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Filomicelle Functionalization and Stability Studies with Applications for Malaria Treatments

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I am submitting herewith a thesis written by Megan Haoyun Yang entitled "Filomicelle Functionalization and Stability Studies with Applications for Malaria Treatments." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Chemical Engineering.

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Filomicelle Functionalization and Stability Studies with Applications for Malaria Treatments

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DEDICATION

I dedicate my dissertation work to my family. My parents, Chih-chun and Yu-chin Yang, supported me financially and provided words of encouragement that helped me through graduate school. My sisters, Hayley and Lindy, were there to help me through possibly the busiest time of my life. Finally, I dedicate this work and give special thanks to my husband, Eric Nalley, for being there for me throughout the entire master's program.

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Thank you all for your help.

ABSTRACT

Malaria is an infectious disease caused by the parasite *Plasmodium* that is transmitted by mosquitoes. It is estimated that malaria causes 1.1 million deaths per year globally. While anti-malarial drugs have been effective in treating infected individuals, new methodologies are needed. Treatments may benefit from approaches that encapsulate drugs in vehicles allowing for more effective delivery. To this end, the use of targeted drug delivery vehicles called filomicelles to treat malaria is proposed.

Certain amphiphilic diblock copolymers self-assemble into filomicelles (long and stable cylindrical micelles), which are capable of carrying hydrophobic drugs in the bodies of rodents. It is shown in this dissertation that the surface of these filomicelles can be covalently modified with peptides. The peptides have been found by other research groups to bind apical membrane antigen 1 (AMA1) of malaria parasites. AMA1 is part of the machinery that allows *Plasmodium* to infect red blood cells.

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CHAPTER I
INTRODUCTION AND GENERAL INFORMATION

1.1 Significance

Malaria is an infection transmitted via mosquitoes carrying the causative protozoan, *Plasmodium*. Much of the early work of the Centers for Disease Control and Prevention (CDC), created in 1946, was to concentrate on the control and elimination of malaria in the United States. With the successful reduction of malaria in the United States, the CDC now focuses on malaria prevention, surveillance, and technical support both domestically and internationally. While no longer a major problem in the United States, malaria infection remains a major threat to half of the world's population. According to the World Health Organization, between the years of 2000 and 2010 malaria caused an estimate of 1.1 million deaths per year, inflicting both adults and children. Current attempts in limiting the infection involve prophylactic treatments, insecticides, environmental measures in eliminating the parasite-bearing mosquitoes, and use of protective netting in shielding against mosquitoes. However, drug resistance has become a major concern with the emergence of both drug resistant mosquitoes and parasite. Chloroquine, for example, is an anti-malarial drug used historically for malaria prophylaxis since the 1940s, but it is no longer recommended by the CDC in travelers to many areas of the world because the parasites have gained resistance to chloroquine [31]. Additionally, other protective measures are not always practical or possible in other countries. As a result, malaria remains a major global health issue. As of 2011, malaria is endemic to more than 100 countries and territories.

1.2 Innovation

Drug delivery vehicles have shown promise in improving the treatments of several diseases. I have developed a drug delivery vehicle based on the template of filomicelles with the aim of improving the treatment of malaria.

Filomicelles are similar to worm-like micelles in their structure and flexibility but are longer (lengths of microns as opposed to tens-of-nanometers) and more stable [8]. They have a widely used poly(ethylene-oxide) (PEO) surface chemistry that can be modified with cell-targeting peptides and can be readily viewed with fluorescent microscopy. As a result of these unique physical properties, filomicelles are emerging as alternate vehicles to more traditional carriers such as liposomes.

Previous work on filomicelles focused on reducing the mass of epithelial-based tumors [6]. In that work, filomicelles were loaded with the anti-cancer drug

paclitaxel (TAX) and injected into mice bearing xenographs. The filomicelles shrunk the size of the tumor over shorter carriers and freely circulating TAX.

In that study, the filomicelles were not targeted to the tumor – that is, no biomolecules were conjugated to the exteriors of the filomicelles that had affinity for the tumor cells. It was postulated that the filomicelles localized to the tumor because of leaky vasculature characteristic of solid mass tumors [20]. Nanoparticles with modification for specific targeting have also been shown to be new promising drug delivery approach [17,23].

The overall goal of this project will be to build upon these achievements by targeting the filomicelles to the malaria parasite (*P. yoelii* 17XNL merozoites, a rodent-specific species of *Plasmodium*). The first step of which is to conjugate AMA1-binding peptides to the surfaces of the filomicelles.

CHAPTER II
LITERATURE REVIEW AND BACKGROUND

This chapter includes reviews of articles on the two main topics of this thesis: malaria and the class of drug delivery vehicles called filomicelles.

2.1 Malaria

2.1.1 Malaria Infection

Five strains of malaria parasites constitute major health risks: *P. falciparum*, *P. vivax*, *P. ovale*, *P. malariae*, and *P. knowlesi* with *P. falciparum* being the most pathogenic and accounting for most malarial mortality [5,18]. The parasites exhibit a life cycle involving both an insect host and a vertebrate host. The sexual stages develop in the female *Anopheles* mosquitoes and the asexual stages take place in the mammalian host.

Identification of infection by *P. falciparum* is usually based on the presence of small ring-stage parasites on blood smears [14]. Infected red blood cells are not enlarged, and multiple infections of a single red blood cell are common [5]. An important difference between *P. falciparum* and other human malarias is that maturing trophozoites are usually sequestered in the capillaries of internal organs, such as the heart, brain, spleen, skeletal muscles, and placenta, thus protecting them from being cleared rapidly by the spleen [33]. Red blood cells infected by *P. vivax* are enlarged and, when stained with Giemsa, often show stippling on the red blood cell membrane, known as Schüffner's dots [34]. All stages of the *P. vivax parasite* are present in the peripheral circulation. *P. ovale* infection produces a fever clinically similar to that of vivax malaria but somewhat less severe. *P. malariae* develop in the mosquito slowly, and infection in humans is not as intense as that caused by the other *Plasmodium* species [35]. Red blood cells infected with *P. malariae* remain the same size throughout schizozony. Infections by *P. malariae* and *P. falciparum* do not relapse. *P.vivax* and *P. ovale* infections produce the classic relapsing malaria, initiated from dormant parasites in the liver that have resumed development after a period of latency [32].

Infected female mosquitoes transfer parasites into the subcutaneous tissue, and less frequently directly into the blood stream, after a mosquito bite [19]. The parasites are sporozoites (motile spore-like) at this stage of the infection, and they localize to parenchymal cells of the liver. The sporozoites then invade liver cells, where they replicate [30]. The length of this asexual division and the number of non-motile progeny (merozoites) produced within each infected cell is a characteristic of the individual species of *Plasmodium*. In general, over a period

of one to two weeks, the *Plasmodium* parasites produce and release thousands of merozoites per liver cell. Specifically, *P. falciparum*, for example, produces about 40,000 merozoites within one week. These merozoites then return to the bloodstream and invade red blood cells. The mechanism of invasion involves initial attachment of the parasite to the red blood cell, apical reorientation of the merozoite, tight junction formation between host and parasite, apical organelle secretion followed by host cell entry utilizing a moving junction between the parasite and the host cell membrane, marked by erythrocyte deformation, and then termination through resolution of ligand-receptor interactions by proteolytic cleavage [21]. In the red blood cells, the parasite begins to grow, first forming the ring-like early trophozoite, and eventually enlarging to fill the cell. The parasite then undergoes asexual division and becomes a schizont composed of merozoites. The parasites are nourished by the hemoglobin within the erythrocytes and produce a characteristic pigment called hemazoin. The cycle is completed when the red blood cell ruptures and releases newly formed merozoites into the bloodstream, leading to clinical infection.

Many attempts to stop invasion have involved targeting merozoite surface proteins. The merozoite surface proteins are very diverse and polymorphic. Of these surface proteins, apical membrane antigen 1 (AMA1) has been identified as an essential gene for parasite invasion and survival within the human host [15]. I focus on targeting this protein.

2.1.2 Apical Membrane Antigen 1

Apical membrane antigen 1 (AMA1) of *Plasmodium* is a surface protein that is expressed in schizont-stage malaria parasite, sporozoites, and merozoites, and plays a crucial role in the invasion of both hepatocytes and red blood cells [12,24].

Several peptides have been identified to bind to AMA1. These peptides include [10]:

1. R1 (VFAEFLPLFSKFGSRMHILK)
2. R3 (PVLRSGRCAELIQIGFRCRA)
3. F1 (GWRLLGFGPASSFSM)

Although AMA1 itself is also very polymorphic, it contains a conserved hydrophobic trough that all three peptides appear to bind [11], blocking merozoite invasion, even after initial attachment to the red blood cell surface [16,26]. In a study with multiple sequence alignment of the AMA1 amino acid sequences from six *Plasmodium* species (*P. berghei, P. yoelii, P. chabaudi, P. vivax, P. knowlesi, and P. falciparum*), 60% of the residues in this region of the protein are conserved across the *Plasmodium* species including *P. yoelii* [1,12]. Therefore, these peptides are attractive candidates for targeting *Plasmodium* to reduce red blood cell invasion.

2.2 Filomicelles

In this study, I propose to modify the surface chemistry of filomicelles with peptides that have been identified to bind malaria parasites. The long-term goal is to hamper the life cycle of malaria parasites by administering these functionalized filomicelles to mice infected with parasites. By functionalizing filomicelles with the above peptides, I aim to achieve specific targeting and retain the previous benefits of delivering hydrophobic drugs. The filomicelles used here have lengths of several microns and diameters of about fifty nanometers [4,27]. The flexibilities of the filomicelles are on the order of the diameter of malaria parasites. The most desirable strength of these fluid filomicelles is that they remain in the circulation of rodents ten-fold longer than spherical analogs and are more persistent than any known synthetic nanoparticle [6], providing an extended opportunity to bind circulating *Plasmodium* parasites.

2.2.1 Degradable Filomicelles

Filomicelles prepared from poly(ethylene oxide) - poly(ε-caprolactone)-copolymers (PEO-PCL) spontaneously shorten to generate spherical micelles due to chain-end hydrolysis of the caprolactone [7]. This characteristic can be used in controlled drug release [28].

2.2.2 Non-degradable Filomicelles

Filomicelles prepared from non-degradable amphiphilic poly(ethylene oxide) - poly(butadiene) copolymers (PEO-PBD) are stable for years at 4°C and can stay in circulation longer than spherical analogs [6], providing an extended opportunity to bind and engulf *Plasmodium* parasites. Both degradable and non-degradable filomicelles may prove effective at different times during the malaria parasite life cycle in infected animals.

CHAPTER III
MATERIALS AND METHODS

3.1 Modified Filomicelles

3.1.1 Filomicelles

The filomicelles used here were assembled from diblock copolymers of poly-ethylene oxide (PEO) with either poly-caprolactone (PCL) or poly-butadiene (PBD) as the hydrophobic block. PEO is FDA-approved for use in the body and PCL is biodegradable. PCL and PBD provide the structural stability of the filomicelles and PEO keeps the filomicelles soluble in aqueous environments. The PEO can also be modified for peptide attachment.

In the beginning stages of my thesis, I perfected and scaled up the formation of PEO-PCL filomicelles. I am now able to obtain 50 ml's of PEO-PCL filomicelles whereas before I had 1 ml of PEO-PCL filomicelles with many assembles that were not cylindrical.

The following procedure was used to form filomicelles having parasite binding peptides. COOH-PEO-PBD was mixed with PEO-PCL where the carboxyl groups were exposed to the aqueous environment. The carboxyl groups react with 1-ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride (EDC) to form an unstable reactive ester intermediate (Figure 1). N-hydroxysulfosuccinimde (Sulfo-NHS) and the peptide are then added to the reaction to form a filomicelle where a certain fraction of the COOH-PEO-PBD diblock copolymers have the new chemistry, peptide-CO-PEO-PBD [9].

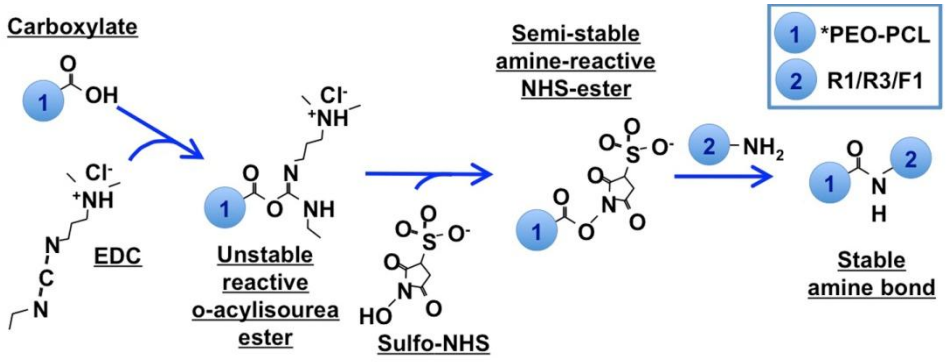

Figure 1. Schematic Diagram for Attaching Primary Amines to PEG.
The symbol * on *PEO-PBD indicates where the carboxylate is located.

3.1.2 AMA1-Targetting Peptides

Three peptides have been identified that bind AMA1 [10]. Of the three, R1 contains lysine, which has the potential to form side products in our reaction scheme, which could reduce its affinity for AMA1 [11,13]. Therefore, I focused on conjugating R3 and F1 peptides to the exteriors of the filomicelles.

3.1.3 Visualization Method

Filomicelles were visualized by fluorescent microscopy using a hydrophobic dye (PKH26) [2,3]. After formation of the filomicelles, 1 µL of 1 mM PKH26 dye in ethanol was added to a 1 mg/ml sample of PEO-PCL filomicelles. Since the dye is hydrophobic, it partitions into the cores of the filomicelles. PKH26 dye remains in the cores of non-degradable PEO-PBD filomicelles for up to one week in the plasma of rodents after tail-vein injection.

3.1.4 Bovine Serum Albumin

Before working with the above peptides, I wished to attach a readily available biomolecule to the filomicelles. Bovine serum albumin (BSA) was chosen because it has multiple exposed lysines and is inexpensive. I attached BSA to the filomicelles using the method described in section 3.1.1. Coomassie staining was used to quantify the amount of BSA bound to the filomicelles.

3.1.5 Electrophoresis

Analysis of SDS-page gels was used to show covalent binding of F1 peptide to the COOH-PEO-PBD copolymer.

3.1.6 Fluorescence Microscopy and Spectroscopy with Alexa Fluor

To determine attachment of R3 to the filomicelles, cysteines on R3 were labeled with a fluorescent maleimide molecule. The fluorescence of the samples was measured after unbound peptides were removed by dialysis.

3.2 PEO-PCL Filomicelle Degradation

3.2.1 Scanning Transmission Electron Microscopy (STEM)

A major question in the field of drug delivery is how the structural integrity of drug delivery vehicles is affected by the deposition of plasma proteins on the surface of the vehicles. To help address this question, I monitored the morphology changes of filomicelles in aqueous media and then in the presence of serum proteins.

Negative staining is a qualitative method for examining the structure of isolated organelles, individual macromolecules and viruses at the nano scale. Here, I imaged filomicelles using negative staining under an electron microscope. Observation of differences on the surface and in shape from degradation was made to qualitatively determine stability of the filomicelles. Since PCL degrades over time, an aging study on the PEO-PCL filomicelles was conducted and the morphology changes of the filomicelles, possibly due to hydrolysis [7], were observed. PEO-PBD filomicelles were used as a control.

3.2.2 Chromatography

Ion-exchange chromatography was used in the separation of filomicelles and proteins contents introduced in the studying of filomicelle stability in presence of high protein environment. Source Q resin was used to separate filomicelles and preliminary tests are done using bovine serum albumin (BSA) in solution.

CHAPTER IV
RESULTS AND DISCUSSION

This chapter includes the results and discussion from the experiments where filomicelles were modified with a test protein, bovine serum albumin (BSA), and then with AMA1-binding peptides, R3 and F1 (section 4.1). Also included are the *in vitro* experiments studying PEO-PCL and PEO-PBD stability using STEM (section 4.2).

4.1 Modified Filomicelles

The chemicals EDC and Sulfo-NHS were used to modify the carboxylic acid ends of the COOH-PEO-PBD copolymers so that the primary amine of a peptide could form a stable covalent bond to the filomicelle. This chemistry is used widely in immobilization procedures and immunogen preparation (attaching a small peptide to a large carrier protein). Several experiments were carried out to test for stable attachment. The morphology of the modified filomicelles was also observed in select cases.

4.1.1 Bovine Serum Albumin (BSA) attachment to filomicelles

BSA was attached to filomicelles using the chemistry scheme shown in Figure 1. Two filomicelle samples were formed. In both samples, 99% of the copolymers were PEO-PCL and 1% were COOH-PEO-PCL. For the first sample, the above chemistry scheme was used to attach BSA through its N-terminus or lysine groups. For the second sample, only BSA was added and EDC and Sulfo-NHS were omitted.

Both samples were stained with Bradford and showed comparable signals (Figure 2). BSA that had not bound the filomicelles was then removed by dialysis overnight. The Bradford signal of the second sample was reduced by a factor of four and was much lower than the signal of the first sample (Figure 2). This suggests that BSA was covalently attached to the modified carboxyl group on the end of the COOH-PEO-PBD copolymer.

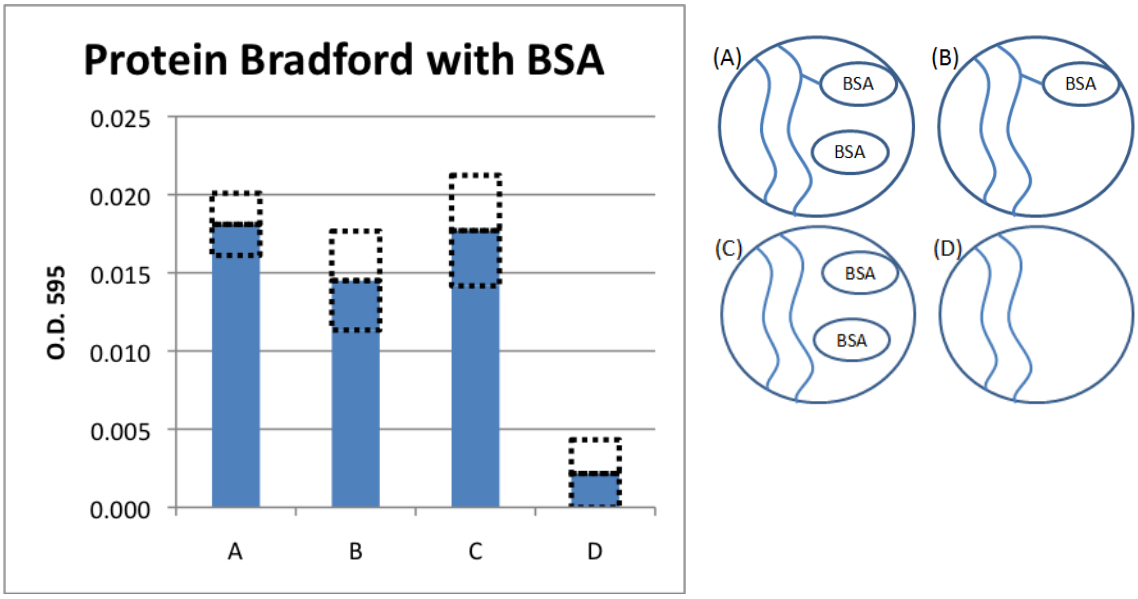

Figure 2. Attachment of BSA to filomicelles.
Modified filomicelles with BSA attachments before dialysis (A), modified filomicelles with BSA attachments after dialysis (B), regular filomicelles with BSA in solution before dialysis (C), and regular filomicelles after dialysis (D). The dashed boxes show 95% confidence interval.

4.1.2 Modified Filomicelles with R3 and F1 peptides

Unfortunately, using Bradford or equivalent stains to determine whether biomolecules are present on filomicelles was unsuccessful because the signal to noise ratio is too low to be detected by spectrophotometers. To overcome this issue, SDS-Page gels were used to determine whether covalent peptide attachment to filomicelles was successful. After performing the chemistry scheme shown in Figure 1 with the F1 peptide, the products were run on an SDS-page gel. The band for unbound peptide can be seen in lane 1; two bands can be seen in lane 2; and a light but broad band can be seen in lane 3 (Figure 3). I postulate that the lower band in lane 2 is peptide conjugated to copolymer and the upper band in lane 2 represents filomicelles that have peptide bound but have not broken up completely. It is not yet understood why the filomicelles appear in lane 3 because they are not charged.

An additional technique that can be used to determine if biomolecules have bound filomicelles is to label the biomolecules with a fluorescent probe. Fluorescent maleimide molecules can be used for this purpose if the biomolecules have solvent-exposed cysteines. I used this scheme to determine if R3 peptide (PVLRSGR**C**AELIQIGFR**C**RA), which contains two cysteines, was covalently attached to the filomicelles after using the chemistry scheme of Figure

1. After formation of two sets of filomicelles with 0.1% COOH-PEO-PBD and 99.9% PEO-PCL, the chemistry scheme described in Figure 1 was used to attach R3 to the modified COOH in the first set of filomicelles. EDC and sulfo-NHS were not added to the second set of filomicelles. A fluorescent maleimide (λ=556) was introduced to both samples. The first set of filomicelles had a 2-fold increase in fluorescence over the control. Fluorescent microscopy images reveal similar results (Figure 4).

It is not clear why the difference in fluorescence between the two samples was so low. One possibility is that the modified COOH group is not well exposed on the PEO brush because the COOH-PEO group is shorter than the PEO group on the other 99.9% of the copolymers. Graduate student Kevin Quigley is currently attempting modify longer PEO groups to PEO-COOH.

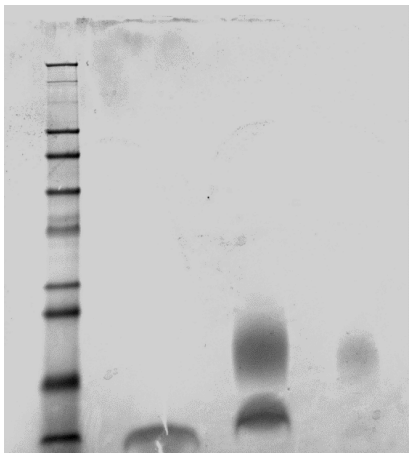

Figure 3. SDS-Page gel showing F1 peptide Attachment.
Ladder, F1 peptide, modified filomicelles with F1 peptide, and regular filomicelles.

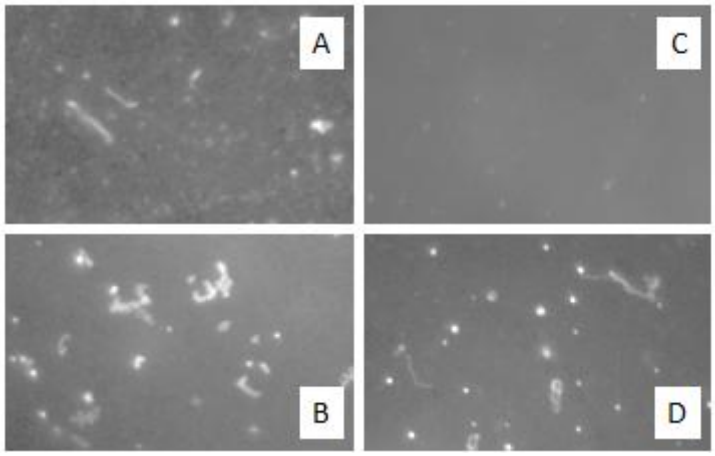

Figure 4. Fluorescent microscopy images showing R3 peptide Attachment.
Filomicelles with R3 covalently bound to the COOH-PEO-PBD groups were incubated with Alexa Fluor maleimide, which binds the cysteines of R3. (A-C) Fluorescent micrographs of (A) R3-filomicelles after incubation with the reactive fluorophore, (B) same as (A) but with PKH26 dye added, (C) Unreactive filomicelles after incubation with the reactive fluorophore (control for (A)), and (D) same as (B) but with unreactive filomicelles.

4.2 Filomicelles as a Model System to Study Drug Delivery Vehicle Degradation

4.2.1 Filomicelle Aging Study

For a drug delivery vehicle to be effective it must maintain its structural integrity in the body. Vehicles that fall apart and leak their contents before reaching the target area in the body are of limited efficacy and can cause side effects. To address this issue, the morphology changes of filomicelles over time were observed using electron microscopy. Electron micrographs showed morphological changes in areas that were degrading presumably due to hydrolysis of the PCL in the filomicelle (Figure 5). Over time, hydrolysis continues to shorten the PCL to the extent that the copolymer then favors spherical conformation [7], which results in the disappearance of cylindrical micelles and appearance of many spherical micelles in the sample by day eleven.

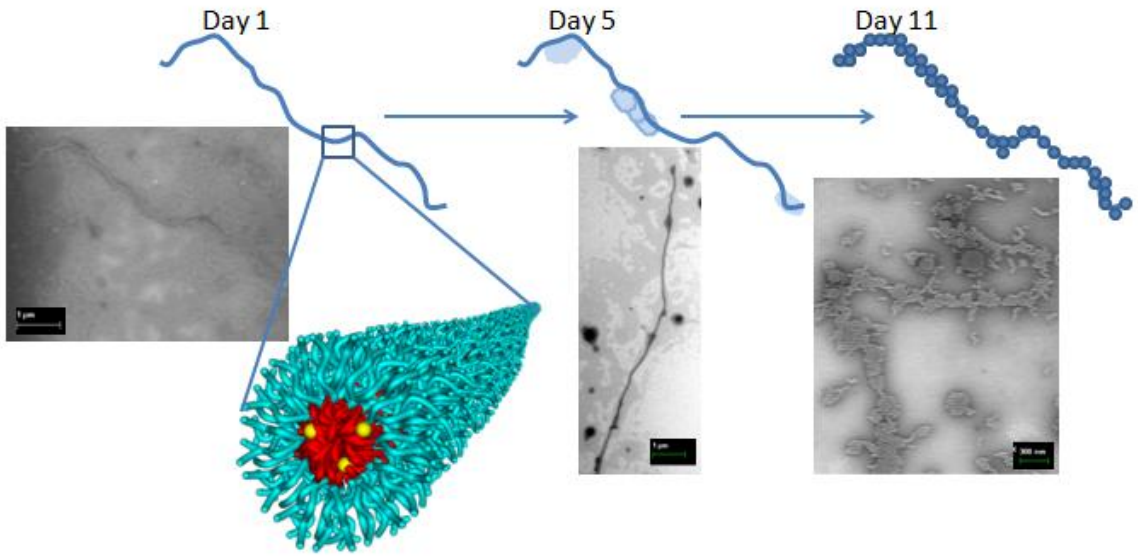

Figure 5. PEO-PCL Filomicelles Degradation.
Filomicelles are represented with blue hydrophilic regions and red hydrophobic regions. The yellow spheres represent dye or drug molecules that can be embedded for delivery. The life of PEO-PCL filomicelles starts with filomicelles with smooth contour (day 1 or t=0 hours). By day 5, t=96 hours, most of the filomicelles show dark areas surrounding edges and corners, which would be the means for the drug (yellow spheres), embedded to leave the carrier. By day 11, t=240 hours, most of the filomicelles have disappeared, leaving spherical micelles.

4.2.2 Accelerated Degradation in High BSA Concentration

I ultimately wish to determine rates of filomicelle morphological changes in physiologically relevant situations. To this end, I incubated filomicelles with serum proteins. Two sets of filomicelles were formed: one set was incubated in 20 mM Tris-HCl buffer, and a second set was incubated in the same buffer with 0.05 mM BSA. Samples were run through Source Q resin to remove unbound proteins (the worms flow past the beads and do not bind). This was done to clear the samples of large proteins, which complicate electron micrograph interpretation. Comparing the two sets of samples suggests that presence of BSA accelerates rate of filomicelle aging by day 3, t=48 hours (Figure 6). The sample with BSA resembles the disappearance of cylindrical micelles and appearance of many spherical micelles in the sample by day eleven from the previous study in water. This implies that the morphology change of the filomicelles would be accelerated when injected and tested *in vivo*; therefore, more studies to determine the effects of protein deposition and shear on filomicelle morphology and stability is needed to prevent premature release of loaded drugs in these degradable micelles.

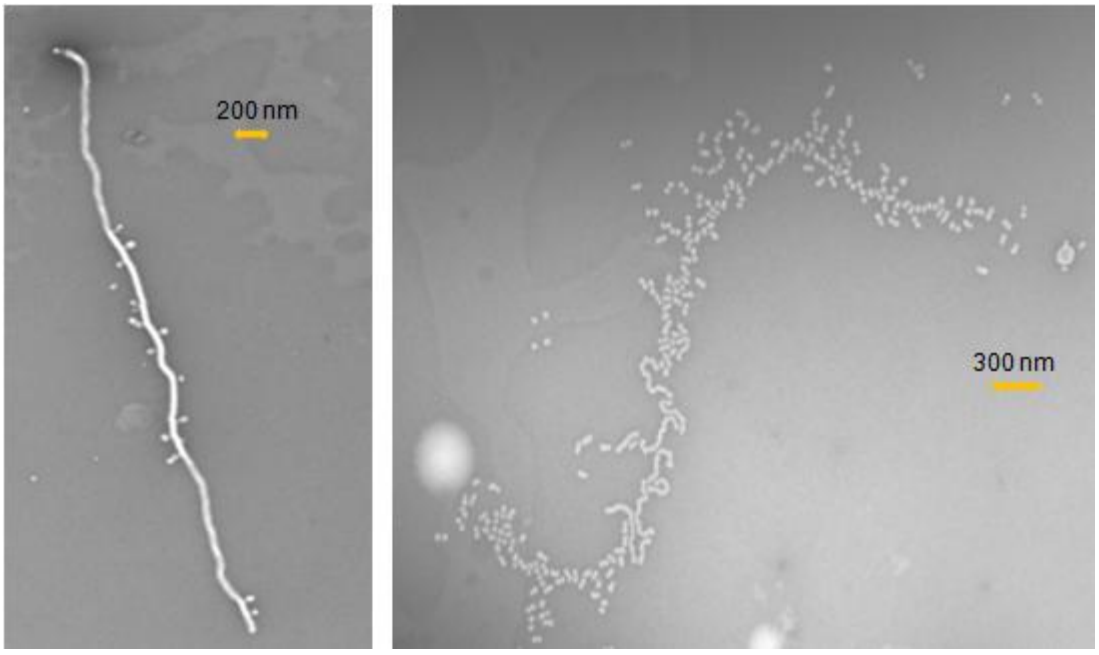

Figure 6. Day 3 PEO-PCL filomicelles in 20 mM Tris-HCl and BSA
Filomicelles in 20 mM Tris-HCl buffer (left pannel). Filomicelles in the presence of 0.05 mM BSA in 20 mM Tris-HCl buffer (right panel).

CHAPTER V
CONCLUSIONS AND RECOMMENDATIONS

My thesis focused on optimizing the covalent conjugation peptides to the exteriors of filomicelles. The peptides were previously identified to bind apical membrane antigen 1 (AMA1), which is expressed on the surface of malaria parasites. I also used high-resolution microscopy techniques to determine changes in filomicelle morphology over time in aqueous media and with the addition of serum proteins.

BSA, R3, and F1 peptides were reacted with the filomicelles and their attachment was checked with Bradford, SDS-Page, and cysteine-maleimide chemistry.

In the second project, electron micrographs of aging PEO-PCL filomicelles reveal unraveling and multi-location degradation over several days in water. Electron micrographs of PEO-PCL filomicelles incubated with BSA concentration shows higher degradation rate, but similar degradation pattern. In the future, this study will extend to monitoring filomicelle structure in plasma and finally live animals.

Two *in vivo* experiments in the Schmidt Lab are proposed for future testing the effectiveness of R3- and F1-filomicelles in the mouse model of the disease. The first is to mix the filomicelles with infected red blood cells (iRBC) and co-inject this mixture into C57BL/6 mice. If the number of parasites is reduced in subsequent blood samples, it will show that filomicelles may be effective in blocking the parasites' replication cycle. The second experiment will be to inject filomicelles into mice that are already infected with the parasite. Again, the number of parasites in circulation will be quantified to determine if filomicelles are appropriate for reducing their replication. In both cases I am hoping for binding of filomicelles to the parasites through the R3-AMA1 and F1-AMA1 interactions (Figure 7).

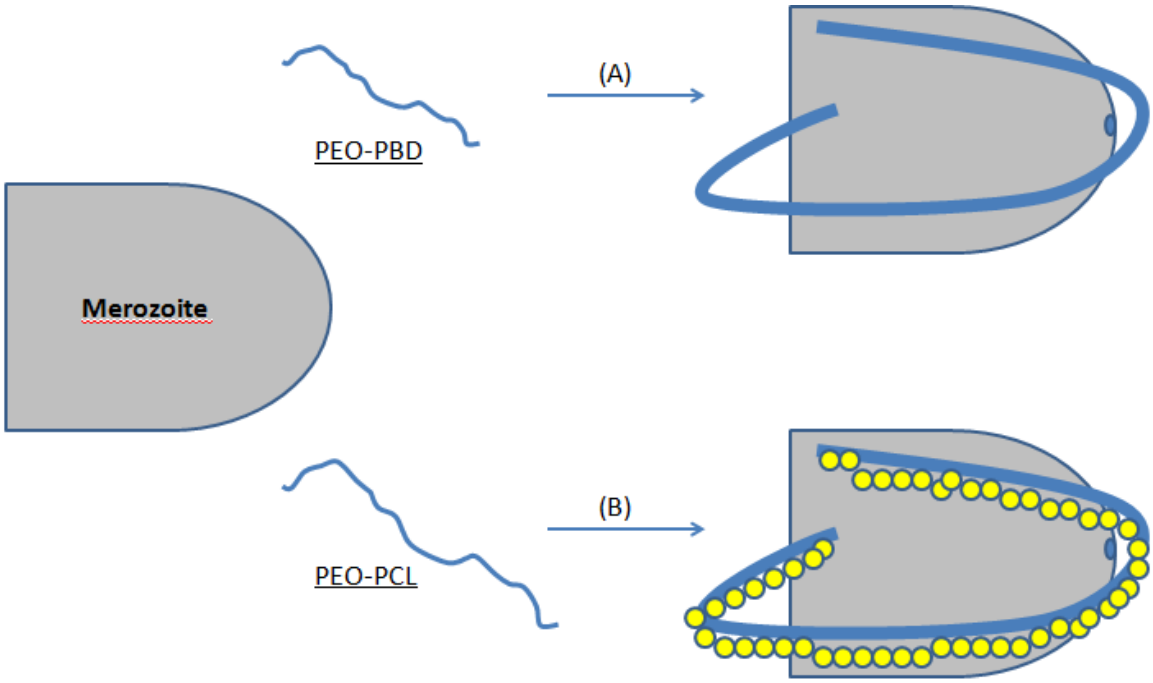

Figure 7. Mechanism of binding malaria parasites by modified filomicelles
The more stable PEO-PBD filomicelles, are expected to bind and ensnare the merozoite (A), and the degradable PEO-PCL filomicelles can be loaded with anti-malarial drugs, and are expected to breakdown and release the drugs (shown in yellow) after localizing near the parasite. These are future projects that were not explored in this work (B).

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APPENDIX

Appendix I. Electron Micrographs of PEO-PCL Hydrolysis in vitro

Each of the samples has been examined as described in section 3.2.1. “Day 1 samples” refer to the samples examined on the day the filomicelles were formed, thus are also samples at t=0 hours. “Day 3 samples” refer to the samples examined on the third day after the filomicelles were formed, thus are also samples at t=48 hours, and so on.

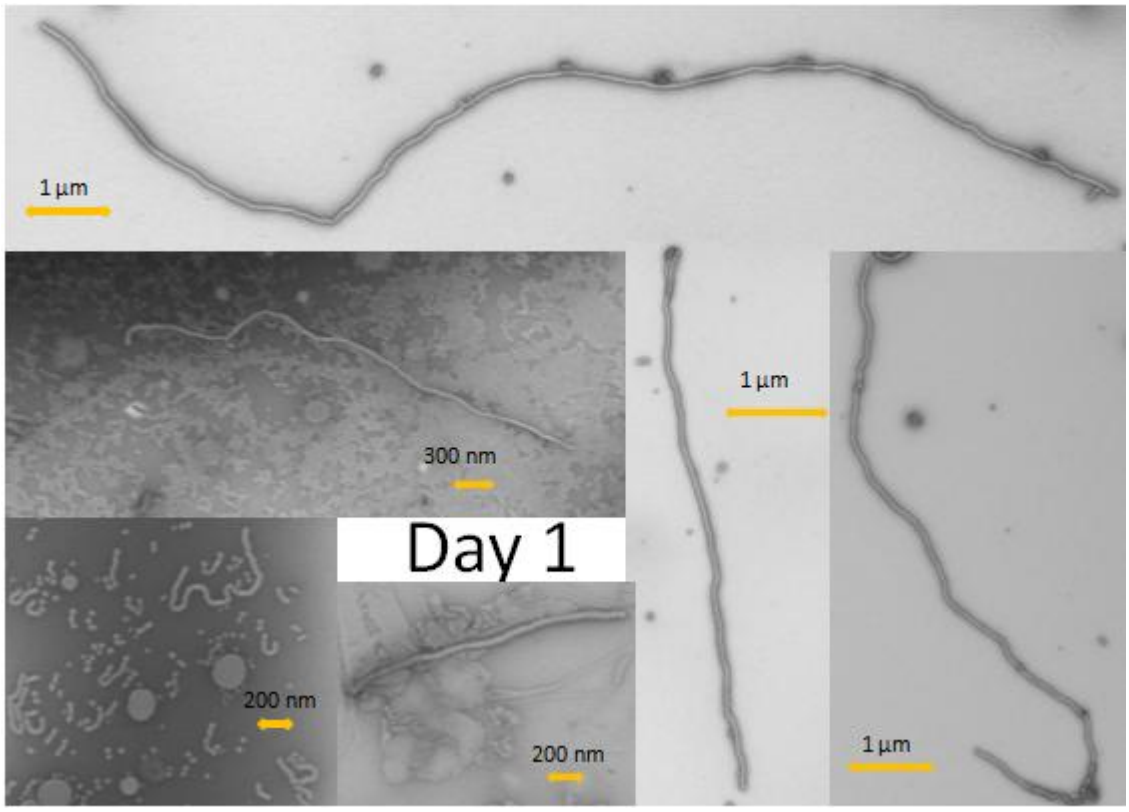

Figure 8. Electron micrographs of PEO-PCL filomicelles in water on day 1.

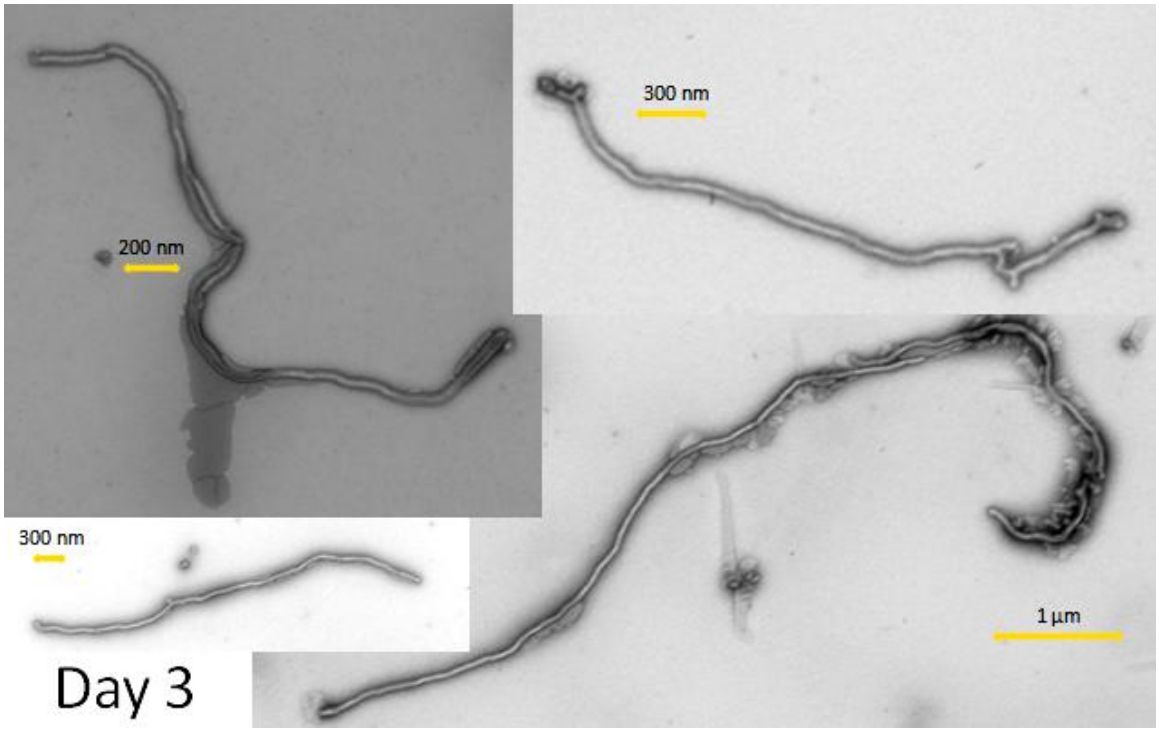

Figure 9. Electron micrographs of PEO-PCL filomicelles in water on day 3.

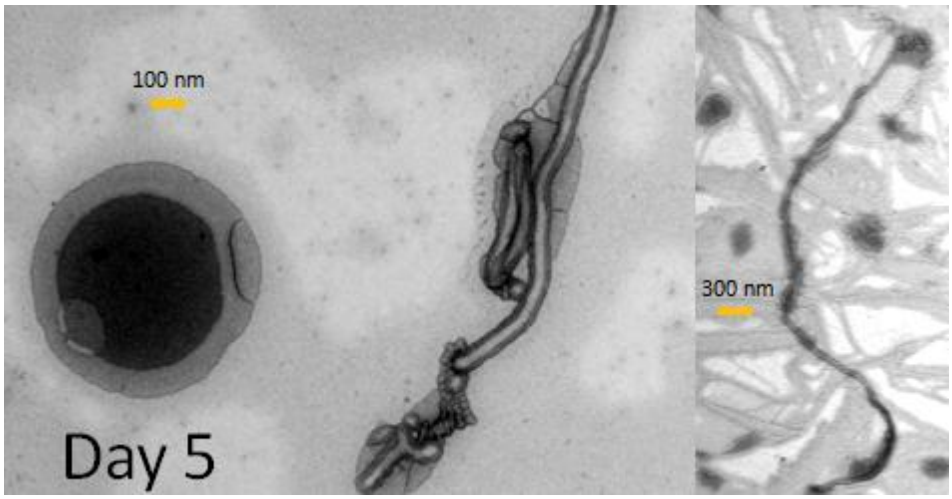

Figure 10. Electron micrographs of PEO-PCL filomicelles in water on day 5.

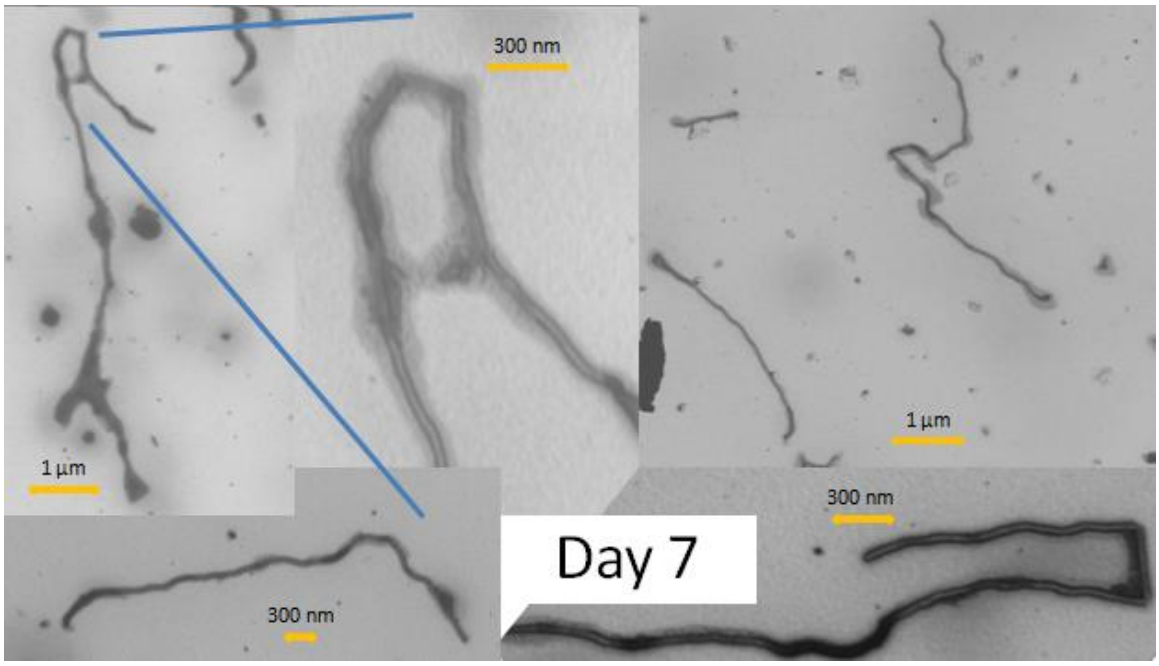

Figure 11. Electron micrographs of PEO-PCL filomicelles in water on day 7.

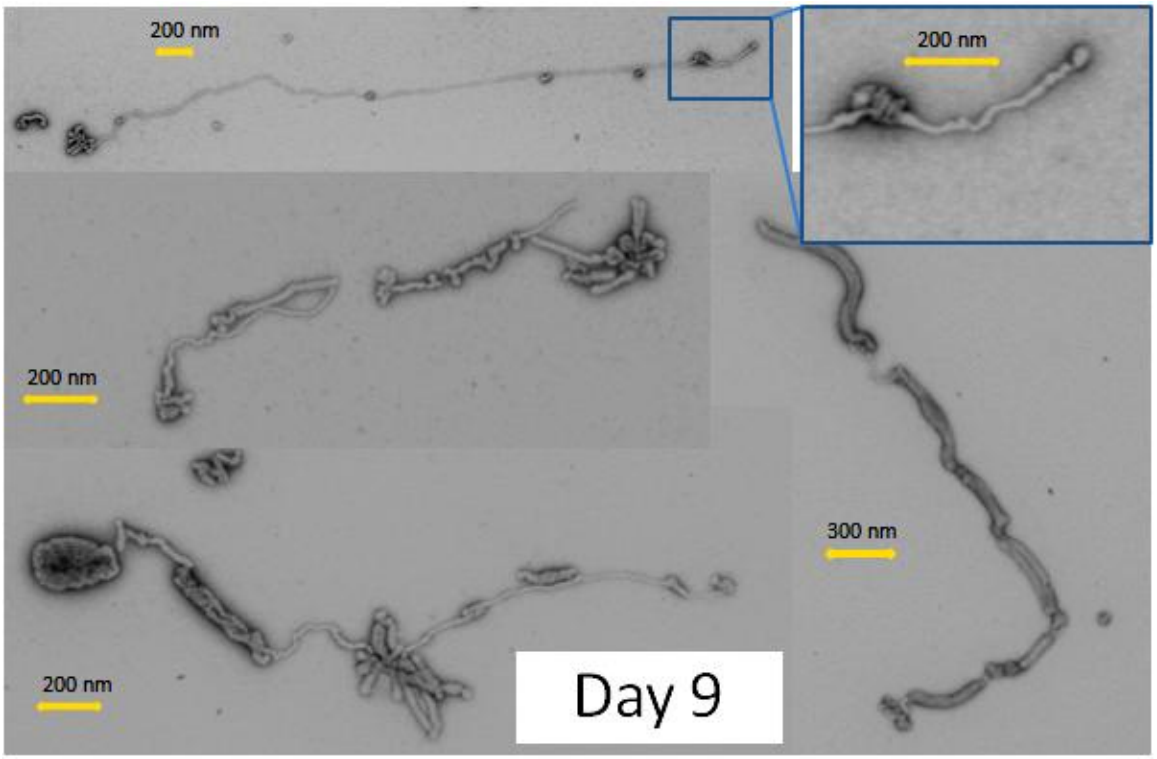

Figure 12. Electron micrographs of PEO-PCL filomicelles in water on day 9.

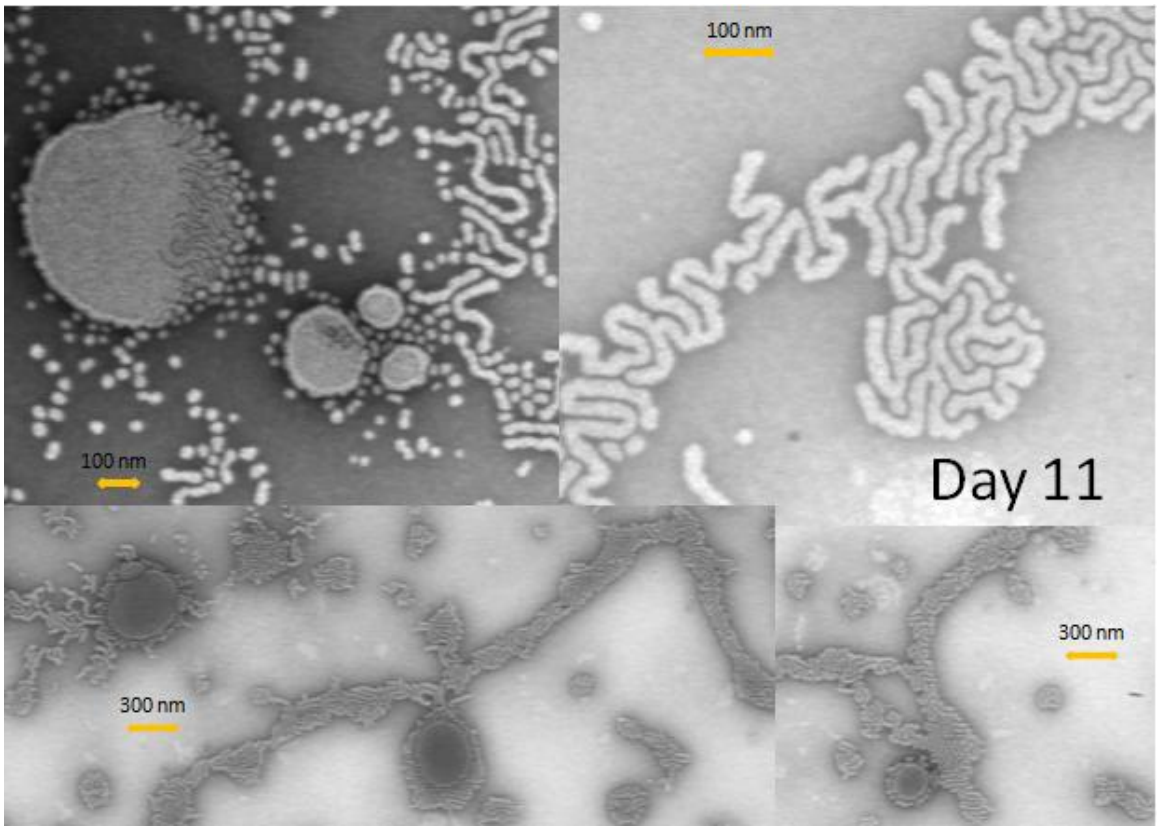

Figure 13. Electron micrographs of PEO-PCL filomicelles in water on day 11.

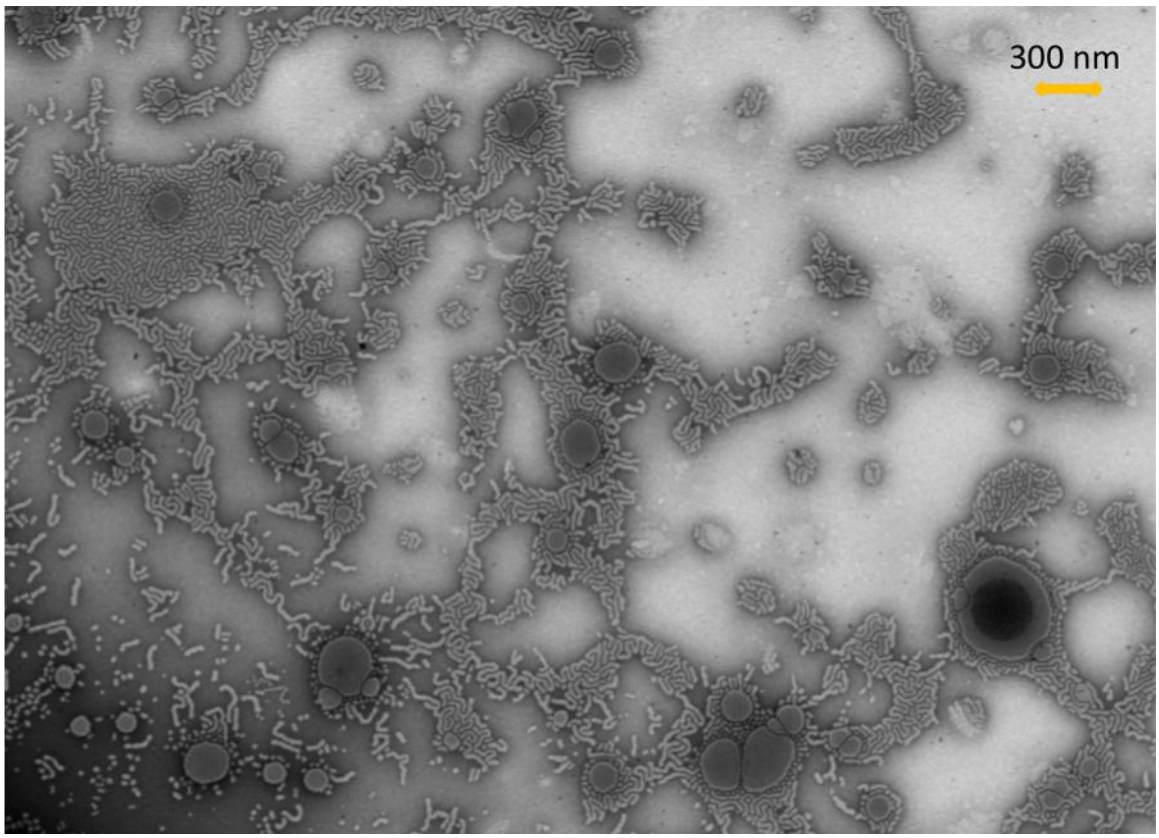

Figure 14. Electron micrograph of PEO-PCL filomicelles in water on day 11.

Appendix II. Supporting Data

Table 1. Protein Bradford Data

	Protein Bradford with BSA Data			
Sample	With/Before	With/After	Without/Before	Without/After
1	0.019	0.014	0.020	0.000
2	0.020	0.015	0.016	0.001
3	0.018	0.015	0.017	0.003
4	0.019	0.016	0.015	0.002
5	0.018	0.013	0.018	0.000
6	0.017	0.012	0.019	0.001
7	0.017	0.016	0.017	0.001
8	0.018	0.017	0.016	0.002
9	0.017	0.014	0.019	0.002
10	0.018	0.013	0.020	0.003
Mean	**0.018**	**0.015**	**0.018**	**0.002**

Table 2. PCL Filomicelle (Worm) Experiment with Alexa Fluor.

Sample	Abs	no PKH 26 (worms/ 5000 μm^2)	with PKH 26 (worms/ 5000 μm^2)
worm with peptide and fluor	0.296	1.92	2.25
plain worm with fluor (control)	0.156	0	2.1

VITA

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