Pluronic-Based Nanoparticles for Gene Therapy Applications

by

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AUTHOR'S DECLARATION

I hereby declare that I am the sole author of this thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners.

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Abstract

Non-viral delivery vectors have potential advantages over the viral systems that currently are used extensively for delivering therapeutic genes of interest. However, non-viral gene therapy has low efficiencies *in vivo*, in part due to the aggregation of the particles in the delivery system associated with serum proteins and other components of the blood. An effective technique for overcoming this problem to use Pluronic™ block copolymers to cover the surfaces of the particles in the delivery system with polyethylene oxide, which decreases their charge density and reduces their interactions with the serum proteins.

The objectives of this project were to characterize a Pluronic-gemini surfactant system to be used as non-viral vectors for gene therapy. Five Pluronics (L44, F68, F87, F108, and F127) were evaluated by studying their physiochemical properties, including particle size and zeta potential. Also, these systems were evaluated in OVCAR-3 cell culture for gene expression and cell viability.

The *in vitro* systems showed small particle sizes (approximately 200 nm) for all Pluronics. The particle sizes in the systems were increased dramatically (up to 2000 nm) by adding dioleylphosphatidylethanolamine (DOPE) to the systems. The zeta potential of these systems shifted the negative zeta potential of DNA (-43 mV) to a positive value (+35 mV). The addition of DOPE had very little effect on zeta potential.

The *in vitro* transfection efficiency in OVCAR-3 showed that all of the Pluronics were able to transfect OVCAR-3 at various DNA/gemini surfactant ratios. The highest transfection efficiency was obtained with Pluronics L44, F87 and F108. PluronicF127 demonstrated the lowest transfection efficiency among the five Pluronics. Adding DOPE did not improve the transfection efficiency in any of the pluronic-gemini surfactant systems.

The viabilities of the cells in these systems were high, and there were greater than the positive control (Lipofectamine 2000). The greatest cell viability (about 60%) was observed when the DNA to

gemini surfactant ratio was 1:2. After adding DOPE, the cell viability decreased in all of the Pluronics except for Pluronic F68.

The results of this investigation indicated that Pluronic block copolymers can transfect OVCAR-3 cell cultures *in vitro* and that they had a low level of cytotoxicity.

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Dedication

To My Parents... Ali Madkhali and Aysha Madkhali

To My Wife... Dikra Haddadi

To My Son... Ayham Madkhali

Table of Contents

AUTHOR'S DECLARATION ii

Abstract iii

Acknowledgements v

Dedication vi

Table of Contents vii

List of Figures x

List of Tables........ xi

List of Abbreviations........ xii

Chapter 1 Introduction........ 1

1.1 Current Gene Therapy 1

1.2 Viral Vectors in Gene Therapy........ 2

1.3 Non-Viral Vectors in Gene Therapy 3

1.4 Gene Delivery and Transfection........ 4

1.4.1 Cationic Liposomes........ 6

1.4.2 Stealth Liposomes 7

1.4.3 Polyplexes 8

1.5 Pluronic Block Copolymers in Gene Therapy........ 10

1.5.1 Introduction 10

1.5.2 Pluronic Block Copolymers for Gene Delivery 12

1.6 Gemini Surfactants in Gene Therapy 15

1.7 Pluronic Block Copolymers and Gemini Surfactants in Gene Therapy 17

1.8 Rationale and the Hypothesis 19

1.9 Objectives of the Research 20

1.9.1 Primary Objective 20
1.9.2 Specific Objectives 20
Chapter 2 Materials and Methods 21
2.1 Purify and extract plasmid DNA to be used in delivery system on OVCAR-3 cell line 21
2.2 Prepare gemini surfactant/pluronic-based systems to be used *in vitro* delivery 21
2.3 Prepare gemini surfactant/pluronic-based systems in combination with DOPE to be used *in vitro* delivery 22
2.4 Evaluate Physiochemical characterization by size and zeta-potential measurements 23
2.4.1 Particle Size Analysis 23
2.4.2 Zeta Potential Analysis 25
2.5 Optimize OVCAR-3 cell culture, transfection, sample collection and detection methods 26
2.6 Fluorescence activated cell sorting (FACS) and cell viability 27
Chapter 3 Results and Discussion 28
3.1 Evaluate Physiochemical characterization of gemini surfactant/pluronics-based nanoparticles and their ability to combine with DNA (Particle size and zeta potential) 28
3.1.1 Particle Size Results 28
3.1.2 Zeta Potential Results 36
3.2 Evaluate the efficiency of GFP expression of gemini surfactant/pluronics-based nanoparticles on OVCAR-3 cell culture in presence and absence of helper lipid (DOPE) 43
3.3 Evaluate the Cytotoxicity of these complexes on OVCAR-3 cell culture in presence and absence of helper lipid (DOPE) by adding Propidium Iodide (PI) 52
Chapter 4 Conclusion and Future Works 58
References 60
Appendix A 69

Appendix B..70

Appendix C 75

List of Figures

Figure 1.1 Chemical structure of DOPE.......... 7

Figure 1.2 Pluronic Block Copolymers Chemical Structure 10

Figure 1.3 A) General Structure of gemini Surfactant; B) structure of the m-s-m gemini surfactant. 15

Figure 3.1 Particle size of the Pluronics at ratio 1: 2 A) Particle size of the Pluronics at 0.1, 1, and 2 CMC in combination with gemini surfactant (16-3-16) and DNA. B) As in A) with DOPE. Results are shown as mean (n=3), errors bars represent standard deviation. 33

Figure 3.2 Particle size of the Pluronics at ratio 1: 5 A) Particle size of the Pluronics at 0.1, 1, and 2 CMC in combination with gemini surfactant (16-3-16) and DNA. B) As in A) with DOPE. Results are shown as mean (n=3), errors bars represent standard deviation. 34

Figure 3.3 Particle Size of the Pluronics at ratio 1: 10 A) Particle size of the Pluronics at 0.1, 1, and 2 CMC in combination with gemini surfactant (16-3-16) and DNA. B) As in A) with DOPE. Results are shown as mean (n=3), errors bars represent standard deviation. 35

Figure 3.4 Zeta Potential of the Pluronics at ratio 1: 2 A) Zeta potential of the Pluronics at 0.1, 1, and 2 CMC in combination with gemini surfactant (16-3-16) and DNA. B) As in A) with DOPE. Results are shown as mean (n=3), errors bars represent standard deviation. 40

Figure 3.5 Zeta Potential of the Pluronics at ratio 1: 5 A) Zeta potential of the Pluronics at 0.1, 1, and 2 CMC in combination with gemini surfactant (16-3-16) and DNA. B) As in A) with DOPE. Results are shown as mean (n=3), errors bars represent standard deviation. 41

Figure 3.6 Zeta Potential of the the Pluronics at ratio 1: 10 A) Zeta potential of the Pluronics at 0.1, 1, and 2 CMC in combination with gemini surfactant (16-3-16) and DNA. B) As in A) with DOPE. Results are shown as mean (n=3), errors bars represent standard deviation. 42

Figure 3.7 Analysis of Transfection experiments using FACS 44

Figure 3.8 *In vitro* Transfection of OVCAR-3 Cells Contain Pluronic L44 47

Figure 3.9 *In vitro* transfection of OVCAR-3 Cells Contains Pluronic F68 48

Figure 3.10 *In vitro* transfection of OVCAR-3 Cells Contains Pluronic F87 49

Figure 3.11 *In vitro* transfection of OVCAR-3 Cells Contains Pluronic F108 50

Figure 3.12 *In vitro* transfection of OVCAR-3 Cells Contains Pluronic F127 51

Figure 3.13 Cell Viability of OVCAR-3 Cells Contain Pluronic L44 53

Figure 3.14 Cell Viability of OVCAR-3 Cells Contain Pluronic F68.. 54

Figure 3.15 Cell Viability of OVCAR-3 Cells Contain Pluronic F87.. 55

Figure 3.16 Cell Viability of OVCAR-3 Cells Contain Pluronic F108.. 56

Figure 3.17 Cell Viability of OVCAR-3 Cells Contain Pluronic F127.. 57

List of Tables

Table 1-1 Physiochemical Characteristics of Pluronics Block Copolymers 18

Table 3-1 Particle Size and Zeta Potential of Pluronic L44+GS and L44+GS+DNA........ 30

Table 3-2 Particle Size and Zeta Potential of Pluronic F68+GS and F68+GS+DNA 30

Table 3-3 Particle Size and Zeta Potential of Pluronic F87+GS and F87+GS+DNA 31

Table 3-4 Particle Size and Zeta Potential of Pluronic F108+GS and F108+GS+DNA 31

Table 3-5 Particle Size and Zeta Potential of Pluronic F127+GS and F127+GS+DNA 32

Table 3-6 Particle Size and Zeta Potential of Pluronic L44+D+GS and L44+D+GS+DNA 37

Table 3-7 Particle Size and Zeta Potential of Pluronic F68+D+GS and F68+D+GS+DNA........ 37

Table 3-8 Particle Size and Zeta Potential of Pluronic F87+D+GS and F87+D+GS+DNA........ 38

Table 3-9 Particle Size and Zeta Potential of Pluronic F108+D+GS and F108+D+GS+DNA........ 38

Table 3-10 Particle Size and Zeta Potential of Pluronic F127+D+GS and F127+D+GS+DNA........ 39

Table 4-1 physical properties of pluronics used in this study. Adapted from product technical information sheets available from the product manufacturer, BASF 69

Table 4-2 Transfection Results of L44±SD........ 70

Table 4-3 Transfection Results of F68±SD........ 71

Table 4-4 Transfection Results of F87±SD........ 72

Table 4-5 Transfection Results of F108±SD........ 73

Table 4-6 Transfection Results of F127±SD........ 74

Table 4-7 Pluronic L44 Anova statistics 75

Table 4-8 Pluronic F68 Anova statistics 77

Table 4-9 Pluronic F87 Anova statistics 79

Table 4-10 Pluronic F108 Anova statistics 81

Table 4-11 Pluronic F127 Anova statistics 83

List of Abbreviations

AAV	Adeno- associated virus
Ad	Adenovirus
CLs	Cationic liposomes
Chol	Cholesterol
CMC	Critical micelle concentration
CMT	Critical micelle temperature
DLC	Dynamic Light Scattering
DNA	Deoxy ribonucleic acid
DOPE	1, 2-dioleyll-snglycerophosphatidlyethanolamine
FACS	Fluorescence activated cell sorting
GFP	Green fluorescent protein
GS	Gemini surfactant
GT	Gene Therapy
HLB	Hydrophilic-Lipophilic Balance
Kb	Kilobase
MPS	Mononuclear Phagocytic System
PAMAM	Polyamidoamine
PEG	Poly-ethylene glycol
PEI	Polyethylenimine
PEO	Polyethylene oxide
PEVP	Poly N-ethyl-4-vinylpyridinium bromide
PG	Plasmid-gemini surfactant
PLD	PEGylated liposomal doxorubicin

PLL	Poly-L-lysine
PPO	Polypropylene oxide
PI	Propidium Idoide

Chapter 1
Introduction

1.1 Current Gene Therapy

Gene therapy (GT) is a technique that is used to correct defective genes responsible for disease development. GT is also a novel and promising approach for the treatment of various diseases with the ultimate goal of developing vectors that are capable of efficient and safe delivery of transgenes to the tissue(s) of interest. ***Gendicine*** (Shenzhen SiBiono Gen Tech), a gene therapeutic for the treatment of head and neck cell squamous carcinoma (HNSCC) was approved for use in humans in China in 2003, and highlights the enormous potential effectiveness of gene therapy [1]. In September of 2005, the Food and Drug Administration of China approved another drug based on gene therapy for the treatment of cancerous tumor in the lungs and other organs, ***Endostar*** [2]. Even though gene therapy offers promising treatment modalities, the difficulty of finding safe and effective ways to transfer genes into the nucleus has not allowed gene therapy to evolve fully as a new treatment or for disease prevention [3]. Gene therapy (GT) depends on using one of two kinds of vectors (viral or non-viral) to deliver genes into a patient's host cells for expression and enable production of proteins to correct or moderate a specific disease.

Pluronic block copolymers (non-viral vectors) have attracted considerable attention for gene deliver [4]. The current research project investigated these copolymers extensively and showed their transfection efficiency as well as their cytotoxicity *in vitro*.

1.2 Viral Vectors in Gene Therapy

Viral delivery, also known as transduction, involves the packaging of DNA (or in some cases RNA) into a virus particle. Gene transfer occurs by the normal viral infection route and is both efficient and cell selective. For this reason, viral delivery is the preferred strategy for *in vivo* gene therapy [5]. About 67% of approved protocols use viral vectors, having developed a considerable number of different viral systems [6]. The most common types of viral vectors that have been used in gene therapy include adenoviral (Ad), retroviral/lentiviral, or adeno associated viral (AAV). The adenovirus (Ad) is a non-enveloped virus with a 36-kilobase (Kb) double-strand DNA genome. Ad has several features that make it an attractive candidate as gene delivery vehicle, including its ability to grow as high-titer recombinant virus, large transgene capacity, and efficient transduction of both dividing and non-dividing cells [7,8]. To date, there are more than 51 human and nonhuman serotypes of Ad have been found to mediate gene delivery to a wide range of tissues, such as respiratory tract, eye, and liver, and urinary tract [9].

Retroviruses are positive sense RNA viruses, containing a single-chain 7-12 kb RNA genome which is converted to DNA by means of a reverse transcriptase encoded by the virus. The resultant viral-DNA is able to integrate in the host's cell genome [5]. Genome integration, which permits stable and prolonged expression of delivered therapeutic genes, makes retroviruses attractive platforms as gene delivery vehicles [10]. However, random insertion of these genes may result in carcinogenesis. In 2002, a young boy developed a leukemia-like condition after being treated with gene therapy using retroviral vector that resulted in gene insertion in an oncogenic site of the genome [11]. In addition, vectors based on Human Immunodeficiency Virus-1 (HIV-1) and other retroviruses, pseudotyped with various envelope proteins, have been used to mediate gene delivery to both dividing and nondividing cells [12].

Another significant viral vector that has been used in clinical trials for gene therapy is adeno- associated virus (AAV). AAV is a nonpathogenic, nonenveloped virus with a 4.7 kb single-stranded DNA genome which requires the presence of certain proteins from a helper virus, usually a member of the adenovirus or herpesvirus family, to complete its lifecycle [13]. To date, more than 100 different serotypes of AAV have been isolated from both human and nonhuman tissues [14,15]. Most studies have focused on AAV serotype 2 (AAV2), but recently several other serotypes, whose sequence variation in the viral capsid confers a broad range of gene delivery properties and options, have shown promising results [12].

The main disadvantage of viral vectors is that they are lacking in safety measures for patients due to potential immune responses, and have the potential of possible integration of the therapeutic gene into the patients' chromosomes which may lead to oncogenesis. In some clinical trials, several patient deaths have been attributed to the use of viral vectors [16]. In addition, viral vectors have limitations in the size of plasmid they can encapsulate, proportion procedures, and the duration of time which they can be stored [17,18].

1.3 Non-Viral Vectors in Gene Therapy

Basically, in order to realize the astonishing potential of gene therapy in the treatment of diseases, non-viral vectors should be used as an alternative method. Non-viral vectors are based on cationic lipids or polymers. They are safe, cheap, and easy to produce in large scale, able to deliver large pieces of DNA that would not fit into the capsid of viral vectors, and are non-toxic/non-immunogenic in most cases [19]. Non-viral transfection vectors consist of cationic lipids or cationic polymers (polycations) which ,through electrostatic interactions, form polyelectrolyte complexes known as "lipoplexes" or "polyplexes", respectively that neutralize and compact the DNA, provide protection from degradation, and enhance transport of the DNA into cell to increase transgene expression [17,4,20].

1.4 Gene Delivery and Transfection

The mechanism of formation of non-viral systems, their binding to DNA, and their entry into the cell have been studied extensively. Gene transfer in eukaryotic cells is a multi-step process including the condensation of DNA, cellular uptake, release from the endosome, nuclear transport and vector unpacking and translation [20]. DNA condensation is a reversible transition favored by the association of the cationic component (s) of the delivery system around the DNA phosphate groups resulting in the formation of polyplexes (cationic polymers) or lipoplexes (cationic lipids) [21]. The excess positive charges of the non-viral vectors interact electrostatically with the negatively charged proteoglycans of the cell membrane [22]. This is followed by non-specific endocytosis, phagocytosis or receptor mediated endocytosis which can be exploited to achieve greater specificity. It is believed that the main entry route of the non-viral vectors to mammalian cells is endocytosis; therefore, any structure facilitating membrane fusion, and allowing DNA release from lipoplexes and escape from endosomes could demonstrate high transfection efficiency [21].

The mechanism of action of gene transfer of lipoplexes has also been studied extensively. Early work suggested that lipoplexes were delivered into the cytoplasm by direct plasma membrane fusion [23], but it is agreed now the lipoplexes are delivered through endocytosis [24, 25]. Following cellular uptake, lipoplexes destabilize the endosomal membrane, resulting in a flip-flop reorganization of phospholipids, which then diffuse into the lipoplex and interact with the cationic lipids causing the DNA to dissociate into the cytoplasm [26].

In contrast, polyplexes do not directly destabilize the endosomal membrane. It is believed that the mechanism of DNA escape from endosomes is associated with the ability of cationic polymers to protonate under the influence of the acidic pH inside the endosome creating a charge gradient resulting in water influx, endosomal swelling, and rupture of the endosome/lysosomes and release of the polyplexes [21].

After their release from the endosomes into the cytosol, the polyplexes must enter the nucleus to undergo transcription. According to Moret et al., although the mechanism of transport of complexes through cytoplasm to nucleus is not completely understood, there is evidence that polycations protect DNA from cytosol nucleases and thus give higher probability for nuclear entry [27]. The transfection efficiency of polyplexes critically depends on the cell cycle and is enhanced by mitotic activity [28]. The best phases of the mitotic cell cycle for transfection are S, (for synthesis, when the DNA replication occurs) or G2, (the "growth" stage following DNA synthesis when protein synthesis occurs) when transfection is facilitated by natural nuclear membrane breakdown [21]. This can be exploited in certain cases such as the treatment of tumors by targeting the dividing tumor cells, while sparing the non-cycling normal cells [21]. By contrast, lipoplexes were never observed in the nucleus indicating that DNA enters probably as free DNA [29]. Disassembly of the polyplexes and lipoplexes to allow the transcription apparatus of the cell to access the DNA efficiently is the final stage in gene expression. Vector unpacking may be a limiting barrier to receptor mediated gene delivery [30]. According to Thomas and Klibanov, significant gene expression has been observed when polyplexes were injected directly into the nucleus suggesting that the dissociation of the complex can actually occur there, possibly mediated by DNA polymerase during transcription in a manner analogous to the stripping of DNA from histone proteins [21]. Release of the DNA from the DNA/liposome complex probably occurs at or before endosomal escape because passage through the nuclear membrane involves only uncomplexed DNA[31]. Passage through the nuclear membrane has to compete with the rapid degradation of uncomplexed DNA by cytoplasmic nucleases [31]. As a result, the vector design has significant effects on the formation of lipoplex, its passage through the cell membrane, and the release of the lipoplex from the endosome and its subsequent dissociation [31].

1.4.1 Cationic Liposomes

Cationic liposomes (CLs or lipoplexes) are considered one of the most efficient non-viral vectors. The cationic liposomes consist of positively charged lipid bilayers that have ability to complex with negatively charged naked DNA through electrostatic interaction resulting in complexes called lipoplexes [32]. In 1987, Felgner described the first application of cationic liposomes to gene therapy [33]. One of the recent applications of lipoplexed delivery was the growth of intratracheally inoculated H-358 human non-small-cell lung carcinoma tumors in nude mice was completely inhibited using an aerosol system administered by intratracheal instillation to deliver wild-type tumor suppressor gene p53 lipoplexes to precancerous and cancerous endobronchial ells [34]. Cationic liposomes have been examined for safety studies through different routes of administration such as intratumoral, intrapulmonary, intracerebral and intravenous with little or no toxicity reported in any of these studies [35]. Liposomes have also other advantages such as simplicity of preparation, ability to produce in large amounts, ability to transfect non-dividing cells, and their versatility for use with any size or type of DNA/RNA [36].

Most lipoplex formulations contain “helper” lipids, such as dioleoylphosphatidyl- ethanolamine (DOPE), or cholesterol (Chol), which provides added stability to the lipoplexes [32]. The helper lipid is generally thought to also improve transfection efficiencies by assisting with escape of the DNA from the endosome after cellular uptake. Combination of cationic lipids and DNA in a micellar or liposomal form leads, generally, to a lamellar organization with DNA molecules sandwiched lipid bilayers. Some systems, for example those that contain the neutral lipid DOPE result in the formation of an inverted hexagonal structure [37] containing DNA in the center of the inverted hexagonal bilayers. According to Zhou and Huang, under physiological conditions, DOPE is a hexagonal phase-forming lipid, which is thought to contribute to its ability to increase

the transfection efficiency of DNA-liposome complexes by destabilizing the lamellar structure of the endsomal membrane lipids [25].

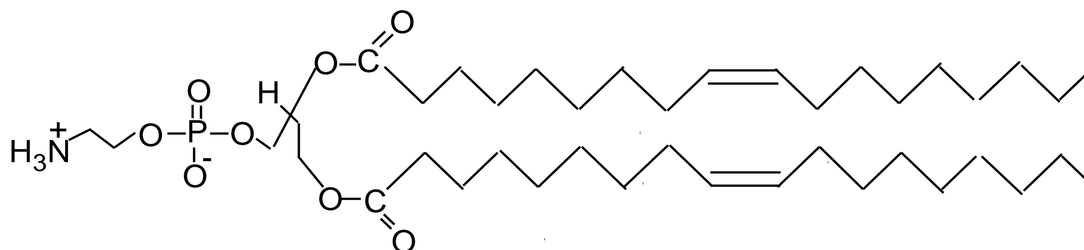


Figure 1.1 Chemical structure of DOPE

Lipoplexes have some drawbackswhich include low transfection efficiency and lack of target specificity [38]. Also, the formation of the lipoplex complex involves interaction among lipid molecules, in addition to that with DNA itself. A major driving force for the complex formation is the release of low-molecular weight counter-ions that makes a large entropic contribution to the free energy of binding [39]. The lipids' hydrophobic segments are determinant in the macroscopic characteristics of the ensuing liposomes, particularly their size, shape, and stability in the dispersed state, as well as interactions with other lipids, cell membranes, and DNA. This, in turn, affects the transfection efficiency of the resulting lipoplexes. Furthermore, liposomal formulations often require an adjuvant, such as DOPE for efficient delivery [40].

1.4.2 Stealth Liposomes

Stealth liposomes are poly-ethylene glycol (PEG)-coated liposomes, and they have been shown to be important for liposomal drug delivery [41]. PEG is a linear polyether diol having several useful properties, such as biocompatibility, solubility in aqueous and organic media[42], lack of toxicity, very low immunogenicity [43], and good excretion kinetics [44]. The molecular weight and structure of PEG can be easily modulated for specific purposes, and it is easier and cheaper to conjugate

the polymer with the lipid. It has been demonstrated that grafting of PEG onto liposomes has many biological and technological advantages. According to Maria et al., the most important properties of PEGylated vesicles are their strongly reduced mononuclear phagocytic system (MPS) uptake and their prolonged blood circulation; consequently, they improve distribution in perfused tissues [41]. In addition, PEG chains on the liposome surface prevent aggregation both with other vesicles and with serum proteins, and thus improve the stability of formulations. PEGylated liposomal doxorubicin (PLD) was the first and still the only stealth liposome formulation to be approved in the USA and Europe for treatment of Kaposi's sarcoma[45], and recurrent ovarian cancer [46].

1.4.3 Polyplexes

In contrast to lipoplexes where the lipids must assemble into vesicle or bilayer structures, polyplexes do not require interaction of the polycation molecules with each other. As a result, polyplexes have greater control of macroscopic properties, and they are quite efficient without adjuvants [21]. In addition, polyplexes are composed of certain repeating structural units which are easily to manipulate by chemical modifications to achieve higher efficiency or cell targeting without the loss of activity [21]. In other words, polyplexes can be synthesized in different lengths, with different geometry, and with substitution or addition of functional groups with relative ease and flexibility [32]. Moreover, polyplexes do not contain a hydrophibic moiety and are completely soluble in water [32]. Basically, polyplexes are comprised of charged complexes of plasmid DNA and a cationic polymer, such as poly-L-lysine (PLL), polyethylenimine (PEI), polyamidoamine (PAMAM); starburst) dendrimers, or chitosan with a net positive charge [28]. There are several polyplexes that have superior transfection efficiency and serum sensitivity in comparison to lipoplexes [47]. For example, Gebhart and Kabanov (2001) demonstrated using a COS-7 cell model transfected for 2 and 4 h at various DNA doses, that linear Polyethyleneimine (ExGenE 500) showed high

transfection in comparison with lipid transfection reagent (LipofectAMINE) [48]. As a result, polyplexes have a promise to be a compelling part for non-viral gene therapy vectors.

The choice of polycation is partly depends on the amphiphlic character or hydrophobic-hydrophilic balance (HLB) of the polycation which affects the endocytosis of the resulting polyplexes and also the transfection efficiency. According to Thomas and Klibanov, attachment of hydrophobic substitutes that can interact with the lipids of the cell membrane could enhance endocytosis of the polyplexes and therefore transfection efficiency [21].

In order to obtain efficient transfection, the correct balance between gaining adequate access of the complexes to the cytoplasm without causing lethal damage to the cell must be achieved. Increasing toxicity has been observed with increasing molecular mass of the lipoplex [21]. This toxicity can be reduced using lower molecular weight polycations with chemical modification to improve transfection efficiency [49].

Cationic lipid and polycations are usually ineffective when they are injected locally into a tissue; therefore, they display low levels of transgene expression in comparison with naked DNA [20]. However, polyplexes formed by biodegradable polymers, are thought to sustain release of DNA at the site of injection as the polycation is hydrolyzed [50,51]. As a result, it is noteworthy to use different group of molecules that display the ability to increase transgene expression upon local administration of DNA in tissues. Non-ionic water-soluble polymers, such as Pluronic block copolymers, represent attractive alternative to the current non-viral gene delivery vectors because of their ability to prevent aggregation of delivery systems, increase transgene expression in a way that does not fit the current non-viral systems as well as they do not condense or bind DNA, which may affect the transfection efficiencies. Therefore, adding gemini surfactant will help in condensing DNA, and thus will improve the transfection efficiency.

1.5 Pluronic Block Copolymers in Gene Therapy

1.5.1 Introduction

Pluronic™ block copolymers are nonionic polymers comprised of two blocks of polyethylene oxide (PEO) separated by a central block of polypropylene oxide (PPO) arranged in A-B-A tri-block structure as illustrated in Figure 1.2 [4]. This arrangement results in an amphiphilic copolymer in which the hydrophobic PO and hydrophilic EO segments can be modified to vary the size, hydrophobicity and lipophilicity of the pluronic. Copolymers with hydrophilic EO and lipophilic PO values are characterized by different hydrophilic–lipophilic balance (HLB), which plays important role in determining the effectiveness of Pluronics [4]. Average diameter of the Pluronics ranges from about 20-80 nm. The aggregation number (number of block copolymer unimers forming one micelle) generally ranges from several to over a hundred. Pluronic™ block copolymers are synthesized by sequential polymerization of PO and EO monomers in the presence of an alkaline catalyst, such as sodium or potassium hydroxide [52].

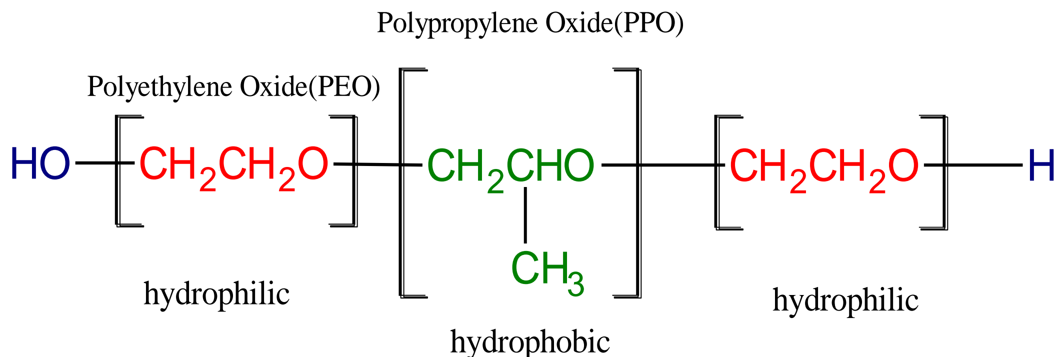


Figure 1.2 Pluronic Block Copolymers Chemical Structure

The most common feature of block copolymers is their ability to form micelles that are characterized by low critical micellar concentrations (CMC). The importance of the CMC can be summarized in two points. First, the CMC determines the stability of micelles against possible dilution of the drug delivery system [53,54]. Second, the CMC specifies the maximal achievable concentration of pluronic unimers to the targeted cells [55]. Block copolymers form micelles with high efficiencies due to the high degree of co-operative interactions within single polymer chains during the self-assembly process [56]. These polymers are known to assemble into nanometer-scale aggregates with a hydrophobic PO core, and a hydrophilic EO shell or corona [4]. It has already been demonstrated that the steric stabilization effect of copolymer micelles reduces unwanted interactions between cells and/ or proteins with the solubilized compounds and increases its circulation time [4,20]. Due to the presence of hydrophobic PO core, the Pluronic unimers can adsorb on surfaces, interact with the hydrophobic and biological membrane, and even translocate inside the cells [57]. The EO corona can also be exploited for drug and gene delivery by incorporating compounds or ligands that are able to bind tissue-specific receptors and to achieve "targeting" of the desired tissue for gene therapy *in vivo*. In addition, polymeric micelles can be used as efficient carriers for compounds that alone exhibit poor solubility and low stability in physiological environment [4].

Polymeric structures often tend to precipitate in water due to a localized hydrophobicity caused by the drug and the hydrophobic portion of polymeric chain. However, with a core/shell structure, the polymer may remain in water-soluble if the number of monomers in the shell-forming block is more than core-forming block [58]. The structure of the block copolymers has profound impacts on the micellization process. The formation of the micelles become more favorable when the length of hydrophobic PO blocks increase resulting in lower values of critical micelle concentration (CMC) and critical micelle temperature (CMT) [59]. On the contrary, an increase in the length of hydrophilic EO block decreases the stability of the micelles. Pluronic block copoly-

mers are generally recognized to be safe for human use, particularly those with high content of PEO [60]. Also, they have molecular weight less than 50,000 g mol^{-1} and can undergo renal clearance; consequently, the toxicity will be reduced [61].

1.5.2 Pluronic Block Copolymers for Gene Delivery

Pluronics have been shown to increase the transfection of nucleic acids with viral and non-viral vectors particularly in combination with polycations at concentrations 500 times lower than established toxicity levels [62]. Scientific literature describes several examples of polyether-polyethyleneimine copolymers as gene transfer agents. These are cationic copolymers linked to nonionic polymers obtained by grafting the PEI with nonionic polyethers such as polyethylene oxide (PEO) or pluronic copolymers.

The research of Sriadibhatla et al. has demonstrated that pluronic block copolymers enhance expression of naked plasmid DNA in muscle, skin, tumor and other tissues, increase expression of genes delivered using polycation-DNA complexes both *in vitro* and *in vivo*, and increase the transfection of cells with adenovirus or lentivirus vectors. Sriadibhatla's studies indicate that copolymers of intermediate hydrophobicity (HLB 9-16) with relatively large hydrophobic blocks (30-69 PO units) were the most effective. The authors compared *in vitro* and *in vivo* gene expression enhancement and found similarities in the magnitude of effects and pattern of activity of different Pluronics [63].

Astafieva et al. compared a synthetic polycation (poly N-ethyl-4-vinylpyridinium bromide) (PEVP) complexed with a plasmid DNA and later the same combination also mixed with 1% Pluronic P85 for DNA intracellular uptake and transgene expression. The role of P85 was to intensify endocytic uptake of the complex into eukaryotic cells, and was to enhance the liberation of macromolecules from the endocytic compartments in the cytoplasm by enhancing internalization and transfection of the DNA-PEVP complex into cells. Both the DNA uptake in the cells as

well as the transgene expression was significantly increased. The authors hypothesized that DNA condensation and recharging due to cationic chains on the surface of the complex, provide for increased binding of the complex to the plasma membrane, facilitating its entrapment into endocytotic vesicles [64]. Yang and colleagues have also reported that P85 promotes transfection enhancement in a promoter-dependent manner, suggesting a signaling pathway activation-dependent mechanism [65]. In addition, co-administration of P85 with DNA in skeletal muscles greatly increased gene expression in the injection tissue site and distinct organs, particularly the draining lymph node and spleen [66]. Most recently, GFP transfection was significantly enhanced when Optison was administered with P85 more than Optison alone-treated mice [67].

In other studies, the receptor-mediated gene delivery to hepatic cell lines, HepG2 using complexes of a plasmid DNA with an asialo-oroso-mucoidpoly-lysine conjugate was increased fourfold in the presence of Pluronic F127 (one of the BASF pluronics being used in this study) [37]. The Pluronic F127 also increased transfection efficiency when the cervical cancer cell line, C-33A was transfected with the polycation/DNA complex [4]. Lemieux et al. have determined that a formulation based on the mixture of the block copolymers, Pluronic L61 and Pluronic F127 increases gene expression 5-20 fold of uncomplexed plasmid DNA in skeletal muscle in mice. The authors found that lower concentrations of SP1017 (which is the block copolymer comprised of both L61 and F127) and at least ten times less DNA were required to achieve a significant increase in gene expression and related physiological response. The high levels of transgene expression observed with SP1017 were sustained for a few weeks as comparison with naked DNA which faded after several days [68].

Pitard and colleagues have discovered that the formulation containing single pluronic copolymers with plasmid DNA also enhanced the gene transfer in the muscle. Particularly, Pluronic

L64 improved the level of transfection efficiency more than the naked DNA in the skeletal and cardiac muscle [69].

Kabanov et al. have investigated the toxicological aspects of injecting both single and multiple doses of various block copolymers in muscle tissue by morphological examination of the muscle tissue and by monitoring creatine phosphokinase levels, and have concluded that the toxicity of the block copolymers was proportional to their lipophilicity; the more lipophilic the copolymer the more severe the lesions. Using intramuscular injection has obvious advantages over myoblast transplantation and intramuscular injection of recombinant viral vectors. The muscles serve as a depot of DNA and could potentially be useful in the treatment of myopathies such as Duchenne's muscular dystrophy or to induce humoral and cell-mediated immune response against infectious diseases and cancers [4].

The mechanism of action of gene expression of Pluronics is not completely understood [70]. However, depending on the applications above, the authors have speculated some mechanism of actions for Pluronics. For example, Pluronics form a gel that may act as a local reservoir for adenovirus release when the concentration is high (15-20 %) [71]. Also, when Pluronics interact with cellular membrane, they facilitate cellular uptake of polyplexes [64] and naked DNA [68]. In addition, Pluronics enhance DNA distribution through the muscle [68], and they increase transport of DNA from the cytoplasm in the nucleus of the muscle cells [69]. Generally, the mechanism of actions in which Pluronics enhance gene expression are different from those of cationic lipids or polycations [13].

According to Kabanov et al., Pluronic block copolymers are promising agents as nonviral vectors for gene therapy applications. Pluronic block copolymers can modify the biological response during gene therapy which leads to enhancement of gene expression and therapeutic effect of transgene. Also, Pluronics block copolymers are able to form novel self-assembling gene de-

livery vectors that have superior effect to current known systems. In addition, Pluronics block copolymers have shown some promise as formulation agents.

1.6 Gemini Surfactants in Gene Therapy

According to Menger and colleagues, gemini surfactants are molecules consisting of two head groups and two aliphatic chains linked by a spacer as shown in Figure 1.3 [72]. Gemini surfactants are synthesized easily at low cost which is an important advantage for industrial drug manufacturing. Gemini surfactants exhibit numerous advantageous properties including low critical micelle concentrations (CMC) and high surface activities. A low value of CMC is very important when considering surfactants as transfection vectors. A high CMC maintains a high monomer concentration that may be necessary to prevent the aggregation of anionic DNA particles, while a low CMC may provide a measure of complex stability, particularly during the delivery process via micellar aggregation [73,74]. Also, gemini surfactants, in general, show very low toxicity and have demonstrated DNA transfection efficiencies as high as 90% [31].

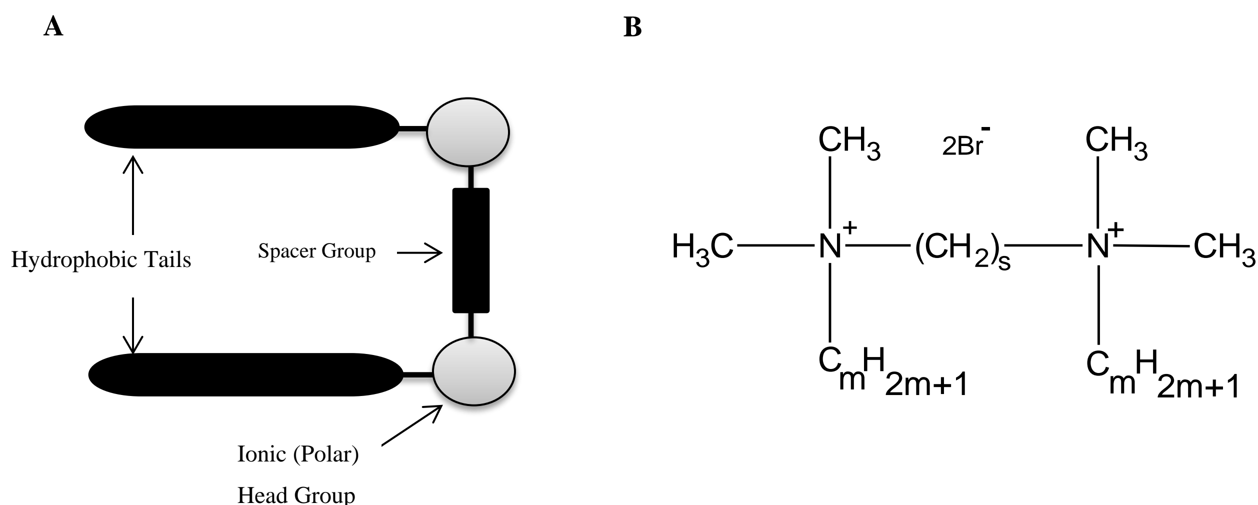


Figure 1.3 A) General Structure of gemini Surfactant; B) structure of the m-s-m gemini surfactant

The effect of variations in both the length of alkyl tails and the size of nature of spacer group of gemini surfactants has been demonstrated in many studies. Variations in the length of alkyl tail affect the properties of surfactant solution almost at the same effect of traditional monomeric surfactants [18]. For example, an increase in the length of alkyl tail of gemini surfactant with a fixed spacer group increases the Kraft temperature and decreases the natural logarithm of the CMC linearly [18]. Low CMC allows gemini surfactant to package or encapsulate DNA, and this is one of the governing factor for low toxicity in gene delivery [75]. The variations of spacer group of gemini surfactants are more complex because they result from steric, electrostatic, and hydrophobic interactions that serve to give rise to a rather rich array of aggregate structure in solution [18].

The cationic *m-s-m* gemini surfactant series is the most commonly studied where *m* and *s* refer to the alkyl tail length and the number of carbon atoms in the polymethylene spacer respectively. The first appearance of *m-s-m* type of gemini surfactant used for transfection, was in 2001 in a study by Rosenzwing and colleagues, who determined that surfactants having C6 spacer and oleyl (cis-9-octadecene) tails resulted in the highest overall transfection and, in particular, that the addition of the neutral helper lipid DOPE (1,2-dioleyl-*sn*-glycer-ophosphatidlyethanolamine) diminished transfection efficiency [76]. Helper lipids work as stabilizing agent for the DNA-gemini surfactant complexes with C10, C12, and C14 tails [77]. Also, the helper lipids are generally thought to improve transfection efficiencies by assisting with escape of the DNA from the endosome after cellular uptake by endocytosis [31]. Badea has determined that transfection efficiencies are greatest for spacer group of size $s \leq 4$ or $s \geq 12$. The short spacing, where s=2, 3 and 4 and their increased transfection efficiency can be explained in terms of optimizing interaction with DNA phosphate groups [78]. Badea also reported that the transfection efficiencies with 16-3-16 (gemini surfactant used in this study) increased when the alkyl tail length increased either in the presence or absence of DOPE [78].

1.7 Pluronic Block Copolymers and Gemini Surfactants in Gene Therapy

As neutral polymers, the Pluronics will not interact with DNA [79]; therefore the systems used in this investigation still require the use of a cationic component to complex and condense the DNA. As a result, the incorporation of a gemini surfactant in the gene delivery nanoparticles should be used. The presence of the gemini surfactant provides the resulting nanoparticles with a positive charge, which is necessary for the nanoparticles to interact with the negatively charged cell membrane. As mentioned above, the Pluronic™ block copolymers are also known to translocate inside cells, possibly facilitate the endosomal escape of DNA during transfection, and may activate cell signaling pathways that aid in the nuclear localization of the DNA. Generally, the interactions between gemini surfactants and neutral triblock copolymer systems are more complex [79]. More details studies are required to see the effective of the pluronics and their dependence on gemini surfactants. As a result, this project examined the replacement of the more extensively studied polycations with nonionic polymers, specifically nonionic block copolymers i.e. Pluronics, while achieving the positive charge afforded in the use of polycations by the use of gemini surfactants which possess a positively charged head group. The project focused on the study of physiochemical properties of gemini surfactants (16-3-16) in combination with Pluronics L44, F68, F87, F108 and F127 as well as it examines the transfection efficiency and toxicity of these systems. The properties of the Pluronics used in this study are listed in Table 1-1.

Table 1-1 Physiochemical Characteristics of Pluronics Block Copolymers [20]

Copolymer	MW[a]	Average no. of EO units(Npo)[b]	Average no. of PO units(Npo)[b]	HLB[c]	Cloud Point in 1% aqueous solution, C[c]	CMC, M[d]
L44	2200	20.00	22.67	16	65	3.6×10^{-3}
F68	8400	152.73	28.97	29	>100	4.8×10^{-4}
F87	7700	122.50	39.83	24	>100	9.1×10^{-5}
F108	14600	265.45	50.34	27	>100	2.2×10^{-5}
F127	12600	200.45	65.17	22	>100	2.8×10^{-6}

[a]The average molecular weight provided by the manufacturer (BASF Co., Parsippany, NJ).

[b]The average numbers of EO and PO units were calculated using the average molecular weights.

[c]HLB values of the copolymers the cloud points were determined by the manufacturer.

[d]CMC values were determined previously using Pyrene probe [80]

1.8 Rationale and the Hypothesis

As a focus for this research, Pluronic block copolymers offer promise for developing non-viral vectors. Their small size and amphiphilic structure facilitate an effective approach as nanoparticles for gene therapy applications. For effective gene therapy, Pluronics must meet two requirements: the ability to efficiently facilitate gene delivery and exert minimal or no toxic effect on their biological host and its cells. As a result, the main goal of this project was to evaluate these Pluronics in combination with DNA and cationic gemini surfactant.

This project will assess the following:

1- Physiochemical properties such as zeta potential and the particle size of Pluronic-GS-DNA

2- Transfection efficiency and cytotoxicity of these systems

The hypothesis for this project is the following;

Non-viral nanoparticles based on a combination of Pluronic block copolymer and gemini surfactant can improve the efficiency of DNA transfection in vitro.

1.9 Objectives of the Research

1.9.1 Primary Objective

The primary objective of the project is to examine the transfection efficiency of five Pluronic block copolymers and test the ability they have for delivering plasmid DNA into a nucleus.

1.9.2 Specific Objectives

1- Purify and extract plasmid DNA to be used in a delivery system on OVCAR-3 cell line.

2- Prepare gemini surfactant/Pluronic-based systems to be used *in vitro* delivery.

3- Prepare gemini surfactant/Pluronic-based systems in addition with DOPE to be used *in vitro* delivery.

4- Evaluate the physiochemical characterization of gemini surfactant/pluronics-based systems either used with DOPE or not by size and zeta-potential measurements.

5- Optimize OVCAR-3 cell culture, transfection, sample collection and detection methods.

6- Evaluate the relationship between the physicochemical properties of Pluronics delivery systems and their efficiency *in vitro*.

7- Set-up and validate fluorescence activated cell sorting (FACS) and cell viability.

Chapter 2
Materials and Methods

2.1 Purify and extract plasmid DNA to be used in delivery system on OVCAR-3 cell line

Plasmids were generated from 4 batches of 50 ml JM109 *Escherichia coli* cultures, harboring the pVGtelRL plasmid, after growing individual colonies in 50 ml of LB + Kan (50 µg/ml) with aeration for approximately 16 hours. Cultures were harvested and plasmid extracted with *E.Z.N.A*. Plasmid Maxi-Prep Kit (Omega, VWR). Plasmid DNA (pDNA) concentration and purity was determined using a NanoDrop spectrophotometer (Thermo Scientific, USA). pDNA extraction was confirmed by gel electrophoresis using a 0.8% agarose gel at 100V for 1 hr followed by gel imaging (AlphaImager, Alpha Innotech).

2.2 Prepare gemini surfactant/pluronic-based systems to be used *in vitro* delivery

The Pluronic-block copolymers (BASF) (L44, F68, F87, F108, and F127) were dissolved in phosphate buffered saline (PBS) at concentrations of 0.1 CMC, 1 CMC, and 2 CMC. Before preparation of the transfection complexes, each Pluronic solution was filtered using a 0.2 µm sterile filter. 0.1, 1, and 2 cmc of Pluronics were added/ well.

Gemini surfactant 16-3-16 (synthesized in the lab) at a concentration of 1.5 mM, was dissolved in mili-Q water and sonicated for 30 minutes until completely dissolved. Before preparation of the transfection complexes, a gemini surfactant solution was filtered using a 0.2 µm sterile filter, and 0.4 µL of gemini surfactant was added/ well.

The plasmid–gemini complexes (PG) were prepared as follows: 0.4 µg plasmid was mixed with an aliquot of gemini surfactant solution added to obtain a 2, 5, or 10 +/- charge ratio and incubated at room temperature for 15 minutes prior to transfection. The plasmid–gemini–Pluronic systems were prepared by mixing the plasmid (0.4 µg/well) with the gemini surfactant solution (2, 5,

or 10 +/- charge ratio) and incubated at room temperature for 15 minutes. To this mixture, 0.1, 1, and 2 cmc of Pluronics were added. Cells were transfected after 30 minutes incubation at room temperature.

2.3 Prepare gemini surfactant/pluronic-based systems in combination with DOPE to be used *in vitro* delivery

Lipid vesicles were prepared using sonication techniques. 1, 2 dioleyl-sn-glycero-phosphatidylethanolamine (DOPE) (Avanti Polar Lipids, Inc.) and α-tocopherol (Sigma-Aldrich) in 5:1 weight ratios were dissolved in 100% ethanol (Commercial Alcohols Inc., Brampton, ON) in a round bottom flask. The solvent was evaporated using rotary evaporation at 100 rpm at 60 ^{o}C, producing a thin film deposited on the walls of the flask. Traces of organic solvent from the lipid were removed with a high vacuum overnight. Glass beads were added to the flask, and the lipid was resuspended in 0.1, 1, and 2 cmc Pluronics (pH= 9). The suspensions were bath-sonicated (Branson 2200, Cleansonic, Orange, VA) for 3 hours at 55 ^{o}C. The suspensions were then filtered using 0.45 µm Acrodisc® filters.

The plasmid-gemini complexes (PG) were prepared as follows: 0.4 µg plasmid was mixed with an aliquot of gemini surfactant solution added to obtain a 2, 5, or 10 +/-charge ratio and then incubated at room temperature for 15 minutes prior to transfection. The plasmid-gemini-Pluronic+DOPE systems were prepared by mixing the plasmid (0.4 µg/well) with the gemini surfactant solution (2, 5, or 10 +/- charge ratio) and incubated at room temperature for 15 minutes. Then, 15 µL of DOPE vesicles were added to this mixture. Cells were transfected after 30 minute incubation at room temperature.

2.4 Evaluate Physiochemical characterization by size and zeta-potential measurements

2.4.1 Particle Size Analysis

Particle size is the diameter of the sphere that diffuses at the same speed as the particle being measured. The Zetasizer system determines size by first measuring the Brownian motion of the particles in a sample using Dynamic Light Scattering (DLS) and then interprets a size by using established theories.

DLS measurements are performed by passing a laser light beam through a sample in aqueous solution. According to quantum field theory, light is emitted as photons that interact with matter; in this case particle size is evaluated using the first principles that govern the processes of diffusion, which depend on particle size and temperature and viscosity of the suspension solvent. The technique relies on the effect of time-dependent light scattering of the random motion of suspended particles (Brownian motion) that depends on particle size. Brownian motion is the movement of particles due to collision with the particles of the liquid that surrounds the particle matter in a water medium in a highly localized manner, but where the probability distributions of these interactions follow a wave-like behavior. The particles in a liquid move randomly and their speed of movement is used to determine the size of the particle [81].

The instrument measures rapid time variations on a scale of microseconds of the scattered light intensity due to interference between light waves scattered by diffusing particles illuminated with a coherent light beam. When a dilute sample is illuminated with a collimated steady monochromatic light source, beginning at $\mathbf{t}_{0}$, the time-dependent irradiance of light scattered by monodisperse spherical particles at a given angle can be measured. The time-dependence of that irradiance comes from the time-dependent interference of light waves scattered by each particle at that angle. The interference is time-dependent because, as the particles are pushed by the fluid mole-

cules away from their positions at time $\mathbf{t}_0$, the conditions for the interference of waves that are scattered by these particles will change.

Single scattering dominates, and particles can diffuse essentially independently of each other, with small particles diffusing faster than large ones. Close to $\mathbf{t}_0$, the instantaneous scattered light intensity is much the same as at $\mathbf{t}_0$. However, as the particles drift further apart, this correlation decays until the scattered light intensity no longer correlates with the initial intensity. The rate at which the correlation between these fluctuations of the light intensity scatter and angle decays with that time delay is thus a measure of the rate of diffusion of the particles and indirectly a measure of particle size.

The translocation diffusion coefficient, once determined from the above process, can be related to the apparent hydrodynamic radius (R_h) using the Stokes's – Einstein equation:

$$R_h = \frac{kT}{6\pi\eta D}$$

Where, **k** is the Boltzmann constant, **T** is the absolute temperature, **η** is the solvent viscosity, and ***D*** is the translational diffusion coefficient [82].

The translational diffusion coefficient, as a function of particle size, depends not only on the size of the particle core, but also on its surface structure as well as the concentration and types of ions in the medium. Ions can affect particle diffusion speed by changing the thickness of the electric layer (the Debye length). A high conductivity medium will suppress the electric double layer and the measured hydrodynamic diameter. Surface structure also affects the diffusion speed. For example, a polymer layer projecting into the medium will slow diffusion speed more than if that polymer is lying flat on the particle surface.

Particles size measurements were performed using disposable Solvent Resistant Micro Cuvette (ZEN0040) in a Malvern Zetasizer NanoZS (Malvern Instruments, Worcestershire, UK), while particle size distributions were calculated using the Malvern DTS software.

2.4.2 Zeta Potential Analysis

Zeta potential (ζ) is the electrostatic potential that exists at the boundary between two layers of ions, namely, the compact layer and the diffuse layer, that surround a particle in solution. It is an important property for understanding colloidal and interfacial behavior. Zeta potential is measured using a combination of the measurement techniques, namely, Electrophoresis and Laser Doppler Velocimetry, sometimes called Laser Doppler Electrophoresis. This method measures how fast a particle moves in a liquid when an electrical field is applied – i.e., its velocity. The zeta potential of the sample will determine whether the particles within a liquid will tend to flocculate (stick together) or not [81]. Zeta potential measurements were performed using Disposable capillary cell (DTS1070) using a Malvern Zetasizer NanoZS; zeta potential distributions were calculated using the Malvern DTS software.

One of the most common techniques used in measuring zeta potential is electrophoresis. When an electric field is applied across an electrolyte, charged particles suspended in the electrolyte are attracted towards the electrode of the opposite charge. Viscous forces acting on the particles will tend to oppose this movement. When equilibrium is reached between these two opposing forces, the particles move with a constant velocity. This velocity will depend on several variables, including the strength of the electric field or voltage gradient, the dielectric constant of the medium, the viscosity of the medium and the zeta potential. The velocity of a particle in a unit electric field is referred to as its electrophoretic mobility.

Zeta potential is related to the electrophoretic mobility using the Henry equation:-

$$UE = \frac{2\,\varepsilon\,\zeta\,f(\kappa a)}{3\eta}$$

where **UE** = electrophoretic mobility, **ζ** = zeta potential, **ε** = dielectric constant, **η** = viscosity and **f (κa)** = Henry's function.

The units of **κ**, termed the Debye length, are a reciprocal length and **κ-1** is often taken as a measure of the "thickness" of the electrical double layer. The parameter **'a'** refers to the radius of the particle and therefore **κa** measures the ratio of the particle radius to the electrical double layer thickness. Electrophoretic determinations of zeta potential are most commonly made in aqueous media and moderate electrolyte concentration [83].

For particle size and zeta potential measurements, the transfection mixture with the pVG-telRL-GFP plasmid (0.4 μg/mL final concentration in the formulation), gemini surfactants (charge ratios of 2, 5 or 10 +/-), Pluronics (0.1, 1, and 2 CMC), and Pluronics+DOPE vesicles were prepared as described earlier for the transfection of OVCAR-3. Samples were run in triplicate, and represented as an average ± SD.

2.5 Optimize OVCAR-3 cell culture, transfection, sample collection and detection methods

OVCAR-3 cells (ATCC) were grown to 90% confluency in 75-cm2 tissue culture flasks in RPMI-1640 medium supplemented with 20% FBS (+FBS, -Ab). The RPMI (+FBS, -Ab) medium was changed to RPMI-1640 medium (without FBS), RPMI (-FBS, -Ab) one hour prior to transfection. The cells were transfected with the following pVGtelRL plasmid using Lipofectamine tm2000 (Invitrogen Life Technologies). For each well, 0.4 μg plasmid was used. The transfection method followed the manufacturer's protocol and was optimized for the OVCAR-3 cells. Briefly, 0.4 μg plasmid was mixed with 0.4 μL lipofectamin tm2000 reagent in 100 uL MEM and incubat-

ed at room temperature for 15 minutes. 0.4 μL of Lipofectamine reagent mixed with 50 μL of MEM was added to the plasmid. After incubating the mixture for 15 minutes at room temperature, it was added dropwise to cells covered with 200 μL of fresh MEM. The gemini surfactants were prepared at 1.5 mM and 0.8, 2, and 4 μL/well were added for the transfection, resulting in a 2:1, 5:1, and 10:1 charge ratio of gemini surfactant to plasmid DNA. Gemini surfactant was mixed with plasmid DNA and incubated at room temperature for 15 minutes. The Pluronic solutions were used at 0.1, 2, and 1 CMC, and the required amount of Pluronic (either with DOPE or not) was added, and incubated for 30 minutes. Then, the mixture was added dropwise to the wells that contained OVCAR-3. After five hours, the transfection mixture in each well was replaced with 500 μL of RPMI (+FBS, -Ab) medium, and the cells were incubated overnight in a tissue culture incubator at 37 °C and 5% CO_2.

2.6 Fluorescence activated cell sorting (FACS) and cell viability

In the next day, 500 μL of RPMI (+FBS, -Ab) medium in each well was aspirated, and 200 uL of triple express (Life-Technology Inc.) was added to detach the cells. Then, 1000 uL of RPMI (+FBS, -Ab) medium was added. Afterwards, the cells were collected in 15 ml tubes and centrifuged at 0°C and 1000 ×g for 5 minutes. GFP expression cells were washed with phosphate buffered saline (PBS) twice after centrifuging. Green fluorescent protein (GFP) expression was quantified using a fluorescence activated cell sorting (FACS) technique. Then, 50,000 cells per sample were analyzed with the FACS instrument (guava easyCyte™ Flow Cytometer) for protein expression and propidium iodide (PI) was examined for cell viability.

Chapter 3
Results and Discussion

3.1 Evaluate Physiochemical characterization of gemini surfactant/pluronics-based nano-particles and their ability to combine with DNA (Particle size and zeta potential)

3.1.1 Particle Size Results

Particle size and charge of the surface measurements of delivery systems are very significant factors in assuring that delivery systems are appropriate for gene delivery. Dynamic Light Scattering (DLS) was used to monitor the polyplexe particle size distributions.

Particle size of gene vector is an important factor that impacts the access and passage of the gene vector through the targeting site [84], and it also impacts the stability of colloidal particles in solution. For efficient endocytosis and gene transfer, particle size of the complex should be below (200 nm) and compact [85]. The particle size depends on many factors, including DNA concentration, sequence of addition of cationic surfactant (gemini surfactant) or DNA to Pluronics during preparation. The particle sizes of polyplexes are closely related to the overall surface charge of the particles. According to Radwan [86], transfection of lipoplexes was directly related to the biophysical properties of lipoplexes. Radwan's team found that lipoplexes of high or low (+/-) charge ratios were small in size, while lipoplexes of near-neutral charge ratios were large.

In this project, the particle size of Pluronic-GS-DNA complex ranged from about 130 to 1450 nm. Tables 3.1- 3.5 and Figures 3.1-3.3 show the particle size data of Pluronics L44, F68, F87, F108, and F127 with three concentrations of Pluronics: below CMC, at CMC, and above CMC. Three ratios (1:2, 1:5, and 1:10) of DNA: gemini surfactant 16-3-16 was added. As shown by this data, the average particle size of Pluronic-GS was variable. It is clear that 0.1 CMC obtained the largest size among all five Pluronics. However, when DNA was added, the average particle size of Pluronic-GS-DNA complex ranged from about 130 to 200 nm. Almost 80 % of

the complexes have particle sizes below 200 nm, except pluronic L44 at ratio 1: 2 at all concentrations (0.1 CMC, 1 CMC, and 2 CMC). This has an overly large particle size that reached approximately 1450 nm. Moreover, Pluronic F127 had a slightly larger particle size at around 250 nm. These results indicate that GS and Pluronics undergo strong compaction and condensation with DNA molecules.

Table 3-1 Particle Size and Zeta Potential of Pluronic L44+GS and L44+GS+DNA

GS=Gemini Surfactant

Pluronic	Size(nm)		ζ-Potential(mV)	
	L44+GS	**L44+GS+DNA**	**L44+GS**	**L44+GS+DNA**
0.1 CMC L44(1:2)	217±28	1458±205	35±29	-25±14
1 CMC L44(1:2)	3.6±0.03	955.5±133	21±2	-21.5±1
2 CMC L44(1:2)	3.8±0.2	1022±24	-1.4±6	-5.7±2.2
0.1 CMC L44 (1:5)	44±14	130.5±0.9	34.1±20	37±1.3
1 CMC L44 (1:5)	7.5±0.2	173.6±1.7	18.5±3	23±0.8
2 CMC L44 (1:5)	3.4±0.3	417±8.2	-0.6±3	18.5±1
0.1CMC L44(1:10)	14±1.2	163.6±1.1	2.3±2	44±6.9
1 CMC L44 (1:10)	6±0.04	192.6±13	8±9.3	26.5±0.2
2 CMC L44 (1:10)	24.5±24	141±2	13±10	23±1.3

Table 3-2 Particle Size and Zeta Potential of Pluronic F68+GS and F68+GS+DNA

GS=Gemini Surfactant

Pluronic	Size(nm)		ζ-Potential(mV)	
	F68+GS	**F68+GS+DNA**	**F68+GS**	**F68+GS+DNA**
0.1 CMC F68(1:2)	290±54	151±3	3.4±5.9	12±0.3
1 CMC F68(1:2)	7.8±1.1	155±2.7	0.4±4.9	-3.11±4.7
2 CMC F68(1:2)	7±0.6	108±7.8	5.8±3.6	-10.8±11.2
0.1 CMC F68 (1:5)	120±8.8	131.7±1.5	26.5±15.7	28.60.9±1.3
1 CMC F68 (1:5)	25±8.7	113±1.6	15.9±0.7	1.98±0.6
2 CMC F68 (1:5)	7.1±0.06	104±7.5	9.3±1.9	1.71±2.34
0.1CMC F68(1:10)	339.5±29.6	174±3.8	31.2±13.3	31±0.3
1 CMC F68 (1:10)	125.7±26.4	116±0.6	21.6±1.42	8.7±1.41
2 CMC F68(1:10)	88.6±2.3	88.6±2.3	8.7±4.6	2.39±0.98

Table 3-3 Particle Size and Zeta Potential of Pluronic F87+GS and F87+GS+DNA

GS= Gemini Surfactant

Pluronic	**Size(nm)**		**ζ-Potential(mV)**	
	F87+GS	**F87+GS+DNA**	**F87+GS**	**F87+GS+DNA**
0.1 CMC F87(1:2)	446±61.6	177.7±8	16.2±9.24	6.40±2.89
1 CMC F87(1:2)	122.8±67	267±15.8	2.07±0.7	-7.07±11.3
2 CMC F87(1:2)	47.7±20	173.7±4.6	2.95±2.77	0.014±0.03
0.1 CMC F87(1:5)	31.9±1.5	168.8±2.6	27.5±13.2	25.4±0.3
1 CMC F87(1:5)	31.15±5	143.3±11.8	1.76±0.9	1.26±0.6
2 CMC F87 (1:5)	17.8±0.6	129.5±7.9	0.39±0.98	0.25±0.7
0.1CMC F87(1:10)	49.3±17	191.4±5.2	28.2±22.2	22.1±2.84
1 CMC F87(1:10)	140±61	114.4±9.3	2.16.6±0.14	3.27±2.08
2 CMC F87(1:10)	58.4±59.6	154.2±4	2.61±1.78	1.59±1.23

Table 3-4 Particle Size and Zeta Potential of Pluronic F108+GS and F108+GS+DNA

GS=Gemini Surfactant

Pluronic	**Size(nm)**		**ζ-Potential(mV)**	
	F108+GS	**F108+GS+DNA**	**F108+GS**	**F108+GS+DNA**
0.1 CMC F108(1:2)	145±50	195.7±7	9.13±5.2	-1.18±9.6
1 CMC F108(1:2)	62±18	156±1.94	0.4±0.9	-1.12±0.9
2 CMC F108(1:2)	79±18	154.5±4.3	-0.7±1.7	-4.13±3
0.1 CMC F108(1:5)	406.5±205	166.9±4	25±13.8	35.6±3.6
1 CMC F108(1:5)	54.6±17.5	177±3.5	2.1±1.3	-0.62±0.2
2 CMC F108 (1:5)	28.5±9	152±6.9	0.13±0.5	-0.8±0.4
0.1CMCF108(1:10)	85±54.7	167±3.9	16.5±9.5	21.8±3
1 CMC F108(1:10)	84±35	190±12.6	0.67±1.7	0.0035±0.6
2 CMC F108(1:10)	22±5.7	176±6.7	0.01±0.7	-0.7±0.5

Table 3-5 Particle Size and Zeta Potential of Pluronic F127+GS and F127+GS+DNA

GS=Gemini Surfactant

Pluronic	**Size(nm)**		**ζ-Potential(mV)**	
	F127+GS	**F127+GS+DNA**	**F127+GS**	**F127+GS+DNA**
0.1 CMC F127(1:2)	93.7±4.5	267±5	-1.67±8	3±1.5
1 CMC F127(1:2)	81±19	243±4	0.13±1	-1±0.7
2 CMC F127(1:2)	80±20	212±4	-0.5±0.5	-1.6±2.3
0.1 CMCF127 (1:5)	71±7	155±1.6	16±7	28±5
1 CMC F127 (1:5)	28±1.4	280±21	0.8±0.5	1±0.9
2 CMC F127 (1:5)	34.7±17	278±16	-0.2±1	-0.9±0.9
0.1CMCF127(1:10)	112±8	187±5	5.8.±6	33±0.6
1 CMC F127 (1:10)	46±2	293±25	0.4±0.5	10±1
2 CMC F127 (1:10)	60±26	167±13	-0.4±1.6	-0.1±0.5

Tables 3.6-3.10 and Figures 3.1-3.3 indicate the particle size of all five Pluronics after adding DOPE. The particle size of all five Pluronics increased dramatically. All Pluronics obtained a size more than 200 nm. Consequently, it is quite evident that there was weak or no complexation between DNA and Pluronic-GS-DOPE.

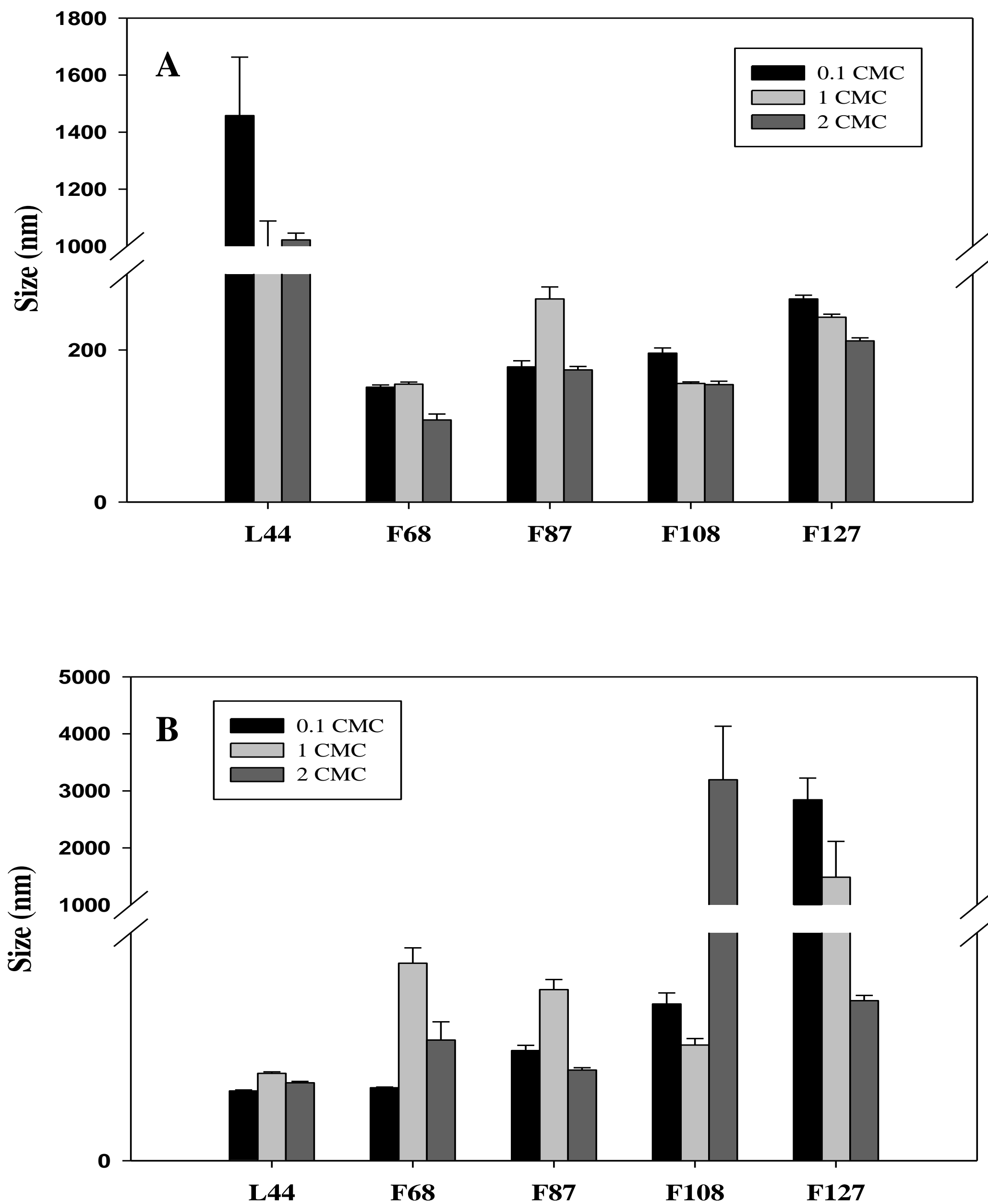


Figure 3.1 Particle size of the Pluronics at ratio 1: 2 A) Particle size of the Pluronics at 0.1, 1, and 2 CMC in combination with gemini surfactant (16-3-16) and DNA. B) As in A) with DOPE. Results are shown as mean (n=3), errors bars represent standard deviation.

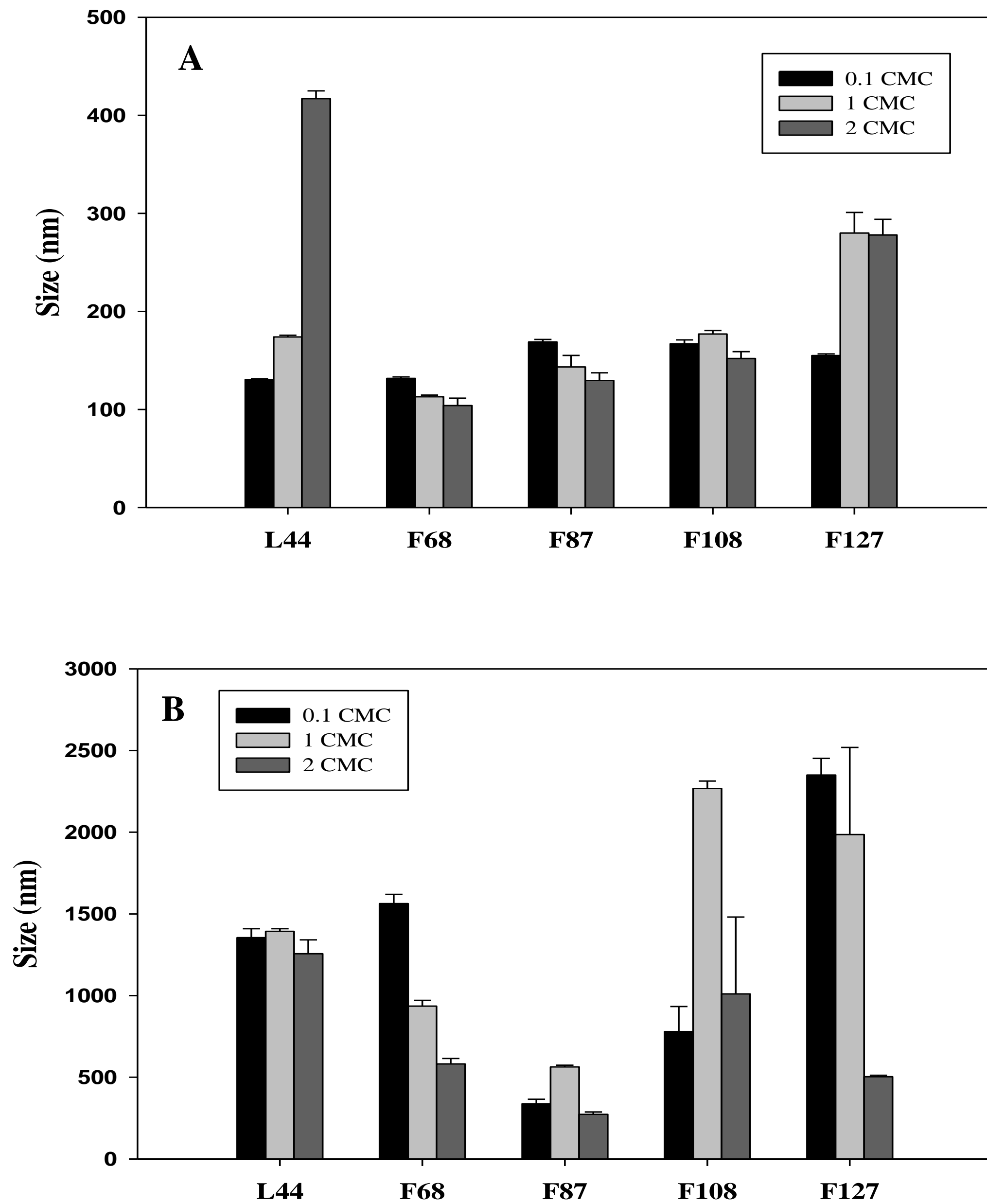


Figure 3.2 Particle size of the Pluronics at ratio 1: 5 A) Particle size of the Pluronics at 0.1, 1, and 2 CMC in combination with gemini surfactant (16-3-16) and DNA. B) As in A) with DOPE. Results are shown as mean (n=3), errors bars represent standard deviation.

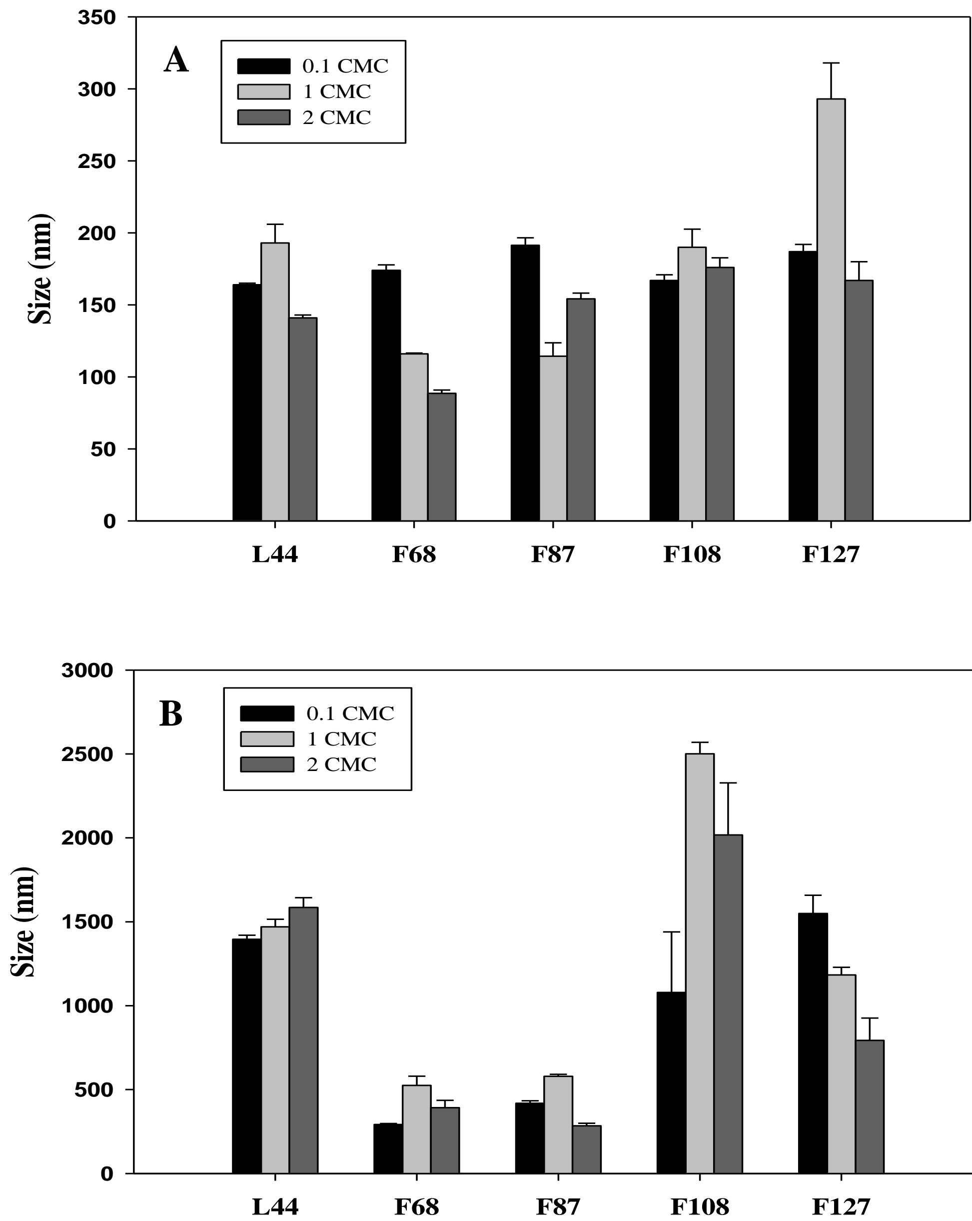


Figure 3.3 Particle Size of the Pluronics at ratio 1: 10 A) Particle size of the Pluronics at 0.1, 1, and 2 CMC in combination with gemini surfactant (16-3-16) and DNA. B) As in A) with DOPE. Results are shown as mean (n=3), errors bars represent standard deviation.

3.1.2 Zeta Potential Results

Zeta potential (ζ) is another important factor for the stability of colloidal delivery systems and DNA transfection. The stability of colloidal systems is determined by a combination of electro-static and steric repulsion. The magnitude of the zeta potential gives an indication of the potential stability of the colloidal system. If all the particles in suspension have a large negative or positive zeta potential then they will tend to repel each other and there will be no tendency for the parti-cles to come together. However, if the particles have low zeta potential values then there will be no force to prevent the particles from coming together and flocculating.

The general dividing line between stable and unstable suspensions is generally taken at either +30 or -30 mV. Particles with zeta potentials more positive than +30 mV or more negative than -30 mV are normally considered stable [81]. However, if the particles have a density different from the dispersant, they will eventually sedimentalize forming a closely packed bed (i.e. a hard cake).

The results of Zeta Potential (ζ) of Pluronic-GS and Pluronic-GS-DNA complex are shown in Tables 3.1-3.5 and Figures 3.4-3.6. Zeta potential measurements indicated electrostatic interaction between Pluronic-GS and plasmid DNA, since the negative charge of plasmid DNA (-43mV) shifted to positive values. It is clear from the data that the zeta potential average of Plu-ronic-GS-DNA complex has a strong positive charge at 0.1 CMC. However, the surface charge at 1 CMC and 2 CMC was neutral or has very weak positive charge. This decrease in surface charge likely resulted from the large amount of Pluronics (have neutral charge) that dilute with gemini surfactant and DNA and led to a decrease in the surface charge of Pluronic-GS-DNA complex.

Adding DOPE to Pluronics maintains the negative charge of Pluronic-GS-DNA complex. Results of zeta potential after adding DOPE are shown in Tables 3.6-3.10 and Figures 3.4-3.6.

Table 3-6 Particle Size and Zeta Potential of Pluronic L44+D+GS and L44+D+GS+DNA

GS=Gemini Surfactant, D=DOPE

Pluronic	Size(nm)		ζ-Potential(mV)	
	L44+D+GS	L44+D+GS+DNA	L44+D+GS	L44+D+GS+DNA
0.1 CMC L44(1:2)	843.6±36.5	214±3	-5±6.5	-29±1.3
1 CMC L44(1:2)	496.7±15	268±4.4	-11±5	-47±3
2 CMC L44(1:2)	569±17	239±4.4	-12±7	-41±3
0.1 CMC L44 (1:5)	942±55	1355±55	0.3±0.2	-16±14
1 CMC L44 (1:5)	506±21	1393±17	-5.5±4	-19±12
2 CMC L44 (1:5)	644±28	1256±85	-6±2	-10±5
0.1CMC L44(1:10)	873±17	1396±24	13.5±6	0.5±7.6
1 CMC L44 (1:10)	1168±67	1470±45	4±5	1.86±9.6
2 CMC L44 (1:10)	668±22	1585±59	6.4±11	1±1.2

Table 3-7 Particle Size and Zeta Potential of Pluronic F68+D+GS and F68+D+GS+DNA

GS=Gemini Surfactant, D=DOPE

Pluronic	Size(nm)		ζ-Potential(mV)	
	F68+D+GS	F68+D+GS+DNA	F68+D+GS	F68+D+GS+DNA
0.1 CMC F68(1:2)	215±3	224±2	-10±7	-27±4
1 CMC F68(1:2)	424±6	606±47	-0.03±0.007	-20±13
2 CMC F68(1:2)	314±55	370±56	-5 ±2	-0.4±0.7
0.1 CMC F68 (1:5)	192±5	1563±57	17±9	-17±15
1 CMC F68 (1:5)	1252±72	936±35	-8±6	-49±8
2 CMC F68 (1:5)	304±28	582±33	-1.8±3	-40±9
0.1CMC F68(1:10)	271±1.7	292±6	22±12	-12±10
1 CMC F68 (1:10)	1955±270	525±55	-0.7±7	-31±26
2 CMC F68 (1:10)	1552±96	392±44	1.24±7	-16±17

Table 3-8 Particle Size and Zeta Potential of Pluronic F87+D+GS and F87+D+GS+DNA

GS=Gemini Surfactant, D=DOPE

Pluronic	Size(nm)		ζ-Potential(mV)	
	F87+D+GS	F87+D+GS+DNA	F87+D+GS	F87+D+GS+DNA
0.1 CMC F87(1:2)	258±8	338±16	-17±4	-0.01±0.05
1 CMC F87(1:2)	200±2.3	525±31	-22±2	-29±0.9
2 CMC F87(1:2)	305±10	278±7.5	-15±1.4	-21±2.5
0.1 CMC F87 (1:5)	463±21	338±28	-15.5±3	-41±5
1 CMC F87 (1:5)	223±20	564±10	-22±1	-27±0.7
2 CMC F87 (1:5)	220±52	273±15	-18±2	-19±3
0.1CMC F87(1:10)	863±14	419±14	-12.6±7	-45±6
1 CMC F87 (1:10)	426±2.7	579±12	-23±3	-26±0.5
2 CMC F87 (1:10)	393±19	284±16	-14±2	-17±2

Table 3-9 Particle Size and Zeta Potential of Pluronic F108+D+GS and F108+D+GS+DNA

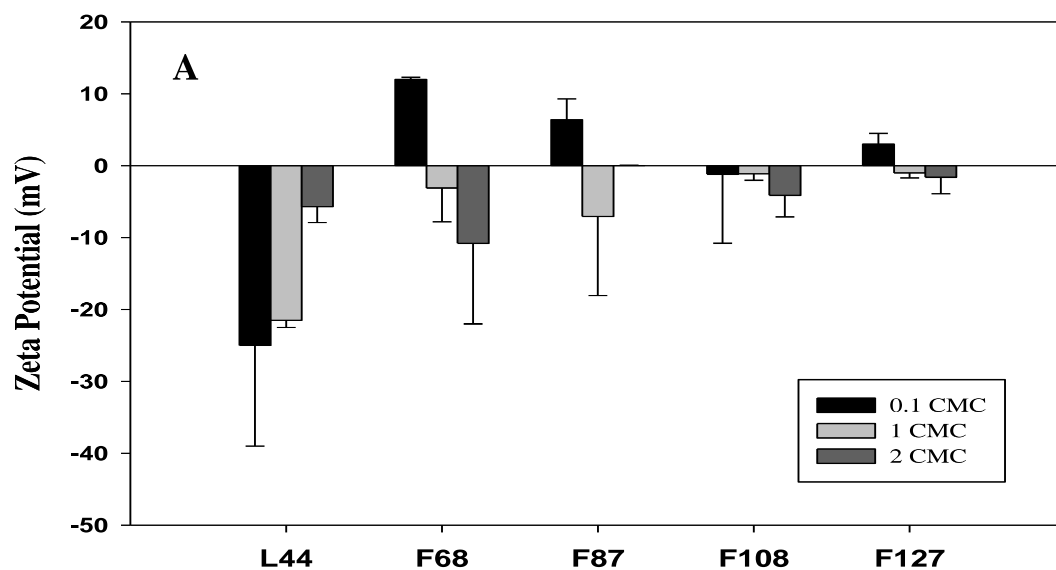
GS=Gemini Surfactant, D=DOPE

Pluronic	Size(nm)		ζ-Potential(mV)	
	F108+D+GS	F108+D+GS+DNA	F108+D+GS	F108+D+GS+DNA
0.1 CMC F108(1:2)	447±25	481±34	-5±0.3	-14.5±1.73
1 CMC F108(1:2)	232±20	355±20	-6±0.7	-2±1.4
2 CMC F108(1:2)	432±37	3196±938	-6±0.8	-15.6±1.9
0.1 CMC F108(1:5)	230±25	780±153	-5±0.5	-16±2.7
1 CMC F108 (1:5)	628±221	2268±45	-5.5±0.5	-8±6.5
2 CMC F108 (1:5)	820±52	1010±471	-6.6±0.9	-2±1
0.1CMCF108(1:10)	767±43	1079±361	-0.01±0.4	0.005±0.4
1 CMC F108 (1:10)	671±0.3	2501±68	-5±0.7	-3±0.9
2 CMC F108 (1:10)	977±78	2017±311	-4.8±0.2	-1.5±0.9

Table 3-10 Particle Size and Zeta Potential of Pluronic F127+D+GS and F127+D+GS+DNA

GS=Gemini Surfactant, D=DOPE

Pluronic	**Size(nm)**		**ζ-Potential(mV)**	
	F127+D+GS	**F127+D+GS+DNA**	**F127+D+GS**	**F127+D+GS+DNA**
0.1 CMC F127(1:2)	785±235	2844±380	-5±1.15	-24±12
1 CMC F127(1:2)	783±35	1487±628	-5±0.13	-18±7
2 CMC F127(1:2)	802±71	491±16	-4±0.7	-11±8
0.1 CMC F127(1:5)	984±125	2350±102	-5±1	-14±5
1 CMC F127 (1:5)	743±67	1986±533	-5.5±0.9	-11±5
2 CMC F127 (1:5)	829±39	504±8	-4.5±0.7	-0.05±0.05
0.1CMCF127(1:10)	1116±94	1549±109	-5.5±0.8	11.4±12
1 CMC F127 (1:10)	960±90	1183±46	-5±0.6	37±1.4
2 CMC F127 (1:10)	1138±348	793±133	-6±1	16±4.5



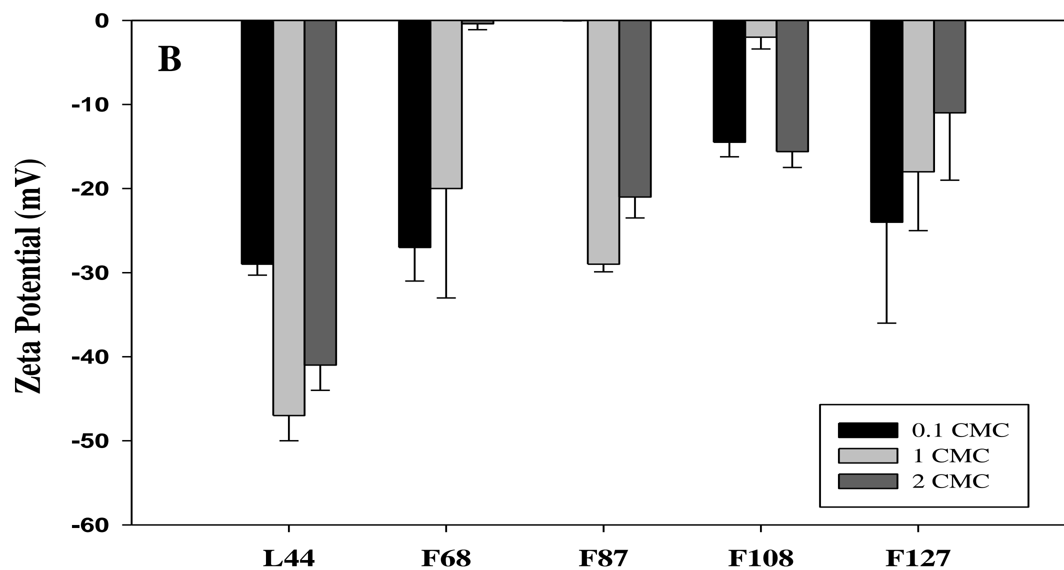


Figure 3.4 Zeta Potential of the Pluronics at ratio 1: 2 A) Zeta potential of the Pluronics at 0.1, 1, and 2 CMC in combination with gemini surfactant (16-3-16) and DNA. B) As in A) with DOPE. Results are shown as mean (n=3), errors bars represent standard deviation.

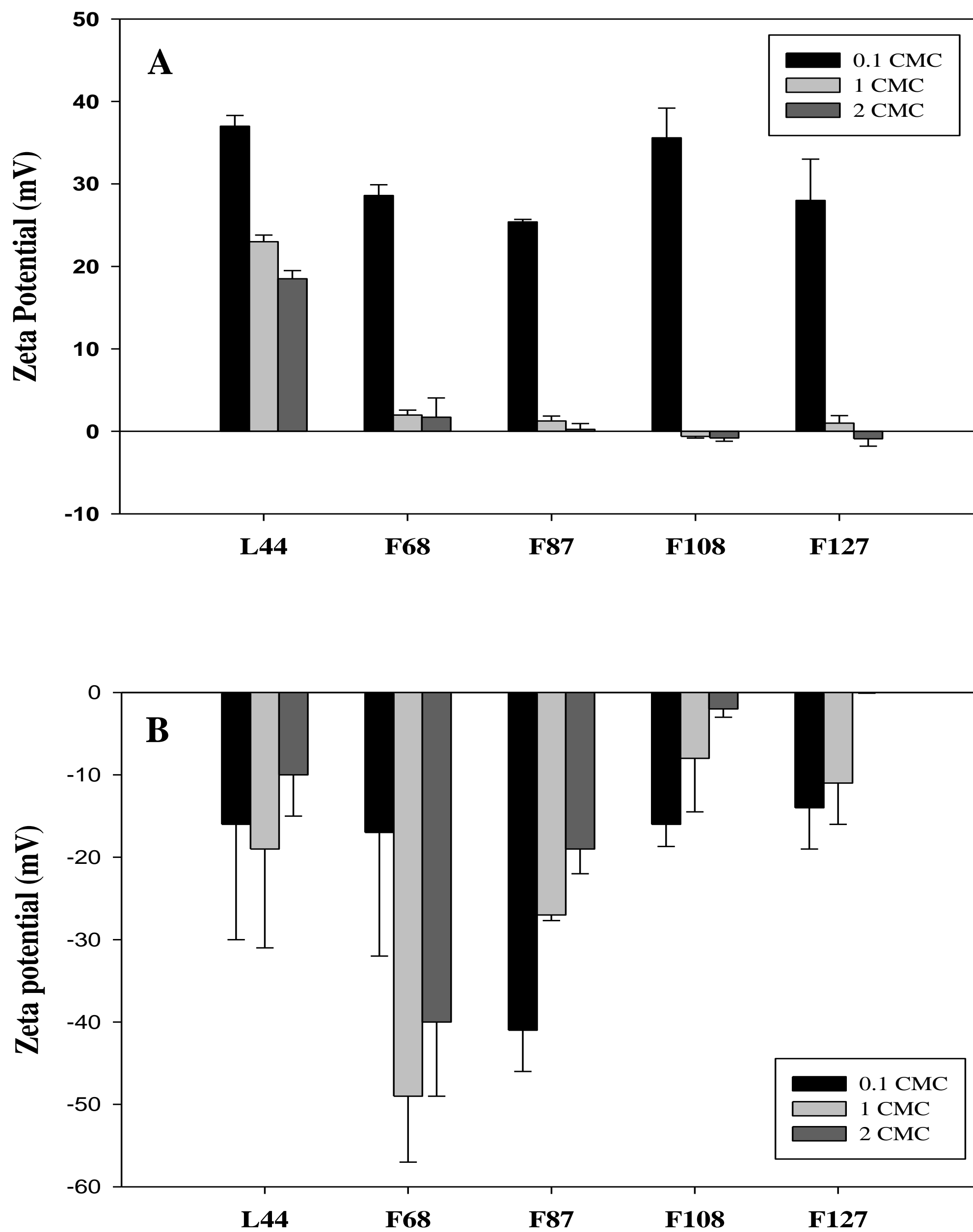


Figure 3.5 Zeta Potential of the Pluronics at ratio 1: 5 A) Zeta potential of the Pluronics at 0.1, 1, and 2 CMC in combination with gemini surfactant (16-3-16) and DNA. B) As in A) with DOPE. Results are shown as mean (n=3), errors bars represent standard deviation.

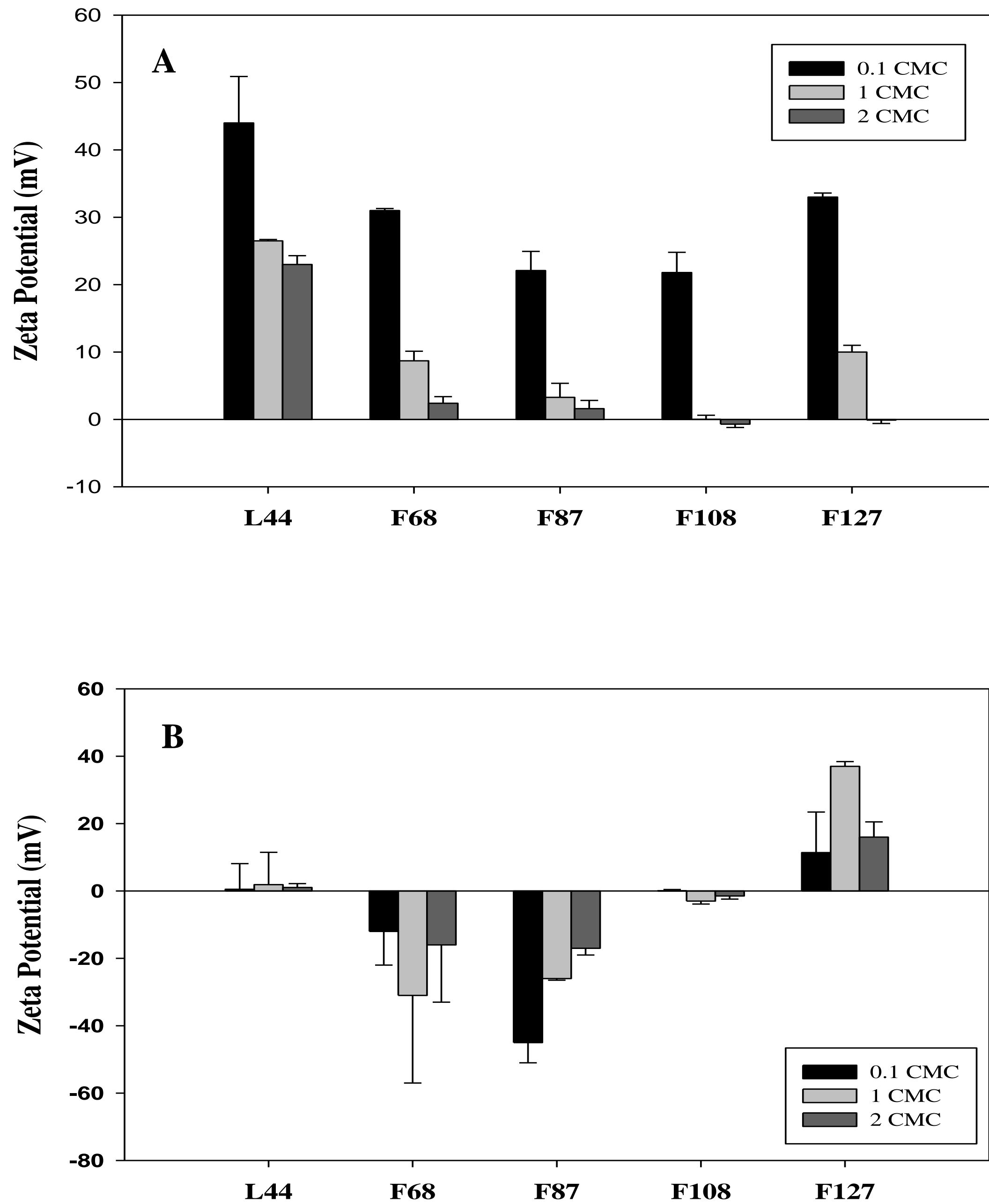


Figure 3.6 Zeta Potential of the the Pluronics at ratio 1: 10 A) Zeta potential of the Pluronics at 0.1, 1, and 2 CMC in combination with gemini surfactant (16-3-16) and DNA. B) As in A) with DOPE. Results are shown as mean (n=3), errors bars represent standard deviation.

3.2 Evaluate the efficiency of GFP expression of gemini surfactant/pluronics-based nanoparticles on OVCAR-3 cell culture in presence and absence of helper lipid (DOPE)

OVCAR-3 cell line was transfected using Pluronic-GS-DNA complex in both absence and presence of DOPE. Three concentrations (0.1, 1, and 2 CMC) of all five Pluronics (L44, F68, F87, F108, and F127) were used in this project. GS 16-3-16 was added at three different ratios to DNA (2:1, 5:1, and 10:1). After that, DOPE was added to Pluronics to examine if it has any effect on transfection efficiency. OVCAR-3 cell line was transfected with these complexes and the results were determined using FACS analysis. Figure 3.7 elucidates the way in which the results were analyzed. The Plot graph is divided into four squares. The upper left corner shows dead cells only. Live cells are shown in the lower left corner. GFP expression is shown in the lower right corner, while the upper right corner represents the transfected dead cells.

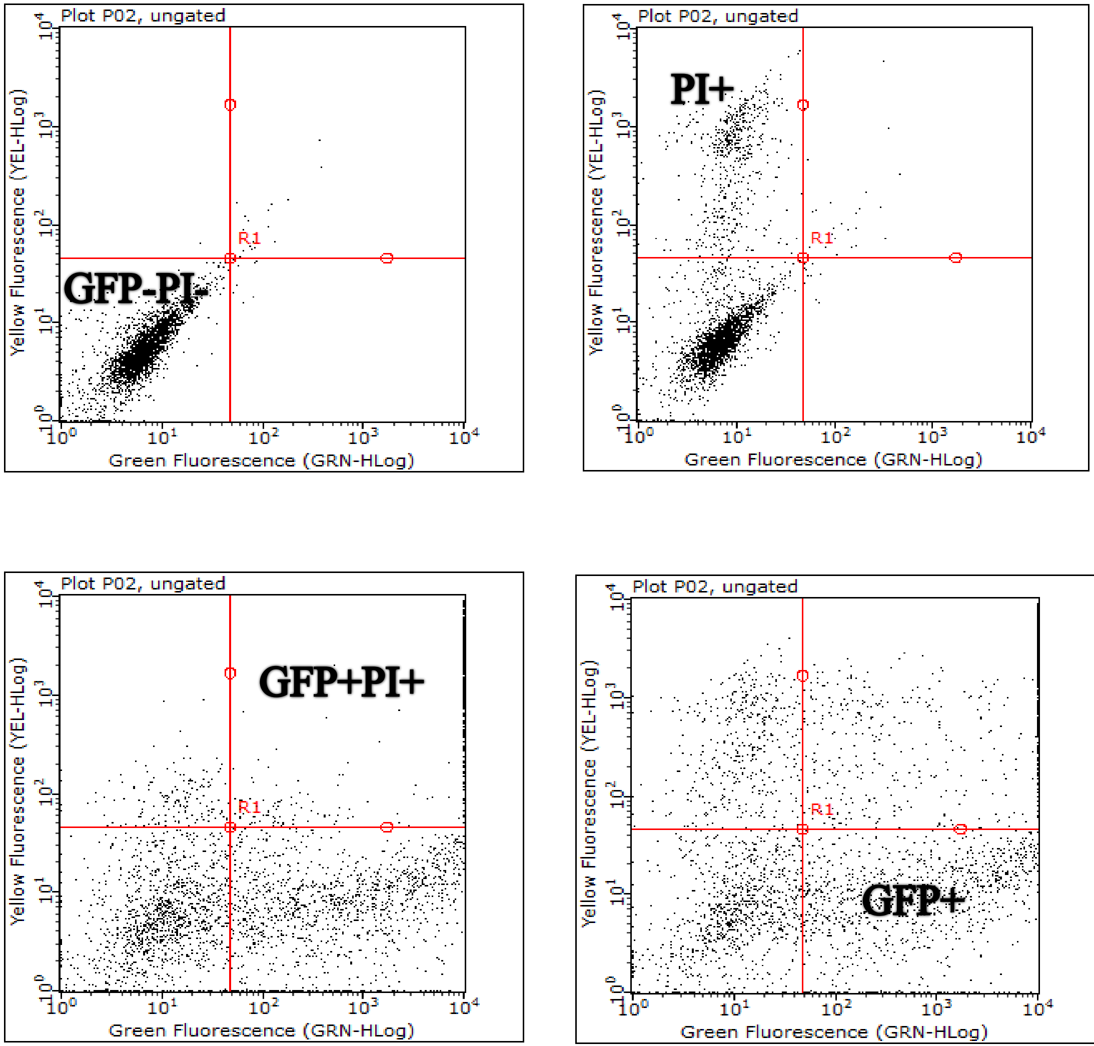


Figure 3.7 Analysis of Transfection experiments using FACS

Bar graphs below indicate the results of these transfections. The maximum fluorescent emission was observed with these three Pluronics (L44, F87, and F108); when the weight ratio of DNA: GS was 1:5; the transfection efficiency reached as much as 15% comparing to Lipofectamine® 2000. Pluronic F127 obtained the lowest transfection efficiency among all Pluronics. Interestingly, the highest transfection efficiency of Pluronic F68 was observed when the ratio DNA: GS was 1:10. It was also clear from these results that the CMC of Pluronics did not have much effect on transfection. It is evident that all CMC (0.1, 1. and 2) have the ability to transfect OVCAER-3 cells at different ratios or Pluronics. Adding helper lipid (DOPE) did not improve the transfection efficiency of the five Pluronics.

No transfection was observed for the combination of Pluronics with DNA alone. That was expected as there was no compaction with DNA (data not shown). As a result, a cationic gemini surfactant plays a very significant role to improve the transfection efficiency of Pluronics. A positive surface charge is necessary for attachment to the anionic cell surface and compaction with DNA, which facilitates the entering of polyplexes into cells by cellular uptake [87]. The most likely reason for the low transfection efficiency of Pluronics with DOPE was because of the poor compaction with DNA as well as with the cell surface.

It is evident from the results is that the highest transfection was noticed with a charge ratio of 1:5 for all Pluronics except Pluronics F68. These results indicated that there was a strong compaction in this ratio. Low transfection efficiency with ratio 1:2 was probably due to the weak interaction between the cationic surfactant and DNA. At the same time, high toxicity at ratio 1:10 occurred because of large additions of gemini surfactant may led to low transfection efficiency.

Generally, to investigate the correlation between molecular weight and transfection efficiency, it is clear from the results that the molecular weight did not influence the transfection results. For example, F108 has the largest molecular weight (Mw=14600) among all five Pluronics;

however, it transfected OVCAR-3 cell line effectively. On the other hand, F127 which also has a large molecular weight (Mw= 12600) obtained the lowest transfection efficiency. Cherve et al. verified these findings when they obtained a similar luciferase expression for three different Pluronics (P85, F68, and F108), although they have three different molecular weights ranging from 4600 to 14600 Da [60].

In addition, the relationship between hydrophilic-lipophilic balance (HLB) and transfection efficiency was studied by Sriadibhatla et al [63]. They found that copolymers that have intermediate HLB (9-16), and relatively large hydrophobic blocks (30-69 PO units) were the most effective. They also found that copolymers with short PO units such as L35 (16 PO units), and hydrophilic copolymers such as F127 (HLB 22), were less effective. In contrast to these results, our findings show that F87 and F108 which have high HLB (24, 27) obtained transfection efficiency similar to L44 (HLB 16). These results were proven by Cherve and colleagues when they found that F68, with the highest HLB (29), was able to increase luciferase expression. This was similar to P85, which has intermediate HLB (16) [60]. They also concluded that Pluronic block copolymers do not enhance nucleic acid transfection at the same level, and they agreed with the previous data obtained by Batrakova et al.[57] which indicated that polymer structure affects the cell membrane interaction behavior and signaling pathway activation.

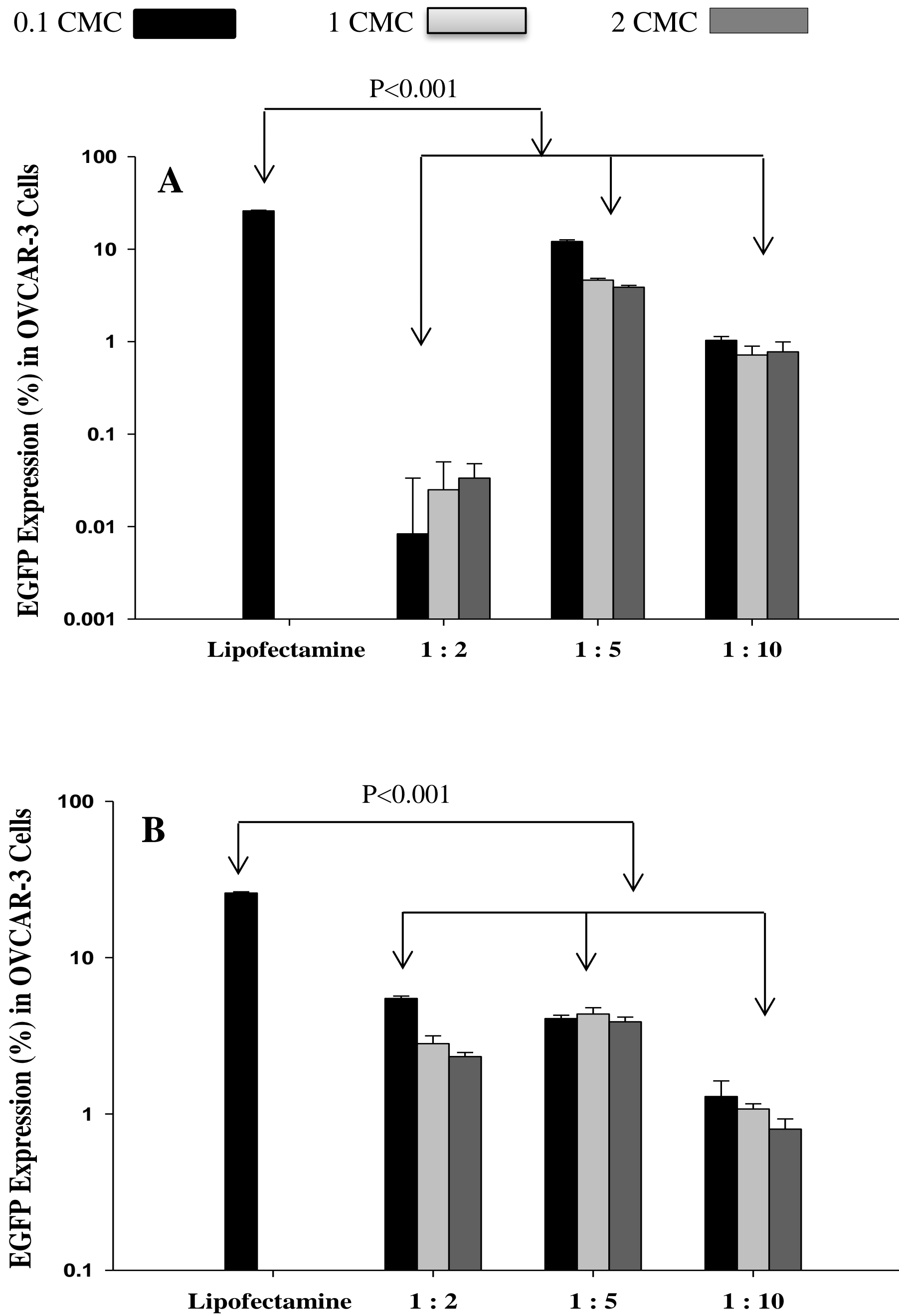


Figure 3.8 *In vitro* Transfection of OVCAR-3 Cells Contain Pluronic L44

A) *In vitro* transfection of OVCAR-3 cells with 0.1, 1, and 2 CMC Pluronic L44 in combination with plasmid DNA and gemini surfactant with ratio 1:2, 1:5, and 1:10. B) As in A) with DOPE. Results are shown as mean (n=3), errors bars represent standard deviation.

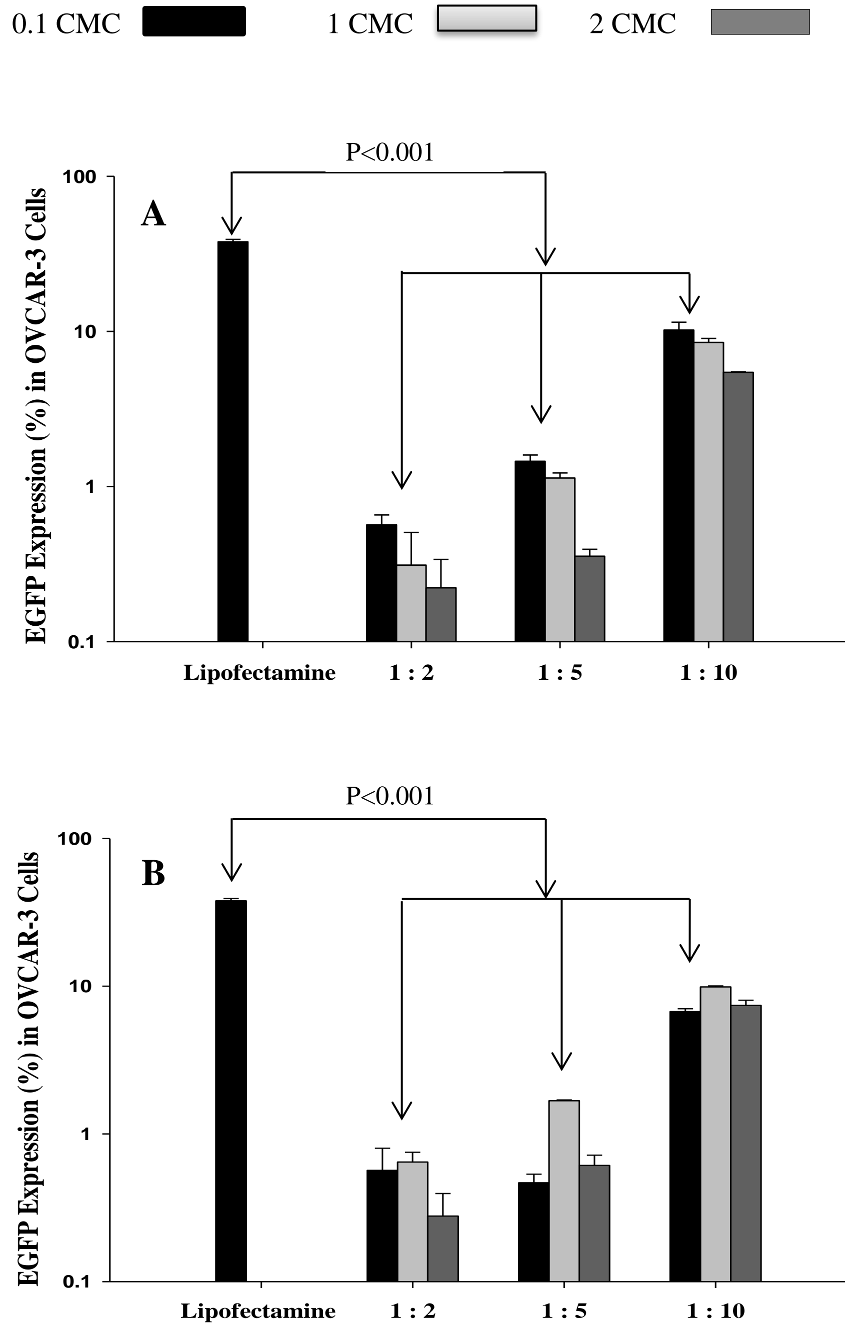


Figure 3.9 *In vitro* transfection of OVCAR-3 Cells Contains Pluronic F68

A) *In vitro* transfection of OVCAR-3 cells with 0.1, 1, and 2 CMC Pluronic F68 in combination with plasmid DNA and gemini surfactant with ratio 1:2, 1:5, and 1:10. B) As in A) with DOPE. Results are shown as mean (n=3), errors bars represent standard deviation.

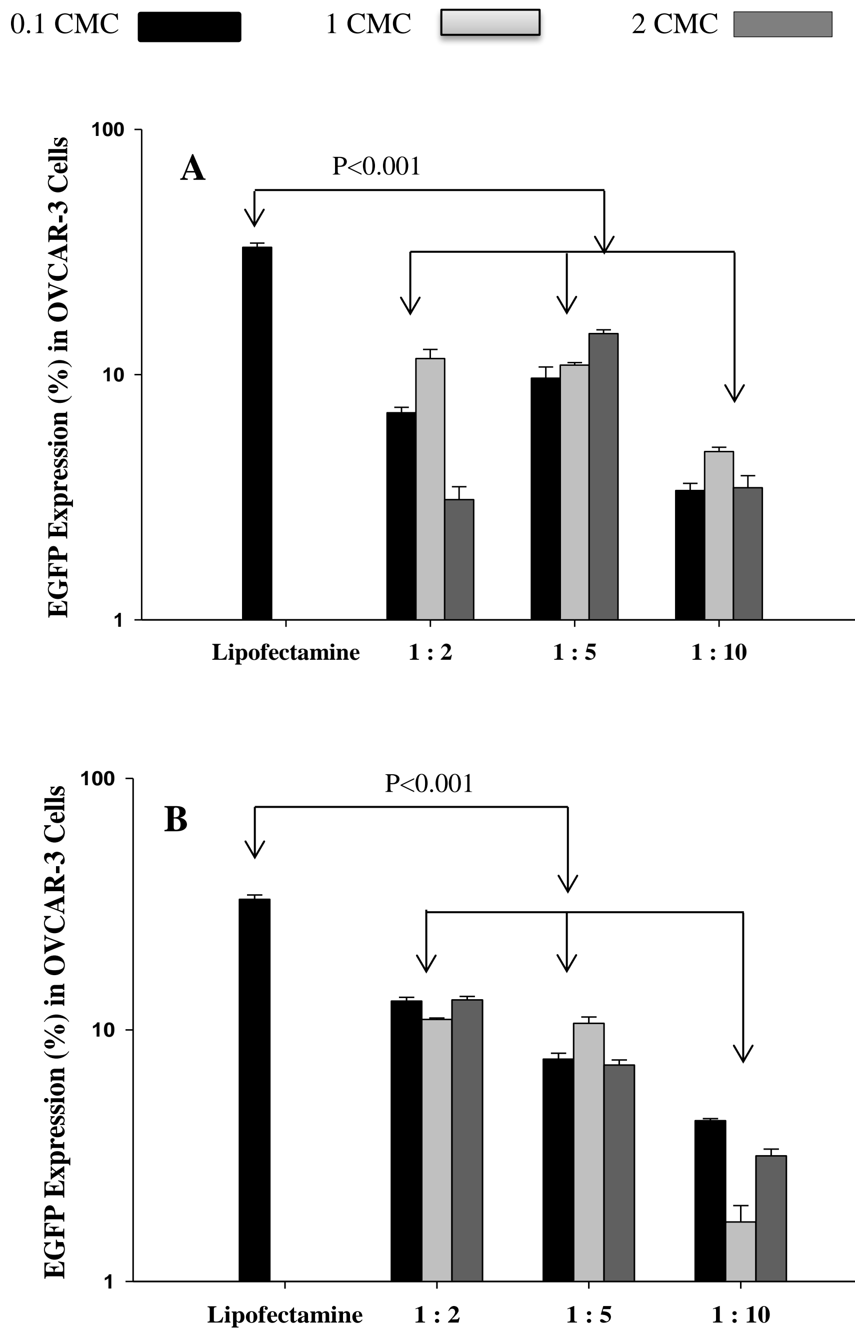


Figure 3.10 *In vitro* transfection of OVCAR-3 Cells Contains Pluronic F87

A) *In vitro* transfection of OVCAR-3 cells with 0.1, 1, and 2 CMC Pluronic F87 in combination with plasmid DNA and gemini surfactant with ratio 1:2, 1:5, and 1:10. B) As in A) with DOPE. Results are shown as mean (n=3), errors bars represent standard deviation.

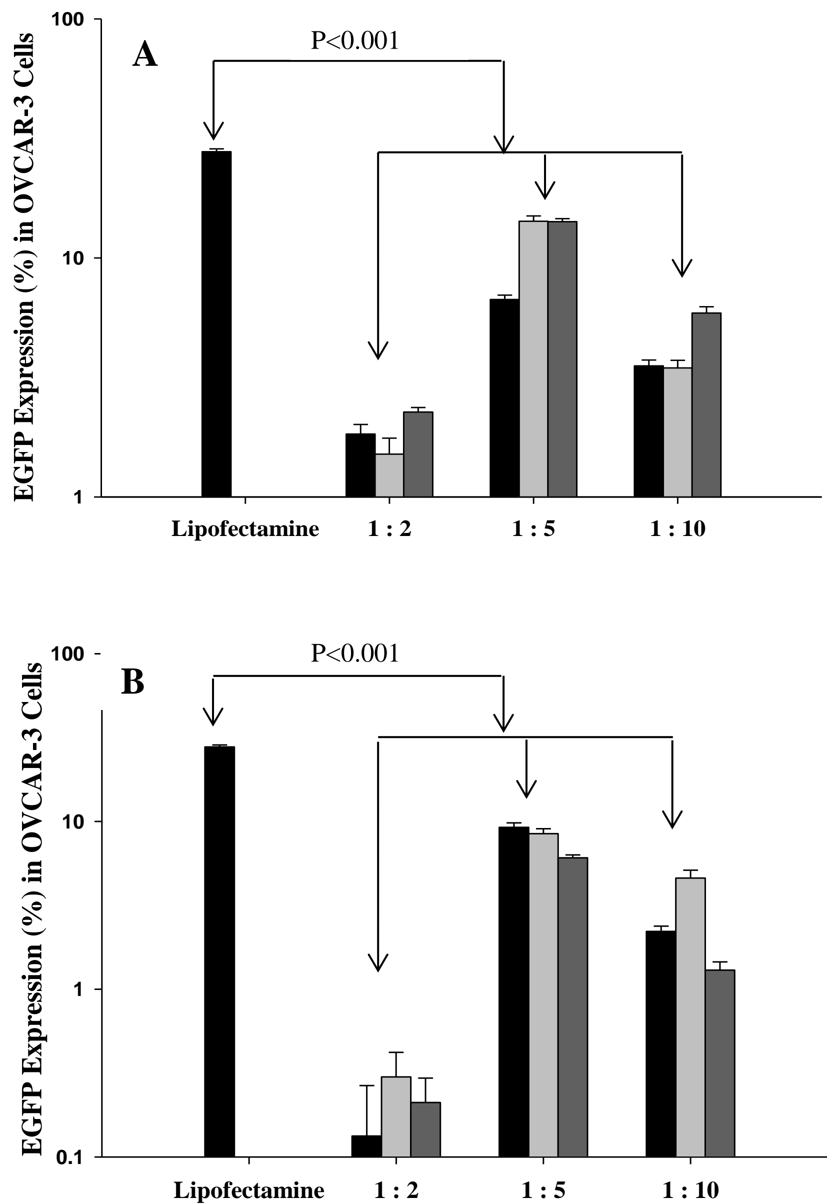


Figure 3.11 *In vitro* transfection of OVCAR-3 Cells Contains Pluronic F108

A) *In vitro* transfection of OVCAR-3 cells with 0.1, 1, and 2 CMC Pluronic F108 in combination with plasmid DNA and gemini surfactant with ratio 1:2, 1:5, and 1:10. B) As in A) with DOPE. Results are shown as mean (n=3), errors bars represent standard deviation

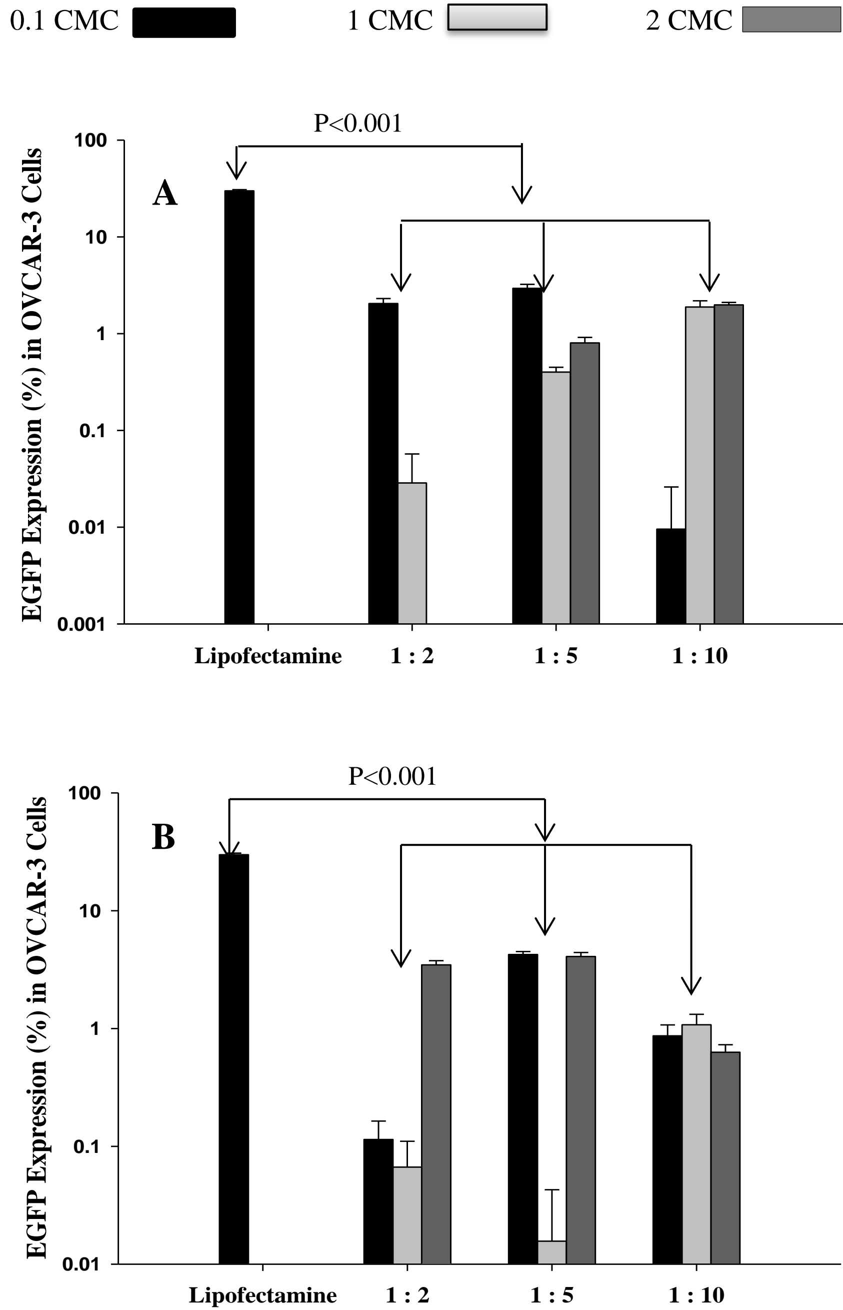


Figure 3.12 *In vitro* transfection of OVCAR-3 Cells Contains Pluronic F127

A) *In vitro* transfection of OVCAR-3 cells with 0.1, 1, and 2 CMC Pluronic F127 in combination with plasmid DNA and gemini surfactant with ratio 1:2, 1:5, and 1:10. B) As in A) with DOPE. Results are shown as mean (n=3), errors bars represent standard deviation.

3.3 Evaluate the Cytotoxicity of these complexes on OVCAR-3 cell culture in presence and absence of helper lipid (DOPE) by adding Propidium Iodide (PI)

Regarding cell viability, this project aimed to study the cytotoxicity of Pluronic-GS-DNA complex after it transfects in OVCAR-3 cell line in the presence and absence of DOPE. The same protocol of transfection was used with cell viability up to the last step. Then, 10 uL of propidium iodide was added to examine the cell death. The results were determined using FACs analysis.

According to Bieber and colleagues, there is a correlation between cytotoxicity and molecular weight [88]. Lungwits et al. also reported that high molecular weight PEI has significantly more cytotoxicity than low molecular weight PEI [89]. Therefore, it can be noticed, as seen in Table 1-1, that Pluronics F108 and F127 have high molecular weights (Mw= 14600 and 12600). As a result, the lowest cell viability was shown with Pluronics F108 and F127, particularly after adding DOPE.

It is also clear, as seen in the bar graphs below, that cell viability decreases when the amount of gemini surfactant increases. In OVCAR-3 cell line, the Pluronic-GS-DNA complex shows about 70% cell viability at ratio 1:2, indicating that Pluronics were essentially non-toxic at the concentration used in gene therapy [60].

Adding DOPE to the Pluronics did not improve cell viability. Bar graphs below show the cell viability after adding DOPE. Pluronics at all three ratios have cell viability higher than Pluronics that contain DOPE, except for Pluronic F68. Positive control (Lipofectamine® 2000) obtained the lowest cell viability for approximately 32%.

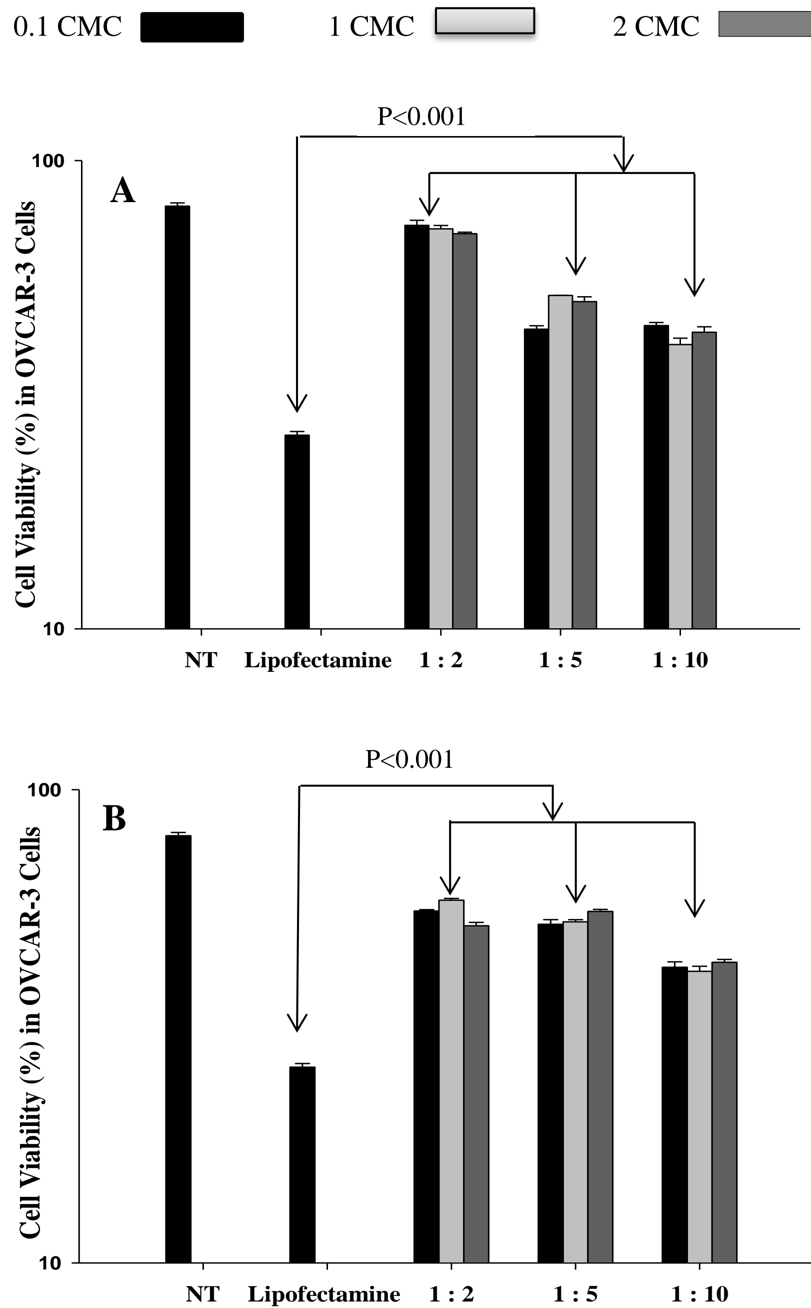


Figure 3.13 Cell Viability of OVCAR-3 Cells Contain Pluronic L44

A) Cell viability OVCAR-3 cells with 0.1, 1, and 2 CMC Pluronic L44 in combination with plasmid DNA and gemini surfactant with ratio 1:2, 1:5, and 1:10. B) As in A) with DOPE. Results are shown as mean (n=3), errors bars represent standard deviation.

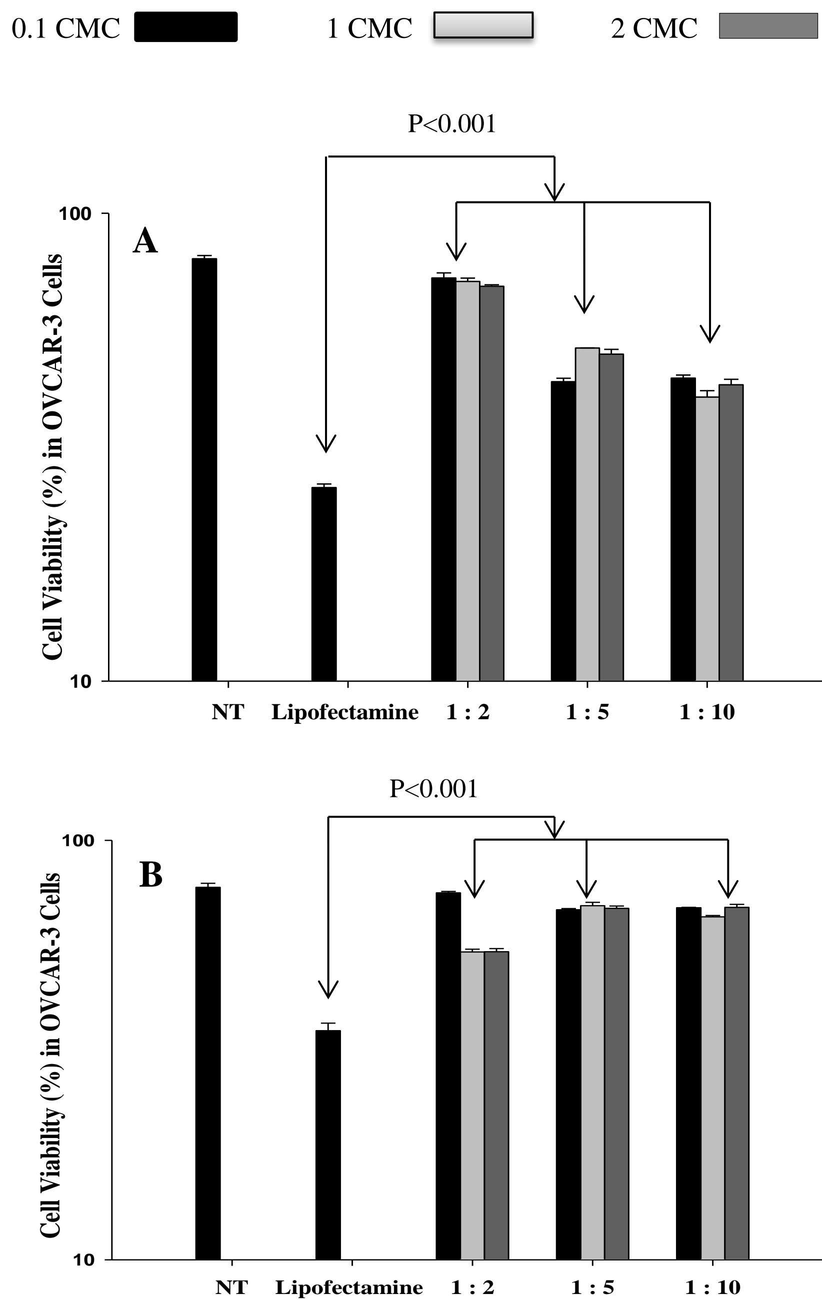


Figure 3.14 Cell Viability of OVCAR-3 Cells Contain Pluronic F68

A) Cell viability OVCAR-3 cells with 0.1, 1, and 2 CMC Pluronic F68 in combination with plasmid DNA and gemini surfactant with Ratio 1:2, 1:5, and 1:10. B) As in A) with DOPE. Results are shown as mean (n=3), errors bars represent standard deviation.

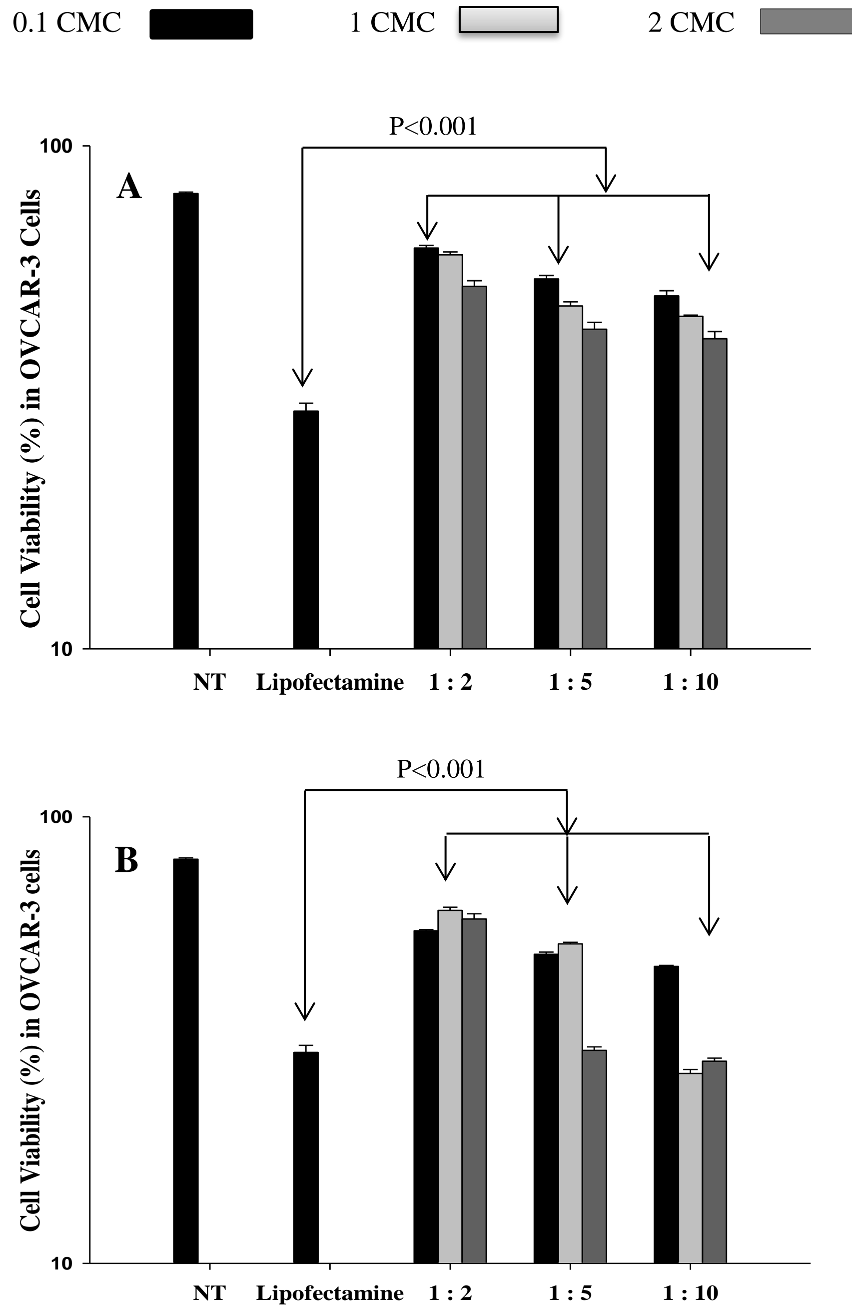


Figure 3.15 Cell Viability of OVCAR-3 Cells Contain Pluronic F87

A) Cell viability OVCAR-3 cells with 0.1, 1, and 2 CMC Pluronic F87 in combination with plasmid DNA and gemini surfactant with Ratio 1:2, 1:5, and 1:10. B) As in A) with DOPE. Results are shown as mean (n=3), errors bars represent standard deviation.

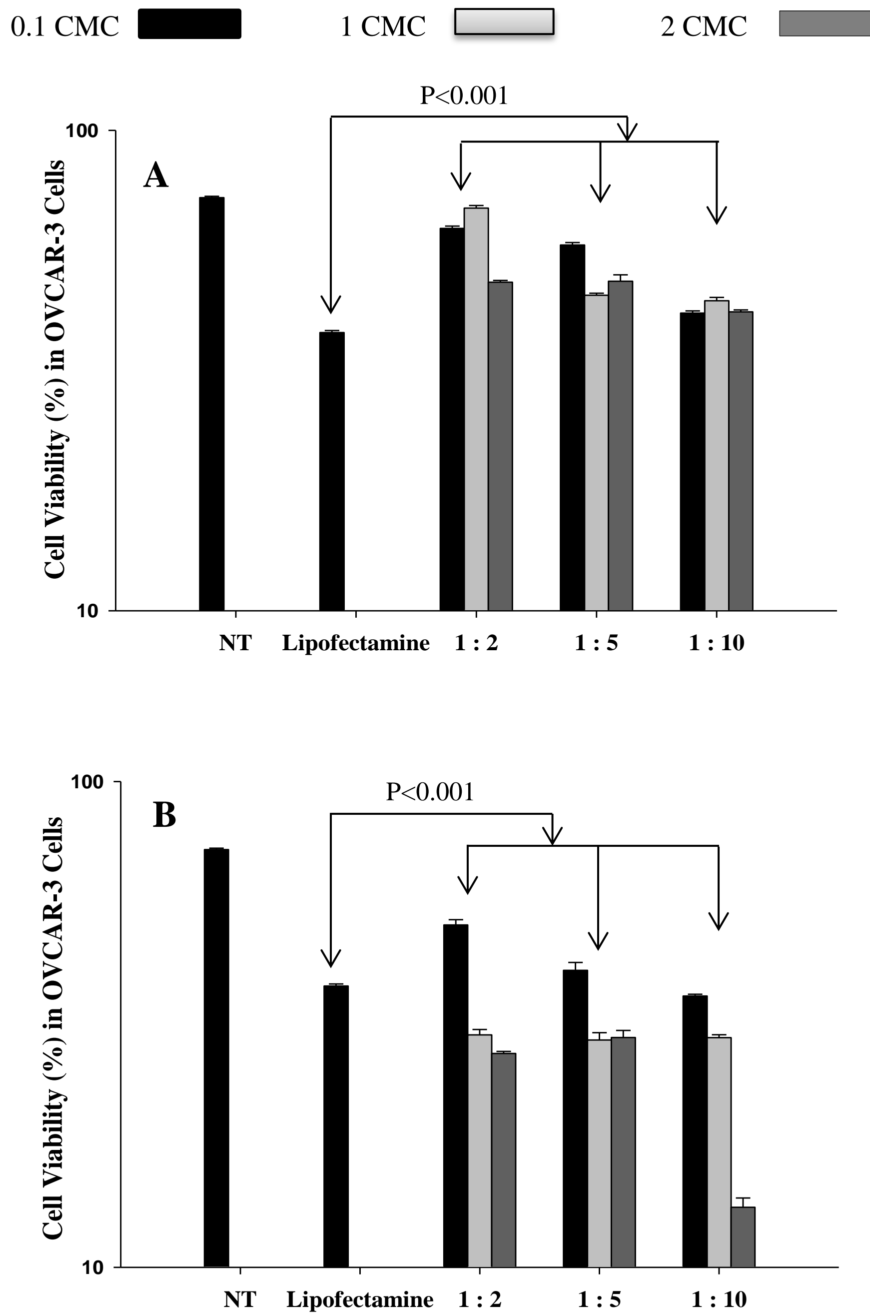
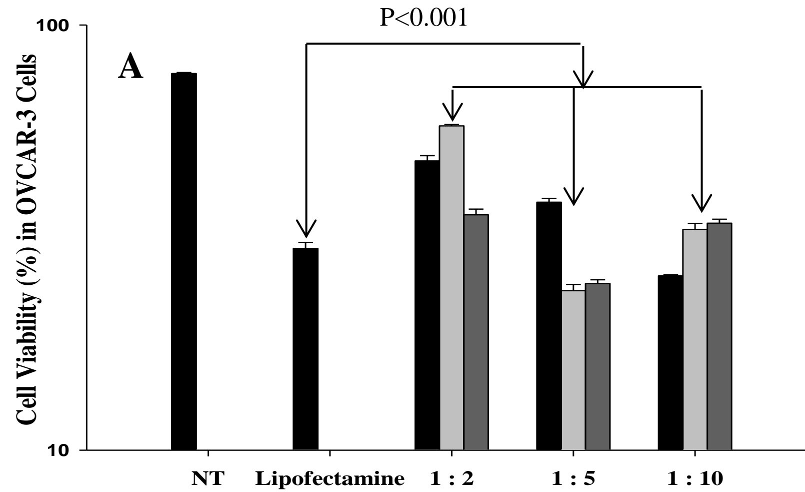


Figure 3.16 Cell Viability of OVCAR-3 Cells Contain Pluronic F108

A) Cell viability OVCAR-3 cells with 0.1, 1, and 2 CMC Pluronic F108 in combination with plasmid DNA and gemini surfactant with Ratio 1:2, 1:5, and 1:10. B) As in A) with DOPE. Results are shown as mean (n=3), errors bars represent standard deviation.

0.1 CMC 1 CMC 2 CMC



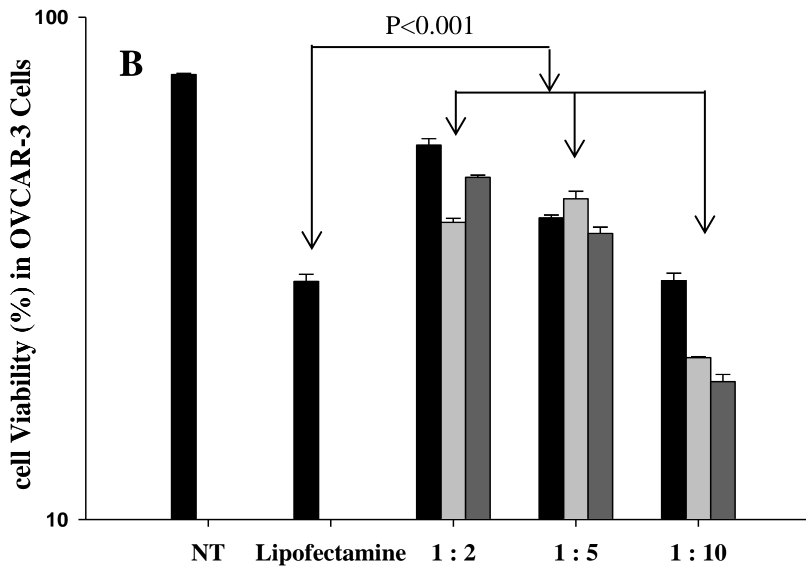


Figure 3.17 Cell Viability of OVCAR-3 Cells Contain Pluronic F127

A) Cell viability OVCAR-3 cells with 0.1, 1, and 2 CMC Pluronic F127 in combination with plasmid DNA and gemini surfactant with Ratio 1:2, 1:5, and 1:10. B) As in A) with DOPE. Results are shown as mean (n=3), errors bars represent standard deviation.

Chapter 4
Conclusion and Future Works

This project investigated the transfection efficiency and cytotoxicity of five Pluronic block copolymers (L44, F68, F87, F108, and F127) in combination with gemini surfactant 16-3-16 at 1.5 mM in OVCAR-3 cell line. Three concentrations of copolymers were used (0.1, 1, and 2 cmc), and three ratios of DNA to gemini surfactant were tested (1:2, 1:5, and 1:10). Physiochemical properties that include particle size and zeta potential of Pluronic-GS-DNA complexe were studied. DOPE was added with all five Pluronics to examine if it has any effect on transfection efficiency.

The findings of this project showed that all five Pluronics were able to transfect OVCAR-3 cell line with various percentages. Ratio 1:5 obtained the highest transfection efficiency for all Pluronics except F68. F127 obtained the lowest transfection efficiency among all five Pluronics. L44, F87, and F108 achieved similar transfection efficiency. Adding DOPE to these Pluronics did not improve transfection efficiency. Cytotoxicity increased with increasing amounts of gemini surfactant, suggesting that copolymers are non-toxic.

Suggesting for future study would be examined more Pluronics in addition to these used in this project. In particular, Pluronics that have intermediate HLB such as L64, P85, and P103 should be examined to verify the findings of Sriadibhatla and colleagues who claimed that Pluronics with intermediate HLB (9-16) are the most effective [63].

Another study that would be worthy is to examine different classes of gemini surfactants in addition to *m-s-m* class that was used in this study. Shawn and colleagues have found that gemini surfactnts that have $s \leq 4$ or $s > 12$ showed the greatest transfection efficiency, e.g. 16-2-16 [18]. Also, the amine-substitute spacer gemini surfactants such as 12-7NH-12 showed very high transfection efficiency versus 12-5N-12, 12-7N12, and 12-8N-12 [18]. It would be worthwhile to examine these gemini surfactants with Pluronics. In addition, phytanyl-substituted gemini surfactants, such as phy-3-16 and

phy-3-18 could be another effective alternatives, demonstrated by Wang and Wettig [90]. These might be used with Pluronics in a future study.

The transfection efficiency did not improve after adding helper lipid (DOPE) in this study. Thus, changing the concentration of DOPE to be appropriate with the concentration of Pluronics might be a helpful technique that leads to transfection improvement. Also, using DOPE with different gemini surfactants other than 16-3-16 may improve transfection efficiency.

Finally, the most important study that will be worthy of investigation is to examine these Pluronics *in vivo,* as many experiments in the existing literature approved the transfection efficiency *in vivo* [60]. Pluronics alone were able to improve transfection efficiency *in vivo*; consequently, trying Pluronics with gemini surfactants or with gemini surfactants and DOPE will constitute another interesting study that might improve the transfection.

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Appendix A

Table 4-1 physical properties of pluronics used in this study. Adapted from product technical information sheets available from the product manufacturer, BASF

Pluronic Surfactant	**Polaxomer name**	**Chemical Structure**	**Specific gravity**	**Melt point**	**Form**	**Solubility in water at 25 ºC**
L44NF	124	$HO(C_2H_4O)_{12}(C_3H_6O)_{20}(C_2H_4O)_{12}H$	1.05	16 ºC	Liquid	>10%
F68NF	188	$HO(C_2H_4O)_{80}(C_3H_6O)_{27}(C_2H_4O)_{80}H$	1.06	52 ºC	Prill	>10%
F87NF	237	$HO(C_2H_4O)_{64}(C_3H_6O)_{37}(C_2H_4O)_{64}H$	1.04	49 ºC	Prill	>10%
F108NF	338	$HO(C_2H_4O)_{141}(C_3H_6O)_{44}(C_2H_4O)_{141}H$	1.06	57 ºC	Prill	>10%
F127NF	**407**	$HO(C_2H_4O)_{101}(C_3H_6O)_{56}(C_2H_4O)_{101}H$	1.05	56 ºC	Prill	>10%

Appendix B

Table 4-2 Transfection Results of L44±SD

	GFP-_PI-	GFP+	PI+	GFP+_PI+		SD	GFP-_PI-	GFP+	PI+	GFP+_PI+
NT	97.9883	0.197798	1.266863	0.547033		**NT**	0.242762	0.039487	0.212842	0.0629
NT+PI	79.95	0.191667	19.275	0.583333		**NT+PI**	1.256732	0.166458	1.002809	0.112731
L+p	67.625	24.64167	1.8	5.933333		**L+P**	0.803508	1.341951	0.108972	0.707254
L+P+PI	32.88333	25.90833	23.21667	17.99167		**L+P+PI**	1.322009	0.465251	1.223809	0.659703
0.1(2)	72.68333	0.008333	27.10833	0.2		**0.1(2)**	1.8842	0.014434	1.846844	0.025
1(2)	71.49167	0.025	28.28333	0.2		**1(2)**	1.167886	0.025	1.075388	0.090139
2(2)	69.81667	0.033333	29.75833	0.391667		**2(2)**	0.483692	0.014434	0.448841	0.072169
0.1(5)	43.625	12.1	33.925	10.35		**0.1(5)**	0.7587	0.520216	0.303109	0.828779
1(5)	51.49167	4.625	41.59167	2.291667		**1(5)**	0.038188	0.2	0.125831	0.112731
2(5)	49.98333	3.875	43.85	2.291667		**2(5)**	1.176418	0.175	1.231868	0.125831
0.1(10)	44.40833	1.033333	53.36667	1.191667		**0.1(10)**	0.687538	0.101036	0.673764	0.062915
1(10)	40.45	0.716667	57.65833	1.175		**1(10)**	1.297112	0.175594	1.499236	0.108972
2 (10)	42.975	0.775	55.375	0.875		**2(10)**	1.167529	0.217945	0.992157	0.132288
D1(2)	55.41667	5.483333	34.55833	4.541667		**D1(2)**	0.361709	0.189297	0.704598	0.401819
D2(2)	58.4	2.816667	36.325	2.458333		**D2(2)**	0.492443	0.34126	0.606733	0.312583
D3(2)	51.58333	2.325	44.00833	2.083333		**D3(2)**	0.837531	0.15	0.929157	0.112731
D1(5)	51.94167	4.075	41.46667	2.516667		**D1(5)**	1.179601	0.204634	1.041733	0.312583
D2(5)	52.56667	4.358333	40.51667	2.558333		**D2(5)**	0.54448	0.425979	1.0153	0.076376
D3(5)	55.31667	3.883333	38.63333	2.166667		**D3(5)**	0.52698	0.284312	1.221253	0.418579
D1(10)	42.14167	1.291667	55.30833	1.258333		**D1(10)**	1.115329	0.337577	0.850123	0.052042
D2(10)	41.31667	1.075	56.69167	0.916667		**D2(10)**	1.006955	0.086603	1.038127	0.209662
D3(10)	43.19167	0.8	55.01667	0.991667		**D3(10)**	0.592839	0.129904	0.65208	0.087797

Table 4-3 Transfection Results of F68±SD

	PI+	GFP+_PI+	GFP-_PI-	GFP+	SD	PI+	PI+_GFP+	PI-_GFP-	GFP+
NT	0	0.011111	99.9333	0.055556	**NT**	0	0.019245	1.74E-14	0.019245
NT+PI	22.56666667	0.122222	77.22223	0.088889	**NT+PI**	1.682594	0.069389	1.756375	0.050917
L+p	0.077777667	6.044447	49.14443	44.73333	**L+P**	0.050917	0.226893	1.302276	1.13335
L+P+PI	11.86666667	15.13333	35.1778	37.8222	**L+P+PI**	0.317981	0.338308	1.486738	1.392979
0.1(2)	24.82223333	0.177778	74.43333	0.566667	**0.1(2)**	0.822163	0.069389	0.737111	0.088192
1(2)	32.8222	0.222222	66.64443	0.311111	**1(2)**	0.523173	0.101835	0.250186	0.195315
2(2)	62.77776667	0.233333	36.76667	0.222222	**2(2)**	0.855285	0.057735	0.856973	0.117063
0.1(5)	51.9889	1.033332	45.52223	1.455557	**0.1(5)**	0.686614	0.272843	0.661946	0.138779
1(5)	43.2778	0.488889	55.1	1.133333	**1(5)**	0.725968	0.019245	0.788111	0.088189
2(5)	32.64446667	0.255556	66.74443	0.355555	**2(5)**	0.661946	0.083887	0.600293	0.03849
0.1(10)	29.19996667	6.08889	54.5	10.21112	**0.1(10)**	1.289703	0.234122	0.433335	1.252708
1(10)	27.2889	4.91111	59.30003	8.5	**1(10)**	1.35168	0.117061	1.457166	0.504427
2 (10)	30.9222	2.655557	60.97777	5.444443	**2(10)**	0.346958	0.101836	0.350131	0.050918
D1(2)	24.35556667	0.122222	74.95553	0.566667	**D1(2)**	0.776249	0.03849	0.567012	0.233333
D2(2)	44.6889	0.488889	54.1778	0.644445	**D2(2)**	0.90207	0.183586	0.816707	0.107152
D3(2)	45.18886667	0.277778	54.25557	0.277778	**D3(2)**	1.07977	0.083887	0.91794	0.117063
D1(5)	30.9889	0.222222	68.3222	0.466667	**D1(5)**	0.504815	0.083887	0.424711	0.066667
D2(5)	27.5778	0.899999	69.84443	1.67778	**D2(5)**	1.160638	0.152751	1.295041	0.019243
D3(5)	30.17776667	0.355556	68.85557	0.611111	**D3(5)**	0.778442	0.083887	0.870041	0.107152
D1(10)	21.73333333	2.455553	69.0889	6.72222	**D1(10)**	0.683961	0.302459	0.101831	0.316811
D2(10)	19.92223333	4.5	65.7	9.877767	**D2(10)**	0.350131	0.218579	0.491059	0.167759
D3(10)	20.64446667	2.73333	69.21113	7.41111	**D3(10)**	0.467047	0.264575	1.128088	0.619439

Table 4-4 Transfection Results of F87±SD

	PI+	GFP+_PI+	GFP-_PI-	GFP+	SD	PI+	GFP+PI+	GFP-_PI-	GFP+
NT	0.077778	0.566667	99.04447	0.311111	**NT**	0.050917	0.145297	0.038509	0.101835
NT+PI	18.42223	0.822222	80.37777	0.377778	**NT+PI**	0.607661	0.226895	0.482257	0.107152
L+p	5.37778	14.84447	40.6889	39.0889	**L+p**	0.183585	0.691501	0.554112	0.81127
L+P+PI	13.65557	23.60003	29.6778	33.06667	**L+P+PI**	0.616775	0.503322	1.098667	1.32961
0.1(2)	24.67777	5.733333	62.59997	6.98889	**0.1(2)**	0.795394	0.384418	0.776745	0.365655
1(2)	19.04447	8.622223	60.7111	11.6222	**1(2)**	0.78624	0.485719	0.802981	1.039428
2(2)	41.97777	2.38889	52.54443	3.08889	**2(2)**	0.976039	0.283498	1.403304	0.397675
0.1(5)	28.25553	7.733333	54.34443	9.666643	**0.1(5)**	0.552099	0.233331	0.872027	1.078562
1(5)	32.66667	8.388887	48.02223	10.92223	**1(5)**	0.491001	0.657719	0.947688	0.269466
2(5)	29.05553	13.05553	43.1889	14.7	**2(5)**	0.81126	0.82754	1.387342	0.523879
0.1(10)	43.1889	3.144447	50.3	3.366667	**0.1(10)**	1.120178	0.279551	1.189331	0.233335
1(10)	45.5222	3.8	45.82223	4.855553	**1(10)**	0.80025	0.43589	0.221973	0.291231
2 (10)	51.4889	3.7	41.35557	3.455557	**2 (10)**	1.512276	0.176383	1.378547	0.416781
D1(2)	21.57777	9.833333	55.56667	13.0222	**D1(2)**	0.633643	0.145297	0.338264	0.443903
D2(2)	21.03337	6.244443	61.7222	11.00003	**D2(2)**	0.64291	0.462282	1.011785	0.152753
D3(2)	21.1	6.733333	59.0111	13.15557	**D3(2)**	1.155153	0.504422	1.607392	0.416812
D1(5)	37.12223	6.01111	49.21113	7.655553	**D1(5)**	0.567954	0.183587	0.567955	0.422074
D2(5)	30.02223	7.455557	51.9111	10.6111	**D2(5)**	0.435077	0.379086	0.459846	0.636238
D3(5)	57.8	4.955557	30	7.244443	**D3(5)**	0.202729	0.455017	0.536469	0.342103
D1(10)	46.22223	3.177777	46.24447	4.355557	**D1(10)**	0.42207	0.134717	0.234143	0.083884
D2(10)	68.77777	2.866667	26.63333	1.722223	**D2(10)**	0.518933	0.260344	0.536463	0.279551
D3(10)	64.9111	3.566667	28.36667	3.155557	**D3(10)**	0.806443	0.378594	0.44099	0.200924

Table 4-5 Transfection Results of F108±SD

	PI+	GFP+_PI+	GFP-_PI-	GFP+	SD	PI+	GFP+_PI+	GFP-_PI-	GFP+
NT	0.044444	0	99.95557	0	**NT**	0.07698	0	0.076961	0
NT+PI	27.55557	0.011111	72.43333	0	**NT+PI**	0.450103	0.019245	0.46665	0
L+p	1.366667	4.5	60.03333	34.1	**L+p**	0.384421	0.33333	0.40549	0.433335
L+P+PI	23.03337	11.24443	37.92223	27.8	**L+P+PI**	1.069268	0.683368	0.389221	0.793725
0.1(2)	35.2111	0.477778	62.4778	1.833333	**0.1(2)**	0.625714	0.101835	0.716714	0.176387
1(2)	29.34443	0.255555	68.8889	1.511113	**1(2)**	0.857842	0.134715	0.883391	0.250183
2(2)	48.59997	0.844444	48.2889	2.26667	**2(2)**	0.550757	0.117063	0.422088	0.1
0.1(5)	32.36667	3.2	57.7333	6.700003	**0.1(5)**	1.065079	0.371183	0.655744	0.288675
1(5)	33.24443	7.166667	45.35557	14.23333	**1(5)**	0.518886	0.80898	0.46229	0.762281
2(5)	31.30003	6.011113	48.51113	14.1778	**2(5)**	1.625833	0.214303	1.526202	0.416761
0.1(10)	53.0222	1.788887	41.65553	3.533333	**0.1(10)**	0.283513	0.423388	0.433778	0.208167
1(10)	50.65553	1.7	44.17777	3.46667	**1(10)**	0.661931	0.317983	0.699508	0.264575
2 (10)	50.16667	2.066667	41.8889	5.877777	**2 (10)**	0.484161	0.384415	0.379064	0.36717
D1(2)	49.13333	0.077778	50.65553	0.133333	**D1(2)**	1.125989	0.050917	1.281188	0.133334
D2(2)	69.3	0.311111	30.0889	0.3	**D2(2)**	0.876224	0.050918	0.774855	0.120185
D3(2)	72.05557	0.177778	27.55553	0.211111	**D3(2)**	0.236502	0.050917	0.267344	0.083887
D1(5)	43.75553	6.144447	40.86667	9.233333	**D1(5)**	0.885914	0.20367	1.549585	0.569604
D2(5)	56.45553	5.722223	29.3667	8.455557	**D2(5)**	0.895267	0.221947	1.044031	0.597524
D3(5)	60.76667	3.455553	29.71113	6.066663	**D3(5)**	0.968371	0.107152	1.014531	0.251661
D1(10)	60.33333	1.28889	36.16667	2.21111	**D1(10)**	0.03335	0.183587	0.328287	0.16443
D2(10)	62.95557	2.744443	29.7	4.6	**D2(10)**	0.907619	0.150308	0.409633	0.523878
D3(10)	84.04147	1.37013	13.2892	1.299173	**D3(10)**	0.430401	0.376792	0.60433	0.154101

Table 4-6 Transfection Results of F127±SD

	GFP-_PI-	GFP+	PI+	GFP+_PI+	SD	GFP-_PI-	GFP+	PI+	GFP+_PI+
NT	96.6406	0.227354	1.96944	1.162605	**NT**	0.467822	0.119496	0.522467	0.174097
NT+PI	76.8762	0.12381	22.13333	0.866667	**NT+PI**	0.412401	0.167413	0.649826	0.11547
L+P	39.61907	43.42857	0.69524	16.25713	**L+P**	1.371808	1.004884	0.380478	0.993435
L+P+PI	29.8	29.85717	16.1619	24.18093	**L+P+PI**	0.974774	0.915616	0.25605	0.934722
0.1 (2)	47.90477	2.04762	46.80953	3.238097	**0.1 (2)**	1.388652	0.256084	1.603668	0.239617
1 (2)	57.89523	0.028571	41.05717	1.019048	**1 (2)**	0.416352	0.028571	0.645846	0.340867
2 (2)	35.78097	0	63.2857	0.933334	**2 (2)**	1.117702	0	1.330621	0.396242
0.1 (5)	38.29523	2.933333	54.06667	4.70476	**0.1 (5)**	0.775846	0.300113	0.474922	0.131965
1(5)	23.71427	0.4	71.6	4.285717	**1(5)**	0.830076	0.049487	1.14139	0.426662
2 (5)	24.6476	0.8	70.73333	3.819047	**2 (5)**	0.518516	0.114286	0.380453	0.174575
0.1(10)	25.69523	0.009524	61.99047	12.30477	**0.1(10)**	0.157344	0.016496	0.512158	0.651697
1 (10)	33.01907	1.885713	59.81903	5.276193	**1 (10)**	1.097773	0.298294	1.36349	0.405406
2 (10)	34.1905	1.980953	59.1905	4.638093	**2 (10)**	0.757195	0.118956	0.657978	0.016495
D1 (2)	55.61907	0.114286	43.09523	1.171431	**D1 (2)**	1.665334	0.049487	1.982827	0.272556
D2 (2)	39.0381	0.066667	60.04763	0.847619	**D2 (2)**	0.73974	0.043644	0.945163	0.185897
D3 (2)	48.00953	3.46667	41.00953	7.514287	**D3 (2)**	0.483494	0.293235	0.462791	0.364778
D1 (5)	39.82857	4.24762	49.9048	6.019043	**D1 (5)**	0.54515	0.246337	0.603618	0.305505
D2(5)	43.50437	0.015657	54.90133	1.578653	**D2(5)**	1.543187	0.027118	1.71568	0.296621
D3(5)	37.10477	4.085713	51.20953	7.6	**D3(5)**	1.105561	0.336851	1.573815	0.347586
D1 (10)	29.8857	0.866667	61.1714	8.07619	**D1(10)**	1.045488	0.206691	0.965963	0.334825
D2 (10)	20.99047	1.07619	72.2	5.733337	**D2(10)**	0.087276	0.242999	0.426654	0.385803
D3 (10)	18.80953	0.628571	72.84763	7.714283	**D3(10)**	0.635274	0.098974	0.71391	0.831522

Appendix C

Table 4-7 Pluronic L44 Anova statistics

TE= Transfection Effeciency

Bonferroni/Dunn for TE
Effect: Column 1
Significance Level: 5 %

	Mean Diff.	Crit. Diff	P-Value	
Lipofectamine, 1:2(0.1CMC)	25.900	.790	<.0001	S
Lipofectamine, 1:2(1CMC)	25.883	.790	<.0001	S
Lipofectamine, 1:2(2CMC)	25.875	.790	<.0001	S
Lipofectamine, 1:5(0.1CMC)	13.808	.790	<.0001	S
Lipofectamine, 1:5(1CMC)	21.283	.790	<.0001	S
Lipofectamine, 1:5(2CMC)	22.033	.790	<.0001	S
Lipofectamine, 1:10(0.1C...	24.875	.790	<.0001	S
Lipofectamine, 1:10(1CMC)	25.192	.790	<.0001	S
Lipofectamine, 1:10(2CMC)	25.133	.790	<.0001	S
1:2(0.1CMC), 1:2(1CMC)	-.017	.790	.9369	
1:2(0.1CMC), 1:2(2CMC)	-.025	.790	.9054	
1:2(0.1CMC), 1:5(0.1CMC)	-12.092	.790	<.0001	S
1:2(0.1CMC), 1:5(1CMC)	-4.617	.790	<.0001	S
1:2(0.1CMC), 1:5(2CMC)	-3.867	.790	<.0001	S
1:2(0.1CMC), 1:10(0.1CMC)	-1.025	.790	<.0001	S
1:2(0.1CMC), 1:10(1CMC)	-.708	.790	.0028	
1:2(0.1CMC), 1:10(2CMC)	-.767	.790	.0014	
1:2(1CMC), 1:2(2CMC)	-.008	.790	.9684	
1:2(1CMC), 1:5(0.1CMC)	-12.075	.790	<.0001	S
1:2(1CMC), 1:5(1CMC)	-4.600	.790	<.0001	S
1:2(1CMC), 1:5(2CMC)	-3.850	.790	<.0001	S
1:2(1CMC), 1:10(0.1CMC)	-1.008	.790	<.0001	S
1:2(1CMC), 1:10(1CMC)	-.692	.790	.0033	
1:2(1CMC), 1:10(2CMC)	-.750	.790	.0017	
1:2(2CMC), 1:5(0.1CMC)	-12.067	.790	<.0001	S
1:2(2CMC), 1:5(1CMC)	-4.592	.790	<.0001	S
1:2(2CMC), 1:5(2CMC)	-3.842	.790	<.0001	S
1:2(2CMC), 1:10(0.1CMC)	-1.000	.790	.0001	S
1:2(2CMC), 1:10(1CMC)	-.683	.790	.0037	
1:2(2CMC), 1:10(2CMC)	-.742	.790	.0019	
1:5(0.1CMC), 1:5(1CMC)	7.475	.790	<.0001	S
1:5(0.1CMC), 1:5(2CMC)	8.225	.790	<.0001	S
1:5(0.1CMC), 1:10(0.1CMC)	11.067	.790	<.0001	S
1:5(0.1CMC), 1:10(1CMC)	11.383	.790	<.0001	S
1:5(0.1CMC), 1:10(2CMC)	11.325	.790	<.0001	S
1:5(1CMC), 1:5(2CMC)	.750	.790	.0017	
1:5(1CMC), 1:10(0.1CMC)	3.592	.790	<.0001	S
1:5(1CMC), 1:10(1CMC)	3.908	.790	<.0001	S
1:5(1CMC), 1:10(2CMC)	3.850	.790	<.0001	S
1:5(2CMC), 1:10(0.1CMC)	2.842	.790	<.0001	S
1:5(2CMC), 1:10(1CMC)	3.158	.790	<.0001	S
1:5(2CMC), 1:10(2CMC)	3.100	.790	<.0001	S
1:10(0.1CMC), 1:10(1CMC)	.317	.790	.1431	
1:10(0.1CMC), 1:10(2CMC)	.258	.790	.2280	
1:10(1CMC), 1:10(2CMC)	-.058	.790	.7817	

Comparisons in this table are not significant unless the corresponding p-value is less than .0011.

Bonferroni/Dunn for Cell viability
Effect: Column 1
Significance Level: 5 %

	Mean Diff.	Crit. Diff	P-Value	
Lipofectamine, 1:2(0.1CMC)	-39.800	3.456	<.0001	S
Lipofectamine, 1:2(1CMC)	-38.608	3.456	<.0001	S
Lipofectamine, 1:2(2CMC)	-36.933	3.456	<.0001	S
Lipofectamine, 1:5(0.1CMC)	-10.742	3.456	<.0001	S
Lipofectamine, 1:5(1CMC)	-18.608	3.456	<.0001	S
Lipofectamine, 1:5(2CMC)	-17.100	3.456	<.0001	S
Lipofectamine, 1:10(0.1C...	-11.525	3.456	<.0001	S
Lipofectamine, 1:10(1CMC)	-7.567	3.456	<.0001	S
Lipofectamine, 1:10(2CMC)	-10.092	3.456	<.0001	S
1:2(0.1CMC), 1:2(1CMC)	1.192	3.456	.2044	
1:2(0.1CMC), 1:2(2CMC)	2.867	3.456	.0050	
1:2(0.1CMC), 1:5(0.1CMC)	29.058	3.456	<.0001	S
1:2(0.1CMC), 1:5(1CMC)	21.192	3.456	<.0001	S
1:2(0.1CMC), 1:5(2CMC)	22.700	3.456	<.0001	S
1:2(0.1CMC), 1:10(0.1CMC)	28.275	3.456	<.0001	S
1:2(0.1CMC), 1:10(1CMC)	32.233	3.456	<.0001	S
1:2(0.1CMC), 1:10(2CMC)	29.708	3.456	<.0001	S
1:2(1CMC), 1:2(2CMC)	1.675	3.456	.0800	
1:2(1CMC), 1:5(0.1CMC)	27.867	3.456	<.0001	S
1:2(1CMC), 1:5(1CMC)	20.000	3.456	<.0001	S
1:2(1CMC), 1:5(2CMC)	21.508	3.456	<.0001	S
1:2(1CMC), 1:10(0.1CMC)	27.083	3.456	<.0001	S
1:2(1CMC), 1:10(1CMC)	31.042	3.456	<.0001	S
1:2(1CMC), 1:10(2CMC)	28.517	3.456	<.0001	S
1:2(2CMC), 1:5(0.1CMC)	26.192	3.456	<.0001	S
1:2(2CMC), 1:5(1CMC)	18.325	3.456	<.0001	S
1:2(2CMC), 1:5(2CMC)	19.833	3.456	<.0001	S
1:2(2CMC), 1:10(0.1CMC)	25.408	3.456	<.0001	S
1:2(2CMC), 1:10(1CMC)	29.367	3.456	<.0001	S
1:2(2CMC), 1:10(2CMC)	26.842	3.456	<.0001	S
1:5(0.1CMC), 1:5(1CMC)	-7.867	3.456	<.0001	S
1:5(0.1CMC), 1:5(2CMC)	-6.358	3.456	<.0001	S
1:5(0.1CMC), 1:10(0.1CMC)	-.783	3.456	.3987	
1:5(0.1CMC), 1:10(1CMC)	3.175	3.456	.0023	
1:5(0.1CMC), 1:10(2CMC)	.650	3.456	.4825	
1:5(1CMC), 1:5(2CMC)	1.508	3.456	.1124	
1:5(1CMC), 1:10(0.1CMC)	7.083	3.456	<.0001	S
1:5(1CMC), 1:10(1CMC)	11.042	3.456	<.0001	S
1:5(1CMC), 1:10(2CMC)	8.517	3.456	<.0001	S
1:5(2CMC), 1:10(0.1CMC)	5.575	3.456	<.0001	S
1:5(2CMC), 1:10(1CMC)	9.533	3.456	<.0001	S
1:5(2CMC), 1:10(2CMC)	7.008	3.456	<.0001	S
1:10(0.1CMC), 1:10(1CMC)	3.958	3.456	.0003	S
1:10(0.1CMC), 1:10(2CMC)	1.433	3.456	.1302	
1:10(1CMC), 1:10(2CMC)	-2.525	3.456	.0116	

Comparisons in this table are not significant unless the corresponding p-value is less than .0011.

Table 4-8 Pluronic F68 Anova statistics

TE=Transfection Efficiency

Bonferroni/Dunn for TE
Effect: Column 1
Significance Level: 5 %

	Mean Diff.	Crit. Diff	P-Value	
Lipofectamine, 1:2 (0.1CM...	37.256	1.929	<.0001	S
Lipofectamine, 1:2 (1CMC)	37.511	1.929	<.0001	S
Lipofectamine, 1:2 (2CMC)	37.600	1.929	<.0001	S
Lipofectamine, 1:5 (0.1CM...	36.367	1.929	<.0001	S
Lipofectamine, 1:5 (1CMC)	36.689	1.929	<.0001	S
Lipofectamine, 1:5 (2CMC)	37.467	1.929	<.0001	S
Lipofectamine, 1:10 (0.1C...	27.611	1.929	<.0001	S
Lipofectamine, 1:10 (1CMC)	29.322	1.929	<.0001	S
Lipofectamine, 1:10 (2CMC)	32.378	1.929	<.0001	S
1:2 (0.1CMC), 1:2 (1CMC)	.256	1.929	.6197	
1:2 (0.1CMC), 1:2 (2CMC)	.344	1.929	.5046	
1:2 (0.1CMC), 1:5 (0.1CMC)	-.889	1.929	.0948	
1:2 (0.1CMC), 1:5 (1CMC)	-.567	1.929	.2769	
1:2 (0.1CMC), 1:5 (2CMC)	.211	1.929	.6815	
1:2 (0.1CMC), 1:10 (0.1C...	-9.644	1.929	<.0001	S
1:2 (0.1CMC), 1:10 (1CMC)	-7.933	1.929	<.0001	S
1:2 (0.1CMC), 1:10 (2CMC)	-4.878	1.929	<.0001	S
1:2 (1CMC), 1:2 (2CMC)	.089	1.929	.8626	
1:2 (1CMC), 1:5 (0.1CMC)	-1.144	1.929	.0353	
1:2 (1CMC), 1:5 (1CMC)	-.822	1.929	.1205	
1:2 (1CMC), 1:5 (2CMC)	-.044	1.929	.9310	
1:2 (1CMC), 1:10 (0.1CMC)	-9.900	1.929	<.0001	S
1:2 (1CMC), 1:10 (1CMC)	-8.189	1.929	<.0001	S
1:2 (1CMC), 1:10 (2CMC)	-5.133	1.929	<.0001	S
1:2 (2CMC), 1:5 (0.1CMC)	-1.233	1.929	.0245	
1:2 (2CMC), 1:5 (1CMC)	-.911	1.929	.0874	
1:2 (2CMC), 1:5 (2CMC)	-.133	1.929	.7952	
1:2 (2CMC), 1:10 (0.1CMC)	-9.989	1.929	<.0001	S
1:2 (2CMC), 1:10 (1CMC)	-8.278	1.929	<.0001	S
1:2 (2CMC), 1:10 (2CMC)	-5.222	1.929	<.0001	S
1:5 (0.1CMC), 1:5 (1CMC)	.322	1.929	.5322	
1:5 (0.1CMC), 1:5 (2CMC)	1.100	1.929	.0422	
1:5 (0.1CMC), 1:10 (0.1C...	-8.756	1.929	<.0001	S
1:5 (0.1CMC), 1:10 (1CMC)	-7.044	1.929	<.0001	S
1:5 (0.1CMC), 1:10 (2CMC)	-3.989	1.929	<.0001	S
1:5 (1CMC), 1:5 (2CMC)	.778	1.929	.1406	
1:5 (1CMC), 1:10 (0.1CMC)	-9.078	1.929	<.0001	S
1:5 (1CMC), 1:10 (1CMC)	-7.367	1.929	<.0001	S
1:5 (1CMC), 1:10 (2CMC)	-4.311	1.929	<.0001	S
1:5 (2CMC), 1:10 (0.1CMC)	-9.856	1.929	<.0001	S
1:5 (2CMC), 1:10 (1CMC)	-8.144	1.929	<.0001	S
1:5 (2CMC), 1:10 (2CMC)	-5.089	1.929	<.0001	S
1:10 (0.1CMC), 1:10 (1CM...	1.711	1.929	.0030	
1:10 (0.1CMC), 1:10 (2CM...	4.767	1.929	<.0001	S
1:10 (1CMC), 1:10 (2CMC)	3.056	1.929	<.0001	S

Comparisons in this table are not significant unless the corresponding p-value is less than .0011.

Bonferroni/Dunn for Cell viability
Effect: Column 1
Significance Level: 5 %

	Mean Diff.	Crit. Diff	P-Value	
Lipofectamine, 1:2 (0.1CM...	-39.256	2.673	<.0001	S
Lipofectamine, 1:2 (1CMC)	-31.467	2.673	<.0001	S
Lipofectamine, 1:2 (2CMC)	-1.589	2.673	.0350	
Lipofectamine, 1:5 (0.1CM...	-10.344	2.673	<.0001	S
Lipofectamine, 1:5 (1CMC)	-19.922	2.673	<.0001	S
Lipofectamine, 1:5 (2CMC)	-31.567	2.673	<.0001	S
Lipofectamine, 1:10 (0.1C...	-19.322	2.673	<.0001	S
Lipofectamine, 1:10 (1CMC)	-24.122	2.673	<.0001	S
Lipofectamine, 1:10 (2CMC)	-25.800	2.673	<.0001	S
1:2 (0.1CMC), 1:2 (1CMC)	7.789	2.673	<.0001	S
1:2 (0.1CMC), 1:2 (2CMC)	37.667	2.673	<.0001	S
1:2 (0.1CMC), 1:5 (0.1CMC)	28.911	2.673	<.0001	S
1:2 (0.1CMC), 1:5 (1CMC)	19.333	2.673	<.0001	S
1:2 (0.1CMC), 1:5 (2CMC)	7.689	2.673	<.0001	S
1:2 (0.1CMC), 1:10 (0.1C...	19.933	2.673	<.0001	S
1:2 (0.1CMC), 1:10 (1CMC)	15.133	2.673	<.0001	S
1:2 (0.1CMC), 1:10 (2CMC)	13.456	2.673	<.0001	S
1:2 (1CMC), 1:2 (2CMC)	29.878	2.673	<.0001	S
1:2 (1CMC), 1:5 (0.1CMC)	21.122	2.673	<.0001	S
1:2 (1CMC), 1:5 (1CMC)	11.544	2.673	<.0001	S
1:2 (1CMC), 1:5 (2CMC)	-.100	2.673	.8882	
1:2 (1CMC), 1:10 (0.1CMC)	12.144	2.673	<.0001	S
1:2 (1CMC), 1:10 (1CMC)	7.344	2.673	<.0001	S
1:2 (1CMC), 1:10 (2CMC)	5.667	2.673	<.0001	S
1:2 (2CMC), 1:5 (0.1CMC)	-8.756	2.673	<.0001	S
1:2 (2CMC), 1:5 (1CMC)	-18.333	2.673	<.0001	S
1:2 (2CMC), 1:5 (2CMC)	-29.978	2.673	<.0001	S
1:2 (2CMC), 1:10 (0.1CMC)	-17.733	2.673	<.0001	S
1:2 (2CMC), 1:10 (1CMC)	-22.533	2.673	<.0001	S
1:2 (2CMC), 1:10 (2CMC)	-24.211	2.673	<.0001	S
1:5 (0.1CMC), 1:5 (1CMC)	-9.578	2.673	<.0001	S
1:5 (0.1CMC), 1:5 (2CMC)	-21.222	2.673	<.0001	S
1:5 (0.1CMC), 1:10 (0.1C...	-8.978	2.673	<.0001	S
1:5 (0.1CMC), 1:10 (1CMC)	-13.778	2.673	<.0001	S
1:5 (0.1CMC), 1:10 (2CMC)	-15.456	2.673	<.0001	S
1:5 (1CMC), 1:5 (2CMC)	-11.644	2.673	<.0001	S
1:5 (1CMC), 1:10 (0.1CMC)	.600	2.673	.4032	
1:5 (1CMC), 1:10 (1CMC)	-4.200	2.673	<.0001	S
1:5 (1CMC), 1:10 (2CMC)	-5.878	2.673	<.0001	S
1:5 (2CMC), 1:10 (0.1CMC)	12.244	2.673	<.0001	S
1:5 (2CMC), 1:10 (1CMC)	7.444	2.673	<.0001	S
1:5 (2CMC), 1:10 (2CMC)	5.767	2.673	<.0001	S
1:10 (0.1CMC), 1:10 (1CM...	-4.800	2.673	<.0001	S
1:10 (0.1CMC), 1:10 (2CM...	-6.478	2.673	<.0001	S
1:10 (1CMC), 1:10 (2CMC)	-1.678	2.673	.0269	

Comparisons in this table are not significant unless the corresponding p-value is less than .0011.

Table 4-9 Pluronic F87 Anova statistics

TE=Transfection Effeciency

Bonferroni/Dunn for TE
Effect: Column 1
Significance Level: 5 %

	Mean Diff.	Crit. Diff	P-Value	
1:2 (0.1 CMC), 1:2 (1 CMC)	-4.633	2.188	<.0001	S
1:2 (0.1 CMC), 1:2 (2 CMC)	3.900	2.188	<.0001	S
1:2 (0.1 CMC), 1:5 (0.1 C...	-2.678	2.188	.0002	S
1:2 (0.1 CMC), 1:5 (1CMC)	-3.933	2.188	<.0001	S
1:2 (0.1 CMC), 1:5 (2CMC)	-7.711	2.188	<.0001	S
1:2 (0.1 CMC), 1:10 (0.1C...	3.622	2.188	<.0001	S
1:2 (0.1 CMC), 1:10 (1CMC)	2.133	2.188	.0014	
1:2 (0.1 CMC), 1:10 (2CMC)	3.533	2.188	<.0001	S
1:2 (0.1 CMC), Lipofectam...	-26.078	2.188	<.0001	S
1:2 (1 CMC), 1:2 (2 CMC)	8.533	2.188	<.0001	S
1:2 (1 CMC), 1:5 (0.1 CMC)	1.956	2.188	.0028	
1:2 (1 CMC), 1:5 (1CMC)	.700	2.188	.2378	
1:2 (1 CMC), 1:5 (2CMC)	-3.078	2.188	<.0001	S
1:2 (1 CMC), 1:10 (0.1CMC)	8.256	2.188	<.0001	S
1:2 (1 CMC), 1:10 (1CMC)	6.767	2.188	<.0001	S
1:2 (1 CMC), 1:10 (2CMC)	8.167	2.188	<.0001	S
1:2 (1 CMC), Lipofectamine	-21.444	2.188	<.0001	S
1:2 (2 CMC), 1:5 (0.1 CMC)	-6.578	2.188	<.0001	S
1:2 (2 CMC), 1:5 (1CMC)	-7.833	2.188	<.0001	S
1:2 (2 CMC), 1:5 (2CMC)	-11.611	2.188	<.0001	S
1:2 (2 CMC), 1:10 (0.1CMC)	-.278	2.188	.6344	
1:2 (2 CMC), 1:10 (1CMC)	-1.767	2.188	.0060	
1:2 (2 CMC), 1:10 (2CMC)	-.367	2.188	.5311	
1:2 (2 CMC), Lipofectamine	-29.978	2.188	<.0001	S
1:5 (0.1 CMC), 1:5 (1CMC)	-1.256	2.188	.0411	
1:5 (0.1 CMC), 1:5 (2CMC)	-5.033	2.188	<.0001	S
1:5 (0.1 CMC), 1:10 (0.1C...	6.300	2.188	<.0001	S
1:5 (0.1 CMC), 1:10 (1CMC)	4.811	2.188	<.0001	S
1:5 (0.1 CMC), 1:10 (2CMC)	6.211	2.188	<.0001	S
1:5 (0.1 CMC), Lipofectam...	-23.400	2.188	<.0001	S
1:5 (1CMC), 1:5 (2CMC)	-3.778	2.188	<.0001	S
1:5 (1CMC), 1:10 (0.1CMC)	7.556	2.188	<.0001	S
1:5 (1CMC), 1:10 (1CMC)	6.067	2.188	<.0001	S
1:5 (1CMC), 1:10 (2CMC)	7.467	2.188	<.0001	S
1:5 (1CMC), Lipofectamine	-22.144	2.188	<.0001	S
1:5 (2CMC), 1:10 (0.1CMC)	11.333	2.188	<.0001	S
1:5 (2CMC), 1:10 (1CMC)	9.844	2.188	<.0001	S
1:5 (2CMC), 1:10 (2CMC)	11.244	2.188	<.0001	S
1:5 (2CMC), Lipofectamine	-18.367	2.188	<.0001	S
1:10 (0.1CMC), 1:10 (1CM...	-1.489	2.188	.0176	
1:10 (0.1CMC), 1:10 (2CM...	-.089	2.188	.8787	
1:10 (0.1CMC), Lipofecta...	-29.700	2.188	<.0001	S
1:10 (1CMC), 1:10 (2CMC)	1.400	2.188	.0244	
1:10 (1CMC), Lipofectamine	-28.211	2.188	<.0001	S
1:10 (2CMC), Lipofectamine	-29.611	2.188	<.0001	S

Comparisons in this table are not significant unless the corresponding p-value is less than .0011.

Bonferroni/Dunn for Cell viability
Effect: Column 1
Significance Level: 5 %

	Mean Diff.	Crit. Diff	P-Value	
1:2 (0.1 CMC), 1:2 (1 CMC)	1.889	3.312	.0423	
1:2 (0.1 CMC), 1:2 (2 CMC)	10.056	3.312	<.0001	S
1:2 (0.1 CMC), 1:5 (0.1 C...	8.256	3.312	<.0001	S
1:2 (0.1 CMC), 1:5 (1CMC)	14.578	3.312	<.0001	S
1:2 (0.1 CMC), 1:5 (2CMC)	19.411	3.312	<.0001	S
1:2 (0.1 CMC), 1:10 (0.1C...	12.300	3.312	<.0001	S
1:2 (0.1 CMC), 1:10 (1CMC)	16.778	3.312	<.0001	S
1:2 (0.1 CMC), 1:10 (2CMC)	21.244	3.312	<.0001	S
1:2 (0.1 CMC), Lipofectam...	32.922	3.312	<.0001	S
1:2 (1 CMC), 1:2 (2 CMC)	8.167	3.312	<.0001	S
1:2 (1 CMC), 1:5 (0.1 CMC)	6.367	3.312	<.0001	S
1:2 (1 CMC), 1:5 (1CMC)	12.689	3.312	<.0001	S
1:2 (1 CMC), 1:5 (2CMC)	17.522	3.312	<.0001	S
1:2 (1 CMC), 1:10 (0.1CMC)	10.411	3.312	<.0001	S
1:2 (1 CMC), 1:10 (1CMC)	14.889	3.312	<.0001	S
1:2 (1 CMC), 1:10 (2CMC)	19.356	3.312	<.0001	S
1:2 (1 CMC), Lipofectamine	31.033	3.312	<.0001	S
1:2 (2 CMC), 1:5 (0.1 CMC)	-1.800	3.312	.0519	
1:2 (2 CMC), 1:5 (1CMC)	4.522	3.312	<.0001	S
1:2 (2 CMC), 1:5 (2CMC)	9.356	3.312	<.0001	S
1:2 (2 CMC), 1:10 (0.1CMC)	2.244	3.312	.0180	
1:2 (2 CMC), 1:10 (1CMC)	6.722	3.312	<.0001	S
1:2 (2 CMC), 1:10 (2CMC)	11.189	3.312	<.0001	S
1:2 (2 CMC), Lipofectamine	22.867	3.312	<.0001	S
1:5 (0.1 CMC), 1:5 (1CMC)	6.322	3.312	<.0001	S
1:5 (0.1 CMC), 1:5 (2CMC)	11.156	3.312	<.0001	S
1:5 (0.1 CMC), 1:10 (0.1C...	4.044	3.312	.0002	S
1:5 (0.1 CMC), 1:10 (1CMC)	8.522	3.312	<.0001	S
1:5 (0.1 CMC), 1:10 (2CMC)	12.989	3.312	<.0001	S
1:5 (0.1 CMC), Lipofectam...	24.667	3.312	<.0001	S
1:5 (1CMC), 1:5 (2CMC)	4.833	3.312	<.0001	S
1:5 (1CMC), 1:10 (0.1CMC)	-2.278	3.312	.0165	
1:5 (1CMC), 1:10 (1CMC)	2.200	3.312	.0201	
1:5 (1CMC), 1:10 (2CMC)	6.667	3.312	<.0001	S
1:5 (1CMC), Lipofectamine	18.344	3.312	<.0001	S
1:5 (2CMC), 1:10 (0.1CMC)	-7.111	3.312	<.0001	S
1:5 (2CMC), 1:10 (1CMC)	-2.633	3.312	.0067	
1:5 (2CMC), 1:10 (2CMC)	1.833	3.312	.0481	
1:5 (2CMC), Lipofectamine	13.511	3.312	<.0001	S
1:10 (0.1CMC), 1:10 (1CM...	4.478	3.312	<.0001	S
1:10 (0.1CMC), 1:10 (2CM...	8.944	3.312	<.0001	S
1:10 (0.1CMC), Lipofecta...	20.622	3.312	<.0001	S
1:10 (1CMC), 1:10 (2CMC)	4.467	3.312	<.0001	S
1:10 (1CMC), Lipofectamine	16.144	3.312	<.0001	S
1:10 (2CMC), Lipofectamine	11.678	3.312	<.0001	S

Comparisons in this table are not significant unless the corresponding p-value is less than .0011.

Table 4-10 Pluronic F108 Anova statistics

TE=Transfection Effeciency

Bonferroni/Dunn for TE
Effect: Column 1
Significance Level: 5 %

	Mean Diff.	Crit. Diff	P-Value	
Lipofectamine, 1:2 (0.1CM...	25.967	1.325	<.0001	S
Lipofectamine, 1:2 (1CMC)	26.289	1.325	<.0001	S
Lipofectamine, 1:2 (2CMC)	25.533	1.325	<.0001	S
Lipofectamine, 1:5 (0.1CM...	21.100	1.325	<.0001	S
Lipofectamine, 1:5 (1CMC)	13.567	1.325	<.0001	S
Lipofectamine, 1:5 (2CMC)	13.622	1.325	<.0001	S
Lipofectamine, 1:10 (0.1C...	24.267	1.325	<.0001	S
Lipofectamine, 1:10 (1CMC)	24.333	1.325	<.0001	S
Lipofectamine, 1:10 (2CMC)	21.922	1.325	<.0001	S
1:2 (0.1CMC), 1:2 (1CMC)	.322	1.325	.3660	
1:2 (0.1CMC), 1:2 (2CMC)	-.433	1.325	.2279	
1:2 (0.1CMC), 1:5 (0.1CMC)	-4.867	1.325	<.0001	S
1:2 (0.1CMC), 1:5 (1CMC)	-12.400	1.325	<.0001	S
1:2 (0.1CMC), 1:5 (2CMC)	-12.344	1.325	<.0001	S
1:2 (0.1CMC), 1:10 (0.1C...	-1.700	1.325	<.0001	S
1:2 (0.1CMC), 1:10 (1CMC)	-1.633	1.325	.0001	S
1:2 (0.1CMC), 1:10 (2CMC)	-4.044	1.325	<.0001	S
1:2 (1CMC), 1:2 (2CMC)	-.756	1.325	.0423	
1:2 (1CMC), 1:5 (0.1CMC)	-5.189	1.325	<.0001	S
1:2 (1CMC), 1:5 (1CMC)	-12.722	1.325	<.0001	S
1:2 (1CMC), 1:5 (2CMC)	-12.667	1.325	<.0001	S
1:2 (1CMC), 1:10 (0.1CMC)	-2.022	1.325	<.0001	S
1:2 (1CMC), 1:10 (1CMC)	-1.956	1.325	<.0001	S
1:2 (1CMC), 1:10 (2CMC)	-4.367	1.325	<.0001	S
1:2 (2CMC), 1:5 (0.1CMC)	-4.433	1.325	<.0001	S
1:2 (2CMC), 1:5 (1CMC)	-11.967	1.325	<.0001	S
1:2 (2CMC), 1:5 (2CMC)	-11.911	1.325	<.0001	S
1:2 (2CMC), 1:10 (0.1CMC)	-1.267	1.325	.0016	
1:2 (2CMC), 1:10 (1CMC)	-1.200	1.325	.0026	
1:2 (2CMC), 1:10 (2CMC)	-3.611	1.325	<.0001	S
1:5 (0.1CMC), 1:5 (1CMC)	-7.533	1.325	<.0001	S
1:5 (0.1CMC), 1:5 (2CMC)	-7.478	1.325	<.0001	S
1:5 (0.1CMC), 1:10 (0.1C...	3.167	1.325	<.0001	S
1:5 (0.1CMC), 1:10 (1CMC)	3.233	1.325	<.0001	S
1:5 (0.1CMC), 1:10 (2CMC)	.822	1.325	.0285	
1:5 (1CMC), 1:5 (2CMC)	.056	1.325	.8749	
1:5 (1CMC), 1:10 (0.1CMC)	10.700	1.325	<.0001	S
1:5 (1CMC), 1:10 (1CMC)	10.767	1.325	<.0001	S
1:5 (1CMC), 1:10 (2CMC)	8.356	1.325	<.0001	S
1:5 (2CMC), 1:10 (0.1CMC)	10.644	1.325	<.0001	S
1:5 (2CMC), 1:10 (1CMC)	10.711	1.325	<.0001	S
1:5 (2CMC), 1:10 (2CMC)	8.300	1.325	<.0001	S
1:10 (0.1CMC), 1:10 (1CM...	.067	1.325	.8502	
1:10 (0.1CMC), 1:10 (2CM...	-2.344	1.325	<.0001	S
1:10 (1CMC), 1:10 (2CMC)	-2.411	1.325	<.0001	S

Comparisons in this table are not significant unless the corresponding p-value is less than .0011.

Bonferroni/Dunn for Cell viability
Effect: Column 1
Significance Level: 5 %

	Mean Diff.	Crit. Diff	P-Value	
Lipofectamine, 1:2 (0.1CM...	-24.556	2.286	<.0001	S
Lipofectamine, 1:2 (1CMC)	-30.967	2.286	<.0001	S
Lipofectamine, 1:2 (2CMC)	-10.367	2.286	<.0001	S
Lipofectamine, 1:5 (0.1CM...	-19.811	2.286	<.0001	S
Lipofectamine, 1:5 (1CMC)	-7.433	2.286	<.0001	S
Lipofectamine, 1:5 (2CMC)	-10.589	2.286	<.0001	S
Lipofectamine, 1:10 (0.1C...	-3.733	2.286	<.0001	S
Lipofectamine, 1:10 (1CMC)	-6.256	2.286	<.0001	S
Lipofectamine, 1:10 (2CMC)	-3.967	2.286	<.0001	S
1:2 (0.1CMC), 1:2 (1CMC)	-6.411	2.286	<.0001	S
1:2 (0.1CMC), 1:2 (2CMC)	14.189	2.286	<.0001	S
1:2 (0.1CMC), 1:5 (0.1CMC)	4.744	2.286	<.0001	S
1:2 (0.1CMC), 1:5 (1CMC)	17.122	2.286	<.0001	S
1:2 (0.1CMC), 1:5 (2CMC)	13.967	2.286	<.0001	S
1:2 (0.1CMC), 1:10 (0.1C...	20.822	2.286	<.0001	S
1:2 (0.1CMC), 1:10 (1CMC)	18.300	2.286	<.0001	S
1:2 (0.1CMC), 1:10 (2CMC)	20.589	2.286	<.0001	S
1:2 (1CMC), 1:2 (2CMC)	20.600	2.286	<.0001	S
1:2 (1CMC), 1:5 (0.1CMC)	11.156	2.286	<.0001	S
1:2 (1CMC), 1:5 (1CMC)	23.533	2.286	<.0001	S
1:2 (1CMC), 1:5 (2CMC)	20.378	2.286	<.0001	S
1:2 (1CMC), 1:10 (0.1CMC)	27.233	2.286	<.0001	S
1:2 (1CMC), 1:10 (1CMC)	24.711	2.286	<.0001	S
1:2 (1CMC), 1:10 (2CMC)	27.000	2.286	<.0001	S
1:2 (2CMC), 1:5 (0.1CMC)	-9.444	2.286	<.0001	S
1:2 (2CMC), 1:5 (1CMC)	2.933	2.286	<.0001	S
1:2 (2CMC), 1:5 (2CMC)	-.222	2.286	.7154	
1:2 (2CMC), 1:10 (0.1CMC)	6.633	2.286	<.0001	S
1:2 (2CMC), 1:10 (1CMC)	4.111	2.286	<.0001	S
1:2 (2CMC), 1:10 (2CMC)	6.400	2.286	<.0001	S
1:5 (0.1CMC), 1:5 (1CMC)	12.378	2.286	<.0001	S
1:5 (0.1CMC), 1:5 (2CMC)	9.222	2.286	<.0001	S
1:5 (0.1CMC), 1:10 (0.1C...	16.078	2.286	<.0001	S
1:5 (0.1CMC), 1:10 (1CMC)	13.556	2.286	<.0001	S
1:5 (0.1CMC), 1:10 (2CMC)	15.844	2.286	<.0001	S
1:5 (1CMC), 1:5 (2CMC)	-3.156	2.286	<.0001	S
1:5 (1CMC), 1:10 (0.1CMC)	3.700	2.286	<.0001	S
1:5 (1CMC), 1:10 (1CMC)	1.178	2.286	.0641	
1:5 (1CMC), 1:10 (2CMC)	3.467	2.286	<.0001	S
1:5 (2CMC), 1:10 (0.1CMC)	6.856	2.286	<.0001	S
1:5 (2CMC), 1:10 (1CMC)	4.333	2.286	<.0001	S
1:5 (2CMC), 1:10 (2CMC)	6.622	2.286	<.0001	S
1:10 (0.1CMC), 1:10 (1CM...	-2.522	2.286	.0004	S
1:10 (0.1CMC), 1:10 (2CM...	-.233	2.286	.7019	
1:10 (1CMC), 1:10 (2CMC)	2.289	2.286	.0011	S

Comparisons in this table are not significant unless the corresponding p-value is less than .0011.

Table 4-11 Pluronic F127 Anova statistics

TE=Transfection Effeciency

Bonferroni/Dunn for TE
Effect: Column 1
Significance Level: 5 %

	Mean Diff.	Crit. Diff	P-Value	
Lipofectamine, 1:2(0.1CMC)	27.810	1.037	<.0001	S
Lipofectamine, 1:2(1CMC)	29.829	1.037	<.0001	S
Lipofectamine, 1:2(2CMC)	29.857	1.037	<.0001	S
Lipofectamine, 1:5(0.1CMC)	26.924	1.037	<.0001	S
Lipofectamine, 1:5(1CMC)	29.457	1.037	<.0001	S
Lipofectamine, 1:5(2CMC)	29.057	1.037	<.0001	S
Lipofectamine, 1:10(0.1C...	29.848	1.037	<.0001	S
Lipofectamine, 1:10 (1CMC)	27.971	1.037	<.0001	S
Lipofectamine, 1:10 (2CMC)	27.876	1.037	<.0001	S
1:2(0.1CMC), 1:2(1CMC)	2.019	1.037	<.0001	S
1:2(0.1CMC), 1:2(2CMC)	2.048	1.037	<.0001	S
1:2(0.1CMC), 1:5(0.1CMC)	-.886	1.037	.0040	
1:2(0.1CMC), 1:5(1CMC)	1.648	1.037	<.0001	S
1:2(0.1CMC), 1:5(2CMC)	1.248	1.037	.0002	S
1:2(0.1CMC), 1:10(0.1CMC)	2.038	1.037	<.0001	S
1:2(0.1CMC), 1:10 (1CMC)	.162	1.037	.5591	
1:2(0.1CMC), 1:10 (2CMC)	.067	1.037	.8092	
1:2(1CMC), 1:2(2CMC)	.029	1.037	.9175	
1:2(1CMC), 1:5(0.1CMC)	-2.905	1.037	<.0001	S
1:2(1CMC), 1:5(1CMC)	-.371	1.037	.1880	
1:2(1CMC), 1:5(2CMC)	-.771	1.037	.0103	
1:2(1CMC), 1:10(0.1CMC)	.019	1.037	.9450	
1:2(1CMC), 1:10 (1CMC)	-1.857	1.037	<.0001	S
1:2(1CMC), 1:10 (2CMC)	-1.952	1.037	<.0001	S
1:2(2CMC), 1:5(0.1CMC)	-2.933	1.037	<.0001	S
1:2(2CMC), 1:5(1CMC)	-.400	1.037	.1577	
1:2(2CMC), 1:5(2CMC)	-.800	1.037	.0082	
1:2(2CMC), 1:10(0.1CMC)	-.010	1.037	.9725	
1:2(2CMC), 1:10 (1CMC)	-1.886	1.037	<.0001	S
1:2(2CMC), 1:10 (2CMC)	-1.981	1.037	<.0001	S
1:5(0.1CMC), 1:5(1CMC)	2.533	1.037	<.0001	S
1:5(0.1CMC), 1:5(2CMC)	2.133	1.037	<.0001	S
1:5(0.1CMC), 1:10(0.1CMC)	2.924	1.037	<.0001	S
1:5(0.1CMC), 1:10 (1CMC)	1.048	1.037	.0010	S
1:5(0.1CMC), 1:10 (2CMC)	.952	1.037	.0023	
1:5(1CMC), 1:5(2CMC)	-.400	1.037	.1577	
1:5(1CMC), 1:10(0.1CMC)	.390	1.037	.1673	
1:5(1CMC), 1:10 (1CMC)	-1.486	1.037	<.0001	S
1:5(1CMC), 1:10 (2CMC)	-1.581	1.037	<.0001	S
1:5(2CMC), 1:10(0.1CMC)	.790	1.037	.0088	
1:5(2CMC), 1:10 (1CMC)	-1.086	1.037	.0007	S
1:5(2CMC), 1:10 (2CMC)	-1.181	1.037	.0003	S
1:10(0.1CMC), 1:10 (1CMC)	-1.876	1.037	<.0001	S
1:10(0.1CMC), 1:10 (2CMC)	-1.971	1.037	<.0001	S
1:10 (1CMC), 1:10 (2CMC)	-.095	1.037	.7304	

Comparisons in this table are not significant unless the corresponding p-value is less than .0011.

Bonferroni/Dunn for Column 1.3
Effect: Column 1
Significance Level: 5 %

	Mean Diff.	Crit. Diff	P-Value	
Lipofectamine, 1:2(0.1CMC)	-18.105	2.719	<.0001	S
Lipofectamine, 1:2(1CMC)	-28.095	2.719	<.0001	S
Lipofectamine, 1:2(2CMC)	-5.981	2.719	<.0001	S
Lipofectamine, 1:5(0.1CMC)	-8.495	2.719	<.0001	S
Lipofectamine, 1:5(1CMC)	6.086	2.719	<.0001	S
Lipofectamine, 1:5(2CMC)	5.152	2.719	<.0001	S
Lipofectamine, 1:10(0.1C...	4.105	2.719	<.0001	S
Lipofectamine, 1:10 (1CMC)	-3.219	2.719	.0002	S
Lipofectamine, 1:10 (2CMC)	-4.391	2.719	<.0001	S
1:2(0.1CMC), 1:2(1CMC)	-9.990	2.719	<.0001	S
1:2(0.1CMC), 1:2(2CMC)	12.124	2.719	<.0001	S
1:2(0.1CMC), 1:5(0.1CMC)	9.610	2.719	<.0001	S
1:2(0.1CMC), 1:5(1CMC)	24.190	2.719	<.0001	S
1:2(0.1CMC), 1:5(2CMC)	23.257	2.719	<.0001	S
1:2(0.1CMC), 1:10(0.1CMC)	22.210	2.719	<.0001	S
1:2(0.1CMC), 1:10 (1CMC)	14.886	2.719	<.0001	S
1:2(0.1CMC), 1:10 (2CMC)	13.714	2.719	<.0001	S
1:2(1CMC), 1:2(2CMC)	22.114	2.719	<.0001	S
1:2(1CMC), 1:5(0.1CMC)	19.600	2.719	<.0001	S
1:2(1CMC), 1:5(1CMC)	34.181	2.719	<.0001	S
1:2(1CMC), 1:5(2CMC)	33.248	2.719	<.0001	S
1:2(1CMC), 1:10(0.1CMC)	32.200	2.719	<.0001	S
1:2(1CMC), 1:10 (1CMC)	24.876	2.719	<.0001	S
1:2(1CMC), 1:10 (2CMC)	23.705	2.719	<.0001	S
1:2(2CMC), 1:5(0.1CMC)	-2.514	2.719	.0022	
1:2(2CMC), 1:5(1CMC)	12.067	2.719	<.0001	S
1:2(2CMC), 1:5(2CMC)	11.133	2.719	<.0001	S
1:2(2CMC), 1:10(0.1CMC)	10.086	2.719	<.0001	S
1:2(2CMC), 1:10 (1CMC)	2.762	2.719	.0010	S
1:2(2CMC), 1:10 (2CMC)	1.590	2.719	.0377	
1:5(0.1CMC), 1:5(1CMC)	14.581	2.719	<.0001	S
1:5(0.1CMC), 1:5(2CMC)	13.648	2.719	<.0001	S
1:5(0.1CMC), 1:10(0.1CMC)	12.600	2.719	<.0001	S
1:5(0.1CMC), 1:10 (1CMC)	5.276	2.719	<.0001	S
1:5(0.1CMC), 1:10 (2CMC)	4.105	2.719	<.0001	S
1:5(1CMC), 1:5(2CMC)	-.933	2.719	.2065	
1:5(1CMC), 1:10(0.1CMC)	-1.981	2.719	.0118	
1:5(1CMC), 1:10 (1CMC)	-9.305	2.719	<.0001	S
1:5(1CMC), 1:10 (2CMC)	-10.476	2.719	<.0001	S
1:5(2CMC), 1:10(0.1CMC)	-1.048	2.719	.1583	
1:5(2CMC), 1:10 (1CMC)	-8.371	2.719	<.0001	S
1:5(2CMC), 1:10 (2CMC)	-9.543	2.719	<.0001	S
1:10(0.1CMC), 1:10 (1CMC)	-7.324	2.719	<.0001	S
1:10(0.1CMC), 1:10 (2CMC)	-8.495	2.719	<.0001	S
1:10 (1CMC), 1:10 (2CMC)	-1.171	2.719	.1169	

Comparisons in this table are not significant unless the corresponding p-value is less than .0011.