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Formulation and characterization of multifunctional polymer modified-iron oxide magnetic nanocarrier for doxorubicin delivery

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ABSTRACT

Chemotherapy is one of the mostly used treatment for neuroblastoma cancer. However, in chemotherapy the drug is transported throughout the body thereby affecting fast growing healthy cells and courses several side effects. Hence, the formulation of multifunctional drug delivery systems (DDSs) as nanocarriers for cancer targeted delivery therapy to specific cancerous tissues has gained considerable attention in the field of nanomedicine. In the current study, a magnetic nanocarrier composed iron oxide magnetic nanoparticles (IONPs) coated with different concentrations of Pluronic F127 for the delivery of doxorubicin (DOX) in neuroblastoma treatment was synthesized. The F127–IONPs were synthetized through coprecipitation method. The HR–TEM images showed spherical and evenly dispersed particles of sizes between 10 and 40 nm. The XRD analyses indicated the crystallization of the prepared nanoparticles. Irrespective of the different F127 concentrations and the sizes of IONPs, all the particles exhibited insignificant coercive force and residual magnetism, signifying their superparamagnetic properties. The MTT assay results showed no significant cytotoxicity of the prepared nanocarrier on treated cells. The *in-vitro* studies of the drug release profile showed a pH-dependent drug release where more DOX was released under acidic environments than in neutral conditions.

1. Introduction

Cancer is the most serious life-threatening disease, which has a high mortality rate around the globe. Statistics indicate that cancer causes about six million deaths yearly, with approximately ten million patients diagnosed with cancer every year [1–3]. It has been reported that by the year 2030 > 30 million people will die of cancer-related infections globally [4,5]. However, during the last five years the death rate has reduced because of the advances in cancer biology, better diagnostic strategies, and improved treatment methods. Neuroblastoma (NB) is a type of malignancy that largely affects children, usually under the age of one. NB causes more than 15% of infant cancer-related deaths with a very low general survival rate of 40% for metastatic tumors after 5 years. NBs begin in the early nerve cells referred to as the neuroblasts of the sympathetic nervous system; therefore, they can be located anywhere along this system [6–8]. The amplification of N-myc proto-oncogene protein (MYCN) expression and mutations in ALK (anaplastic lymphoma kinase) are the major causes of NB [9–11]. Despite extensive research, conventional chemotherapy is still the major cancer treatment approach. Nevertheless, the effectiveness of chemotherapy is restricted because of its incapacity to distinguish between tumor and normal cells, thus resulting in the death of normal healthy cells [10–12].

To overcome the limitations of chemotherapy, several studies have been focusing on the innovation of novel DDSs using non-toxic and biocompatible materials. Recently, nanotechnology has gained more attention in the Biomedical Engineering field. It affords great opportunities to develop various nanoparticles that vary in size, shape, surface properties, and compositions. Owing to their small sizes that are similar to cellular components, nanoparticles can penetrate through cell membrane by cellular endocytosis

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mechanisms, particularly pinocytosis [13,14]. Their small size and large surface area enable more drug molecules to be closer to the surface of the nanoparticle compared to a larger carrier, a phenomenon that help to attain faster drug loading and release [15,16].

In addition, magnetic iron oxide nanoparticles (IONPs), also referred to as superparamagnetic iron oxide nanoparticles (SPIONs), have been explored for a comprehensive application in DDSs, magnetic resonance imaging (MRI), hyperthermia therapy, locating of cells *in-vivo*, and cells separation [17]. The IONPs are nontoxic and their biocompatibility is very high, hence, the Food and Drug Administration (FDA) accepted ferumoxytol, which is one of the Fe_3O_4 nanoparticles, as an iron supplement. The FDA also approved Feridex® (ferumoxides), which are dextran-coated IONPs used as an imaging contrast agent for locating liver lesions [14,18–23]. Due to their magnetic properties, IONPs have a strong susceptibility to aggregate. In addition, because they easily oxidize in the air and contain high chemical activity, IONPs tend to lose magnetism and dispersibility [24,25].

Therefore, introducing a polymer coating on the surface of IONPs can improve its properties. Polymers have been extensively used for coating IONPs to increase their colloidal stability when dispersed in a solution, avoid aggregation, and prevent the oxidation and degradation of IONPs core [26]. Furthermore, polymer selection is important for successful coating and developing an effective drug delivery system, that is capable of enhancing the drug antitumor efficacy [26–28]. In order to overcome the limitations, IONPs were coated with the amphiphilic Pluronic F127. The Pluronic micelles denote a unique form of a polymer that can improve the solubility of doxorubicin (DOX), prolong the time of circulation and the targeted release of the drug at the right tissues/cells. This polymer could modify the biodistribution of the drug. Pluronic polymers are stimuli-responsive, temperature and pH-sensitive polymers and have been successfully utilized as DDSs [29–31]. Moreover, several studies have reported that the extracellular polymeric substance (EPS) and soluble microbial products (SMP) are extracellular carbohydrate polymers produced and secreted by microorganisms, which collect outside the cells. The capability of such hydrophilic polymers to forming hydrogels, cross-linked 3D network structures keeping a lot of water while left insoluble, renders them useful in drug delivery as carriers. These gels can encapsulate drugs/genes into their inner structures, thereby adsorbing the therapeutic particles onto their external surfaces and enter cells and tissues openings to reach at targeted organs [32–34].

Additionally, cell viability determines the influence of nanoparticles on cell growth and survival. This is an important aspect when developing an effective DDS. Numerous studies have investigated the cytotoxicity of iron oxide based nanocarriers on cell. Most studies suggested that toxicity slightly rose with the increased concentration of IONPs. However, after coating the IONPs with biocompatible polymer materials, it was confirmed that the nanocarrier exhibit no toxicity towards cells. For example, Vu-Quang et al. reported that F127 coated IONPs and F127-Folate coated IONPs were nontoxic to cells with a long incubation period (24 h). Moreover, Wang et al. confirmed that iron oxide at various concentrations coated with carbon indicated that the nanocarrier was nontoxic to the tested cells at concentrations up to 100 μg/mL. These results are an indicative that upon coating, IONPs will not exhibit any significant cytotoxicity towards cells, therefore they are safe to use as nanocarriers for DDS [27–30,43].

Additionally, DOX is one of the most useful cancer drugs for breast, lung, and neuroblastoma cancer therapy. The DOX drug is one of the most active chemotherapeutic agents [35]. It is one of the widely used cancer drugs in the world, which functions by distracting gene expression [36,37]. Unfortunately, DOX application is limited by several side effects, such as vomiting, diarrhea, and loss of hair [39–41]. Therefore, the objective of this study was to formulate a multifunctional DDS capable of carrying and delivering DOX for neuroblastoma cancer treatment. The loading of DOX into nanocarriers made of iron oxide nanoparticles and Pluronic F127, and the release of the drug from the DDS were studied. The IONPs were synthesized and characterized for the fabrication of DOX-loaded F127–IONP nanocarrier. The prepared nanocarrier in this study can physically load and deliver drug nanocrystals to a tumor site, possibly preserving the activity of the original drug. Nevertheless, low capacity and prompt release of the loaded drug affects the DDS. Certainly, dissolution in bodily fluids is the main restraint for the physical loading approach, which can result to uncoupling of the carrier into monomer and drug precipitation. Favorably, DOX particles are compatible with F127 since it contains both hydrophobic and hydrophilic properties, thus making it suitable for encapsulation both hydrophobic and hydrophobic cancer drug [33,40,47]. This carrier also boasts of being non-toxic, biocompatible, biodegradable, pH, and temperature sensitive, which makes it more favorable to be used for delivering cancer drugs to human body. Moreover, due to the magnetic property of the system, it can be able to become MR imaging contrast agents. A major hurdle linked with the use of IONPs in the system is their performance *in-vivo*. The effectiveness of the system is usually restricted because of the blockage of the nanocarrier by the reticuloendothelial system (RES) before it reaches the target tissues and its incapability to prevail over biological barriers, like the blood brain barrier and vascular endothelium. The success of the system upon entering the body depends on dispersibility, charge, surface composition, morphology, and size. These physicochemical features of the nanocarrier determine its bio-distribution and pharmacokinetics [32,33,40,47]. Hence, this study has employed several characterization techniques such as polymer coating, improved dispersion, and reduced particle size to enhance the effectiveness and blood circulation time of the nanocarrier, thus increasing its possibility to reach the targeted tissues. This work aimed at investigating the prospects of F127 coated IONPs to deliver DOX towards neuroblastomat treatment. Several reported research works have delved on the use of F127–IONPs for the delivery of cancer drug, however, there are no detailed research works focusing on neuroblastoma cancer treatment using F127–IONP–DOX system.

2. Materials and methods

2.1. Preparation of the Pluronic F127–IONPs core-shell

The IONPs were synthesized based on a method reported by Mdlovu et al. [47] with some minor modifications. Briefly, 2.4 g of $FeCl_3{\cdot}6H_2O$ and 0.83 g $FeCl_2{\cdot}4H_2O$ at the ratio of Fe^{2+}:Fe^{3+} = 1:2, were dissolved in 110 mL deionized (DI) water in the presence of 0.6 g sodium citrate. Ammonia (33%) solution was added dropwisely to the solution under an argon atmosphere followed by continuous stirring for 2 h. Magnetic separation was used to isolate the IONPs. To remove the supernatants, the obtained particles were washed several times using ethanol and deionized water then were vacuum-dried overnight. The preparation of polymer-coated IONPs was achieved through the addition of 3.8 N sodium hydroxide to the obtained mixture in order to adjust to different pH values. The solution was stirred for 2 h then washed numerous times using acetone, ethanol, and DI water. The Pluronic solution was prepared at different concentrations of 1, 0.5, and 0.3 wt%, by adding them into DI water and sonication for 30 min. The mixture was then sonicated for 1 h then added into a sealed Teflon-lined stainless-steel autoclave under the condition of 170 °C for 10 h. A magnetic filtration was used to collect the obtained solid solution, which was then washed 5 times using DI water and ethanol to

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