Assembling a Dextran-Based Nanoparticle Platform for Uptake by Cancerous Cells

Rahul Shankar

under the direction of
Prof. Mansoor Amiji and Dr. Arun Iyer
Northeastern University

Research Science Institute
July 26, 2011
Abstract

The development of a versatile nanocarrier platform that can encapsulate a variety of drugs has proven to be a formidable challenge. In this study, a novel dextran-based nanoparticle platform was developed to encapsulate rhodamine and fluoroscien isothiocyanate (FITC). Using click chemistry, lipid-, thiol-, and poly(ethylene glycol)-conjugated dextran molecules were synthesized. Structures were confirmed by $^1$H-NMR spectroscopy and dynamic light scattering determined nanocarrier size. When incubated with free dyes and nanoparticles with dyes, cancer cells with nanoparticles had better fluorescent signals, showing a better method of uptake than the free dye. This dextran-based nanocarrier platform is promising for drug encapsulation for applications like cancer.

Summary

Current cancer treatment methods result in the unwanted death of both healthy and cancerous cells. A less toxic treatment method that uses nanocarriers as a drug delivery system is currently being investigated. The nanocarrier must encapsulate and carry a drug, pass biological barriers, release the drug at a sustained rate, and kill the cancerous cells. Nanocarrier components were synthesized separately and then combined to form nanocarrier molecules. By using fluorescent dyes as model drugs, a nanocarrier platform was developed and successfully encapsulated dyes with varying hydrophobicities. These dextran (type of sugar molecule) nanocarriers were tested on pancreatic cancer cells to determine the nanoparticle efficiency and cellular uptake. As is evident from microscopy, using nanocarriers results in a higher cellular uptake by the cancerous cells compared to the free dye.
1 Introduction

1.1 Nanotechnology and Cancer

Nanoparticles, generally defined to be particles between 1 and 100 nanometers in size [6], have shown significant potential as drug delivery systems, especially for cancer [15]. Current cancer treatment methods like surgery, radiation, and chemotherapy result in toxicity and death for both healthy and cancerous cells.

Nanoparticles that can pass through biological barriers in the human body, carry a drug to the targeted location, and release the drug at a sustained rate to eliminate solely cancerous cells may offer better treatment options. An added benefit of small size is relatively higher intracellular uptake and reduced detection, and consequently elimination, by the immune system [15]. There are several effective drugs available in the market, like paclitaxel and doxorubicin, that have been shown to kill cancerous cells in vitro, but they are ineffective in vivo because of poor encapsulation, delivery, and residency of the drug, and high levels of cytotoxicity and multi-drug resistance (MDR) development [7]. However, due to the fact that many novel anti-cancer agents have very different physicochemical properties, like molecular weight, charge, and hydrophobicity, there is a need to develop a versatile platform of nanoparticles capable of encapsulating a variety of payloads to become efficient therapeutic reagents [6].

1.2 Nanocarriers

Nanocarriers are nanoparticles used to transport drugs to target sites and are composed of several elements. In general, a polymeric substance forms the backbone of the nanocarrier to create the outer shell. Fatty acids and amines can be attached to the backbone to create a liposome-like particle. A hollow cavity allows for the encapsulation of a payload, usually anti-cancer therapeutic agents. In this study, dextran was used as the backbone, and two fluorescent dyes, rhodamine and fluoroscien isothiocyanate (FITC), were the payloads en-
capsulated by the lipid layer. The effectiveness of a nanocarrier can in part be predicted by values that measure the surface charge and solubility of the nanoparticle.

1.2.1 Dextran

Dextran is a glucan with variable chain lengths [2]. A short fragment of dextran is shown in Figure 1. Unlike other commonly used backbones such as hyaluronic acid, dextran has a neutral charge [7]. This means that forming the nanocarriers is less difficult since the charge does not need to be accounted for in regards to cytotoxicity of the nanocarrier. Another benefit of using dextran is its ability to bond with fluorescent molecules for imaging and fatty acids and amines to create self-assembled nanoparticles with the help of thiolated dextran molecules. To avoid detection by the immune system, the nanoparticles in this study are coated with poly(ethyleneglycol) (PEG), providing increased stealth and longer circulation in the bloodstream [2]. An added benefit to PEG is the ability to attach targeting moieties to the end of the PEG molecule [7].

Figure 1: Dextran Polymer Fragment [2]. The dextran fragment shows three monomer units that have bonded together to form a polymer. More monomer units were attached until the dextran’s molecular weight was 40 kDa, to create the dextran used in this study. Other moieties, like fatty acids or amines, are bound at the hydroxyl sites on dextran.

1.2.2 Fatty Acids and Amines

Fatty acids and fatty amines are composed of hydrocarbons with terminal carboxylic acid and amine groups, respectively. Their hydrophobicity is directly proportional to carbon chain length. Fatty amines possess a positive charge on the amine nitrogen, and the charge must be
taken into account when determining the $\zeta$-potential values (measure of the surface charge of nanoparticle) and cytotoxicity of the nanoparticle [7]. The carbon chain length of the fats, number of fats attached to dextran, and nitrogen content can be varied [1]. The size of the nanoparticle decreases as the carbon chain length increases, due to the increased van der Waals attraction between the hydrophobic molecules.

### 1.2.3 Payloads: Fluorescein isothiocyanate (FITC) and Rhodamine

Doxorubicin and paclitaxel are commonly used cancer therapeutics due to their ability to induce cellular apoptosis, but they have low specificity for cancerous cells [2]. To enhance their delivery to cancer cells, a nanoparticle platform must first be created to encapsulate these drugs. Two fluorescent dyes, FITC and rhodamine, were used as model drugs, in the sense that they are capable of being encapsulated by the dextran-based nanoparticle and can be detected in cells with confocal microscopy. These two dyes were chosen for this study because they encompass most of the spectrum of current dyes in terms of hydrophobicity. Rhodamine is highly hydrophilic, whereas FITC is highly hydrophobic [7]. If a nanocarrier platform can encapsulate both extremely hydrophobic and extremely hydrophilic dyes, most other dyes within that hydrophobicity range are expected to be well-encapsulated as well. The hydrophobicity of these drugs is measured using the partition coefficient ($logP$ value). The $logP$ value for rhodamine is 1.2 compared to -2.0 for FITC [7]. Hydrophobicity also impacts how the drugs disperse when passing through the human body, which determines therapeutic efficiency [11].

### 1.2.4 Molecular Self-Assembly

Molecular self-assembly occurs when a polymer conjugated to fatty acids or amines comes into contact with water. The hydrophobic fatty acids repel the water and tend to clump together, curling into a globular shape that forms the interior of the nanocarrier molecule. The exterior is composed of the hydrophilic dextran backbone, which is surrounded by wa-
ter. Upon submersion in an aqueous environment, these dextran-based nanocarrier building blocks self-assemble to form nanocarrier molecules, with an inner liquid core with drugs encapsulated by a polymeric membrane [15].

1.2.5 Specificity

Targeting only cancerous cells is in theory possible given the unique properties of the tumor vasculature. Lipid envelopes, which possess relatively similar exteriors compared to nanocarriers, are too large to pass through healthy blood vessels into normal cells, but can fit in pores of the vasculature. Tumor blood vessels have larger pores because the tumors alter the vasculature to obtain more oxygen and blood to function and grow [7]. These carriers can escape the bloodstream and accumulate next to tumor cells. This process is known as passive targeting of cancerous cells and is a result of the enhanced permeability and retention (EPR) effect [2].

Passive targeting, however, is not very effective since only a small percentage of the drug circulating in the bloodstream finds the tumor before the body washes it out. Active targeting involves the modification of the nanocarrier surface to allow preferential accumulation within tumor cells. For example, if PEG is coated on a biodegradable polymer (the dextran backbone), its physical properties supplement the increased hydrophilicity of the nanoparticle’s exterior. Being hydrophilic will allow for a systemic method of delivery through the bloodstream to cells when nanocarriers are used to deliver therapeutic agents [7].

2 Methods and Materials

2.1 Materials and Procedure Overview

All reagents used in the experiments were purchased from Sigma-Aldrich and used without additional purification, unless otherwise specified. 40 kDa dextran from Leuconostoc mesenteroides, stearyl amine (99% pure), cystamine, pyridine, sodium periodate (NaIO₄), sodium
cyanoborohydride (NaCNBH₃), potassium sulfate (K₂SO₄) and azo-bis-isobutyronitrile were purchased from Sigma-Aldrich Chemical Co (St. Louis, MO). Rhodamine-conjugated PTX was supplied by Natural Pharmaceuticals (Beverley, MA) and 6 well plates were purchased from Promega Corporation (Madison, WI). Dehydrated dimethylformamide (DMF) and dimethylsulfoxide (DMSO) were purchased from Acros Organics (Parsippany, NJ). PANC-1 human pancreatic carcinoma cells were purchased from American Type Culture Collections (Manassas, VA).

The flowchart displayed in Figure 2 shows the overall procedure used in this study. Three different dextran molecules were synthesized, which self-assembled to form the nanocarrier. A Michael-Addition approach was attempted to synthesize the 3 dextran-modified molecules, beginning with lipid-dextran, but ultimately did not work (Appendix A). Instead, click synthesis was used to synthesize the lipid-, thiol-, and PEG-modified dextran molecules. These components were self-assembled in an aqueous environment to encapsulate the dyes.

![Methodology Flowchart](image)

Figure 2: **Methodology Flowchart.** The flowchart shows the steps involved to synthesize the different types of dextran necessary for self-assembly. Two types of syntheses were used: Click and Michael-Addition. Both syntheses followed the same overall process, but the products from each reaction were slightly different.
2.2 Synthesis of O-Pentynyl Dextran

Dextran (MW = 40 kDa, 1 g, 6.17 mM) was dissolved in dry DMSO (40 mL). Once a clear solution was formed from mixing, 1.5 M MeLi (18.5 mL, 1.5 eq.) was added, and the mixture was placed in an ice bath to cool. 5-chloro-1-pentyne (0.6 mL, 0.3 eq.) was added slowly and this mixture was stirred for 24 hours under a nitrogen atmosphere. The product was precipitated and washed once with 350mL of ethanol and twice with 50 mL of ethanol. Each wash was followed by centrifuging the samples (15 min, 2400 g). The product was purified via dialysis against demineralized water and then freeze dried (1.25 g, whitish solid) with a degree of substitution (DS) of about 10 percent. The DS was determined using $^1$H-NMR.

2.3 Click Synthesis of Lipid-Modified Dextran

Experiments were carried out with O-pentynyl dextran and alkyl bromides ($C_nH_{2n+1}Br$, $n = 4, 8$). In a representative experiment, O-pentynyl dextran (DS 10%, 250 mg) was dissolved in water (25 mL) and added to a round-bottom flask containing bromoethane (2g, 18.35 mM), sodium azide (2.38 g, 36.71 mM), sodium ascorbate (19 mg, 0.129 mM) and copper (II) sulfate pentahydrate (8 mg, 0.032 mM). After 24 hours of stirring at room temperature, the mixture’s product was purified via dialysis against demineralized water and subsequently freeze-dried (180 mg, pale green solid).

2.4 Click Synthesis of Thiol-Modified Dextran

Sodium azide (NaN$_3$, 0.5 g, 3.17 mM) was added to a solution of 1-bromo-3-chloropropane (0.2 g, 3.17 mM) in 15 mL of DMF at room temperature. The reaction was stirred overnight and then phase-separated between ether and water, and the organic layer was washed with water, dried over Na$_2$SO$_4$ and concentrated to give 1-azido-3-chloropropane (0.3 g, 92%) as a colorless, viscous liquid. A solution of cysteamine (0.19 g, 2.5 mM) in THF (15 mL) was added to a stirred suspension of 1-azido-3-chloropropane (0.3 g, 2.5 mM) in THF (15 mL).
After stirring under a nitrogen atmosphere for 3 days at room temperature, the solvent was evaporated in vacuo and the yellow solid residue was washed with THF/hexane (1/5 ratio). This product was dissolved in 25 mL water and added to a round-bottom flask containing O-pentynyl dextran (DS 10%, 250 mg), copper (II) sulfate pentahydrate (8 mg, 0.032 mM), and sodium ascorbate (19 mg, 0.129 mM). After stirring at room temperature for 24 hours, the product was purified by dialysis against demineralized water and freeze-dried (110 mg, pale yellow solid).

2.5 Click Synthesis of PEG-Modified Dextran

Methoxypolyethylene glycol azide 2000 (250 mg) and O-pentynyl dextran (DS 10%, 250 mg) were dissolved in 25 mL water in a round-bottom flask. Copper (II) sulfate pentahydrate (8 mg, 0.032 mM) and sodium ascorbate (19 mg, 0.129 mM) were added and the mixture was stirred at room temperature for 24 hours. The product was purified by dialysis against demineralized water and freeze-dried (320 mg, pale green solid).

2.6 Preparation of Drug-Encapsulated Self-Assembled Nano-Structures

Drug-loaded nanoparticles were prepared by three different processes: nano-precipitation, solvent evaporation, and dialysis. The nano-precipitation method was applied as follows: Dex-lipid (20 mg), Dex-thiol (15 mg), and Dex-PEG (15 mg) were dissolved together in 5 mL of water and the dye (0.5 mL) in either ethanol or dimethyl sulfoxide (DMSO) (1 mL) was added and vortexed for 5 minutes. Another 5 mL of water was added and vortexed for 5 minutes, then the mixture underwent dialysis. The resulting liquid was then lyophilized and the solid material obtained was dissolved in 5 mL of water. Rhodamine-loaded dextran nanoparticles were also prepared by solvent evaporation method. Briefly, 0.5 mg of rhodamine dissolved in 0.5 mL of ethanol was added to 5 mL of water containing Dex-lipid (20 mg) and stirred vigorously. The organic solvent was evaporated by gaseous nitrogen evaporation at
room temperature. Then, Dex-thiol (15 mg) and Dex-PEG (15 mg) were added to the mixture and vortexed for 5 minutes. The mixture was then lyophilized and the solid material obtained was dissolved in 5 mL water. Preparation of FITC and rhodamine loaded-nanoparticles was carried out by a dialysis process. The self-assembly procedure is outlined in Figure 3.

![Figure 3: Self-Assembly Procedure](image)

**Figure 3: Self-Assembly Procedure** [1]. This image depicts how nanocarrier building block elements interact to form a self-assembled nanoparticle.

## 2.7 Characterization of Self-Assembled Nano-Structures

Nuclear magnetic resonance (NMR) spectroscopy was used as a basis to determine if dextran was conjugated to other molecules, for example the fatty amines or acids. The nanoparticle sizes and size distributions were determined by dynamic light scattering using a ZETASIZER (3000HS, Malvern Co., UK) at a 90° fixed angle at 25°C. The nanoparticle solution was diluted for particle size analysis with deionized distilled water (1 mL), and the average hydrodynamic diameter and the polydispersity index (PDI) were determined. The PDI is a ratio generated that determines the homogeneity of a sample, where an ideal value would be close to 1. Based on the sizes and PDIs calculated, it is possible to determine a rough estimate for the percentage of molecules that actually encapsulated.
2.8 Cellular Uptake and Intracellular Drug Distribution

Cell Preparation. The PANC-1 human pancreatic carcinoma cell line was grown in RPMI medium supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin. Cell cultures were maintained in a humidified 95% O₂/5% CO₂ atmosphere at 37°C. For the subculture, cells growing as monolayer were detached from the tissue culture flasks by treatment with 0.05% trypsin/EDTA.

Standard Curve. In order to determine the amount and concentration of dye needed to add to the PANC-1 cells, standard curves were calculated for both FITC and rhodamine. A serial dilution for each dye was tested for absorbance levels using a UV-vis instrument. The standard curve for rhodamine is shown Figure 4. The standard curve was linear and the coefficient of determination was approximately 0.9979. When the fluorescence of the cells is detected in a later step (microscopy), the images generated should not have oversaturated or undetectable fluorescence.

Adding Nanocarriers. Drug-loaded dextran nanoparticles were prepared as previously described for the cellular uptake test. The cells were seeded in two 6-well plates, one for FITC and another for rhodamine samples, and incubated for 24 hours. Glass coverslips were placed at the bottom of each well to collect cells for microscopy. The cells were exposed to a medium containing the free drug or nanoparticles loaded with drug for further 1 and 3 hour incubation periods. To counter-stain nuclei, the cells were incubated with 1 µg/mL of DAPI (Invitrogen, Carlsbad, CA) for 5 minutes. The cells were washed with RPMI medium 3 times.

Microscopy. The coverslip was removed from the bottom of wells and flipped onto a slide. Brightfield and fluorescence microscopy images at varying magnifications were obtained on a Zeiss confocal microscope. Transmission electron microscopy (TEM) analysis was used to determine the dye’s distribution in the cells as well.
Figure 4: **Rhodamine Standard Curve.** Rhodamine was dissolved in ethanol and underwent a serial dilution to change concentration values. Based on the output of a UV-vis instrument, the absorption (AU) was plotted with the concentration to make the standard curve. A linear trend shows a strong correlation, so the curve can be used to determine the concentration and amount of rhodamine needed to add to the cancerous cells.

# 3 Results and Discussion

Dextran was first purified by washing with ethanol and the NMR spectroscopy was used to determine the sample’s purity. As is evident in Figure 8, there seem to be few impurities away from the main peaks. Following dextran polymer oxidation, the NMR spectrum shows additional right-shifted peaks that indicate the addition of carbon atoms to form \(O\)-pentynyl dextran (Appendix B, Figure 9).

Initially, two methods for synthesizing the dextran components in the nanocarrier were explored. For click synthesis, the initial reactants are volatile but stabilize during the reactions to form dextran. On the other hand, Michael-Addition reactions become more volatile since energy must be inputted into the system in order to synthesize the dextran. The
Michael-Addition reactions did not work well because the lipid-modified dextran particles were insoluble. These molecules became insoluble and were not pursued in further reactions. The click synthesis reactions were much simpler to perform and eventually resulted in successful encapsulation.

Once the lipid-modified dextran was synthesized, NMR was performed again to check for the conjugation and the purity of the sample. Additional peaks from the newly-joined carbon atoms are visible in Figure 10 on the right side of the spectrum, meaning that the fat molecules were able to attach to the reactive hydroxyl groups on the dextran polymer.

In order for the reactants to click, the triple bond on the end of the O-pentynyl dextran has to react with another molecule, for example the nitrogen, capable of bonding to the site. The nitrogen-containing azide clicks with the O-pentynyl dextran and the thiolated dextran was formed. To synthesize PEG-modified dextran nanoparticles, a different azide is required to click with the O-pentynyl dextran. The click reactions were simple, yet reliable and effective at synthesizing the lipid-, thiol-, and PEG-modified dextran components.

The components of the nanocarriers were mixed together to encapsulate the dyes. At first both FITC and rhodamine were prepared using the nano-precipitation method. However, during the dialysis, the rhodamine in the rhodamine samples washed out of the dialysis membrane. This could be attributed to the fact that rhodamine is very hydrophilic, and was not as attracted to the fatty acids and amines on dextran compared to FITC. A new approach to encapsulating rhodamine was thus required, and resulted in the successful encapsulation of rhodamine samples. By creating a new method for the thiol-modified dextran, the thiol-dextran produced was more yellow, a sign that more sulfur was present, thus increasing the ability for the molecules to bond together. A Zetasizer instrument was used to determine the size of the nanoparticles, as shown in Table 1.

After the cells had been stained with the DAPI dye, fluorescence microscopy was used to determine the cellular uptake and dye distribution in the cells. In Figure 5, a split-channel view of the image produced is shown. The merged, color image (Figure 5a) was split into
<table>
<thead>
<tr>
<th>Sample</th>
<th>Size (nm)</th>
<th>Polydispersity Index (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C4 Lipid-Dextran</td>
<td>75.41</td>
<td>1.000</td>
</tr>
<tr>
<td>C8 Lipid-Dextran</td>
<td>122.2</td>
<td>0.316</td>
</tr>
<tr>
<td>C4 Lipid-Dextran FITC</td>
<td>193.1</td>
<td>0.397</td>
</tr>
<tr>
<td>C8 Lipid-Dextran FITC</td>
<td>171.1</td>
<td>0.230</td>
</tr>
</tbody>
</table>

Table 1: The size of the elements in each different sample was calculated using a Zetasizer instrument, which uses dynamic light scattering. Both the size and polydispersity index (PDI) are outputted from the instrument.

Table 1 above shows the size and polydispersity index of different samples used in the study. The data indicates that the size of the nanoparticles ranges from 75.41 nm to 193.1 nm, with the polydispersity index ranging from 1.000 to 0.230. These values suggest a relatively narrow size distribution for the samples.

The bright-field (Figure 5b, DAPI-colored (Figure 5c), and dye-colored images (Figure 5d). A free dye sample was used as a control to see how the non-encapsulated dye distributes itself in the cell compared to the nanocarrier particles. In Figures 5a–d, the free dye for FITC at 60× magnification at the 1 hour time point is displayed.

Similarly, the dextran polymers that reacted with the bromobutane (4 carbons) and bromooctane (8 carbons) encapsulated the dyes and were checked for fluorescence. The 1 hour trial for FITC with C4 lipid-dextran is shown in Figure 10a–b.

The rhodamine C8 lipid-modified dextran nanocarriers after 3 hours of incubation are shown in the images in Figure 7a–c. The method of encapsulation had to be changed in order for the rhodamine nanocarriers to be imaged. The first attempt at encapsulation resulted in the washing out of most of the sample.

The pictures from the fluorescence microscopy were visually determined that the nanoparticles resulted in a stronger fluorescence from the cells. There were some problems with detecting the different fluorescence signals, especially between the DAPI and FITC. A slight overlap in wavelengths exists between DAPI and FITC, so often mixed signals emitted from the images. This effect was minimized by using computer software to readjust the colors based on the dye.

In theory, the nanoparticles were able to enter the cell through endocytosis and achieve a higher efficiency. Although nanoparticles do not travel into the cell via a specific pathway, the majority have been shown to enter through macropinocytosis [12]. In macropinocytosis,
Figure 5: **1 hour 60× FITC Free Dye.** The free dye was added to the cells and diffused across the cell. Figure 5a shows the combined fluorescent image, 5b shows the bright field, 5c shows the DAPI nucleus stains, and 5d shows the location of FITC in the cells.

A portion of the cell’s lipid bilayer pinches off to surround the incoming nanocarriers. This vesicle then travels into the cytoplasmic matrix and then merges with other vesicles in endosomes or lysosomes. If nanocarriers enter the lysosomes, they will hydrolyze and break down. Many nanocarriers are designed with timed-release mechanisms, and dextran-based nanocarriers are able to open in acidic environments. The acidic environment in a lysosome will allow the dextran shell to deteriorate and release its contents into the cell. Nanoparticles have also been located in the endosomes of a cell [11]. From this point, it is difficult to predict what would happen to the nanocarrier, but they might go to the plasma membrane of a cell, or to the lysosome if it is a late endosome. Further research regarding the pathways of nanoparticle entry into cells will help to design nanoparticles capable of targeting subcellular regions within cells.
Figure 6: **1 hour C4 FITC Lipid-Dextran.** The nanocarriers with C4 lipid-dextran were used to encapsulate the FITC dye. Figure 10a shows the merged image of the 1 hour fluorescence, and Figure 10b shows the same image with a different color scheme.

### 4 Conclusion

#### 4.1 Successful Assembly and Cellular Uptake of Nanocarriers

The dextran-based nanocarriers were able to encapsulate both the rhodamine and the FITC dyes. A new method of encapsulation was applied to rhodamine. As a result, the rhodamine was not washed out during dialysis and was internalized by the nanocarrier particles. In addition, the nanocarriers showed higher concentrations and uptake into the cells in comparison to the free dye. This research has demonstrated a promising method for actively targeting and delivering drugs to diseased areas of the body without affecting healthy tissue and cells, the most detracting aspect of current surgical and radioactive treatment approaches.
Figure 7: **3 hour C8 Rho Lipid-Dextran.** Rhodamine was encapsulated in C8 lipid-dextran nanocarriers for 3 hours. Figure 7a was the bright field, 7b was the image with DAPI stains, and 7c was the fluorescence given off by the rhodamine.

### 4.2 Future Work: Molecular Dynamics

Numerous factors affect the polymeric composition, drug absorption, biodistribution pattern, and elimination of the nanoparticles. Some factors include the hydrophobicity ($logP$ value) and surface charge ($\zeta$-potential value) of the polymer and the molecular weight and charge of the incorporated dye or drug. A new nanocarrier is formed when all the nanocarrier building blocks self-assemble. The properties of the final products can be predicted using molecular dynamics software. Specific physical properties of the final nanocarriers, especially the $logP$ and the $\zeta$-potential values, will aid in reducing cytotoxicity and other biological barriers that are typically encountered during *in vivo* tests [7].

By employing molecular dynamics computer programs, the theoretical compositions,
physical properties, and 3-dimensional structures of the nanocarriers can be rendered, and their elements’ interactions are visualized using Newtonian mechanics. A theoretical model can significantly decrease the amount of time required for experiments because a computer can test thousands of combinations and concentrations of nanocarrier elements in far less time to create a combinatorial library. This helps to identify promising combinations for in vitro and in vivo testing. The top matches will be compared with the experimental data obtained from this study to determine how well the program worked and the most effective nanocarrier building materials combination. New combinations can be predicted by the program, which will then be validated experimentally.

5 Acknowledgments

I am extremely grateful and appreciative of my mentors, Prof. Mansoor Amiji and Dr. Arun Iyer of Northeastern University, for their guidance on this project. I would like to express my sincerest gratitude towards the Research Science Institute, Center for Excellence in Education, Massachusetts Institute of Technology, the Leonetti/O’Connell Family Foundation, Mr. Jonathan A. Marcus, Mr. and Ms. Michael J. Miller, the Kenneth and Myra Monfort Charitable Foundation Inc., and all RSI staff and support for making this program possible and giving me the opportunity to work in Boston this summer. I would also like to thank Annie Ouyang, Vinay Tripuraneni, Varoon Baskyakarla, Shuyu Wang, Joseph Dexter, Laurie Rumker, Hadass Inbar, and Albert Wu for the suggestions and support they had to offer.
References


A  Michael-Addition Synthesis of Lipid-Modified Dextran

The paper by Zhang et al. was used as a basis for the dextran acrylate synthesis [17]. In a round bottom flask, dextran (MW = 40 kDa, 2g) was dissolved in a LiCl/DMF (50mL) solvent mixture. The flask was placed in an oil bath and the temperature was increased to 120°C over 2 hours. The reaction resulted in a golden homogenous solution. After cooling to room temperature, 500 µL of pyridine was added and stirred. The flask was then placed in an ice bath and acryloyl chloride (1-1.5 molar excess) was added dropwise for 1 to 2 hours via an addition funnel. The reaction was continuously stirred overnight. The dextran-acrylate product was precipitated using cold ethanol, and was washed three times with ethanol.

Approximately 200 mg of the produced dextran acrylate was dissolved in dry DMF and stirred with 5-10% stearylamine and a 0.01 mole % catalyst AlCl₃. After being heated for 24 hours in an oil bath, the resulting stearyl-modified dextran was precipitated and washed in ethanol. The lipid-dextran was dissolved in a small amount of deionized water and lyophilized (pale yellow solid). NMR spectroscopy confirmed the conjugation between the lipid and dextran molecules.

B  ¹H-NMR

The spectrum in Figure 8 shows the nuclear magnetic resonance for dextran. This spectrum was used as a basis to determine if the dextran was changed during reactions.

The purified dextran was then oxidized to produce O-pentynyl dextran as seen in Figure 9. The peaks that resulted from the reaction are circled in red.

After the dextran was oxidized, the click synthesis process began. The dextran reacted with alkyl bromides to form the dex-lipid product. For example, the resulting spectra for the reaction with bromobutane is shown in Figure 10.
Figure 8: $^1$H-NMR

Figure 9: $^1$H-NMR of O-Pentynyl Dextran
Figure 10: $^1$H-NMR of C4 Dex-Lipid