

The Formation of Doxorubicin Loaded Targeted Nanoparticles using Nanoprecipitation, Double Emulsion and Single Emulsion for Cancer Treatment

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Abstract

Chemotherapeutic agents lead to the damage of healthy cells because they react with cells which are dividing at a high rate by inhibiting the DNA synthesis and interfering with the process of cell division. "Therapeutic drug delivery allows for an increase in the efficacy of the chemotherapeutic agent while minimizing the interaction with nontumour sites in the body by controlling the release rate of the therapeutic agent and having targeted sites on the nanoparticle which will allow the nanoparticle to release its contents at targeted sites."

There are three main methods of creating nanocarriers for drug delivery that will be considered in this paper, nanoprecipitation, single emulsion and double emulsion. N Single emulsion is the formation of nanoparticles through oil in water emulsion.

Another way to decrease the toxic effect of the drug loaded nanoparticle on the cells is by having specific ligands attached to the nanoparticle that specifically targets a cancer cells or have magnetic particles that one can move to the area where the tumour is located by using a magnet

Keywords: Doxorubicin; Nanoparticles; Nanoprecipitation; Emulsion; Nanocarrier

Introduction

Chemotherapeutic agents lead to the damage of healthy cells because they react with cells which are dividing at a high rate by inhibiting the DNA synthesis and interfering with the process of cell division [1]. "Therapeutic drug delivery allows for an increase in the efficacy of the chemotherapeutic agent while minimizing the interaction with non-tumour sites in the body by controlling the release rate of the therapeutic agent and having targeted sites on the nanoparticle which will allow the nanoparticle to release its contents at targeted sites [1]. The polymer that will be used as a nanocarrier is poly(lactic-co-glycolic acid) (PLGA) which is hydrophilic allowing for it to stay in the body longer without it being removed by the liver and the therapeutic agent is Doxorubicin, a well-known cancer drug.

There are three main methods of creating nanocarriers for drug delivery that will be considered in this paper, nanoprecipitation, single emulsion and double emulsion. Nanoprecipitation includes adding an organic phase to an aqueous phase which results in the spontaneous precipitation of nanoparticles. The organic phase was PLGA, acetone and doxorubicin which was added into an aqueous phase containing a stabilizer [1]. Single emulsion is the formation of nanoparticles through oil in water emulsion. The oil phase includes the organic PLGA which was emulsified in the water phase which is DOX in buffer, with polyvinyl alcohol [2]. Double emulsion is emulsifying DOX aqueous solution into PLGA and then adding it into PVA solution to have a W/O/W emulsion [2]. The results concluded that when it comes to entrapment efficiency values for double emulsion were lower than single emulsion. The entrapment efficiency of the single emulsion technique is 95% while the entrapment efficiency of double emulsion is 67% [2]. The release percentage after four hours for double emulsion nanoparticles is six times higher than the single emulsion particles meaning that the porosity for the double emulsion nanocarriers were greater than for single emulsion [2]. After twenty four hours 1.5 percent of the drug was released by double emulsion while 6.5 percent was

released by single emulsion [2]. For nanoprecipitation 70% and 60% cumulative drug release occurred for 2.9% and 5% loading of the drug respectively [1]. The entrapment efficiency for each percentage is 75% and 79% respectively. The cumulative release vs. time is significantly higher than single and double emulsion. In order to reduce the toxicity of the drug on cells that are healthy one wants a slow release this means that single emulsion is the best technique to use in the creating of nanoparticles. The single emulsion technique also has a low cumulative drug release (only 1.5% of the drug entrapped in the nanocarrier was release), which will reduce the amount of dose administrations by a patient since the drug will be in the body for a longer duration of time in comparison to the other techniques. Another way to decrease the toxic effect of the drug loaded nanoparticle on the cells is by having specific ligands attached to the nanoparticle that specifically targets a cancer cells or have magnetic particles that one can move to the area where the tumour is located by using a magnet.

Chemotherapeutic agents that are used for cancer research lead to the damage of healthy cells due to their action of rapidly proliferating cells which inhibit DNA synthesis and interfere with cell division and metabolism. A way to effectively treat cancer but reduce the harmful effects on healthy non-cancerous cells is by incorporating the drug in a nanocarrier which will allow for a controlled release of the drug from the carrier to the tissue or systemic circulation.

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This report will discuss how to create a nanocarrier which incorporates a therapeutic drug for the treatment of a cancerous tumor. The nanocarrier will have to be able to circulate through capillaries, passively target tumor tissue as a result of its permeability and then to enter the cells for intracellular drug delivery. The nanocarrier will have to be able to do this without being removed by the body's removal system in order for the carrier to directly release the drug to the targeted cell.

Polyactic-glycolic acid (PLGA) is a biodegradable copolymer it degrades by hydrolysis of its ester bonds into lactic and glycolic acids which are further metabolized into carbon dioxide and water through the Krebs cycle. Biodegradable nanoparticles are able to circulate through capillaries and enter cells for intracellular drug delivery. I will be using this polymer for my nanocarrier.

Doxorubicin blocks DNA synthesis and transcription by intercalating between DNA nucleotides and inhibiting topoisomerase II and generates damaging radicals from its metabolism. This drug will be used in my design for the treatment of tumor cells.

Methods

There are three different ways to create drug loaded nanocarriers. The three main ways are single emulsion, double emulsion and nanoprecipitation. In this section I will talk about how each method is completed and then determine which method will be used for the design of my drug loaded nanocarriers.

In the first article that I will be discussing is called "Doxorubicin-loaded PLGA nanoparticles by nanoprecipitation: preparation, characterization and in vitro evaluation," it discusses how to create nanocarriers loaded with doxorubicin which I will discuss below.

Nanoprecipitation

Materials

- The materials use for both sections are
- PLGA with 50/50 lactide/glycolide molar percent and carboxylic acid end group
- Doxorubicin hydrochloride
- Bovine serum albumin
- Phosphate buffer saline
- 3,3 dimethyl glutaric acid

Preparation of Doxorubicin loaded nanoparticles

Doxorubicin (DOX) was dissolved in methanol at a concentration of 2.2, 4.2 and 5.8 mg/ml for 2.1, 3.9, 5.3 wt.% targeted loadings. One hundred mg of PLGA were dissolved in 3 ml of acetone. The organic phase was formed by combining DOX solution and 3 ml of PLGA solution. This was added to 10 ml of aqueous phase containing 10 mg/ml bovine serum which is a suspension stabilizer. This was followed by sonication for thirty seconds. Nanoparticles were formed as a result of the migration of the water miscible acetone and methanol into the water phase and then the precipitation of PLGA in the form of nanoparticles. The solvent was then removed by stirring under vacuum for 45 minutes at room temperature. The nanoparticles were then recovered by centrifugation. The particles were washed three times by suspending the pellet in BSA solution followed by centrifugation. Supernatant was collected and then absorbance was found using the UV spectrometer.

The morphology of the nanoparticles was studied using scanning electron microscope. The transmission electron microscope was used to determine the nanoparticle size and to study their internal structure features. The surface charge of the Nanoparticles was studied using zeta potential measurements.

The entrapment efficiency and loading percentage were determined using the following equations by quantifying the amount of DOX recovered from wash supernatants and assuming that the rest of the drug was encapsulated.

$$EE = \frac{M(\text{mass of drug loaded nanoparticles}) - M(\text{mass of Dox in supernatants}) * 100\%}{M(\text{mass of drug loaded nanoparticles})}$$

$$\text{Drug Loading: } \frac{\text{Mass of Doxorubicin in nanoparticles} * 100\%}{\text{Mass of Nanoparticles}}$$

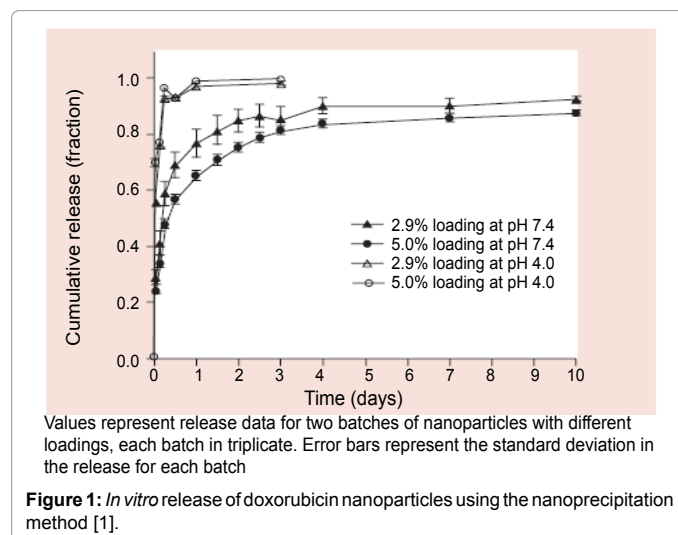
In vitro doxorubicin release

Drug release studies were performed with 10 mM PBS (pH 7.4). PBS was used to mimic the conditions in which the drug loaded nanoparticles would be exposed to during circulation in the body. A known amount of DOX-loaded nanoparticles of a certain mass was suspended in the buffer solution. At specific times the sample was centrifuged for ten minutes and the supernatant containing the released drug was removed for UV analysis and replaced with a known amount of buffer solution. Absorbance of the supernatant was found at 480 nm of supernatant and was used to determine the amount of DOX released.

Results

Nanoprecipitation

As seen in the SEM image the nanoparticles were spherical. The average diameter was 230 nm. The average zeta potential was -45 mV while the zeta potential of the uncoated PLGA nanoparticles without the bovine stabilizing agent was 40.3 mV which means that the bovine serum allowed for the nanoparticles to be stable as colloidal suspensions since columbic repulsion forces arise from surface charge and can overcome the Vander wall attractive forces that would allow for coalescing of nanoparticles. As one can see from the Tables 1 and 2 below the maximum drug loading of doxorubicin in the nanoparticle was an average of 5.3 wt.% and an encapsulation efficiency of 80 percent. The drug release rate as one can see from Figure 1 below shows that 20-30 percent of the drug was released in the first hour while only



Values represent release data for two batches of nanoparticles with different loadings, each batch in triplicate. Error bars represent the standard deviation in the release for each batch

Figure 1: In vitro release of doxorubicin nanoparticles using the nanoprecipitation method [1].

Group	Targeted loading (wt%)	Number of batches (n)	Size ± SD (nm)	Polydispersity ± SD	Zeta-potential ± SE (mV)
Blank NPs	0	4	225 ± 249	3 ± 1.3	-44.7 ± 1.7
DOX NPs 1	2.1	1	249	3	-46.5 ± 1.5
DOX NPs 2	3.9	1	173	3	-46.4 ± 1.5
DOX NPs 3	5.3 ± 0.1	6	239 ± 27	3 ± 0.7	-44.9 ± 1.4

Values represent the average ± standard deviation for n batches of equivalent targeted drug loading.
DOX: Doxorubicin; NP: Nanoparticles; SD: Standard deviation; SE: Standard error.

Table 1: Size and zeta potential of doxorubicin loaded nanoparticles using nanoprecipitation [1].

Targeted loading (wt%)	Number of batches (n)	Encapsulation efficiency (wt%)		Drug loading (wt%)		Mass of DOX accounted for (%)
		Supernatant analysis	Dissolved NPs	Supernatant analysis	Dissolved NPs	
2.1	1	80.9	95.4	1.7	2.0	114.5
3.9	1	75.6	72.7	2.9	2.9	97.1
5.3 ± 0.1	3	79.5 ± 6.9	65.4 ± 4.6	4.3 ± 0.4	4.7 ± 1.1	85.8 ± 10.7

Results represents the average ± standard deviation for n batches of equivalent targeted drug loading.
DOX: Doxorubicin; NP: Nanoparticle

Table 2: Doxorubicin encapsulation efficiency and loading using nanoprecipitation [1].

65-75% was released in the first day. The doxorubicin drug continued to be released from the formulation at a slower rate for 3 days at pH 7.4. If the pH is more acidic the drug is released at a faster rate because the degradation of the PLGA is accelerated.

Critique

As one can see the nanoprecipitation technique allows for an entrapment efficiency of maximum 80 percent with a drug loading percentage of 5.3%. The nanoprecipitation technique has a release of a minimum eighty percent after day one of the drug at 5% loading at pH 7.4. The release rate is quite high for this technique, which means that the dose that the person will need to take more medication in order to maintain a certain amount of drug content in their body will. Also if the drug loaded nanoparticles are in contact with other cells the toxicity of the drug will affect the normal cells since a high concentration of drug is being released after one day.

The critique for this article is that the particle size, uniformity, and distribution are not mentioned. It is noted in articles that different organic solvents lead to different particle sizes [3]. Also the type of stabilizer and the concentration of the stabilizer used affects the particle size as well [3], with the higher concentration of the stabilizer leading to a smaller size of particle [3].

The second article that describes how to create doxorubicin loaded nanocarriers using single emulsion and double emulsion techniques called: "Comparative study of doxorubicin loaded poly (lactide-co-glycolide) nanoparticles prepared by single and double emulsion methods". The methods are described below.

Double Emulsion and Single Emulsion

Materials

- DOX hydrochloride salt
- Poly(D,L-lactic-co-glycolic acid) with a lactic/glycolic molar ratio of 50/50 and a weight average of 40-75 kDa

Preparation of doxorubicin loaded nanoparticles

Double emulsion: Aqueous DOX solution was emulsified in 2 ml of methyl chloride solution containing 100 mg of PLGA by sonication for 15 s in an ice bath. Then 4 ml of 3% (w/v) PVA solution was added and sonicated for 30 s to make a water/oil/water emulsion. The double

emulsion was diluted in 50 ml of PVA aqueous solution and MC was evaporated under a vacuum. The nanoparticles were collected, washed and freeze dried.

Single emulsion: DOX extraction in methyl chloride was carried out by dissolving one milligram of DOX in 3 ml of borate buffer pH 8.6. The aqueous phase was shaken with 50 ml of methyl chloride during 24 hours at 25 degrees Celsius. Then methyl chloride was evaporated under vacuum until one ml was reached. One hundred micrograms of PLGA was dissolved in one millimeter of DOX solution. This phase was emulsified in 4 ml of aqueous PVA poly (vinyl alcohol) by sonication for thirty seconds in an ice bath to obtain an oil/water emulsion. Nanoparticles were washed three times with deionized water by centrifugation for twenty minutes. Nanoparticles were then freeze dried. Nanoparticle morphology was analyzed using a transmission electron microscope.

The nanoparticles were evaluated in terms of drug encapsulation efficiency. A known amount of freeze dried doxorubicin loaded nanoparticles was dissolved in DMSO (dimethyl sulfoxide) and the drug absorbance was measured using UV visible spectrometer at 480 nm. All experiments were performed in triplicates.

In vitro Doxorubicin release: DOX release experiments were performed using dialysis. Dialysis was achieved using a membrane in a 20 ml acceptor volume of phosphate buffer of pH 7.4 stirred at 37 degrees Celsius. At regular time intervals solution was sampled.

Results

Single and double emulsion

Using TEM one can see the shape of the nanoparticles and the mean diameter was found to be 280 nm and 316 nm for particles formed by single and double emulsion methods.

The entrapment efficiencies for single emulsion were 95% and 67% for double emulsion, which can be found in the Table 3. Looking at the Cumulative release vs time in Figure 2 can see that after twenty four hours only 1.5% of doxorubicin was released through single emulsion and 6.5% doxorubicin for double emulsion.

Critique

As one can see from the results Single emulsion allows for a greater entrapment efficiency of 95% in comparison to 67% for double

Method of preparation	Initial amount of polymer (mg)	Initial amount of DOX (mg)	Theoretical DOX loading (% w/w)	Experimental DOX loading (% w/w)	Entrapment efficiency (% w/w)
Simple emulsion	100	1.00	0.97	0.92 ± 0.06	95 ± 6
Double emulsion	100	1.00	0.97	0.65 ± 0.10	67 ± 10

Characteristics of doxorubicin-loaded nanoparticles

Table 3: Entrapment efficiency and drug loading for single and double emulsion methods [2].

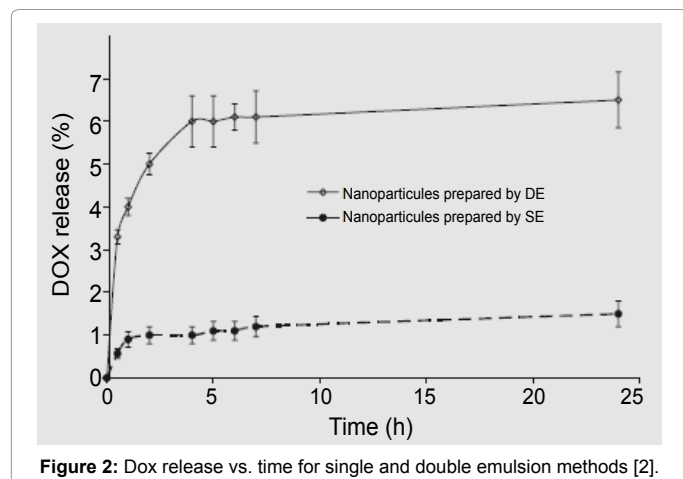


Figure 2: Dox release vs. time for single and double emulsion methods [2].

emulsion. Single emulsion after day one allows for a doxorubicin release of 1.5% in comparison to 6.5% for double emulsion. This means that single emulsion allows for a slow release, which means less doses for patients since the drug will be circulating in the system longer in comparison to double emulsion. Also less normal cells will be affected by the doxorubicin drug since a lower amount of it is being released leading to less harmful effects on normal cells. Single Emulsion is also beneficial for cancer treatment because for the treatment of cancer a sufficient low dose over a long period of time is better than a single high dose exposure. This is because a low dose of cancer treatment drug over a long period of time leads to greater apoptosis of cancer cells [4,5].

Another critique is that they do not mention the size of the nanoparticles that were created, their uniformity and size distribution. It is stated that in order to create nanoparticles using single and double emulsion one should consider the sonication time in order to create the emulsification the longer the sonication time [4] e.g. 20 minutes the smaller the particle size you will get. With a sonication time of twenty minutes one will get mean diameter of 256 nm with a polydispersity index of 0.22 and a size distribution of 100% (all the nanoparticles will be of a size of 256 nm) [4]. As one increases the drug content the size distribution percentage increases and the particle size increases while the polydispersity decreases. The organic solvent and aqueous phase volume also determines the size distribution, particle size and uniformity. The greater the volume or the w/v% the smaller the particle size, the lower the polydispersity index and the greater the uniformity (size distribution percentage) [4].

Ligands and Magnetic Nanoparticles in Order to Improve Therapeutic Efficacy

In order to ensure that all the nanocarriers with loaded drug gets to the targeted site one can use magnetic nanoparticles or ligands added onto the nanocarriers. This also ensures that normal cells have less interaction with the doxorubicin drug upon release leading to less damage to normal cells. Once can accumulate the drug to the tumor

through magnetic control the drug must be immobilized at the site of the tumor by interacting the cancer cells. Target molecules could be attached to the nanocarrier surface to allow for interaction of drug loaded nanocarriers with cancer cells. Drugs that have a tumour specific molecule conjugated to it will allow for the molecules to release when the drug approaches the tumour. Most of the linkers are usually peptidase cleavable. Monoclonal antibodies which are able to bind to specific tumor antigens, with these antigens being able to be expressed on all tumor cells but not on host cells and most targeted cancer treatments using antibodies for specific cancer types are FDA approved. This combined with the use of ferrous fluids incorporated into the nanoparticle can allow for a greater efficiency in drug targeted delivery. The iron oxide would be incorporated in the organic phase in the methods described above which will allow one to use a magnet to allow for the drug to be moved to a predetermined site. It has been found through studies that magnetic nanoparticles do allow for the particles to be moved to a specific site of interest through magnetic resonance imaging and histological studies [5,6].

The third article describes how to create magnetic nanoparticles in order to allow for targeted drug delivery. This article is called "Magnetic nanoparticles encapsulated into biodegradable microparticles steered with an upgraded magnetic resonance imaging system for tumor chemoembolization."

Creation of Magnetic Nanoparticles

Materials

- FeCO₂
- Oleic acid
- PLGA

FeCo nanoparticle synthesis

CO₂, (CO)₈ and Fe(CO)₅ were cannulated into a solution of oleic acid and tris-n-octyl-phosphine oxide in DCB at 200 degrees Celsius under reflux and inert atmosphere.

After 35 minutes at 285 degrees Celsius the suspension was cooled down to room temperature and passed through a filter. The FeCo nanoparticles were washed by centrifugation with ethanol and then annealed in an oven. The nanoparticles were suspended in a solution of oleic acid in dichloromethane. The nanoparticles were sonicated and homogenized. The sonication and homogenization steps were repeated five times. The nanoparticles were then washed by centrifugation three times.

Encapsulation of nanoparticles into PLGA microparticles

PLGA was added to FeCo in DCM. FeCo nanoparticles and PLGA were emulsified in aqueous solution. At the end of the emulsion PVA solution was added to the dispersion. DCM was evaporated under rotation in vacuum for fifty minutes. FeCo-PLGA microparticles were collected on a filter after being washed three times. The microparticles were freeze dried until use.

MRI Steering

The setup is composed of a MRI scanner, MRI camera above the phantom, steering coils, syringe pumps and Plexiglas hepatic artery phantom with a rectangular cross section. An aqueous solution of bovine serum was used. The flow in the phantom was 8 mL/min in order to allow FeCo-PLGA microparticles to be detected by the camera. The steering efficiency was determined by video analysis; microparticles collected during each steering assay were degraded in solution of HNO₃. Iron and cobalt concentrations in each vial were quantified by AAS (atomic absorption spectrometer). The steering efficiency was determined by the ratio of the concentration of metallic ions collected in the vial placed at the outlet of the targeted channel divided by the sum of metallic ions collected in the two vials.

Results

FeCo nanoparticles

FeCo nanoparticles were synthesized and then annealed under inert atmosphere at 650 degrees Celsius to improve magnetic properties. TEM images show that the mean diameter of annealed FeCo nanoparticles increased to 182 nm with a broad distribution which can be explained by the aggregation of nanoparticles occurring upon the degradation of surfactant during the annealing process.

FeCo-PLGA nanoparticles

FeCo-PLGA nanoparticles had an average diameter of 58 micrometers which fits the size requirement for embolization. The loading capacity was 40 percent with AAS. The FeCo-PLGA microparticles display ferromagnetic behaviour with an Ms of 61 emu g⁻¹ which can be seen in Figure 3.

FeCo-PLGA microparticle MRI magnetic steering

The MRI steering ability of the synthesized microparticles were tested in a phantom mimicking the hepatic artery with a flow in the same order of magnitude as that of the hepatic artery flow. In the MRI tunnel, FeCo-PLGA microparticles formed aggregates in the direction of the magnetic field which can be seen in Figure 4. The steering efficiency was calculated and it was shown that with a magnetic gradient of plus or minus 400 mT m⁻¹ the steering efficiency was 86%. With no magnetic gradient the particles were equally distributed in both channels of the phantom.

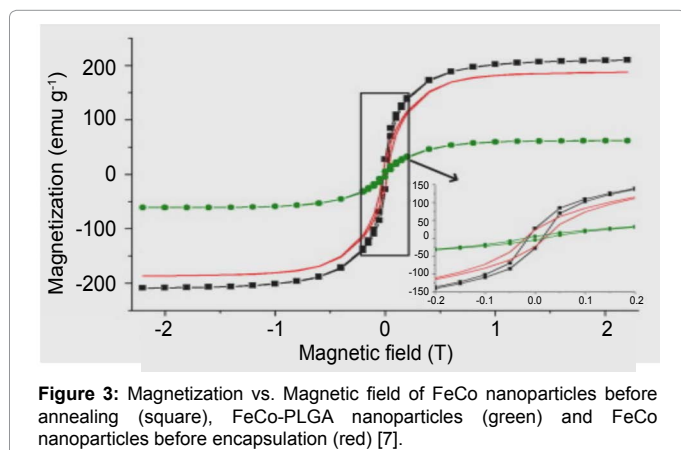


Figure 3: Magnetization vs. Magnetic field of FeCo nanoparticles before annealing (square), FeCo-PLGA nanoparticles (green) and FeCo nanoparticles before encapsulation (red) [7].

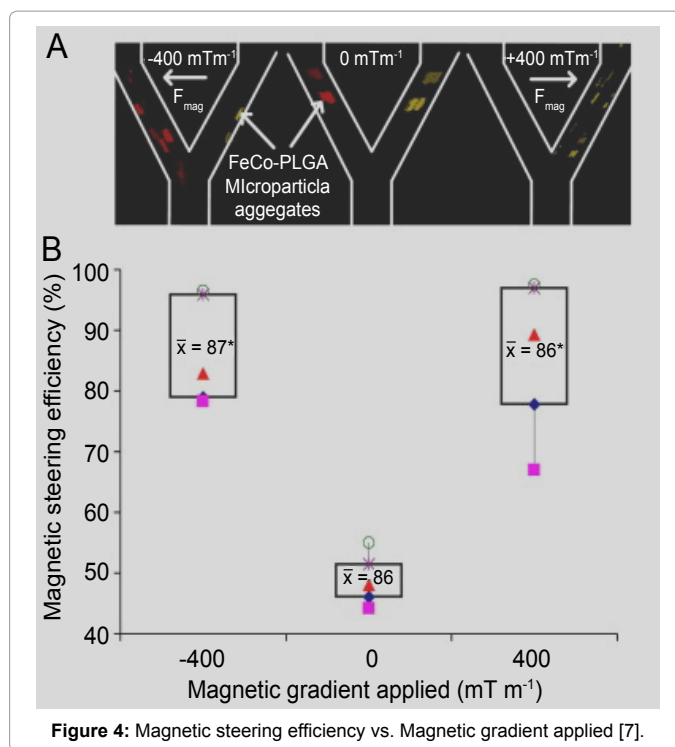


Figure 4: Magnetic steering efficiency vs. Magnetic gradient applied [7].

Critique

This journal article mentions how to create magnetic PLGA nanoparticles for chemoembolization. In this article it shows that magnetic fields are able to steer the magnetic particles in the direction of the magnetic field. The model that they used is an animal model of a rabbit in which they used a phantom mimicking the hepatic artery of a rabbit.

The problems with this journal article are that they only encapsulated a magnetic nanoparticle inside PLGA and determined whether steering took place when a magnetic field was used. However they did not encapsulate an anticancer drug into the nanoparticle to determine if this had an effect on its ability to be steered by a magnetic field and if its magnetization was affected when a drug was encapsulated into the PLGA nanoparticle.

Another problem with this paper is that a rabbit model was used. Instead of using parameters of a human the blood flow of a rabbit was used. The blood flow of the rabbit is 8.5 mL/min but because the MRI could not detect this flow a lower flow of 8 mL/min was used. Therefore one does not know what the actual steering efficiency of the rabbit is, nor does one know the steering efficiency of a human model.

Another critique is that no in vivo tests were conducted. In order to do this one can inject these magnetic particles with a fluorescent tag and put it into a rabbit with cancer and use a magnet to steer the drug to a particular part of the body. One can dissect the rabbit and by removing the part of the body that one wanted the drug to go to and see if a significant amount was at that source.

The fourth article describes how to covalently or noncovalently bond a monoclonal antibody (to treat cancer) on a PLGA nanoparticle. The article is called "Targeting cancer cells using PLGA nanoparticles surface modified with monoclonal antibody."

Creation of Targeted Nanoparticles using Monoclonal Antibodies

Materials

- PLGA
- PVA (polyvinyl alcohol)
- Alexa Fluor 546 labelled goat anti mouse immunoglobulin and Blue Cell Tracker from Molecular process
- Bovine serum albumin

Monoclonal antibody preparation

The mAb from mouse hybridoma cell lines was isolated; it was then purified from the hybridoma culture medium by affinity chromatography. The mAb recognizes cytokeratin's expressed in breast epithelial cell lines and breast tumour cells. The mAb was labelled with Alexa Fluor 546 dye and purified on a resin and stored.

Adsorption

After the creation of PLGA nanoparticles using a double emulsion technique nanoparticles were dispersed in PBS at pH 5, mAb was added to the solution and then was adsorbed onto the nanoparticles at four degrees Celsius for twenty four hours then centrifuged at 1000 rpm for 15 minutes to separate immune nanoparticles from free mAb. The sediment was washed in PBS and redispersed in PBS.

Covalent Binding

EDC (1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide) reagent was added to the nanoparticles and mAb solution. The reaction was stirred for two hours at room temperature. Excess linking reagent and soluble products were separated by centrifugation for ten minutes and the sediment was washed three times with PBS solution. EDC was used to conjugate the primary amine group of maybe with the carboxylic end of PLGA nanoparticles forming an amide bond.

Fluorescence microscopy

Localization of fluorescent dyes was observed using fluorescence microscopy

Cell cultures

Human breast epithelial cells (MCF-7 and MCF-10A) were used to determine cellular uptake of covalent and non covalent PLGA nanoparticles

Results

As one can see in Figure 5 cell lysates were used to test the ability of mAb to bind to cell type specific antigens. Nanoparticles with adsorbed mAb bind to the cell lysates and the extent of the binding is one third higher than the control. The control had no adsorbed mAb on it. Covalent coupling of mAb resulted in a loss of binding affinity to cytokeratin's in cell lysates and was significantly smaller than that of unmodified nanoparticles (control).

Cellular uptake of immune-nanoparticles by MCF-10A neoT cells

The uptake of nanoparticles was evaluated by fluorescence microscopy and confirmed the internalization of non-coated and immuno nanoparticles. The uptake was detected after one hour of incubation and increased progressively over the next twenty four

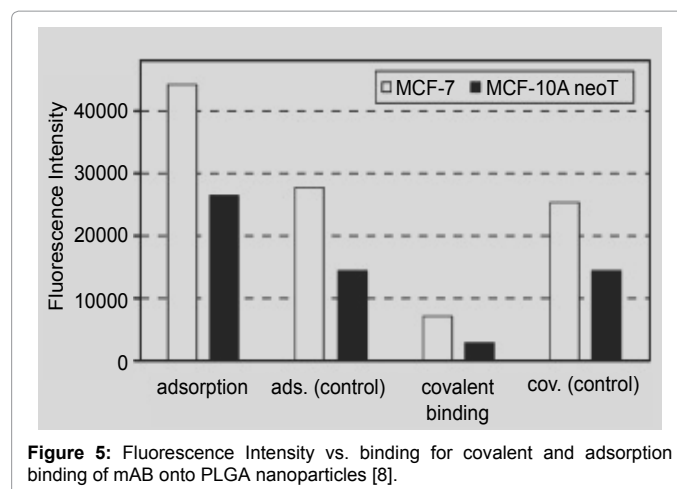


Figure 5: Fluorescence Intensity vs. binding for covalent and adsorption binding of mAb onto PLGA nanoparticles [8].

hours. After one hour 65% and 59% of cells had internalized immune-nanoparticles and non-coated nanoparticles [8]. After four hours the fractions increased to 81% and 77% [8] and after twenty four hours 94 percent of cells internalized immuno-nanoparticles and 94% non-coated nanoparticles [8].

Critique

This journal did a great job in comparing covalently and adsorption bonding of antibodies onto PLGA nanoparticles. However one of the results showed that the amount of covalently bonded nanoparticles onto cell lysates was lower than its control with no covalently bonded ligands. I believe that this journal should either find a new way to covalently bond ligands to the PLGA nanoparticles or recheck its process of forming an amide linkage in order to see if this did occur. One can do this using XRAY analysis or FTIR to see there is mAb antibodies on the nanoparticle surface.

The main purpose of adding antibodies onto nanoparticles is to allow targeting of nanoparticles in this case to cancer cells, so that healthy cells are not subject to cancer drugs. One should test these nanoparticles linked with antibodies on healthy cells and see how many of these antibody nanoparticles are taken into healthy cells by fluorescence microscopy, in order to determine if these particles served its purpose of targeting only cancer cells.

Conclusion

From the results described above one can see that single emulsion has a higher entrapment efficiency and a lower cumulative DOX release. A higher entrapment efficiency means that each nanocarrier has a higher drug content which means less amount of drug will have to be taken per day in order to maintain the dose rate needed in the patient's body. Also in order to reduce the damage to non-cancerous cells a lower release which the single emulsion technique takes into consideration. In cancer treatment a low sustained release of drug leads to a greater amount of cell apoptosis then a high single release of drug. Therefore it is better to use single emulsion.

In order to control the size of the nanoparticle, the uniformity and the distribution there are several techniques that can do such as increase sonication time and increase aqueous and organic phase which leads to a controlled nanoparticle size, a small distribution of particle sizes and greater uniformity.

In order to allow for more specific targeting of drug to specific area of the body one should use magnetic nanoparticles which means adding

ferric oxide into the organic phase of the methods described below. Magnetic nanoparticles as well as ligands targeting specific cancer cells allow for the nanocarriers containing drugs to be moved to a specific site of the body and the ligands allow for the drug to be released when the ligand is in contact with certain hormones on the cancer or specific cells. Adsorption of ligands onto nanoparticles seems to allow for greater adherence to cancer cells, therefore this method should be chosen instead of covalent bonding (amide linkages) of ligands onto nanoparticles. In order to determine if magnetic nanoparticles will lead to a high steering rate in human cells, one should use a human model as well as in vivo experiments to determine if the magnetic nanoparticles can be steered to specific parts of the animal's body.

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