A Technical and Economic Evaluation of Novel pH-Responsive Core-Shell Nanoparticles: Delivering Innovation from Laboratory to Market

by

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Submitted to the Department of Materials Science and Engineering on July 09, 2008 in partial fulfillment of the requirements for the degree of Master of Engineering in Materials Science and Engineering

Abstract

Many potentially powerful therapeutic strategies for the treatment of disease require the delivery of drugs into the cytosolic or nuclear compartments of cells. Members of the Irvine laboratory have developed a novel pH-responsive core-shell nanoparticle system that can achieve efficient and non-cytotoxic drug delivery into the cytosol. Another advantage is that the shell can be easily modified to bind to different types of drug agents and incorporate ligands for specific cell targeting. Experimental analysis of the newly synthesized nanoparticles with various shell structures has demonstrated that modification of the shell does not compromise their cytosolic delivery. These nanoparticles, if successful, will improve the therapeutic potential of a wide range of drugs. However, critical issues on the research side need to be resolved, and an appropriate intellectual property strategy should be initiated in the near future.

Applications to siRNA delivery and vaccines have been examined in depth, as cytosolic delivery is one of the main challenges in these fields. Partnerships with large pharmaceutical companies are critical in order to acquire key patents on siRNA/antigen. Even though the market is competitive, there is a strong demand for innovative delivery platforms; provided that the overall profile of the core-shell nanoparticles is comparable to that of emerging drug delivery systems, and a strong intellectual property portfolio is developed, the Irvine technology should be able to compete in the market.

After analyzing risks on the business side, including the FDA approval process, a suggested business strategy is outlined, through which value can be successfully obtained throughout the existing pharmaceutical supply chain from the novel drug delivery system. The Irvine technology company will develop formulations, contract manufacturers will produce the nanoparticles, and pharmaceutical companies will concentrate on clinical trials, late-stage development and sales and marketing. A case study on the liver cancer market has demonstrated that commercial development of the Irvine nanoparticles can be a financially successful endeavor.

Thesis Supervisor: Darrell J. Irvine
Title: Eugene Bell Career Development Associate Professor of Tissue Engineering

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This thesis is dedicated to my parents. Without their unyielding encouragement and support, I would never have been able to achieve what I have. Last, but not least, I would like to thank my sister and friends for their love and support.

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1.0 Introduction

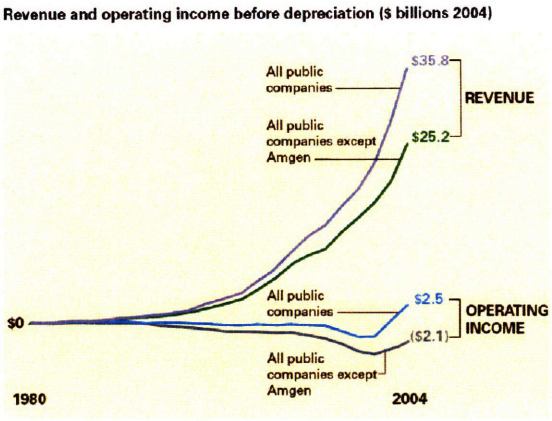


Figure 1: Profitless growth of the biotechnology industry[2].

Despite massive efforts into drug discovery fueled by revolutionary technologies, such as combinatorial chemistry, recombinant DNA and high-throughput screening, productivity in the pharmaceutical and biotechnology industry has been decreasing. Over the past few years, new chemical entities accounted for only one in four products approved, with the majority of approvals being combinations of already approved products[1]. Most biotechnology companies have yet to make operating income (Figure 1[2]). Some of the recurring problems in drug development include low water solubility, instability, poor pharmacokinetics and insufficient cell uptake of macromolecules, such as proteins. Many scientists from diverse fields around the world have turned to advanced drug delivery system technology, especially nanotechnology, to solve these challenges. Recent developments in the Irvine laboratory have demonstrated that, through use of novel pH-responsive core-shell nanoparticles, cytosolic delivery of drug molecules can be achieved. As many potentially powerful therapeutics strategies for the treatment of disease, such as gene therapy mediated by plasmid DNA, gene silencing or RNAi interference by oligonucleotides and therapeutic protein delivery, depend on efficient cytosolic delivery, the technology has the potential to have a huge impact on the healthcare industry.

2.0 Background

2.1 Cytosolic Delivery

Although small non-polar molecules can permeate through cell membranes, hydrophilic macromolecules are internalized through three major types of endocytosis. Phagocytosis is an active process performed by specialized cells via specific or non-specific receptors. Receptor-mediated endocytosis is another active mechanism to uptake specific macromolecules and involves clathrin-coated pits. On the other hand, macropinocytosis is a continuous, receptor-independent and passive ingestion[3]. These processes confine molecules in endosomes or

phagosomes where the pH is progressively decreased to 5.5-6.5. Upon fusion of these vesicles with lysosomes, pH is further lowered to 4.5. The low pH environment and degradation machinery of the vesicles often lead to rapid destruction of therapeutic agents without any release into the cytosol (Figure 2[4]). Therefore, cytosolic delivery presents a major challenge.

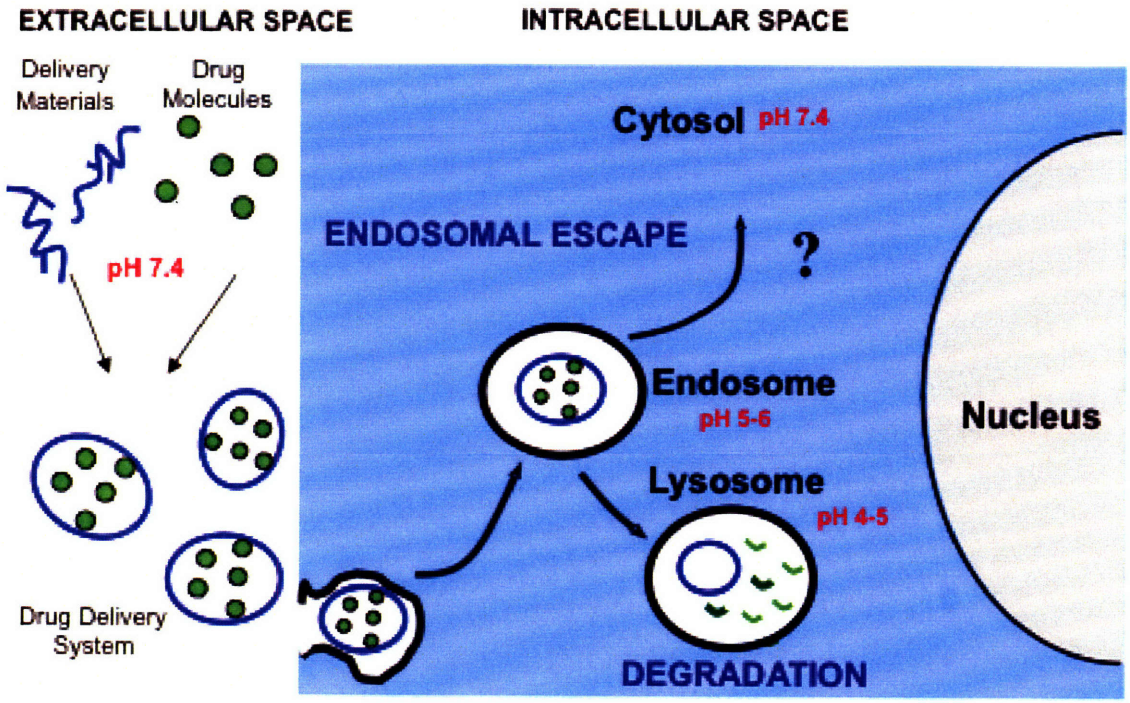


Figure 2: Typical pathway for intracellular drug delivery[4].

2.2 Barriers to Cytosolic Delivery

In addition to the endolysosomal escape mentioned in section 2.1, there are several barriers to efficient delivery of drug molecules to the cell cytosol. First, a practical drug delivery system should be able to detect the disease area and target specific cell types. This capability would prevent widespread biodistribution of the drug delivery system after administration, limiting immune responses and side effects from affecting normal tissues. Conjugation of targeting moieties or antibodies specific to receptors expressed by target cells can not only achieve specific cell targeting but also improve cellular uptake via receptor-mediated endocytosis, which is another obstacle to cytosolic delivery[5]. Size and surface charge of the drug delivery system are additional critical parameters for cellular uptake. Studies have shown that positive surface charge is advantageous since the cell membrane possesses slightly negative charge[6]. After endolysosomal escape, drug molecules should be released from the drug delivery

system to the cytosol. Without unpacking of the drug cargos from their carriers, effective dosage would be low. Although not essential as long as biocompatibility is met, biodegradability of the drug delivery system to improve the safety profile is also of interest. For example, research on polymeric carriers that can undergo hydrolysis or enzymatic degradation is ongoing[7-9].

In summary, an ideal intracellular drug delivery system should be able to target specific cell types, easily be taken up by cells, perform endolysosomal escape, release drug molecules and undergo degradation to non-toxic components.

2.3 Past Approaches to Cytosolic Delivery and Their Limitations

As the importance of cytosolic delivery to increase therapeutic potential of drug agents has been known for a long time, numerous approaches to the problem have been reported. Physical methods, such as microinjection, exist, but they are highly invasive and cannot be used for in vivo application. Therefore, most efforts have focused on developing drug delivery systems that encapsulate or coat drug agents and deliver them to target cells. Although viral vectors are highly efficient, immunogenicity and safety concerns are significant[10]. On the other hand, liposomes are non-toxic, but not stable in vivo.

Polymeric carriers are promising because size, charge density and chemistry can be tailored to achieve a desired functionality. In addition, polymers are stable and can be engineered to have extended circulation time in the body[11]. One of the most widely studied polymer-based delivery systems are based on hydrolytically degradable polyesters, such as poly(lactic-co-glycolic acid) copolymers (PLGA). Although they are biodegradable and biocompatible, they present several major limitations. First, hydrolysis of the ester backbone leads to an acidic microenvironment that can denature drug molecules[12]. Furthermore, these materials cannot perform endolysosomal escape. Most of the drug molecules are eventually degraded by low pH and enzymes in endosomes before they can be released into the cytosol. Thus, these systems are not suitable for intracellular drug delivery.

One of the earlier studies involved the use of polyethleneimine (PEI), polyhistidine and polylysine systems that have the ability to escape endosomes through the "proton sponge" effect, which will be described in section 2.4. However, significant cytotoxicity due to high cationic density, which leads to perforation of cell membranes, is a major issue[13-15]. Also, strong electrostatic binding between these polycationic materials and negatively charged therapeutic

agents prevents efficient release into the cytosol. Another approach has been directed at developing synthetic chaperones that can facilitate transport of drug molecules across the cell membrane. Examples include cell membrane penetrating peptides (CPPs) and pathogen-derived pore-forming proteins[16]. While many of these approaches show promise, a drug delivery system that promotes efficient cytosolic delivery while avoiding cytotoxicity is still sought.

2.4 Nanoparticle Description

Members of the Irvine laboratory have developed novel crosslinked hydrogel nanoparticles with core-shell structure that respond to the pH environment. As demonstrated in Figure 3[17], the core of the nanoparticles is formed from polymerization of 2-diethylamino ethyl methacrylate (DEAEMA) with poly(ethylene glycol) dimethacrylate (PEGDMA) as a crosslinker. Because DEAEMA possesses a tertiary amine with a pK_b of 7.0-7.3, the core is pH sensitive. The amine groups become protonated during transition from the extracellular pH of 7.4 to the endolysosomal pH of 5, which is responsible for a significant chloride accumulation in order to maintain charge neutrality. The increase in osmotic pressure drives water uptake and swelling of the particles, referred to as the "proton sponge" effect, and leads to disruption of the endosomes, and release of the nanoparticles to the cytosol. The pH-insensitive outer shell is created from polymerization of 2-aminoethyl methacrylate (AEMA). AEMA contains a primary amine group that would normally be protonated. The outer shell is hydrophilic and therefore, mediates drug/cell binding with low toxicity by shielding the hydrophobic core inside.

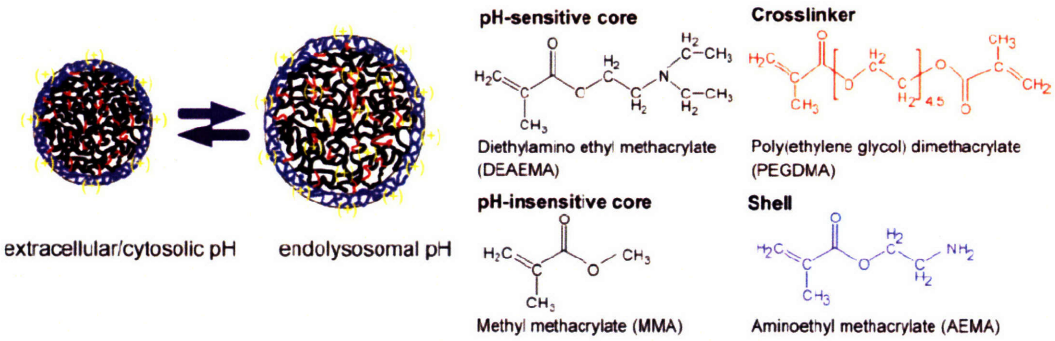


Figure 3: Schematic structure and chemical composition of pH-responsive core-shell nanoparticles[17].

2.5 Small Scale Production of Nanoparticles

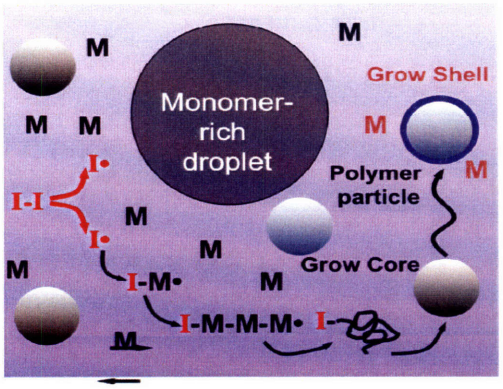


Figure 4: Theory of emulsion polymerization[4].

Surfactant-free emulsion polymerization, which involves an oil-in-water (o/w) emulsion, is used to produce the nanoparticles. Droplets of monomer are emulsified in a continuous phase of water. Although slightly hydrophobic, small amounts of monomer diffuse through the water and undergo radical polymerization in the presence of water-soluble initiators to form growing particles. As these latex particles are water-insoluble and possess similar surface charge, they are prevented from coagulating with each other, leading to monodispersity (Figure 4[4]). The surfactant-free emulsion polymerization is a very simple synthetic process to generate particles suitable for biological applications. In addition to control over size, surfactant-free aspect allows the particles to be easily purified.

First, DEAEMA (1mL, 4.97mmol) premixed with PEGDMA 200 (10μL, 0.03mmol) are dispersed in 9mL of water with stirring and equilibrated at 70°C for 15 minutes before adding ammonium peroxodisulfate (10mg) as the initiator. The emulsion polymerization is allowed to proceed for 3 hours to grow the particle core, followed by injection of AEMA (40mg, 0.24mmol) to grow the particle shell for an additional 1.5 hours. The nanoparticles are purified by dialysis in deionized water for 3 days, followed by ultrafilitration and centrifugation at 15,000rpm. The nanoparticles are stored in phosphate buffered saline (PBS) solution at 4°C until needed[17].

2.6 Distinguishing the Irvine Nanoparticles

The Irvine nanoparticles are highly monodisperse, with the size being approximately 200nm at pH of 7.4 and temperature of 37°C. The swelling transition occurs at a narrow range of pH (6.8 to 7.0 at 37°C), and the diameter and volume changes between pH 5 and 7.4 are 2.8 and 22 fold, respectively (Figure 5[17]). The Irvine group has demonstrated that the nanoparticles are stable, efficiently deliver ovalbumin as model drug molecules to the cytosol and have low cytotoxicity. The proposed mechanism of cytosolic delivery is confirmed through multiple experimental data.

Irvine nanoparticles offer many advantages over existing technologies for the cytosolic delivery. Synergy of these properties is responsible for novelty of the research.

- Efficient cytosolic delivery of membrane-impermeable molecules
- Low cytotoxicity through the core-shell structure
- Control over the particles size, chemical groups and stability
    - Optimal size for cellular uptake
    - Monodispersity
    - Ease of targeting ligand introduction
    - Ability to load many types of drug agents with the outer shell modification
- Inexpensive process

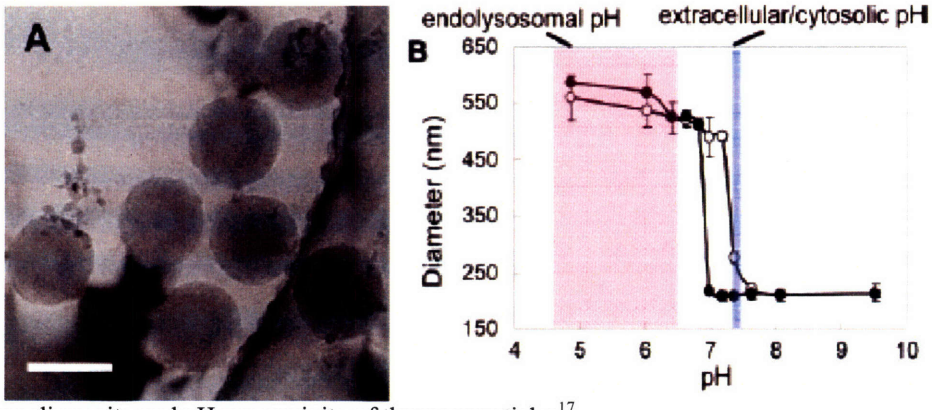


Figure 5: Monodispersity and pH responsivity of the nanoparticles[17].

2.7 Hype over Nanoparticle Drug Delivery Systems

Since the early to late 1990s, nanoparticle technology has seen an explosion in terms of research in the pharmaceutical industry. Many scientists believe that nanotechnology offers an excellent opportunity to address the challenging needs and requirements of the newer drug moieties in order for them to exhibit their targeted therapeutic effect. This has led to introduction of new terms, such as nanomedicine and nanobiotechnology. More than 1,650 articles related to nanotechnology and drug delivery since 2005 indicate the extent of interest in this field. Government funding dedicated to nanotechnology is abundant. The National Science Foundation has predicted that by 2010, nanotechnology based-drug delivery applications will account for half of all the pharmaceutical sales. Industry analysts predict that annual drug revenue in the nano-pharma market will grow by $80 to 200 billion by the year 2015 to 2020[1].

However, many of the current nano drug delivery systems, including the Irvine nanoparticles, are remnants of conventional drug delivery systems that happen to be in the nanometer range[18]. In fact, nanoparticles were first developed approximately 35 years ago[19]. Despite the hype created by the industry and market, formidable challenges do remain. Therefore, it is important for one to critically analyze all the risks involved in transferring the technology from laboratory to market. Through an iterative process, one can assess whether the Irvine technology will be able to be commercialized and generate a profitable business model. Some of the risks are listed in Table 1 and will be discussed throughout the thesis. Adaptability due to broad research and broad applications can mitigate these risks.

Risks Involved in Commercializing the Irvine Technology	
Research Side	Commercialization & Market Side
Loading of the actual drug molecules	Human clinical trials
Incorporating biodegradability	Long time horizon
Specific cell targeting	Manufacturing process
Route of administration	Overestimation of the market

Table 1: Risks involved in commercializing the Irvine nanoparticles.

3.0 Nanoparticles with Different Shell Structures (Research)

3.1 Purpose of the Study

One of the advantages of the Irvine nanoparticles is that emulsion polymerization allows composition of the shell to be separately tuned to facilitate particle targeting, cell binding, and drug binding. To analyze whether modifications of the shell structure compromise the endosome-disrupting function of the pH-responsive core, various analogs of the core-shell particles were synthesized. This section summarizes the design of these nanoparticles and discusses their pH-responsivity, surface charge and cytosolic delivery assay results.

3.2 Materials and Methods

3.2.1 Materials

All reagents were used as received without further purification. 2-aminoethyl methacrylate hydrochloride (AEMA, 90%), methacrylic acid (MAA, 99%), calcein and ammonium peroxodisulfate (APS) were purchased from Sigma-Aldrich Chemical Co.

Poly(ethylene glycol) dimethacrylate (PEGDMA, MW_{PEO} = 200g/mol) and poly(ethylene glycol) monomethyl ether monomethacrylate (PEGMA, MW_{PEO} = 1,000g/mol) were acquired from Polysciences Inc. Cy3 mono-NHS ester was from GE Healthcare UK Limited. RPMI 1640, DMEM (with 4.5g/L glucose) and Trypsin EDTA (0.25% trypsin/2.21mM EDTA in HBSS without sodium bicarbonate, calcium and magnesium) were purchased from Mediatech Inc., and fetal bovine serum (FBS) was purchased from Hyclone.

3.2.2 Methods

3.2.2.1 Synthesis of the Core-Shell Nanoparticles

The procedure outlined in section 2.3 was followed to form the particle core. In order to assess whether variation of AEMA concentration and addition of PEGDMA crosslinker during the shell growth had an impact on the structure of the nanoparticles, pH-responsivity and endosome-escaping capability, four samples were prepared (Table 2). Monomer concentrations in AEMA_H and AEMA_H_X samples were higher than those of AEMA_L and AEMA_L_X samples by a factor of 10. For AEMA_L_X and AEMA_H_X samples, the molar ratio of the crosslinker to the monomer was maintained at 0.03 to 4.97, identical to that during the core growth. Total weights of the shell reagents in AEMA_L_X and AEMA_H_X samples were kept at 40mg and 400mg, respectively.

	Monomer	PEGDMA	Water
AEMA_L	40mg (0.24mmol)	None	50μL
AEMA_L_X	39.5mg (0.24mmol)	0.5μL (1.44μmol)	50μL
AEMA_H	400mg (2.40mmol)	None	300μL
AEMA_H_X	394.9mg (2.38mmol)	4.8μL (14.39μmol)	300μL

Table 2: Samples prepared with different AEMA concentrations with or without PEGDMA crosslinkers. L: low concentration of AEMA; H: high concentration of AEMA; X: addition of PEGDMA crosslinker.

To synthesize the core-shell particles with different shell monomers and surface charges, six samples were prepared as listed in Table 3. Weights of the shell monomer in AEMA,

PEGMA and MAA samples were 200mg, and the molar ratio of the crosslinker to the monomer was maintained at 0.03 to 4.97. Total weights of the shell reagents excluding the crosslinker in AEMA_P, PEGMA_P and MAA_P samples were 40mg. The ratio of the shell monomer to PEGMA in AEMA_P and MAA_P samples was 1 to 2 by weight.

	Monomer	PEGMA	PEGDMA	Water
AEMA	200mg (1.20mmol)	None	2.4μL (7.24μmol)	100μL
AEMA_P	13.3mg (0.08mmol)	24.2μL (0.02mmol)	0.5μL (1.52μmol)	50μL
PEGMA	200mg (0.18mmol)	N/A	0.4μL (1.11μmol)	100μL
PEGMA_P	40mg (0.04mmol)	N/A	0.5μL (1.52μmol)	50μL
MAA	0.197mL (2.32mmol)	None	4.6μL (14.02μmol)	100μL
MAA_P	13.1μL (0.15mmol)	24.2μL (0.02mmol)	0.5μL (1.52μmol)	50μL

Table 3: Samples prepared with different shell monomer with or without PEGMA. P: addition of PEGMA.

After 3 hours of core synthesis, above reagents were injected to grow the particle shells for an additional 1.5 hours. The nanoparticles were purified by dialysis (3,500 MWCO Slide-A-Lyzer® Dialysis Cassettes, Pierce Chemical Co.) in deionized water for 3 days, followed by centrifugation (3X, 1mL PBS buffer (pH 7.4), at 15,000rpm for 15 minutes each). Purified particles were stored in PBS buffer at 4°C until needed.

3.2.2.2 Characterization of the Core-Shell Nanoparticles

To characterize the pH sensitivity of the newly synthesized core-shell nanoparticles, the hydrodynamic diameters, determined by dynamic light scattering (DLS, Brookhaven 90Plus instrument, 6 runs with 30 seconds of duration), were measured for particles equilibrated in 100mM phosphate buffers of different pHs at 25°C. Surface charges of the nanoparticles were analyzed from zeta potential measurements (Brookhaven ZetaPALS instrument, Smoluchowski model, 10 cycles with 15 seconds of duration). The purified particles were equilibrated in 5mM sodium chloride solution at 25°C, and pH was adjusted using 0.1M sodium hydroxide and 0.1M

hydrogen chloride solutions. Finally, concentrations of the particles were measured by their dry weight following lyophilization. After centrifuging 0.5mL of each sample at 15,000rpm for 5 minutes, the particles were flash-frozen in liquid nitrogen and lyophilized over 2 days.

3.2.2.3 Cell Culture

DC2.4 cells, a dendritic cell clone originally derived by Shen *et al.*[20], were a gift from Professor Kenneth Rock. These cells were cultured and passaged in complete RPMI 1640 medium containing 10% FBS, 50μM 2-mercaptoethanol, 5mM L-glutamine, 10mM HEPES, and penicillin/streptomycin.

3.2.2.4 Cytosolic Delivery of Calcein

The particles were fluorescently labeled by incubating Cy3 mono-NHS ester (5μL of 1mg/mL solution in DMSO) with 100μL of each sample in 395μL of PBS buffer overnight at 4°C. Unconjugated dye was removed by centrifugation (3X 0.5mL PBS buffer, at 15,000rpm for 15 minutes each). The labeled particles were resuspended and stored in PBS buffer at 4°C.

Calcein, a membrane-impermeant fluorophore, was used as a model drug molecule and tracer to monitor the stability of endosomes/phagosomes following particle uptake[21]. DC2.4 cells were plated in Lab-Tek™ chambers (Nunc 8-well chambered coverglasses, 1.2 x 10^5 cells/well), and then calcein was added to the cells (150μg/mL, 0.24mM) with or without 25μg/mL of different core-shell nanoparticles in complete medium for 1 hour at 37°C. After three washes with medium to remove extracellular calcein/particles, the cells were imaged live by Zeiss LSM 510 Confocal Laser Scanning Microscopy (CLSM) at 37°C.

3.3 Results and Discussion

3.3.1 Variation of AEMA Concentration and Addition of PEGDMA Crosslinker

3.3.1.1 Characterization of the Core-Shell Nanoparticles

Variation of AEMA concentration and addition of PEGDMA crosslinker during the shell growth were expected to influence the surface structure of the nanoparticles. Increasing concentration of AEMA on a scale comparable to that of DEAEMA would induce faster growth of the shell, enlarging the particles. On the other hand, introduction of PEGDMA would cause the shell to undertake hydrogel structure instead of a brushy layer. Without additional PEGDMA,

AEMA monomers or linear chains of AEMA would crosslink to unconjugated ends of PEGDMA of the core.

However, as shown in Figure 6, modification of the shell did not impact the pH-responsivity and monodispersity of the core-shell nanoparticles. Similar to the original nanoparticles, they were largely deswollen at elevated pH but swelled abruptly between pH 7.4 and 7.0 at 25°C. With pK_b of approximately 11, the primary amines of the AEMA groups in the particles shells should remain highly ionized across the relevant pH range. Only the tertiary amines of the particle cores, which possess pK_b of 7.0-7.3, respond to the changes in pH. The swelling transition of AEMA_H and AEMA_H_X nanoparticles occurred at slightly higher pH compared to AEMA_L and AEMA_L_X nanoparticles. Since the swelling response of DEAEMA-containing nanoparticles have shown to be sensitive to temperature, the swelling transition of these particles is expected to shift to lower pH at physiological temperature (37°C)[17].

From the DLS data, it is difficult to conclude that higher AEMA concentration increased the hydrodynamic diameters of the nanoparticles. Although AEMA_H particles were bigger than AEMA_L particles, AEMA_H_X particles were slightly smaller than AEMA_L_X particles. First, the reaction conditions for different samples were not identical. With use of a multi-stir plate, stir rate could have been different, influencing size of the core and the shell. Temperature of the reaction solution could have been not uniform and varied from sample to sample. This led to formation of a membrane at the top, making injection and dissolution of the shell reagents difficult. In addition, size of the core particles before the shell growth was not measured. It is possible that the difference in the particles size could have resulted from the core, not the shell. One should also note that all the nanoparticles, including the positive control (AEMA_L), were larger than expected. The original particles exhibited the diameters of 208±4nm at 37°C in PBS buffer[17]. Since particle size is a critical parameter for cellular uptake, it is important to have reproducible experimental control over particle size. Nevertheless, the particles displayed a similar change in diameter and volume on moving from the extracellular/cytosolic pH of 7.4 to an endolysosomal pH of 5 compared to the original ones.

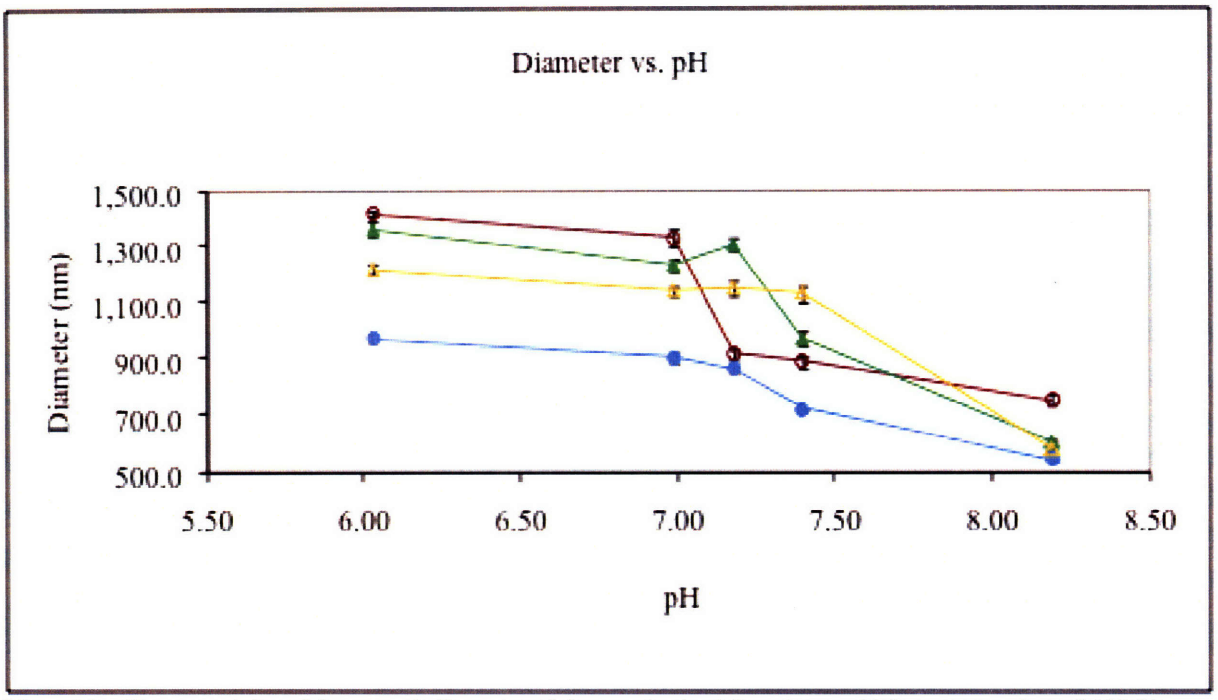


Figure 6: pH-responsivity of the core-shell nanoparticles synthesized from low or high concentration of AEMA with or without PEGDMA crosslinkers during the shell growth. (•) AEMA_L; (○) AEMA_L_X; (▲) AEMA_H; (△) AEMA_H_L.

Zeta potential measurements of AEMA_H and AEMA_H_X nanoparticles were expected to be higher than those of AEMA_L and AEMA_L_X nanoparticles. With higher concentration of AEMA to increase their incorporation on the shell, surface charge would be more positive. According to Table 4, such conclusion cannot be made. Around pH 9, values for AEMA_H and AEMA_H_X particles were bigger than those of AEMA_L and AEMA_L_X. However, observation was the opposite at pH of 7.4. Determination of the shell structure is needed in order to explain the data. Finally, concentrations of these nanoparticles after purification and lyophilization were between 0.02g/mL and 0.04g/mL.

Zeta Potential Measurements

Sample	Zeta Potential (pH 7.4)	Zeta Potential (pH 9)
AEMA_L	37.10±2.55mV	3.18±0.82mV
AEMA_L_X	36.23±1.26mV	8.59±1.14mV
AEMA_H	36.05±1.77mV	14.59±0.84mV
AEMA_H_X	29.41±0.92mV	16.54±1.99mV

Table 4: Zeta potential measurements of the core-shell nanoparticles synthesized from low or high concentration of AEMA with or without PEGDMA crosslinkers during the shell growth.

3.3.1.2 Cytosolic Delivery of Calcein

It was hypothesized that the pH sensitivity of the newly synthesized core-shell nanoparticles would facilitate endosome/phagosome disruption through the "proton sponge" effect and swelling of the particles. Because of the interest in delivering membrane-impermeable molecules into dendritic cells for vaccines and antiviral drug delivery, the uptake of the nanoparticles by a dendritic cell clone DC2.4 was investigated. As shown in Figure 7A,F, cells treated with calcein alone showed a punctuate distribution of fluorescence indicative of endolysosomal compartmentalization of the dye. In contrast, cells co-incubated with calcein and the core-shell nanoparticles exhibited calcein fluorescence throughout the cytosol and nucleus (Figure 7B-E and Figure 7G-J). The confocal images show that the particles were taken up by the phagocytic DC2.4 cells, leading to the phenomenon. The nanoparticles deliver calcein to the cytosol of cells by co-endocytosis of calcein and particles, followed by particle disruption of endosomes and escape of the dye into the cytosol/nucleus (note that calcein that reaches the cytosol is also able to freely enter the nucleus by diffusion). Since the pH-responsive core is responsible for the cytosolic delivery of the particles, it was expected that modification of the shell structure would have minimal impact.

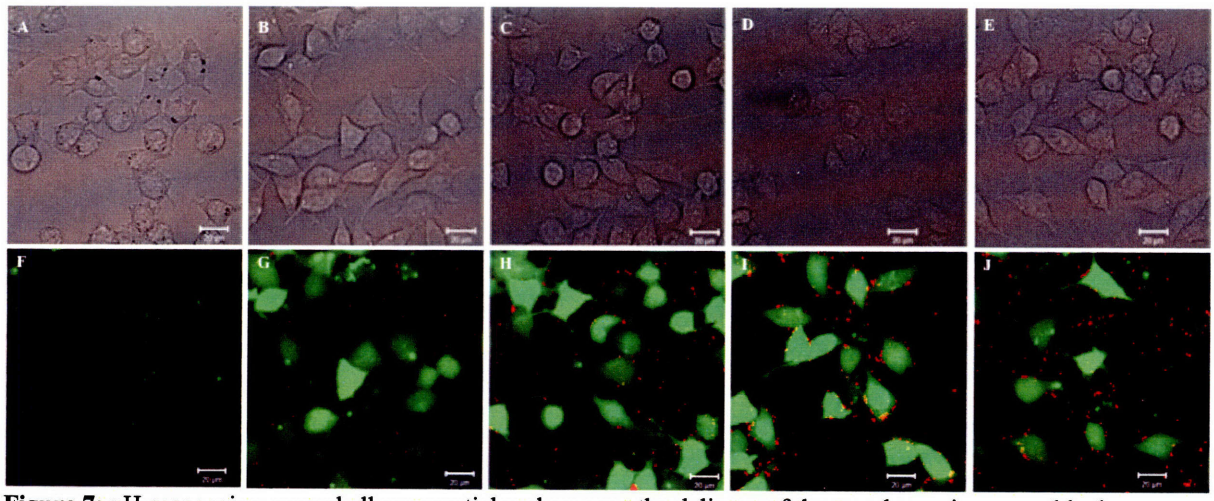
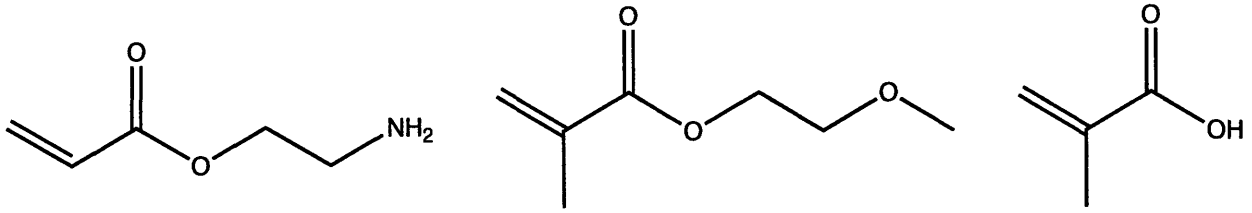


Figure 7: pH-responsive core-shell nanoparticles chaperone the delivery of the membrane-impermeable dye molecule calcein into the cytosol of dendritic cells. (A-J) CLSM images at 40x. (A-E) Bright-field images. (F-J) Fluorescence overlays (red, nanoparticles; green, calcein). (A,F) Cells were treated with calcein alone. (B,G) Cells were co-incubated with calcein and AEMA_L nanoparticles. (C,H) Cells were co-incubated with calcein and AEMA_L_X nanoparticles. (D,I) Cells were co-incubated with calcein and AEMA_H nanoparticles. (E,J) Cells were co-incubated with calcein and AEMA_H_X nanoparticles. Scale bar: 20μm.

3.3.2 Variation of the Shell Monomer and Surface Charge

3.3.2.1 Characterization of the Core-Shell Nanoparticles



AEMA PEGMA MAA

Figure 8: Shell monomers used to form different core-shell nanoparticles.

Nanoparticles with various shell structures that can bind to different types of drug molecules were synthesized. AEMA particles can associate with negatively charged molecules whereas PEGMA and MAA particles can complex with non-ionic polar and positively charged molecules, respectively (Figure 8). PEGMA monomers have been introduced to AEMA_P and MAA_P samples in order to prevent aggregation of the particles. In addition to improving stability of the particles, the poly(ethylene glycol) (PEG) moieties in PEGMA can also improve drug delivery properties by inhibiting protein binding that has been known to lead to opsonization and phagocytosis. Numerous studies of coupling PEG chains to decrease immune system recognition and subsequent clearance have been reported[22].

As shown in Figure 9, modification of the shell structure and surface charge did not influence the pH-responsivity and monodispersity of the core-shell nanoparticles. Similar to the original nanoparticles, they were largely deswollen at elevated pH but swelled abruptly between pH 7.4 and 7.0 at 25°C. The swelling transition of the particles should occur at lower pH at 37°C.

With incorporation of PEGDMA crosslinkers, these nanoparticles possessed hydrogel structure in the shell. AEMA and PEGMA particles, synthesized from 200mg of monomers, exhibited similar hydrodynamic diameters. DLS data for MAA particles are not shown in Figure 9, as they could not be completely purified; centrifugation did not yield solid pellets. In addition, MAA particle solution was viscous and slightly transparent after dialysis, unlike milky solutions of AEMA and PEGMA particles. However, it is expected that MAA sample would display similar particle size, monodispersity and pH responsivity. Total weights of the shell reagents excluding the crosslinker were 40mg for AEMA_P, PEGMA_P and MAA_P samples. Therefore, the hydrodynamic diameters of AEMA_P and PEGMA_P particles were less than those of

AEMA and PEGMA particles, respectively. However, effects of shell monomer variation and PEGMA addition on the particle size cannot be determined from the DLS data for the reasons stated in section 3.3.1.1.

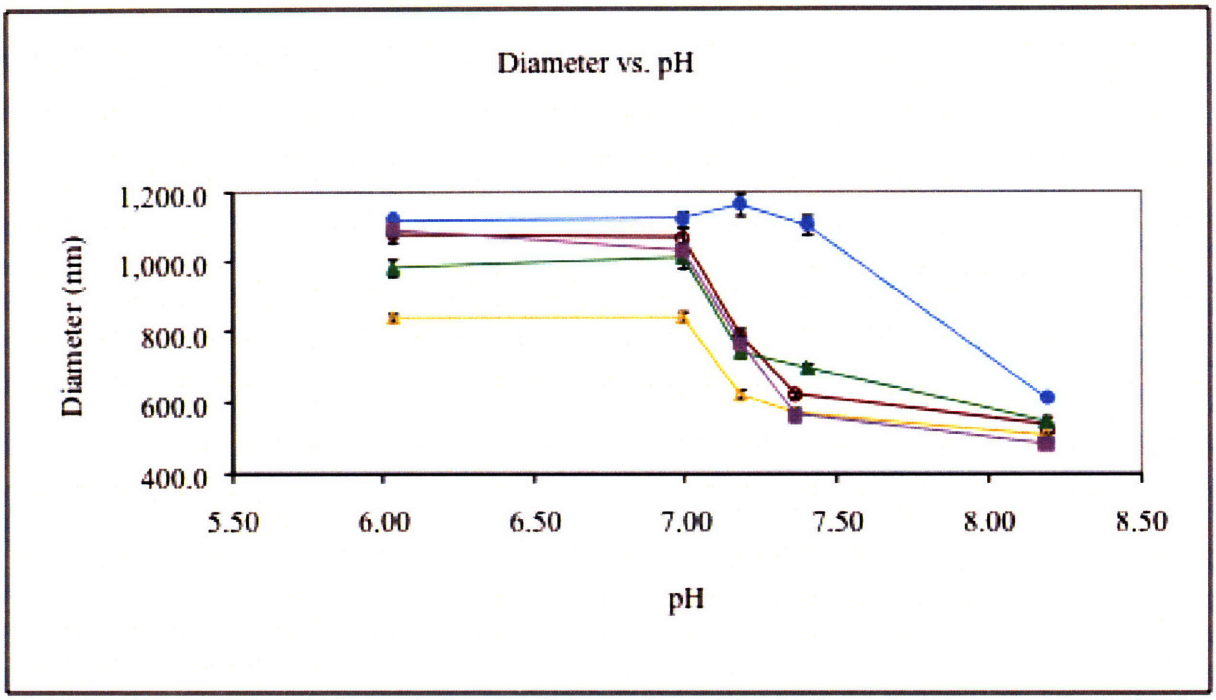


Figure 9: pH-responsivity of the core-shell nanoparticles synthesized from different shell monomers and PEGDMA crosslinkers with or without PEGMA during the shell growth. (●) AEMA; (○) AEMA_P; (▲) PEGMA; (△) PEGMA_P; (■) MAA_P.

Zeta potential measurements for the newly synthesized nanoparticles are shown in Figure 10. At physiological conditions, values for the particles synthesized from AEMA were expected to be positive whereas values for the particles synthesized from MAA were expected to be negative (note that AEMA and MAA possess pK_b and pK_a of approximately 11 and 5, respectively). On the other hand, with non-ionic shell monomers, PEGMA and PEGMA_P particles were anticipated to display near-neutral zeta potential values. In addition, thicker shells of AEMA and MAA particles were expected to increase the magnitude of the values compared to AEMA_P and MAA_P particles. However, all the nanoparticles possessed positive values at low pH and negative values at high pH. In general, the isoelectric point was around pH 9. The values were very similar for AEMA, PEGMA and MAA particles, and the isoelectric point of PEGMA particles was lower than that of AEMA and MAA particles. The values for AEMA_P, PEGMA_P and MAA_P particles were bigger at low pH.

One possible source of error was impurity of MAA particles, as previously mentioned. Also, 3,500 MWCO cassettes instead of 10,000 MWCO cassettes were used for purification. This could have made dialysis less efficient, and side products and unused reactants could have remained in the particle solutions. Lack of data points could have prevented accurate interpretation of the results. Another possibility was that the zeta potential machine was reading the surface charge of the core, which was much thicker than the shell. The group of Armes has shown that DEAEMA-containing latex particles exhibit the isoelectric point around pH 9, similar to that of the newly synthesized core-shell particles[23,24]. In addition, the shell could have been formed from both the shell monomer and DEAEMA, which was evidenced by Nuclear Magnetic Resonance (NMR) analysis. The molar ratio of the shell monomer to DEAEMA after the second stage of synthesis was 1 to 2 (data not shown). Presence of DEAEMA on the shell could explain why all the particles displayed positive zeta potential values at physiological conditions.

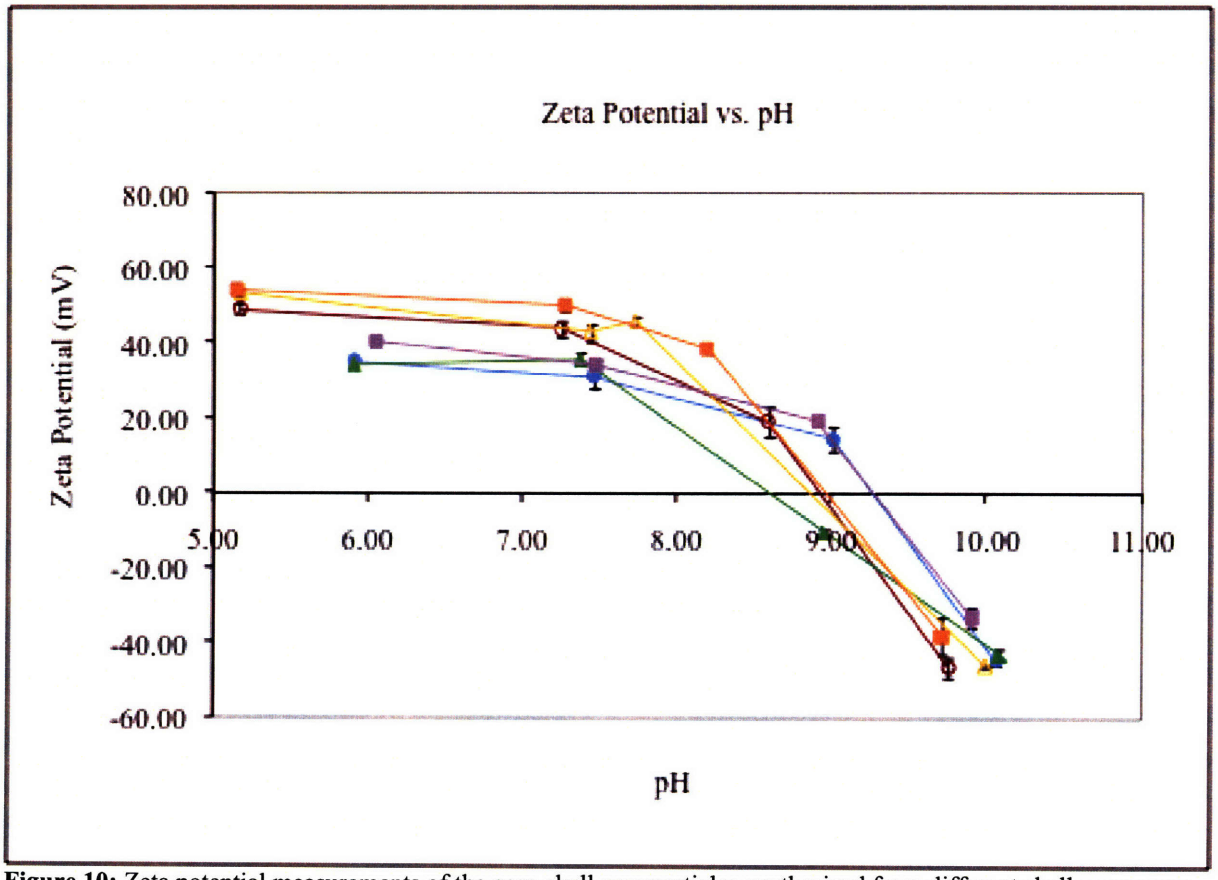


Figure 10: Zeta potential measurements of the core-shell nanoparticles synthesized from different shell monomers and PEGDMA crosslinkers with or without PEGMA during the shell growth. (●) AEMA; (○) AEMA_P; (▲) PEGMA; (△) PEGMA_P; (■) MAA; (□) MAA_P.

3.3.2.2 Cytosolic Delivery of Calcein

Similar to the results from section 3.3.1, cells co-incubated with calcein and the core-shell nanoparticles exhibited fluorescence throughout the cytosol and nucleus (Figure 11B-D and Figure 11F-H). As displayed by the confocal images, the particles were internalized by the phagocytic DC2.4 cells. The nanoparticles deliver calcein to the cytosol of cells by co-endocytosis of calcein and particles, followed by particle disruption of endosomes and escape of the dye into the cytosol/nucleus. Since the pH-responsive core is responsible for the cytosolic delivery of the particles, it was expected that variation of the shell monomer and surface charge would have minimal impact.

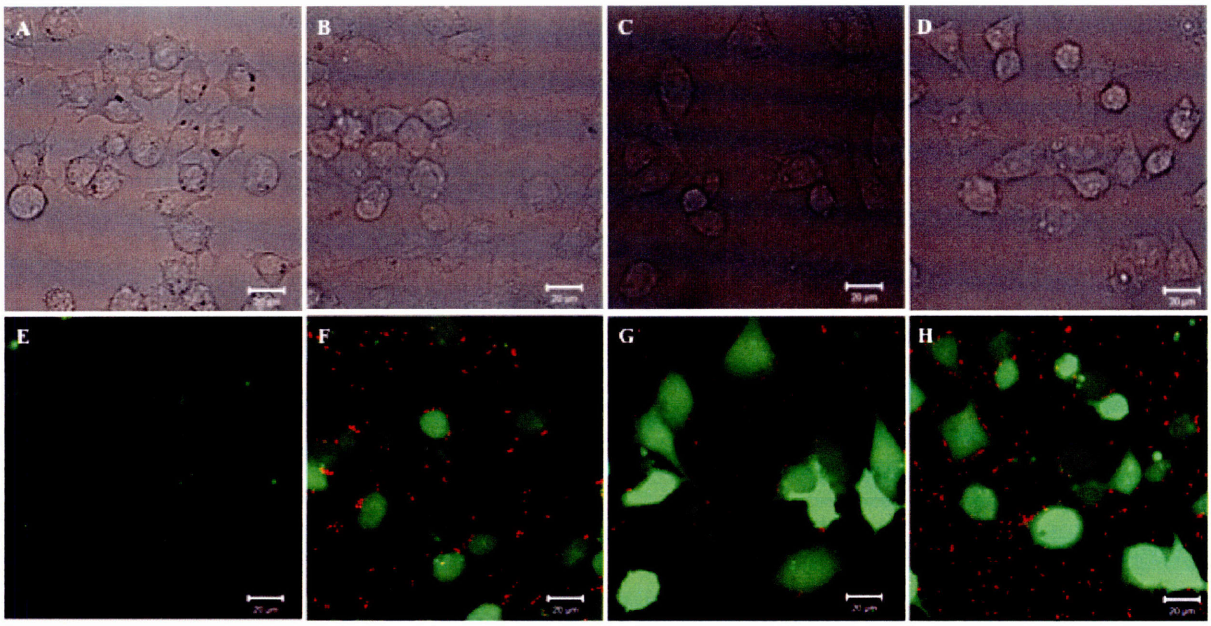


Figure 11: pH-responsive core-shell nanoparticles chaperone the delivery of the membrane-impermeable dye molecule calcein into the cytosol of dendritic cells. (A-H) CLSM images at 40x. (A-D) Bright-field images. (E-H) Fluorescence overlays (red, nanoparticles; green, calcein). (A,E) Cells were treated with calcein alone. (B,F) Cells were co-incubated with calcein and AEMA nanoparticles. (C,G) Cells were co-incubated with calcein and PEGMA nanoparticles. (D,H) Cells were co-incubated with calcein and MAA nanoparticles. Scale bar: 20μm.

3.4 Conclusions

Different types of pH-sensitive core-shell nanoparticles have been synthesized and analyzed. Variation of the surface structure by increasing the monomer concentration, adding PEGDMA crosslinker and changing the monomer during the shell growth did not influence the pH-sensitivity and cytosolic delivery of membrane-impermeable calcein to dendritic cells. The results demonstrate flexibility and broad applicability of the nanoparticles, as they can bind and

deliver different types of drug agents. However, further research needs to be conducted to directly determine the shell structure. Ovalbumin binding assay to differentiate the nanoparticles is under investigation. Since the protein is negatively-charged, the particles synthesized from AEMA are expected to bind to ovalbumin more effectively than the particles synthesized from PEGMA and MAA. It might also be necessary to find a strategy to prevent incorporation of DEAEMA on the shell to decrease cytotoxicity and to improve binding efficiency between the core-shell particles and drug molecules.

4.0 Additional Improvements to the Irvine Nanoparticles

Although the fundamental issue of cytosolic delivery has been solved, critical short-term barriers on the research side, including the ones discussed in section 3, must be overcome for the technology to be viable for commercialization. First, loading of the actual drug molecules needs to be performed. As shown in Figure 12, electrostatic interaction between oppositely charged drug agents and the shells of the nanoparticles has been proposed[4]. One concern is that binding competition with endogenous proteins could decrease the bound drug molecules on the nanoparticles before endocytosis. In addition, ribonucleases and proteases could access the drug molecules on the shells and degrade them. Encapsulation of the therapeutic agents in the shell, in the core, or between the shell and the core could be a potential method for drug loading and protection. This could reduce the drug dosage and avoid frequency of drug treatments.

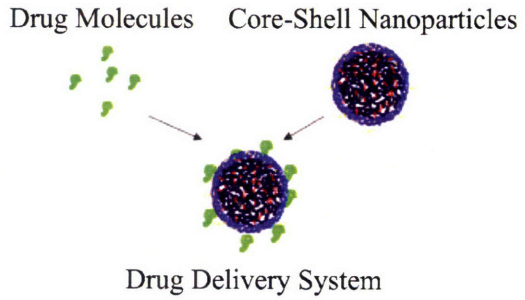


Figure 12: Electrostatic interaction between the core-shell particles and the drug molecules[4].

As implied above, sufficient electrostatic interaction between the drug molecules and the nanoparticles is necessary to withstand binding competition with other proteins in the body. However, overly strong electrostatic force could inhibit efficient unpacking of the drug agents. Initial mRNA knockdown experiments demonstrated that the Irvine nanoparticles achieve less

siRNA delivery than commercially available lipid transfection agents under certain conditions (Figure 13[4]). Strong electrostatic interaction might inhibit siRNA release from the shells once in the cytosol. Incorporating digestible crosslinkers to the hydrogel, such as bis(acryloyl) cystamine (BAC) that can be degraded in the presence of intracellular level of glutathione, is a possible solution. This approach not only has the potential to increase intracellular release of siRNA, but also improve the safety profile of the nanoparticles. Lack of biodegradability with the current drug delivery system could be of great concern since biodistribution of the nanoparticles in the bloodstream and accumulation in the kidney and spleen might induce immune response and long-term toxicity[25]. Since DEAEMA homopolymer breakdown products could be toxic to cells, incorporation of hydrophilic PEG methacrylate in the core is being investigated[26]. After cleavage of the BAC crosslinks, the PEG chains would extend into solution, forming a unimolecular micelle and hiding hydrophobic DEAEMA backbone units. It is hypothesized that such a micellar breakdown product could be safely eliminated from cells and eliminated from the body.

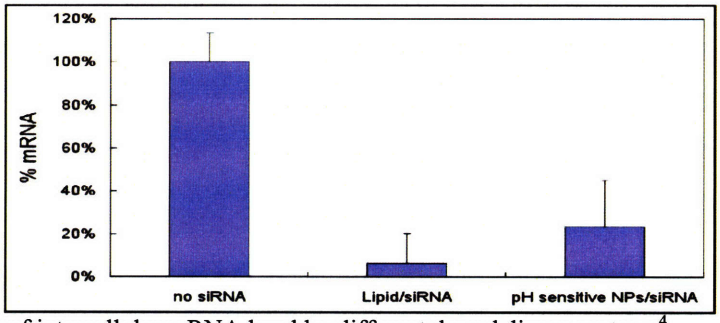


Figure 13: Knockdown of intracellular mRNA level by different drug delivery systems[4].

Another important issue to be addressed in the near future is determining the route of administration. It would depend on specific application, but intravenous delivery is most plausible. Although more invasive than other methods, it offers several advantages. First, the nanoparticles, when thus administered, can potentially reach disease that is either disseminated, making local injection difficult, or too small to be detected[27]. Oral delivery is not feasible since degradation machinery of the stomach and the gastrointestinal tract can destroy the nanoparticles. In addition, the acidic pH environment will enlarge the nanoparticles, making their absorption even more challenging. Parental and nasal passages are other possibilities, but bioavailability can be significantly limited. Another attractive feature of systemic administration is that enhanced permeability and retention (EPR) effect can be fully utilized for passive targeting of tumor cells.

As tumors grow and begin to outstrip the available supply of oxygen and nutrients, they release cytokines and other signaling molecules that recruit new blood vessels, in a process called angiogenesis. Angiogenic blood vessels have gaps as large as 600 to 800nm between adjacent endothelial cells, and the nanoparticles can easily extravasate into the tumor interstitial space[28].

As mentioned in section 2.6, size of the nanoparticles is around 200nm at physiological conditions. The particles are expected to display relatively long half-life. This is because the kidney removes particles smaller than 2 to 3nm whereas the reticuloendothelial system (RES) physically captures less than 70nm particles in its tissues and clears those greater than 200nm in diameter. One potential drawback is that these particles would have difficulty infiltrating target tissues since only small particles of 1 to 3nm in size can escape vasculature, except for the leaky blood vessels of tumors. A possible solution is to slightly reduce the size of the particles and sacrifice their half-life, provided that it would have minimal impact on their cellular uptake and endosome-disrupting function. This can also increase their biocompatibility, as smaller particles can be excreted more easily.

Furthermore, conjugation of ligands to the shell should be investigated to target specific cell types and prevent widespread biodistribution of the nanoparticles. Finally, in vivo data is required before clinical trials can be conducted. Critical issues like the ones described in this section indicate that the technology is still at the university research stage. As described in Figure 14, technological barriers of complexity that might be greater than the fundamental issue of cytosolic delivery do remain. Resolving them in the near future will not only reduce the degree of risk on the research side but also improve patentability of the technology.

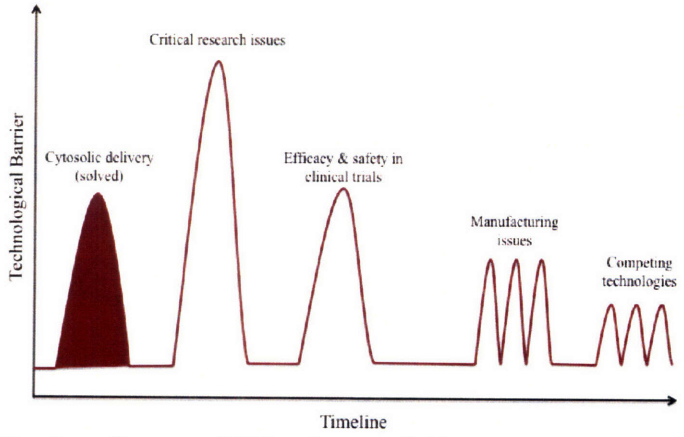


Figure 14: Technological barriers of commercializing the core-shell nanoparticles.

5.0 Initial Patent Analysis

5.2 Patentability of the Irvine Nanoparticles

If one is to pursue commercialization of this technology, the components and the process of producing the nanoparticles have to be patented regardless of the market sectors and business strategies. Otherwise, there are no means of protecting the technology due to simple experimental protocol and publication of the relevant data in 2007. In order to assess patentability, there must be no prior art information. A patent search was performed with regards to different aspects of the technology.

5.2.1 Patents Related to pH-Responsive Polymers

Although several intellectual property rights on pH-responsive polymers exist, none of the systems use DEAEMA. Professor Robert Langer at Massachusetts Institute of Technology holds a number of patents in this area. U.S. Patent No. 6,998,115 claims biodegradable and biocompatible nanoparticles formed from various poly(β-amino esters) that become hydrophilic and soluble at acidic pH. They can be used to deliver surface-complexed polynucleotides or encapsulated drugs[29]. However, chemical structures of the nanoparticles are significantly different. In addition, Professor Langer's U.S. Patent No. 6,692,911 covers biodegradable and biocompatible drug delivery systems that utilize the "proton sponge" mechanism. As the patent claims systems comprising of polyhistidine and polylysine, it does not appear to cover the compositions used in the Irvine nanoparticles[30].

5.2.2 Patents Related to Synthesis of the Irvine Nanoparticles

Because emulsion polymerization has existed for a long time, there should not be any patents preventing the process of synthesizing the Irvine nanoparticles. For completeness, a patent search was conducted. One patent, assigned to Rohm and Haas, deserves particular attention. It claims a process for continuous production of crosslinked polymeric nanoparticles of 1 to 100nm in diameter. Although the size of the current Irvine nanoparticles is not within the proposed range, it may limit the extent of the technology. However, the patent is not yet issued, and the broad claims may become limited[31].

5.2.3 Additional Relevant Patents

One of the patents that appear to be most troublesome for the Irvine nanoparticles is assigned to Instituto Superiore Di Santa in Italy. It describes core-shell nanoparticles for delivery of proteins and nucleotides obtained through emulsion polymerization, where the core is formed from water-insoluble polymers, and the shell is formed from hydrophilic polymers. The description of the components and proposed applications is very similar to those of the Irvine nanoparticles. It claims DEAEMA as a possible monomer for the core and also covers nanoparticles that comprise of at least one pharmacologically active agent adsorbed on the surface. Fortunately, the nanoparticles claimed possess corona structure without any use of crosslinkers instead of the hydrogel structure. In addition, the patent does not claim AEMA for the shell monomer[32]. U.S. Patent No. 7,129,293, assigned to Japan Science and Technology Agency, is another intellectual property that describes core-shell nanoparticles formed from DEAEMA. However, it claims copolymer structure with addition of polyethylene glycol segment and covers completely different core-shell structures[33]. To ensure that improvements to the current technology, described in the previous section, are patentable, further search was conducted. Even though one patent claims reversible hydrogel system that contains disulfide crosslinkers, including BAC, it is synthesized from different monomers[34].

5.2.4 Literature Search

Because publicly disclosed data can also obstruct a patent from being issued, literature search was also performed. There is a possibility that an author has recently made his/her data public, but has not yet filed a patent. Focus was on the pH-responsive drug delivery systems that consist of DEAEMA, published in primary literature. DEAEMA hydrogels for drug delivery purposes do exist, but their mechanisms of action and chemical structures are significantly different from the Irvine nanoparticles[35,36].

5.2.5 Preliminary Intellectual Property Strategy

From the in-depth patent and literature search, the Irvine nanoparticles and their improvements should be patentable, including the ones with different shell structures and biodegradable crosslinkers. However, it is important to keep in mind that a patent gives the right to prohibit somebody from using a technology, but does not automatically endow freedom to

operate. Therefore, additional patent search is required to address intellectual property issues with complexing the Irvine nanoparticles with specific drug agents, such as siRNAs and protein antigens. This will be covered section 5.

A preliminary intellectual property strategy is proposed, and the course of action depends on whether the critical issues on the research side are resolved or not in the near future. First of all, with the current technology, there is no eligibility in foreign countries as the data were published in September of 2007. In the U.S., there is a one-year grace period, and a provisional patent, which can be as cheap as $105, on the publicly disclosed information should be filed as soon as possible. There are two scenarios.

Scenario A: If the critical issues, such as drug loading/unloading and biodegradability, are resolved a few months before September of 2008, an additional provisional patent should be filed on the same day of public disclosure of the new improvements to obtain foreign rights. This is an important issue since Europe and Japan are significant markets, as will be discussed later. Then, sources of funding other than venture capital, such as angel investors, Small Business Innovative Research (SBIR) and other government funding, should be identified before filing non-provisional patents. Although the cost of obtaining a patent can vary greatly depending on its complexity, these investments should be sufficient to cover the necessary fee and to form a start-up company.

Scenario B: If the critical issues are not overcome before September of 2008, one should forfeit the original priority date and file another provisional application, if allowed, to obtain another year. If this is not possible, the provisional patent should be forfeited and wait for the problems to be solved. If they cannot be solved after significant time investment, one should reconsider commercializing the technology.

6.0 Potential Applications

6.1 Overview

Because many therapeutic strategies for the treatment of disease depend on efficient delivery of drugs into the cytosolic or nuclear compartments of cells, the number of applications for the technology is virtually unbounded. By modifying the shell structure, numerous types of therapeutic agents can be loaded on the nanoparticles. The thesis will focus on two applications: siRNA delivery and vaccines. As will be discussed, cytosolic delivery is one of the main

challenges in these fields, and overcoming the barrier can lead to treatment of deadly diseases, including cancer, HIV, malaria and hepatitis C virus. Delivering DNA for treating chronic diseases and genetic disorders is another possibility, but it requires an additional mechanism for nuclear uptake. In addition, despite years of research, safety concerns due to genetic disruption still reside. Since 1990, scientists have led more than 1,300 gene therapy trials worldwide, but only one, in China, has so far yielded a marketable product[37]. For small molecules, which can easily permeate through cell membranes, other tactics of delivery improvement can easily be employed.

6.2 siRNA Market Analysis

6.2.1 Introduction to siRNA

Since the completion of the Human Genome Project, nucleic acid-based approaches for gene silencing in a sequence-specific manner have become powerful tools. There are three main technologies to silence genes responsible for diseases. Antisense technology was the first one to be developed, and it involves single-stranded RNA molecules that bind to mRNAs to prevent translation. Although there are many antisense drugs in clinical trials, its success has been limited, especially with weak Phase III results of Affinitak, a lung cancer antisense drug being developed by Isis Pharmaceuticals and Eli Lilly. Ribozyme, which is a catalytic RNA molecule that can recognize and cut specific RNA molecules, is another gene silencing approach. RNA interference (RNAi) technology is the one that is receiving the most attention from the scientific community and market. There are several advantages that RNAi offers over others. It is more specific and efficient in targeting with less side effects and toxicity.

Within RNAi, three subtypes exist. Small interference RNA (siRNA) is double stranded whereas microRNA (miRNA) is processed from single-stranded RNA and shows partial complementarity to its targets. Short hairpin RNA (shRNA) contains a high degree of secondary structure. Although the Irvine nanoparticles can load all these types of RNAi, siRNA delivery will be focused as miRNA and shRNA research is still in infancy.

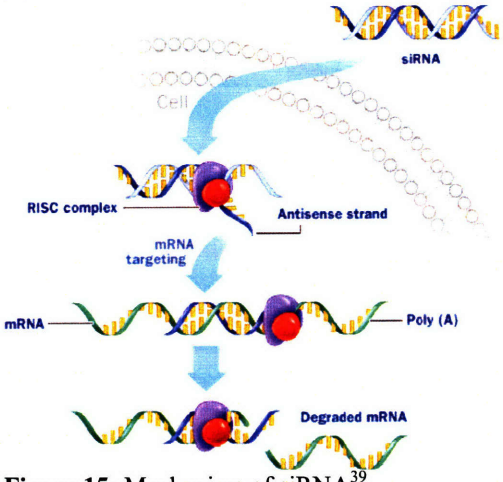


Figure 15: Mechanism of siRNA[39].

Since siRNA prevents translation of faulty proteins, its mechanism has been described as "stopping the flood by turning off the faucet instead of mopping up the floor"[38]. Once a double-stranded RNA (dsRNA) is introduced to the cytosol, a ribonuclease, called dicer, cleaves it into 21 to 25 nucleotide siRNAs. These siRNA molecules assemble with the RNA-induced silencing complex (RISC), unwinding in the process. Activated RISC binds to complementary transcripts by base pairing interactions between the siRNA antisense strand and the mRNA. Finally, the bound mRNA is cleaved, and sequence-specific degradation of mRNA results in gene silencing (Figure 15[39]).

6.2.2 Global siRNA Market

A breakthrough in siRNA research in the recent past led to extreme interest in the field. Some of the indications include siRNA being named "The Technology of the Year" in 2002 by Science magazine, "The Next Billion Dollar Breakthrough" in 2003 by Fortune and "The Most Important Breakthrough in the Past Decade" by MIT. At the end of 2007, PubMed search on siRNA yielded more than 20,000 entries. In 2006, Professor Andrew Fire from Stanford University and Professor Craig Mello from University of Massachusetts Medical School won the Nobel Prize in Medicine for their siRNA research.

The global RNAi revenue is estimated to be $447 million in 2006, and is projected to grow to $0.9 billion in 2010, with compound annual growth rate (CAGR) of 19%[40]. Although the market is relatively small, with the success of RNAi therapy, the technology has the potential to capture 10% of the pharmaceutical industry, according to Nature Biotechnology. Key end user groups currently include pharmaceutical and biotechnology companies as well as academic and government institutions.

The market is largely divided into three sectors by application, with target validation occupying the biggest share (Figure 16[41]). The target validation step of the drug discovery process aims to prove that a given target is directly involved in disease and can be used for development of a therapeutic drug. Since traditional validation methods are tailored for a

particular gene and require individualized, time-consuming, and often expensive studies, siRNA is becoming increasingly accepted by the pharmaceutical industry. On the other hand, RNAi therapeutic market is the smallest one, as there are no commercial products and until recently, pharmaceutical companies viewed the technology as a risk rather than an opportunity. However, this sector is rapidly growing with exponential interest from the pharmaceutical industry. The Irvine nanoparticles will be applicable to all these segments of the market because drug delivery systems are required in efficient knockdown of mRNA.

In terms of geography, North America possesses a major share of the market with key RNAi-focused companies, world-class universities and research environment, and readily available funding for biotechnology and start-up companies (Figure 17[41]). Europe and Japan are significant markets, and RNAi market in China and India is rapidly growing.

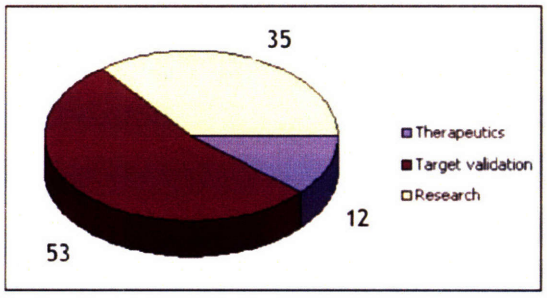


Figure 16: Breakdown of siRNA market by application (2003)[41].

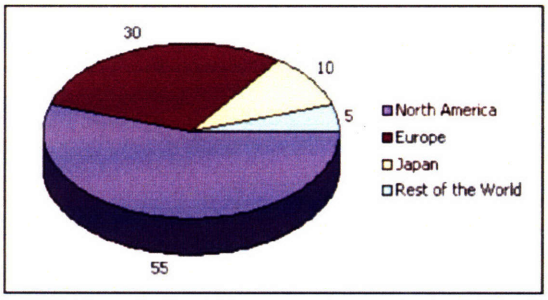


Figure 17: Breakdown of siRNA market by geography (2003)[41].

The global RNAi market is moderately competitive with 50 companies participating. Dharmacon, Qiagen, Ambion and Invitrogen control more than 70% of the market. The degree of competition is expected to increase with entrance of pharmaceutical companies. These companies are not direct competitors of the Irvine technology. As will be explained later, they are potential customers.

A summary of market drivers and market restraints for the RNAi technology is displayed in Table 5. Some of the issues have been already addressed, and others will be discussed further. The most notable fact is that in vitro and in vivo delivery of siRNA is the most challenging problem, which is widely agreed across the industry. Since siRNA is too big and negatively charged to cross the cell membrane, cytosolic delivery is essential. Also, drug delivery systems are needed to protect siRNA, which is unstable and rapidly cleared in vivo. Therefore, the

potential for the market is heavily reliant on the efficiency and reliability of delivery tools. The Irvine technology is addressing the right problem; if successful, it will be able to penetrate the market.

Global RNAi Market	
Market Drivers	Market Restraints
■ Useful for a large range of diseases, including cancer and neurological diseases ▪ Can target virtually any proteins	■ Expensive RNAi reagents ▪ siRNA 10 to 15 times more expensive than DNA
■ Adoption of siRNA for target validation by pharmaceutical companies ▪ Transgenics can be very expensive & time-consuming	■ Failure of key antisense drugs
■ RNAi technology minimizes the risk of toxicity and off-target effects	■ Selection and design of appropriate siRNAs
■ Increasing number of partnerships and M&A activities from the pharmaceutical industry	■ Polymorphism in the population
■ A number of products entering clinical trials	■ Big pharmaceutical companies view RNAi as a risk rather than an opportunity
	■ Unclear IP landscape
	■ In vitro and in vivo delivery of siRNA

Table 5: Market drivers and market restraints for RNAi technology.

6.2.3 U.S. RNAi Delivery Tools Market

Several methods of siRNA delivery exist. Chemical transfection is the most widely used approach and is dominated by lipid-based systems. Although they are versatile and non-toxic, they are difficult to design for in vivo use. Electroporation is highly efficient, but is also not suitable for in vivo use due to cell membrane damage. Plasmid and viral vectors are very effective. However, potential to induce immune responses has limited their use. CPPs have established their application in RNAi delivery, but as mentioned previously, research is relatively new. Finally, nanoparticles are anticipated to grow at the fastest rate (Figure 18[42]). Some of the advantages of polymeric nanoparticles include potential of size and charge density tailoring, stability, extended circulation time, biodegradability and biocompatibility.

U.S. RNAi delivery tools market is moderately competitive with 20 participants. Invitrogen holds the biggest market share with its leading product, Lipofectamine 2000, which

was the first to penetrate the market. This further indicates that significant improvements have not been made in this field. As the product is not suitable for in vivo use, the company is focused on providing products for life science research instead of therapeutics. Nucleofactor, Amaxa's electroporator, is another significant product (Figure 19[42]). Based on the past analysis, there is not much customer loyalty in the market. Fortunate for the Irvine technology, there is ample room for innovative companies and innovative technologies.

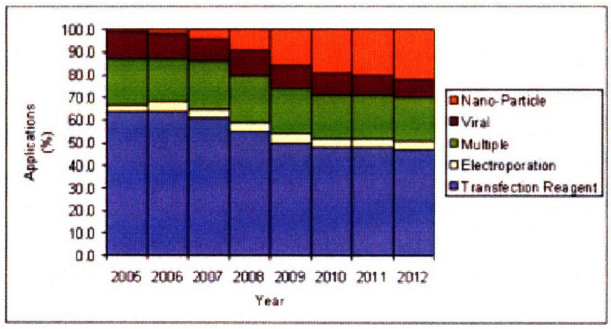


Figure 18: Breakdown of U.S. RNAi delivery tools market (2006)[42].

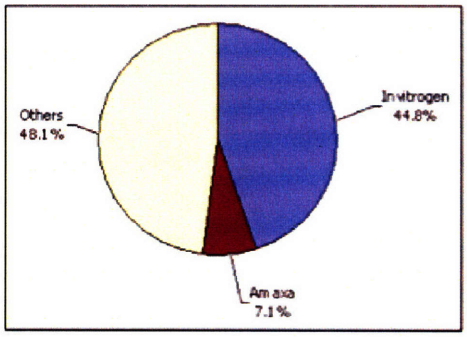


Figure 19: Market share of U.S. RNAi delivery tools market (2006)[42].

6.2.4 Emerging Competitive RNAi Delivery Systems

Novel RNAi delivery systems for clinical and research use are being developed by several companies. Provita Biotherapeutics, based in Canada, have developed stable nucleic acid lipid particles (SNALP) that can encapsulate in their aqueous core and deliver siRNA duplexes. By coating the particles with polyethylene glycol, they remain in the bloodstream over 24 hours. With maximum silencing above 90%, Provita Biotherapeutics claim that SNALP has 100-fold better efficiency than other in vivo delivery systems. It was also the first non-viral vector for siRNA showing activity in non-human primates[43].

Mirus Bio Corporation's Dynamic PolyConjugates (DPC) is another potential competitor to the Irvine nanoparticles, and mimics the natural viral targeting disassembly process for cytosolic delivery. It includes endosomolytic polymer, charge-masking agents, environmental responsive linkage chemistry, targeting ligand and siRNA. Its maximum silencing has been reported to be over 90% as well[44].

Calando Pharmaceuticals, cofounded by Professor Mark Davis at California Institute of Technology, has developed a two-component siRNA delivery system. The first part is composed of cyclodextrin-containing polycations that bind to the anionic backbone of siRNA. Terminal

admantane groups prevent aggregation, enhance stability, enable systemic administration and target tissue of interest. The nanoparticles are 50nm in size and stealthy to the immune system. They have shown low toxicity in non-human primate studies, and have entered Phase I of clinical trials[45,46]. As will be discussed later in the thesis, these companies have successfully leveraged their technologies to partner with bigger pharmaceutical and biotechnology companies to come up with therapeutic siRNA products. Because siRNA therapy is expected to be much more effective than conventional approaches, such as chemotherapy, these technologies will be more relevant competitors to the Irvine nanoparticles.

6.2.5 Patents Related to siRNA Applications

Patentability of the Irvine nanoparticles has already been established. However, RNAi technology requires an intellectual property portfolio comprising of patents on RNAi content, targets, drug delivery systems and methods of complexing RNAi with drug delivery systems. Since siRNA technology is relatively new, intellectual property landscape used to be somewhat ambiguous. It is now becoming clearer what the key siRNA patents are.

6.2.5.1 Patents Related to siRNA Content and Targets

This field is the most competitive and still hotly debated over who owns what. Thomas Tuschl, from Max-Planck Institute, owns two key patents. U.S. Patent No. 7,056,704 broadly covers methods of making siRNAs for any target with or without chemical modifications important for siRNA activity. It includes siRNAs with 3'-overhangs at one or both ends of the double-stranded RNAs and claims siRNA length of 19 to 25 nucleotides that falls within the size range optimal for RNAi[47]. U.S. Patent No. 7,078,196 is a development of the previous patent and claims siRNAs with phosphorothioates, 2'-O-methyl and/or 2'-fluro modifications, which are important for achievement of drug-like properties for RNAi therapeutics, such as stability and delivery[48]. Both patents have been exclusively licensed to Alnylam Pharmaceuticals. Thomas Tuschl has filed another patent that describes 21 to 23 nucleotide RNAs and their use for specifically inactivating gene functions[49]. It is still awaiting decision, and the claims may become limited. U.S. Patent No. 6,506,559 is one of the earlier siRNA patents, assigned to Nobel laureates, Andrew Fire and Craig Mello. It describes a method of inhibiting gene expression of a target gene in many types of cells through dsRNAs comprising of at least 25 nucleotides[50].

Lastly, U.S. Patent No. 5,898,031 and 6,107,094, assigned to Isis Pharmaceuticals, are referred to as the "Crooke" series. They claim oligomeric compounds that have subsequences of 2-pentoribofuranosyl nucleosides that activate ribonuclease for target mRNA degradation. They further include substituent groups for increasing binding affinity of siRNAs to complementary nucleic acid strands as well as groups for increasing nuclease resistance[51,52]. Both patents have been licensed to Alnylam Pharmaceuticals (Table 6).

Strategic RNAi Patent Landscape

Patent	Inventors	Assignee	Filing Date	Licensure
US7,056,704	Tuschl *et al.*	Max Planck Society	04/27/04	Alnylam (exclusive)
US7,078,196	Tuschl *et al.*	Max Planck Society	04/27/04	Alnylam (exclusive)
US2003/0108923	Tuschl *et al.*	Whitehead Institute	09/26/02	Alnylam, CytRx, Merck/Sirna
US5,898,031	Crooke *et al.*	Isis	06/06/96	Alnylam
US6,107,094	Crooke *et al.*	Isis	06/06/97	Alnylam
US6,506,559	Fire *et al.*	Carnegie Institute	12/18/98	Non-exclusive
EP1,144,623	Kreutzer *et al.*	Ribopharma	01/29/00	Alnylam (exclusive)
EP1,214,945	Kreutzer *et al.*	Ribopharma	01/29/00	Alnylam (exclusive)

Table 6: Key siRNA patents. Details of each patent are described in section 5.2.5.1 and 5.2.5.2.

6.2.5.2 Patents Related to siRNA and Drug Delivery System Complexation

"Kreutzer-Limmer" series, which Alnylam Pharmaceuticals has gained exclusive access with its acquisition of Ribopharma AG, is also important for siRNA applications. European Patent No. 1,144,623 describes a method for inhibiting the expression of a given target gene in a cell of a mammalian in vitro through the use of 15 to 21 nucleotide dsRNAs[53]. European Patent No. 1,214,945 is similar to the previous one, except it claims longer dsRNAs (15 to 49 nucleotides), and is still hotly contested[54]. It further covers dsRNAs enclosed by micellar structures, such as liposomes, and synthetic or natural viral capsids. Polymeric systems are not covered, and the Irvine nanoparticles will not be infringing upon these methods of complexation (Table 6). One patent, assigned to Intradigm Corporation, appears problematic for the Irvine technology. It describes methods to silence target gene expression in vivo by RNAi and claims composition that comprises a polymeric carrier[55]. If approved, numerous preceding patents would be infringing upon this application. It is unlikely that such broad claims will be accepted without modifications.

6.2.5.3 siRNA Patent Strategy

As a drug delivery system-focused company, it will be difficult to invest money and time to independently develop siRNA molecules and determine their targets. In addition, key patents, which are mostly owned by Alnylam Pharmaceuticals, present formidable barrier to entry. Therefore, it will be crucial to obtain licenses to patents on siRNAs and their targets, which will be further explained in section 6. On the other hand, there are no patents obstructing complexation of siRNAs with the Irvine nanoparticles. Intellectual property portfolio covering methods of complexing various RNAi molecules with appropriate nanoparticles should be developed continuously.

6.2.6 Collaboration with the Pharmaceutical Industry

Platform Alliances

RNAi company	Partner	Announced	Scope	Upfront	Other revenues
Alnylam	MERCK	09/2003	■ 5-year technology & therapeutics alliance	■ $2mm in cash ■ $5mm equity investment	■ Research reimbursements & milestones for demonstration of RNAi in live animals
Alnylam	NOVARTIS	09/2005	■ 3-year research collaboration	■ $10mm in cash ■ $58.5mm equity investment at 16% premium	■ Up to $700mm for development milestones ■ Royalties on product sales
Sirna Therapeutics	gsk GlaxoSmithKline	04/2006	■ Respiratory disease	■ $6mm in cash ■ $6mm equity investment at 24% premium	■ Up to $700mm for development milestones ■ Royalties on product sales
Sirna	MERCK	10/2006	■ Acquisition	■ $1.1bn at 102% premium	
Alnylam	Roche	07/2007	■ Nonexclusive access to 4 therapeutic areas ■ Acquisition of Alnylam Europe	■ $273.5mm in cash ■ $42.6mm in equity investment at 41% premium	■ Up to $700mm for development milestones ■ Royalties on product sales

Table 7: Partnerships between big pharma/biotech companies and siRNA focused companies[56].

Growing interest in the RNAi technology from the pharmaceutical companies is indicated by the increase in the number of their platform alliances with the RNAi companies (Table 7). To

gain access to the promising market, Merck & Co. decided to acquire Sirna Therapeutics for $1.1 billion at 102% premium. In return for nonexclusive access to four therapeutic areas and acquisition of Alnylam Europe, Roche has paid $273.5 million in cash in addition to $42.5 million equity investment at 41% premium. Alnylam Pharmaceuticals is also eligible for up to $700 million for development milestones and royalties on product sales[56]. The deal is considered to be the largest one of its kind in biotechnology history.

Delivery Collaborations

RNAi company	Partner	Announced	Scope	Upfront	Other revenues
Sirna Therapeutics	Provita Biotherapeutics	02/2005	■ Sirna gains access to Provita's SNALP siRNA delivery system ■ Provita gains access to Sirna's siRNA chemistry and 3 targets under Sirna IP	■ $500,000 in cash ■ $488,000 in Sirna stock to Provita	■ Mutual royalties on product sales involving other party's IP
Alnylam	Medtronic	02/2005	■ Technology codevelopment for neurodegenerative disease ■ Alnylam provides siRNAs & Medtronic provides device technology	■ None	■ Up to $21mm equity investment by Medtronic in Alnylam upon completion of certain milestones
Alnylam	Tekmira	03/2006	■ Alnylam gains exclusive access to Tekmira's SNALP siRNA delivery system ■ Tekmira receives 3 targets from Alnylam	■ $8mm of Alnylam stock to Tekmira ■ $4mm R&D funding to Tekmira	■ Up to $13mm in milestones for each successful product ■ Royalties on product sales
Mirus	Pfizer	01/2007	■ 2-year collaboration involving 2 of Mirus' intravenous delivery methods	■ Not disclosed	■ Not disclosed

Table 8: Partnerships between big pharma/biotech companies and siRNA drug delivery system companies[56].

Significant number of collaborations with delivery system companies exists as well since cytosolic delivery is regarded as the most challenging issue in RNAi. In 2005, Sirna Therapeutics, a subsidiary of Merck & Co., has gained access to Provita Biotherapeutics' SNALP technology. In exchange, Provita now has access to Sirna's siRNA chemistry as well as three targets, and received $500,000 in cash and $488,000 in equity. Each party will acquire mutual royalties on products sales involving other party's intellectual property. Pfizer has also made an intent to

enter the RNAi market by signing a two-year collaboration involving Mirus Bio Corporation's delivery methods. Detailed terms are not disclosed, but the deal is expected to be worth multimillion dollars[56] (Table 8).

6.2.7 Summary from siRNA Market Analysis

RNAi market is very attractive for the Irvine technology. First of all, abundant sources of funding exist, including SBIR funded by the National Institutes of Health (NIH), and $1 billion Massachusetts biotech initiative. Since cytosolic delivery of siRNA molecules is the most challenging issue, the Irvine nanoparticles can make a significant impact. Although the market is relatively competitive with emerging technologies, such as Provita Biotherapeutics' SNALP technology, there is room for novel drug delivery systems. For instance, Sirna Therapeutics is especially interested in delivery vehicles with the following characteristics[57]:

- Novel polymers and lipids for encapsulation
- Low toxicity and biodegradable
- Amenable to use of process development and manufacturing platforms
- Amenable to molecular targeting strategies
- Long-term storage stability
- Delivery vehicles that improve endosomal escape
- Assemblies of or less than 200nm in diameter

The Irvine nanoparticles meet all of these criteria, assuming that the critical issues are resolved. Because key patents on siRNA content and targets must be obtained, partnerships are critical. If strong intellectual property portfolio is established, the Irvine technology has the potential to earn capital in the form of venture capital, initial public offering (IPO) proceeds, upfront and milestone payments, royalties on product sales and merger and acquisition activities, as evidenced by other RNAi companies.

6.3 Subunit and DNA Vaccine Market Analysis

6.3.1 Introduction to Immune System and Vaccines

Protective immunity against pathogens is mediated by the early reactions of innate immunity and the later responses of adaptive immunity. Adaptive immunity is the primary target for vaccines. When an antigen, or a component of a foreign substance, is recognized and

internalized by an antigen presenting cells (APC), it is processed and displayed on the surface through major histocompatibility complexes (MHCs). APCs travel to lymph nodes, where helper T lymphocytes, or CD4+ T_H cells, bind to these complexes through T cell receptors (TCRs), and secrete cytokines that lead to differentiation and proliferation of B and T lymphocytes. An additional stimulus, referred to as an adjuvant, is required for this activation step to ensure that immune responses are induced when they are needed and not against harmless substances. These effector cells travel to site of infection. Cytotoxic T lymphocytes (CTLs), also known as CD8+ T_C cells, mediate termination of pathogen-infected cells through lysis. B lymphocytes release antibodies that eliminate extracellular pathogens though various mechanisms. Although effector cells are short-lived, memory cells confer long-term immunity[58]. The principle behind vaccination is to introduce an antigen from a pathogen to develop immunity without causing infection.

6.3.2 Different Types of Vaccines

Since Edward Jenner's first successful vaccination against smallpox in 1798, many vaccines of various types have been developed. Vaccines are largely divided into two classes; prophylactic vaccines endow immunity against future exposure to pathogens whereas therapeutic vaccines are used to treat ongoing conditions, such as cancer and HIV.

Several types of traditional vaccines exist. First, killed pathogens are previously virulent microorganisms killed with chemicals or heat, and examples include vaccines against flu, cholera and hepatitis A virus. On the other hand, live, attenuated pathogens are cultivated under conditions that disable their virulent properties. Although these vaccines induce strong immunological response, manufacturing and characterization challenges as well as safety concerns are significant issues. Subunit vaccines utilize a fragment of an inactivated or attenuated microorganism. Due to the absence of intact pathogens, adjuvants are required in most systems. As opposed to the first two types, subunit vaccines are safe and easy to manufacture and characterize, but immune responses are not as robust.

DNA vaccines, which involve insertion of antigen-encoding viral or bacterial DNA into human cells, are an emerging technology that has been receiving much attention. Unlike gene therapy, DNA vaccines do not require lifelong production of antigens. Therefore, change in the human genome is not a major concern. Some of the advantages include ease of production and

characterization and low cost. However, as demonstrated by clinical trials of DNA vaccines, efficacy and safety in humans need to be improved[59].

6.3.3 Intracellular Delivery to Dendritic Cells

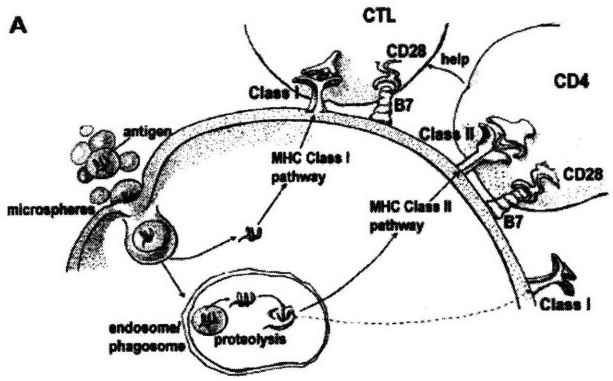


Figure 20: Activation of T cells through dendritic cells[61].

Two of the key challenges in vaccine and immunotherapy technology are to increase the potential defenses against chronic diseases that evade the immune system, and to develop effective immunity after single injections of vaccine. Recent strategies for developing preventive and therapeutic vaccines have focused on the ability to deliver antigens to dendritic cells (DCs), which are the most effective APCs and crucial in initiating T-cell mediated immunity[60]. After antigens are internalized and proteolysed in endosomes, most are loaded onto MHC class II molecules that activate CD4+ T_H cells. Through cross-presentation, for which the mechanism is still unclear, some are presented on MHC class I molecules that are recognized by CD8+ T_C cells (Figure20[61]). However, this does not lead to strong activation of CTLs. Since these effector cells play an important role in termination of infected cells and tumor cells, delivery of antigens to the cytosol, where the intracellular machinery can load them efficiently onto class I MHC molecules, is required.

6.3.4 Global Vaccine Market

Although vaccine market accounts for only 2% of the global pharmaceutical industry, it has been growing at an impressive rate led by development of vaccines against cancer, HIV and hepatitis C virus. The global revenue is estimated to be $10 billion in 2005, and is projected to grow to $20 billion in 2012, with CAGR of 10.5%[62]. North America and Europe possess more than 80% of the market, and the trend is expected to remain stable (Figure 21[62]). With more than 200 companies participating, the market is highly competitive. GlaxoSmithKline, Sanofi-Pasteur, Wyeth, Merck & Co. and Chiron control more than 85% of the global market, which is expected to remain stable, leaving minimal space for other market participants (Figure 22[62]). These

companies are not direct competitors of the Irvine technology. As will be explained later, they are potential customers.

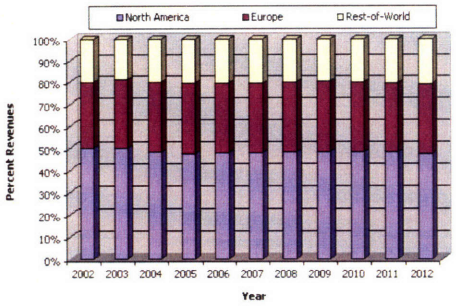


Figure 21: Breakdown of global vaccine market by geography (2005)[62].

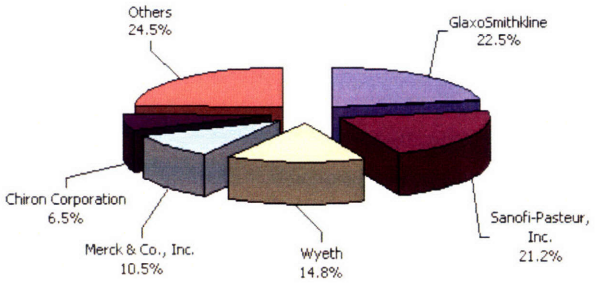


Figure 22: Market share of global vaccine market (2005)[62].

Strengths, Weakness, Opportunities, Threats (SWOT) analysis on the vaccine industry is displayed in Table 9. Benefits of vaccines are substantial. For instance, its economic value is unparalleled, with the cost of vaccination being much less than the cost of treatment. Therefore, vaccine research is heavily supported by the government. Similar to the siRNA market, there are opportunities for innovative delivery platforms, as efficient delivery presents a major challenge for many types of vaccines. Whereas intravenous injection is the prominent approach in siRNA, various routes of administration exist for vaccines, and less invasive and more patient-friendly methods, such as transdermal and oral delivery, are being developed. For instance, PowerMed has invented Particle Mediated Epidermal Delivery (PMED) technology, and Iomai Corporation's liposome carriers, coupled with antigens and adjuvants, target Langerhans cells underneath the skin[63,64].

Since killed pathogens and live, attenuated vaccines cannot be applied to cancer, HIV and hepatitis C virus, subunit and DNA vaccines are being developed. The Irvine technology is addressing the major challenge in these vaccines, which is cytosolic delivery of antigens and DNA to increase CTL responses. Even though numerous antigens have been identified that can generate immunity, the development of subunit and DNA vaccines has had limited success because of the cytosolic delivery challenge. Discussion on subunit vaccines will be focused in this thesis since DNA vaccines require an additional mechanism to deliver DNA molecules to the cell nucleus. The application potential of the Irvine nanoparticles has been confirmed by

measuring the activity of CD8+ T_C cells that have internalized the nanoparticles surface-complexed with model antigens through interferon-γ. Compared to soluble antigens, the core-shell nanoparticles lowered the required dose by more than 100 fold[17]. However, further improvements are needed in addition to the ones discussed in Table 1 and section 4.1. Most importantly, kinetics of antigen release from the nanoparticles must be tailored so that the subsequent processing and presentation coincide with the biological timetable of APC-mediated adaptive immunity in vivo. Immediate antigen release is not optimal in generating an immune response because premature antigen presentation by APCs is known to induce tolerance[65]. Adjuvants may also be needed to increase the strength of immune responses. If these issues are resolved, and the resulting profile is competitive to emerging technologies, the Irvine nanoparticles will be able to penetrate the market.

SWOT Analysis	
Strengths	Weaknesses
■ Increasing awareness of benefits of vaccines	■ Weak supply security ▪ Shortfall in the manufacturing capacity
■ Strong pipeline of vaccines ▪ > 15 vaccines in phase III/registration phase	■ Limited vaccination budgets of emerging markets
■ Support from aid agencies and governments	■ Absence of structured healthcare systems in emerging markets
Opportunities	Threats
■ Development of more effective combination vaccines ▪ Increase patient adherence	■ Domination by top 5 companies
■ Emergence of regional vaccine manufacturers	■ Anti-vaccination group
■ Rising costs & limited vaccine budgets for the major vaccine manufacturers	■ High-cost of clinical trials
■ Untapped market potential in emerging markets	
■ Novel delivery platforms & technologies	

Table 9: SWOT analysis of the vaccine industry.

6.3.5 Emerging Competitive Subunit and DNA Vaccine Delivery Systems

Novel subunit and vaccine delivery systems are being developed in many academic institutions and companies. The Langer laboratory has synthesized microparticles composed of

pH-sensitive poly(β-amino esters) and poly(lactic-*co*-glycolic acid). In extracellular pH, poly(β-amino esters) are uncharged and water-insoluble. In endolysosomal pH, tertiary amine groups become protonated, leading to rapid dissolution of the microparticles and release of antigens. The resulting osmotic pressure gradient and the "proton sponge" effect are responsible for cytosolic delivery[66,67]. Professor David Putnam at Cornell University has molecularly engineered poly(ortho ester) microspheres with optimal kinetics of antigen-encoding DNA release. Cytosolic delivery is mediated through rapid dissolution of the polymers at acidic pH, but does not seem to involve the "proton sponge" mechanism[65]. Unlike conventional poly(lactic-co-glycolic acid) microspheres, these systems do not generate acidic environment that would compromise the bioactivity of antigens. Another advantage is that their size is optimal for preferential uptake by APCs. When used as vaccines in vivo, these microparticle formulations induce antigen-specific rejection of transplanted syngenic tumor cells. On the other hand, Professor Jeffrey Hubbell from EPFL in Switzerland is utilizing ultra-small nanoparticles (25nm) synthesized from Pluronic-stabilized polypropylene sulfide (PPS) to target lymphatic capillaries and lymph nodes, rich in DCs. These biodegradable nanoparticles can function as an adjuvants as well[68]. Finally, Professor Niren Murthy at Georgia Institute of Technology has developed microgel particles, 200 to 500nm in diameter, with antigens bound to acid-degradable crosslinkers. Degradation of the particles leads to osmotic pressure gradient and endolysosomal escape[69]. One disadvantage of this approach is that synthesis is not trivial.

CytRx Corporation's TranzFect technology is one of the most successful delivery platforms for DNA vaccines on the market. Its microparticulate micelles are formed from surface-active agents that can associate with DNA molecules and facilitate their delivery into cells. The micelles themselves act as adjuvants to stimulate an optimized immune response[70].

Advantages of subunit and DNA vaccines with delivery improvements have already been discussed. These vaccines will be much more effective against cancer, HIV and hepatitis C virus compared to conventional therapies, such as chemotherapy and highly active antiviral therapy (HAART). Therefore, emerging technologies described above will be more relevant competitors to the Irvine nanoparticles. When comparing different vaccines, several properties must be addressed. Efficacy in humans is the most important. However, most of the described technologies, including the Irvine nanoparticles, have not entered clinical trials. Predictive measurements, such as increase in cytosolic delivery and the activity of antigen-specific CTLs in

vitro or in vivo, can be used. Many systems show slightly less cytosolic delivery than Lipofectamine 2000. This does not mean that they are worse than Lipofectamine 2000 because the latter is not suitable for in vivo use. Cytotoxicity is another essential measurement. The Irvine nanoparticles possess similar non-cytotoxicity compared to the emerging delivery systems, which should be improved upon incorporation of biodegradability. In vivo stability, ability to target APCs, routes of administration and cost of synthesis contribute to the fate of a new vaccine technology. As long as the Irvine nanoparticles display comparable or better overall profile, they will be able to successfully compete with the emerging technologies.

6.3.6 Patents Related to Vaccine Applications

Similar to the siRNA application, vaccine technology requires an intellectual property portfolio comprising of patents on antigens, adjuvants, targets, drug delivery systems and methods of complexing these different components with the nanoparticles.

6.3.6.1 Patents Related to Vaccines and Targets

May important antigens and their targets specific to pathogens have already been patented. For instance, U.S. Patent No. 5,864,027, assigned to Genentech, describes a method for preparation of vaccines based on HIV envelope polypeptide gp120. More specifically, it claims a specific DNA sequence of less than 5kb encoding gp120[71]. Oncogen owns a patent that covers protein antigens related to melanoma P97 with specific size and nucleotide sequence. These antigens can be produced in large quantities via recombinant DNA techniques and/or chemical synthesis. It further claims these antigens complexed with any kind of pharmaceutical carriers[72]. Another example is U.S. Patent No. 5,709,995 that claims novel hepatitis C virus-derived peptides that are recognized by patient CTLs[73].

6.3.6.2 Patents Related to Complexing Antigens and Adjuvants with Delivery Systems

There are no issued patents that prevent the Irvine nanoparticles from being complexed with antigens and/or adjuvants. However, one patent application deserves particular attention. Patent No. 2006/0189554 describes a vaccine delivery system composed of nanoparticles complexed with adjuvants and an immunogenic antigen or nucleic acid encoding an immunogenic antigen. It claims a broad class of nanoparticles and virtually all antigens[74].

However, it is highly likely that the claims will be more limited since numerous preceding patents would be infringing upon this application, if approved. In addition, the Irvine nanoparticles may not require additional adjuvants to invoke strong CTL responses.

6.3.6.3 Vaccine Patent Strategy

Again, it will be difficult to invest money and time to independently develop antigens and determine their targets as a drug delivery system-focused company. Therefore, it will be important to collaborate with pharmaceutical and biotechnology companies to obtain licenses to patents on specific antigens, adjuvants and targets. On the other hand, there are no patents obstructing complexation of these components with the Irvine nanoparticles. Intellectual property portfolio covering methods of complexing various antigens and adjuvants with appropriate nanoparticles should be developed continuously. Based on previous patents, such as U.S. Patent No. 5,709,879, there is a possibility of claiming a broad class of antigens with specific drug delivery systems[75].

6.3.7 Collaboration with the Pharmaceutical Industry

There is a large number of collaborations between small companies and large pharmaceutical companies in the vaccine industry. For instance, Merck & Co. has received exclusive rights to Idera Pharmaceuticals' agonist compounds to develop prophylactic vaccines. In exchange, Idera has acquired $30 million in cash and is eligible for up to $425 million for development milestones as well as royalties on product sales[76]. In 2007, Novartis partnered with Intercell Smart Vaccines to gain access to over 10 vaccine projects, including adjuvant IC31 for flu vaccines. In return, Intercell received €270 million and eligibility of milestone and royalty payments[77].

Significant number of collaborations with drug delivery system companies to develop subunit and DNA vaccines exists since delivery issue is regarded as the main hurdle. Merck and Co. acquired the right to use CytRx's TranzFect technology in DNA vaccines targeted to four infectious diseases, including HIV. CytRx received upfront payment of $2 million and will be entitled to $4 million for development milestones and royalties on product sales. In addition, Vical paid $3.75 million in cash to CytRx in order to gain exclusive rights to use TranzFect technology in areas other than the four diseases mentioned[70]. In 2004, MGI Pharma made a $50

million acquisition of Zycos to capture their products including ZYC300, which is a PLGA microparticle-based vaccine[78] (Table 10).

Delivery Collaborations

Company	Partner	Announced	Scope	Upfront	Other revenues
CytRx	MERCK	11/2000	■ Merck receives the right to use TranzFect technology in DNA-based vaccines targeted to four infectious diseases, including HIV	■ $2mm in cash	■ Up to $4mm for development milestones ■ Royalties on product sales
CytRx	Vical	12/2001	■ Vical gains exclusive rights to use TranzFect technology except for the above cases	■ $3.75mm in cash	■ Milestone & royalty payments
PEPSCAN THERAPEUTICS	cytos biotechnology	12/2004	■ Collaboration to generate protein vaccines ■ Cytos Biotechnology's Immunodrug carriers	■ Undisclosed	■ Undisclosed
ZYCOS	MGI PHARMA	09/2004	■ Acquistion ■ Zyco's products include ZYC300, PLGA microparticle vaccine	■ 50mm	

Table 10: Partnerships between big pharma/biotech companies and vaccine drug delivery system companies.

6.3.8 Summary from Vaccine Market Analysis

Vaccine market, especially for cancer, HIV and hepatitis C virus treatment, is also very attractive for the Irvine technology. In addition to SBIR, Bill and Melinda Gates Foundation serves as an abundant source of funding. Since cytosolic delivery of antigens is one of the most challenging issues for subunit and DNA vaccines, the Irvine technology has the potential to make a significant impact. Emerging delivery platforms to enhance CTL responses are arising, but with further improvements, the Irvine nanoparticles will be able to compete with them. Again, partnerships are required in order to gain access to antigens and their targets. With strong intellectual property portfolio to cover application of the delivery systems to a broad class of diseases, and a proper business model, the Irvine technology can generate hefty profit.

7.0 Business Model

7.1 Supply Chain in the Pharmaceutical Industry

A general diagram of the pharmaceutical supply chain suitable for the Irvine nanoparticles is depicted in Figure 23[79]. After identifying an active pharmaceutical ingredient (API), which is the drug substance, and developing formulations after years of research, a pharmaceutical company manufactures it into its final packaged form, using its own manufacturing facilities or contract service manufacturing entities. Starting materials, such as chemicals and polymers, are usually obtained from reagent companies. Then, retail and wholesale distributors distribute the product to end-customers, including hospitals and patients.

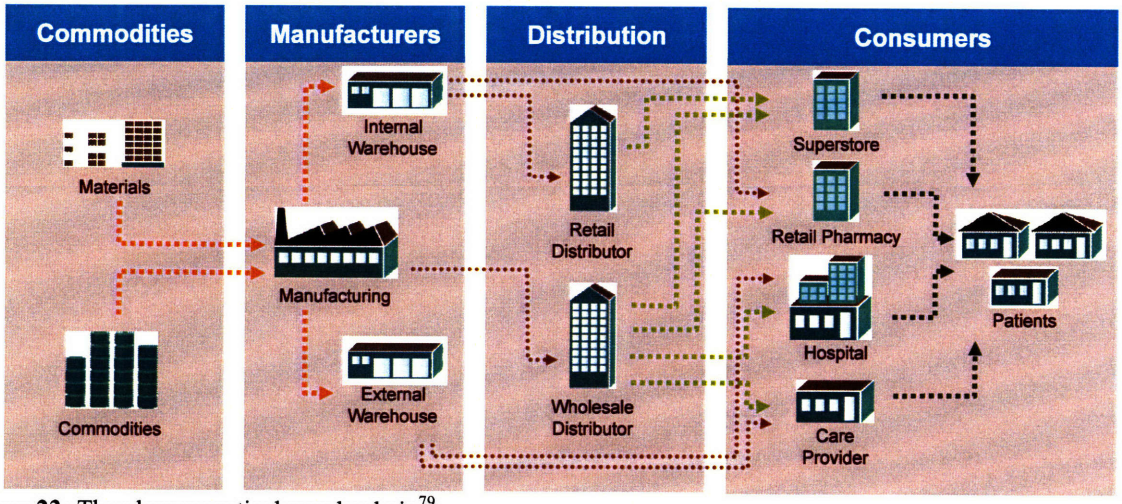


Figure 23: The pharmaceutical supply chain[79].

The pharmaceutical supply chain is becoming increasingly disaggregated. Although contract manufacturing has been largely confined to small pharmaceutical and biotechnology companies in the past, large organizations intend to outsource most of their drug manufacturing activities. One of the key drivers is that the big pharmaceutical companies are operating several of their manufacturing plants at utilization rates of 20 to 30%. This has had a negative impact on their operating margins. In addition, building or upgrading new facilities, associated costs, and training personnel to incorporate advances or changes in manufacturing technology have been increasing at an enormous rate, forcing pharmaceutical companies to outsource such processes rather than investing in fixed assets. Uncertainty over success of new products, capacity issues, and short time-to-market are other reasons for this trend. As a result, the global pharmaceutical contract manufacturing market is expected to grow from $13.4 billion in 2005 to $25.7 billion in

2011, with CAGR of 11%[80]. Pharmaceutical companies are also partnering or buying small biotechnology companies that have the expertise to develop innovative medicines, allowing themselves to focus on clinical trials, late-stage development and sales and marketing[56].

7.2 Proposed Business Model for the Irvine Technology

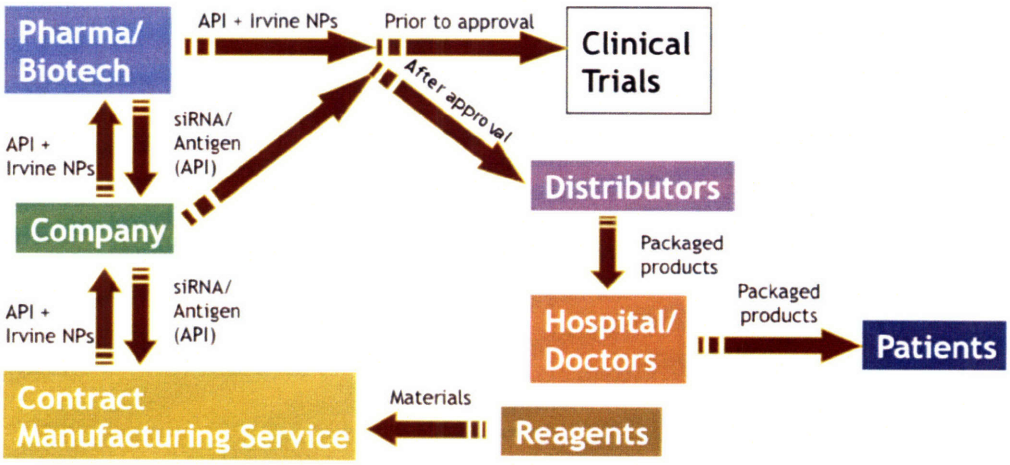


Figure 24: Proposed business model for the Irvine technology.

A proper business model for an innovative technology is essential in achieving financial success in the market. Integrating all the information about the technology, potential markets and supply chain, a business model for the Irvine technology is proposed and shown in Figure 24, with focus on therapeutic applications. The Irvine technology company (referred to as the Company) will adopt a hybrid of intellectual property and manufacturing business model. The Company first needs to partner with large pharmaceutical and biotechnology companies to gain access to the infrastructure and obtain their APIs, such as protein antigens and siRNA molecules. After receiving APIs, the Company will develop formulations by modifying the nanoparticles so that the final products possess optimal drug properties. Then, the Company partners with an appropriate contract manufacturer that has the expertise and proper facilities to develop the core-shell nanoparticles. Since emulsion polymerization has existed for a long time in the industry, this should not be challenging. The pharmaceutical and biotechnology companies will lead the clinical trials after receiving the Irvine nanoparticles complexed with APIs. The Company will collaborate during the process to make necessary changes in formulations. Once they are approved by the Food and Drug Administration (FDA), the contract manufacturer will produce

commercial volume of the final packaged products. The pharmaceutical and biotechnology companies will market their products, and distributors will distribute them to hospitals, where patients can finally receive treatments.

The business model is optimal for the Irvine technology as it maximizes return and minimizes risk, and allows each player to focus on what it does best. The Company will grant non-exclusive rights to pharmaceutical and biotechnology companies to use the Irvine nanoparticles for specific applications so that its customers are not limited. In addition to upfront payments, The Company will be eligible for development milestone payments and royalties on product sales. The Company will not have to invest in expensive clinical trials, where the drug molecules have high likelihood of being disapproved. As demonstrated by previous deals, this type of partnership is common. Contract manufacturing service also reduces risks for all the reasons stated in the previous section. Even if the Company does possess the capital to establish its own manufacturing facilities, it will not be wise to do so since there is no guarantee that the products will be commercialized.

7.3 Value Added across the Supply Chain

One of the most important factors for an innovative technology to succeed in the market is the ability to add value to all the major players in the supply chain. The pharmaceutical supply chain has existed for a long time, and if the technology poses any negative effects on one the major players with strong balance sheets, there will be immense pressure to drive the company out of business. The Irvine technology has the potential to add significant value across the supply chain with minimal disruption. The Company must create knowledge of these benefits and facilitate virtual vertical integration.

Pharmaceutical and biotechnology companies have much to gain from the technology, as their APIs will be equipped with novel nanoparticles that can solve their delivery issues. Without proper delivery systems, APIs cannot be commercialized. As will be demonstrated later in the thesis, profits from product sales will make up for the payments to the Company. Another significant benefit is increased share price from publicity associated with the profits and success of addressing unmet medical needs. For siRNA therapy, Alnylam Pharmaceuticals and Sirna Therapeutics are potential partners since they hold a larger number of key siRNA patents. For

vaccines, GlaxoSmithKline, Merck & Co. and Novartis, which have recently shown strong earnings, are possible candidates.

The Company will sign non-exclusive or exclusive license agreements for contract manufacturers to use the patented methods for producing the nanoparticles. Contract manufacturers will benefit from profit margin, which is the difference between the selling price and the total cost of production. In addition, they will gain early exposure to innovative products, allowing them to establish know-how in manufacturing the nanoparticles. Brookwood Pharmaceuticals and Oakwood Laboratories, which have experience in manufacturing polymeric drug delivery systems, such as microparticles, are potential candidates[81,82].

Distributors, such as McKesson Corporate and Cardinal Health, are minimally affected by the Irvine technology, and will generate revenues from the same process as before. In addition to profits, hospitals can obtain value from the Irvine nanoparticles because they require less time per patient and are much more effective than standard treatments. Finally, patients who receive the therapy will value it as a result of its high efficacy and fewer side effects.

7.4 Proposed Timeline

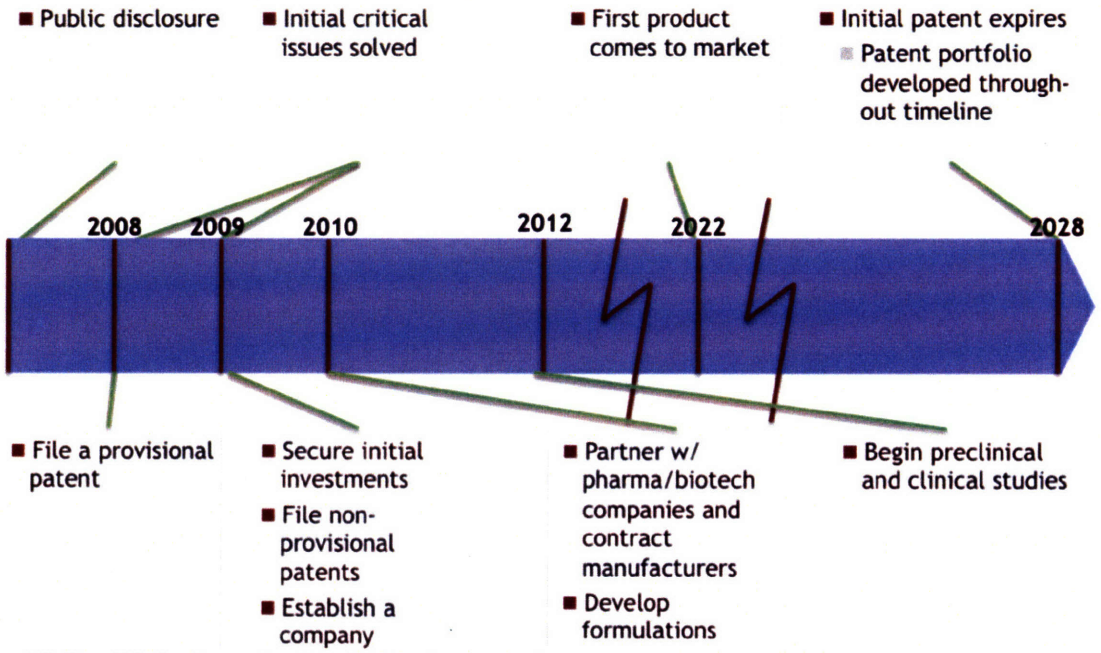


Figure 25: Possible business timeline for development of the first product.

A possible timeline for development of the first product is displayed in Figure 25. An initial intellectual property strategy has been discussed in section 4. Assuming that the short-term critical issues on the research side are resolved in the near future, the Company would be established with initial investments. After setting up a laboratory with scientists and necessary equipments, and coming up with a prototype, the Company would seek out to pharmaceutical/biotechnology companies and contract manufacturers for partnerships. Upfront and milestone payments can be used for additional investments, such as development of patent portfolio. The earliest possible date predicted for the first product to come to market is in 2022. Afterwards, stable cash flow would be generated through royalties on product sales. Initial patent would expire in 2028, but intellectual property portfolio developed through the timeline can continue to prevent other companies from using the Irvine technology.

7.5 Risks during and after Commercialization of the Irvine Nanoparticles

Analysis performed in this section and the next two sections is based on an assumption that the Irvine nanoparticles are successfully commercialized. However, besides critical issues on the research side covered in section 4.1, risks on the business side are significant as well. First, there are potential issues with large-scale development of the Irvine nanoparticles. Particles generated from the manufacturing facilities could display different properties, such as size and monodispersity. Although the particles have shown to be stable at least one week in neutral saline, complexation with siRNAs and protein antigens could dramatically lower their stability and shelf-life[4]. Additional storage requirements, such as lower temperature and anhydrous environment, could increase the cost of producing the nanoparticles. Contract manufacturers that have the expertise in large-scale production of polymeric drug delivery systems have the ability to solve these problems.

The FDA approval process of a pharmaceutical product can be very challenging. Of five candidate drugs that make it to human clinical trials, only one is approved for sale[83]. As seen with the recently failed Merck's HIV vaccine, promising in vitro and animal study results do not necessarily translate into successful clinical outcomes[84]. Although it varies case by case, about eight years pass from the time a cancer drug enters clinical trials until it receives approval from the FDA. Drugs for other diseases have similar timelines[85]. Because of this intensive, long-term, high-risk investment, coupled by the overwhelming odds of failure, report by the Tufts Center

for the Study of Drug Development estimated the average cost to develop a new prescription drug to be $802 million[83]. At this point, it is difficult to predict what the cost of clinical trials will be for the Irvine nanoparticles complexed with APIs. It will be clearer once in vivo data are obtained. Although the FDA approves the pharmaceutical product as a whole, not individual components, one should note that the synthetic materials used to produce the core-shell nanoparticles have not been approved. Compared to drug delivery systems using materials that are FDA-approved as components of various other drugs and devices, the process could be more challenging. However, as described in previous sections, the Irvine nanoparticles are likely to address high-risk groups, such as patients with cancer, and an additional level of toxicity is often considered acceptable to them if the benefit of the pharmaceutical product is substantial. Furthermore, regardless of the adverse effects that ensue, the nanoparticle-formulated APIs will still prove to be safer than traditional treatments, including chemotherapy, surgery and radiation. This analysis reemphasizes the importance of partnering with pharmaceutical companies that have the expertise and financial ability to conduct clinical trials.

A long time horizon to commercialize the Irvine nanoparticles, mostly due to the FDA approval process, increases the likelihood of competing technologies described in previous sections to be on the market, leaving less market share for late entrants. In addition, it decreases a period of market exclusivity prior to the expiration of the initial patent. However, the proposed business model as well as flexibility and broad applicability of the research minimizes the risks involved in commercializing the core-shell nanoparticles,

8.0 Cost Model

Although the contract manufacturers would be producing the nanoparticles for clinical trial and commercial purposes, lab-scale production is still needed to develop appropriate formulations with APIs. A general diagram of the process using two Continuous Stirred Tank Reactors (CSTRs) is shown in Figure 26. CSTRs provide uniform composition and temperature of solution in an inert gas environment to maximize yield and purity of the nanoparticles. Aqueous solution of DEAEMA and PEGDMA is introduced to a CSTR, followed by APS to initiate emulsion polymerization. After 3 hours of core synthesis, the whole volume is transferred to another CSTR, where AEMA is introduced to form the shell for 1.5 hours. The process is

continued so that a batch of the nanoparticles can be obtained every 1.5 hours. The desired products are isolated, unreacted monomers are recovered, and side products are removed.

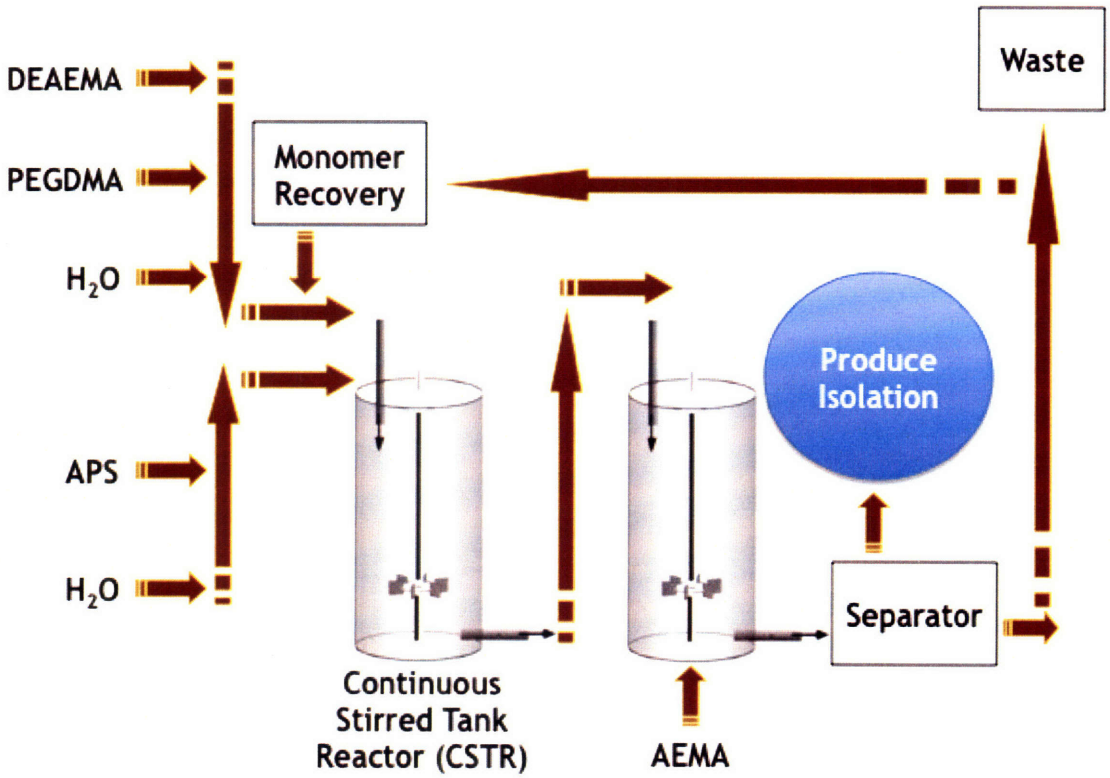
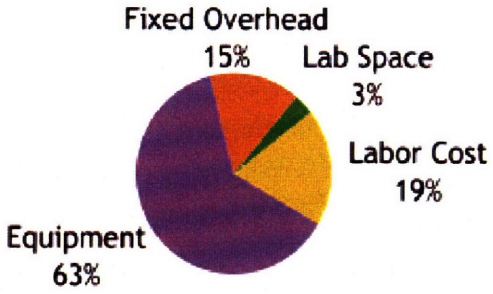


Figure 26: General diagram of the lab-scale production of the Irvine nanoparticles.

Assuming that it takes one year to establish proof-of-concept of the technology and partner with a pharmaceutical company, amount of initial investment required to start and operate the Company is calculated. Approximately 63% of total costs are made up by the laboratory equipments, as confocal microscope, transmission electron microscope (TEM) and scanning electron microscope (SEM) are very expensive[86-88]. Renting a 2,000 square feet incubator laboratory space in Massachusetts costs about $50,000 per year[89]. Labor cost is based upon hiring two scientists ($100,000 per year) and two research assistants ($50,000 per year), and the fixed overhead is estimated to be 15% of the total costs. It is assumed that one of the cofounders will be acting as a Chief Executive Officer in the beginning and will not need to be paid in salary. Materials used to produce the nanoparticles are very cheap, and therefore, material cost is insignificant[90,91]. In total, approximately $1.6 million is required (Figure 27). In addition to investment by the cofounders, SBIR is a potential source of funding. Phase I and Phase II

award up to $100,000 and $750,000, respectively[92]. The Company can seek funding from other agencies, such as Massachusetts Biotech Initiative and Bill and Melinda Gates Foundation. Venture capital investment should be avoided since it is an expensive source of capital, and the business model does not require large upfront capital requirements.



Category	Amount in USD
Materials	$464.50
Lab Space	$50,000.00
Labor Cost	$300,000.00
Equipment	$982,150.00
Fixed Overhead	$235,167.26
Total:	**$1,567,781.76**

Figure 27: Breakdown of total costs for the first year of the Company.

9.0 Case Study (Liver Cancer Market)

9.1 Introduction

The market potential of the Irvine nanoparticles has already been established from section 5. To analyze extent of the benefits that the Company and pharmaceutical/biotechnology companies can extract from the technology through partnerships, it is necessary to concentrate on a specific disease application, as the type of API used, the competing market and the cost analysis will vary from one type to another. A case study on the liver cancer market is performed in this section. Therapeutic area is focused here, but one should note that in addition to being applicable in a broad range of diseases, the Irvine nanoparticles could be used in research as well as target validation, which are currently bigger markets as mentioned previously.

Primary liver cancer is one of the most common cancers worldwide with 600,000 patients diagnosed every year. Secondary liver cancer, which is the metastatic version, has incidence of 500,000 patients[38]. siRNA therapy holds much promise in this area since genes involved in the growth and development of liver tumors have been identified recently. Assuming that the Irvine nanoparticles can be marketed worldwide, the market is expected to be 1,100,000 patients per year. In the following sections, the core-shell nanoparticles complexed with siRNAs will be referred to as siRNA therapy.

9.2 Pharmaceutical/Biotechnology Company Perspective

To estimate how much costs are incurred to a pharmaceutical/biotechnology company to come up with siRNA therapy, several assumptions have been made. First of all, total costs are calculated from costs of producing siRNAs and conducting the preclinical and clinical trials. The nanoparticles are obtained from the Company without a fee. Price of siRNA, which is commercially available at $250 for 0.25mg, is multiplied by 0.75 to discount the profit margin and to back-calculate the actual cost of producing siRNAs[93]. Amount of siRNAs required for each dose depends on various factors, such as the disease, type of drug delivery system and route of administration, but 1mg/kg, which was the siRNA dosage of SNALP-encapsulated siRNAs introduced to nonhuman primates, is used (dosage information regarding liver cancer treatment are not yet available)[43]. Assuming 70kg weight for an average patient, each dose would cost approximately $52,500. If three doses are needed for complete treatment, based on several siRNA preclinical data, the cost of producing siRNAs to treat a patient is $157,500 (Figure 28)[43,94].

- **1. Cost of siRNA: $250/0.25mg x 0.75 = $750/mg**
- **2. Required dosage: 1mg/kg**
- **3. Cost of siRNA per dosage: $750/mg x 1mg/kg x 70kg = $52,500/dose**
- **4. Cost of siRNA per patient: $52,500/dose x 3 doses = $157,500/patient**

Figure 28: Calculation of siRNA cost per patient.

As will be seen in the analysis, siRNA content is the most expensive component of siRNA therapy by far. Without proper drug delivery systems to increase efficacy, each dose may require more than 10mg/kg, making siRNA therapy untenable[94]. Another important assumption is that research and development costs are approximately $800mm, although they can vary case by case. The number is based on the average cost to develop a new prescription drug[83]. Lastly, low demand of 1% is assumed at the outset since it is more expensive than conventional treatments. In comparison, chemotherapy generally costs the patient between $25,000 and $65,000 for complete treatment, although it is not uncommon for costs to exceed that range. Surgery can cost upwards of $10,000. Radiation can range in price from $5,000 to $40,000 depending upon the type and duration of treatment[95]. If research and development costs are

annualized over 10 years, the total costs of siRNA therapy per patient come out to be about $164,773 (Figure 29). Since siRNA content is very expensive, larger production volume does not significantly lower the total costs.

- **1. Liver cancer market: 1,100,000 patients/year**
- **2. Predicted market share: 1% x 1,100,000 patients/year = 11,000 patients/year**
- **3. R&D costs: $800,000,000 (annualized over 10 years)**
- **4. Cost of siRNA therapy per patient: [($157,500/patient x 11,000 patients/year) + ($800,000,000/10 years)]/11,000 patients/year = $164,773/patient**

Figure 29: Calculation of siRNA therapy cost per patient.

9.3 Company Perspective

- 1. Contract manufacturing
  - $15mm to set up and operate a manufacturing plant
  - 15 years of lifespan and $0 of scrap value
  - 15% of profit margin
  - Material cost
    - 100mg of nanoparticles/dose x 33,000 doses = 3,300,000mg of nanoparticles
    - 100% efficient scale-up
    - Total cost: $116,183
  - Total cost: 1.15 x [($15,000,000/15 years) x 1 year + $116,183)] = $1,287,660
- 2. Formulation
  - 15 years of lifespan and $0 of scrap value
  - 3 years of utilization for a particular product
  - Total cost: $1,466,935
- 3. Total cost of nanoparticles per patient: [$1,287,660 + ($1,466,935)/10 years]/ 11,000 patient/year = $130/patient

Figure 30: Calculation of nanoparticle cost per patient.

Several assumptions have been made to estimate cost of producing the core-shell nanoparticles, as most of the data involving contract manufacturing service are confidential. Assuming 100mg of nanoparticles per dose (1:10 (wt/wt) siRNA/nanoparticle[94]), and 100% efficient scale-up from laboratory production, contract manufacture service costs approximately $1.28 million, which is reasonable based on previous deals. If the Company dedicates three years

into developing a formulation for the core-shell particles complexed with particular APIs, and if involved costs are annualized over 10 years, the total costs of the nanoparticles per patient is $130 (Figure 30). This is much lower than the cost of siRNA content. Even though the nanoparticles make siRNA therapy feasible by significantly lowering the required dosage and improving various drug properties, they add minimal costs.

Contract manufacturing service occupies bulk of the cost of the nanoparticles at 47%. Since material cost is minimal, the unit cost of the core-shell particles can fall rapidly with increase in production volume (Figure 31). In general, if fewer than 30,000 doses are produced, a substantial amount is added to the final cost of a single dose. Even though the siRNA therapy will most likely capture a fraction of the 1.1 million liver cancer population initially, production volume of the nanoparticles is still above 30,000 doses. However, regardless of the production volume, the cost of the Irvine nanoparticles is significantly less than that of siRNA content.

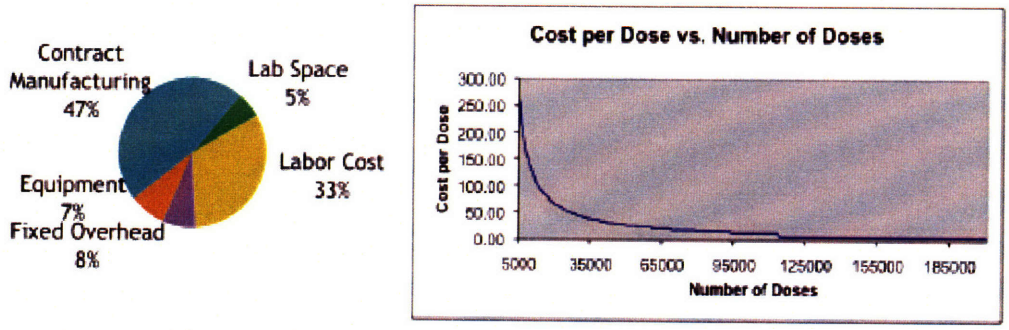


Figure 31: Cost breakdown of the Irvine nanoparticles, and cost per dose of the nanoparticles vs. production volume.

9.4 Cost Analysis

Several factors can impact the cost of siRNA therapy. Although the unit cost was assumed to be constant in section 8.2, economies of scale can significantly lower the cost of siRNA content. If initial market share turns out to be higher than predicted, larger production volume will decrease the cost of siRNA therapy. The location of the companies and manufacturing facilities is another issue. Lower wages in emerging countries, such as China and India, can reduce the cost as well. However, political and marketing concerns involved with manufacturing the nanoparticle-formulated siRNAs outside the United States are significant. This is because stringent Good Manufacturing Practice (GMP) regulations need to be met to guarantee that the pharmaceutical product is produced in a consistent manner and have the

appropriate safety qualifications necessary for its intended use. Next, number of doses per labeled pack can affect the production costs. Single-dose vials require the same equipment and labor as multi-dose vials, but yield fewer doses. Therefore, using single-dose packaging as opposed to multi-dose packaging can increase the production price. Because the overall costs of siRNA therapy are very high compared to the production price, the increase in cost should be insignificant. Furthermore, use of single-dose packing offers several advantages for safety reasons, such as avoiding contamination from needle reuse.

9.5 Pricing siRNA Therapy

As a result of the high costs associated with development of siRNA therapy, higher prices per dose must be charged to break even. Utility analysis can be performed to determine what the price needs to be in order to compete with other cancer treatments. Price of $170,000 per patient is taken as an example, which is above the cost of siRNA therapy for the companies to make profit. Even though it is much more expensive than standard cancer treatments, improved treatment efficacy, site-specific administration and reduced adverse side effects that would be associated with siRNA therapy would make it viable in the market. Because patients' lives are involved, if siRNA therapy significantly increases survival rate and improves quality of life compared to other alternatives, higher prices will be accepted. Annual profit for the pharmaceutical/biotechnology company is $57.5 million. If royalty is assumed to be 5%, the Company would receive $2.9 million every year in addition to upfront payments and development milestone payments. The case study only concerns the liver caner market. There is no question how profitable the Irvine technology and the business model can be, if the nanoparticles are successfully commercialized.

10.0 Conclusion

The technical and economic evaluation of a novel drug delivery system has been performed throughout this paper. Developed in the Irvine laboratory, the pH-responsive core-shell nanoparticles utilize the "proton sponge" effect to mediate cytosolic delivery of membrane-impermeable molecules. Compared to other intracellular drug delivery systems, these nanoparticles offer several advantages, including efficient cytosolic delivery and low cytotoxicity. Another advantage is that emulsion polymerization, which is the process used to synthesize the Irvine nanoparticles, allows composition of the shell to be separately tuned to facilitate drug binding and particle targeting. Variation of the shell structure did not compromise the endolysosomal-escaping capability of the pH-responsive core. These experimental results demonstrate flexibility and broad applicability of the nanoparticles, as they can bind to and deliver various types of drug agents with modification of the shell. However, critical issues on the research side remain and indicate that the technology is still in early stage development. From detailed patent and literature search, the Irvine nanoparticles should be patentable, and a preliminary patent strategy has been outlined.

Since many potentially powerful therapeutic strategies for the treatment of disease depend on cytosolic delivery, numerous applications of the nanoparticles exist. Applications to siRNA delivery and vaccines have been examined in depth. Cytosolic delivery is one of the main challenges in these examples. Therefore, multimillion dollar deals between pharmaceutical companies and drug delivery system-focused companies have already been established. Because key patents on siRNA/antigen must be obtained, partnerships with large pharmaceutical and biotechnology companies are crucial. If overall profile of the core-shell nanoparticles is comparable to that of emerging drug delivery systems, and a strong intellectual property portfolio is developed, the Irvine technology should be able to compete in the market.

A hybrid of intellectual property and manufacturing business model that maximizes return and reduces risk has been proposed in this thesis. It allows each player to focus on what it does best; the Irvine technology company will develop formulations, contract manufacturers will produce the nanoparticles, and pharmaceutical companies will concentrate on clinical trials, late-stage development and sales and marketing. The model also adds value across the existing supply chain. However, risks on the business side, including the FDA approval process, are significant as well. As with any pharmaceutical products, there is no guarantee that the

nanoparticles will be commercialized. Finally, a case study on the liver cancer market is performed to analyze the extent of benefits that the Irvine nanoparticle company and pharmaceutical companies can extract from the technology through partnerships. Even though the core-shell particles themselves are inexpensive, siRNA content can make the therapy very costly. Because patients' lives are involved, if siRNA therapy significantly increases survival rate and improves quality of life compared to other alternatives, higher prices will be accepted. A simplistic cost analysis has shown that commercial development of the Irvine nanoparticles can be a successful endeavor.

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Biographical Note

EDUCATION

09/07 – 09/08 **Massachusetts Institute of Technology**, Cambridge, MA
Master of Engineering Candidate in Materials Science and Engineering

09/02 – 06/06 **Stanford University**, Stanford, CA
Bachelor of Science: Biological Sciences. Minor: Chemistry

09/98 – 06/02 **Phillips Exeter Academy**, Exeter, NH

RESEARCH EXPERIENCE

09/07 – 09/08 **Massachusetts Institute of Technology** Cambridge, MA
Research Assistant
- Researched pH-responsive core-shell nanoparticles that can deliver various types of drug agents to the cell cytosol/nucleus
- Designed and synthesized nanoparticles with different shell structures that possess endosome-escaping capabilities
- Investigated technical and economic aspects of commercializing the technology

07/05 – 09/05 **Genentech, Inc. (Medicinal Chemistry)** South San Francisco, CA
Research Intern
- Developed the synthetic pathways and reaction conditions to construct various analogs of powerful anticancer agents that suppress Inhibitors of Apoptosis commonly expressed in tumor cells
- Interpreted cell-based assay, pharmacokinetics and toxicology data to assess the potency and efficacy of the new drug candidates, and conducted reports to the project members

06/05 – 07/05 **Seoul National University** Seoul, Korea
Research Intern
- Worked under leading experts in stem cell and cloning
- Assisted the professor during animal surgeries and embryo transfer
- Acquired the techniques of aspiration, somatic cell nuclear transfer, in vitro fertilization, and etc.

04/04 – 08/04 **Bing Foundation Research Program in Chemistry** Stanford, CA
Research Assistant
- Participated in Professor Chaitan Khosla's Coeliac Sprue project, utilizing knowledge of organic chemistry and biology to synthesize inhibitors of the Coeliac Sprue disease
- Planned logical approaches to the problem and analyzed graphs and data to evaluate the potency of the new inhibitors

06/03 – 09/03 **Korea Institute of Science and Technology** Seoul, Korea
Research Intern
- Researched Nuclear Pore Complex and investigated honeycomb mesh structure formed by monoolein and poly-L-lactic acid

AWARDS

06/06	Order of Omega Honor Society
06/06	Graduation with Distinction
04/04	Winner of Bing Summer Fellowship
09/01	Early Cum Laude Award
06/01	Rensselaer Medal for Science and Mathematics
05/01	National Merit Scholar

PUBLICATIONS

Choi, K., Siegel, M., Piper, J.L., Yuan, L., Cho, E., Strnad, P., Omary, B., Rich, K.M., and Khosla, C. (2005). Chemistry and biology of dihydroisoxazole derivatives: selective inhibitors of human transglutaminase 2. *Chemistry and Biology, 12*, 469-475.

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