Diagnosis and Treatment of Ovarian Cancer using Magnetic Nanoparticles

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Abstract

Nanotechnology is an emerging field that has high potential for improving human medicine giving various opportunities for research and development, one such promising field involves magnetic nanoparticles in cancer detection and treatment.Ovarian cancer(OC) is one of the most common cancers seen in women around the world. To overcome the inefficient side effects, nanotechnology has seen several spotlights in this type of cancer. This paper reviews how to improve the current screening methods and discusses the advances in the diagnosis and treatment of ovarian cancer using magnetic nanoparticles.We have discussed improvements in the detection of CA-125 as well as proposed two new methods using Immunoassay and MRI Imaging for the diagnosis of OC. The review discusses the potential of magnetically induced hyperthermia in chemotherapy, the use of MNPs for targeted tumor cell removal, and the development of site-targeted drug delivery systems using folate receptors. It also discusses a biomimetic RBC vesicle-coated DOX vesicle system (magnetic targeting) for ovarian cancer treatment. The review also explores future developments in this field.

1. Introduction

Nanotechnology is a wide upcoming field that has shown an immense amount of applications in the medical field mainly including targeted drug delivery, implants, pharmaceutical products, tissue engineering, and even the detection and treatment of various diseases including Cancer. Nanotech, in particular nanoparticles, are particles under the range between 1 to 100 nanometers that exhibit different physical and chemical properties compared to the larger materials. Researchers have found that magnetic nanoparticles have been widely applied for in vitro diagnosis and other applications due to their easy functionalization.Their biocompatibility and chemical stability can be improved by altering their surfaces with biocompatible polymers and other functional nanostructures.Nanoparticles are thus designed to mimic or be vastly similar to the molecule present in our body that can easily and safely reach the targeted cells while not causing unwanted damage to the surrounding cells. This can be obtained due to their ability to control their distal location or thermal activation by applying varying magnetic fields to the nanoparticles.

Ovarian cancer is one of the leading causes of death worldwide among women in developed countries. More than 313,959 patients are diagnosed with ovarian cancer out of which more than 207,252 deaths are recorded each year[1]. These terrible statistics are because this cancer is identified only at advanced stages- at stage III or later in more than 60% of the diagnoses where the 5-year survival rate is just 46% whereas if identified at stage I, the 5-year survival rate is 92%[2,3] . By identifying cancer early, it is more likely to respond to treatment, resulting in a better chance of survival as well as a lower cost of treatment. Early detection of cancer and avoiding delays in treatment can significantly improve the lives of cancer patients. The Current screening methods involve detection of cancer antigen-125(CA- 125 or mucin 16(MUC16)) by serum analysis through blood test and transvaginal ultrasonography. But these tests have low sensitivity and false positive rates as CA-125 do not appear in detectable levels in the serum until advanced stages and transvaginal ultrasonography always cannot tell the difference between ovarian cancer and other common conditions(ovarian cysts, endometriosis)[4].

Chemotherapy is the most common treatment plan for any type of cancer. The cytotoxic drugs used in the treatment are nonselective and can have side effects such as damaging the normal cells along with the targeted cancer cells.
Treatment for ovarian cancer usually involves a combination of surgery and chemotherapy or targeted therapies, Targeted therapies involve drugs targeted to the growth or spread of cancer cells depending on the stage.
Magnetic nanoparticles can be used as a drug delivery system loaded with anticancer drugs and can be carried to the targeted cancer cell by an external magnetic field, heating it by oscillating the applied magnetic field, thus releasing the drug
In addition to these recent findings, studies have been conducted on the preparation of magnetic nanoparticles as a theranostic tool, simultaneous diagnosis and therapy.
The main advantage of using MNP is their ability to localize the heat treatment when used as a hyperthermia tool.

2. Pathology of Ovarian Cancer and clinical features

A malignant neoplasm in one or both ovaries are referred to as ovarian cancer (OC).It has the highest death rate among gynecological cancers and is one of the most prevalent. Any kind of cell that makes up the ovary can develop into ovarian cancer. Epithelial, germ cell, stromal, and other types (mesothelial–mesenchymal, mixed cell, and secondary tumors) are distinguished from one another.[6]

Over 90% of ovarian neoplasms arise from the epithelial surface of the ovary, the rest from germ cells or stromal cells. The epithelial neoplasms are classified as serous (30–70%), endometrioid (10–20%), mucinous (5–20%), clear cell (3–10%), and undifferentiated (1%).The serous histotype is the most common type of ovarian carcinoma. It is classified as low grade serous

carcinoma(LGSOC) or high grade serous carcinoma(HGSOC) based on the extent of nuclear atypia and mitosis.[7]

3.Diagnosis of OC with MNPs

Magnetic nanomaterials (NMs) have the advantages of easy recycling, low pollution, economy, environmental protection, high electro-catalytic activity, large specific surface area, and easy functionalization[10]. Utilizing magnetic MNPs in MRI provides a high contrast for generating the most detailed imaging.Magnetic iron oxide (Fe3O4) nanoparticles have been used for MR imaging of the lungs because of their excellent biocompatibility and magnetization as well as their proper drug uptake and release.[8]

3.1 Using Magneto-Nanosensors as Immunoassays

3.1.1 Improving the detection of CA-125

Y. Yue et al. in his research tried to improve on the existing diagnosis method of detecting CA-125 on the serum by overcoming the low specificity , developed a new affinity based assay which uses magnetically self induced assembly technology to construct an electrochemical biosensor. He tried to develop a label-free electro-chemical biosensor based on magnetic $Mg_{0.5}Cu_{0.5}Fe_2O_4$-Au nanocomposites for the sensitive detection of cancer antigen 125 (CA125).

Magnetic $Mg_{0.5}Cu_{0.5}Fe_2O_4$ NPs have super-paramagnetism, which can be magnetically separated by an external magnetic field to achieve pre-enrichment of the object to be measured. Here $Mg_{0.5}Cu_{0.5}Fe_2O_4$ NPs is modified by coating it with Au,since Au-coated nanoparticles have good stability, electrochemical, optical, electromagnetic,and catalytic properties[11].This is magnetically self assembled onto a magnetic glassy carbon electrode (MGCE) without adding any crosslinking agent, which is simple and fast to operate.
MGCE is chosen because of high electrical conductivity, good stability to chemicals, no gas passing through the electrode, high purity,wide potential window, and cheap and easy to obtain. The main advantages of MGCE are reflected in regeneration and easy use, therefore the surface of the electrode can be cleaned by magnetic separation and reused, thus greatly reducing the cost and providing the possibility for a clinical point of care test(POCT).
The biosensor utilizes the DNA-aptamer molecule for specifically recognizing the target substance[9]. Moreover,Au and sulfhydryl modified DNA-aptamer can be assembled via the Au-S bond, thus creating a biocompatible environment.

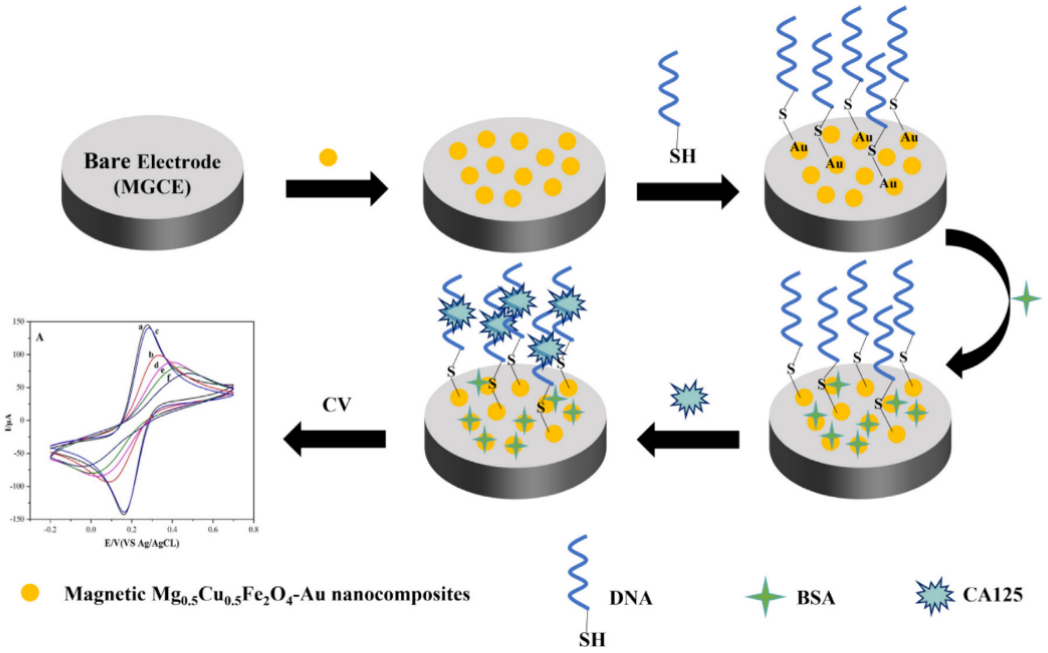


Fig. 1 Construction mechanism diagram of the label-free electrochemical biosensor based on magnetic Mg0.5Cu0.5Fe2O4-Au nanocomposites.

Once the fabrication process of the electrochemical nanosensor is completed, the CA125 antigen was incubated at 37 °C and dropped onto the surface of the modified MGCE for the label free determination of CA-125. The target molecule(CA-125) would automatically bind to the DNA on the electrode surface because of a specific selection of aptamer and antigen. To investigate the detection performance for the nanosensor, Differential Pulse Voltammetry(DPV) is used, which gives the relation between concentration of the molecule and peak current under optimal conditions.

To test the detection performance, DPV responses of the MGCE/Mg0.5Cu0.5Fe2O4-Au/DNA-aptamer/BSA electrochemical biosensor with the increase of CA125 concentration (5–125 U/mL) were investigated, the clinical levels of ovarian cancer patients were covered.It could be noted that the DPV peak current decreased with the increase of CA125 concentration,under the optimal experimental conditions as shown in Fig 2. This kind of response is due to the steric hindrance and non-conductivity of the protein, the electron transfer was reduced and the current signal was reduced as well[12]. Selectivity, stability, and reproducibility were necessary factors to evaluate the efficiency of biosensors. They tested the biosensors for the above mentioned factors, the results affirms the efficiency of the biosensor.

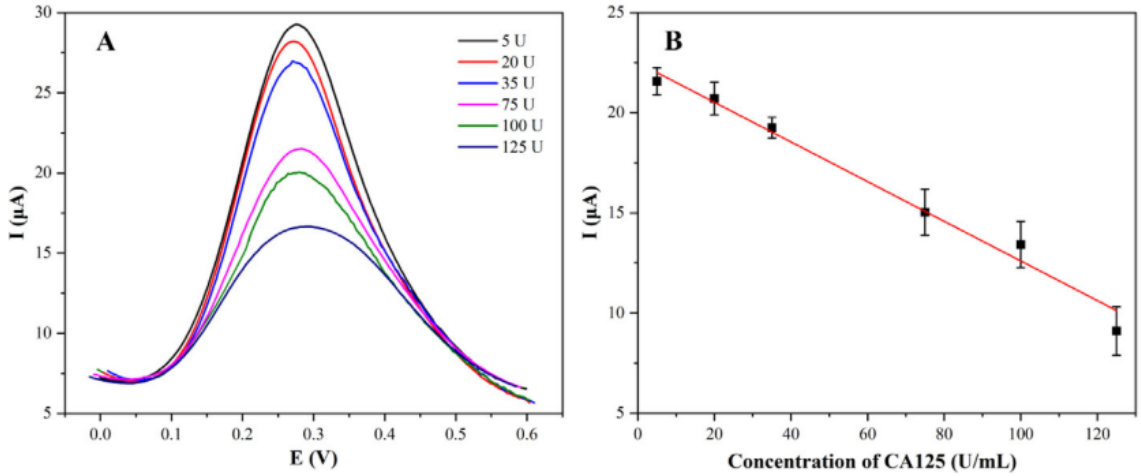


Fig2; DPVs current responses of CA125 at six concentrations (5 U/mL, 20 U/mL, 35 U/mL, 75 U/mL, 100 U/mL, and 125 U/mL) (A),calibration graph of the response current vs different CA125 antigen concentrations (B) in 0.1 M of KCl containing 5 mM [Fe(CN)6]3-/4 under optimal experiment conditions at 25 °C.

3.1.2 Using Autoantibody for detection of OC

A.Gani et al. in his research tried to explore a new way to diagnose the onset of OC[21]. In this approach the target molecule(biomarker) is an autoantibody instead of antigen.Cancer autoantibody is an antibody produced by the host's immune system in response to tumor antigen.It is produced at a much earlier disease stage before other biomarkers can be observed, circulates in the serum for a relatively long period of time, and is present at higher concentrations than antigen biomarkers[13].For the detection,the diagnosis platform used is a Magneto-Nanosensor since it has shown superior sensitivity and dynamic range compared to the current gold standard ELISA (Enzyme-linked Immunosorbent Assay)[14].

The target molecule which is an antibody for the early detection of OC used in this research is Anti- Selenium Binding Protein 1(Anti-SBP1).Studies showed that concentration levels of SBP1 are associated with cancer progres-
sion [15].

A Magneto-nanosensor(MN) is used because of its many advantages like,
The obtained signals in magneto-nanosensors are not interfered with background signals, and thus have higher signal-to-noise ratio.[16]It is also relatively easy to integrate with electric

read-out systems which is quite advantageous for the development of hand-held devices[17]. The chip consists of 80 individually-addressable Giant Magnetoresistive(GMR) spin valve sensors.The SBP1 capture probe was immobilized on GMR sensors, capturing anti-SBP1 that binds specifically to the probe. Biotinylated detection antibody was added, forming a sandwich structure. Streptavidin-coated magnetic nanoparticles created a magnetic fringe field, affecting GMR sensor resistance. Electronic readout circuits measured resistance changes in real time.
For the experiment,they used the capture SBP1-coated sensors(green),Bovine Serum Albumin (BSA)-coated sensors(red), and electrical reference sensors (black) to get the readings are shown in the Fig 3;Sensors coated with SBP1 shows specific binding to anti-SBP1 to the sensor surface whereas the BSA coated sensors acts as negative control sensors that produce background/noise signals since anti- SBP1 does not bind to it.
The standard curve measured using MN was obtained by measuring the signals from various concentrations of spiked anti-SBP1 (similar to Figure 3), then subtracting the signals from the background signals from BSA-coated sensors to remove the effect of nonspecific binding. The standard curve measured using this MN and ELISA is plotted in a graph as shown in Fig 4;. The comparison proves that MN is capable of detecting anti-SBP1 down to 25X lower concentration than ELISA as MN, anti-SBP1 assay achieves sensitivity of 4 ng/ml. Compared to ELISA whose sensitivity is 100 ng/ml.

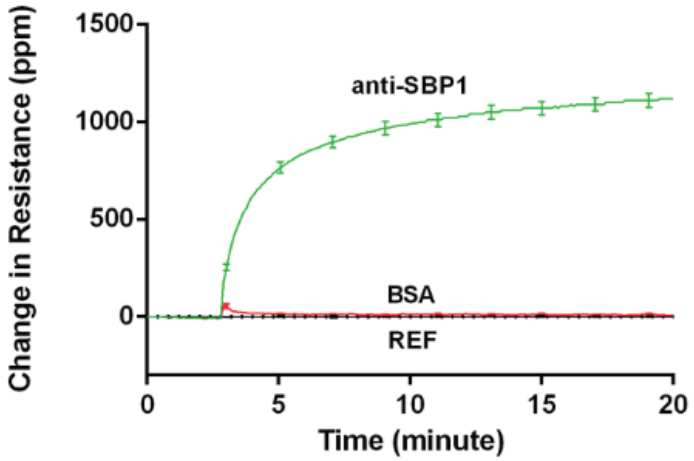


Fig. 3. A real-time binding curve from magnetic immunoassay of 1000 ng/ml of anti-SBP1 using Magneto-Nanosensor. The measured signal is highly specific, with very low background/noise signal.

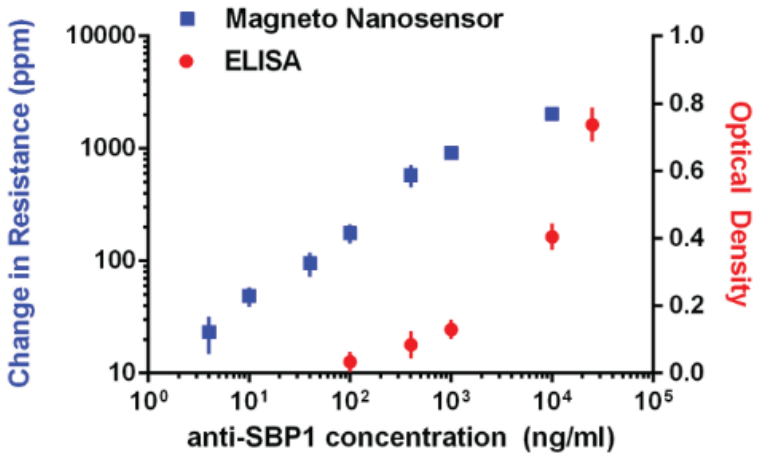


Fig. 4. Anti-SBP1 standard curves measured using Magneto-Nanosensor (blue squares) and ELISA (red circles). With much higher sensitivity, Magneto-Nanosensor can measure anti-SBP1 down to 25X lower concentration than ELISA.

Anti-SBP1 is significantly higher even in the case of infertility in women[20]. So the ability of Anti-SBP1 to differentiate between infertility and OC is questionable, but if we use a multiplexed approach associating Anti-SBP1 with CA-125, detecting these with established immunoassays will provide better accuracy during diagnosis.

3.2 Using Magnetic Nanoparticles as Contrast agents for MR Imaging

3.2.1 FA-targeted Fe_3O_4 NPs as T2-negative contrast agents for in vivo MR imaging

Iron Oxide (Fe_3O_4) nanoparticles are the most widely used magnetic materials for a variety of biomedical applications. Recent developments in nanoscience and nanotechnology have made it possible to develop a variety of contrast agents for MR imaging applications.[18]

H. Zhang et al. in his research proposed a way to use Fe_3O_4 Nanoparticles to target Folic Acid molecules in the ovary to diagnose OC[22]. Folic acid (FA) receptors as single-chain glycoproteins with high specific affinity for FA are highly overexpressed on various malignant tumors, including human ovarian cancer[19]. In his experiment, he followed a intraperitoneal xenograft tumor
Model where he implanted Skov-3 cells in mice. He prepared FA-targeted Fe_3O_4 NPs and Non-targeted Fe_3O_4 NPs to be injected into the test subject.To qualitatively confirm the cellular

uptake of Fe_3O_4 NPs by Skov-3 cells, the cells was stained with Prussian blue.The results showed that the Skov-3 cells treated with FA-targeted Fe_3O_4NPs had more obvious blue staining than the cells treated with non-targeted Fe_3O_4 NPs at the same Fe concentration as shown in **Fig 5**. MR scans were performed before injection and 0.5, 1, 2, and 4 h after injection of the particles by 1.5 T clinical MR system was used with a custom-built rodent receiver coil whose **Fig 6**
The T2-weighted MR effect of the NPs were evaluated by the MR system whose result is shown in **Fig 7**; as a chart.It should be noted that the T2 signals intensity of the mice treated with FA-targeted Fe_3O_4NPs was significantly lower than that of the mice treated with non-targeted Fe_3O_4NPs at 2 h post injection.However, both in vitro and in vivo imaging results (as shown in Figs. 5 and. 6) proved FA-targeted ligands can enable the tumor uptake through more active pathway, thus making the tumors look like more dark compared with non-FA targeted group.This results suggested that the prepared FA-targeted Fe_3O_4 NPs have a great potential to be used as contrast agents for targeted MR imaging to diagnosis the ovarian tumors.

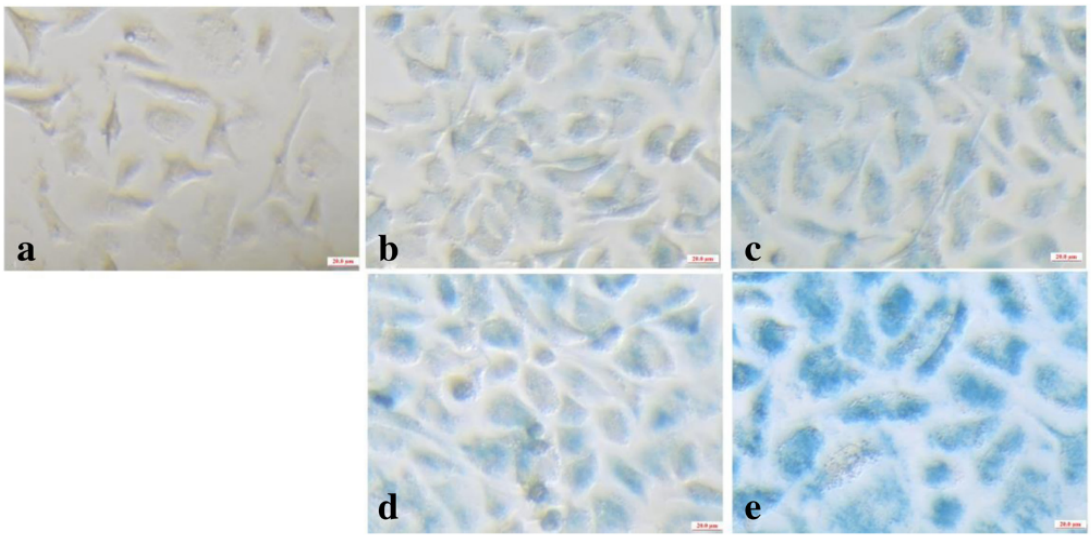


Fig 5; Prussian blue-stained Skov3 cells incubated with PBS (a), non-targeted Fe3O4 NPs (b, c) and FA-targeted Fe_3O_4NPs (d, e) in given Fe concentration of 0.2 mM (b, d) and 0.4 mM (c, f). Scale bar = 20 µm

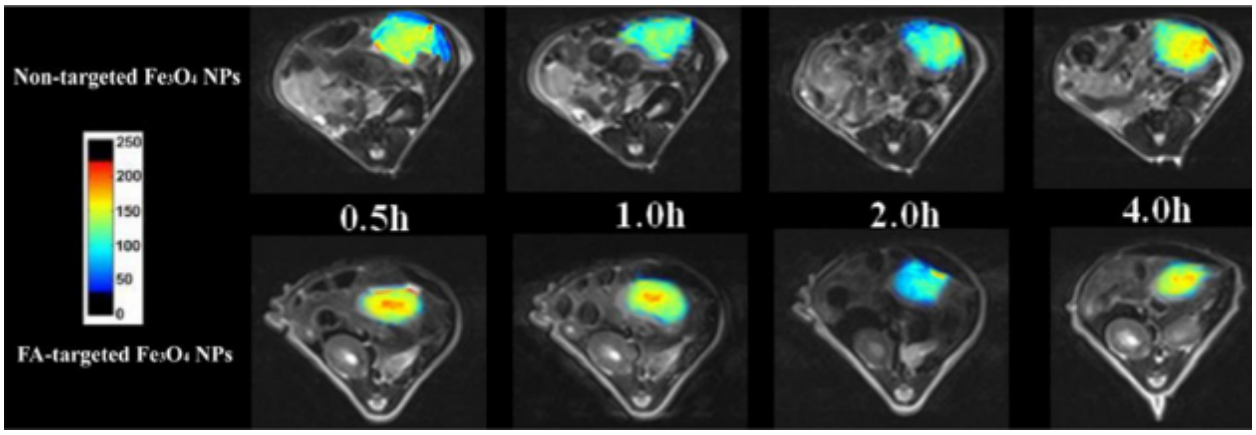


Fig 6, In vivo MR imaging of intraperitoneal tumor after intravenous injection of FA-targeted Fe_3O_4 NPs or non-targeted Fe3O4 NPs (0.6 mg Fe) at different time points

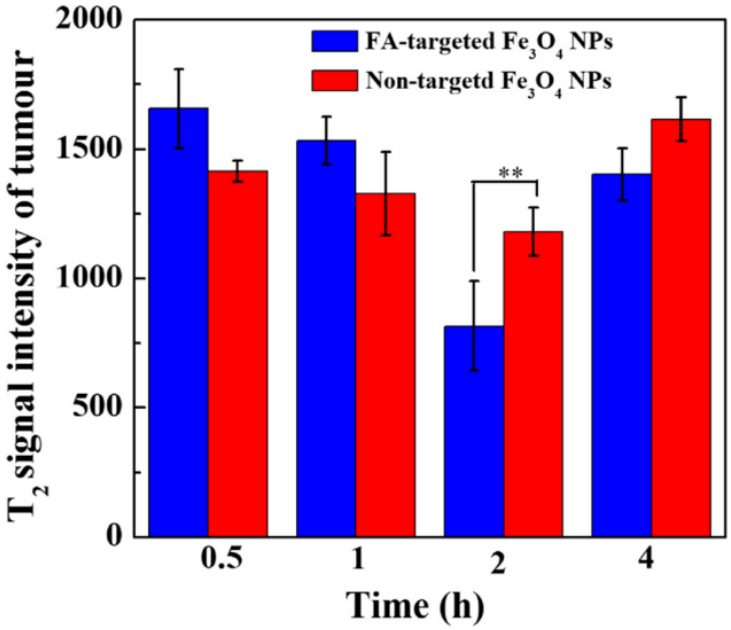


Fig.7 Measurements of T2 signals intensity of intraperitoneal tumor in nude mice after intravenous injection of FA-targeted Fe_3O_4 NPs or non-targeted Fe_3O_4 NPs (0.6 mg Fe) at different time points

There are certain limitation to this research as tumors at an earlier stage or smaller might not be detected because of overlapping neighboring

organs (such as gut, kidney, or bladder) and background which could be improved by the use of bimodal magnetic nanoprobes with fluorescent materials incorporated into Fe_3O_4 NPs. Secondly,since FA receptors are overexpressed in most malignant tumors, the FA targeting ligand we used may not be specific for detecting ovarian cancer.

4. Therapy for Ovarian Cancer using Magnetic Nanoparticles

4.1 Magnetic Nanoparticle-Induced Hyperthermia for Cancer Therapy

Hyperthermia is another widely used method for cancer therapy where particular targeted areas are heated for more effective results after chemotherapy, the magnetic application of the nanoparticles is used in this type of treatment as an alternating magnetic field is implemented to excite the magnetic nanoparticles to generate heat killing the cancer cells.
Hyperthermia is not widely available across the world along with chemotherapy and radiation therapy but is used to treat advanced types of cancers.[33][41]

As we can see magnetic nanoparticles have become a vital and significant tool in cancer treatment, the ideal structure consists of a magnetic core to direct the nanoparticles to the targeted cancer cells and for hyperthermic application, a layer with efficient receptors and recognition molecules and a therapeutic load, many having just the first two.
MNPs of 11 nanometers mean diameter with 2000 MW polyethene (PEG) coating have showcased preferable accumulation in tumor cells through an enhanced permeability and retention (EPR) effect, trapping the nanoparticles within the tumor cells. [39]

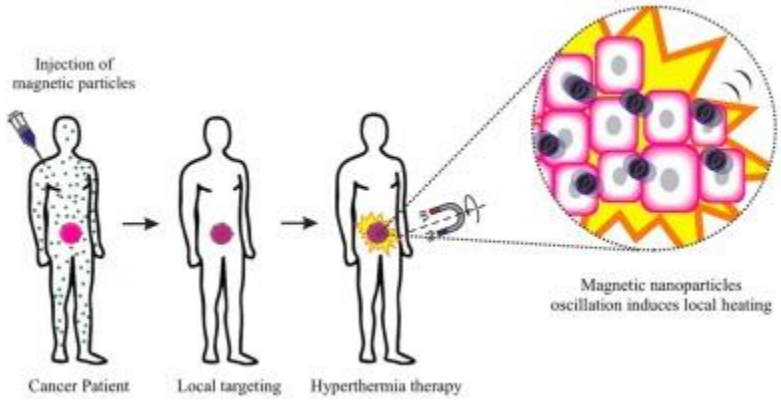


Fig 8 : the above figure shows the illustrative representation of how magnetic nanoparticles help in hyperthermia-centered therapy [39]

In vivo and in vitro experiments have shown great results in cancer cell apoptosis and complete tumor regression in mice upon treatment with a combination of nanoparticles and alternating magnetic field [39]
Core–shell nanoparticles containing a magnetite core of 20 nm in diameter, a 20 nm-thick mesoporous silica shell and a covalently attached thermo-sensitive polymer, poly[(ethylene glycol)-*co*-(L-lactide)] (P(EO-*co*-LLA)) coating, have showcased efficient treatment of cancer by AMF-induced hyperthermia and thermo/pH-responsive drug delivery.[43]

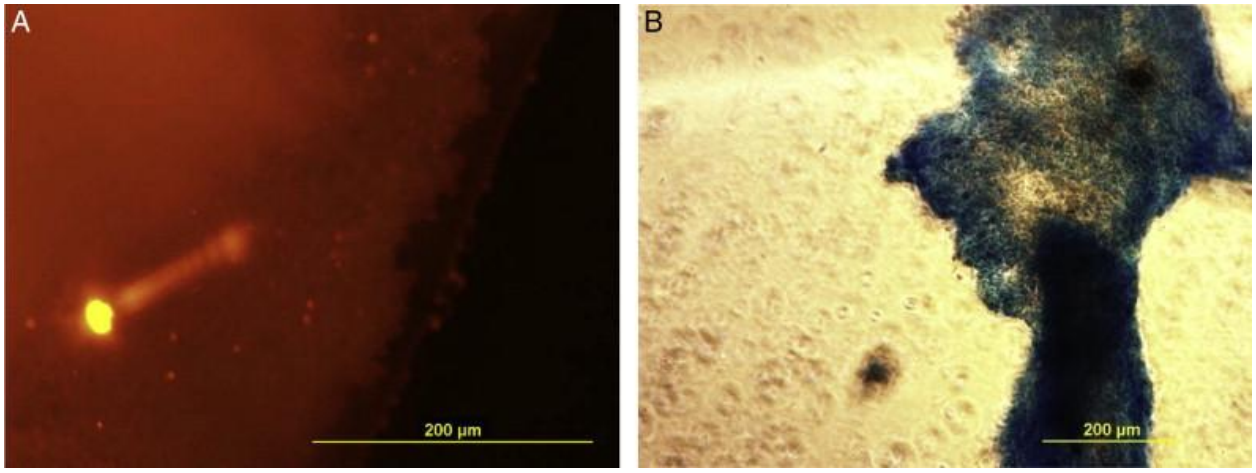
Many studies have been conducted on the possibility of using Super Paramagnetic Iron Oxide Nanoparticles (SPIONs) as theranostic agents for cancer treatment. Drug delivery, therapeutic uses, and diagnostic imaging can all benefit from the use of SPIONS. Because of their non-invasive properties.Fe3O4 due to its heat abilities through the induction of cancer can be used for killing cancer cells by coating them on nanoparticles.[25]
In 2009, superparamagnetic particles of iron oxide were experimented on to check their ability to enhance MRI in combination with diffusion magnetic nanoparticles, which can be used for cancer diagnosis and treatment, thus enhancing the capability for photothermal therapy of cancer cells.[33]

4.2 OC Cell Removal with MNPs

The majority of ovarian cancer metastases are caused by the shedding of malignant cells from the primary tumor into the abdominal cavity, resulting in free-floating cells in the internal organs and other tissues.[33] Fluid accumulation in the peritoneal and pleural cavities causes ovarian cancer. The peritoneal cavity epithelial cells are responsible for the majority of this fluid accommodation. These can be isolated from peritoneal fluids using magnetic nanoparticles containing ligands that have a high affinity for EphA2 tyrosine kinase receptors. This could reduce the amount of chemotherapy or radiation therapy administered, as well as any negative side effects that are frequently connected to ovarian cancer therapy.[29]

Human epithelial antigen (HEA) antibody Ber-EP4, the extracellular domain of the MUC16 cell surface protein, CA125, and a receptor tyrosine kinase EphA2 have all been found to be highly selective markers for free-floating ovarian cancer cells.

Fluorescence microscopy was used to confirm the GGGGYSAYPDSVPMMSK peptide conjugation to nanoparticles on magnetically aggregated particles that had been cleansed in multiple washes with PBS. Figure A depicts the aggregation of magnetic nanoparticles with rhodamine-conjugated peptides. The aggregate's red fluorescence was interpreted as proof that the peptides had been successfully linked. [50]



In the above FIG. B, the nanoparticle aggregate appears blue, suggesting the adherence of dead cells. The removal of whole cells was evidenced by analyzing particle size distributions in ascites samples from each patient (Multisizer 3; Beckman Coulter, Fullerton, California)

4.3 Delivery of Anti- Cancer Drug by MNPs

MNPs can transport anti-cancer drugs to tumor cells by using an external magnetic field.
Many of the magnetic nanoparticles have been coated with PEG for their soluble property in a variety of both soluble and non-soluble solvents, enhancing their therapeutic potential (PEGylation)
Folate is necessary for DNA nucleotide synthesis and cell division, but in cancer, it is present in uncontrollably high concentrations, ensuring high affinity for folate receptors for accurate and faster targeting and delivery of magnetic nanoparticles for anti-cancer drug delivery.
Many cancer treatments have serious side effects, including cardiotoxicity, hair loss, hand-foot syndrome, and drug resistance.[50][41]

Nanoparticles are biocompatible and biodegradable, allowing for precise and painless drug delivery.
Through controlled release, the use of magnetic nanoparticles for drug delivery ensures targeted drug release and increases drug concentration in the target tissue.
Drug resistance can be overcome by modifying systems with a covalent or non-covalent attachment of a targeting ligand on the surface of nanoparticles, allowing them to recognize specific antigens or receptors on tumor cells and allowing the particles to be engulfed via endocytosis. (30) Over 90 percent of ovarian carcinomas overexpress folate receptors, thus making it very useful in ovarian cancer treatment.
For example, in a study conducted by Güliz Ak, Habibe Yilmaz, Aybike Güneş, and Senay Hamarat Sanlier, they focused on the anti-cancer drug Doxorubicin and developed a biomimetic RBC vesicle-coated DOX carrying magnetic nanoparticle system for ovarian cancer treatment.[50]

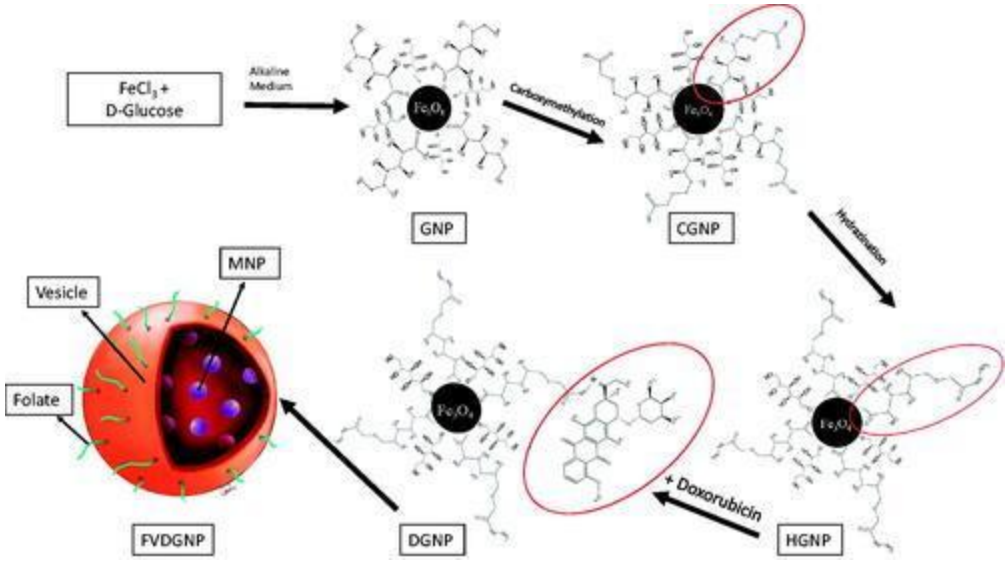


Fig: depicts the reaction of DGNP formation in the experiment mentioned.

First, magnetic nanoparticles (GNP) coated with glucose or gluconic acid and loaded with DOX were synthesized and studied. These magnetic nanoparticles enable system targeting to a desired location. [50]
Next, RBCs and a folate ligand tethered onto cell membrane vesicles were used to create ghost cells. To add a biomimetic aspect, drug-loaded magnetic nanoparticles were subsequently enclosed into vesicles. [50]
The final phases involved conducting ex vivo, in vitro, and in vitro experiments to assess the effectiveness of the nanocarrier treatment. The findings indicate that magnetic drug delivery systems with biomimetically tailored folate ligand attachment have the potential to be used in targeted therapy for ovarian cancer[50]
Since these nanocarrier systems can be generated from the patient's erythrocytes when erythrocytes are supplied from ovarian cancer patients, this technology may enable tailored ovarian cancer therapy.[50]

The developed DGNP Loaded and Folate Aached Erythrocyte Vesicles (FVDGNP) and folate-attached erythrocyte vesicles (VDGNP) were studied and it was found that due to the biomimetic property of the vesicular forms, distribution of each vesicle in liver and lungs were reduced in other studies free DOX mostly accumulated only in liver, spleen and lungs, whereas DOX carrying magnetic nanoparticles accumulated relatively lower in lungs and liver.
In ovaries, however, due to the Folate receptor interaction, the distribution of FVDGNP was more than VDGNP.[50]

5.Challenges and Future Scope

Nevertheless, the number of medications based on nanocarriers that are now authorized for clinical usage is quite low. Some of the challenges that must be overcome in the clinical application of these nanomedicines are regulatory issues, safety concerns, and the physicochemical characterizations of the drugs (size, shape, drug loading, surface distribution, biodegradability, surface chemistry, etc.). also include manufacturing issues.We must tackle this issue with new painless and more advanced treatment methods.Nanoparticle drug delivery system is being further developed to distribute and carry smaller doses of chemotherapeutic agents in the most effective and controlled form.New technological advancements in the medical field have converged science and engineering to develop more innovative ways to favor a targeted, more effective less toxic and more biocompatible ways be it in the diagnostic or the treatment aspect of cancer.

6.Conclusion

Nanotechnology in itself is a vast growing field and is undergoing an immense amount of research in its application in the medical field. It has helped researchers and scientists to revolutionize medicine and improve various treatments and diagnostic tools. The application of magnetic nanoparticles are widely researched in the field of medicine due to their easy functionalization.In India, the estimated incidence of ovarian cancer is the second highest, next to China, among the world population. India accounts for 76.5% of incidence and 77.5% of mortality of Ovarian Cancer patients among the south central Asian countries.The integration of these nanoparticles into various aspects of cancer care holds the potential to improve early detection, enhance treatment precision, and ultimately contribute to better outcomes for patients with ovarian cancer. Through this review paper we have discussed various approaches in which these MNPs could be used for diagnosis and treatment of Ovarian Cancer.

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