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Sunitinib-Loaded MPEG-PCL Micelles for the Treatment of Age-Related Macular Degeneration

by

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A thesis submitted in partial fulfillment of the requirements for the degree of
Master of Science in Pharmaceutical Nanotechnology
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DEDICATION

This thesis is dedicated to my loving parents, Mable & Johnny Streets, and my sister, Terryn Streets for their continued support, encouragement, and prayers throughout this journey thus far. I also would like to dedicate my work to Dr. Kerry J. Lee, Program Coordinator & Associate professor at Florida Gulf Coast University for being the first to introduce me to the field of nanotechnology.
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ABSTRACT

Age-related Macular Degeneration, or AMD will be responsible for the vision impairment of more than 5 million late-aged adults in the next 30 years. Current treatment options include frequent intravitreal injections which report high levels of pain resulting in a low patient compliance. However, there are methods of drug delivery that can decrease the amount of intravitreal injections by sustaining drug release. Polymeric micelles, specifically MPEG-PCL have reported superb biocompatibility and more efficient ways of delivering anti-VEGF drugs such as Sunitinib to the posterior segment of the eye. In this study, the novel micellar formulation exhibited an average DLS particle size of 132.7 nm with a ZP of -0.159 mV. TEM imaging further confirmed the nanoscopic size of the micelles with sizes ranging from 86.76 to 114.51 nm. In addition to their miniscule size, the SM-MPEG-PCL formulation showed minimal cytotoxicity onto ARPE-19 human retinal pigment epithelial cell line reporting a % viability of more than 88% for all concentrations at time intervals of 24 and 48h. The SM-MPEG-PCL micelles also exhibited exceptional performance during an anti-VEGF ELISA that decreased the overall VEGF protein expression in the cells across a 24-72 hour period. Furthermore, it can be concluded that this type of polymeric vehicle is a promising solution to symptoms caused by AMD and improving the management of those suffering from AMD.
CHAPTER I: INTRODUCTION

1.1 Introduction

Age-Related Macular Degeneration, or AMD is an eye disease that gradually deteriorates the macula and is the leading causes of vision failure among late-aged adults (2,17). The macula is located in the posterior segment of the eye near the retinal center and it is a key component for central vision processing using its photoreceptor cells (1). Macular degradation as a result of AMD disrupts the process of normal vision processing in the optic nerve causing blurry and distorted vision. It is projected that by 2050, more than 5 million Americans will have some form of AMD (2).

Vascular Endothelial Growth Factor, or VEGF is a protein that causes the formation of blood vessels within the eye as a result of AMD. Anti-VEGF drugs inhibit the expression of VEGF at the angiogenic site to prevent central vision damage induced by hyperoxic conditions (22). Generally, anti-VEGF drugs such as Axitinib, Pazopanib, and Sorafenib are available for use in cancer treatments, but recently have been propitious agents for treating AMD-induced neovascularization (11-12). Despite the success of these angiogenic inhibitors, a newer drug named Sunitinib has proved to be a notable contender for the treatment of ocular diseases (14-15).

Sunitinib malate is a tyrosine kinase inhibitor that exhibits anti-VEGF and anti-HIF properties (16). Originally approved for treatment of renal cell carcinoma, Sunitinib has displayed usefulness in the treatment of ocular diseases like AMD that depend on VEGF or
PDGFR receptors (3). Sunitinib can specifically inhibit all three types of VEGF receptors (VEGFR1-VEGFR3) along with seven other important components of cell proliferation (3). Notably, VEGFR1, VEGFR2, and platelet-derived growth factor beta, or PDGFR(beta) are the main integrants of angiogenesis in the body. Additionally, Sunitinib can inhibit eight total tyrosine kinase receptor proteins, such as Kit, Flt-3, and CSF-1R (28). Because Sunitinib is able to terminate tyrosine kinase binding, it can potentially inhibit ocular angiogenesis, a common symptom of AMD (4). Nonetheless, it is noted that the use of sunitinib is restricted due to its toxicity among higher dosages (14-15).

However, there are significant physical barriers including the blood-retinal barrier to reaching the posterior segment of the eye where the retina and macula are housed (5, 23-24). Notably, Poly(ethylene glycol)-block-poly (ε-coprolactone), or PEG-PCL copolymers have been recognized for their low toxicity, biocompatibility, and ability to aid in sustained drug release (6). PEG, along with PEG derivatives like PEG-PCL have been approved by the FDA for medical uses (7). Optimal biodistribution of PEG-PCL nanoparticles was observed in vivo and ex vivo studies using parameters between 20-200nm in size and molecules with a negative zeta potential indicating that circulation time of drugs carried by PEG-PCL copolymers can be greatly extended reducing the frequency of administration (8). PEG-PCL micelles for delivery of anti-VEGF drug Axitinib also expressed its usefulness in inhibiting angiogenesis while displaying exceptional cytocompatibility (9,27). These advancements are promising in fulfilling the need to decrease frequent intravitreal injections and significantly improve patient compliance (18, 25-26).

Consequently, MPEG-PCL micelles in conjunction with Sunitinib appears to be a viable option considering its ability to inhibit eight receptors in the body responsible for cell
proliferation and angiogenesis (10, 27). This study will examine the effectiveness of sunitinib-loaded MPEG-PCL micelles by evaluating their morphological characterization, in vitro drug release, cytotoxicity and VEGF expression.
CHAPTER 2: MATERIALS AND METHODS

2.1 Materials

Sunitinib Malate was purchased from Selleck Chemicals, Houston, TX, USA. All (2k-2k, 5k-2k, 5k-10k) Methoxy poly(ethylene glycol)-block-poly (ε-coprolactone) polymers were purchased from Sigma-Aldrich, St. Louis, MO, USA. Organic solvents used such as methanol, ethanol, dimethyl sulfoxide (DMSO), acetone were purchased from Thermo Fischer Scientific, Waltham, MA, USA. The dialysis membrane (Mw 10000) was purchased from Sigma Aldrich (St. Louis, MO, USA). Cell culturing materials such as Fetal bovine serum, streptomycin-penicillin antibiotic (10,000 U/ml) was purchased from Gibco Thermo Fischer Scientific, USA. ARPE-19 (ATCC© CRL2302™), a human retinal pigment epithelial cell line along with DMEM F12 medium was purchased from American Type Culture Collection, or ATCC (VA, USA). Trypsin (0.05%) was obtained from Thermo Fischer Scientific (Lansing, MI). Whereas, 1X phosphate buffer saline solution, or PBS was obtained from Corning Cellgro (Manassas, VA). MTT reagent, 3-(4,5 dimethylthiazol-2-yr)-2,5-diphenyltetrazolium bromide salt was purchased from Sigma Aldrich (St. Louis, MO, USA). Invitrogen™ eBioscience™ Human VEGF ELISA Kit was purchased from Fischer Scientific (Lansing, MI).

2.2 Cell Culture

Cell line studies were carried out using ARPE-19 (ATCC© CRL2302™) cells derived from human retinal epithelial cell line. Nourishment for the cells were provided from complete
media, a combination of DMEM F12 medium, 10% v/v Fetal Bovine Serum (FBS), 1% 10000 u/ml penicillin antibiotic. Additionally, cells were stored in a moisture-controlled CO₂ incubator set at 37°C.

2.3 Formulation of SM-MPEG-PCL Micelles

A mass of 2 mg of Sunitinib malate and 200μl of DMSO were dissolved into a 5 ml centrifuge tube. This mixture is sonicated for eight minutes to achieve full dissolution of the drug into the organic solvent. Afterwards, 1.8 ml of acetone along with 20 mg of Methoxy poly(ethylene glycol)- block-poly (ε-caprolactone), or MPEG-PCL polymer is added to the DMSO/sunitinib mixture and sonicated again to fully dissolve the drug. Mixture is then vortexed to mix the two solvents as this will be the 2 ml organic solvent component. The accompanying aqueous phase is 4 ml of filtered distilled water. Under magnetic stirring, the 2 ml organic phase is added dropwise using a 23G syringe to the aqueous phase. The mixture is then stirred magnetically for 24 hours at 700 rpm to allow for adequate precipitation of organic solvents. Following 24 hours, the formulation is then centrifuged at 5000 rpm for 15 minutes to extrapolate any free drug or polymer as seen in Figure 1C.
Figure 1: (A) Structure of MPEG-PCL Polymer (B) Structure of Sunitinib (C) Preparation Scheme for SM-MPEG-PCL Micelles

2.4 Particle Size and Zeta Potential

Size and zeta potential of the SM-MPEG-PCL micelles were measured using Dynamic Light Scattering, or DLS via the Zeta Sizer Nano ZS 90 instrument (Malvern Instruments Ltd., UK, Zeta Sizer Software Ver. 7.10). The parameters for characterizing nanoparticles included a 90 degree measuring angle along with 25°C temperature for each sample. Each cuvet consisted of 900µl filtered distilled water and 100 µl of formulation (31). The ZetaSizer reported characterization data in triplicates along with an average Polydisperity Index, or PDI which measures the biomolecular distribution of nanoparticles within a solution.

Using a zeta cuvette cell, zeta potential was measured with 1 ml total of sample diluted with filtered distilled water. Zeta potential utilizes the dynamics of the Smoluchowski equation which determines the motility of the particles electrically in conjunction with a back-scatter of 90° (32). Similar to size characterization, zeta potential data is also measured in triplicates.
2.5 Differential Scanning Calorimetry

Differential scanning calorimetry analysis, or DSC was executed using a DSC Q 20 instrument (TA Instruments, New Castle, DE, USA, Q series Q20—2288-DSC software). Four samples were analyzed beginning with polymer, pure sunitinib drug, physical mixture of sunitinib and MPEG-PCL polymer, and SM-MPEG-PCL formulation. Five milligrams of each sample was taken and placed inside of an aluminum pan specially made for DSC analysis (29). Samples were then heated to approximately 30-300°C at a rate of 10°C while simultaneously under a nitrogen purge at a rate of 50 mL/min (30).

2.6 Transmission Electron Microscopy

Morphology of the SM-MPEG-PCL micelles were measured using a Transmission Electron Microscope, or TEM. To prepare the formulation for imaging, the sample was inserted onto a 200 Cu film square grid. The sample was then allowed to settle for approximately 10 minutes. After the sample was air dried, it is prepared for negative staining using 2% w/v phosphotungstic acid (PTA). Furthermore, images were obtained at 80,000X magnification at an accelerating voltage of 120kV.

2.7 Entrapment Efficiency

The SM-MPEG-PCL formulation was centrifuged at 5000 rpm for 15 minutes to separate any excess drug or polymer. Furthermore, the supernatant was collected and centrifuged once more at 18000 rpm for approximately 20 minutes to transform the nanoparticles into pellet form for further analysis. In order to prepare the formulation for UV spectrophotometer analysis, the pellet was washed three times with filtered distilled water to further ensure the removal of
unentrapped drug. The nanoformulation was paired with methanol as a solvent and analyzed using a UV Spectrophotometer at a wavelength of 432 nm (λmax). From this data, the amount of drug within the sample was calculated and used in the equations below to determine the entrapment efficiency and loading efficiency percentages.

\[
\% \text{ Entrapment Efficiency} = \frac{\text{Actual amount of drug loaded in NPs}}{\text{Actual amount of drug used for preparation}} \times 100
\]

\[
\% \text{ Loading Efficiency} = \frac{\text{Amount of Sunitinib in nanoparticles}}{\text{Total amount of NPs}} \times 100
\]

2.8 In Vitro Drug Release Study

The in vitro drug release included a comparison of pure SM drug solution in DMSO and SM-MPEG-PCL formulation by the use of a dialysis bag (MWCO 10000 Da) (33). In preparation for loading of both sunitinib in DMSO and the SM-MPEG-PCL formulation, release medium was prepared by the addition of Phosphate buffer saline, or PBS with a pH adjusted to 7.4 along with Tween 80 solution (0.1% v/v). The release medium was further optimized under magnetic stirring at 100 rpm with a constant temperature of 37°C. Approximately 200μl of each formulation was taken and subsequently replaced with 200μl release medium at time intervals beginning at 0.25, 0.5, 1, 2, 3, 4, 5, 6 hours for the initial day and every 24 hours for seven days. Samples were diluted with methanol in preparation to be read using a UV Spectrophotometer at a wavelength of 432 nm.
2.9 Cytotoxicity Study

Cytotoxicity of the SM-MPEG-PCL formulation and pure drug solution were measured using an MTT Assay analysis on ARPE-19, a human retinal pigment epithelial cell line. In a 96 well-plate, cells were seeded at a density of 5000 cells per well supplemented with incomplete medium consisting of 200μl of DMEM and 10% FBS solution. Cells were then allowed to grow in a 5% atmosphere 37°C CO₂ incubator for 24 hours to ensure proper attachment of the cells to the bottom of the well plates (34). Following initial incubation, cells were then treated with dilutions of 0.001, 0.01, 0.1, 1, 10, 20 μM for each formulation in quadruplicates. Similarly, in the remaining two rows, incomplete and complete mediums and Triton X100 were used as positive and negative controls (35).

After treatment, cells were allowed to incubate for 24 and 48 hours. Subsequently, the treatments were removed and cells were then washed once thoroughly with sterile 1X PBS solution. Afterwards, cells were supplemented with fresh complete medium and were incubated for 24 and 48 hours. After 24 hours, complete media was removed from the cells and replaced with 100μl (1mg/1mL) of MTT reagent into each well and incubated for 4 hours. After 4 hours has elapsed, complete media is removed and replaced with 100μl of dimethyl sulfoxide (DMSO) to dissolve the formazan crystals that indicate cell viability by displaying a distinct purple color. The plate was then read using a UV spectrophotometer at a wavelength of 595 nm to obtain the absorbances of each well. For comparison, cells treated with DMEM-F12 were the negative control and 0.1% Triton X100 was the positive control respectively.
2.10 Anti-VEGF ELISA

In a sterile 96-well plate, 48 wells were seeded with a density of 5,000 cells per well and treated with complete media to induce confluency. After 24 hours, complete medium was removed and cells received treatment. Two treatments were used in the ELISA beginning with free drug solution and the SM-MPEG-PCL formulation. Complete and incomplete medium was used as negative controls and two treatments each at 10μM were treated into quadruplicates for 24, 48, and 72 hours (36). After 72 hours of incubation, VEGF expression was assessed using an Invitrogen Human VEGF-A Platinum ELISA Kit. Quantification of cell protein content was measured using a Pierce BCA Protein Assay Kit following the collection of cell lysate. Quantitative analysis of samples were read using an ELISA plate reader at absorbances of 450 nm. The differences between the two absorbances were noted and used to determine the inhibitory effects of VEGF secretion using a standard curve.

2.11 Statistical Analysis

In order to quantize all data reported in this study, an extensive statistical analysis was done using the mean, standard deviation, and t-test.
CHAPTER 3: RESULTS AND DISCUSSIONS

3.1 Differential Scanning Calorimetry

The physiochemical properties of pure SM drug, pure MPEG-PCL polymer, physical mixture of polymer and SM, and SM-MPEG-PCL micelle formulation were analyzed using DSC analysis. Figure 2 displays the thermal analysis of pure SM, physical mixture of SM and MPEG-PCL polymer, MPEG-PCL polymer, SM-MPEG-PCL formulation. DSC is useful in providing evidence of the drug’s natural, physiochemical state and also its physical state within the MPEG-PCL formulation. Sunitinib alone exhibited an endothermic peak at a temperature of 202.53℃ indicative of its melting point (Figure 2). In contrast, the DSC spectra for the SM-MPEG-PCL formulation did not exhibit a peak at this melting point, rather at a peak near 105.26℃ affirming the entrapment of SM in MPEG-PCL micelles and SM in an amorphous form (13). Furthermore, an endothermic peak shown at ~201℃ is seen in the physical mixture of SM and MPEG-PCL.

Figure 2: DSC Spectra of (A) Pure Drug, Sunitinib (B) SM-MPEG-PCL Micelle Formulation (C) SM & MPEG-PCL Physical Mixture
3.2 Size and Zeta Potential

Using a 1:10 drug to polymer ratio, DLS analysis reported the mean size of the 2k-2k sunitinib micelles were 167.8 nm with an average PDI of 0.211 (Table 1). Whereas, the zeta potential for 2k-2k sunitinib micelles was +4.34 mV. 5k-2k SM-MPEG-PCL micelles with a 1:10 drug to polymer ratio were seen to have an average size of 134.2 nm with an average PDI of 0.160. The zeta potential reported for this ratio was -0.159 mV (Figure 3A). Because the 5k-2k MPEG-PCL polymer provided a more desirable size, a 1:7 ratio was also tested in hopes of a smaller particle size. The 1:7 ratio reported a mean particle size of 170.8 nm, ZP of +0.842 mV and a PDI of 0.104. Furthermore, a 1:15 ratio of the 5k-2k polymer was tested and showed an average size of 120.9 nm, ZP of +10.5 mV and a PDI of 0.149 (Table 1). The low PDI values indicate the homogeneity and monotonous distribution of the nanoparticles within the formulation (19). It is concluded that the 1:10 ratio of the 5k-2k polymer was the optimal molecular ratio to use for this experiment as the positive zeta potential values indicate the possibility of aggregation within the body (20). Table 2 displays the size difference between the 2k-2k & 5k-2k MPEG-PCL blank micelles for size comparison to compare the weightiness of SM drug onto the micelles.

Table 1: Comparison Chart of Physiochemical Characterization of Formulations and Ratios

| Drug-loaded Polymer       | Ratio | DLS Average Size (nm) | Zeta Potential (mV) | Polydispersity Index (PDI) |
|---------------------------|-------|-----------------------|---------------------|---------------------------|
| 2k-2k MPEG-PCL           | 1:10  | 167.8 nm              | +4.34 mV            | 0.211                     |
| 5k-2k MPEG-PCL           | 1:7   | 170.8 nm              | +0.842 mV           | 0.104                     |
| 5k-2k MPEG-PCL           | 1:10  | 132.7 nm              | -0.159 mV           | 0.160                     |
| 5k-2k MPEG-PCL           | 1:15  | 120.9 nm              | +10.5 mV            | 0.149                     |
### Table 2: Size Comparison of Blank MPEG-PCL Micelles

| Polymer           | DLS Size | Polydisperity Index (PDI) |
|-------------------|----------|---------------------------|
| 2k-2k MPEG-PCL    | 120.7 nm | 0.304                     |
| 5k-2k MPEG-PCL    | 115.1 nm | 0.172                     |

#### 3.3 Transmission Electron Microscopy

TEM images reported particle sizes ranging from 86.76 nm to 114.51 nm in contrast to the mean particle size of 134.2 nm reported from DLS (Figure 3B). The discrepancy between the difference in particle sizes among both DLS and TEM are due to the differences in the way the formulation is processed. TEM analyzes the nanoparticles within a fixed copper grid, whereas DLS utilizes the hydrodynamic diameter of the micelles to report a mean particle size (21). TEM imaging of SM-MPEG-PCL micelles affirmed the size uniformity of the nanoparticles coinciding with the low PDI of the 5k-2k formulation. Figure 3B also exhibits the physical characteristics of the nanoparticles having a spherical structure and monotonous surface. It is also accurate to infer from TEM imaging that the particles are in agreement with the negative zeta potential value and less likely to agglomerate.

![Figure 3](image-url)

**Figure 3:** (A) Z-Average of MPEG-PCL Micelles (B) TEM Image of SM-MPEG-PCL Micelles with 80,000X at accelerating voltage of 120 kV (scale bar 200 nm)
3.4 Entrapment Efficiency

Entrapment efficiency was evaluated by centrifugation of the formulation at 5000 rpm for 15 minutes. The supernatant was then collected and diluted with methanol by a factor of 20 (1.9 ml of methanol & 0.1 ml formulation). This dilution was then read at $\lambda_{max}$ of 432 nm using a UV Spectrophotometer and calculated according to absorbances obtained from the plate reader in conjunction with the previous equation $y=0.0458x-0.0035$ and $r^2$ value of 0.999. The 1:10 ratio of the 5k-2k SM-MPEG-PCL formulation reported an entrapment efficiency of 64.3%, whereas the entrapment efficiency for the 1:15 ratio was 45.6%. Hence, the 1:15 ratio was not feasible to use for further studies despite its smaller particle size.

3.5 In Vitro Drug Release

The in vitro drug release study using the dialysis method compared the release capacity of SM drug solution, SM-MPEG-PCL, SM-PLGA nanoparticles, and SM-NP gel formulations over a 7-day period. Release medium was prepared using PBS (pH 7.4) and Tween 80 (0.1% v/v) at an approximate temperature of 37°C for 7 days. The drug solution exhibited a complete expulsion in the release media at approximately 12 hours after initial administration. In contrast, the SM-MPEG-PCL formulation saw a full release of drug from the formulation at approximately 168h, or 7 days as seen in Figure 4. Because of this, we can infer that the MPEG-PCL formulation is only capable of releasing the drug at 7 days. Within 2 hours of the initiation, the SM formulation displayed a burst release of 11.8%. Whereas, after 24 hours the formulation had a burst release of 39.44±4.1%. Furthermore, the formulation displayed a sustained release profile of 95.56±2.7% after the seven-day period. Hypothetically, this can be classified as
sustained drug release, but further optimization of the formulation is needed to extend the release of the drug further than 7 days.

Figure 4: Drug release profile of Sunitinib from Sunitinib solution and SM-MPEG-PCL Micelle Formulation up to 15 days at 37°C in phosphate buffer at pH 7.4

3.6 Cytotoxicity

The SM-MPEG-PCL, blank MPEG-PCL, and SM drug solution were evaluated for cytotoxicity by the use of an MTT Assay performed on ARPE-19, human retinal epithelium cell line seen in Figure 5. Cells were first treated with incomplete medium and distributed according to concentrations of 0.001, 0.01, 0.1, 1, 10, and 20μl for each formulation in quadruplicates for 24 and 48 hours. Cell viability of the formulations were compared to the positive control, complete medium that obtained a standard percentage of 100% viability. It is concluded that the cell viability of the SM-MPEG-PCL, blank MPEG-PCL micelles were found to be greater than 88% for all respective concentrations. The optimal cell viability percentage in the SM-MPEG-PCL formulation at 24h was observed at a both concentrations of 10 μM and 20 μM of whereas in the blank micelles, the highest viability was at the concentration of 0.01 and 0.1μl at a
percentage of 99.25% viability (Figure 5A). The viability of the drug solution was 68% for 24h and 61% inferring that the solution is toxic to the cells in higher concentrations.

Results for 48 hours also showed that the SM-MPEG-PCL formulation, along with the blank micelles are viable with percentages above 95% for all six concentrations (Figure 5B). The MTT assay analysis reaffirmed the SM-MPEG-PCL and blank micellar formulations ability to show no significant cytotoxicity within the ARPE-19 cell line indicating that the formulation is suitable for further comprehensive studies.

Figure 5: % Cell Viability at Various Concentrations of Sunitinib drug solution and SM-MPEG-PCL formulation at (A) 24 h & (B) 48 h on ARPE-19 Human Retinal Cell Line (mean ± SD, n=4)
Figure 6: % VEGF Expression in ARPE-19 cells treated with Sunitinib drug solution and MPEG-PCL formulation at 24, 48, and 72 h timepoints using ELISA (mean ± SD, n=4)

3.7 Anti-VEGF ELISA

An anti-VEGF ELISA was used to determine the percentage of VEGF protein within cells after treatment with SM drug solution and SM-MPEG-PCL micelles. Following initial treatment and incubation, samples were obtained at time intervals of 24, 48, and 72 hours. These samples were then analyzed by the aid of a Human VEGF-A ELISA kit. At 100% VEGF expression, the SM drug solution served as the control group and was compared to the VEGF expression by the SM-MPEG-PCL formulation (Figure 6). It is seen that VEGF expression is decreased in the 24, 48, and 72 hour periods for the free SM drug solution. Whereas the SM-MPEG-PCL formulation also decreased the overall VEGF expression (p < 0.05) across 24, 48, and 72 hours of exposure to a 10µM concentration of the formulation.

However, there was not a significant percent difference in the SM drug solution and formulation at 72 h. This can be attributed to the need for a longer period of observation, indicative of repeating the experiment for a longer period of time. Ideally, an observation period of 144h may suffice in showing an increase in the SM free drug solution and subsequently a
steady VEGF percentage decrease in the formulation. In summation, both the free SM drug solution and the SM-MPEG-PCL formulation both showed an overall decrease in VEGF expression across a 24-72 hour period. This infers that the SM drug and the formulation are both suitable in decreasing the overall expression of VEGF expression within ARPE-19 cells.
CHAPTER 4: CONCLUSIONS

4.1 Conclusions

Sunitinib-loaded 5k-2k MPEG-PCL Micelles exhibited an average size of 132.7 nm with an average zeta potential of -0.159 mV with a PDI of 0.160. With an entrapment efficiency of 64.3%, the SM-MPEG-PCL formulation was studied for biocompatibility within ARPE-19, a human retinal pigment epithelial cells to demonstrate its biological interactions in vitro to treat symptoms caused by AMD. SM-MPEG-PCL formulation exhibited a sustained release profile up to seven days with an overall release percentage of 95.56±2.7%. SM-MPEG-PCL micelles showed a percent viability of above 88% for all six concentrations for 24h and above 95% for 48h demonstrating that the SM-MPEG-PCL formulation and blank MPEG-PCL micelles were not toxic to the cells. The anti-VEGF ELISA confirmed that the drug solution and the formulation were effective at reducing the overall expression of VEGF protein in ARPE-19 human retinal pigment epithelial cell line across a 24-72hr period. In summation, the SM-MPEG-PCL micelles demonstrated exceptional preliminary performance to be further studied in vivo for providing a more effective treatment of AMD.
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