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The Study of PLGA Drug Delivery Systems: Implications for Management of Crohn’s Disease

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I just graduated with my bachelors degree in Chemical Engineering this past May. I am originally from Indonesia, and my journey in the University of Kentucky started in Spring, 2001. During my studies, I was a runner-up in the Physical and Engineering Sciences category of the 2006 Oswald Research and Creativity Program. Also, I was member of Tau Beta Pi, the Engineering Honor Society, and the American Institute of Chemical Engineers (AIChE). In the Summer of 2005, the Nanoscale Engineering Certificate Program (NECP) granted me a summer fellowship that has partially supported the research reported here.

Based on my two previous research experiences and my future plan to attend graduate school in Pharmaceutical Science, I was looking for another research opportunity after I came back from my co-op, a year-long internship at ISP Chemical Inc., Calvert City, KY. At that time, a new faculty member in the department of Chemical and Material Science, Dr. J. Zach Hilt, offered me an opportunity to take part in his joint project with Dr. Razvan Arsenescu in the department of Internal Medicine and Gastroenterology. The project involves developing a polymer-based (PLGA) drug delivery system for Crohn’s disease. Through support from my mentors and the continuation of this project, I was able to work on this research independently for one and a half years and also obtain a strong foundation for the drug delivery-focused PhD program in Pharmaceutical Science that I am about to pursue in Fall, 2006. Moreover, from the preliminary data obtained during this research, we are currently in the process of filing a patent application on this novel device.

Outside of my books, I enjoy playing musical instruments, but mostly keyboard. During the school year, I usually play keyboard at the International Christian Fellowship (ICF). Besides being a worship leader in ICF, I am also involved with lots of other activities that are oriented mostly to international students. These activities include but are not limited to serving as secretary for the International Student Council in 2002-2003 and volunteering in several welcome weeks, activities that are put together to welcome new international students who have just arrived on campus.

Abstract

The purpose of this research is to develop biodegradable drug delivery systems. One of the delivery systems that we have developed is a suture-like structure for the treatment of Crohn’s disease that utilizes a biodegradable polymer, poly(DL-lactide-co-glycolide) acid (PLGA). Crohn’s disease is an inflammatory bowel condition. Aside from the gut involvement, perianal complications develop in the form of enterocutaneous fistulas. Medical management of Crohn’s disease consists of various drugs with immunosuppressive properties. Complicated disease manifestation requires surgical interventions in the form of bowel resection or fistula drainage with Setons. Setons are suture-like devices that help to drain associated abscesses and close secondary fistulous tracts. They are often removed prematurely to prevent entrapment as the proximal and distal ends of the fistula heal. In their current form, Setons are not degradable and are not able to deliver drugs. Thus, the patients typically receive their drugs systemically.

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I am pleased to be writing this endorsement on behalf of Christin Pramudiati. Her submission for publication in Kaleidoscope is original research that she completed within my laboratory in collaboration with Dr. Razvan Arsenescu (Internal Medicine, Gastroenterology). Christin is an exceptional student whose meticulous methods and outstanding work ethic in the laboratory have separated her from her peers. The specific research presented in this submission is novel and has led to a patentable technology that promises to have application in the controlled delivery of therapeutic agents from degradable Setons.
The novel device, biodegradable Setons from PLGA, that we developed promises superior treatment by providing adequate drainage, uniform healing, and a lower level of immunosuppression. Currently, there is a patent application filed on this novel device.

The chronic inflammation is driven by cells of the innate and acquired immune system. Macrophages and dendritic cells (innate immune system cells) sample luminal (gut) antigens and trigger more specialized acquired immune responses. Targeting these cells may break a vicious cycle that creates a chronic state of inflammation within the gut. Drug incorporated-biodegradable nanoparticles can mimic inert particulate matter present in the lumen of the gut. Thus, they may be actively sampled by dendritic cells. Most of these cells remain within the lamina propria of the gut or travel to the gut-associated lymph nodes. By targeting these antigen-presenting cells, we anticipate preventing ongoing activation of B and T cells (acquired immunity) and limiting the systemic exposure to immunosuppressive drugs.

These innovative methods present a more efficient and safe drug delivery that addresses both the chronic gut inflammation (via nanoparticles) and the associated perianal complications (via Setons).

1. Introduction

1.1. Crohn’s Disease

Crohn’s disease is an inflammatory bowel disease that results from a deregulated immune response to the commensal intestinal flora. Aside from the gut involvement, perianal complications develop in the form of enterocutaneous fistulas. Medical management of Crohn’s disease consists of various drugs with immunosuppressive properties. Complicated disease manifestations require surgical interventions in the form of bowel resection or fistula drainage with Setons.

Figure 1.
Types of fistulas based on anatomical locations

Figure 2.
Seton placed in a perianal fistula

The cumulative frequency of perianal fistulas in patients with Crohn’s disease has been reported to range from 14% to 38% in referral centers [1], with 17% to 28% of patients requiring surgery [2]. For treatment of the fistulas, Tacrolimus and Infliximab are the only drugs shown to be effective in prospective, randomized, placebo-controlled studies. The former can be used orally and topically. In addition to side effects associated with large dose administrations, recurrence after treatment cessation is a major problem with both drugs. In other cases, anatomically complex and medication refractory fistulas require surgical treatment. Continuous drainage with surgically placed Setons (see Figure 1 and 2), is an effective, less invasive, complementary, or stand-alone management. However, for treatment with current Setons, the caveats are patient discomfort, associated with long term placement of these non-resorbable sutures, and absence of complete closure.

Therefore, creating a drug eluting, biodegradable Seton from a PLGA system promises great benefits: (i) the incorporated drug will be slowly and continuously released over time while providing targeted delivery of a lower dose, (ii) uniform healing, because the Seton will be present in the fistulous tract even after the distal and proximal ends (see Figure 2) have closed, (iii) Seton entrapment is no longer an impediment, given the biodegradable nature of these polymers. Overall, this novel device will translate to long-lasting therapeutic success, improved safety, and quality of life for these patients.

1.2. Poly (DL-Lactic-Glycolic) Acid (PLGA)

There has been a great amount of research conducted on controlled drug delivery using biodegradable polymer systems. Compared to other biodegradable polymers, poly (D,L-lactide-co-glycolide) acid (PLGA) systems, copolymers of poly(lactic acid)
(PLA) and poly(glycolic acid) (PGA), have generated great interest due to their favorable properties, including excellent biocompatibility, biodegradability, and mechanical strength. Also, PLGA systems have been approved by the Food and Drug Administration (FDA) for use in drug delivery applications. PLGA is advantageous because it biodegrades into lactic and glycolic acids, which can then be metabolized and eliminated from the body as carbon dioxide and water [3].

The degradation of PLGA systems is dependent on their physical properties (i.e., the molecular weight and polydispersity index) and molar ratio of the individual monomer components (lactide and glycolide) (see Figure 3) in the copolymers [3]. PLGA polymers with a 50:50 ratio of lactic acid and glycolic acids are hydrolyzed much faster than those containing a higher ratio of either monomer [4] (see Figure 4).

For degradation analysis purposes, we are mainly focusing on three different ratios of PLGA: 75:25, 65:35, and 50:50. From figure 4, it can be seen that within the range of PLA ratios from 50% to 100%, copolymers with higher ratios of lactide exhibit a higher half-life, thus degrading more slowly. Therefore, as expected, PLGA with a ratio of 50:50 degrades first, followed by 65:35 and 75:25. Degradation of this biodegradable polymer results in a mass loss due to the resorption or dissolution of the material, accompanied by a reduction in molecular weight and changes in mechanical properties such as strength and stiffness.

1.3. PLGA Nanoparticles

The chronic inflammation of Crohn’s disease is driven by cells of the innate and acquired immune systems. Macrophages and dendritic cells (innate immune system cells) sample luminal (gut) antigen and trigger more specialized acquired immune responses. The significant roles that macrophages and dendritic cells play in the regulation of immune responses in the gastrointestinal tract as antigen-presenting cells are well known [5,6,7]. Targeting these cells may break a vicious cycle that creates a chronic state of inflammation within the gut [8]. Drug eluting, biodegradable nanoparticles, can mimic inert particulate matters present in the lumen of the gut. Thus, they may be actively sampled by dendritic cells. Most of these cells remain within the lamina propria of the gut or travel to the gut associated lymph nodes. By targeting these antigen-presenting cells, we can prevent the ongoing activation of B and T cells (acquired immunity) and limit the systemic exposure of immunosuppressive drugs.

In order to target these specific cells, the drug delivery system needs to be designed in the nanoscale range. Particles that are less than 500 nm can cross the microfold (M) cells in the Payer’s patch or be actively sampled by dendritic cells [9]. Therefore, based on the previous research of Tabata [10,11,12,13] and Nakase [8] that has proven the efficient uptake of microparticles by activated macrophages, we attempted to tailor a controlled drug release from PLGA nanoparticles with size of less than 1 μm.

PLGA nanoparticles were obtained by using an oil-in-water emulsion method. With the help of a surfactant, polyvinyl alcohol (PVA), and stirring the emulsion at about 7900 rpm for 2 minutes, 150-250 nm particles can be obtained by using acetone as the solvent. The size of the nanoparticles acquired depends on several different variables, including (i) solvent choice, (ii) PLGA concentration in the solvent, (iii) the concentration, volume, and type of surfactant, (iv) the volume ratio of PLGA and surfactant, and (v) speed of stirring.

1.4. Polyphenols (Green Tea)

Green tea polyphenols are natural plant antioxidants found in tea leaves. Much research has been done to prove that antioxidants can prevent damage caused by free radicals to DNA and other molecules [14]. In addition to several cancer preventive properties, these compounds may reduce abnormal cell growth and inflammation, free the body from cancer causing agents, and restore communication between different cells in the body. Green tea polyphenols have also been shown to be effective in murine models of Crohn’s disease. Polyphenols were used as a model drug system in these experiments.

2. Experimental Method

2.1. Preparation of the PLGA films

Three ratios of PLGA films were prepared by casting a solution of PLGA (75:25, 65:35, or 50:50) and acetone into either aluminum or Teflon molds. Films were dried for at least 48 hours before being peeled and circled with a cork borer into discs with an average diameter of 8.4 mm (see Figure 5). Films with different thicknesses were obtained by dissolving PLGA with acetone. Drug-loaded PLGA films with different ratios (75:25, 65:35, and 50:50) were prepared in a similar manner.
2.2. Degradation Study

A degradation study of different ratios was carried out by analyzing the mass loss of PLGA discs at six time points: 1, 2, 3, 4, 6, and 8 weeks, and that of different thickness of PLGA film done with 10 time points. For error analysis, samples were prepared in triplicate. PLGA discs were immersed in PBS (Phosphate Buffered Saline) (see Figure 6). At each time point, discs were taken from the water bath for mass analysis, while the PBS in the remaining samples was replaced weekly with fresh PBS. The mass of the discs at the various time points was then compared to their initial mass. Then, the degradation (mass at time point divided by initial mass) was calculated.

2.3. Quantification of Polyphenols’ Concentration

Prior to calibration, solutions of green tea polyphenols and PBS pH 7.4 were prepared in various concentrations: 0.0005, 0.001, 0.005, 0.010, and 0.015 mg/ml. Calibration was carried out by measuring the absorbance of each concentration in a UV-Visible Spectrophotometer over the wavelength range of 200 to 250 nm. Polyphenols have their strongest absorbance at 207 nm.

2.4. Drug Release Study

Discs loaded with the drug were immersed in PBS. Samples for each ratio of PLGA (75:25, 65:35, and 50:50) were prepared in quadruplicate. For infinite sink conditions, the PBS solution in each vial was replaced at each time point with a fresh solution. The absorbance of each solution was measured using a UV-Visible Spectrophotometer at 207 nm. Then, the absorbance data was translated to the concentration by using the calibration curve.

2.5. PLGA Nanoparticles

Currently, the most optimized nanoparticles were obtained by the following method: PLGA was dissolved in acetone in a centrifuge tube. If polyphenols or another compound was to be encapsulated, it was added after the polymer was dissolved. To this solution, a surfactant, poly(vinyl alcohol) (PVA), was added, and the resulting emulsion was very briefly hand shaken before being homogenized. The emulsion was then transferred to a vacuum with additional water used as a rinse. To remove the residual acetone, the emulsion was stirred while under a modest vacuum. While stirring continued, a small volume of emulsion was removed from the flask for sizing. All the materials used in the procedure were simply multiplied proportionally by a factor of 1/4, 1/2, 2, 5, etc, to obtain various quantities of nanoparticles. To remove the drug present in the surfactant, the nanoparticles were separated using centrifugation. The supernatant was collected and analyzed using a UV Visible spectrophotometer (at 207 nm) to detect the amount of polyphenols that was not encapsulated in the nanoparticles. The pellets of nanoparticles were combined and re-suspended in PVA solution for release study purposes. Currently, we are still in the process of developing a better, more efficient way to synthesize these nanoparticles and to evaluate the polyphenols released from this system.
3. Results and Discussion

3.1. Degradation Study of Different Ratios of PLGA

The degradation and drug release of PLGA varies depending on the ratio of the PLA to PGA. The 50:50 ratio starts to degrade in 2 weeks, followed by 65:35 and 75:35. Often, we performed molecular weight analyses instead of mass loss analyses to obtain a more accurate degradation study. However, because this research was conducted with a target of in vivo drug delivery, mass loss analysis can give a good representation of the degradation and a rough estimation of the residence time of the system in the body. To obtain a preliminary estimate of the PLGA degradation in vitro, we carried out the study using the PLGA discs. Wu et al. [15] reported the study of various ratios of PLGA in the form of rods. The difference in the geometries (between discs and rods) might give a slightly different degradation response. However, an in vivo study using Seton-like structures is being planned to be carried out in the near future. Thus, more accurate degradation of PLGA and its inflammatory response will be studied further.

The summary of the preliminary PLGA degradation study based on different ratios is presented in Figure 7. The relatively large error bars associated with the figures were caused by several factors, including variance of PLGA weight percent in the system and mass loss during solution replacement in the experimental methods. The use of a filter and mesh could be a way to reduce the error. Also, by making larger PLGA films, all the discs used for the experiment will be somewhat more homogenous, thus, decreasing the error.

3.2. Degradation Study of Different Thicknesses of PLGA Film

Figure 8 indicates that the degradation of PLGA also varies depending on the thickness of the film. The thicker PLGA film degrades a lot faster compared to the thin one. This is due to the fact that the thicker PLGA film tends to lower the pH of the media, causing the film to be even easier to degrade. Due to difficulties in handling and weighing the discs, large errors were generated with the thinnest film.

3.3. Quantification of Polyphenols Concentration

Figure 9 shows the correlation between the concentration of the polyphenols and their absorbances, obtained from a UV-Visible spectrophotometer. The absorbance data used was normalized at 207 nm, where polyphenols absorbance is at its maximum. By determining the absorbance of five different concentrations (listed in section 2.2), the constant value of $e_b$, 169.49, can be obtained from the linear plot.

| Table 1. Summary of particle average size and the standard deviation due to the effects of various variables |

<table>
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<tr>
<th>Mass of PLGA (mg)</th>
<th>Concentration of PVA (mg/mL)</th>
<th>Volume of PVA (mL)</th>
<th>Ratio of PLGA and PVA</th>
<th>Polyphenol ratio to acetonitrile (mg/mL)</th>
<th>Homogenization Speed (rpm)</th>
<th>Homogenization Time (min)</th>
<th>Avg Size (mm)</th>
<th>Std Dev (mm)</th>
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<td>3</td>
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following Beer’s law. In the release study, the polyphenols incorporated in the discs were slowly released to the PBS solution in which they were incubated. At its time point, the absorbance of these PBS solutions was measured using a UV-Visible Spectrophotometer. Thus, by utilizing the attained \( e_b \) value, the only unknown variable, concentration \( (c) \), can be calculated from the measured solution absorbance \( (A) \).

3.4. Release Study

From the release study data summary (see Figure 10), it can be seen that the release of drug is directly related to the physical properties of the PLGA system. Here, the ratio of the mass released at time \( t \), \( M_t \), over mass released at time infinity, \( M_{\infty} \), is plotted versus time. The PLGA with a ratio of 50:50 released the polyphenols first, followed by 65:35 and 75:25. Using this release data, we can possibly tailor the release of the drug in a similar dosage over a period of up to 4 to 5 months. The errors in the release study data were partially generated by the inhomogeneous distribution of polyphenols in the PLGA films, which results in a slightly different loading concentration in each disc being studied.

3.5. Nanoparticles

Table 1 shows some values of the particle average size along with standard deviations due to the combinations of different variables. There are many factors that determined the size of the nanoparticles, including: the ratio of the PLGA over the surfactant, the amount of PLGA in the solvent, concentration and volume of the surfactant, homogenizing time and speed, solvent and surfactant choices, and many more. By utilizing acetone as the solvent to dilute the PLGA and PVA as the surfactant, the smallest size of particles obtained during experiments were from a ratio volume of PLGA and PVA of 3:5. At this volume ratio, nanoparticles with the size of 150-250 nm have been successfully synthesized.

Figure 11 shows the size of the particles, 202.15 \( \pm \) 41.9 nm, that were used for the release study. The size of the particles was confirmed using a scanning electronic microscope (Figure 12). The “strings” observed in Figure 12 were most probably formed due to excessive PVA that still had not been removed from the system. PVA was kept in the system to avoid particles aggregating. Again, we are in the process of developing a better, more efficient method to synthesize these nanoparticles, and the release study of nanoparticles still also needs to be further developed.

4. Conclusions

Through this research, we have successfully developed a degradable Seton from PLGA that also will be able to release drugs locally. Because the PLGA degradation rate in any form of device can be tailored based on its dependency on the physical properties, such as ratio, molecular weight, and polydispersity index, this device promises a better therapeutic system for Crohn’s disease patients. With these preliminary experiments, we were able to determine the degradation rate of the PLGA at different ratios (75:25, 65:35, and 50:50) and thicknesses using mass loss analysis. Although there were errors associated with the mass loss analysis, trends were observed. The release of polyphenols in PLGA discs with different ratios was quantified using the calibration data obtained. A Phase I clinical study for patients with fistulizing Crohn’s disease is in the process of being developed. We are also attempting to optimize the nanoparticle production and ways to analyze the release of a model drug (e.g., polyphenols). Overall, we believe that these drug delivery methods present a more efficient and safe drug delivery that addresses both the chronic gut inflammation (via nanoparticles) and the associated perianal complications (via Setons).
Acknowledgements

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