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Incorporation of Tetracycline Hydrochloride into Electrospun Fibrinogen: a study of mechanical properties and time release

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INCORPORATION OF TETRACYCLINE HYDROCHLORIDE INTO ELECTROSPUN FIBRINOGEN: A STUDY OF MECHANICAL PROPERTIES AND TIME RELEASE

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Biomedical Engineering at Virginia Commonwealth University.

by

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Acknowledgement

I could never say that I accomplished this alone. I could write another thesis with nothing but appreciations, but it would appear as a cross between a high school yearbook inside cover and an Academy Award speech. Nonetheless, great thanks go to Dr. Gary Bowlin, my advisor and my friend. Thanks for making sure I could afford to eat. Dr. David Simpson always knew what to say when I had a question – it just wasn't always the answer, but it was always appreciated. Dr. Gerald Miller offered incredible advice and counsel, and excellent fashion and hygiene commentary. Dr. Clive Baumgarten fielded my questions and offered answers replete with new questions to ask. Dr. Glenn Van Tuyle was very patient to have a strange graduate student ask strange, often unintelligible questions on his lunch hour. Thanks all around.

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My parents have been so patient with my days of whining and of exhilaration. I know they remain incredulous of why someone would want to be in school this long, and still want more. I promise to figure it out soon.

Most importantly, I thank Nathalie. Her timing and presence are perfect. Nothing else I could write could do her justice.

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List of Abbreviations

HFP	1,1,1,3,3,3-Hexafluoro-2-propanol
mg/mL	Milligrams per Milliliter
ECM	Extracellular matrix
PGA	Polyglycolic acid
kDa	Kilodaltons
FRPA	Fibrinopeptide A
FRPB	Fibrinopeptide B
FDA	Food and Drug Administration
10 X MEM	Minimal Essential Medium
SEM	Scanning Electron Microscopy
mL/hr	Milliliters per Hour
kV	Kilovolts
mm	Millimeters
rpm	Revolutions per Minute
mm/s	Millimeters per Second
cm	Centimeters
mm/min	Millimeters per Minute
Hz	Hertz

4000x	Magnification Factor 4000
PBS	Phosphate Buffered Saline
µL	Microliters
µg/mL	Micrograms per Milliliter
ANOVA	Analysis of Variance
w/w	Percentage by Weight
µm	Micrometers
μm^2	Square Micrometers
SD	Standard Deviation
Tetra	Tetracycline Hydrochloride
min	Minutes
110 w/ 2.5%	110 mg/mL fibrinogen with 2.5% Tetracycline hydrochloride by weight
110 w/ 5%	110 mg/mL fibrinogen with 5% Tetracycline hydrochloride by weight
110 w/ 10%	110 mg/mL fibrinogen with 10% Tetracycline hydrochloride by weight
120 w/ 2.5%	120 mg/mL fibrinogen with 2.5% Tetracycline hydrochloride by weight
120 w/ 5%	120 mg/mL fibrinogen with 5% Tetracycline hydrochloride by weight
120 w/ 10%	120 mg/mL fibrinogen with 10% Tetracycline hydrochloride by weight
130 w/ 2.5%	130 mg/mL fibrinogen with 2.5% Tetracycline hydrochloride by weight

130 w/ 5%	130 mg/mL fibrinogen with 5% Tetracycline hydrochloride by weight
130 w/ 10%	130 mg/mL fibrinogen with 10% Tetracycline hydrochloride by weight

Abstract

INCORPORATION OF TETRACYCLINE HYDROCHLORIDE INTO ELECTROSPUN FIBRINOGEN: A STUDY OF MECHANICAL PROPERTIES AND TIME RELEASE

By Charles Dudley Anderson, Jr., B.S.

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Biomedical Engineering at Virginia Commonwealth University.

Virginia Commonwealth University, 2004.

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Electrospinning has the capacity to create fibers of natural or synthetic polymers with dimensions that are similar to analogous fibers in native tissue. Mats consisting of fibers of these sub-micron dimensions have shown promise in provoking little immune response and in offering a habitable environment for cell proliferation. Fibrinogen is a natural protein capable of being

electrospun and offers the benefit of existing as part of the natural coagulation cascade. Mats of fibrinogen could be utilized as possible hemostatic dressings or as an early scaffold for cell migration for either wound repair or tissue engineering. The addition of antibiotic into such a dressing/scaffold could prevent infection during healing/incorporation. The goal of this study was to determine any effect that the addition of the antibiotic tetracycline hydrochloride (0%, 2.5%, 5%, 10% by weight) would have on the mechanical properties of electrospun fibrinogen (110 mg/mL, 120 mg/mL, and 130 mg/mL concentrations). Also, the time release of tetracycline from electrospun fibrinogen was investigated. The results show no significant effect of tetracycline loading on the mechanical properties of electrospun fibrinogen under the conditions of this study. The results of the release study demonstrate that initial tetracycline release is dependent upon loading percentage. The release data also demonstrate that the amount of tetracycline released is approximately 20-30% of the tetracycline in the original solution and that the release occurs within approximately 4 hours, with no significant release thereafter. This study demonstrates the feasibility of tetracycline in electrospun fibrinogen

for the purposes of short term drug release in fibrinogen-based technologies.

Introduction and Background

The goal of tissue engineering is to create analogues to native tissue for the purpose of replacement or for the facilitation of repair. Depending on application, the structure and composition of these analogues does differ. Regardless of application, the products of tissue engineering should not provoke a damaging immune response, should offer a successfully attractive framework for new or native cell establishment, and should possess mechanical and chemical properties similar to the replaced tissue. The purpose of this study was to incorporate tetracycline hydrochloride into a fibrinogen scaffold possibly to be used for *in vivo* wound repair or as a basis for the *in vitro* development of a tissue engineering scaffold. The incorporation of an antibiotic such as tetracycline should assist in the resistance to infection of a maturing wound dressing or tissue scaffold, but the effects of its addition on the mechanical properties of the plain fibrinogen scaffold must be explored. In addition, the

release of the tetracycline from the fibrinogen in solution should be of sufficient concentration and duration to elicit therapeutic effects.

Tissue Engineering

Tissue engineering is devoted to creating new tissue that can be accepted and utilized by the body. A critical focus of tissue engineering is the framework with which the cells coexist, the extracellular matrix (ECM). Communication between the ECM and the cells guides growth, repair, and morphology[1].

ECM analogues that more closely resemble native ECM will be better tolerated and incorporated *in vivo*. One characteristic of ECM analogue closely related to success *in vivo* is fiber size. When the size of fiber approaches that of the size of natural components of ECM, cells attach more easily, readily, and with better success. Other critical aspect of successful biomimicking scaffolds is the minimization of an immune response. This is affected greatly by the material from which the scaffold is constructed. Although some synthetic polymers can provoke a negligible immune response, and others still can be

degraded and metabolized by the body, greater success is found in the use of natural polymers such as collagen, elastin, or fibrinogen. Collagen is the major component of native ECM, and elastin exists in some various tissues of differing mechanical properties (i.e. blood vessels). Fibrinogen exists in tissues in the early stages of the repair of damaged tissue, and it yields fibrin, which may be thought as a 'provisional matrix'.

The above tissue engineering concepts could be utilized to create or recreate native tissue such as skin, cornea, or liver. For these applications, the most successful cell scaffold (collagen, elastin, fibrinogen, or blends) would be the best choice. If the creation of a wound dressing is the target, fibrinogen is the obvious choice. Not only would it have the advantage of being a native protein and possibly very acceptable to the native tissue, but fibrinogen also has the advantage of existing in the natural coagulation cascade, and so for a bleeding wound would have both wound healing and hemostatic effects. The possible incorporation of antibiotic into a wound dressing might offer better success in healing due to a reduction in infection.

Electrospinning

Electrostatic spinning, or electrospinning, is a process that utilizes electrostatic forces to create small diameter fibers from the solution of a polymer. The first successful development of electrospinning was in 1934 by Anton Formhals, who received a patent on the technology. Formhals electrospun small fibers of cellulose ester from a solvent of acetone and alcohol.[2] The process can generate generous amounts of fibers at the sub-micron level, smaller in diameter than any standard extrusion process.

Electrospinning is based on the electrostatic repulsion within a polymer solution, and the subsequent electrostatic attraction of the polymer solution (or its solute) to a grounded electrode. It is a variant of electrospraying, where droplets of charged solution are attracted to a grounded electrode by an external electric field. Electrospinning succeeds in the creation of fibers, rather than droplets that leave the charged solution. During electrospinning, a solution of polymer forms a droplet at the end of a capillary tube, or syringe needle, in this setup. The droplet is maintained by the surface tension within the solution, and the applied electric field

to the solution forms the droplet into a "Taylor cone", as the electric repulsion begins to overcome the surface tension. When the electric repulsion within the solution reaches a critical value, a charged jet of the solution leaves the Taylor cone.[3] This jet, attracted to the grounded electrode, becomes more concentrated in both solute and charge density as the solvent evaporates. Progressively, a thin charged fiber is left behind that travels to the ground electrode. A diagram of an electrospinning apparatus is shown below (Figure 1).

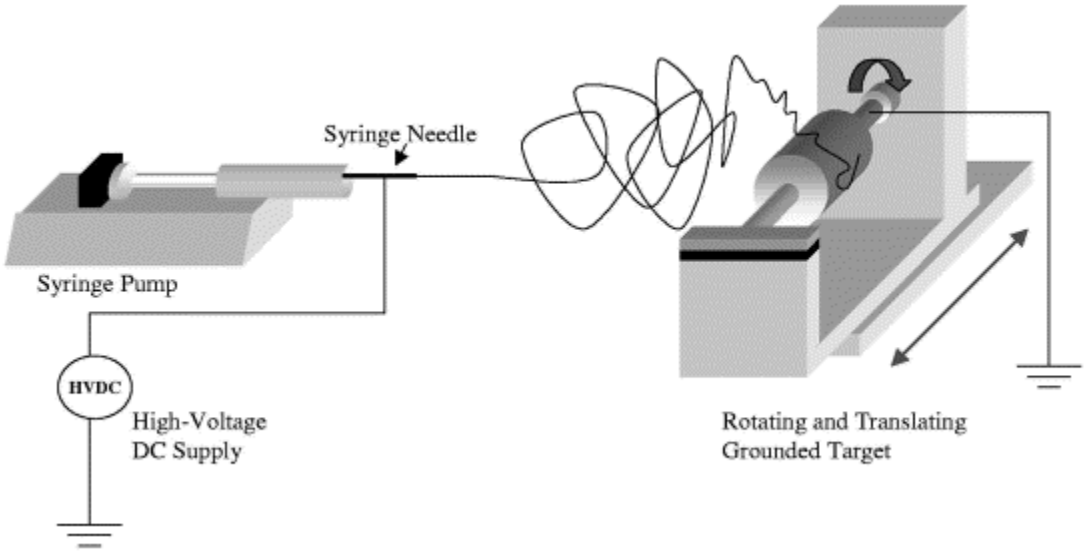

Figure 1 : Schematic of electrospinning apparatus

The efficacy of this process, as well as the final fiber product, are affected by a litany of factors, including, but not limited to solution polymer concentration, viscosity of solution, voltage between solution and ground electrode, the distance between the Taylor cone and the ground electrode, and environmental conditions such as humidity and temperature.[4]

Electrospun fibers, because of their small diameters, have seen much interest not only in the textile field, but also in that of biomedical research. The small diameter fiber is more attractive to cell attachment, because of its similarity in size to native ECM components, which allow for the cell to attach to several fibers in a more natural geometry, rather than the singular flattened orientation that an attached cell would experience on a large diameter fiber. Much research had been conducted with synthetic resorbable polymers, such as PGA, and natural polymers, such as collagen and fibrinogen.[5,6,7] These studies have shown the utility of electrospun polymers as scaffolds for tissue engineering.

In addition, an electrospun mat of synthetic or natural polymer could be used as a vehicle for drug delivery. The target drug could be incorporated into the

polymer solution to be spun, and should be uniformly distributed with the final mat product. Other technologies for drug delivery have proven useful (films, slow-release pelletized formations, polymer disks), but none of these technologies would enjoy the full cell-friendly benefits of electrospun materials.[8,9] A study by Kenawy et al. utilized electrospun (and film deposited) synthetic polymers with incorporated tetracycline and compared the release characteristics of the same to a film based commercial product.[10] In this study, two synthetic polymers, poly(ethylene-co-vinylacetate) and poly(lactic acid), and a blend of the two, were electrospun with 5% tetracycline hydrochloride. Samples of these mats were placed in a buffered solution, and the concentration of tetracycline in that solution was determined using spectroscopy. These polymers were chosen for their success in medical products.

The use of a natural polymer, such as fibrinogen, should remove much concern of immunogenicity and offer a more favorable attachment scaffold for *in vivo* infiltration of cells in such an application.

Fibrinogen

Fibrinogen Structure

Fibrinogen is a large protein of mean molecular weight 33600 kDa, with 95% of the molecular weight attributed to six amino acid chains (2 Aα, 2 Bβ, and 2 γ chains). These six amino chains are arranged in two identical halves, each half consisting of a single Aα, single Bβ, and single γ chain, with disulfide bonds linking these chains and the two AαBβγ halves together (a total of 29 disulfide bonds). This arrangement often leads to fibrinogen described as a dimer of the AαBβγ monomer[11]. The Aα chain is the largest of the three chains, with 610 amino acid residues. The Bβ chain and γ chain have 461 and 411 residues, respectively. The Bβ chain and γ chain possess sites of bound carbohydrates, which account for approximately 5% of the molecular weight of fibrinogen[12]. Extensive data regarding the sequencing and variance of inter-species fibrinogen exists, and is beyond the scope of this study, yet, further research may show some effect of varying sequences on biochemical activity.

Fibrinogen Function and Activity

Fibrinogen is a crucial component of the coagulation cascade. When vascular injury occurs, the pathway leading to blood coagulation is activated. There exist two different pathways of coagulation, the intrinsic and extrinsic pathways. Both of the pathways involve sequential activation of different blood components, yet both arrive at the same destination, the activation of Factor X to Factor Xa. It is here that the pathways converge, and where the common pathway begins. The common pathway will be discussed in modest detail, for it is here where fibrinogen plays an important part as substrate. A diagram of the common pathway is presented in Figure 2.

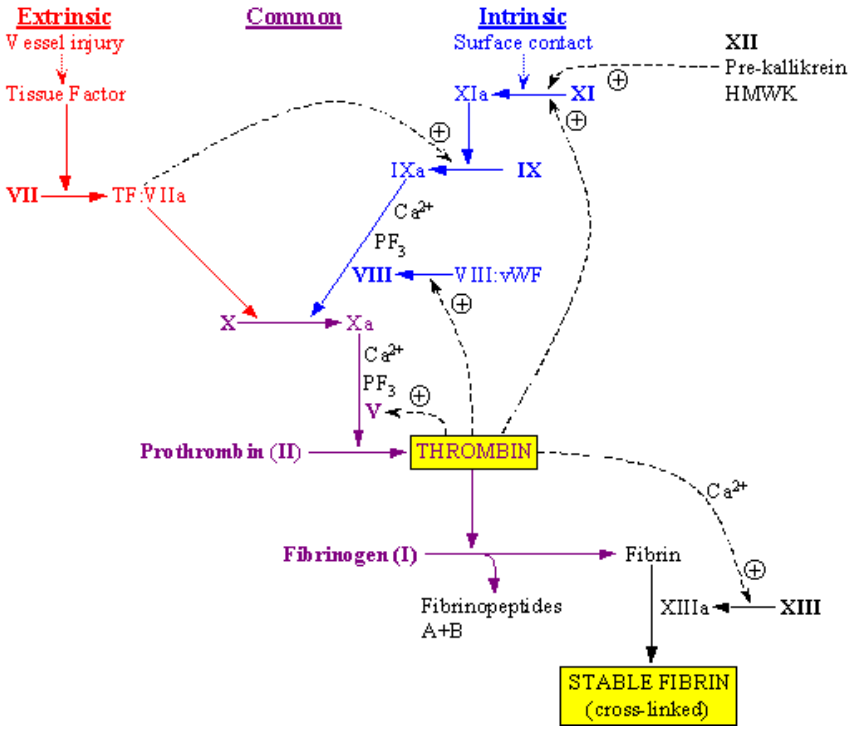

Figure 2 : Diagram of coagulation cascade [13]

Factor X is activated (Factor Xa) while attached to the membrane of platelets at the injury site. The biochemical function of Factor Xa is that of a serine protease, a chemical capable of breaking the amino acid backbone of a protein at a specific location. Platelet-attached Factor Xa forms a complex with another blood-borne component, Factor Va, and, in the presence of calcium, converts prothrombin to thrombin. The protease action of Factor Xa is responsible for this conversion, assisted by

the cofactors Va and free calcium. It is thrombin that truly controls coagulation, through two distinct actions. Thrombin activates yet another inactive blood component, Factor XIII to Factor XIIIa. Thrombin also cleaves small peptide chains from the fibrinogen molecule to form soluble fibrin.[12]

The action of thrombin on fibrinogen is targeted at portions of the amino acid backbone of the N-terminal end of the Aα and Bβ chains of fibrinogen, releasing fibrinopeptides A and B (FRPA and FRPB). These fibrinopeptides are used as indicators of thrombin activity in laboratory tests. The absence of these fibrinopeptides exposes regions on the original fibrinogen molecule (now fibrin) that form weak hydrogen bonds with like molecules, forming a loose clot at the injury site.[12] The presence of Factor XIIa (the other thrombin-activated component) creates covalent bonds between fibrin molecules through transglutaminase activity, forming an insoluble fibrin clot[14]. The now insoluble fibrin clot traps platelets as the clot accumulates at the injury site.

Fibrinogen in Tissue Engineering

Because of the native appearance of fibrinogen to cells of the body, it is possible that fibrinogen could be utilized as a possible scaffold material for tissue engineering. Upon exposure to thrombin, fibrinogen will yield fibrin, which is the first ECM that healing tissue will possess. The fibrin clot offers stability and structure to the reforming tissue. The fibrinopeptides cleaved from fibrinogen signal immune and endothelial cells to the site of injury, beginning the healing process via inflammatory response and angiogenesis[15]. Other invading cells begin to digest the provisional fibrin plug and deposit permanent ECM materials such as collagen.[16] More so than collagen or elastin, and certainly any synthetic polymer, fibrin should be the ideal healing scaffold for a wound, since it is the material that tissue would lay down naturally. Many studies have been documented showing the effectiveness of the fibrin matrix is supporting cell growth and tissue development in musculoskeletal, epithelial, and urological applications.[15]

Fibrin-Based Technologies

The use of fibrin in wound treatment is storied, with reporting of such dating to 1909 by Bergel, who used dried plasma to arrest surgical bleeding. Patches of pure fibrin have documented use in the decade to follow. Early uses of fibrin included aid to skin grafting and nerve repair.[17] The usage of fibrin/fibrinogen products is somewhat clouded today due to possible transmission of viruses from a natural blood product, which led to an FDA ban on usage of pooled fibrinogen concentrates in 1978, leaving American clinicians with screened blood-bank and autologous blood as the sole source for fibrin products.[18]

The current fibrin-based products come in two forms, dry and liquid. Both involve simultaneous application of fibrinogen and thrombin, sometimes with the addition of Factor XIII and calcium. The dry product contains fibrinogen and thrombin, in a freeze-dried or frozen state, to prevent their reacting. Stored in air-tight packaging, this product will yield fibrin upon exposure to air, and more appropriately, to a wound. The United States Army has funded and overseen development of a technology similar to this for use on the battlefield.[19] The liquid product is delivered as a simultaneous spray upon the wound of

fibrinogen and thrombin, forming a layer of fibrin that immediately clots the bleed. Both technologies show great efficacy in their ability to deliver concentrations of these proteins at levels much higher than in blood.[20] Both of these products, however, have their drawbacks. The dry sealant has difficulty in handling because of its activation upon exposure to moisture (even humid air will begin the reaction). The liquid version has a long preparation time because of the necessary dissolution of components, and therefore would not be optimal in an emergency setting.

Possible Fibrinogen-Based Technology

If fibrinogen alone is used as the substrate for fibrin, the stability, ease of use, and longevity of the product is increased. Without simultaneous thrombin administration, the fibrinogen-based product is reliant upon the thrombin of the wound for the formation of fibrin. However, the rapid attainment of fibrin with better handling could make such a product more viable and practical. With the technology of electrospinning, an electrospun fibrinogen wound dressing would have the added benefit of small fiber diameter which could accelerate

wound healing. Wnek et al. demonstrated the feasibility of electrospinning fibrinogen through the determination of an appropriate addition (10 X MEM) to the common electrospinning solvent HFP.[10] Once the appropriate solvent was determined, Wnek et al. electrospun different concentrations of bovine fibrinogen and measured the fiber diameters of the final product using SEM microscopy and image processing software. Their results indicated that fiber diameter could be controlled by the adjustment of the concentration of the spinning solution.

Tetracycline

Structure and Usage

Tetracyclines encompass a large group of antibiotics of similar structure and action. The basic structure is one of four fused six carbon rings, with several positions that carry different functional groups depending on the particular tetracycline species. The first isolated tetracycline was chlortetracycline (aureomycin), which was isolated from *Streptomyces aureofaciens* in 1948 by Benjamin Duggar. The removal of the chlorine from the 7 position of chlortetracycline yielded tetracycline. Tetracycline is considered the first drug that outperformed the natural drug source from which it originated. This isolation is credited to Lloyd Conover in 1955.[21] Other members of the family include oxytetracycline, doxycycline, and demeclocycline.[22] The structure of tetracycline is shown in Figure 3. It exists in use as a hydrochloride salt to increase solubility. Tetracycline alone is a basic compound. Widely used as an oral antibiotic, tetracyclines had the largest antimicrobial index of known antibiotics until the 1950s.[23]

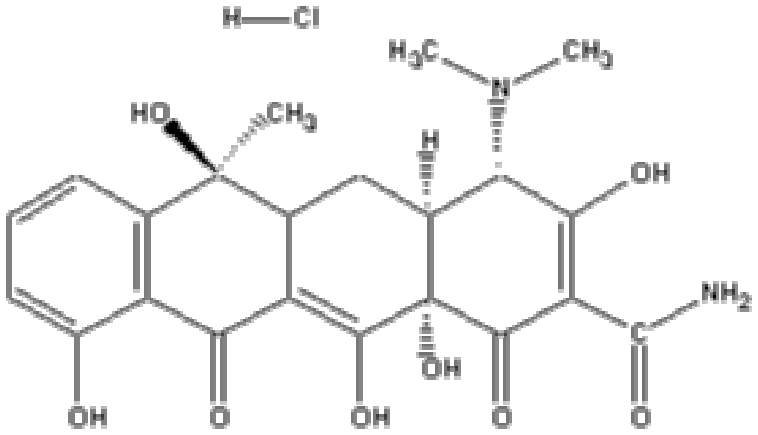

Figure 3 : Tetracycline structure[24]

Method of Action

The tetracyclines are mostly bacteriostatic, damaging the ability of bacteria to reproduce, and are therefore only effective on those bacteria in stages of reproduction. Tetracyclines work by inhibiting protein synthesis in bacteria by binding to the 30S ribosome, thereby halting the progressive translation of proteins. Considered very effective against *E. Coli*, bacterial ulcers and many topical infections, tetracyclines find use today in prophylaxis of acne, topical anti-inflammatory applications and in dental implants.[25]

Project Synopsis

This project explores the effect of tetracycline hydrochloride on the mechanical properties of electrospun fibrinogen, and the release of tetracycline from the same. Tetracycline was added to the solutions at the beginning of the electrospinning process, and the products of the electrospinning were subjected to mechanical testing. Also, time studies were conducted of independent samples of various preparations of fibrinogen/tetracycline to observe the release of the drug into a buffered saline solution. The intended outcomes of this research were to observe and quantify any change in mechanical properties because of tetracycline loading, as well as to observe and report the effects of different fibrinogen concentrations and tetracycline loading on the release of the drug from the electrospun mats.

Materials and Methods

Solution Preparation

Due to the small mass of tetracycline hydrochloride to be added to fibrinogen solutions, stock mixtures of bovine fibrinogen (Sigma-Aldrich) and tetracycline (Sigma-Aldrich) in appropriate proportions were prepared in advance. These mixtures offered greater precision in solution preparation. All necessary calculations insured the desired concentration of fibrinogen in each preparation. Solutions of fibrinogen in concentrations of 110 mg/mL, 120 mg/mL, and 130 mg/mL were prepared with tetracycline weight percentages of 2.5%, 5%, and 10%. In addition, pure fibrinogen solutions were prepared for mechanical analysis comparison and release controls. The fibrinogen/tetracycline mixture was dissolved in a solution of 90% HFP and 10% 10 X MEM. The volume of solvent for each preparation was 6 mL (5.4 mL HFP + 0.6 mL 10 X MEM).

Electrospinning

In this setup, the solutions of fibrinogen with varying weight percentages of tetracycline were placed in 5 mL syringes with 18 gauge blunt needles. A syringe pump (KD Scientific) was used to maintain a measured flow rate. The flow rate of the pump was set at 2.0 mL/hr, with minor adjustments during individual spins for the purpose of optimizing the spinning process and minimizing waste. A high voltage power supply (Spellman CZE100R, Spellman High Voltage Electronics Corporation) imparted a voltage of 22 kV at the syringe needle. The electrospun fibers were collected on a rotating stainless steel mandrel of length 75 mm, width 25 mm, and thickness 4 mm, which also served as the ground electrode as mentioned earlier. The mandrel was rotated at a rotational velocity of approximately 340 rpm, and was also translated before the needle tip with a rectilinear velocity of approximately 75 mm/s. The distance from the needle tip to the mandrel was 10 cm.

The spinning process was conducted in a low-light environment because of the light-sensitive nature of tetracycline. Fibers were collected on the mandrel until the syringe was devoid of solution, and the

fibrinogen/tetracycline mats were removed from the mandrel, wrapped in heavy foil to minimize light exposure, and stored at -20 degrees C until needed.

Materials Testing

For materials testing, samples of the preparation were trimmed into a "dogbone" profile in order to minimize grip effects, and to create a region of probable failure of known geometry for calculation of modulus and peak stress. The samples were 18.6 mm in length and 6.2 mm in width at their ends (grip attachment), narrowing to width 2.8 mm in the center of the sample (probable failure region). The samples from each preparation (including pure fibrinogen) were subjected to uniaxial tensile testing using a MTS Bionix 200 testing station with a strain rate of 10 mm/min, and with a sampling rate of 20 Hz. Samples were tested in both a dry state, and after immersion in PBS for twenty minutes. Modulus, breaking strain, and peak stress were recorded.

Materials Characterization

Samples of mats spun from each preparation were prepared for scanning electron microscopy (SEM). The samples of each mat were affixed to an electron microscopy stud. As the mats are completely dry when spun, the only necessary step was sputter coating for the imaging process to be conducted. SEM images were obtained with a JEOL JSM-820 microscope at 4000x magnification. The images obtained from SEM were scanned into digital computer images. UTHSCSA ImageTool Version 2.0 was used to determine the diameters of sixty sample fibers in each preparation for comparison across concentrations and loading percentages. In addition, the area of thirty pores was determined with ImageTool. These values were averaged and standard deviations calculated.

Short Term and Long Term Release Protocol

To study release, samples of each preparation were obtained. These samples were harvested from each mat using a hole punch with diameter 6.35 mm. The shape of every sample was identical, but the thickness varied slightly with each spin, and within each mat. An average mass for a single sample punch was determined using the total weight of samples from a mat, and the number of sample punches. The samples were placed in 1 mL of PBS at 20 degrees Celsius. All samples were independent – a given sample was studied at only one time point. There were two time courses studied for release properties – a short time course of 128 min (8 time events) and a long time course of 7 days (8 time events). The short time course allowed for the collection of data regarding the initial surface dissolution and early release of tetracycline from the mats, a behavior overlooked when the first time sample is at after such dissolution has taken place. From this study, that period of rapid dissolution was approximately an hour in length. For the short time course, data were collected at 1, 2, 4, 8, 16, 32, 64, and 128 minutes. For the longer time course, data were collected at 1, 4, 14, 24, 48, 72, 120, and 168 hours. For all release studies,

there existed triplicate independent samples for each time event. This was done to minimize any error from non-uniformity in the electrospun mats from which the samples came. Also, for each short term time event, three 200 μL samples of the release solution were obtained, in an effort to avoid any collection error. Four samples were obtained from each long term time event. This offered nine (or twelve) values for each preparation at a given release time. All solution samples for spectrophotometry were placed in a 96 well culture plate.

Tetracycline concentration in the solutions was determined using spectrophotometry. A Molecular Devices Spectramax Plus spectrophotometer was used to obtain absorbances. A standard curve was generated from known concentrations of tetracycline (0 μg/mL to 150 μg/mL) in PBS and corresponding absorbances at 360 nanometers. This allowed for calculation of tetracycline concentration in unknown samples. From the concentration (via absorbance standard curve), the defined volume in each sample well (1 mL), and the spectrophotometer sample volume (200 microliters), the amount of tetracycline released into solution by the samples could be determined. With the

assumption that the weight percentage of tetracycline would be the same in the sample as it were in solution (i.e., if no disproportionate loss of tetracycline occurred between syringe to sample), the percentage release could be calculated with the mass of tetracycline in solution and the original mass of the sample. The percentage release, even with the usage of the average mass calculation mentioned above, offers a better comparison of behaviors between preparations, as all spins were not identical, leading to variation among and within the sample groups. Such variation was minimized through identical geometry of samples (6.35 mm circles) and through screening of obvious incongruities within and among samples (i.e. portions of mats that were egregiously thin or damaged were not sampled).

Analytical Methods

Mechanical Data

Analysis of Variance (ANOVA) was utilized to observe any statistical differences between the mechanical properties of modulus, breaking strain, and peak stress across different loading percentages for each concentration. These properties for given concentrations of fibrinogen and tetracycline were compared using analysis of variance. Analysis of variance is a statistical test that compares the variation within a group to the variation among groups. A positive ANOVA indicates that the differences among groups are more substantial than would be expected by chance alone. If differences among the sample groups were found, Tukey's W was used to determine which values were significantly different. Comparisons were made among preparations of the same fibrinogen concentration with different tetracycline loading, and among the average values of preparations with the same fibrinogen

concentration. An alpha level of 0.05 was selected *a priori* for all statistical tests.

Materials Characterization

ANOVA was also used to determine any difference in average diameter between preparations of identical fibrinogen concentrations with different tetracycline loading percentages. Again, Tukey's W was used to determine which preparations, if any, differed from each other. In addition, average fiber diameters of all preparations of a given fibrinogen concentration were analyzed to determine any difference among samples of differing fibrinogen concentrations. The same procedures were conducted regarding pore size.

Release

From each time event, the absorbance at 360 nm was recorded. A standard curve of absorbance of known tetracycline concentrations at 360 nm allowed for the calculation of concentration from absorbance (Figure 39). Also, from the volume of solution introduced into each plate well, the mass of tetracycline released into solution could be determined. With the mass of tetracycline

released and the mass of the sample itself, the percentage of tetracycline released could be calculated.

For the short term release portion of the study, each of the triplicate samples for a time event was sampled three times, creating nine data values for each time event. For the long term release portion of the study, each of the triplicate samples was sampled four times, creating twelve data values. This variation allowed for more precise measurements to be taken during a period of relatively lower release. These values were averaged, and standard deviations were calculated.

The concentration values of each time event were compared across different tetracycline loading percentages for a given fibrinogen concentration. ANOVA was used to determine if these samples at given time index did differ significantly – if so, Tukey's W test was used to assess which values were significantly different at $\alpha = 0.05$. Also, for a given run of release, the data values at all time indices for a given preparation were analyzed using ANOVA. This would indicate if any significant release was occurring. Upon significance of differing values, Tukey's W test was again analyzed to determine which contiguous data points did not significantly differ, thereby

indicating the absence of release at that time, and possibly beyond.

In an effort to normalize the release curves, the percentage of tetracycline released was calculated. This allowed for a more illustrative comparison between different preparations, rather than simple concentration in the release solution. The percentage data was used to compare all preparations to each other (using ANOVA and possible ancillary tests), as these data were independent of the mass of tetracycline in the sample.

Those fibrinogen samples without tetracycline showed no change over time in release solution absorbance in preliminary testing. Also, the standard curve for absorbance was obtained with solutions of known concentration of tetracycline in PBS. Therefore, the data obtained by spectrophotometry were already controlled for presence of fibrinogen and PBS.

Results

Fiber Diameter and Pore Area

After electrospinning, a small sample of each preparation was obtained for scanning electron microscopy (SEM). Sample photographs follow (Figures 4a-4l).

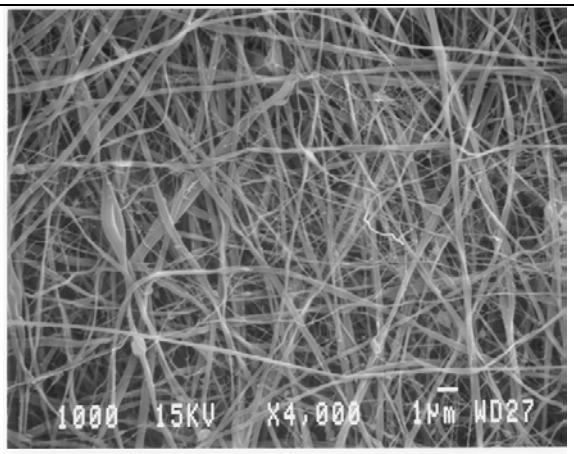

Figure 4a : SEM micrograph at 4000x magnification of 110 mg/mL fibrinogen with 0% tetracycline by weight

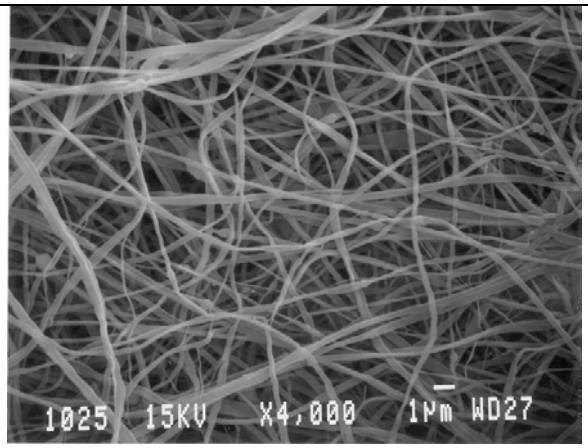

Figure 4b : SEM micrograph at 4000x magnification of 110 mg/mL fibrinogen with 2.5% tetracycline by weight

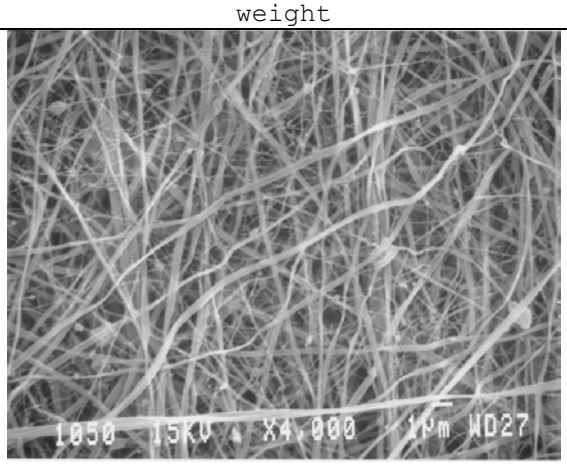

Figure 4c : SEM micrograph at 4000x magnification of 110 mg/mL fibrinogen with 5% tetracycline by weight

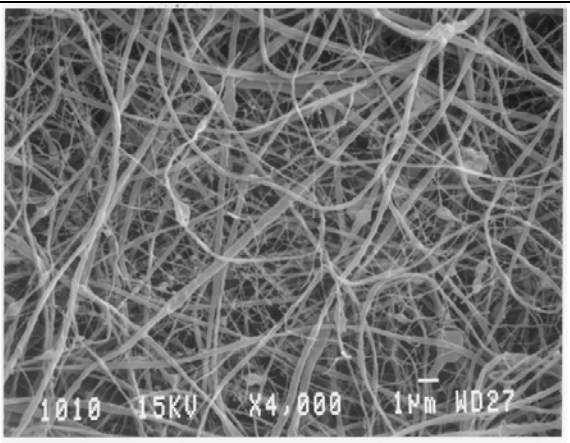

Figure 4d : SEM micrograph at 4000x magnification of 110 mg/mL fibrinogen with 10% tetracycline by weight

Figure 4e : SEM micrograph at 4000x magnification of 120 mg/mL fibrinogen with 0% tetracycline by weight

Figure 4f : SEM micrograph at 4000x magnification of 120 mg/mL fibrinogen with 2.5% tetracycline by weight

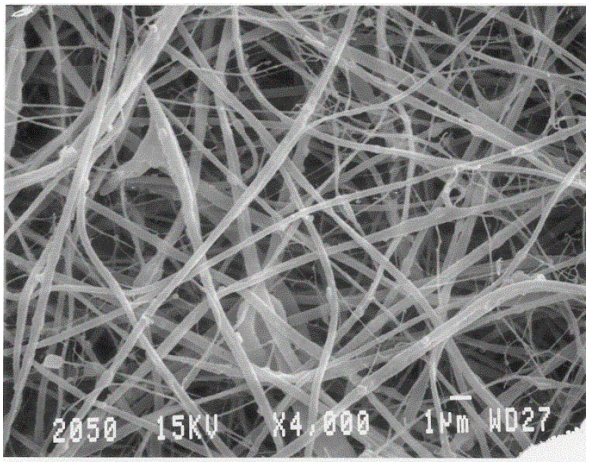

Figure 4g : SEM micrograph at 4000x magnification of 120 mg/mL fibrinogen with 5% tetracycline by weight

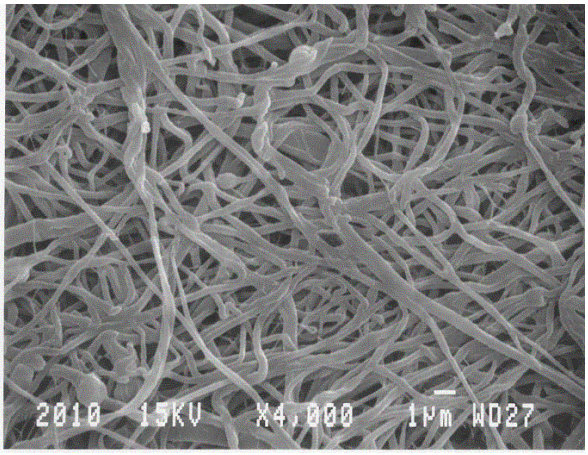

Figure 4h : SEM micrograph at 4000x magnification of 120 mg/mL fibrinogen with 10% tetracycline by weight

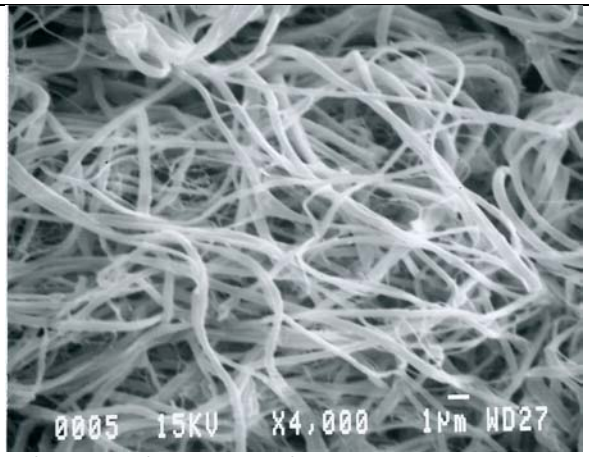

Figure 4i : SEM micrograph at 4000x magnification of 130 mg/mL fibrinogen with 0% tetracycline by weight

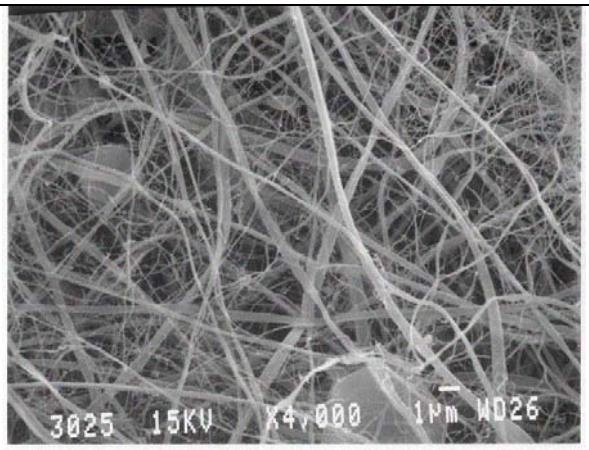

Figure 4j : SEM micrograph at 4000x magnification of 130 mg/mL fibrinogen with 2.5% tetracycline by weight

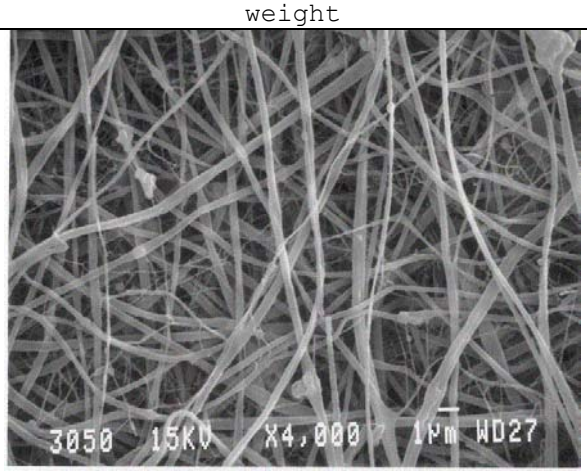

Figure 4k : SEM micrograph at 4000x magnification of 130 mg/mL fibrinogen with 5% tetracycline by weight

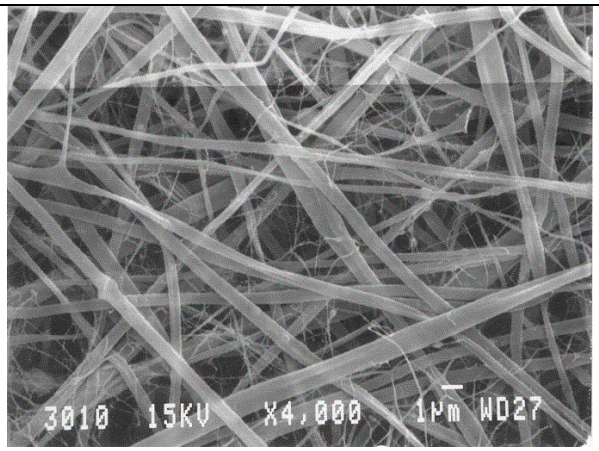

Figure 4l : SEM micrograph at 4000x magnification of 130 mg/mL fibrinogen with 10% tetracycline by weight

Figures 4a-4l: SEM micrographs of electrospun fibrinogen with and without tetracycline

From these micrographs, values for average fiber diameter and pore size were determined using UTHSCSA ImageTool. Tables of fiber diameter data (Table 1) and

pore area (Table 2) are provided. Average fiber diameter, average pore area, and standard deviations are provided.

	Fiber Diameter (μm) Mean ± SD		
	Fibrinogen 110 mg/mL	Fibrinogen 120 mg/mL	Fibrinogen 130 mg/mL
0 % Tetracycline	0.345 ± 0.086	0.479 ± 0.155	0.695 ± 0.227
2.5 % Tetracycline	0.304 ± 0.112	0.448 ± 0.195	0.669 ± 0.219
5 % Tetracycline	0.299 ± 0.114	0.442 ± 0.157	0.660 ± 0.234
10 % Tetracycline	0.307 ± 0.104	0.452 ± 0.146	0.702 ± 0.238

Table 1 : Fiber diameter data for fibrinogen/tetracycline preparations

	Pore Area (μm^2) Mean ± SD		
	Fibrinogen 110 mg/mL	Fibrinogen 120 mg/mL	Fibrinogen 130 mg/mL
0 % Tetracycline	2.475 ± 1.126	5.188 ± 2.037	7.198 ± 1.956
2.5 % Tetracycline	2.919 ± 1.331	5.066 ± 2.272	6.737 ± 2.938
5 % Tetracycline	2.483 ± 1.001	5.237 ± 2.685	6.571 ± 2.963
10 % Tetracycline	2.686 ± 0.910	5.500 ± 2.225	6.740 ± 2.946

Table 2 : Pore area data for fibrinogen/tetracycline preparations

The following figures present the data in graphical form. Error bars account for one standard deviation.

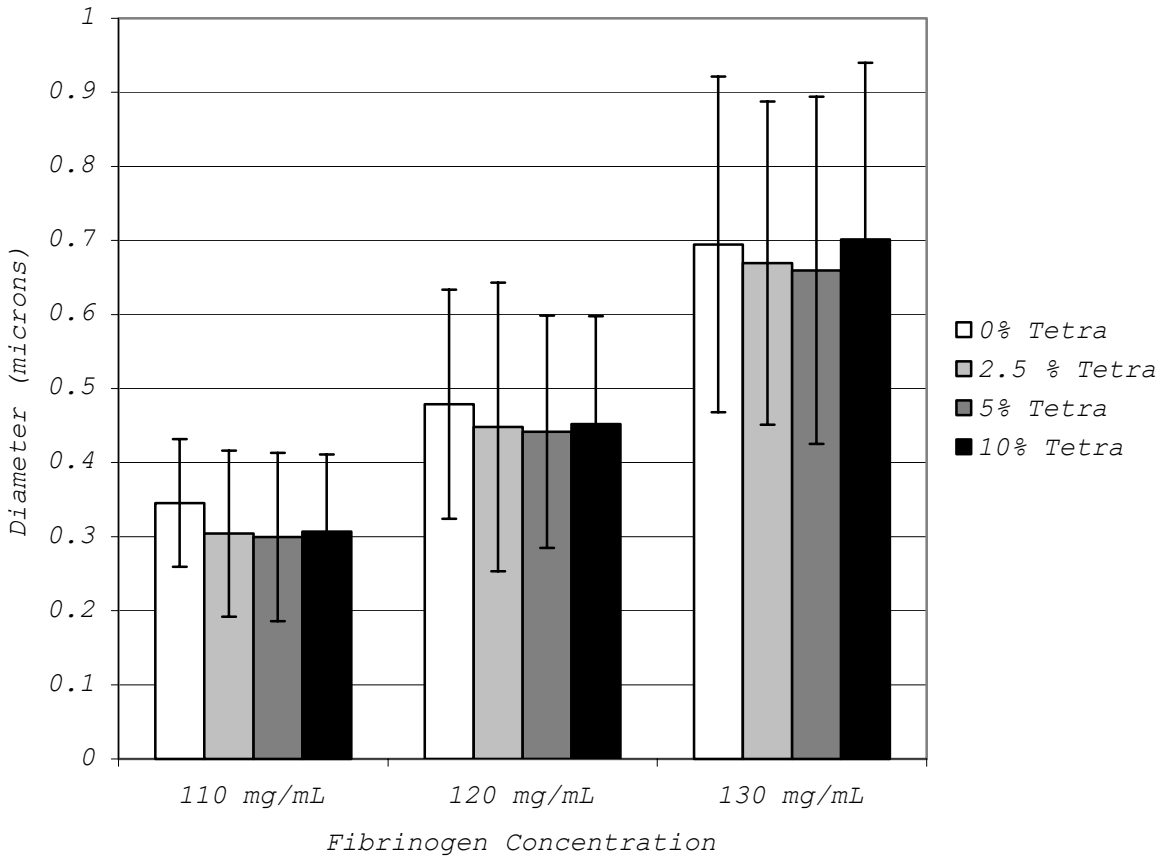

Figure 5 : Fiber Diameter by fibrinogen concentration and tetracycline weight percentage

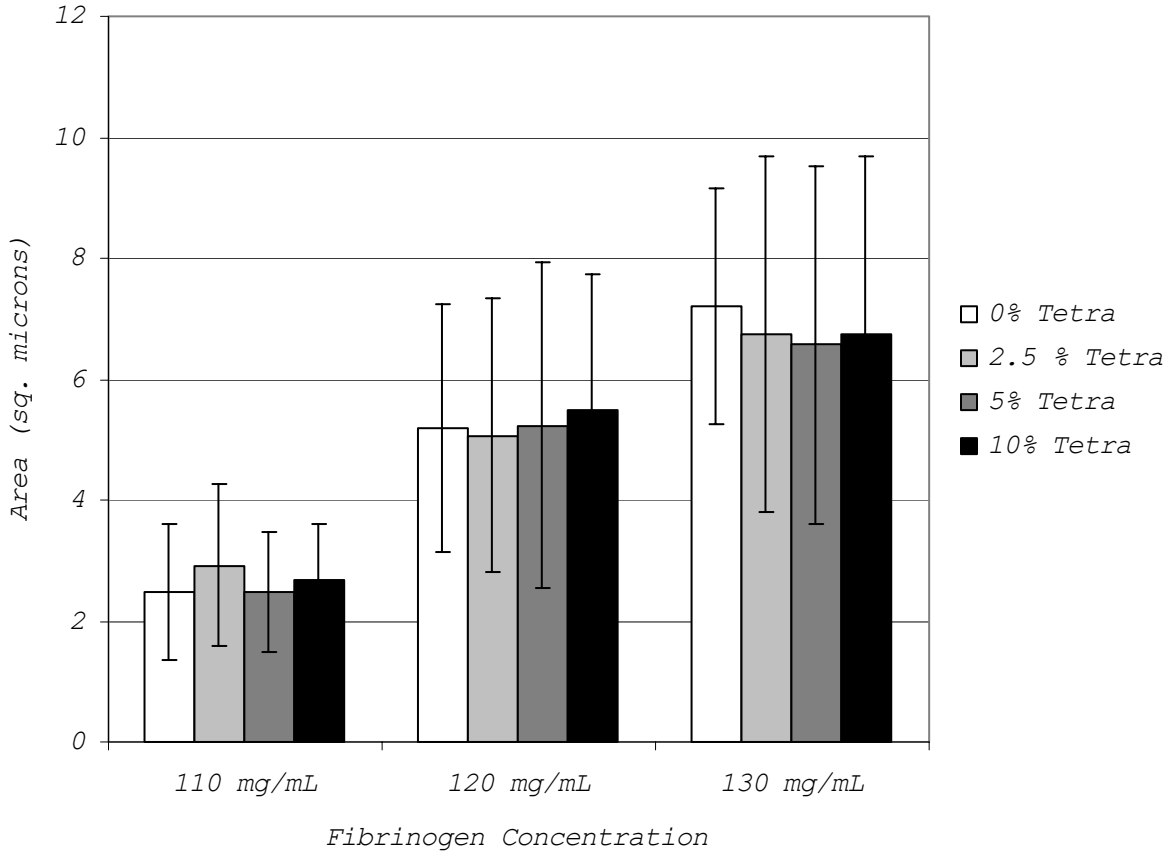

Figure 6 : Pore area by fibrinogen concentration and tetracycline weight percentage

An analysis of variance indicated no significant difference ($\alpha = 0.05$) in either fiber diameter (Table 1) or pore area (Table 2) for varying tetracycline loading percentages at a given fibrinogen concentration. However, an analysis of variance of average values of fiber diameters for a given fibrinogen concentration showed

significant differences across concentrations ($P < 0.001$). A Tukey's W showed that each fiber diameter mean was significantly different pairwise, for each pair of concentrations (All $P < 0.001$). Similarly, an analysis of variance showed significant differences in the average pore area values for the differing fibrinogen concentrations ($P < 0.001$), and Tukey's W indicated that each fibrinogen concentration pore area mean was statistically different from the others.

Mechanical Data

From each preparation, samples were obtained for mechanical testing, under both dry and wet (saturated in PBS) conditions. Modulus, breaking strain, and peak stress were recorded. Stress is the quotient of the force acting upon a object and the cross-sectional area of the object, and provides a normalized force for use in comparing mechanical behaviors among different materials and differing shapes. Peak stress is the highest stress experienced until the material begins to fail. Strain is a measure of deformation of an object while acted upon by an external force. Breaking strain is the maximum deformation of a material before it ruptures. Modulus is a quantity that relates the response of a material (strain) to the application of outside stress. It is a description of how stiff or flexible the material is. The following graphs illustrate the data. Confidence level for all statistical tests is $\alpha = 0.05$.

Moduli of Dry Samples

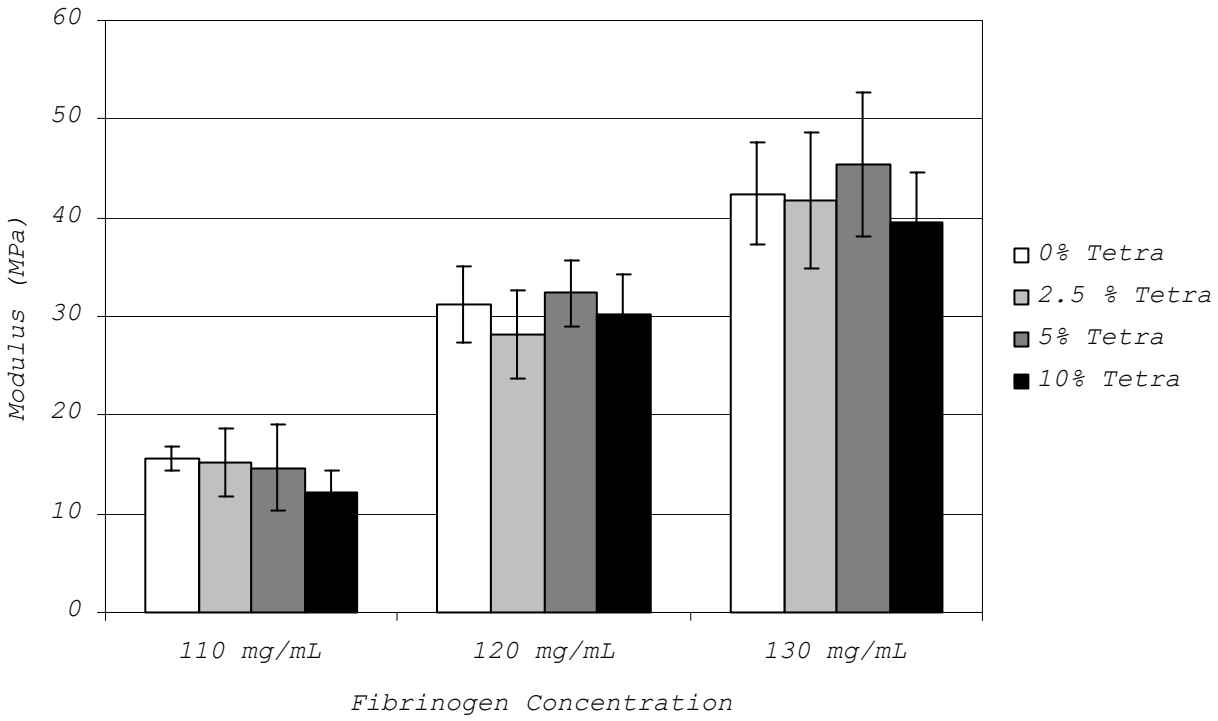

Figure 7 : Modulus by Concentration and Percentage Tetracycline (Dry Sample)

An analysis of variance showed no significant difference in dry moduli (Figure 7) among different tetracycline loading percentages for a given fibrinogen concentration. However, average values of dry moduli for a given fibrinogen concentration were significantly different from each other ($P < 0.001$). Tukey's W demonstrated significant differences among all average values of

fibrinogen concentrations ($P < 0.001$). These significant differences indicate that the 130 mg/mL preparations possess higher moduli than the 120 mg/mL preparations, which in turn possess higher moduli than the 110 mg/mL preparations.

Breaking Strains of Dry Samples

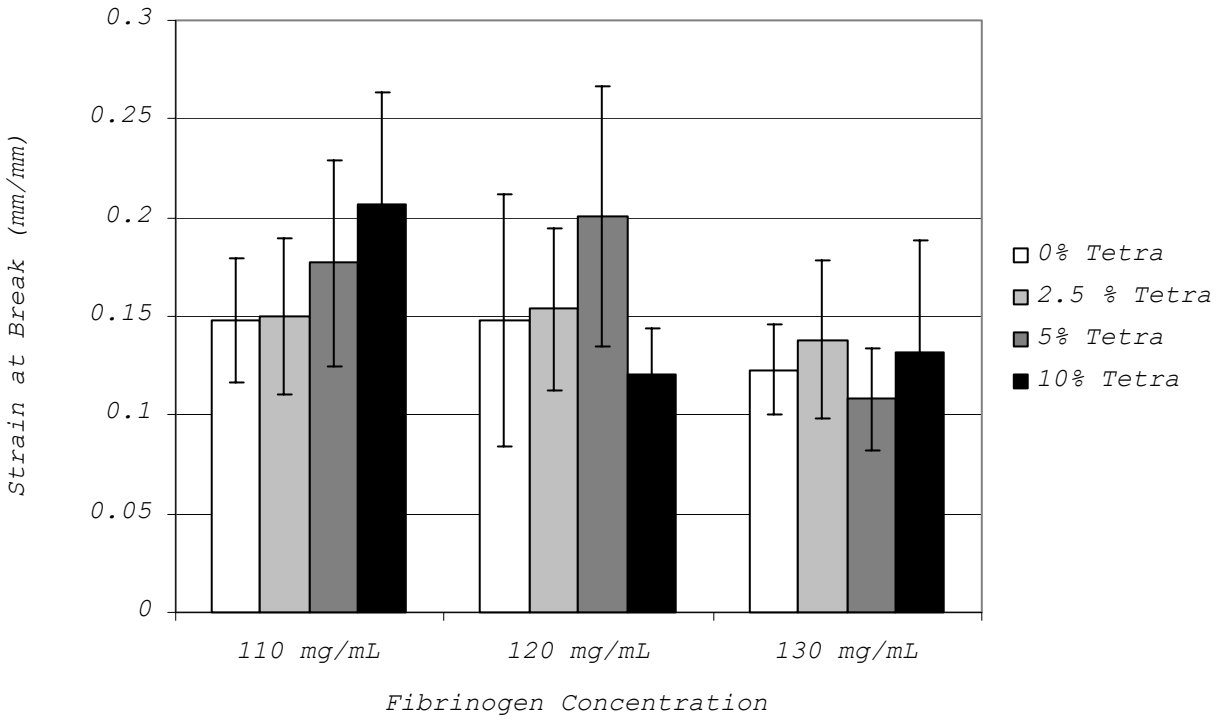

Figure 8 : Breaking Strain by Concentration and Percentage Tetracycline (Dry Sample)

An analysis of variance of dry breaking strain data (Figure 8) showed no significant differences in breaking strain across tetracycline loading percentages for a given fibrinogen concentration, nor any significant differences in breaking strain for differing fibrinogen concentrations.

Peak Stresses of Dry Samples

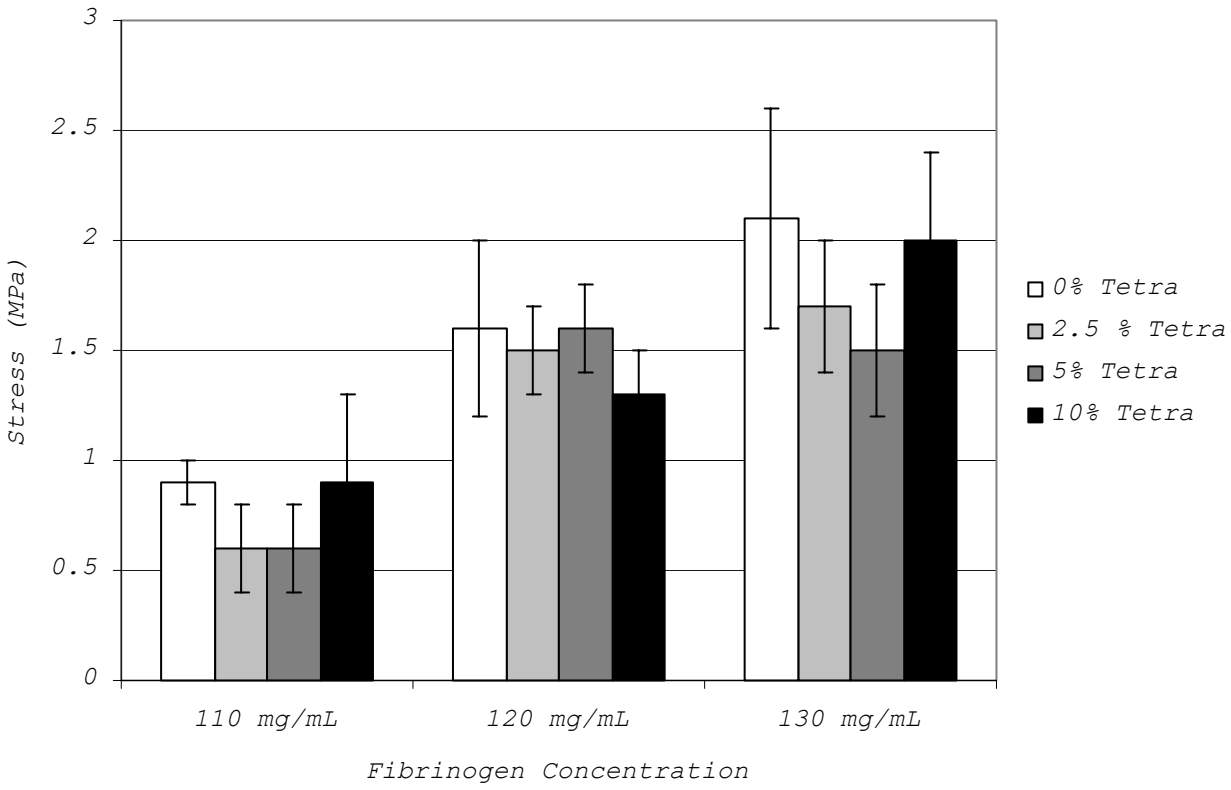

Figure 9 : Peak Stress by Concentration and Percentage Tetracycline (Dry Sample)

An analysis of variance showed no significant differences in peak stress due to differing percentages of tetracycline (Figure 9). However, ANOVA returned a significant difference in average peak stress values among fibrinogen concentrations regardless of loading ($P < 0.001$), and Tukey's W found differences between 130 mg/mL preparations and 110 mg/mL preparations ($P < 0.001$), as

well as between 120 mg/mL preparations and 110 mg/mL ($P < 0.003$). In each case, the peak stress of dry 110 mg/mL fibrinogen concentrations was lower.

Moduli of Wet Samples

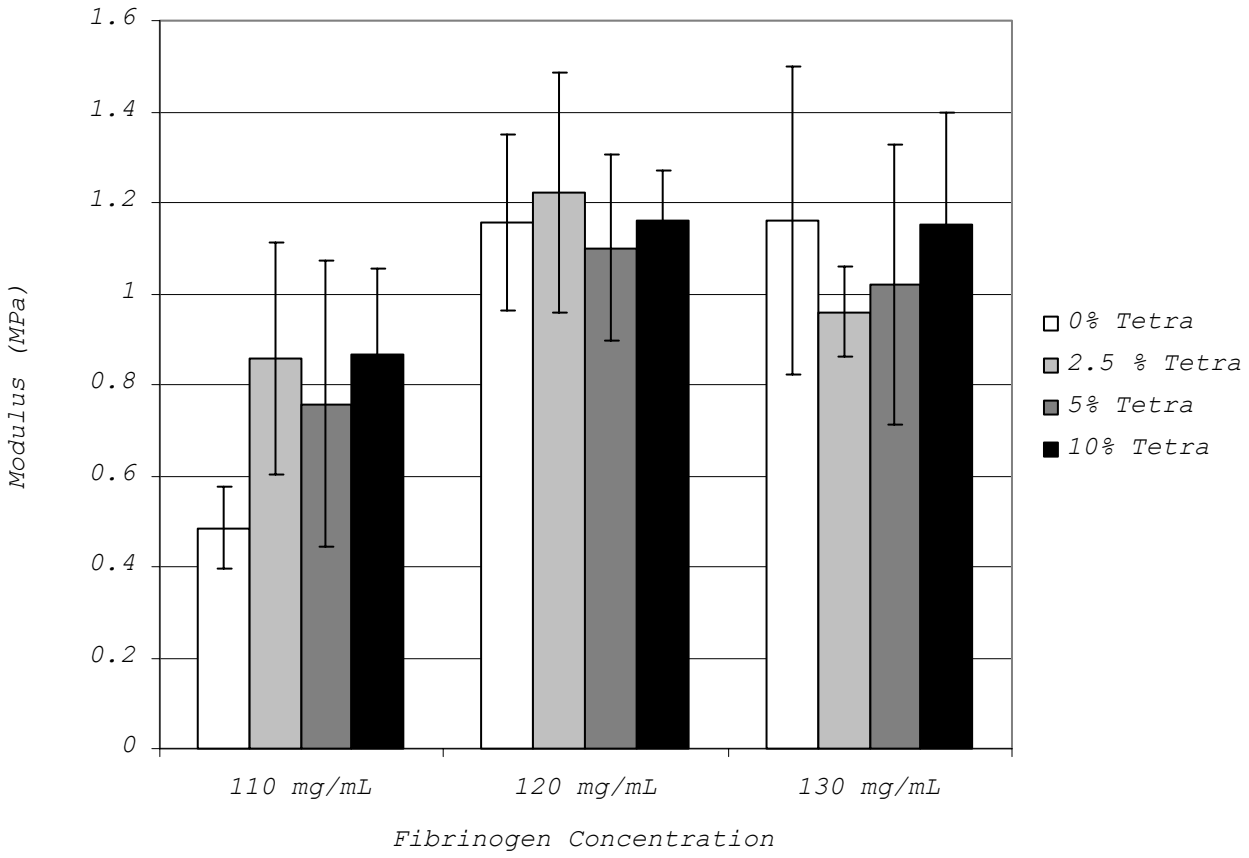

Figure 10 : Modulus by Concentration and Percentage Tetracycline (Wet Sample)

An analysis of variance showed no significant differences in modulus due to differing percentages of tetracycline for wet samples (Figure 10). However, ANOVA returned a significant difference in average modulus among fibrinogen concentrations regardless of loading (P = 0.002), and Tukey's W found differences between 120 mg/mL

preparations and 110 mg/mL preparations (P = 0.002), as well as between 130 mg/mL preparations and 110 mg/mL (P = 0.01). In each case, the modulus of wet 110 mg/mL fibrinogen concentrations was lower.

Breaking Strains of Wet Samples

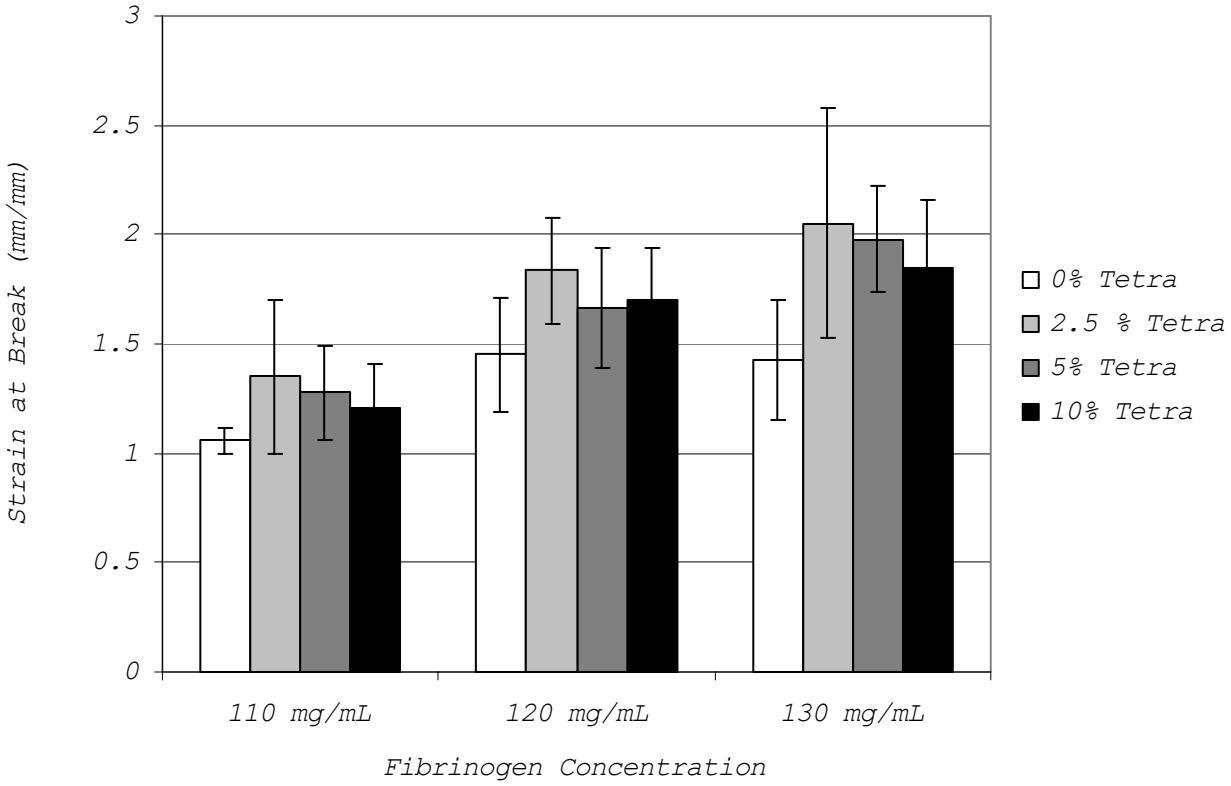

Figure 11 : Breaking Strain by Concentration and Percentage Tetracycline (Wet Sample)

An analysis of variance showed no significant differences in breaking strain due to differing percentages of tetracycline for wet samples (

Figure 11). However, ANOVA returned a significant difference in average breaking strain among fibrinogen concentrations regardless of loading (P = 0.005), and Tukey's W found differences between 120 mg/mL preparations

and 110 mg/mL preparations (P = 0.03), as well as between 130 mg/mL preparations and 110 mg/mL (P = 0.005). In each case, the breaking strain of wet 110 mg/mL fibrinogen concentrations was lower than the other concentrations.

Peak Stress of Wet Samples

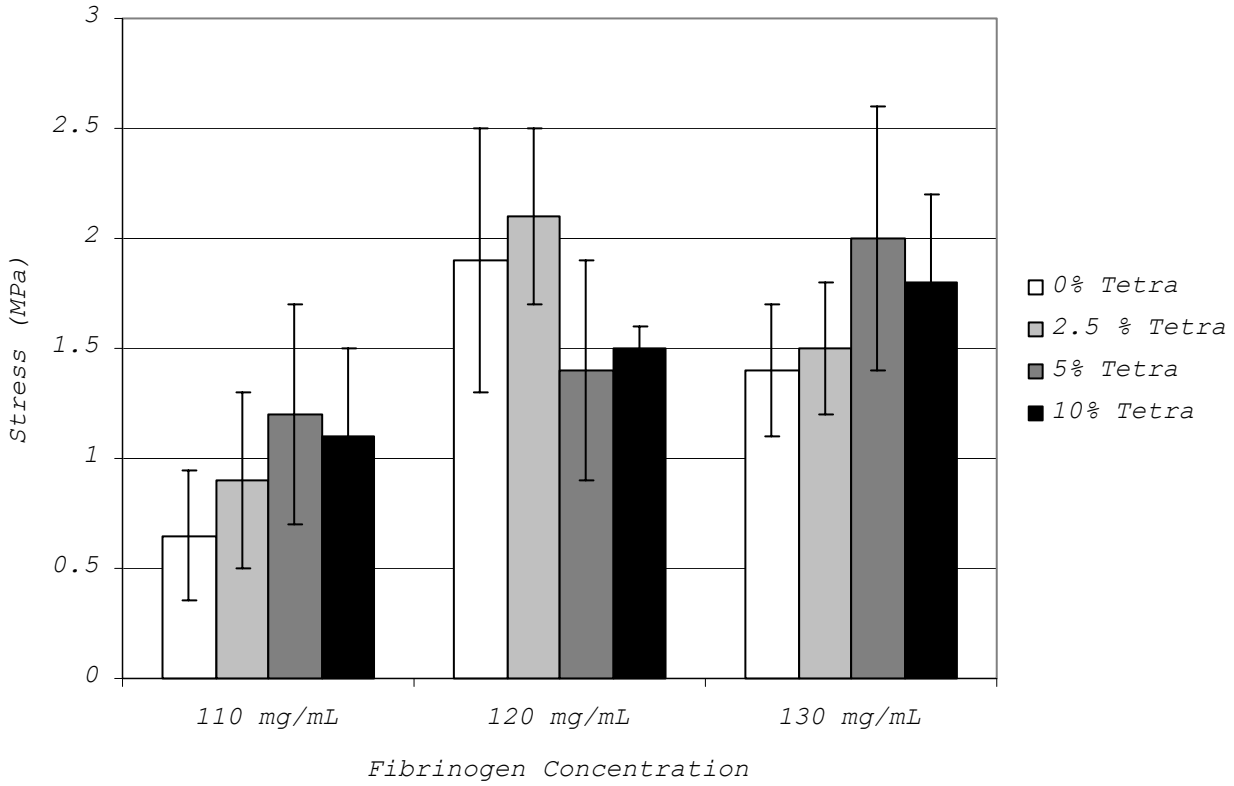

Figure 12 : Peak Stress by Concentration and Percentage Tetracycline (Wet Sample)

An analysis of variance showed no significant differences in peak stress due to differing percentages of tetracycline for wet samples (Figure 12). However, ANOVA returned a significant difference in average peak stress among fibrinogen concentrations regardless of loading (P =

0.007), and Tukey's W found significant differences between 130 mg/mL preparations and 110 mg/mL preparations (P = 0.016), as well as between 120 mg/mL preparations and 110 mg/mL (P = 0.011). In each case, the peak stress of wet 110 mg/mL fibrinogen was lower.

Short Term Release

The results for short term release follows for all preparations of fibrinogen with tetracycline. For each fibrinogen concentration (110 mg/mL, 120 mg/mL, and 130 mg/mL), there exist three different preparations, each with different loading percentages of tetracycline (2.5% w/w, 5% w/w, 10% w/w). The results first report differences in release profiles for different tetracycline loading of a given fibrinogen concentration. Then, concentration over the time course of the release run (1 min to 128 min) is analyzed to determine when significant release occurred. The percentage releases among the three preparations are compared to observe any differences among different loading of tetracycline. Then the results are grouped by tetracycline loading to observe the effects of varying fibrinogen concentrations. All analyses will utilize ANOVA and Tukey's W, with the *a priori* significance level of $\alpha = 0.05$.

Release from 110 mg/mL Fibrinogen Preparations

Concentration

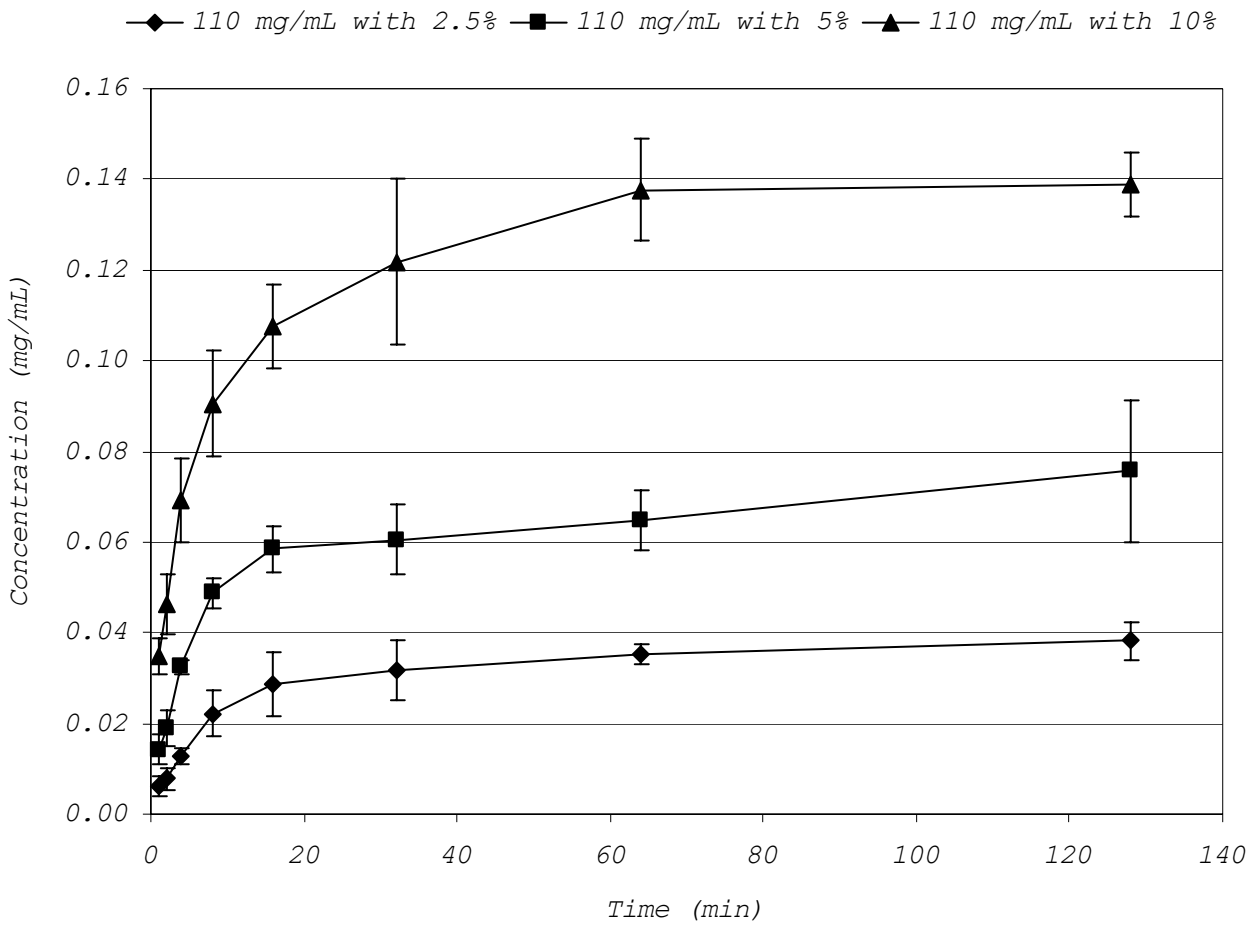

Figure 13 : Concentration versus time 110 mg/mL fibrinogen with tetracycline hydrochloride (1 min to 128 min)

Figure 13 shows the change in tetracycline concentration in the release solution over time for 110 mg/mL fibrinogen samples. Three different loading percentages of tetracycline are shown. Analysis of variance of the tetracycline concentrations at each time point showed significant difference among the three tetracycline loading concentrations (all $P < 0.001$). Tukey's W found that, for each time point, the release concentration of the 10% preparation was significantly higher than the 5% preparation (all $P < 0.001$), and that the release concentration of the 5% preparation was higher than that of the 2.5% preparation (all $P < 0.001$). In other words, the three profiles in Figure 13 are significantly different.

For the 110 mg/mL fibrinogen with 2.5% tetracycline, ANOVA revealed that the concentrations from 1 min to 128 min were significantly different ($P < 0.001$). However, Tukey's W indicated that the only significant difference between two successive values existed between 4 min and 8 min ($P < 0.001$). Therefore, any release after 8 min can not be considered significant. (See Table 20 in the Appendix for all pairwise P-values)

For the 110 mg/mL fibrinogen with 5% tetracycline, ANOVA revealed that the concentrations from 1 min to 128 min were significantly different ($P < 0.001$). However, Tukey's W found that the only significant differences in concentration observed between successive time points were between times 2 min and 4 min ($P = 0.004$), 4 min and 8 min ($P < 0.001$), and between 64 min and 128 min ($P = 0.042$). Therefore, there was significant release from 2 min to 8 min and from 64 min to 128 min. (See Table 21 in Appendix for all pairwise P-values)

For the 110 mg/mL fibrinogen with 10% tetracycline, ANOVA revealed that the concentrations from 1 min to 128 min were significantly different ($P < 0.001$). The significant differences in concentration observed between contiguous time points by Tukey's W were between times 2 min and 4 min ($P < 0.001$), 4 min and 8 min ($P = 0.001$), 8 min and 16 min ($P = 0.02$), and 32 min and 64 min ($P = 0.042$). This indicated that significant release occurred from time 2 min to 16 min. There was further significant release from 32 min to 64 min. (See Table 22 in Appendix for all pairwise P-values)

Release from 110 mg/mL Fibrinogen Preparations

Percentage Release

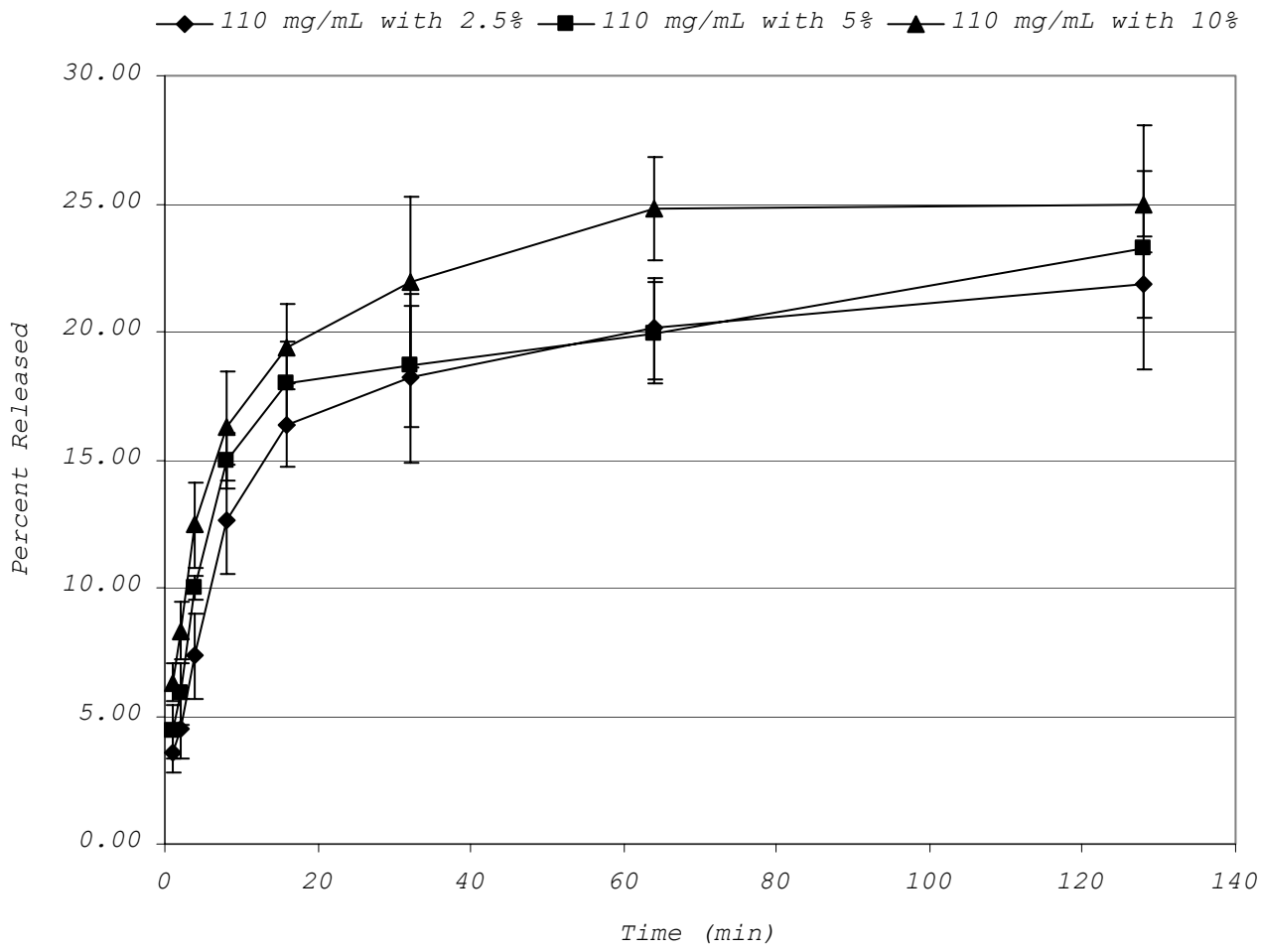

Figure 14 : Percentage released versus time for 110 mg/mL fibrinogen with tetracycline hydrochloride (1 min to 128 min)

Figure 14 shows the percentage release over time from preparations of 110 mg/mL fibrinogen with three different tetracycline loading concentrations. These percentage release profiles are identical in shape to the concentration graphs of Figure 13. The concentrations have been converted to percentage of tetracycline released by using the average mass of the sample, and the concentrations of Figure 13 as indicated in the Methods. The calculation of percentage release serves as a normalized view of release, independent of mass, and, therefore allows for a better comparison of release from different preparations.

Analysis of variance at each time event indicated that at all time points other than 16 min and 128 min, there existed significant difference among the samples. These results are provided below in Table 3. At time points where ANOVA indicated differences among the percentage values, Tukey's W was used to find which pairs of percentages were different. These results are also provided in Table 3. In short, the percentage release from the preparation with 10% tetracycline was significantly higher than the 2.5% preparation for all times when significant differences were observed. Also, the 10% preparation also

was significantly higher than the 5% preparation at times 1 min, 2 min, and 64 min. At time 4 min, all percentage release values were significantly different.

Time	Difference among samples (ANOVA)	Pairwise Differences (Tukey's W)
1 min	Yes, P < 0.001	10% > 5% (P < 0.001) 10% > 2.5% (P = 0.001)
2 min	Yes, P < 0.001	10% > 5% (P < 0.001) 10% > 2.5% (P = 0.001)
4 min	Yes, P < 0.001	10% > 5% (P < 0.001) 10% > 2.5% (P < 0.001) 5% > 2.5% (P < 0.001)
8 min	Yes, P = 0.006	10% > 2.5% (P = 0.004)
16 min	None	None
32 min	Yes, P = 0.049	10% > 2.5% (P < 0.001)
64 min	Yes, P < 0.001	10% > 5% (P < 0.001) 10% > 2.5% (P < 0.001)
128 min	None	None

Table 3 : P – values for ANOVA and Tukey's W for 110 mg/mL samples (1 min to 128 min)

Release from 120 mg/mL Fibrinogen Preparations

Concentration

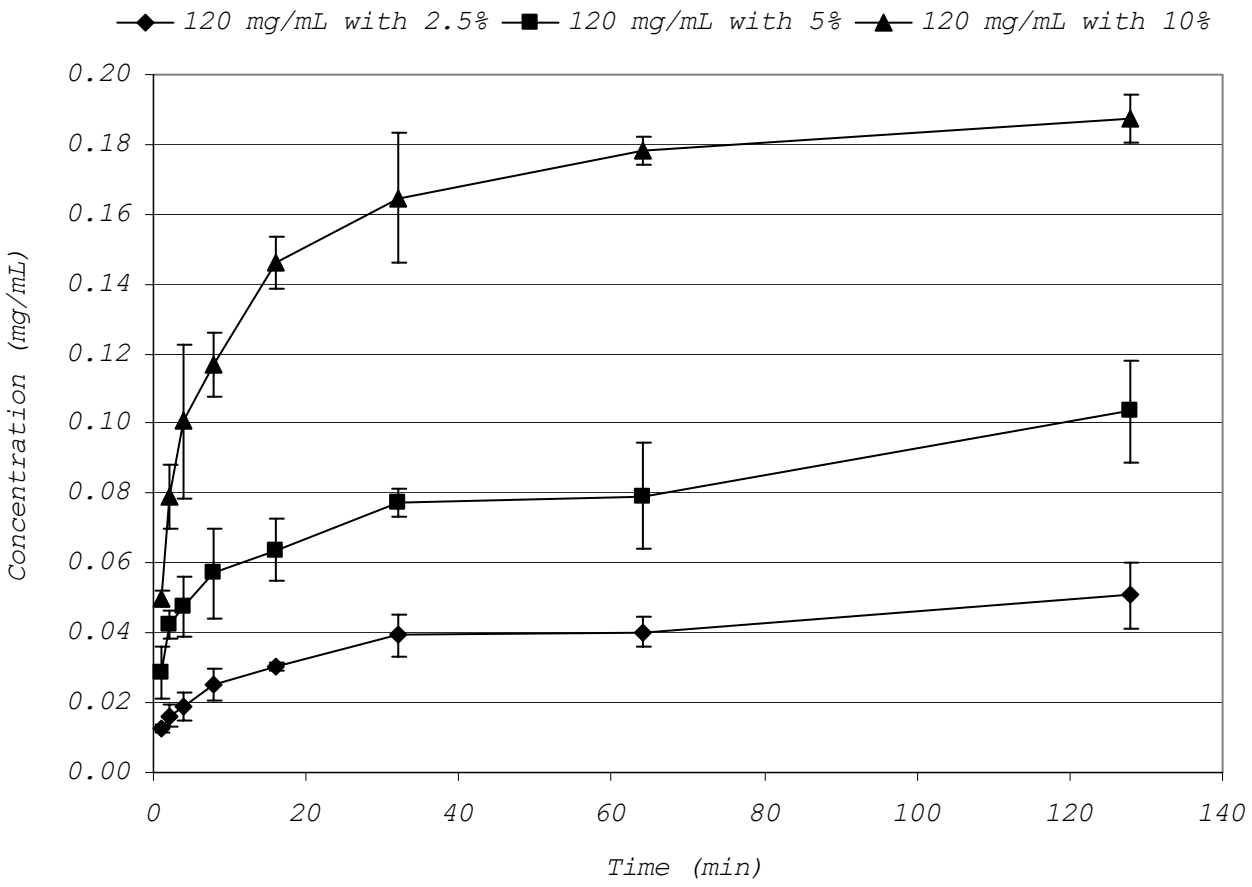

Figure 15 : Concentration versus time 120 mg/mL fibrinogen with tetracycline hydrochloride (1 min to 128 min)

Figure 15 shows the change in tetracycline concentration in the release solution over time for 120 mg/mL fibrinogen samples. Three different loading

percentages of tetracycline are shown. Analysis of variance of the tetracycline concentrations at each time point showed significant difference among the three tetracycline loading concentrations (all $P < 0.001$). Tukey's W found that, for each time point, the release concentration of the 10% preparation was significantly higher than the 5% preparation (all $P < 0.001$), and that the release concentration of the 5% preparation was higher than that of the 2.5% preparation (all $P < 0.001$). In other words, the three profiles in Figure 15 are significantly different.

For the 120 mg/mL fibrinogen with 2.5% tetracycline, ANOVA revealed that the concentrations from 1 min to 128 min were significantly different ($P < 0.001$). However, Tukey's W indicated that only significant difference between two successive values existed between 4 min and 8 min ($P < 0.001$). Therefore, significant release only occurred between 4 min and 8 min. (See Table 23 in the Appendix for all pairwise P-values)

For the 120 mg/mL fibrinogen with 5% tetracycline, ANOVA revealed that the concentrations from 1 min to 128 min were significantly different ($P < 0.001$). However, Tukey's W found that the only significant differences in

concentration observed between successive time points were between times 2 min and 4 min ($P = 0.004$), 4 min and 8 min ($P < 0.001$), and between 64 min and 128 min ($P = 0.042$). Therefore, there was significant release from 2 min to 8 min and from 64 min to 128 min. (See Table 24 in Appendix for all pairwise P-values)

For the 120 mg/mL fibrinogen with 10% tetracycline, ANOVA revealed that the concentrations from 1 min to 128 min were significantly different ($P < 0.001$). The significant differences in concentration observed between contiguous time points by Tukey's W were between times 1 min and 2 min ($P < 0.001$), 2 min and 4 min ($P < 0.001$), 4 min and 8 min ($P = 0.001$), 8 min and 16 min ($P = 0.02$), and 32 min and 64 min ($P = 0.042$). This indicated that significant release occurred from time 1 min to 16 min. There was further significant release from 32 min to 64 min. (See Table 25 in Appendix for all pairwise P-values)

Release from 120 mg/mL Fibrinogen Preparations

Percentage Release

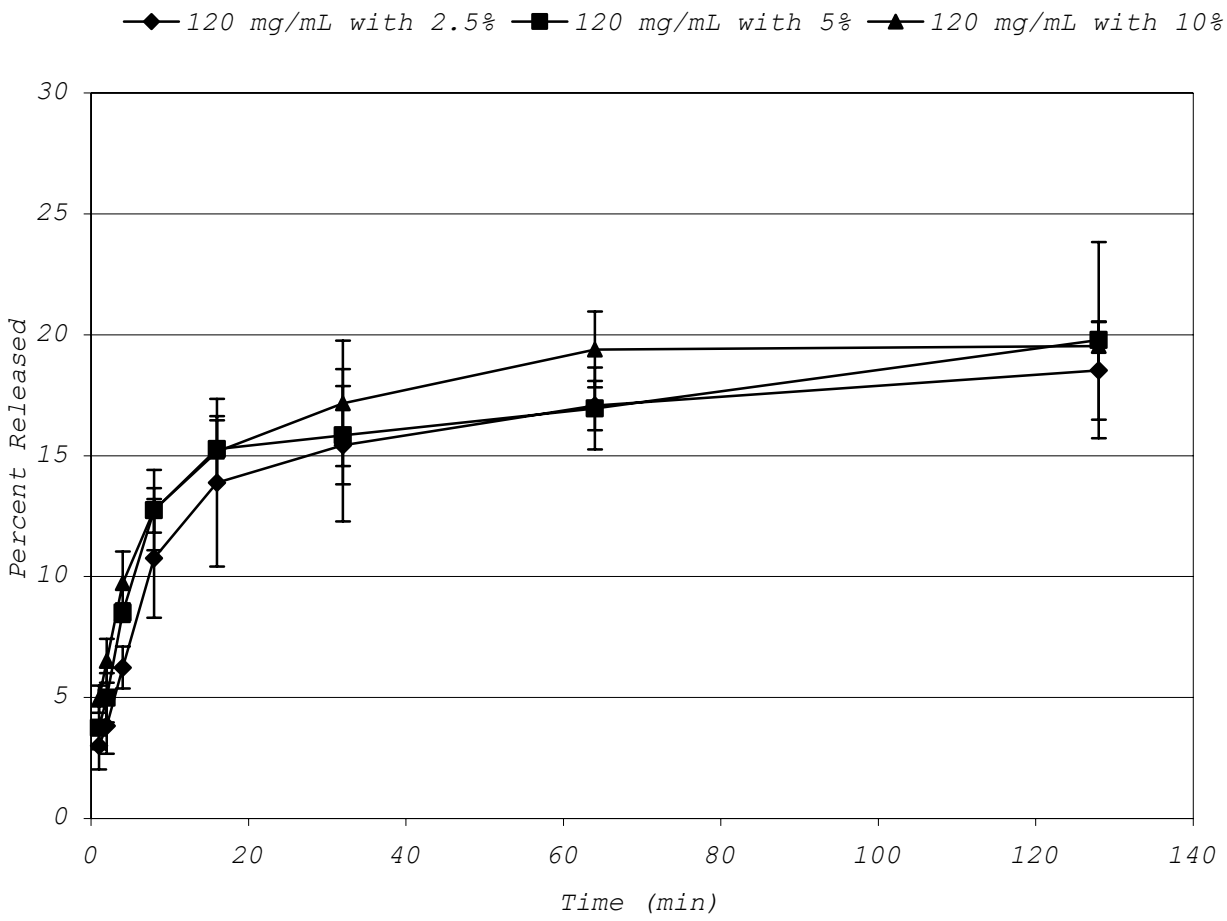

Figure 16 : Percentage released versus time 120 mg/mL fibrinogen with tetracycline hydrochloride (1 min to 128 min)

Figure 16 shows the percentage release over time from preparations of 120 mg/mL fibrinogen with three different

tetracycline loading concentrations. These percentage release profiles are identical in shape to the concentration graphs of Figure 15. The concentrations have been converted to percentage of tetracycline released by using the average mass of the sample, and the concentrations of Figure 15 as indicated in the Methods. The calculation of percentage release serves as a normalized view of release, independent of mass, and, therefore allows for a better comparison of release from different preparations.

Analysis of variance at each time event indicated that at all time points other than 1 min and 128 min, there existed significant difference among the samples. These results are provided below in Table 4. At time points where ANOVA indicated differences among the percentage values, Tukey's W was used to find which pairs of percentages were different. These results are also provided below in Table 4. In summary, the percentage release from the preparation with 10% tetracycline was significantly higher than the 2.5% preparation for all times when significant differences were observed, with the exception of time 2 min. Additionally, the 10% preparation also was significantly higher than the 5% preparation at times 2

min, 32 min, and 64 min. The 5% preparation was significantly higher than the 2.5% preparation at 2 min and 4 min. At time 16 min, all percentage release values were significantly different, with preparation of higher tetracycline percentages demonstrating greater release.

Time	Difference among samples (ANOVA)	Pairwise Differences (Tukey's W)
1 min	None	None
2 min	Yes, $P < 0.001$	10% > 5% ($P < 0.001$) 5% > 2.5% ($P < 0.001$)
4 min	Yes, $P < 0.001$	10% > 2.5% ($P < 0.001$) 5% > 2.5% ($P = 0.023$)
8 min	Yes, $P = 0.004$	10% > 2.5% ($P = 0.004$)
16 min	Yes, $P < 0.001$	10% > 5% ($P < 0.001$) 10% > 2.5% ($P < 0.001$) 5% > 2.5% ($P = 0.031$)
32 min	Yes, $P = 0.001$	10% > 5% ($P = 0.038$) 10% > 2.5% ($P = 0.003$)
64 min	Yes, $P = .001$	10% > 5% ($P < 0.001$) 10% > 2.5% ($P < 0.001$)
128 min	None	None

Table 4 : P – values for ANOVA and Tukey's W for 120 mg/mL samples (1 min to 128 min)

Release from 130 mg/mL Fibrinogen Preparations

Concentration

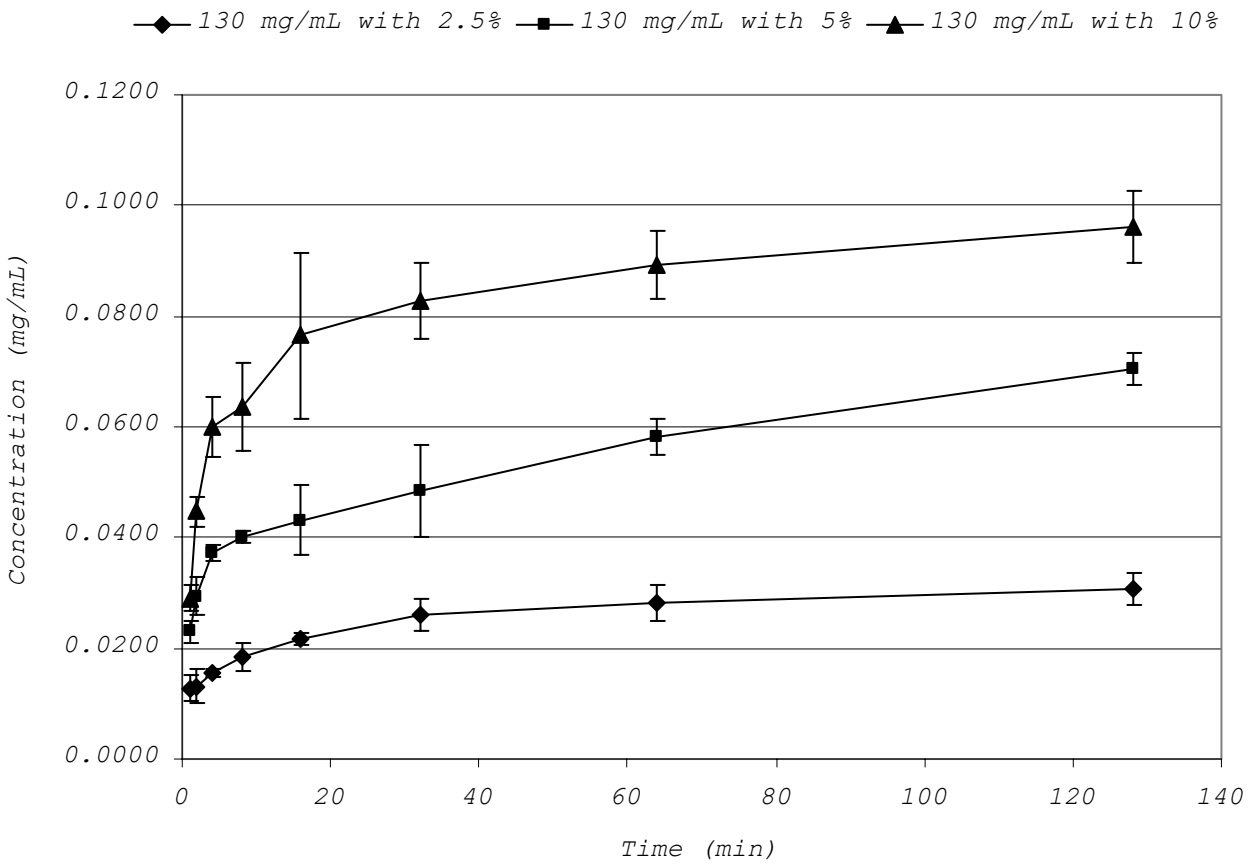

Figure 17 : Concentration versus time 130 mg/mL fibrinogen with tetracycline hydrochloride (1 min to 128 min)

Figure 17 shows the change in tetracycline concentration in the release solution over time for 130 mg/mL fibrinogen samples. Three different loading

percentages of tetracycline are shown. Analysis of variance of the tetracycline concentrations at each time point showed significant difference among the three tetracycline loading concentrations (all $P < 0.001$). Tukey's W found that, for each time point, the release concentration of the 10% preparation was significantly higher than the 5% preparation (all $P < 0.001$), and that the release concentration of the 5% preparation was higher than that of the 2.5% preparation (all $P < 0.001$). In other words, the three profiles in Figure 17 are significantly different.

For the 130 mg/mL fibrinogen with 2.5% tetracycline, ANOVA revealed that the concentrations from 1 min to 128 min were significantly different ($P < 0.001$). However, Tukey's W indicated that only significant difference between two successive values existed between 16 min and 32 min ($P < 0.01$). Therefore, significant release only occurred between 16 min and 32 min, and no significant release occurred thereafter. (See Table 26 in the Appendix for all pairwise P-values)

For the 130 mg/mL fibrinogen with 5% tetracycline, ANOVA revealed that the concentrations from 1 min to 128 min were significantly different ($P < 0.001$). However,

Tukey's W found that the only significant differences in concentration observed between successive time points were between times 2 min and 4 min ($P = 0.006$), 32 min and 64 min ($P < 0.001$), and between 64 min and 128 min ($P < 0.001$). Therefore, there was significant release from 2 min to 8 min and from 32 min to 128 min. (See Table 27 in Appendix for all pairwise P-values)

For the 130 mg/mL fibrinogen with 10% tetracycline, ANOVA revealed that the concentrations from 1 min to 128 min were significantly different ($P < 0.001$). The significant differences in concentration observed between contiguous time points by Tukey's W were between times 1 min and 2 min ($P < 0.001$), 2 min and 4 min ($P = 0.004$), 8 min and 16 min ($P < 0.001$), and 16 min and 32 min ($P = 0.018$). This indicated that significant release occurred from time 1 min to 4 min. There was further significant release from 8 min to 32 min, but no significant release thereafter. (See Table 28 in Appendix for all pairwise P-values)

Release from 130 mg/mL Fibrinogen Preparations

Percentage Release

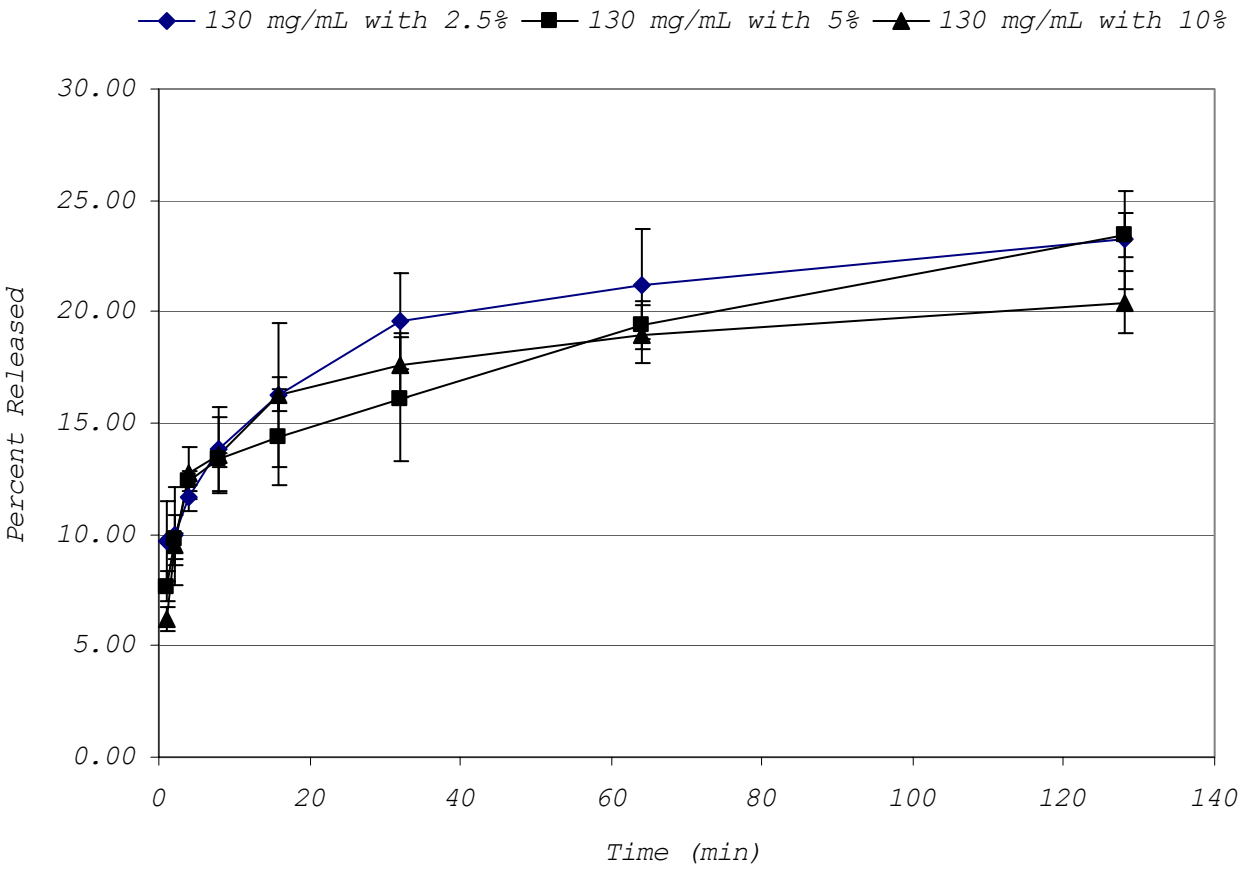

Figure 18 : Percentage released versus time 130 mg/mL fibrinogen with tetracycline hydrochloride (1 min to 128 min)

Figure 18 shows the percentage release over time from preparations of 120 mg/mL fibrinogen with three different tetracycline loading concentrations. These percentage release profiles are identical in shape to the

concentration graphs of Figure 17. The concentrations have been converted to percentage of tetracycline released by using the average mass of the sample, and the concentrations of Figure 17 as indicated in the Methods. The calculation of percentage release serves as a normalized view of release, independent of mass, and, therefore allows for a better comparison of release from different preparations.

Analysis of variance at each time event indicated that at all time points other than 2 min, 8 min, and 128 min, there existed significant difference among the samples. These results are provided below in Table 5. At time points where ANOVA indicated differences among the percentage values, Tukey's W was used to find which pairs of percentages were different. These results are also provided below in Table 5. In summary, the percentage release from the preparation with 10% tetracycline was significantly higher than the 2.5% preparation at times 1 min, 4 min, 64 min, and 128 min, as well as higher than the 5% preparation at times 2 min, and 128 min. The 5% preparation was significantly different from the 2.5% preparation at 1 min and 32 min. At 1 min, all percentage release values were significantly different.

Time	Difference among samples (ANOVA)	Pairwise Differences (Tukey's W)
1 min	Yes, P < 0.001	10% > 2.5% (P < 0.001) 10% > 5% (P = 0.029) 5% > 2.5% (P= 0.003)
2 min	None	None
4 min	Yes, P = 0.018	10% > 2.5% (P < 0.001) 5% > 2.5% (P = 0.023)
8 min	None	None
16 min	None	None
32 min	Yes, P = 0.01	5% > 2.5% (P = 0.007)
64 min	Yes, P = 0.025	10% > 2.5% (P = 0.028)
128 min	Yes, P < 0.001	10% > 2.5% (P = 0.002) 10% < 5% (P = 0.003)

Table 5 : P – values for ANOVA and Tukey's W for 130 mg/mL samples (1 min to 128 min)

Release from 2.5% Tetracycline Preparations

Concentration

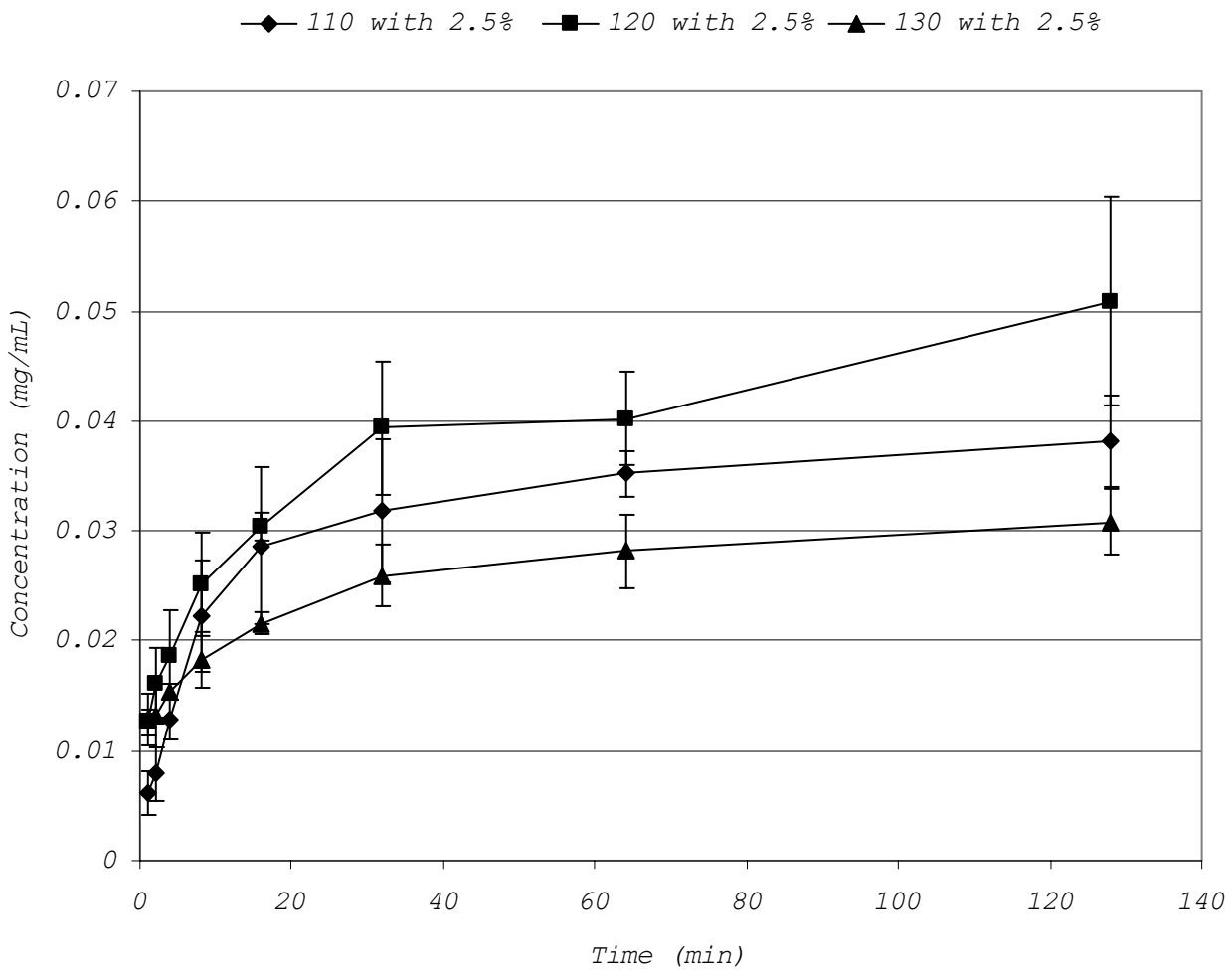

Figure 19 : Concentration versus time for preparations with 2.5% Tetracycline (1 min to 128 min)

Figure 19 shows the change in tetracycline concentration in the release solution over time for samples with 2.5% tetracycline loading. Three different fibrinogen concentrations are shown. Analysis of variance of the

tetracycline concentrations at each time point showed significant difference among the three fibrinogen concentrations (all $P < 0.01$). Tukey's W found significance differences between pairs of concentrations at all times. These results are summarized in Table 6. In summary, the 130 mg/mL and 120 mg/mL preparations exhibited significantly higher release than the 110 mg/mL preparations at times 1 min and 2 min. From that point on, the general trend shows significantly higher release from the 120 mg/mL preparations. The 110 mg/mL preparations also exhibited greater release than the 130 mg/mL preparations at 8 min, 16 min, 64 min, and 128 min.

Time	Difference among samples (ANOVA)	Pairwise Differences (Tukey's W)
1 min	Yes, P < 0.001	130 mg/mL > 110 mg/mL (P < 0.001) 120 mg/mL > 110 mg/mL (P < 0.001)
2 min	Yes, P < 0.001	130 mg/mL > 110 mg/mL (P < 0.001) 120 mg/mL > 110 mg/mL (P < 0.001)
4 min	Yes, P = 0.009	120 mg/mL > 130 mg/mL (P = 0.006)
8 min	Yes, P < 0.001	120 mg/mL > 130 mg/mL (P < 0.001) 110 mg/mL > 130 mg/mL (P = 0.005)
16 min	Yes, P < 0.001	120 mg/mL > 130 mg/mL (P < 0.001) 110 mg/mL > 130 mg/mL (P = 0.005)
32 min	Yes, P < 0.001	120 mg/mL > 110 mg/mL (P = 0.018) 120 mg/mL > 130 mg/mL (P < 0.001)
64 min	Yes, P < 0.001	110 mg/mL > 130 mg/mL (P < 0.001) 120 mg/mL > 110 mg/mL (P = 0.011) 120 mg/mL > 130 mg/mL (P < 0.001)
128 min	Yes, P < 0.001	110 mg/mL > 130 mg/mL (P = 0.048) 120 mg/mL > 110 mg/mL (P < 0.001) 120 mg/mL > 130 mg/mL (P < 0.001)

Table 6 : P – values for ANOVA and Tukey's W for release concentration of samples with 2.5 % tetracycline loading (1 min to 128 min)

Release from 2.5% Tetracycline Preparations

Percentage Release

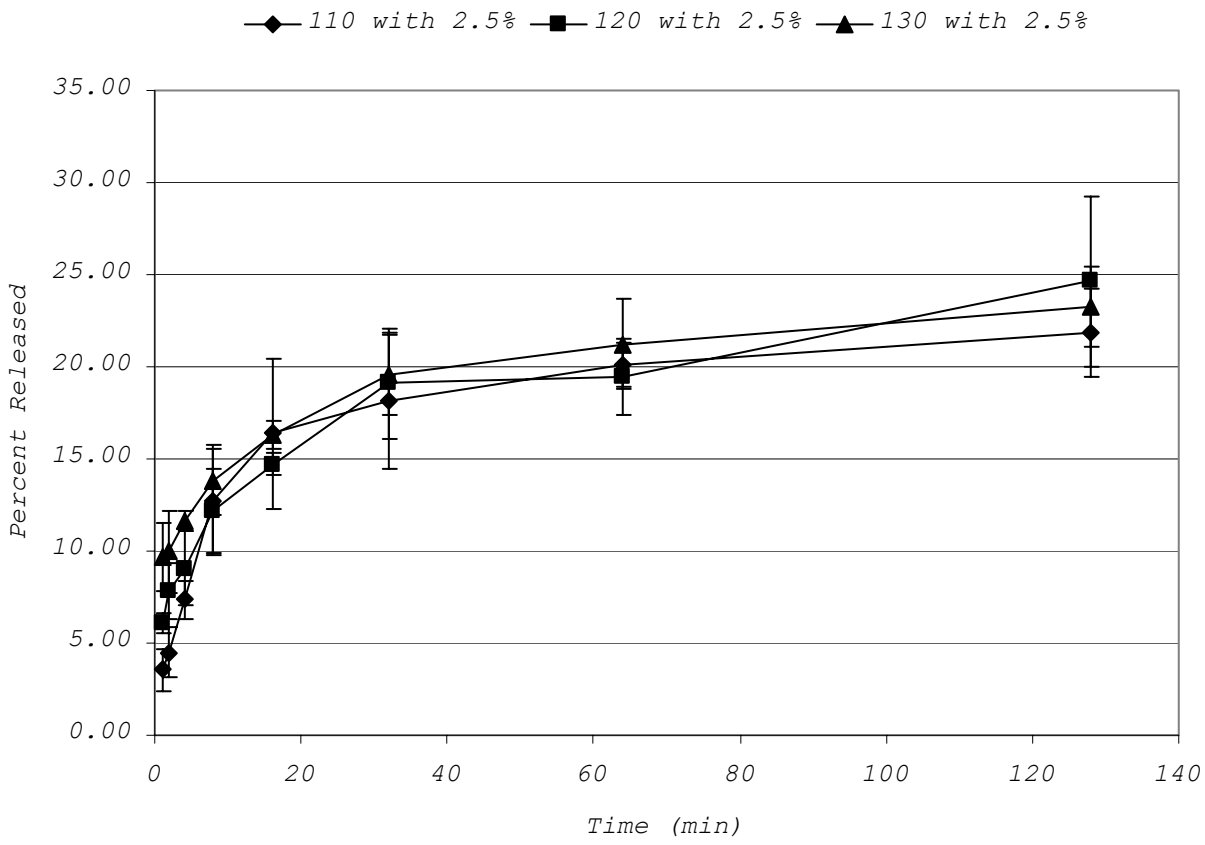

Figure 20 : Percentage released versus time for preparations with 2.5% tetracycline (1 min to 128 min)

Figure 20 shows the percentage release over time from preparations with 2.5% tetracycline loading for three different fibrinogen concentrations. These percentage release profiles are identical in shape to the concentration graphs of Figure 19. The concentrations have

been converted to percentage of tetracycline released by using the average mass of the sample, and the concentrations of Figure 19 as indicated in the Methods. The calculation of percentage release serves as a normalized view of release, independent of mass, and, therefore allows for a better comparison of release from different preparations.

Analysis of variance at each time event indicated that only at time points 1 min and 2 min, there existed significant differences among the samples. These results are provided below in Table 7. At time points where ANOVA indicated differences among the percentage values, Tukey's W was used to find which pairs of percentages were different. In short, the percentage release from the preparation with 130 mg/mL fibrinogen exceeded that of 120 mg/mL fibrinogen preparation at times 1 min and 2 min. In addition the percentage release from the preparation with 120 mg/mL fibrinogen was greater that of 110 mg/mL fibrinogen at those times. From 4 min, the percentage release from the three preparations was not significantly different.

Time	Difference among samples (ANOVA)	Pairwise Differences (Tukey's W)
1 min	Yes, P < 0.001	130 mg/mL > 110 mg/mL (P < 0.001) 130 mg/mL > 120 mg/mL (P < 0.001) 120 mg/mL > 110 mg/mL (P < 0.001)
2 min	Yes, P < 0.001	130 mg/mL > 110 mg/mL (P < 0.001) 130 mg/mL > 120 mg/mL (P = 0.040) 120 mg/mL > 110 mg/mL (P = 0.001)
4 min	None	None
8 min	None	None
16 min	None	None
32 min	None	None
64 min	None	None
128 min	None	None

Table 7 : P – values for ANOVA and Tukey's W for percentage release of samples with 2.5 % tetracycline loading (1 min to 128 min)

Release from 5% Tetracycline Preparations

Concentration

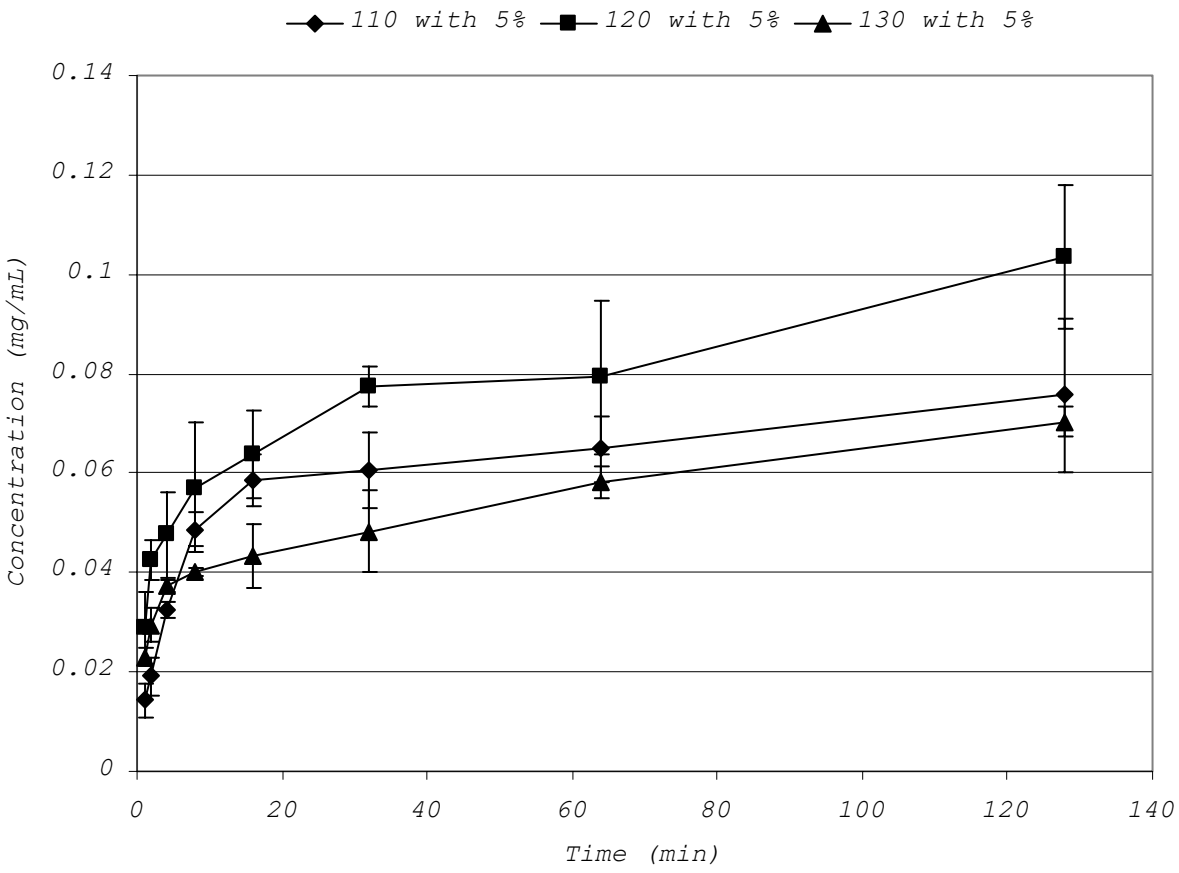

Figure 21 : Concentration versus time for preparations with 5% tetracycline (1 min to 128 min)

Figure 21 shows the change in tetracycline concentration in the release solution over time for samples with 5% tetracycline loading. Three different fibrinogen concentrations are shown. Analysis of variance of the tetracycline concentrations at each time point showed significant difference among the three fibrinogen

concentrations (all $P < 0.001$). Tukey's W found significance differences between pairs of concentrations at all times. These results are summarized in Table 8. In summary, at times 1 min and 2 min, the 130 mg/mL and 120 mg/mL fibrinogen preparations exhibited greater release than the 110 mg/mL preparation, with the release from 120 mg/mL exceeded the 130 mg/mL preparation as well. From that point on, the general trend shows significantly higher release from the 120 mg/mL preparations. The 110 mg/mL preparations also exhibited greater release than the 130 mg/mL preparations at 16 min and 32 min.

Time	Difference among samples (ANOVA)	Pairwise Differences (Tukey's W)
1 min	Yes, P < 0.001	130 mg/mL > 110 mg/mL (P = 0.003) 120 mg/mL > 110 mg/mL (P < 0.001) 120 mg/mL > 130 mg/mL (P = 0.050)
2 min	Yes, P < 0.001	130 mg/mL > 110 mg/mL (P < 0.001) 120 mg/mL > 110 mg/mL (P < 0.001) 120 mg/mL > 130 mg/mL (P < 0.001)
4 min	Yes, P < 0.001	120 mg/mL > 110 mg/mL (P < 0.001) 120 mg/mL > 130 mg/mL (P < 0.001)
8 min	Yes, P < 0.001	120 mg/mL > 130 mg/mL (P < 0.001)
16 min	Yes, P < 0.001	120 mg/mL > 130 mg/mL (P < 0.001) 110 mg/mL > 130 mg/mL (P < 0.001)
32 min	Yes, P < 0.001	120 mg/mL > 110 mg/mL (P < 0.001) 120 mg/mL > 130 mg/mL (P < 0.001) 110 mg/mL > 130 mg/mL (P = 0.003)
64 min	Yes, P < 0.001	120 mg/mL > 110 mg/mL (P = 0.013) 120 mg/mL > 130 mg/mL (P < 0.001)
128 min	Yes, P < 0.001	120 mg/mL > 110 mg/mL (P < 0.001) 120 mg/mL > 130 mg/mL (P < 0.001)

Table 8 : P – values for ANOVA and Tukey's W for release concentration of samples with 5 % tetracycline loading (1 min to 128 min)

Release from 5% Tetracycline Preparations

Percentage Release

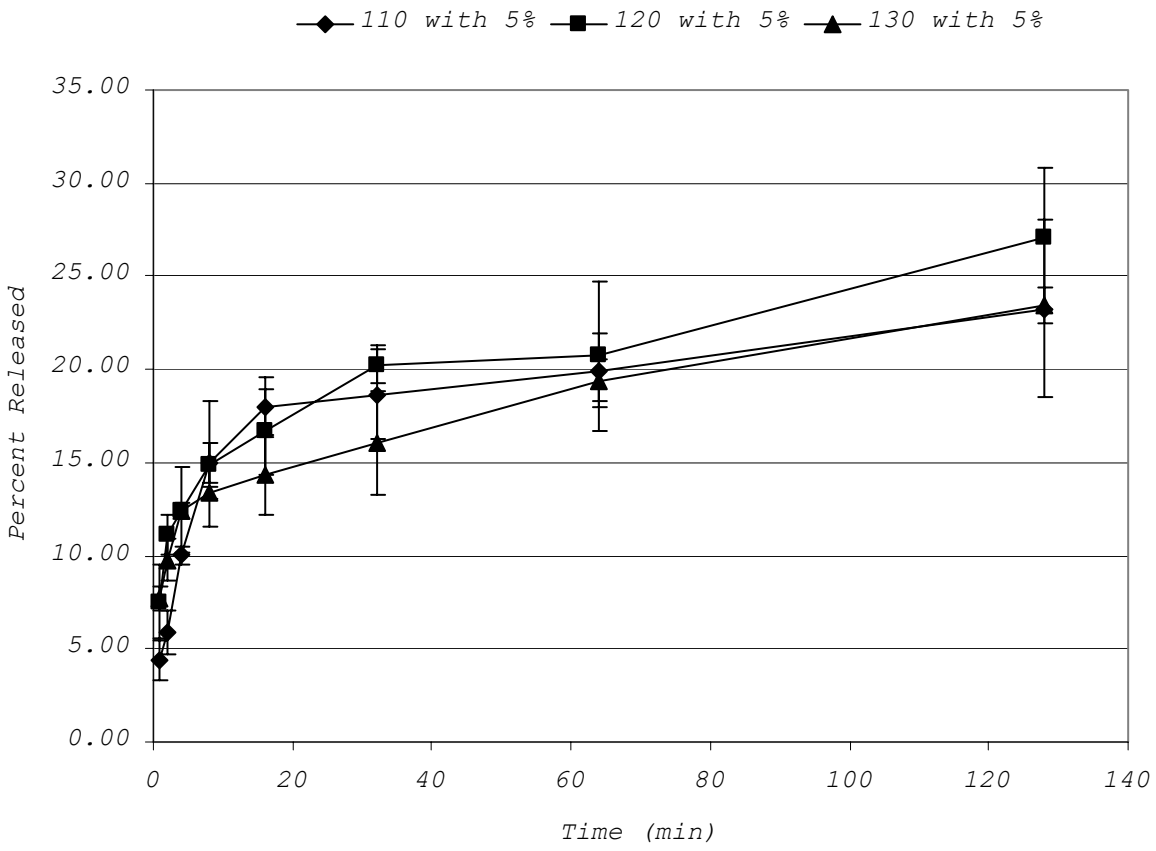

Figure 22 : Percentage released versus time for preparations with 5% Tetracycline (1 min to 128 min)

Figure 22 shows the percentage release over time from preparations with 5% tetracycline loading for three different fibrinogen concentrations. These percentage release profiles are identical in shape to the concentration profiles of Figure 21. The concentrations

have been converted to percentage of tetracycline released by using the average mass of the sample, and the concentrations of Figure 21 as indicated in the Methods. The calculation of percentage release serves as a normalized view of release, independent of mass, and, therefore allows for a better comparison of release from different preparations.

Analysis of variance at each time event indicated that at all times other than 64 min and 128 min, there existed significant difference among the samples. These results are provided below in Table 9. At time points where ANOVA indicated differences among the percentage values, Tukey's W was used to find which pairs of percentages were different. In short, the percentage release from the preparation with 130 mg/mL fibrinogen exceeded that of 110 mg/mL fibrinogen preparation at times 1 min, 2 min and 4 min. In addition the percentage release from the preparation with 120 mg/mL fibrinogen was greater that of 110 mg/mL fibrinogen at those times. At 8 min and 16 min, the percentage release from the 110 mg/mL was significantly greater than from the 130 mg/mL. At 32 min, the release from 120 mg/mL exceeded 130 mg/mL. There were no significant differences in percentage release after 32 min.

Time	Difference among samples (ANOVA)	Pairwise Differences (Tukey's W)
1 min	Yes, P < 0.001	130 mg/mL > 110 mg/mL (P < 0.001) 120 mg/mL > 110 mg/mL (P < 0.001)
2 min	Yes, P < 0.001	130 mg/mL > 110 mg/mL (P < 0.001) 120 mg/mL > 130 mg/mL (P = 0.050) 120 mg/mL > 110 mg/mL (P < 0.001)
4 min	Yes, P = 0.001	130 mg/mL > 110 mg/mL (P < 0.001) 120 mg/mL > 110 mg/mL (P < 0.001)
8 min	Yes, P = 0.004	110 mg/mL > 130 mg/mL (P = 0.003)
16 min	Yes, P = 0.002	110 mg/mL > 130 mg/mL (P = 0.002)
32 min	Yes, P = 0.002	120 mg/mL > 130 mg/mL (P = 0.050)
64 min	None	None
128 min	None	None

Table 9 : P – values for ANOVA and Tukey's W for percentage release of samples with 5 % tetracycline loading (1 min to 128 min)

Release from 10% Tetracycline Preparations

Concentration

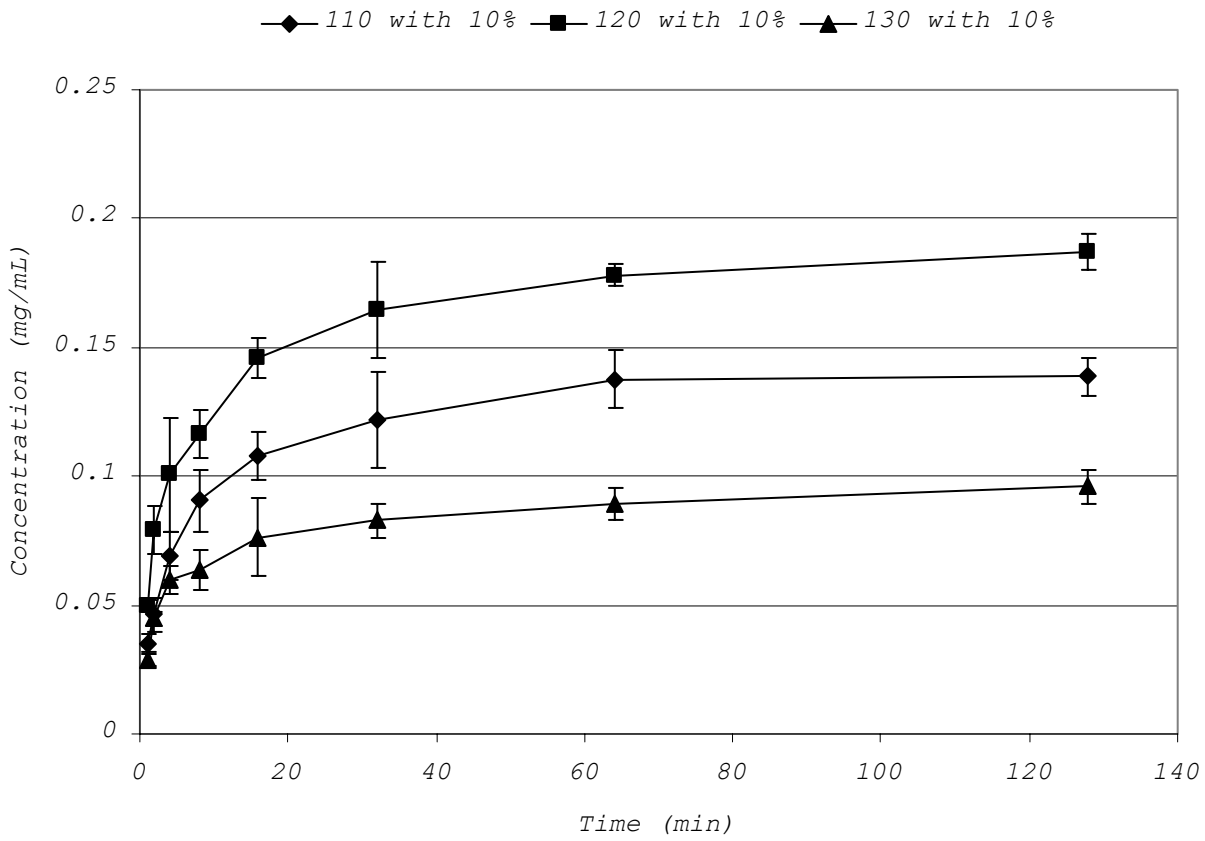

Figure 23 : Concentration versus time for preparations with 10% Tetracycline (1 min to 128 min)

Figure 23 shows the change in tetracycline concentration in the release solution over time for samples with 10% tetracycline loading. Three different fibrinogen concentrations are shown. Analysis of variance of the tetracycline concentrations at each time point showed

significant difference among the three fibrinogen concentrations (all $P < 0.001$). Tukey's W found significance differences between pairs of concentrations at all times. These results are summarized in Table 10. In summary, the 120 mg/mL fibrinogen preparations exhibited significantly greater release at all times. In addition, at all times other than 2 min and 4 min, the 110 mg/mL fibrinogen preparations exhibited greater release than the 130 mg/mL preparations.

Time	Difference among samples (ANOVA)	Pairwise Differences (Tukey's W)
1 min	Yes, P < 0.001	120 mg/mL > 130 mg/mL (P < 0.001) 120 mg/mL > 110 mg/mL (P < 0.001) 110 mg/mL > 130 mg/mL (P = 0.001)
2 min	Yes, P < 0.001	120 mg/mL > 110 mg/mL (P < 0.001) 120 mg/mL > 130 mg/mL (P < 0.001)
4 min	Yes, P < 0.001	120 mg/mL > 110 mg/mL (P < 0.001) 120 mg/mL > 130 mg/mL (P < 0.001)
8 min	Yes, P < 0.001	120 mg/mL > 130 mg/mL (P < 0.001) 120 mg/mL > 110 mg/mL (P < 0.001) 110 mg/mL > 130 mg/mL (P = 0.001)
16 min	Yes, P < 0.001	120 mg/mL > 130 mg/mL (P < 0.001) 120 mg/mL > 110 mg/mL (P < 0.001) 110 mg/mL > 130 mg/mL (P < 0.001)
32 min	Yes, P < 0.001	120 mg/mL > 130 mg/mL (P < 0.001) 120 mg/mL > 110 mg/mL (P < 0.001) 110 mg/mL > 130 mg/mL (P < 0.001)
64 min	Yes, P < 0.001	120 mg/mL > 130 mg/mL (P < 0.001) 120 mg/mL > 110 mg/mL (P < 0.001) 110 mg/mL > 130 mg/mL (P < 0.001)
128 min	Yes, P < 0.001	120 mg/mL > 130 mg/mL (P < 0.001) 120 mg/mL > 110 mg/mL (P < 0.001) 110 mg/mL > 130 mg/mL (P < 0.001)

Table 10 : P – values for ANOVA and Tukey's W for release concentration of samples with 10 % tetracycline loading (1 min to 128 min)

Release from 10% Tetracycline Preparations

Percentage Release

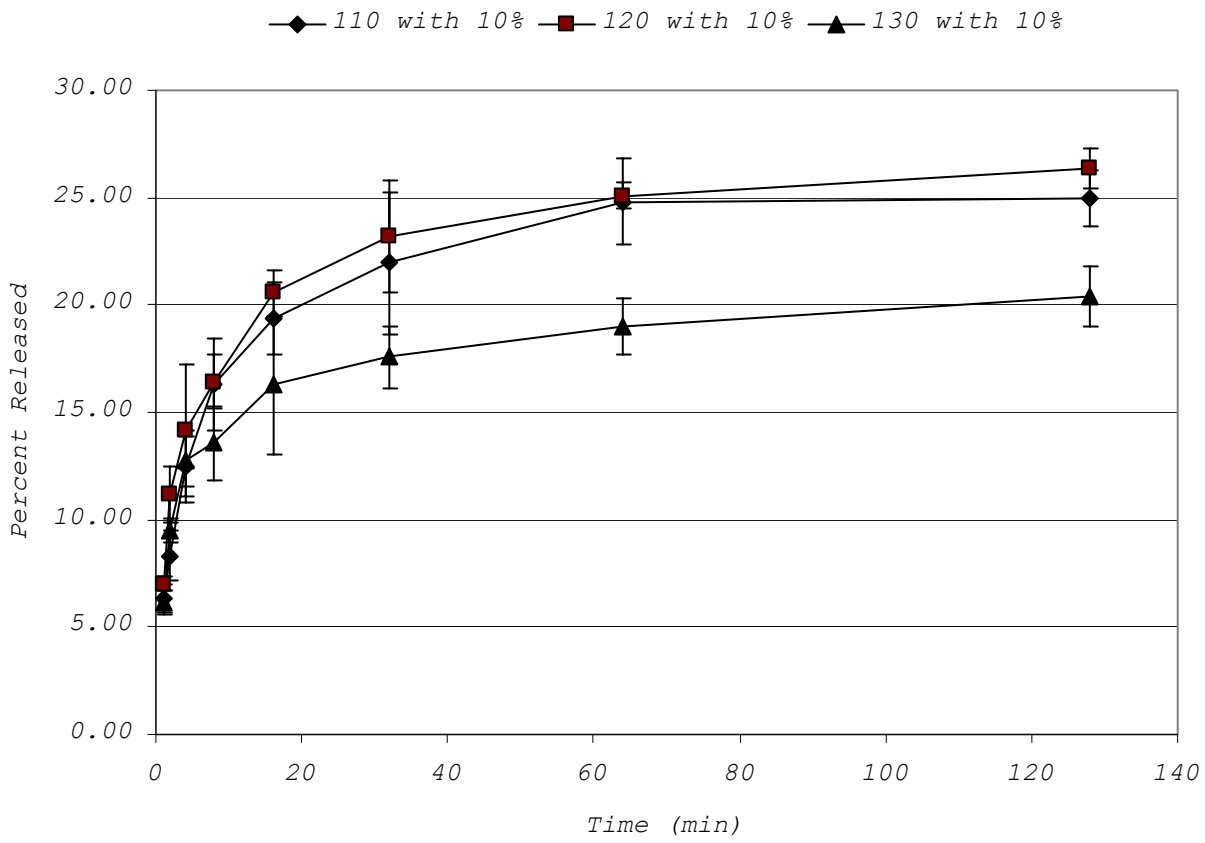

Figure 24 : Percentage released versus time for preparations with 10% tetracycline (1 min to 128 min)

Figure 24 shows the percentage release over time from preparations with 10% tetracycline loading for three different fibrinogen concentrations. These percentage release profiles are identical in shape to the concentration profiles of Figure 23. The concentrations

have been converted to percentage of tetracycline released by using the average mass of the sample, and the concentrations of Figure 23 as indicated in the Methods. The calculation of percentage release serves as a normalized view of release, independent of mass, and, therefore allows for a better comparison of release from different preparations.

Analysis of variance at each time event indicated that at all times other than 64 min and 128 min, there existed significant difference among the samples. These results are provided below in Table 11. At time points where ANOVA indicated differences among the percentage values, Tukey's W was used to find which pairs of percentages were different. In short, the percentage release from the preparation with 120 mg/mL fibrinogen exceeded that of 110 mg/mL and 130 mg/mL fibrinogen preparation at times 1 min and 2 min. At 8 min and beyond, the percentage releases from the 110 mg/mL and 120 mg/mL were significantly greater than from the 130 mg/mL, but not significantly different from each other. There were no significant differences in percentage release at 4 min.

Time	Difference among samples (ANOVA)	Pairwise Differences (Tukey's W)
1 min	Yes, P = 0.007	120 mg/mL > 130 mg/mL (P = 0.009) 120 mg/mL > 110 mg/mL (P = 0.025)
2 min	Yes, P < 0.001	120 mg/mL > 110 mg/mL (P < 0.001) 120 mg/mL > 130 mg/mL (P = 0.008)
4 min	None	None
8 min	Yes, P = 0.002	120 mg/mL > 130 mg/mL (P = 0.005) 110 mg/mL > 130 mg/mL (P = 0.007)
16 min	Yes, P < 0.001	120 mg/mL > 130 mg/mL (P = 0.001) 110 mg/mL > 130 mg/mL (P = 0.014)
32 min	Yes, P < 0.001	120 mg/mL > 130 mg/mL (P < 0.001) 110 mg/mL > 130 mg/mL (P = 0.004)
64 min	Yes, P < 0.001	120 mg/mL > 130 mg/mL (P < 0.001) 110 mg/mL > 130 mg/mL (P < 0.001)
128 min	Yes, P < 0.001	120 mg/mL > 130 mg/mL (P < 0.001) 110 mg/mL > 130 mg/mL (P < 0.001)

Table 11 : P – values for ANOVA and Tukey's W for percentage release of samples with 10% tetracycline loading (1 min to 128 min)

Long Term Release

The data for long term release (1 hr to 168 hr) follows for all preparations. As with the short term release, there exist three fibrinogen concentrations (110 mg/mL, 120 mg/mL, and 130 mg/mL), each with three different loading percentages of tetracycline (2.5% w/w, 5% w/w, 10% w/w). First any differences in release profiles for different tetracycline loading of a given fibrinogen concentration are reported. Then, concentration over the time course of the release run (1 hr to 168 hr) is analyzed to determine when significant release occurred. Then the results are grouped by tetracycline loading to observe the effects of varying fibrinogen concentrations. All analyses will utilize ANOVA and Tukey's W, with the *a priori* significance level of $\alpha = 0.05$.

Concentration Results (Long Term)

All Preparations

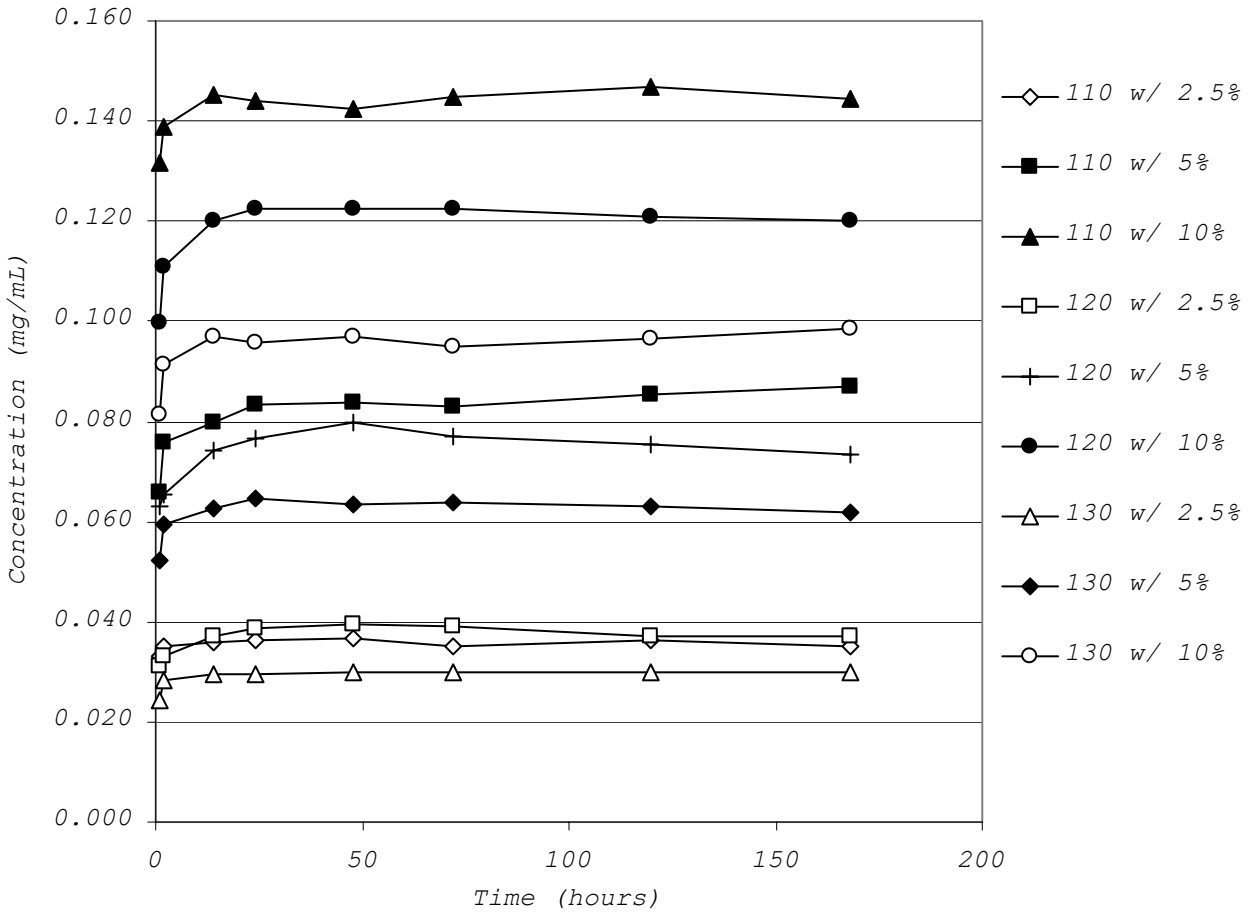

Figure 25 : Concentrations of all preparations over time 168 hours

Figure 25 shows release solution concentrations of all preparations over time 168 hours. The error bars have been omitted for clarity. The general trend shows that concentrations at time events increase with increasing tetracycline loading, and decrease with increasing

fibrinogen concentration (fiber diameter). However, the statistics only validate one of these assertions. ANOVA observed differences among concentrations at all times (all $P < 0.05$) – however, Tukey's W only showed significant differences between concentration values of pairs that were of different tetracycline concentrations. For example, two preparations of identical tetracycline loading (110 mg/mL with 5% tetracycline and 120 mg/mL with 5% tetracycline) would not have significantly different concentrations in their release solutions at any particular time point in the run. Significant differences were only seen when the tetracycline weight percentages differed. Tables replete with P-values of these comparisons can be found in the Appendix. Individual figures for each fibrinogen concentration and each tetracycline percentage follow.

Release from 110 mg/mL Fibrinogen

Concentration

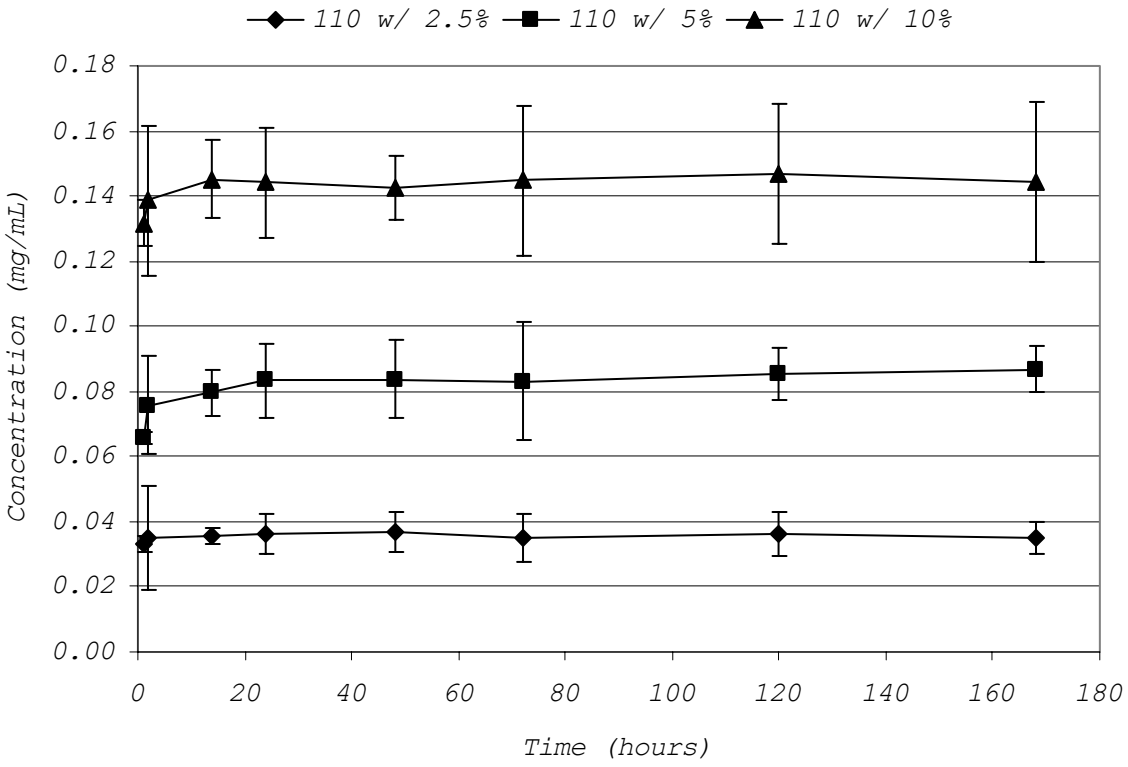

Figure 26 : Concentration versus time 110 mg/mL fibrinogen with tetracycline hydrochloride (1 hr to 168 hr)

Figure 26 shows the change in tetracycline concentration in the release solution over time for 110 mg/mL fibrinogen samples. Three different loading percentages of tetracycline are shown. Analysis of variance of the tetracycline concentrations at each time point showed significant difference among the three tetracycline loading concentrations (all $P < 0.001$).

Tukey's W found that, for each time point, the release concentration of the 10% preparation was significantly higher than the 5% preparation (all $P < 0.001$), and that the release concentration of the 5% preparation was higher than that of the 2.5% preparation (all $P < 0.001$). Therefore, the three profiles in Figure 26 are significantly different.

For the 110 mg/mL fibrinogen with 2.5% tetracycline, ANOVA revealed that the concentrations from 1 hr to 168 hr were not significantly different. This indicates no significant release during the duration of this run (no release after 1 hour).

For the 110 mg/mL fibrinogen with 5% tetracycline, ANOVA revealed that the concentrations from 1 min to 128 min were significantly different ($P < 0.001$). However, Tukey's W found that the only significant differences in concentration were observed between 1 hour and all times greater than 24 hours. Since no concentrations after 24 hours were judged significantly different from the 24 value, no significant release can be reported after 24 hours. (See Table 29 in Appendix for all pairwise P-values)

For the 110 mg/mL fibrinogen with 10% tetracycline, ANOVA revealed that the concentrations from 1 hr to 168 hr

were not significantly different. This indicates no significant release during the duration of this run (no release after 1 hour).

Release from 110 mg/mL Fibrinogen

Percentage Release

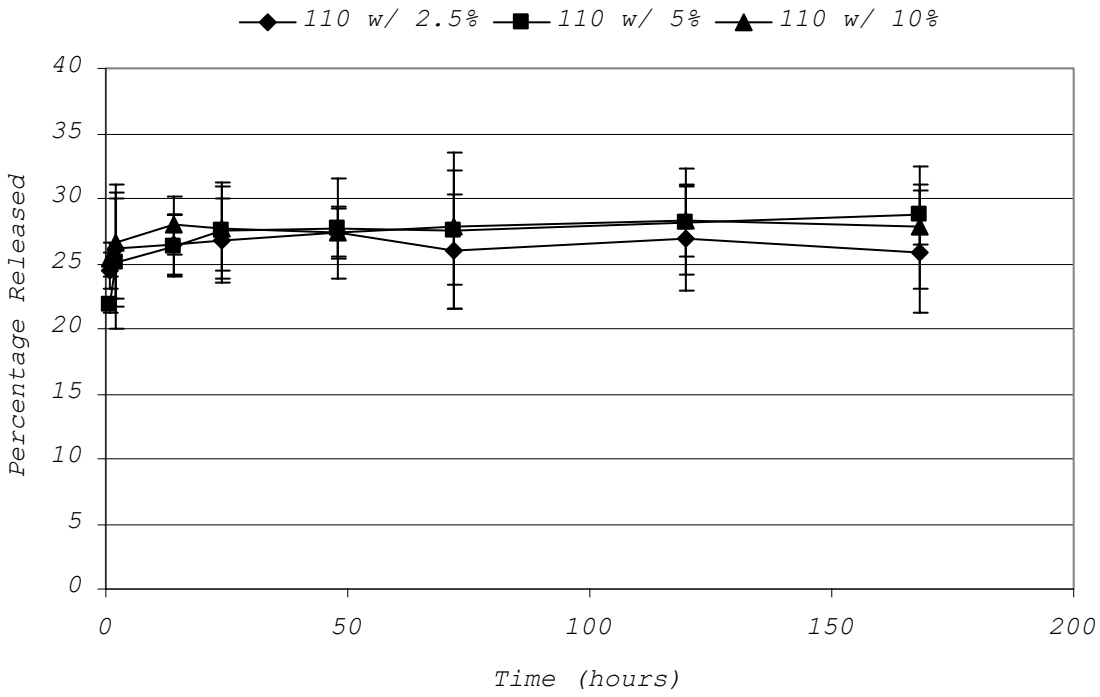

Figure 27 : Percentage released versus time for 110 mg/mL fibrinogen with tetracycline hydrochloride (1 hr to 168 hr)

Figure 27 shows the percentage release over time from preparations of 110 mg/mL fibrinogen with three different tetracycline loading concentrations. These percentage release profiles are identical in shape to the concentration profiles of Figure 26. The concentrations have been converted to percentage of tetracycline released by using the average mass of the sample, and the

concentrations of Figure 26 as indicated in the Methods. The calculation of percentage release serves as a normalized view of release, independent of mass, and, therefore allows for a better comparison of release from different preparations.

Analysis of variance at each time event indicated that at only time point 1 hour, there existed significant difference among the samples. These results are provided below in Table 12. At time points where ANOVA indicated differences among the percentage values, Tukey's W was used to find which pairs of percentages were different. These results are also provided in Table 12. The percentage release between 110 mg/mL preparations of different tetracycline loadings differed only at 1 hour. At 1 hour, the 2.5% tetracycline and 10 % tetracycline preparations exhibited greater release than the 5% tetracycline preparation.

Time	Difference among samples (ANOVA)	Pairwise Differences (Tukey's W)
1 hr	Yes, P < 0.001	2.5% > 5% (P < 0.001) 10% > 5% (P < 0.001)
4 hr	None	None
14 hr	None	None
24 hr	None	None
48 hr	None	None
72 hr	None	None
120 hr	None	None
168 hr	None	None

Table 12 : P – values for ANOVA and Tukey's W for 110 mg/mL samples of different tetracycline loading (1 min to 128 min)

Release from 120 mg/mL Fibrinogen

Concentration

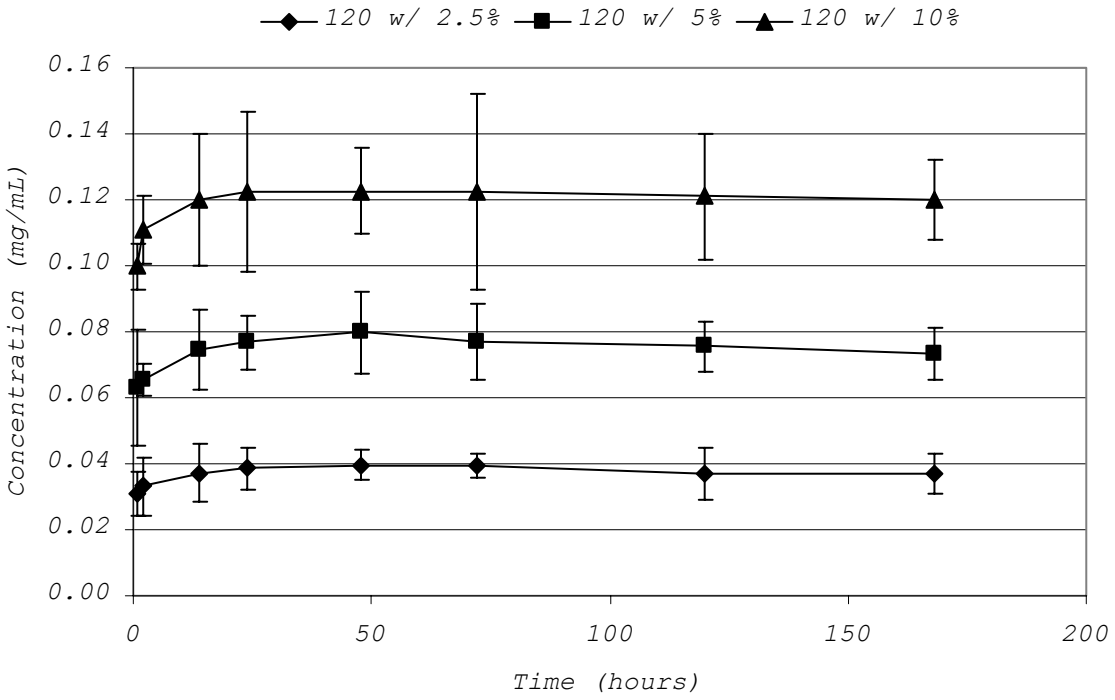

Figure 28 : Concentration versus time 120 mg/mL fibrinogen with tetracycline hydrochloride (1 hr to 168 hr)

Figure 28 shows the change in tetracycline concentration in the release solution over time for 120 mg/mL fibrinogen samples. Three different loading percentages of tetracycline are shown. Analysis of variance of the tetracycline concentrations at each time point showed significant difference among the three tetracycline loading concentrations (all $P < 0.001$).

Tukey's W found that, for each time point, the release concentration of the 10% preparation was significantly higher than the 5% preparation (all $P < 0.001$), and that the release concentration of the 5% preparation was higher than that of the 2.5% preparation (all $P < 0.001$). Therefore, the three profiles in Figure 28 are significantly different.

For the 120 mg/mL fibrinogen with 2.5% tetracycline, ANOVA revealed that the concentrations from 1 hr to 168 hr were significantly different ($P = 0.035$). However, Tukey's W returned no significant differences between any values. This indicates no significant release can be inferred over the duration of this run (no release after 1 hour).

For the 120 mg/mL fibrinogen with 5% tetracycline, ANOVA revealed that the concentrations from 1 min to 128 min were significantly different ($P < 0.001$). However, Tukey's W found that the only significant differences in concentration were observed between 48 hours and 1 hour. Since no concentrations after 48 hours were judged significantly different from the 48 hour value, no significant release can be reported after 48 hours. (See Table 30 in Appendix for all pairwise P-values)

For the 120 mg/mL fibrinogen with 10% tetracycline, ANOVA revealed that the concentrations from 1 hr to 168 hr were significantly different (P = 0.031). However, Tukey's W returned no significant differences between any values. This indicates no significant release can be inferred over the duration of this run (no release after 1 hour).

Release from 120 mg/mL Fibrinogen

Percentage Release

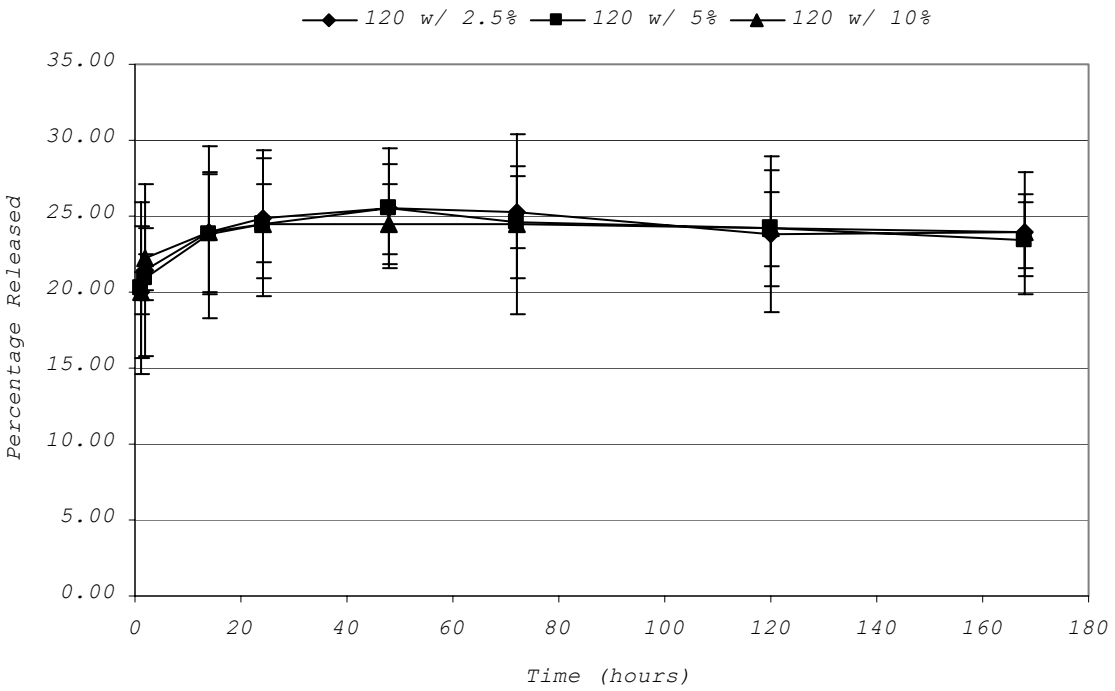

Figure 29 : Percentage released versus time for 120 mg/mL fibrinogen with tetracycline hydrochloride (1 hr to 168 hr)

Figure 29 shows the percentage release over time from preparations of 120 mg/mL fibrinogen with three different tetracycline loading concentrations. These percentage release profiles are identical in shape to the concentration profiles of Figure 28. The concentrations have been converted to percentage of tetracycline released by using the average mass of the sample, and the

concentrations of Figure 28 as indicated in the Methods. The calculation of percentage release serves as a normalized view of release, independent of mass, and, therefore allows for a better comparison of release from different preparations.

Analysis of variance at each time event indicated that at no time points did there exist significant differences among the samples. Therefore, all 120 mg/mL fibrinogen preparations exhibited similar percentage release.

Release from 130 mg/mL Fibrinogen

Concentration

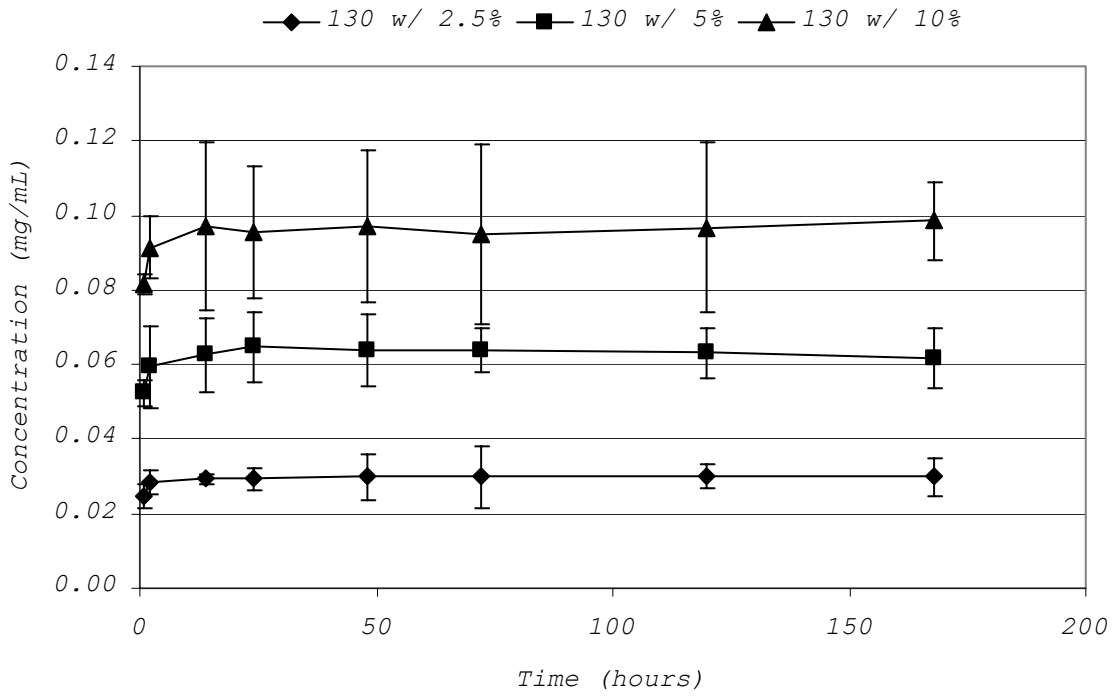

Figure 30 : Concentration versus time 130 mg/mL fibrinogen with tetracycline hydrochloride (1 hr to 168 hr)

Figure 30 shows the change in tetracycline concentration in the release solution over time for 130 mg/mL fibrinogen samples. Three different loading percentages of tetracycline are shown. Analysis of variance of the tetracycline concentrations at each time point showed significant difference among the three

tetracycline loading concentrations (all $P < 0.001$). Tukey's W found that, for each time point, the release concentration of the 10% preparation was significantly higher than the 5% preparation (all $P < 0.001$), and that the release concentration of the 5% preparation was higher than that of the 2.5% preparation (all $P < 0.001$). Therefore, the three profiles in Figure 30 are significantly different.

For the 130 mg/mL fibrinogen with 2.5% tetracycline, ANOVA revealed that the concentrations from 1 hr to 168 hr were significantly different ($P = 0.045$). However, Tukey's W returned no significant differences between any values. This indicates no significant release can be inferred over the duration of this run (no release after 1 hour).

For the 130 mg/mL fibrinogen with 5% tetracycline, ANOVA revealed that the concentrations from 1 min to 128 min were significantly different ($P < 0.001$). However, Tukey's W found that the only significant differences in concentration were observed between times after 24 hours and 1 hour. Since no concentrations after 24 hours were judged significantly different from the 24 hour value, no significant release can be reported after 24 hours. (See Table 30 in Appendix for all pairwise P-values)

For the 130 mg/mL fibrinogen with 10% tetracycline, ANOVA revealed that the concentrations from 1 hr to 168 hr were significantly different (P = 0.031). This indicates no significant release can be inferred over the duration of this run (no release after 1 hour).

Release from 130 mg/mL Fibrinogen

Percentage Release

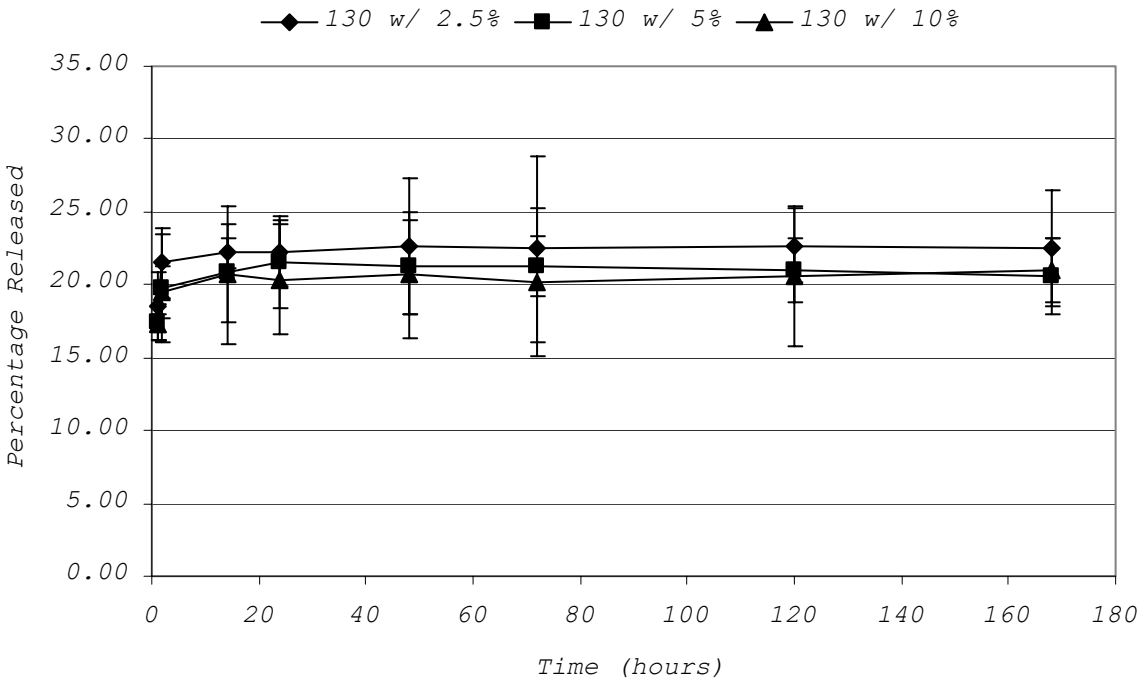

Figure 31 : Percentage released versus time for 130 mg/mL fibrinogen with tetracycline hydrochloride (1 hr to 168 hr)

Figure 31 shows the percentage release over time from preparations of 130 mg/mL fibrinogen with three different tetracycline loading concentrations. These percentage release profiles are identical in shape to the concentration graphs of Figure 30. The concentrations have been converted to percentage of tetracycline released by using the average mass of the sample, and the

concentrations of Figure 30 as indicated in the Methods. The calculation of percentage release serves as a normalized view of release, independent of mass, and, therefore allows for a better comparison of release from different preparations.

Analysis of variance at each time event indicated that at no time points did there exist significant differences among the samples. Therefore, all 130 mg/mL fibrinogen preparations exhibited similar percentage release.

Long Term Release

Release from 2.5% Tetracycline Preparations

Concentration

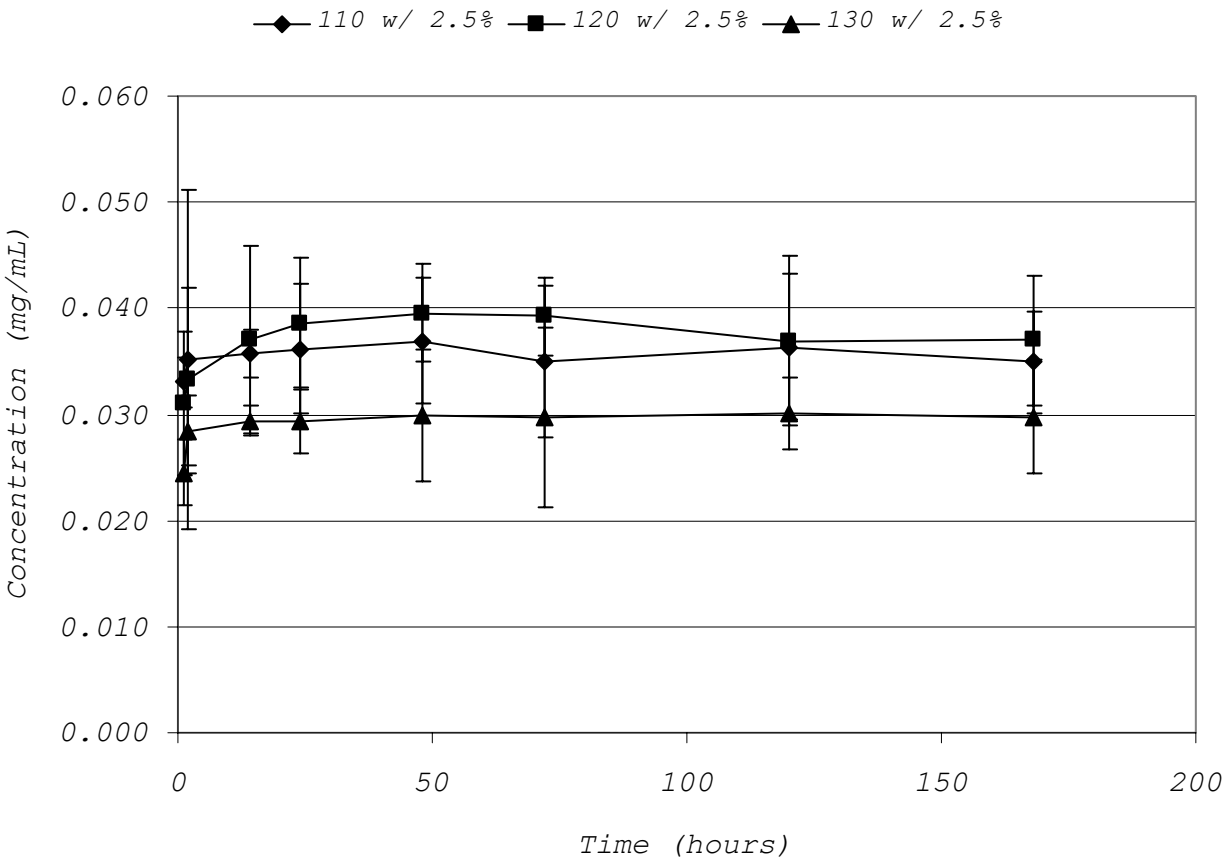

Figure 32 : Concentration versus time for preparations with 2.5% tetracycline over 168 hours

Figure 32 shows the tetracycline concentration of the release solution over time 168 hours for fibrinogen preparations with 2.5% tetracycline loading. Three

different fibrinogen concentrations are shown. Analysis of variance of the tetracycline concentrations at each time point showed significant difference among the three fibrinogen concentrations at all times other than 4 hours (all $P < 0.05$). Tukey's W found significance differences between pairs of concentrations at all times other than 4 hours. These results are summarized in Table 13. In summary, the 120 mg/mL fibrinogen preparations exhibited significantly greater release than 130 mg/mL fibrinogen at all times other than 4 hours. In addition, at 14 hours, the 120 mg/mL fibrinogen preparation expressed significant greater release than the 110 mg/mL preparation. Also, at 1 hour, 24 hours, and 48 hours, the 110 mg/mL fibrinogen preparation expressed significantly higher release than the 130 mg/mL preparations.

Time	Difference among samples (ANOVA)	Pairwise Differences (Tukey's W)
1 hr	Yes, P < 0.001	120 mg/mL > 130 mg/mL (P = 0.005) 110 mg/mL > 130 mg/mL (P < 0.001)
4 hr	None	None
14 hr	Yes, P = 0.002	120 mg/mL > 110 mg/mL (P = 0.003) 120 mg/mL > 130 mg/mL (P = 0.013)
24 hr	Yes, P < 0.001	120 mg/mL > 130 mg/mL (P < 0.001) 110 mg/mL > 130 mg/mL (P = 0.008)
48 hr	Yes, P < 0.001	120 mg/mL > 130 mg/mL (P < 0.001) 110 mg/mL > 130 mg/mL (P = 0.013)
72 hr	Yes, P = 0.006	120 mg/mL > 130 mg/mL (P = 0.005)
120 hr	Yes, P = 0.023	120 mg/mL > 130 mg/mL (P = 0.034)
168 hr	Yes, P = 0.008	120 mg/mL > 130 mg/mL (P = 0.008)

Table 13 : P – values for ANOVA and Tukey's W for release concentration of samples with 2.5 % tetracycline loading (1 hr to 168 hr)

Release from 2.5% Tetracycline Preparations

Percentage Release

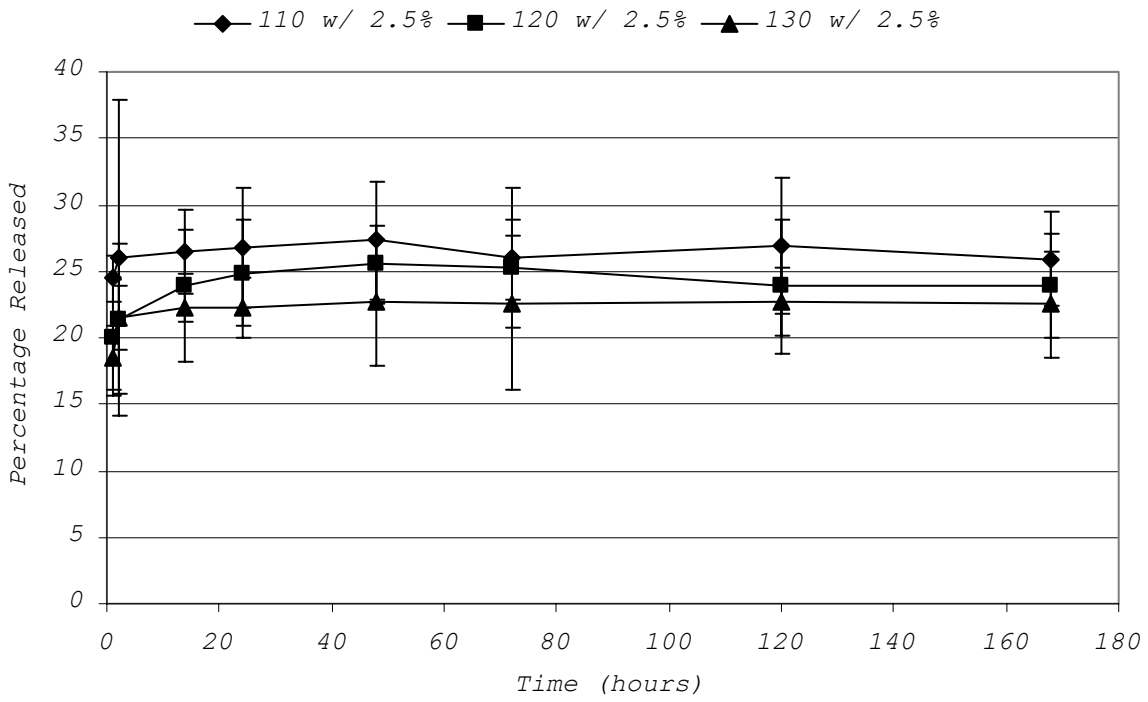

Figure 33 : Percentage release versus time for preparations with 2.5% tetracycline over 168 hours

Figure 33 shows the percentage release over time from preparations with 2.5% tetracycline loading for three different fibrinogen concentrations. These percentage release profiles are identical in shape to the concentration graphs of Figure 32. The concentrations have been converted to percentage of tetracycline released by using the average mass of the sample, and the concentrations of Figure 32 as indicated in the Methods.

The calculation of percentage release serves as a normalized view of release, independent of mass, and, therefore allows for a better comparison of release from different preparations.

Analysis of variance at each time event indicated that at times 1 hour, 14 hours, 24 hours, and 48 hours, there existed significant difference among the samples. These results are provided below in Table 14. At time points where ANOVA indicated differences among the percentage values, Tukey's W was used to find which pairs of percentages were different. In short, significant differences in percentage released were seen at 1 hour, 14 hours, 24 hours, and 48 hours. At each of these times, the 110 mg/mL preparations exhibited higher release than the 130 mg/mL preparations. In addition, at time 1 hour, the 110 mg/mL preparations had significantly higher release than the 120 mg/mL preparations. No other significant differences were found.

Time	Difference among samples (ANOVA)	Pairwise Differences (Tukey's W)
1 hr	Yes, P < 0.001	110 mg/mL > 120 mg/mL (P = 0.003) 110 mg/mL > 130 mg/mL (P < 0.001)
4 hr	None	None
14 hr	Yes, P = 0.017	110 mg/mL > 130 mg/mL (P = 0.013)
24 hr	Yes, P = 0.018	110 mg/mL > 130 mg/mL (P = 0.013)
48 hr	Yes, P = 0.028	110 mg/mL > 130 mg/mL (P = 0.022)
72 hr	None	None
120 hr	None	None
168 hr	None	None

Table 14 : P – values for ANOVA and Tukey's W for percentage release of samples with 2.5 % tetracycline loading (1 hr to 168 hr)

Concentration Results (Long Term)

Release from 5% Tetracycline Preparations

Concentration

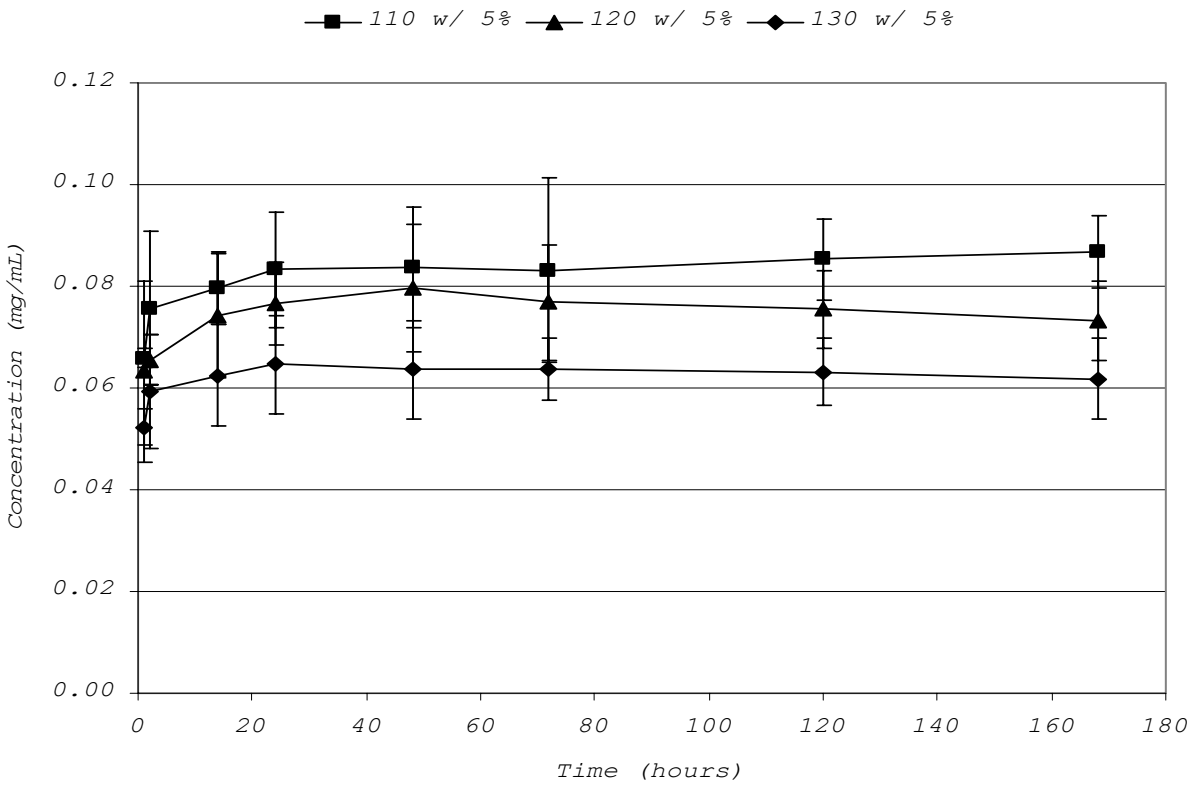

Figure 34 : Concentration versus time for preparations with 5% tetracycline over 168 hours

Figure 34 shows the tetracycline concentration of the release solution over time 168 hours for fibrinogen preparations with 5% tetracycline loading. Three different fibrinogen concentrations are shown. Analysis of variance of the tetracycline concentrations at each time point

showed significant difference among the three fibrinogen concentrations at all times (all $P < 0.05$). Tukey's W found significance differences between pairs of concentrations at all times. These results are summarized in Table 15. In summary, the 110 mg/mL fibrinogen preparations exhibited significantly greater release than 130 mg/mL fibrinogen at all times. In addition, at all times other than 4 hours, the 120 mg/mL fibrinogen preparations expressed significant greater release than the 130 mg/mL preparations. Also, at 120 hours and 168 hours, the 110 mg/mL fibrinogen preparation expressed significantly higher release than the 120 mg/mL preparations.

Time	Difference among samples (ANOVA)	Pairwise Differences (Tukey's W)
1 hr	Yes, P = 0.008	120 mg/mL > 130 mg/mL (P = 0.042) 110 mg/mL > 130 mg/mL (P = 0.008)
4 hr	Yes, P = 0.004	110 mg/mL > 130 mg/mL (P = 0.003)
14 hr	Yes, P < 0.001	120 mg/mL > 130 mg/mL (P = 0.018) 110 mg/mL > 130 mg/mL (P < 0.001)
24 hr	Yes, P < 0.001	120 mg/mL > 130 mg/mL (P = 0.015) 110 mg/mL > 130 mg/mL (P < 0.001)
48 hr	Yes, P < 0.001	120 mg/mL > 130 mg/mL (P = 0.005) 110 mg/mL > 130 mg/mL (P < 0.001)
72 hr	Yes, P = 0.003	120 mg/mL > 130 mg/mL (P = 0.044) 110 mg/mL > 130 mg/mL (P = 0.002)
120 hr	Yes, P < 0.001	120 mg/mL > 130 mg/mL (P < 0.001) 110 mg/mL > 120 mg/mL (P = 0.008) 110 mg/mL > 130 mg/mL (P < 0.001)
168 hr	Yes, P < 0.001	120 mg/mL > 130 mg/mL (P = 0.002) 110 mg/mL > 120 mg/mL (P < 0.001) 110 mg/mL > 130 mg/mL (P < 0.001)

Table 15 : P – values for ANOVA and Tukey's W for release concentration of samples with 5 % tetracycline loading (1 hr to 168 hr)

Release from 5% Tetracycline Preparations

Percentage Release

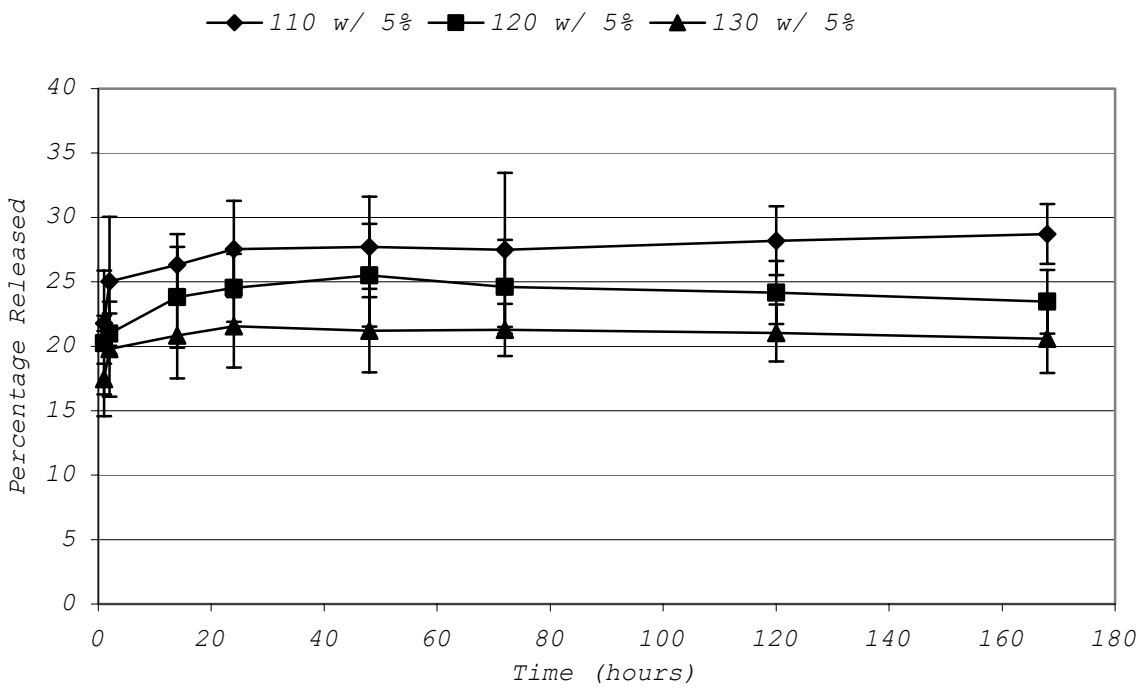

Figure 35: Percentage release versus time for preparations with 5% tetracycline over 168 hours

Figure 35 shows the percentage release over time from preparations with 5% tetracycline loading for three different fibrinogen concentrations. These percentage release profiles are identical in shape to the concentration profiles of Figure 34. The concentrations have been converted to percentage of tetracycline released by using the average mass of the sample, and the concentrations of Figure 34 as indicated in the Methods.

The calculation of percentage release serves as a normalized view of release, independent of mass, and, therefore allows for a better comparison of release from different preparations.

Analysis of variance at each time event indicated that there existed significant difference among the samples at all times. These results are provided below in Table 16. At time points where ANOVA indicated differences among the percentage values, Tukey's W was used to find which pairs of percentages were different. In short, significant differences in percentage released were seen at all times. At each of these times, the 110 mg/mL preparations exhibited higher release than the 130 mg/mL preparations. In addition, at time 4 hours, 120 hours, and 168 hours the 110 mg/mL preparations had significantly higher release than the 120 mg/mL preparations. Also, at times 48 hours, 120 hours, and 168 hours, the 120 mg/mL preparations had significantly greater release than the 130 mg/mL preparations.

Time	Difference among samples (ANOVA)	Pairwise Differences (Tukey's W)
1 hr	Yes, P = 0.012	110 mg/mL > 130 mg/mL (P = 0.009)
4 hr	Yes, P = 0.004	110 mg/mL > 120 mg/mL (P = 0.004) 110 mg/mL > 130 mg/mL (P = 0.030)
14 hr	Yes, P = 0.001	110 mg/mL > 130 mg/mL (P < 0.001)
24 hr	Yes, P < 0.001	110 mg/mL > 130 mg/mL (P < 0.001)
48 hr	Yes, P < 0.001	110 mg/mL > 130 mg/mL (P < 0.001) 120 mg/mL > 130 mg/mL (P = 0.021)
72 hr	Yes, P = 0.004	110 mg/mL > 130 mg/mL (P = 0.003)
120 hr	Yes, P < 0.001	110 mg/mL > 130 mg/mL (P < 0.001) 110 mg/mL > 120 mg/mL (P < 0.001) 120 mg/mL > 130 mg/mL (P = 0.010)
168 hr	Yes, P < 0.001	110 mg/mL > 130 mg/mL (P < 0.001) 110 mg/mL > 120 mg/mL (P < 0.001) 120 mg/mL > 130 mg/mL (P = 0.022)

Table 16: P – values for ANOVA and Tukey's W for percentage release of samples with 5 % tetracycline loading (1 hr to 168 hr)

Release from 10% Tetracycline Preparations

Concentration

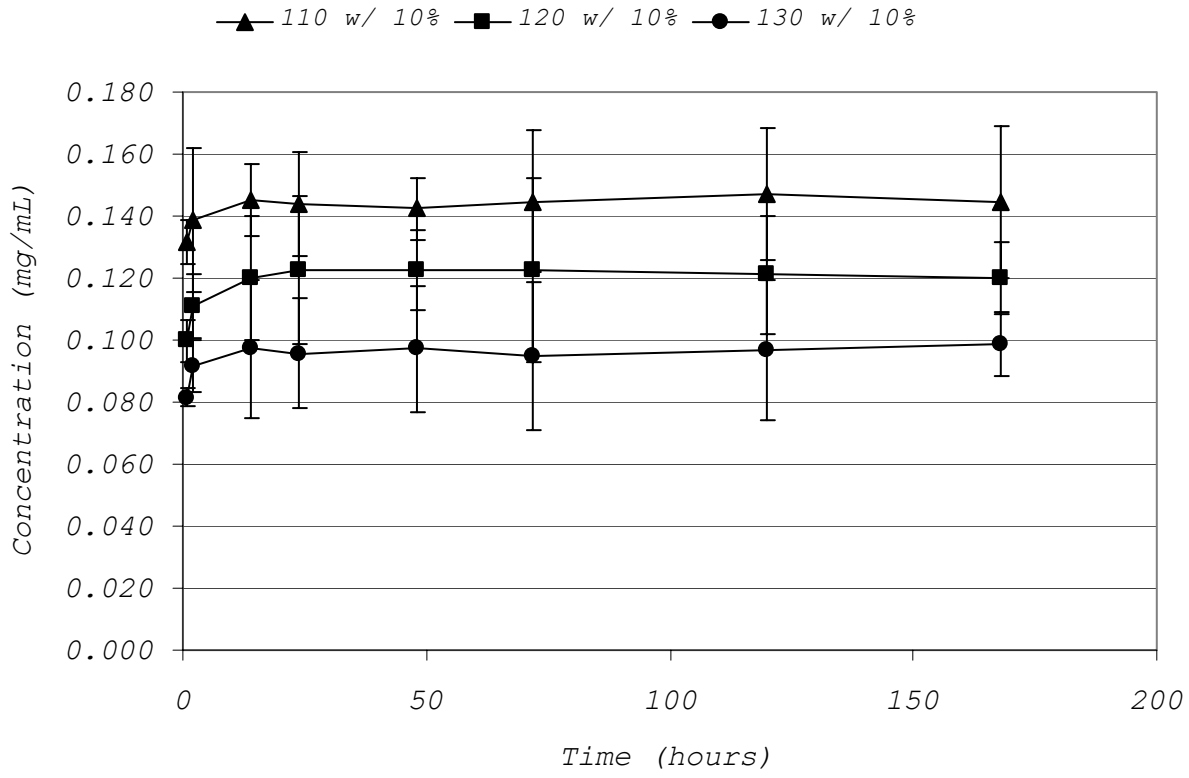

Figure 36 : Concentration versus time for preparations with 10% tetracycline over 168 hours

Figure 36 shows the tetracycline concentration of the release solution over time 168 hours for fibrinogen preparations with 10% tetracycline loading. Three different fibrinogen concentrations are shown. Analysis of variance of the tetracycline concentrations at each time point showed significant difference among the three

fibrinogen concentrations at all times (all $P < 0.05$). Tukey's W found significance differences between pairs of concentrations at all times. These results are summarized in Table 17. In summary, there is a significant trend across all times. At all times except 72 hours, the 110 mg/mL preparations exhibited significantly higher release than the 120 mg/mL preparations, and the 120 mg/ml preparations exhibited significantly higher release than the 130 mg/mL preparations. At 72 hours, the release from 110 mg/mL and 120 mg/mL were not significantly different, but were both significantly higher than that of the 130 mg/mL preparations.

Time	Difference among samples (ANOVA)	Pairwise Differences (Tukey's W)
1 hr	Yes, P < 0.001	120 mg/mL > 130 mg/mL (P < 0.001) 110 mg/mL > 120 mg/mL (P < 0.001) 110 mg/mL > 130 mg/mL (P < 0.001)
4 hr	Yes, P < 0.001	120 mg/mL > 130 mg/mL (P = 0.010) 110 mg/mL > 120 mg/mL (P < 0.001) 110 mg/mL > 130 mg/mL (P < 0.001)
14 hr	Yes, P < 0.001	120 mg/mL > 130 mg/mL (P = 0.015) 110 mg/mL > 120 mg/mL (P = 0.007) 110 mg/mL > 130 mg/mL (P < 0.001)
24 hr	Yes, P < 0.001	120 mg/mL > 130 mg/mL (P = 0.006) 110 mg/mL > 120 mg/mL (P = 0.029) 110 mg/mL > 130 mg/mL (P < 0.001)
48 hr	Yes, P < 0.001	120 mg/mL > 130 mg/mL (P < 0.001) 110 mg/mL > 120 mg/mL (P = 0.008) 110 mg/mL > 130 mg/mL (P < 0.001)
72 hr	Yes, P < 0.001	120 mg/mL > 130 mg/mL (P = 0.035) 110 mg/mL > 130 mg/mL (P < 0.001)
120 hr	Yes, P < 0.001	120 mg/mL > 130 mg/mL (P = 0.021) 110 mg/mL > 120 mg/mL (P = 0.014) 110 mg/mL > 130 mg/mL (P < 0.001)
168 hr	Yes, P < 0.001	120 mg/mL > 130 mg/mL (P = 0.009) 110 mg/mL > 120 mg/mL (P = 0.003) 110 mg/mL > 130 mg/mL (P < 0.001)

Table 17 : P – values for ANOVA and Tukey's W for release concentration of samples with 10 % tetracycline loading (1 hr to 168 hr)

Release from 10% Tetracycline Preparations

Percentage Release

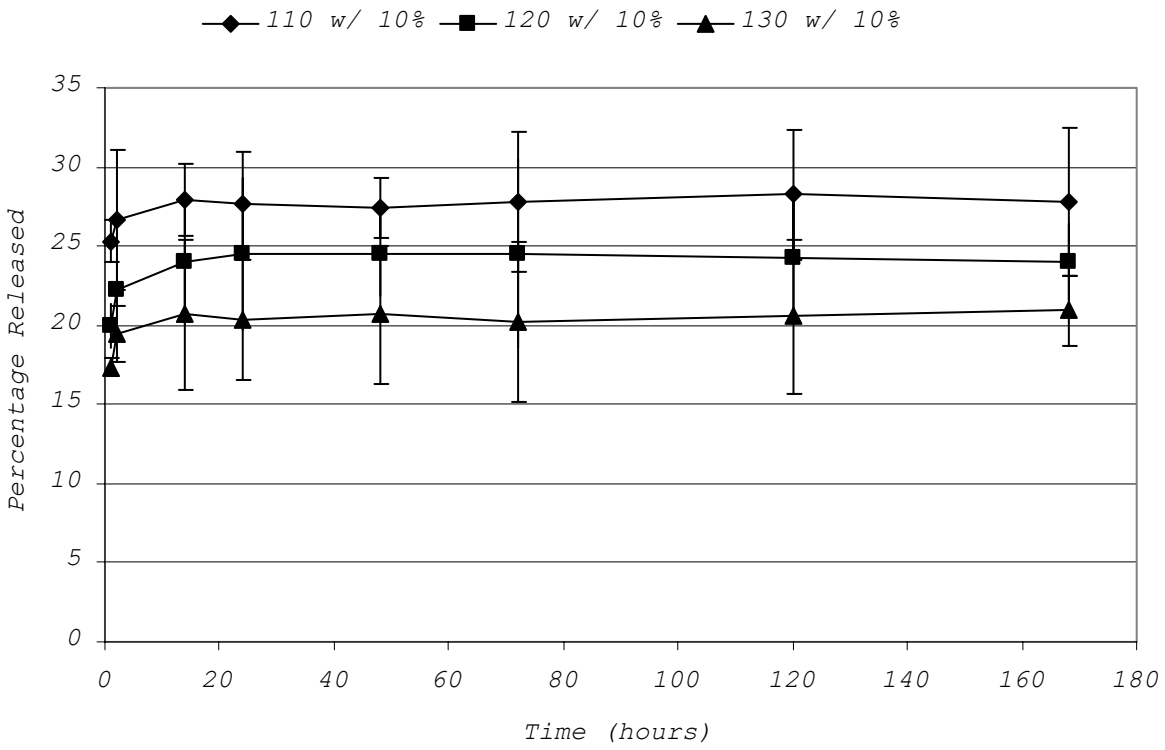

Figure 37: Percentage release versus time for preparations with 10% tetracycline over 168 hours

Figure 37 shows the percentage release over time from preparations with 10% tetracycline loading for three different fibrinogen concentrations. These percentage release profiles are identical in shape to the concentration profiles of Figure 36. The concentrations have been converted to percentage of tetracycline released by using the average mass of the sample, and the

concentrations of Figure 36 as indicated in the Methods. The calculation of percentage release serves as a normalized view of release, independent of mass, and, therefore allows for a better comparison of release from different preparations.

Analysis of variance at each time event indicated that there existed significant difference among the samples at all times. These results are provided below in Table 18. At time points where ANOVA indicated differences among the percentage values, Tukey's W was used to find which pairs of percentages were different. In short, significant differences in percentage released were seen at all times. At each of these times, the 110 mg/mL preparations exhibited higher release than the 130 mg/mL preparations. In addition, at time 1 hour, 4 hours, 14 hours, and 168 hours the 110 mg/mL preparations had significantly higher release than the 120 mg/mL preparations. Also, at times 4 hours, 24 hours, and 48 hours, the 120 mg/mL preparations had significantly greater release than the 130 mg/mL preparations.

Time	Difference among samples (ANOVA)	Pairwise Differences (Tukey's W)
1 hr	Yes, P < 0.001	110 mg/mL > 130 mg/mL (P < 0.001) 110 mg/mL > 120 mg/mL (P < 0.001) 120 mg/mL > 130 mg/mL (P < 0.001)
4 hr	Yes, P < 0.001	110 mg/mL > 130 mg/mL (P < 0.001) 110 mg/mL > 120 mg/mL (P = 0.003)
14 hr	Yes, P < 0.001	110 mg/mL > 130 mg/mL (P < 0.001) 110 mg/mL > 120 mg/mL (P = 0.043)
24 hr	Yes, P < 0.001	110 mg/mL > 130 mg/mL (P < 0.001) 120 mg/mL > 130 mg/mL (P = 0.040)
48 hr	Yes, P < 0.001	110 mg/mL > 130 mg/mL (P < 0.001) 120 mg/mL > 130 mg/mL (P = 0.013)
72 hr	Yes, P = 0.004	110 mg/mL > 130 mg/mL (P = 0.003)
120 hr	Yes, P < 0.001	110 mg/mL > 130 mg/mL (P < 0.001)
168 hr	Yes, P < 0.001	110 mg/mL > 130 mg/mL (P < 0.001) 110 mg/mL > 120 mg/mL (P = 0.021)

Table 18: P – values for ANOVA and Tukey's W for percentage release of samples with 10 % tetracycline loading (1 hr to 168 hr)

Percentage Release (All Preparations)

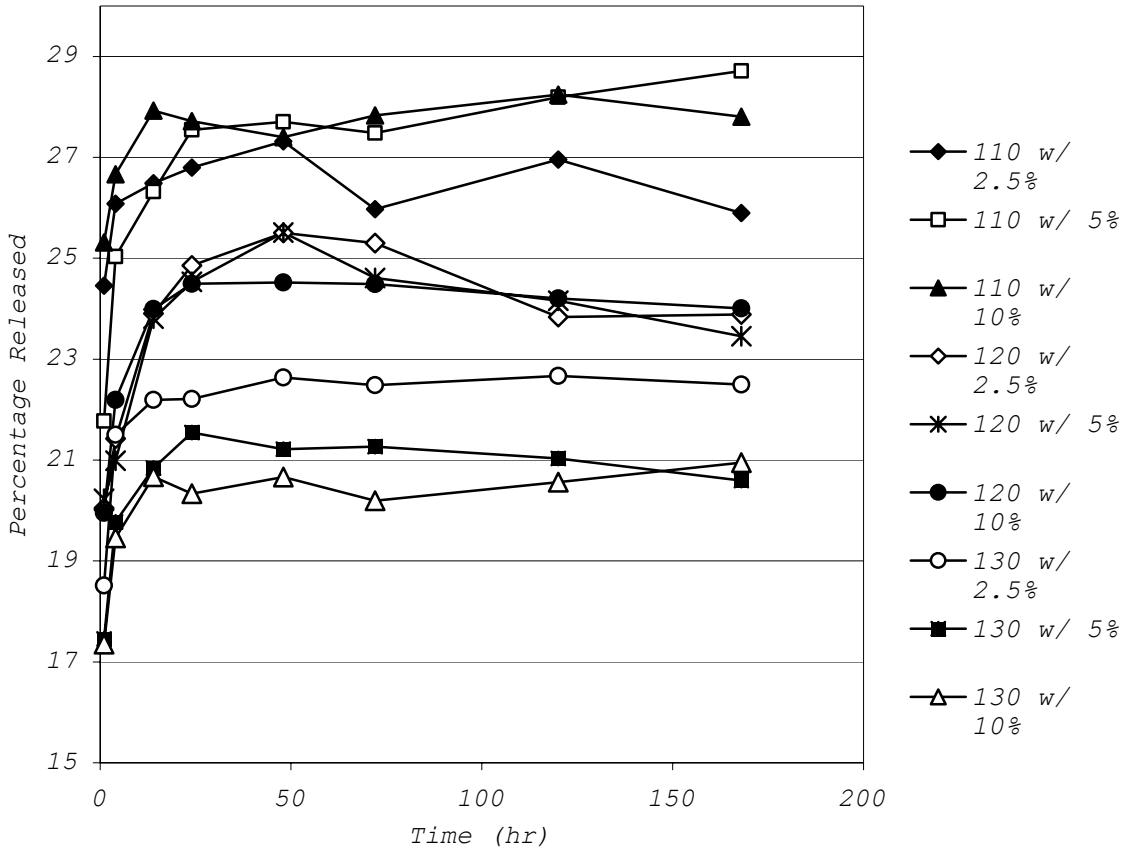

Figure 38 : Percentage Release over time for all preparations (error bars excluded for clarity)

Figure 38 presents percentage release data for all preparations over the long term 168 hour release study. Error bars have been submitted for clarity. The graph suggests several trends. First, preparations of the same fibrinogen concentrations are grouped closely and exhibit similar release. Secondly, upon viewing these fibrinogen

concentrations, those preparations of lower fibrinogen concentration tend to release a greater percentage of tetracycline over time (110 mg/mL higher than 120 mg/mL, 120 mg/mL higher than 130 mg/mL). Lastly, the ultimate percentage released for all preparations is roughly 20% to 30%.

Percentage Release Statistics

Analyses of variance were performed at each time value for the percentage release of all preparations. All showed significant difference among the preparations. However, with two noted exceptions, Tukey's W only showed pairwise differences between preparations of fibrinogen concentration 110 mg/mL and those of 120 mg/mL or 130 mg/mL fibrinogen. These differences were not found in every comparison, but with 36 pairwise comparisons for each time event, and relatively few significant differences, individual statements of release differences would be expansive. A summary of these differences are listed in the Appendix. The exceptions to the above trend occurred at 1 hour (110 w/ 10% > 110 w/ 5%) and 48 hours (120 w/ 5% > 130 w/ 10% and 120 w/ 2.5% > 130 w/ 10%). The data for percentage release by fibrinogen concentration and by tetracycline loading was offered separately in earlier sections. This analysis is supplied only to offer a more global perspective.

Discussion

Fiber Diameter and Pore Area

As shown in the results, the addition of tetracycline in weight percentages of up to 10% has no significant effect on fiber diameter. Fiber diameter of electrospun fibrinogen has been shown to be a function of solution concentration and these results demonstrate this as well[7]. In addition, tetracycline had no significant effect on pore area, as this is often controlled by fiber diameter.

Mechanical Data

Regardless of the mechanical property measured, the addition of tetracycline showed no significant effect. The modulus of dry samples was dependent on the original fibrinogen concentration, with higher fibrinogen concentrations in the electrospinning solution yielding electrospun mats with stiffer moduli. This is probably due to the increase in fiber diameter of the resulting mats (see fiber diameter data). The testing of modulus for wet

preparation showed a similar trend, with differences between the 110 mg/mL and 120 mg/mL and between 110 mg/mL and 130 mg/mL being significant. In each case, the greater fibrinogen concentration yielded a higher modulus. A greater sample size may have allowed for more significance to be seen between 120 mg/mL and 130 mg/mL. The modulus of wet fibrinogen was consistently seen as lower than that of the dry preparation.

There was no significant difference among preparations in breaking strain. Although there may appear to be a trend of increasing breaking strain with increasing fibrinogen concentration (and fiber diameter), the variation among samples and the incongruities within electrospun fibrinogen may have occluded any possible statistically significant differences. Wet breaking strain did exhibit a trend, with increasing fibrinogen concentration causing an increasing in the strain to break. The significant difference between 110 mg/mL and 120 mg/mL and 110 mg/mL and 130 mg/mL lend evidence to the dependence between breaking strain and fibrinogen concentration. An amazing property of fibrinogen is demonstrated in the possible ten fold increase in breaking strain when fibrinogen mats are hydrated. Although the modulus

decreases with hydration, the material becomes ultimately more flexible.

The peak stresses of dry preparations were statistically different, and the 130 mg/mL preparations were statistically greater than the 110 mg/mL preparations. A similar result was found in the hydrated preparations, with significantly higher peak stresses in the 120 mg/mL and 130 mg/mL preparations, as compared to the 110 mg/mL.

Since the addition of tetracycline had little effect on the mechanical properties, it can be inferred that the fibrinogen concentration has a great effect upon the mechanical properties, for it has a large contribution to fiber diameter. A larger diameter fiber will be stronger in tension and should increase modulus, peak stress, and breaking strain of materials. Since all other variables of the fabrication process were controlled (distance to mandrel, spinning voltage, mandrel rotation and geometry), the changes in fiber diameter (and mechanical properties) should be able to be attributed to solution concentration.

The greater flexibility of hydrated fibrinogen is more interesting. The hydration of fibrinogen in PBS has an obvious effect, increasing flexibility, but the cause is speculative. If the technology had been available to

observe fibers in this wet state, perhaps a change in fiber geometry, size, or orientation could address this issue. The difference in wet fibrinogen may also address the relatively short period of release.

Short Term Release

The effects of tetracycline loading on release can be easily summarized. An increase in loading offers a higher concentration of tetracycline in the release solution. This is probably because of a greater mass of tetracycline on the surface of the fibrinogen, which readily dissolves into solution. This increase in release concentration was seen in all preparations. Upon viewing the short term release by tetracycline loading percentage, it was seen that the 120 mg/mL preparations showed greater release than other preparations. This result is possibly due to some incongruities between samples, as the 110 mg/mL preparations showed higher release than larger diameter preparations in the long term study. In summary, higher tetracycline loading percentage led to higher release because of increased mass of available tetracycline, and smaller diameter fibers (except for 120 mg/mL exceptions) also led to greater release. The smaller diameter fibers

offer more surface area for tetracycline to exist and be readily dissolved.

However, regardless of preparation, the release is short-lived, often reaching a plateau within minutes, with no significant release thereafter. This was also seen in the longer term study. With the differing mechanical behavior of hydrated fibrinogen, a possible explanation is also the swelling of fibers.

As the fibrinogen hydrates, the individual fibers could swell, and their increase in diameter could narrow whatever pores that were available for the tetracycline in the inner part of the mat to diffuse into solution. Since the short term study showed that no more than 25% of the tetracycline in the mat entered solution, this sealing of pores and possible sequestration of tetracycline happens rapidly. In addition, the higher release percentages seen from preparation of smaller fiber diameters suggests that smaller fibers take more time and hydration to reach the point where their increased size prevents further release from the interior of the sample.

This relatively low release of tetracycline could have another cause. The calculation of percentage release requires an unsupportable assumption – that the entire

amount of tetracycline in the electrospinning solution is incorporated into the final product. This seems reasonable, but could not have been verified without destruction of the sample. This is a recommendation for a later study.

The "missing" tetracycline may not exist as a potential drawback – it could be a benefit. If the tetracycline is indeed trapped within the fibrinogen, then the degradation of fibrinogen would lead to further release. Since fibrinogen is insoluble in PBS, this was not observed. However, in applications as implantable tissue scaffolding or wound dressing, there is a possible rediscovery of the tetracycline. Upon exposure to thrombin in blood, the fibrinogen will be cleaved to fibrin, forming a clot, the "provisional matrix". As the tissue remodels this matrix, replacing it with collagen, the fibrin will be exposed to enzymes that degrade it. As the fibrin is degraded, and the matrix replaced, any tetracycline within could be liberated.

Long Term Release

The long term results are similar in profiles to those of the short term release. The reliance on loading percentages is again illustrated. The data suggests that concentration is dependent on increasing loading and on decreasing fibrinogen concentration (fiber diameter). Although not judged significant at every time point, a decrease in fiber diameter (per concentration change), did offer more fiber surface area, so that the early surface release could be higher.

In many of the preparations, no significant difference in tetracycline concentration existed between any time values from 1 hour and 168 hours. Therefore, no release can be confirmed after 1 hour. A purpose of the short term study was to show the rapid onset of concentration, and it is apparent that most release occurs before an hour. There were samples that showed a significant change (release) up to 48 hours, yet the general trend was that release had a shorter duration. This could also be attributed to the possibly swelling of the fibers.

Recommendations for Further Study

This study was not rife with weakness, but in retrospect, many things could have been improved. One major concern is the lack of uniformity with the samples taken from preparations. Although each sample had the same diameter, and was obtained with the same instrument, there were variations. The electrospun fibrinogen mat does vary in thickness, so pieces with identical cross-sectional area would have different masses. Using the average mass of the samples introduced error, as the percentage calculation was dependent upon mass. If a particular sample had a mass quite different from the sample, the variation would be seen in the percentage results. Since the release of tetracycline has been suggested to be restricted to the surface of the mat (quick cessation of release), the thickness of the mat is not of the same concern. These problems could be remedied by the use of larger sample pieces for the release study.

Also, a fundamental change in design could address the above issue, but comes with a price. This study was conducted with independent samples. A given sample of a preparation offered data at only one time event. A design with a larger sample that was repeatedly measured for

release would reduce variation, but this design would demand the replacement of harvested release solution, constant recalculation, and different statistics.

A larger sample size for all facets of the experiment would undoubtedly reduce variation and show more significance. A less conservative ancillary to ANOVA (the author's choice was Tukey's W) might have given more significant results. Human error could be lessened if the spectrophotometry could be accomplished without disturbing the sample, that is, if the absorbance could be continually measured while the sample releases its tetracycline.

Also, a method for observing the tetracycline within the mat would address the surface dissolution theory. Cross-sections of the mat could possibly be viewed with TEM or fluorescence microscopy to "see" where the tetracycline is. Or, the enzymatic breakdown of fibrinogen mats would release the tetracycline that is conceivably trapped.

Conclusion

In summary, the presence of tetracycline has minimal effect on mechanical properties (modulus, peak stress, breaking strain) and material geometry (fiber diameter and pore size) of electrospun fibrinogen. The release data suggests that release concentration is dependent upon tetracycline loading percentage (available release material). The release concentration is also dependent upon fiber diameter, as smaller fibers allow for more release because of their overall increased surface area. The incomplete release of tetracycline from electrospun fibrinogen could be a result of the swelling of the fibers, occluding paths of release from the interior of the mat. This theory is supported by the higher release percentages of smaller diameter electrospun mats, which will undergo more swelling over a longer time period to reach altered diameters than can prevent release. These results showed that tetracycline is a viable addition to applications of electrospun fibrinogen, and that the release of

tetracycline from the same can be controlled by the factors of fibrinogen concentration and tetracycline loading percentage.

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Appendix

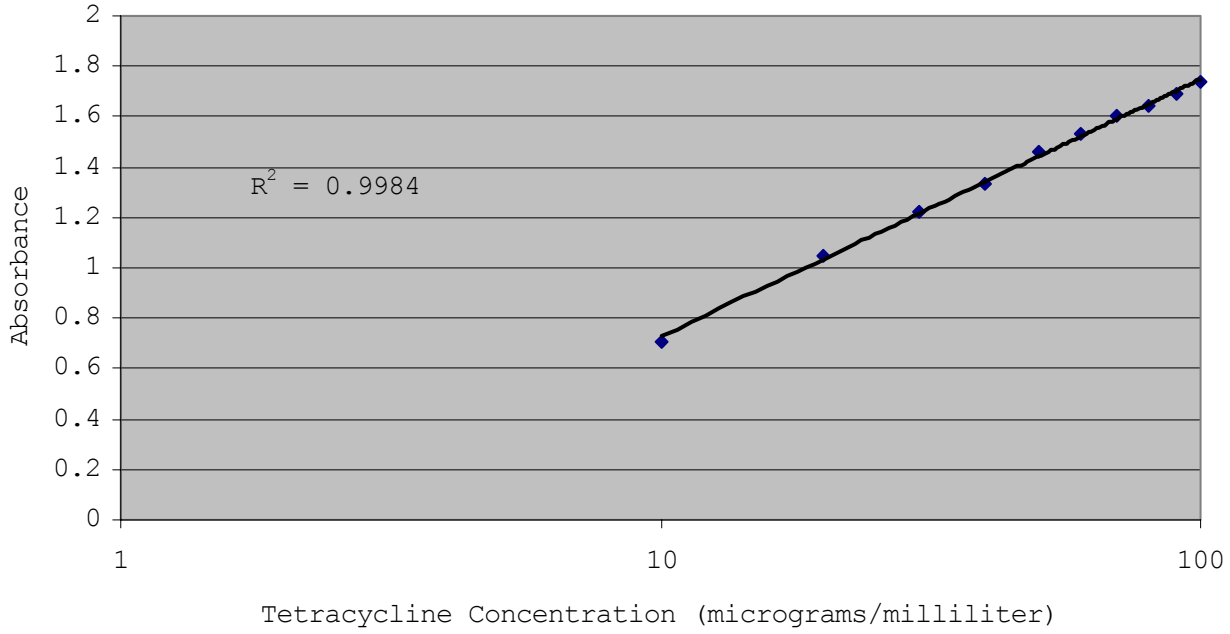

Figure 39 : Standard Curve of Absorbance versus tetracycline concentration

Time(hours)	Pairwise Differences with P-values	
1	110 w/ 10% > 130 w/ 10%	(P < 0.001)
	110 w/ 10% > 130 w/ 5%	(P < 0.001)
	110 w/ 10% > 130 w/ 2.5%	(P < 0.001)
	110 w/ 10% > 120 w/ 10%	(P < 0.001)
	110 w/ 10% > 120 w/ 2.5%	(P < 0.001)
	110 w/ 10% > 120 w/ 5%	(P < 0.001)
	110 w/ 10% > 110 w/ 5%	(P = 0.044)
	110 w/ 2.5% > 130 w/ 10%	(P < 0.001)
	110 w/ 2.5% > 130 w/ 5%	(P < 0.001)
	110 w/ 2.5% > 130 w/ 2.5%	(P < 0.001)
	110 w/ 2.5% > 120 w/ 10%	(P = 0.003)
	110 w/ 2.5% > 120 w/ 5%	(P = 0.007)
	110 w/ 2.5% > 120 w/ 2.5%	(P = 0.003)
	110 w/ 5% > 130 w/ 10%	(P = 0.005)
	110 w/ 5% > 130 w/ 5%	(P = 0.005)
4	110 w/ 10% > 130 w/ 10%	(P < 0.001)
	110 w/ 10% > 130 w/ 5%	(P < 0.001)
	110 w/ 10% > 120 w/ 2.5%	(P < 0.001)
14	110 w/ 10% > 130 w/ 10%	(P < 0.001)
	110 w/ 10% > 130 w/ 5%	(P < 0.001)
	110 w/ 10% > 130 w/ 2.5%	(P = 0.004)
	110 w/ 2.5% > 130 w/ 10%	(P = 0.003)
	110 w/ 2.5% > 130 w/ 5%	(P = 0.005)
	110 w/ 5% > 130 w/ 10%	(P = 0.005)
	110 w/ 5% > 130 w/ 5%	(P = 0.007)
24	110 w/ 10% > 130 w/ 10%	(P < 0.001)
	110 w/ 10% > 130 w/ 5%	(P = 0.002)
	110 w/ 10% > 130 w/ 2.5%	(P = 0.011)
	110 w/ 5% > 130 w/ 10%	(P < 0.001)
	110 w/ 5% > 130 w/ 5%	(P = 0.004)
	110 w/ 5% > 130 w/ 2.5%	(P = 0.016)
	110 w/ 2.5% > 130 w/ 10%	(P = 0.001)
	110 w/ 2.5% > 130 w/ 5%	(P = 0.019)
48	110 w/ 10% > 130 w/ 10%	(P < 0.001)
	110 w/ 10% > 130 w/ 5%	(P = 0.002)
	110 w/ 10% > 130 w/ 2.5%	(P = 0.049)
	110 w/ 5% > 130 w/ 10%	(P < 0.001)
	110 w/ 5% > 130 w/ 5%	(P = 0.001)
	110 w/ 5% > 130 w/ 2.5%	(P = 0.028)
	110 w/ 2.5% > 130 w/ 10%	(P < 0.001)
	110 w/ 2.5% > 130 w/ 5%	(P = 0.003)
	120 w/ 5% > 130 w/ 10%	(P = 0.042)

	120 w/ 2.5% > 130 w/ 10%	(P = 0.042)
72	110 w/ 10% > 130 w/ 10%	(P = 0.006)
	110 w/ 10% > 130 w/ 5%	(P = 0.031)
	110 w/ 5% > 130 w/ 10%	(P = 0.010)
120	110 w/ 10% > 130 w/ 10%	(P < 0.001)
	110 w/ 10% > 130 w/ 5%	(P < 0.001)
	110 w/ 10% > 130 w/ 2.5%	(P = 0.015)
	110 w/ 5% > 130 w/ 10%	(P < 0.001)
	110 w/ 5% > 130 w/ 5%	(P < 0.001)
	110 w/ 5% > 130 w/ 2.5%	(P = 0.017)
	110 w/ 2.5% > 130 w/ 10%	(P = 0.003)
	110 w/ 2.5% > 130 w/ 5%	(P = 0.007)
168	110 w/ 5% > 130 w/ 10%	(P < 0.001)
	110 w/ 5% > 130 w/ 5%	(P < 0.001)
	110 w/ 5% > 130 w/ 2.5%	(P < 0.001)
	110 w/ 5% > 120 w/ 10%	(P = 0.017)
	110 w/ 5% > 120 w/ 5%	(P = 0.004)
	110 w/ 5% > 120 w/ 2.5%	(P = 0.013)
	110 w/ 10% > 130 w/ 10%	(P < 0.001)
	110 w/ 10% > 130 w/ 5%	(P < 0.001)
	110 w/ 10% > 130 w/ 2.5%	(P = 0.004)
	110 w/ 10% > 120 w/ 5%	(P = 0.038)
	110 w/ 2.5% > 130 w/ 5%	(P = 0.004)
	110 w/ 2.5% > 130 w/ 10%	(P = 0.010)

Table 19 : Pairwise differences at times for long term release

Time (min)	1	2	4	8	16	32	64	128
1	XX	No	0.039	<0.001	<0.001	<0.001	<0.001	<0.001
2		XX	No	<0.001	<0.001	<0.001	<0.001	<0.001
4			XX	<0.001	<0.001	<0.001	<0.001	<0.001
8				XX	No	<0.001	<0.001	<0.001
16					XX	No	0.045	<0.001
32						XX	No	No
64							XX	No
128								XX

Table 20 : P-values for Tukey's W for pairwise comparison of concentrations over time for 110 mg/ml with 2.5% tetracycline

Time (min)	1	2	4	8	16	32	64	128
1	XX	No	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
2		XX	0.004	<0.001	<0.001	<0.001	<0.001	<0.001
4			XX	<0.001	<0.001	<0.001	<0.001	<0.001
8				XX	No	0.018	<0.001	<0.001
16					XX	No	<0.001	<0.001
32						XX	No	<0.001
64							XX	0.042
128								XX

Table 21 : P-values for Tukey's W for pairwise comparison of concentrations over time for 110 mg/ml with 5% tetracycline

Time (min)	1	2	4	8	16	32	64	128
1	XX	No	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
2		XX	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
4			XX	0.001	<0.001	<0.001	<0.001	<0.001
8				XX	0.02	<0.001	<0.001	<0.001
16					XX	No	<0.001	<0.001
32						XX	0.042	0.025
64							XX	No
128								XX

Table 22 : P-values for Tukey's W for pairwise comparison of concentrations over time for 110 mg/ml with 10% tetracycline

Time (min)	1	2	4	8	16	32	64	128
1	XX	No	0.039	<0.001	<0.001	<0.001	<0.001	<0.001
2		XX	No	<0.001	<0.001	<0.001	<0.001	<0.001
4			XX	<0.001	<0.001	<0.001	<0.001	<0.001
8				XX	No	<0.001	<0.001	<0.001
16					XX	No	0.045	<0.001
32						XX	No	No
64							XX	No
128								XX

Table 23 : P-values for Tukey's W for pairwise comparison of concentrations over time for 120 mg/ml with 2.5% tetracycline

Time (min)	1	2	4	8	16	32	64	128
1	XX	No	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
2		XX	0.004	<0.001	<0.001	<0.001	<0.001	<0.001
4			XX	<0.001	<0.001	<0.001	<0.001	<0.001
8				XX	No	0.018	<0.001	<0.001
16					XX	No	No	<0.001
32						XX	No	<0.001
64							XX	0.042
128								XX

Table 24 : P-values for Tukey's W for pairwise comparison of concentrations over time for 120 mg/ml with 5% tetracycline

Time (min)	1	2	4	8	16	32	64	128
1	XX	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
2		XX	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
4			XX	0.001	<0.001	<0.001	<0.001	<0.001
8				XX	0.02	<0.001	<0.001	<0.001
16					XX	No	<0.001	<0.001
32						XX	0.042	0.025
64							XX	No
128								XX

Table 25 : P-values for Tukey's W for pairwise comparison of concentrations over time for 120 mg/ml with 10% tetracycline

Time (min)	1	2	4	8	16	32	64	128
1	XX	No	No	<0.001	<0.001	<0.001	<0.001	<0.001
2		XX	No	0.001	<0.001	<0.001	<0.001	<0.001
4			XX	No	<0.001	<0.001	<0.001	<0.001
8				XX	No	<0.001	<0.001	<0.001
16					XX	0.010	<0.001	<0.001
32						XX	No	0.003
64							XX	No
128								XX

Table 26 : P-values for Tukey's W for pairwise comparison of concentrations over time for 130 mg/ml with 2.5% tetracycline

Time (min)	1	2	4	8	16	32	64	128
1	XX	0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
2		XX	0.002	<0.001	<0.001	<0.001	<0.001	<0.001
4			XX	No	<0.001	<0.001	<0.001	<0.001
8				XX	0.015	<0.001	<0.001	<0.001
16					XX	No	0.015	<0.001
32						XX	No	0.01
64							XX	No
128								XX

Table 27 : P-values for Tukey's W for pairwise comparison of concentrations over time for 130 mg/ml with 5% tetracycline

Time (min)	1	2	4	8	16	32	64	128
1	XX	<0.001	0.039	<0.001	<0.001	<0.001	<0.001	<0.001
2		XX	0.004	<0.001	<0.001	<0.001	<0.001	<0.001
4			XX	No	<0.001	<0.001	<0.001	<0.001
8				XX	<0.001	<0.001	<0.001	<0.001
16					XX	0.018	<0.001	<0.001
32						XX	No	0.002
64							XX	No
128								XX

Table 28 : P-values for Tukey's W for pairwise comparison of concentrations over time for 130 mg/ml with 10% tetracycline

Time (hr)	1 hr	4 hr	14 hr	24 hr	48 hr	72 hr	120 hr	168 hr
1 hr	XX	No	No	0.006	0.004	0.006	0.001	<0.001
4 hr		XX	No	No	No	No	No	No
14 hr			XX	No	No	No	No	No
24 hr				XX	No	No	No	No
48 hr					XX	No	No	No
72 hr						XX	No	No
120 hr							XX	No
168 hr								XX

Table 29 : P-values for Tukey's W for pairwise comparison of concentrations over time for 110 mg/ml with 5% tetracycline

Time (hr)	1 hr	4 hr	14 hr	24 hr	48 hr	72 hr	120 hr	168 hr
1 hr	XX	No	No	No	0.009	No	No	No
4 hr		XX	No	No	No	No	No	No
14 hr			XX	No	No	No	No	No
24 hr				XX	No	No	No	No
48 hr					XX	No	No	No
72 hr						XX	No	No
120 hr							XX	No
168 hr								XX

Table 30 : P-values for Tukey's W for pairwise comparison of concentrations over time for 120 mg/ml with 5% tetracycline

Time (hr)	1 hr	4 hr	14 hr	24 hr	48 hr	72 hr	120 hr	168 hr
1 hr	XX	No	No	0.013	0.031	0.027	0.048	No
4 hr		XX	No	No	No	No	No	No
14 hr			XX	No	No	No	No	No
24 hr				XX	No	No	No	No
48 hr					XX	No	No	No
72 hr						XX	No	No
120 hr							XX	No
168 hr								XX

Table 31 : P-values for Tukey's W for pairwise comparison of concentrations over time for 130 mg/ml with 5% tetracycline

Vita

Charles Dudley Anderson, Jr. was born on Monument Avenue, Richmond, Virginia on October 31, 1974. He graduated from Lee-Davis High School in Mechanicsville, Virginia in 1992. He graduated from the University of Richmond in 1996 with a Bachelor of Science in Physics, working under the late Dr. R. Wayne Major. He attended the College of William and Mary to complete his Master of Arts in Education coursework, earning his Secondary Education Teacher Licensure. After four years of teaching at Douglas Southall Freeman High School in Richmond, he enrolled at Virginia Commonwealth University in August 2002. Charles will receive a Master of Science degree in Biomedical Engineering in December 2004, and will pursue a Doctor of Philosophy degree in Physiology at Virginia Commonwealth University.