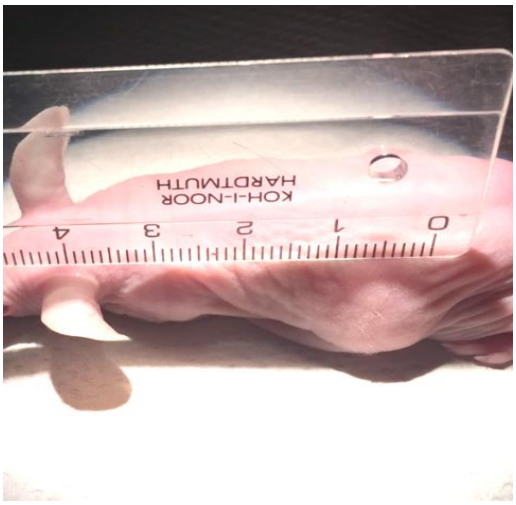

Figure 3.52. Appearance of the tumor 7 days after injection of a solution of γ-Fe_2O_3_5-FU and application of a magnetic field obtained after application of a voltage of 12V.

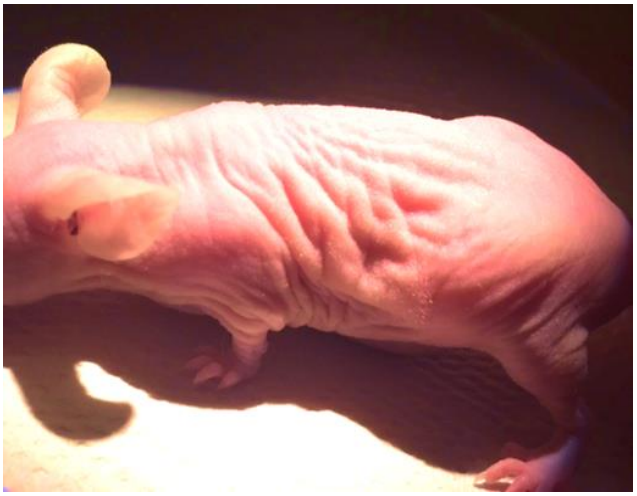

Figure 3.53. Appearance of the tumor 14 days after injection of a solution of γ-Fe_2O_3_5-FU and application of a magnetic field obtained after application of a voltage of 12V.

Two weeks after starting treatment with γ-Fe_2O_3 _5-FU, a decrease in tumor size of approximately 0.5-0.9 cm was observed in all laboratory animals treated with γ-Fe_2O_3 _5-FU and γ-Fe_2O_3 _5-FU and magnetic field obtained after applying a voltage of 8V and 12V, respectively. We also noticed that the animals in the groups that received treatment with γ-Fe_2O_3 _5-FU and simple 5-FU, respectively, recorded a weight loss of 3-5 g, compared to the standard, which kept its weight relatively constant. In terms of survival, no differences were observed throughout treatment. At 14 days all mice were sacrificed and their organs were analyzed histopathologically. No macroscopic changes were found for any of the 8 groups analyzed.

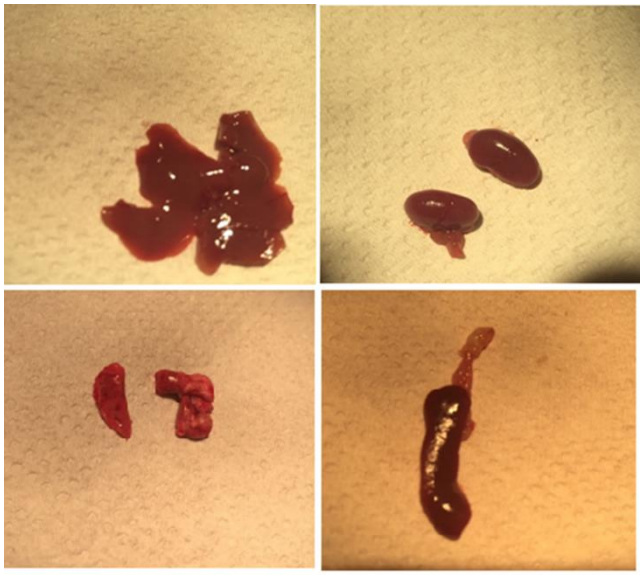

Figure 3.54. Macroscopic appearance of the kidney, liver, and lung 14 days after injection of a solution of γ -Fe_2O_3_5-FU and application of a magnetic field obtained after application of a voltage of 12 V

Anatomopathological tests performed on the kidney, liver, lung and splenic tissues did not identify irreversible changes. Also, unlike the animals in which we administered simple Doxorubicin, in this case we did not observe the appearance of metastases for any of the tested suspensions. The only difference between the administration of 5-FU and γ-Fe_2O_3 _5-FU, together with the magnetic field obtained after the application of a voltage of 8V and 12V, respectively, was represented by the difference in the decrease of the tumor size at the level of the groups. For the groups in which simple 5-FU was administered, the decrease in tumor size was 0.3-0.5 cm, while for γ-Fe_2O_3 _5-FU, administered together with the magnetic field, the decrease in tumor size was between 0.5-1cm.

CHAPTER 4: CONCLUSIONS AND PERSONAL CONTRIBUTIONS

Colorectal cancer is the third most common neoplastic disease in the world, and the number of new patients is constantly growing. Although significant efforts that have been made to improve both surgical treatment and especially chemotherapy, there are currently a number of shortcomings that we have discussed extensively in this PhD thesis. Surgical treatment has made significant progress, with oncology surgeries having a high survival rate.

The present study tried to find a solution in order to improve the effects of the chemotherapeutic medication currently used, and especially to reduce the side effects due to the administration of these drugs. Thus, iron oxide particles at nanometric dimensions and spherical morphology were obtained by an adapted coprecipitation method. Suspensions based on iron oxide functionalized with 5FU and doxorubicin were also obtained.

In the EDX spectra's obtained on the analyzed samples, the presence of the main constituent chemical elements of both 5FU and those of iron oxide is observed, namely: Carbon (C), Fluorine (F), Nitrogen (N), Oxygen (O) and Iron (Fe). Furthermore, the results also showed that samples P1 and P3 did not show high toxicity related to all cell types studied (Caco 2, HeLa and osteoblasts cell line), for any of the time intervals studied (24 and 48 hours) compared to control. In contrast, sample P2 (5 Fluorouracil) showed toxicity for all the studied types of cell and for all the time intervals studied.

The determination of the bicompatibility of γ-Fe_2O_3 nanoparticles was performed by inoculating them, to nude rats by various routes of administration. These studies showed that, both in the case of intraperitoneal and intratracheal administration of γ-Fe_2O_3, the laboratory animals showed no clinical signs of toxicity or changes in the analyzed blood parameters. Also, no irreversible histopathological changes in the tissues were noticed for any of the tested concentrations. In this way we demonstrated that γ-Fe_2O_3 nanoparticles are biocompatible and do not produce macroscopic or microscopic changes in organs. Once the biocompatibility of these nanoparticles has been established, an attempt has been made to determine their efficacy when functionalized with various chemotherapeutics, namely 5-FU and Doxorubicin. Tumors were obtained by injecting nude rats in the anterior region of the thigh with HT 29 cells.

Suspensions of γ-Fe_2O_3 functionalized with 5-FU and Doxorubicin, respectively, were injected at these tumors, at different concentrations and at different time intervals. The influence

of applying a magnetic field on these nanoparticles was also analyzed in order to improve the antitumor effects. The results obtained were encouraging, also demonstrating that nanoparticles functionalized with various chemotherapeutic drugs reduce the size of tumors, without affecting other organs and systems, as shown by the clinical evolution of laboratory animals, histopathological analysis of organs taken from these animals, as well as from the analyzed biological determinations. We have shown that both the functionalization of nanoparticles and their targeting with the aid of a magnetic field have guaranteed a high efficiency of the drug, while reducing side effects. Unlike laboratory animals in which chemically functionalized γ-Fe_2O_3 was administered and a magnetic field was applied, animals that received simple chemotherapy (Doxorubicin) had secondary pulmonary and hepatic determinations. Considering the results of the studies presented in this PhD thesis, we can say that the objectives of the thesis were 100% achieved due to the fact that were obtained nanometer-sized particles with a superior biocompatibility demonstrated by both *in vitro* and *in vivo* studies. .

We believe that the results of our studies could be the basis for future phase I and II clinical trials to determine the efficacy of these nanoparticles in both the treatment and diagnosis of colon cancer in human subjects.

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