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Folate receptor mediated in vivo targeted delivery of human serum albumin coated manganese ferrite magnetic nanoparticles to cancer cells

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Abstract. Manganese ferrite nanoparticles (MnFe_2O_4) have received increasing attention due to their remarkable magnetic properties and have been used for various biomedical applications. They have potential applications in magnetic resonance imaging and hyperthermia for cancer. Both novel applications require a delivery system that will allow nanoparticle to move easily and localization of nanoparticle to the target tissue. In our work, we developed human serum albumin coated manganese ferrite magnetic nanoparticles (HSA-MF NPs). The nanoparticles were prepared using solvothermal method and modified with folic acid for targeted delivery. Structure and morphology of manganese ferrite nanoparticle were characterized by X-ray diffraction (XRD) pattern and transmission electron microscopy (TEM). The size of folic acid conjugated HSA-MF NPs (HSA-MF-FA NPs) were studied by dynamic light scattering (DLS). In the in vivo study, we used benzopyrene-induced cancer in mice. We successfully delivered HSA-MF-FA NPs through intravenous tail injection after induction of the tumour. We found that 54% of initial HSA-MF-FA NPs which previously injected localize in the target tissue. While obtained p-value from independent T-test is 0.013 which shows that there is a difference between the control group (HSA-MF NPs) and the treated group (HSA-MF-FA NPs)

1. Introduction

Cancer is one of the leading causes of mortality worldwide, it is also one of the most difficult puzzles to solve. Based on International Agency for Research on Cancer (IARC), there were more than 14 million new cases of cancer and 8 million cancer death worldwide in 2012 [1]. Traditional treatment method of cancer such as surgery, radiation therapy, and chemotherapy or a combination of these therapies. Over the years these methods have improved and managed to increase the number of patients healing. However, this method of treatment is still not perfect. The invasive nature of the surgery, providing a high degree of risk to patients both before and after the procedure. Radiation therapy have a short-term



side effects such as nausea, itchiness, dryness and long-term side effects such as vascular damage, atrophy, and fibrosis due to radiation [2]. While chemotherapy causes systemic side effects. Drugs used in anti-cancer chemotherapy destroys cells that divide rapidly, either cancerous or healthy cells. Therefore, technology that can direct anti-cancer drugs right to the location of the tumour is strongly needed, thus the risk of chemotherapy side effects can be made as low as possible.

The current technological developments allow us to develop targeted therapy that can be an option for cancer treatment. Many scientists have done research to develop a drug delivery system (DDS) that can deliver the drug to the right place, localize the drug and reduce the risk of drugs side effects. One of the strong candidate for DDS is Magnetic nanoparticle with modified surface.

This study develops manganese ferrite nanoparticles (MnFe_2O_4) encapsulated in Human Serum Albumin (HSA). HSA was used because it can make nanoparticle system stable, it also acts as a phase transfer agent. The encapsulated MnFe_2O_4 magnetic nanoparticles system is expected to have stable, water-soluble properties and can be used for DDS.

Some targeting agent such as polyethylene glycol (PEG), lactic-co-glycolic acid (PLGA), human epidermal growth factor receptor (EGFR), folic acid, silica, and somatostatin / growth hormone (GH) has been developed. For cancer therapy, folic acid is very suitable for cancer targeting agent due to its low level of toxicity and long biodegradable, folic acid also has a high specificity towards cancer cells [3]. For these reasons, in this study manganese ferrite nanoparticles will use folic acid as targeting agent for cancer.

2. Materials and Methods

2.1. Nanoparticles and surface preparation

All materials used in this experiment were purchased from Sigma-Aldrich and used as-received without further purification. Manganese ferrite nanoparticles (MF NPs) were synthesized chemically using solvothermal method. For this method, an aqueous solution was prepared by dissolving 2 mmol Iron (II) acetylacetonate and 1 mmol Mangan (III) acetylacetonate in 15ml benzyl ether ($\text{C}_{14}\text{H}_{14}\text{O}$) and 15ml oleylamine ($\text{C}_{18}\text{H}_{35}\text{NH}_2$). Then the solution mixture was heated to 110 °C for 1 hour following by heated to 300 °C also for 1 hour. After solution temperature back to room temperature, the solution was washed with ethanol and separated by means of centrifugation at 6000 rpm for 10 minutes. MF NPs obtained from this procedure is in black powder form.

The HSA transfer was done by dissolve MF NPs with 1 ml of hexene. In another container, HSA were dissolved in 10 ml of aqua bidestilation. Both nanoparticles and HSA then mixed together and undergone ultrasonication process at 20 kHz for 2 minutes. The mixture is then left for 24 hours. From this procedure, we get human serum albumin coated manganese ferrite magnetic nanoparticles (HSA-MF NPs). The procedure is then repeated for the attachment of folic acid to obtain folic acid conjugated HSA-MF NPs (HSA-MF-FA NPs).

2.2. Samples characterization

The X-ray diffraction (XRD) patterns of prepare manganese ferrite nanoparticles sample was characterized by PAN Analytical Philips X'Pert diffractometer with $\text{CuK}\alpha$ (0.154 nm) radiation to generate XRD patterns from samples at room temperature in a 2θ range of 10°-70°. The morphology of the prepared sample is observed by transmission electron microscopy and size distribution of nanoparticles was determined using dynamic light scattering (DLS) method.

2.3. Cancer Cells Induction and in vivo Procedure

Benzopyrene-induced cancer in wistar mice was done by injecting benzopyrene solution to breast area of mice. Benzopyrene solution was prepared by dissolve benzopyrene powder in olive oil at a ratio of 1:1. Cancer induction was done by injecting mice with benzopyrene solution of 0.3ml/kg two times a week subcutaneously in the breast area for 4 weeks.

In vivo procedure consists of two group, one group of mice administered injection of HSA-MF NPs (positive control group) and other group of mice administered injection of HSA-MF-FA NPs

(intervention group). Both of group administered injection of 1 cc solution intravenously from the tail. After 8 hours, cancer tissue of mice was taken for voltammetry characterization to determine the manganese metal content

3. Results and Discussion

3.1. X-ray diffraction, morphology, and size characterization

The XRD pattern of prepared MF NPs sample is shown in Fig. 1. The pattern of diffraction shows peaks which corresponds to Bragg reflections from (220), (311), (222), (400), (422), (333), (440) planes. Peaks of pattern in Fig. 1 is in good agreement with the standard XRD pattern of MnFe_2O_4 (JCPDS cards No. 74-2403, 10-0319) which indicate the samples had a face-centered cubic crystal structure.

From XRD data, average crystallite size of MF NPs sample was calculated using Scherrer's formula [4]. The result of calculation using full width at half maximum intensity for (311) plane shows that average crystallite size of sample is 4.6 nm.

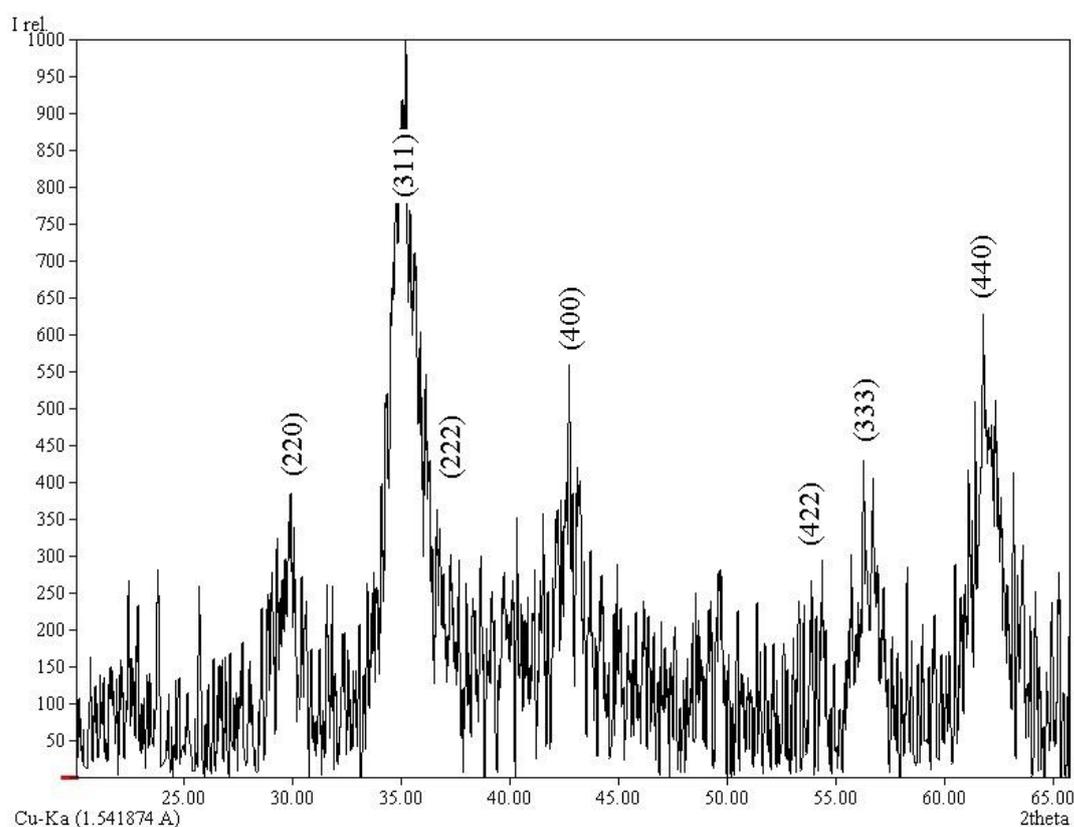


Figure 1. XRD pattern of prepared MF NPs.

The size of prepared MF NPs was studied by TEM which is shown in Fig. 2. The TEM result reveals that nanoparticles have almost spherical shape and quite homogeneous with a size between 10-20 nm. This result indicating that the synthesis method adopted is successful in obtaining homogeneous MF NPs.

Size distribution characterization result of HSA-MF-FA NPs using DLS show that coated nanoparticles has average size of 227.3 ± 114.5 nm. Addition size of MF NPs is due to coating and agglomeration. The size of HSA-MF-FA NPs is determined by concentration ratio of HSA with the

nanoparticle. The greater the concentration of HSA used for coating procedure, the smaller size of HSA-MF-FA NPs [5].

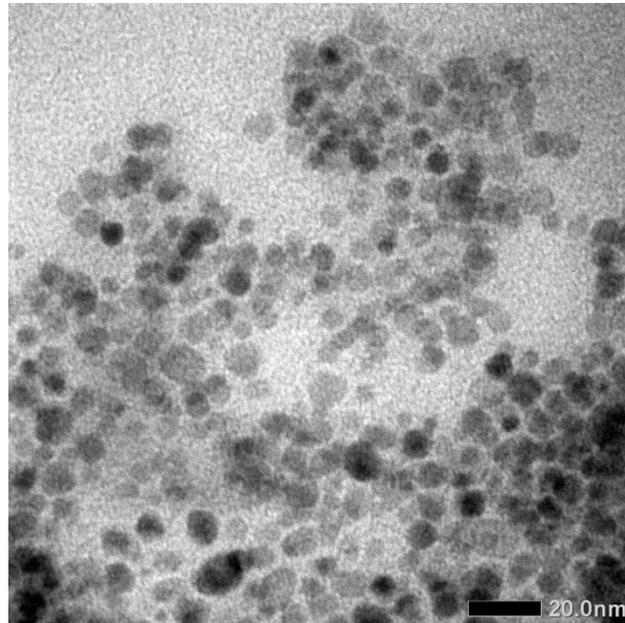


Figure 2. TEM micrograph of MF NPs.

3.2 *In Vivo* and voltammetry analysis

Voltammetry Analysis was conducted to determine the concentration of HSA-MF-FA NPs in cancer tissue. Standard model was generated to determine the concentration of manganese (Mn) using multiple solutions with different concentrations (1, 2, 4, and 8 ppm). The result shows that for intervention group, there is 54% of HSA-MF-FA NPs injected intravenously in wistar mice reached the cancer cells.

Based on the test results of Shapiro-Wilk, it is known that p-value in the positive control group was 0.081 and in the intervention group was 0.166. It shows that the data are normally distributed due to the p-value $> \alpha$ (0.05). Analyse using independent t-test show that p-value of 0.203, this is suggesting that the variance of the data is homogeneous due to the p-value $> \alpha$ (0.05). The complete statistical result is shown in Table 1

Group	Mean	Median	SD±	P
Intervention group	0,0038	0,0038	0,0008	0,013
Positive control group	0,0024	0,0019	0,0011	

Based on the analysis results of independent t-test, p-value obtained was $0.013 < \alpha$ (0.05) this shows that there are differences between (HSA-MF-FA NPs) and (HSA-MF NPs) as a delivery system media against cancer cells in wistar mice.

In vivo results in wistar mice show that HSA-MF-FA NPs have potential to be used as a DDS. Voltammetry analysis show that there was a 54% concentration of HSA-MF-FA NPs distributed in cancer cell of mice 8 hours after injected with 1cc HSA-MF-FA NPs intravenously. It happens due to

the nano-size of HSA-MF-FA NPs making it possible to enter the cell through the leakage of blood vessels in the cancer cells. The nanoparticles also have a good ability to penetrate the interstitial part of blood vessel of the cancer cells with higher retention times than in healthy cells. Also, the uncontrollable cancer cells growth will always require faster vascularization and increases the permeability of that vascular so that it can easily be accessed by nanoparticles. This result is similar with study results by Hui S Huang in 2013 which revealed that the concentration of the nanoparticles identified in the tumour cells in the first 1 hour of 23%, 3 hours of 35%, 8 hours of 35%, 24 hours of 52%, and 96 hours of 20.58% [6].

In addition, HSA also play a role in the process of MF NPs entry into the cancer cells, it happens due to the differences in pH between healthy cells and cancer cells, where the high level of acidity in the environment of cancer cells will bring MF NPs move towards the cancer cells [7]. The change of pH in cancer cells will make the HSA structure opens and stimulates the release of folate from MF NPs so that MF NPs can enter cancer cells.

Delivery system media using HSA-MF-FA NPs are more effective accompanied by folic acid as a targeting agent that will move towards folate receptors on cancer cells. Some studies show promising results in accuracy and sensitivity detection of folate against cancer cells [3,8]. In our case folate receptor would normally distributed in the epithelium of healthy cells and increases in the epithelium of malignant cells. Folate receptor also activates the endocytosis progress so that HSA-MF-FA NPs can easily get into the cells' cytoplasm. This allows the use of targeted agents in the form of folic acid that have greater concentrations in cancer cells.

4. Conclusion

HSA-MF-FA NPs were successfully delivered through intravenous tail injection after induction of the cancer. It is found that 54% of initial HSA-MF-FA NPs which previously injected localize in the target tissue. Obtained p-value from independent T-test is 0.013 which shows that there is a difference between the control group (HSA-MF NPs) and the treated group (HSA-MF-FA NPs). It is indicating that HSA-MF NPs are less effective to be used as a delivery system media against cancer cells of wistar mice. From these results, it is concluded that the material of HSA-MF-FA NPs have potential to be used as a delivery system media against cancer cells in wistar mice.

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