



Image analysis of novel biomaterials : effectiveness at inhibiting bacterial colonization with unique polymer coatings and/or the controlled release of ciprofloxacin
by Sara Kirsten Hendricks

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Abstract:

Millions of dollars are spent every year in the U.S. on biomedical implants ranging from commonplace uses, such as contact lenses to applications as rare as total artificial hearts. One of the main stumbling blocks in the long term usage of these devices is bacterial infection which can only be rectified by the removal of the implant, resulting in increased costs and trauma to the patient. Consequently, four different polymer formulations were studied for their efficacy at preventing bacterial colonization. The polymer under investigation was placed in a parallel plate flow cell, challenged with fluid containing *Pseudomonas aeruginosa* for six hours, and then exposed to a fluid of nutrients only for the remainder of a twenty four hour run. Two different sets of test polymers subjected to this protocol were examined by image analysis. One set consisted of a BioSpan™/ polyetherurethane (PEU) base matrix (BP) coated with triethylene glycol dimethyl ether (triglyme), while the other set had an additional coating of poly(butyl methacrylate), polyBMA. Each set also had one formulation to which a known amount of the antibiotic ciprofloxacin™ had been added. The coatings of triglyme and triglyme+BMA cut bacterial colonization in half when compared to the control BP material. While the additional factor of the controlled release of ciprofloxacin™ from the materials resulted in more than a two fold reduction in bacterial colonization when compared to the control BP material. These polymers therefore hold promise in decreasing the risk of infection encountered during the use of biomedical implants.

IMAGE ANALYSIS OF NOVEL BIOMATERIALS: EFFECTIVENESS AT
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COATINGS AND/OR THE CONTROLLED RELEASE OF CIPROFLOXACIN

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ABSTRACT

Millions of dollars are spent every year in the U.S. on biomedical implants ranging from commonplace uses, such as contact lenses to applications as rare as total artificial hearts. One of the main stumbling blocks in the long term usage of these devices is bacterial infection which can only be rectified by the removal of the implant, resulting in increased costs and trauma to the patient. Consequently, four different polymer formulations were studied for their efficacy at preventing bacterial colonization. The polymer under investigation was placed in a parallel plate flow cell, challenged with fluid containing *Pseudomonas aeruginosa* for six hours, and then exposed to a fluid of nutrients only for the remainder of a twenty four hour run. Two different sets of test polymers subjected to this protocol were examined by image analysis. One set consisted of a BioSpanTM/ polyetherurethane (PEU) base matrix (BP) coated with triethylene glycol dimethyl ether (triglyme), while the other set had an additional coating of poly(butyl methacrylate), polyBMA. Each set also had one formulation to which a known amount of the antibiotic ciprofloxacinTM had been added. The coatings of triglyme and triglyme+BMA cut bacterial colonization in half when compared to the control BP material. While the additional factor of the controlled release of ciprofloxacinTM from the materials resulted in more than a two fold reduction in bacterial colonization when compared to the control BP material. These polymers therefore hold promise in decreasing the risk of infection encountered during the use of biomedical implants.

INTRODUCTION

The National Institutes of Health have defined a biomaterial "as any substance (other than a drug) or combination of substances, synthetic or natural in origin, which can be used for any period of time, as a whole or as a part of a system which treats, augments, or replaces any tissue, organ, or function of the body". Thus, biomaterials will have an impact on virtually everyone at some point in their life.

Biomaterials may be used for long term applications such as; central nervous system shunts, extended wear contact lenses, or hemodialysis systems. They may be employed in short term applications like; contact lenses, needles for phlebotomy or vaccination, cardiopulmonary bypass systems, or wound healing devices. Or biomaterials may be utilized in permanent implants such as; heart valves, periodontal restorative devices, intraocular lenses, or orthopedic devices.(NIH Consens, 1982)

All biomedical implants are susceptible to bacterial colonization and subsequent biofilm formation. Biofilms are three dimensional gelatinous structures consisting of adherent bacteria and insoluble polysaccharides secreted by the bacterial cells. Bacteria use the biomaterial as a substratum to which they attach and adhere resulting in a biomaterial centered infection. Biofilm infections are extremely difficult to eradicate. The biofilm gel matrix cannot only keep the host defense mechanisms from reaching and/or recognizing the adherent bacteria, but biofilms can also lower the efficacy of antibiotics. Usually the only way to deal with a device-centered infection is to remove the infected implant which is costly as well as traumatic to the patient. Therefore, it is desirable to develop a material that will inhibit bacterial colonization.

The objective of the research presented in this thesis was to ascertain the effectiveness of four different formulations of a biomedical grade polyurethane, Biospan™, at inhibiting bacterial colonization under flow conditions. The scope of this work

included: (1) evaluation of four Biospan™/polyethylene glycol (PEG) matrix biomaterials, two of which contained the antibiotic, ciprofloxacin, (2) development of a flow cell system to evaluate the potential for bacterial adhesion and biofilm formation, and (3) development of a novel staining technique to allow for the visualization of bacteria against an opaque surface without interfering with normal cell behavior.

LITERATURE REVIEW

Background and Significance

Biomaterials have been used in the human body since the early 1900's when bone plates were introduced to stabilize fractures and speed healing. By the 1950's experimentation into the replacement of blood vessels had begun, and by the 1960's artificial hip joints and heart valves were under development. As science and engineering has advanced so has the clinical use of biomaterials.(Blanchard, 1996, NIH Consens, 1982)

Biomedical implants are no longer used just in life-threatening situations. They are now utilized in every major body system for three general purposes: (1) to preserve life or limb, (2) to restore or improve function, and (3) to restore or improve shape. The first category includes most neurosurgical and cardiovascular implants such as pacemakers and hydrocephalus shunts. Dental implants and joint replacements are included in the second category, while biomaterials used in reconstructive surgeries are placed in the last class.(NIH Consens, 1982)

Estimates in 1982 placed biomaterial implant use in the United States for that year at several million. The demand for biomaterials is said to grow by 5 to 15 percent a year and will only increase as the population ages and the expectations of maintaining a good quality of life increase. What was a multi-million dollar per year industry in the U.S. now exceeds \$10 billion a year. This figure is especially remarkable given that the U.S. only represents about 10 percent of world demand. (Blanchard, 1996, NIH Consens, 1982, Bricannica Online, 1997)

One of the major risks encountered in the extended use of implants is the susceptibility of biomaterials to bacterial attachment and adhesion resulting in biomaterial-

centered infections. The three most common bacterial species implicated in these infections are *Pseudomonas aeruginosa*, *Staphylococcus epidermidis*, and *Staphylococcus aureus*. *Escherichia coli*, *Proteus mirabilis*, and beta hemolytic *Streptococcus spp.* have also been isolated from contaminated implants. (Gristina, 1987, Gristina, 1994)

Once bacteria begin adhering to implant material, they can form a biofilm that is extremely difficult to eradicate. The biofilm can not only prevent the host defense mechanisms from reaching and/or recognizing the bacteria, but it can also lower the efficacy of antibiotics. Usually the only way to deal with biofilm infections is by removal of the implant which can be costly as well as traumatic to the patient (Blanchard, 1996, Gristina, 1987, Gristina, *et al*, 1993). Therefore it is desirable to develop a biomaterial that will inhibit bacterial colonization.

There are two common approaches to preventing bacterial colonization. The first is to modify the substratum's surface chemistry rendering it non-adhesive, and the second is to design a material which will slowly release an antibacterial agent killing the bacteria before they reach the surface. (Bryers, 1997)

Processes Governing Biofilm Formation

The establishment of a biofilm (**figure 1**) involves many steps: (1) pre-conditioning of the surface by adsorption of organic molecules (eg. protein) from the fluid phase; (2) cell transport to the substratum; (3) cell adsorption/desorption; (4) permanent adhesion to the surface; (5) cellular metabolism (growth, replication, death); and (6) biofilm removal (detachment and sloughing). Pre-conditioning involves coating the surface with host- derived proteins such as; fibronectin, human serum albumin, and platelets (Tebbs, Sawyer, and Elliot, 1994; Carballo, Ferreirós, and Criado, 1991; and Yu, *et al*, 1995). Cell transport to the surface may occur through passive processes, such

as diffusion or fluid flow, or active processes, such as flagellar movement. Reversible adsorption (adsorption/desorption) of cells to the substratum can be explained in terms of the laws of colloid chemistry, such as the Derjaguin-Landau and Verwey-Overbeek

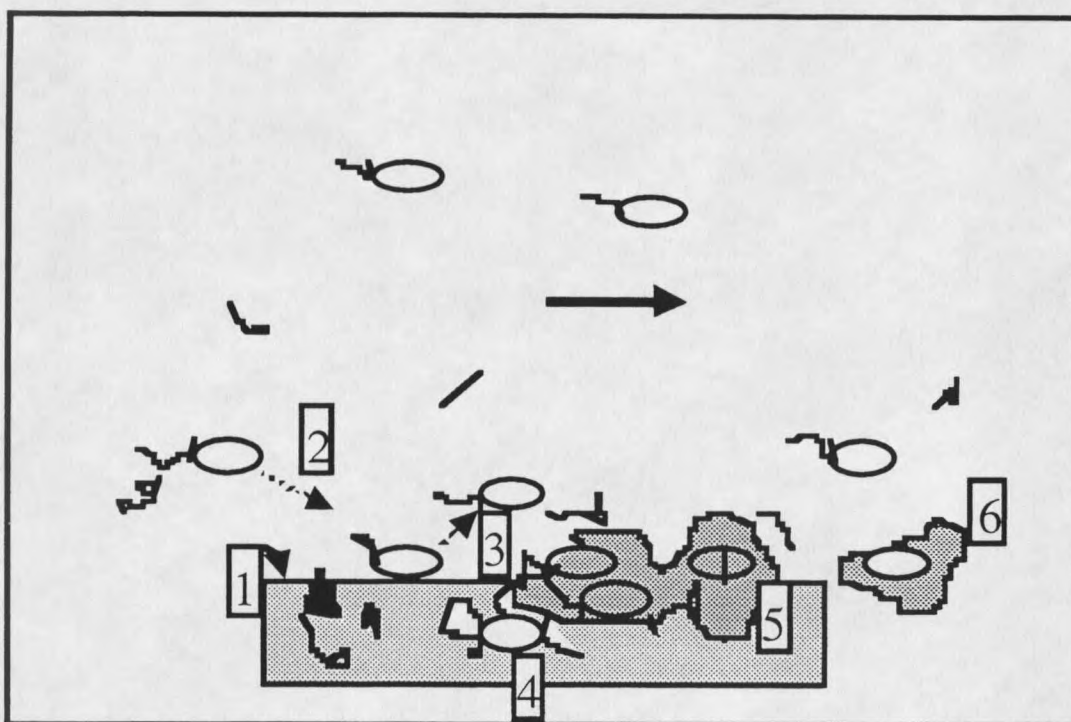


Figure 1. Biofilm formation: (1) pre-conditioning, (2) cell transport to surface, (3) cell adsorption/desorption, (4) permanent adhesion, (5) proliferation, and (6) removal.

(DLVO) theory. Electrostatic forces and London-van der Waals forces combine to bring particles and/or cells to a surface by helping to overcome energy barriers allowing the cells to form a loose attachment with the substratum (Eginton, 1995; Characklis and Marshall, 1990; Weber and DiGiano, 1996). Within a certain distance of the substratum, permanent adhesion becomes possible through such mechanisms as specific binding to proteins in the conditioning film or hydrogen bonding. Once at the surface colonization can begin. Cells begin to produce exopolysaccharides (EPS) literally gluing themselves to the surface.

Within this EPS matrix the cells continue to grow, divide, and die. Occasionally, chunks of the biofilm will detach as a result of either shear forces or weaken of the bonds holding them to the substratum (Characklis, 1990).

Surface Modification

Every natural and synthetic surface has unique physical and chemical properties that can influence cellular adhesion. By modifying the biomaterial surface the fundamental processes governing biofilm formation can be altered. Several review articles discuss these processes in detail (Gristina, *et al*, 1994; Gristina, Naylor, and Myrvik, 1992; Bryers, 1988; Characklis, 1990; Dankert, Hogt, and Feijen, 1986) and acknowledge the similarities between bacterial colonization and tissue integration. In many situations host cells are actually competing with bacteria to colonize the biomaterial surface. Thus, many surface modifications are aimed at intentionally promoting natural tissue adhesion, while others are directed at preventing bacterial adhesion.

Modifications: Host Cell Adhesion

Techniques focused on promoting tissue integration range from endothelial cell sodding to glow plasma discharge modification (Williams, *et al*, 1992; Massia and Hubbell, 1991). The coating of silicone rubber membranes with poly (2-hydroxy ethyl methacrylate) (poly HEMA) by glow plasma discharge has shown encouraging results *in vitro* with regard to the attachment and growth of corneal epithelial cells, bringing the development of an artificial cornea one step closer to reality (Lee, *et al*, 1996).

Hydroxylapatite (HA) coatings of bone implants have already proven effective at promoting faster and greater bone adaptation and improving implant fixation. Better techniques of applying the calcium phosphate to the implant are being examined, including plasma spray, heat-treated plasma spray, and magnetron-sputter (Hulshoff, *et al*, 1996). Carbon deposition, excimer laser ablation, and photochemical coatings are being examined as ways of promoting and controlling endothelial cell proliferation (Kaibara, *et al*, 1996, Doi, Nakayama, and Matsuda, 1996). Surface modifications may also decrease the binding of host proteins, such as thrombin and antithrombin III, thereby lowering the potential to form blood clots which can promote bacterial adhesion. A common method of accomplishing this is by immobilizing heparin on the surface (Byun, Jacobs, and Kim, 1996, Paulsson, Gouda, Larm, and Ljungh, 1994, Lindhout, *et al*, 1995).

Modifications: Bacterial Cell Adhesion

Methods for inhibiting bacterial colonization range from antithrombogenic coatings to the incorporation of antimicrobial substances such as silver or quaternary amine salts (Wang, Anderson, and Marchant, 1993; Ryu, *et al*, 1994; Jansen and Kohnen, 1995). Heparin is only one of the substances used to coat surfaces which have been shown to decrease bacterial adhesion due to protein-mediated adhesion. Poly(vinyl pyrrolidone) (PVP) coatings which inhibit the adsorption of fibrinogen to the surface have also exhibited decreased bacterial adhesion (Francois, *et al*, 1996). Reductions in bacterial adhesion to silastic catheters coated with salicylic acid, a nonsteroidal anti-inflammatory drug, have been demonstrated *in vitro* (Farber and Wolff, 1993). Coatings of hydrophilic polymers such as polyethylene glycol (PEG), have shown potential for inhibiting implant-related infections (Portoles, Refojo, and Leong, 1994). Latex catheters coated with

glycomethacrylate gel, another hydrophilic polymer, have also demonstrated the ability to reduce infection. The reduction was enhanced by the incorporation cephalothin, an antibiotic (Lazarus, et al, 1971). The elution of an antimicrobial from the implant is one of the most popular and oldest methods of dealing with biomaterial-centered infections, illustrated by the use of antibiotic impregnated bone cement (Strachan, 1995; Seyral, *et al*, 1994). As already seen, however, the incorporation of antibiotics and other antimicrobials has also been studied with regard to various polymer systems (Rushton, *et al*, 1989; Ackart, *et al*, 1975; Golomb and Shpligelman, 1991; Greenfeld, *et al*, 1995). As knowledge about the immune system increases so does the potential for developing new substances that can coat biomaterials or be released from them to provide protection against bacterial colonization, as witnessed by development of passive local immunotherapy (Gristina, 1997).

Controlled Release

Controlled release of antibiotics at the site of implantation is one of the more common approaches to preventing infection and is being used successfully in orthopedic surgery. A recent U.S. survey showed that 27% of responding hospitals commonly use antibiotic impregnated bone cement for joint replacement surgery (Strachan, 1995). However, the use of bone cement is not recommended in younger, active people. Consequently, the controlled release of antibiotics from coated implants is under investigation (Price, *et al*, 1995). Thus, it can be seen that there are several different types of controlled release systems.

Controlled Release Systems

Controlled release devices are classified by the method that controls the release of the substance of concern. The most common classifications are: diffusion controlled systems, chemical reaction systems, and solvent activated systems. There are several excellent books and articles that cover this subject in depth (Langer and Wise, 1984; Kost and Langer, 1984; Fan and Singh, 1989; Baker, 1987; Lohmann, 1995; Kydonieus, 1992; Robinson and Lee, 1987) so only a brief overview will be given here.

Controlled release systems can be designed to produce release rate profiles that enhance the efficacy of the desired agent. This is in contrast to sustained release devices which allow the substance of concern to be effective longer but are dependent on environmental factors when it comes to the amount and the rate of release.

The three most common release profiles achieved by controlled release devices are (1) zero-order release, (2) first-order release, and (3) $t^{-1/2}$ release, shown graphically in **figure 2**. Zero-order release is the most desired release rate and is most easily obtained by using diffusion controlled release systems. (Kost and Langer, 1984; Lohmann, 1995)

Diffusion Controlled Devices. There are two general types of diffusion controlled systems: (1) matrix, or monolithic and (2) reservoir. The rate limiting step in both systems is the diffusion of the drug through the polymer matrix. which may be described by (1) Fick's first law of diffusion and (2) Fick's second law of diffusion, also called the diffusion equation (Bird, Stewart, and Lightfoot, 1960). For a one dimensional system they may be written as:

Release rate kinetic:

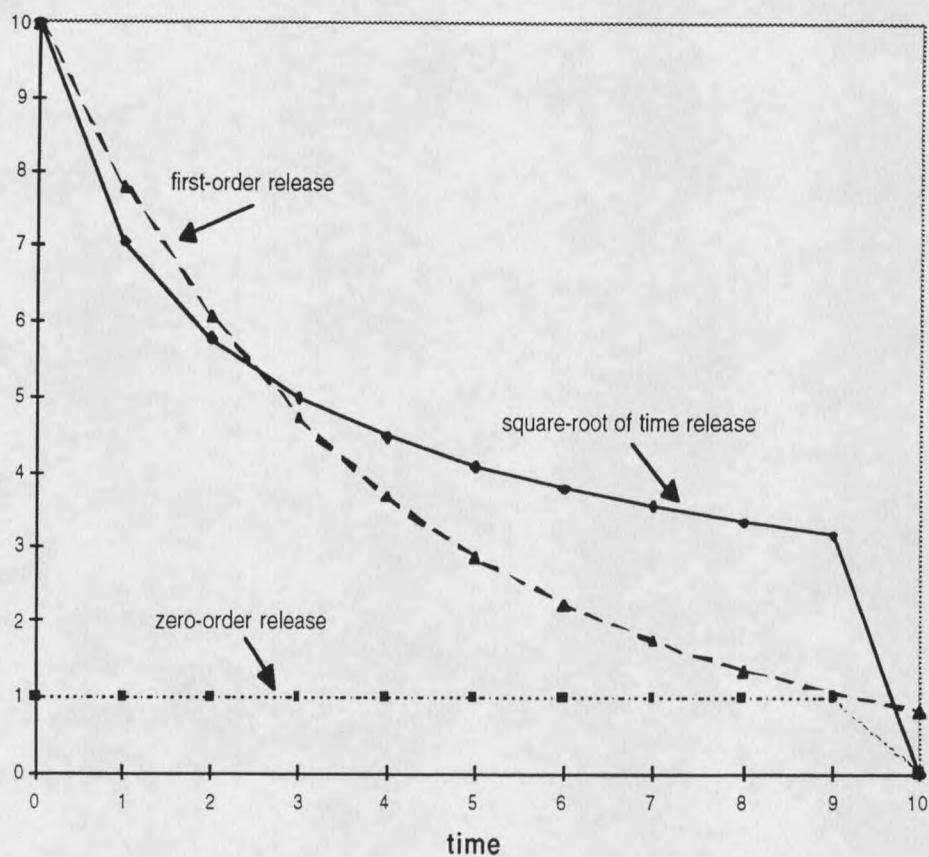


Figure 2. Zero-order, first-order, and square-root of time, release patterns from controlled-release devices.

$$(1) \quad j_i = -D_{ip} * dc_i/dx$$

$$(2) \quad \partial c_i / \partial t = D_{ip} * \partial^2 c_i / \partial x^2$$

j_i - mass flux of solute (drug) i

c_i - concentration of solute i

x - position of release

t - time

D_{ip} - solute diffusion coefficient through the polymer

Equation (1) is commonly used to simulate the release rates from reservoir devices. The diffusion coefficient is a measure of the mobility of the individual solute molecules through the reservoir membrane and is considered concentration independent. The concentration gradient in the membrane is represented by dC/dx . The negative sign reflects the movement of solute down the concentration gradient toward more dilute regions. If the membrane is saturated with solute a burst effect may be seen which causes an initial spike of solute. A lag effect occurs when the solute must first permeate the membrane before it is released (Baker, 1987; Langer and Peppas, 1981).

Equation (2) is obtained by combining equation (1) with the continuity equation for mass transfer, assuming no reaction and zero velocity. Equation (2) describes transient diffusion in membranes. The diffusion coefficient is considered to be concentration dependent. It results in the square root of time release rate for simple geometries, such as a one-dimensional slab (Baker, 1987; Langer and Peppas, 1981). Detailed discussions on the applications, assumptions, and solutions of both these equations can be found in the literature (Langer and Wise, 1984; Kost and Langer, 1984; Fan and Singh, 1989; Baker, 1987; Lohmann, 1995; Kydonieus, 1992; Robinson and Lee, 1987; Langer and Peppas, 1981).

Monolithic devices are made by mixing the substance of concern with the polymeric material to form a solution from which the finished product is manufactured. The drug to be released may be dissolved or dispersed (supersaturated) in the resulting polymer depending on how much is "loaded" into the carrier polymer. Examples of monolithic devices include flea collars and antibiotic loaded bone cement.

Examples of reservoir systems include nitroglycerin skin patches, Norplant™, a subcutaneous birth control device, and Ocusert™, a contact lens like device used to treat glaucoma. In these types of systems, the substance of interest is surrounded by an inert

membrane which may be either porous or non-porous. If the membrane is porous, the drug simply diffuses through the pores, but if the membrane is non-porous the drug must first dissolve in the membrane structure before it can diffuse along and between the segments of the membrane. Reservoir systems are known for the ease with which they can be designed to achieve zero-order release rates.

Both monolithic and reservoir diffusion controlled systems are rate limited by the diffusion of the drug through the polymer. The choice of polymer and the resulting effect on the diffusion and partition coefficient of the substance of concern as well as the geometry of the device will influence drug release rates (Kost and Langer, 1984; Lohmann, 1995).

Chemically Controlled Systems. When the substance of concern is encased in a biodegradable non-diffusive polymer, the resulting reservoir device is classified as a chemical reaction system. As seen in the diffusion controlled devices, there are two basic types of biodegradable devices; (1) reservoir and (2) monolithic. The release rates of these systems are strongly influenced by the erosion of the polymer. In the ideal case, surface erosion would be the only factor affecting the release rate, however, this system has yet to be designed (Kost and Langer, 1984; Lohmann, 1995).

A second category of chemical reaction controlled release is chemical immobilization. In this type of system the substance of concern may be chemically bound to the polymer carrier backbone or it may be part of the backbone. The release rate is then influenced by the enzymatic or hydrolytic cleavage of the appropriate bonds (Kost and Langer, 1984; Lohmann, 1995).

Solvent Activated Systems. The last class of controlled release devices to be mentioned here are the solvent controlled systems. These fall into two categories, (1) swelling controlled and (2) osmotically regulated. In both types, the substance of concern is either dissolved or dispersed within the polymer, but it is unable to diffuse through the polymer matrix until activated. In the case of the swelling controlled system, the solvent is absorbed by the matrix causing the polymer to swell and allowing the active agent to diffuse out. In the osmotic system, the solvent permeates the polymer-drug system due to osmotic pressure, which promotes release. Release may occur by either simple Fickian diffusion or by non-Fickian diffusion. Detailed analysis of these and other controlled release systems can be found in the references cited previously.

Advantages. Controlled release systems offers several advantages over conventional drug delivery systems. The major advantage when dealing with biomaterial centered infections is increased efficacy of the drug. The drug can be administered at the biomaterial site, thereby allowing the therapeutic levels to be maintained locally while decreasing the systemic drug level. This will minimize side effects and improve pharmacokinetics. In addition, the effective drug level can be sustained for an extended period of time (Kost and Langer, 1984; Lohmann, 1995).

The use in joint replacement surgery of antibiotic impregnated bone cement combined with a polymer coating, such as poly-L-lactic acid polymer (PLLA), has proven popular for its ability to prevent infections as well as aid in joint fixation (Strachan, 1995). Controlled release of antibiotics from biomaterials is less common however in operations involving other types of implants. Nonetheless research is being done into this method of inhibiting bacterial colonization. A British study showed that antibiotic impregnated silicone rubber coatings of implanted stimulator devices significantly decreased

postoperative infection when compared with systemic antibiotic prophylaxis (Rushton, *et al*, 1989). One of the newer antibiotics receiving attention as a treatment for biomaterial-centered infections is ciprofloxacin.

Ciprofloxacin

Ciprofloxacin is a fluorinated quinolone antimicrobial agent. It is active against a broad range of bacteria, ranging from aerobic gram-negative bacteria to aerobic gram-positive bacteria. Anaerobic bacteria, however, are not affected by ciprofloxacin. It was approved by the Food and Drug Administration in October 1987 (Wolfson and Hooper, 1989; cponline, 1997).

Like other quinolone agents, ciprofloxacin is a synthetic antimicrobial which mainly effects DNA gyrase, the bacterial topoisomerase II. The gyrase is a two subunit enzyme responsible for regulating the supercoiling of DNA during replication and transcription. The A subunit of the gyrase introduces nicks in the DNA that allow the B subunit to twist the single stranded DNA (ssDNA) around its complementary strand of DNA. The A subunit of the gyrase then seals the nicks. It is believed that ciprofloxacin and the other quinolones interfere with the A subunit, preventing it from sealing the nicks. Bactericidal levels of quinolones do not affect mammalian topoisomerase enzymes. The exact bacterial killing mechanism is not known, but detailed discussions about the mechanisms of quinolones action can be found in Wolfson and Hooper (1989). It is known that both slow growing and fast growing organisms are inhibited by ciprofloxacin, and that a prolonged post-antibiotic effect is exhibited by fluoroquinolones (Wolfson and Hooper, 1989; cponline, 1997; Craig and Ebert, 1991; Campa, Bendinelli, and Friedman, 1993).

Ciprofloxacin has the lowest minimum inhibitory concentration (MIC) of the quinolones. The MIC⁹⁰ for *P. aeruginosa in vitro* is between 0.25 µg/ml and 1 µg/ml. Thus ninety percent of the *P. aeruginosa* strains will be inhibited at these low concentrations (Craig and Ebert, 1991; Campa, Bendinelli, and Friedman, 1993). Due to its wide antimicrobial range and low MIC, ciprofloxacin was chosen as a model antibiotic for incorporation into the test PEU polymers.

MATERIALS AND METHODS

Bacteria and Cultures

Pseudomonas aeruginosa (ERC-1) was obtained from the National Science Foundation Engineering Research Center for Biofilm Engineering, Montana State University, Bozeman, MT. Cultures of *P. aeruginosa* were stored as frozen stocks maintained in a solution of 2% peptone and 20% glycerol at -70°C.

Solutions

Medium

A minimal salts medium with glucose as the sole carbon source was used for culturing bacteria. A one liter solution consisted of: 0.5g glucose, 2.56g Na₂HPO₄, 2.08 g KH₂PO₄, 1.0g NH₄Cl, 0.1g CaCl₂, 0.5g MgSO₄, and 0.02ml of a trace metals solution. The trace metal solution was composed of: 0.5% CuSO₄·5H₂O, 0.5 % ZnSO₄·7H₂O, 0.5% FeSO₄·7H₂O, and 0.2% MnCl₂·4H₂O all in a weight per volume ratio dissolved in 10% concentrated HCl (Manual of Industrial Microbiology and Biotechnology, 1986). The trace metals solution was autoclaved separately from the nutrient medium, as was a 5M CaCl₂ solution and a 2.5M MgSO₄ solution. The glucose, trace metals, CaCl₂, and MgSO₄ were all added to the sterile medium by injection through a septum after the medium had been brought to room temperature. All solutions were filter sterilized through a 0.2 µm sterile, syringe tip filter (Corning) before addition to the medium. All solutions were made using Nanopure water (Barnstead, ultrapure water system, Nanopure system) and autoclaved in glass containers at 121°C for 15 minutes per liter.

Cytological Stains

A 0.05% solution of Acridine Orange used for epifluorescent total cell counts was filter sterilized using an autoclaved filter apparatus. Hoechst 33342 (Sigma) was obtained in 100mg quantities and stored in a 0°C freezer until a fresh solution was needed. Then 10ml of sterile nanopure water were added through a 0.2 µm sterile, syringe tip filter to dissolve the powder. Once the Hoechst solution was made it was kept in the refrigerator. All stains were stored in light sensitive bottles and made at fresh at least once a month.

Ciprofloxacin

Ciprofloxacin hydrochloride was obtained from Miles, Inc. as 867 µg ciprofloxacin/mg. A 10,000 µg/ml stock solution was made by dissolving 11.53 mg of powder in 10 ml of sterile nanopure water. The stock solution was stored in a light sensitive glass container in the refrigerator for up to one year, as per the supplier's recommendations.

Detection. A Milton Roy Spectronic 1201 spectrophotometer was used to determine the proper wavelength for detecting ciprofloxacin. Effluent samples were collected from the flow cell, filtered through a 0.2 µm sterile syringe tip filter (Corning), and stored in amber microcentrifuge tubes kept in a 0°C freezer until analysis. A Milton Roy Spectronic 601 set at 339 nm was used to determine the concentration of ciprofloxacin in the effluent from the flow cell.

Reactors and Flow Cell Systems

Continuously-stirred Tank Reactor (CSTR)

A CSTR was designed to provide a constant supply of *Pseudomonas aeruginosa* at room temperature ($23^{\circ}\pm 3^{\circ}\text{C}$) in exponential growth. A 125 ml filter flask was equipped with; an inlet, outlet, aerator, and injection port to build the CSTR. The arm of the filter flask was situated so that overflow occurred when a volume of 128 ml was reached. **Table 1** lists the parameters used for sizing the CSTR. At steady-state operation the specific growth rate, μ , is assumed to be equivalent to the dilution rate, D . **Figure 3** shows a schematic of the chemostat setup. The outlet tube from CSTR was connected to a

Specific growth rate (μ)	$0.467 \pm 0.025 \text{ hr}^{-1}$ (Appendix A)
Volume (V)	128 ml
Volumetric flow rate (Q)	1.0 ml/min.

Table 1. Parameters used for sizing of biologic reactor

waste jug, unless cells were needed for an experiment, in which case the CSTR was connected to a collection vessel. A fresh, clean, sterile collection flask was used for every experiment. The setup was dismantled, cleaned and sterilized at least once every two weeks.

Flow Cell

A schematic of the flow cell used to evaluate the test materials can be seen in **figure 4**. The polymer sample (P) was placed on a clear polycarbonate base which had entrance and exit stainless steel portals milled into it. A white FDA vinyl/nitrile rubber (D) gasket

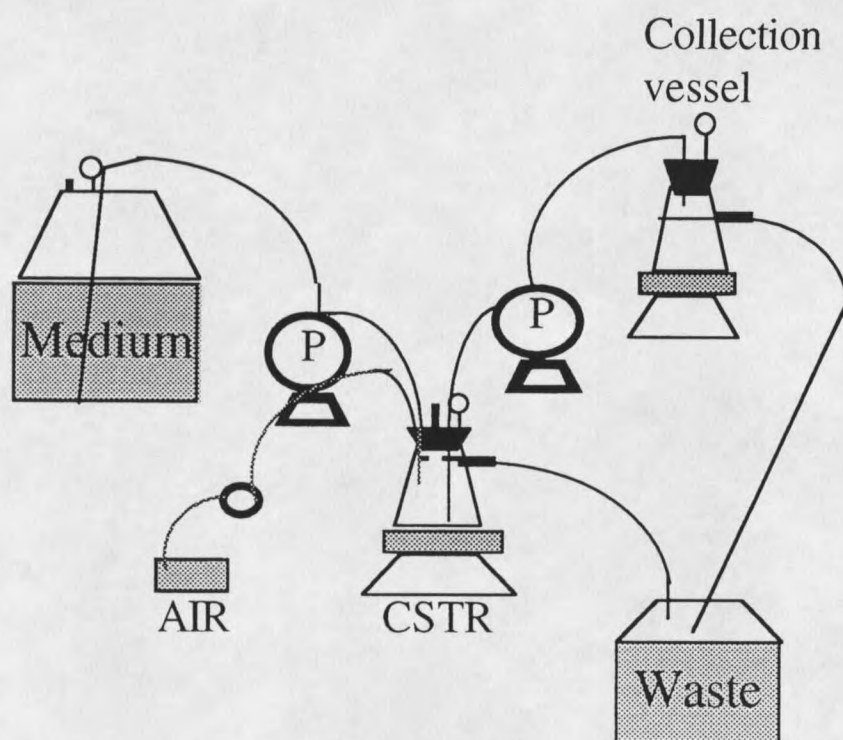


Figure 3. CSTR setup used to grow *P. aeruginosa* continuously

was used to equalize sealing pressure around the polymer sample as the assembly was screwed together. A thin gauge, natural latex rubber (C) gasket was clamped between the test sample and coverglass (B) to form the flow channel. The coverglass was glued over a hole in the clear polycarbonate cover using the denture adhesive Fasteeth®. **Table 2** lists the dimensions of the flow channel and various hydrodynamic parameters. All of the materials used in the flow cell were sterilized by dipping the components in 70% ethyl

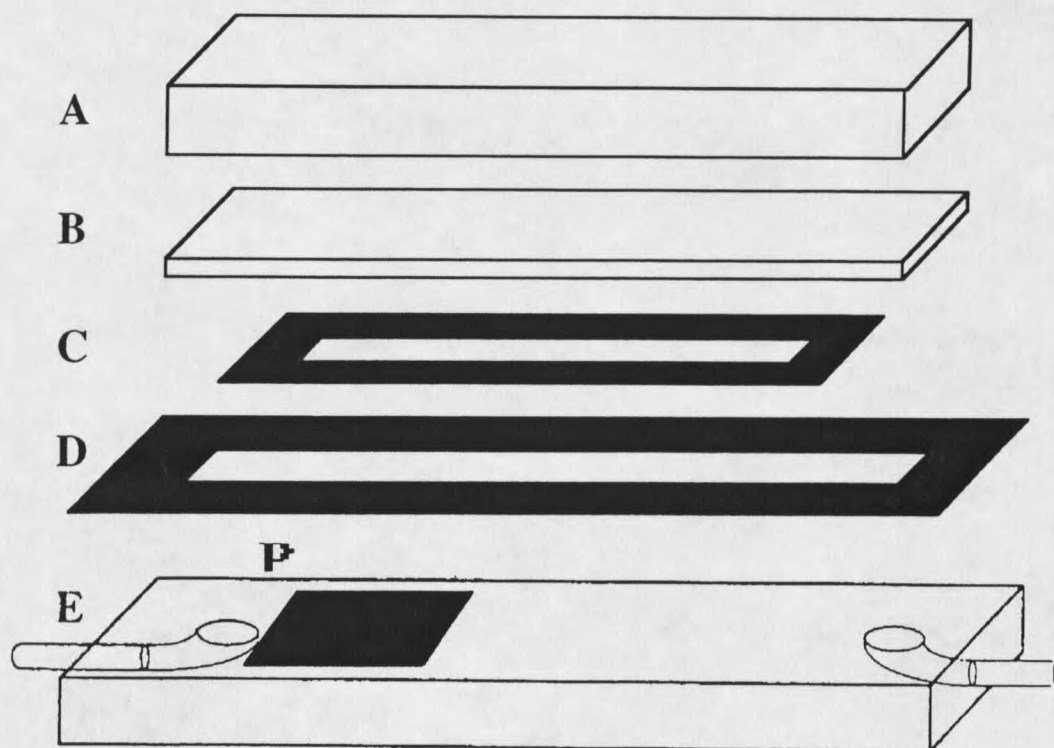


Figure 4. Flow cell schematic: (A) clear polycarbonate cover with hole through center for viewing, (B) # 2 glass coverslip, (C) thin gauge, natural latex gasket, (D) white FDA vinyl/nitrile rubber sealing gasket, (E) clear polycarbonate base, (P) "test" polymer.

alcohol, rinsing with sterile nanopure water, and exposing to UV light for at least 15 minutes. The test polymers were not exposed to UV. The flow cell was assembled under a laminar flow hood to ensure sterility.

Table 2. Flow channel dimensions and hydraulic parameters

width (w)	16 mm
length (l)	44 mm
depth (d)	0.8 mm
area (A)	12.8 mm ²
wetted perimeter (P)	33.6 mm
hydraulic radius (R_h)	0.4 mm
Reynolds number (Re)	2.1
entrance length (L_e)	0.1 mm
wall shear stress (J_w)	20.3 dyne/cm ²

Microscope Setup and Techniques

Polymer Analysis

The polymer samples were tested for bacterial adhesion and growth using an image analysis system seen in **figure 5**. For the first six hours of the experiment approximately 5×10^8 cells/ml of stained *Pseudomonas aeruginosa* were flowed over the test polymer at a rate of 1.0 ml/min. For the remainder of the experiment complete medium without bacteria was pumped through the flow cell.

Hoechst 33342 Staining Procedure. *P. aeruginosa* in log growth were collected and stained with 10 μ g/ml of Hoechst 33342. Once the stain was added to the cells the flask containing the mixture was wrapped in foil and put on an insulated stir plate for three hours. After three hours the suspension was poured into a sterile centrifuge bottle and centrifuged at 10,000 xg for 15 minutes in a 20°C centrifuge (Sorvall Instruments, Dupont, model RC5C, GSA rotor). The liquid was then poured off and the cells were resuspended in sterile complete medium minus the glucose. The cells were washed twice

more before placing them in a sterile 125 ml Erlenmeyer flask with a stir bar, at a concentration of approximately 5×10^8 cells/ml. This procedure was repeated every two hours until the flow cell feed was switched to complete medium only, at hour six.

Cell Visualization. At $t=0$ the flow cell was placed on the stage of an Olympus BH-2 Epi-Illumination UV upright microscope equipped with Olympus filter combinations encompassing the ultra-violet, violet, blue, and green regions of the spectrum. A 40x Nikon water immersion objective was used since the flow channel was

greater than 0.100 mm in depth. A minimum of three fields was captured by an Optronics OPDEI-4700T cooled color CCD camera. The resulting image was processed by a Targa 64+ analog digital converter (ADC) card and stored on computer until the image could be analyzed using Image Pro PlusTM. Images were taken every two hours until $t=6$ hours when the feed was switched from the cell suspension to sterile complete medium. The remaining four time points captured were; (1) $t=7$ or 8 hours, (2) $t=16$ or 18 hours, (3) $t=20$ or 21 hours, and (4) $t=24$ hours.

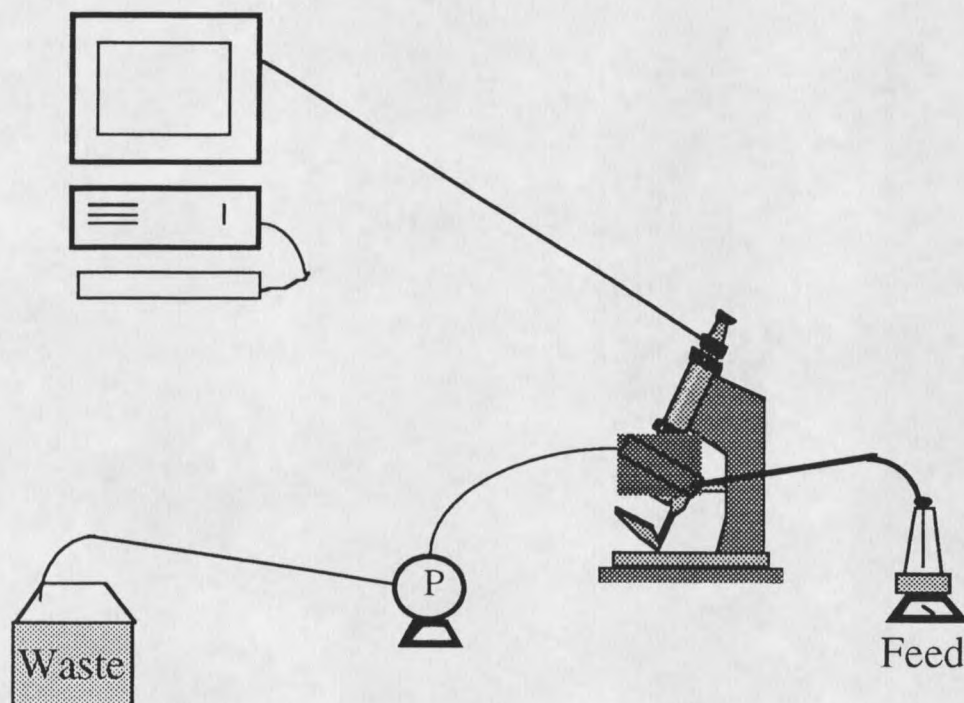


Figure 5. Experimental setup. Feed container = cell suspension $t=0-6$ hrs, after $t=6$ hrs. feed = complete medium. Flow rate through flow cell 1.0 ml/min..

Cell density calculations. The software system Image Pro Plus was used to determine the density of cells on the polymer surface. At the beginning each experiment an image of a micrometer was taken to allow for the calibration of cell size and image area. Actual cell counts could be obtained at the early time points. Cell density calculations were then simply a matter of dividing cell count by the viewing area. At time points greater than 8 hours the area occupied by cells was used to give a range for the cellular density. This was accomplished by dividing the area covered by the high and low published values for *P. aeruginosa* cell area (Holt, 1994) to calculate cell numbers. This method proved to be valid as the cells numbers obtained at the early time points fell within the high and low range of cell numbers calculated using area covered.

In addition, samples from both the inlet and outlet of the flow cell were collected for total cell counts and viable cell counts. Total counts and viable counts were also conducted on a sample of the solution in which the polymer was placed at the end of the experiment. Before any sample was removed for these tests the polymer was sonicated for 30 seconds to remove adherent bacteria. This allowed for the calculation of cell density on the polymer using total cell counts and viable cell counts.

Total Cell Counts. A portion of each sample collected from the inlet, outlet, and sonicated polymer solution was used to determine total cell counts. One milliliter of 0.05% Acridine orange was combined with one milliliter of 2% glutaraldehyde and the appropriate dilution of sample. This solution was poured over a black polycarbonate membrane (pore size 0.22 μm and 25 mm diameter, Fisher Scientific) placed in a cell free glass Millipore filter apparatus. Suction was applied to trap the cells on the membrane. The membrane was mounted on a slide by putting a drop of oil (non-drying immersion oil type FF) on a microscope slide, placing the membrane cell side up on top of the oil drop,

followed by a second drop of oil, and covering with a glass cover slip. The slide was examined under fluorescent microscopy. Bacteria were counted using an Reichert-Jung Microstar IV model UV microscope with a mercury lamp, and a Reichert 100x oil immersion objective. Total counts (# cells/grid) were then converted to total cells/ml using the following conversion:

$$\frac{\text{total cells}}{\text{ml}} = \frac{(\text{cell count}) * (\text{dilution}) * (\text{conversion factor})}{\text{sample volume}}$$

where the conversion factor was 2.27×10^5 .

Viable Counts. A part of the cell suspension sample was used to make serial dilution for plating onto plate count agar (Difco Laboratories) plates. A 100 µl multipipetor (Rainin, epd 2) was used to dispense ten, 10 µl drops of properly diluted cell suspension onto a plate. This procedure was performed in triplicate for each dilution plated. Plates were incubated at room temperature for 24 hours. Drops that contained between 3 and 30 colonies were counted and converted to colony forming units (cfu)/ml by taking into account the dilution factor (Miles, *et al.*, 1938).

Susceptibility and Adhesion Effect Studies

Ciprofloxacin

Growth curves, carried out in batch, were used to determine the susceptibility of increasing concentrations of ciprofloxacin on *P. aeruginosa*. These tests were not carried out as most traditional MIC assays. Studies here were performed as per the procedures of

Nodine and Siegler, 1964; Lennette, *et al*, 1985, starting with a cell concentration of 10^6 cells/ml. *P. aeruginosa* was grown in separate batch cultures which contained various concentrations of ciprofloxacin. At time points ranging from $t=0$ to $t=24$ hours samples were taken for viable counts.

Hoechst Stain (33342)

Susceptibility studies were also conducted on Hoechst 33342 to ensure that the stain did not interfere with normal cell growth. The studies were conducted in the same manner as the ciprofloxacin tests. Concentrations of Hoechst stain examined ranged from $0\mu\text{g/ml}$ (control) to $50\mu\text{g/ml}$.

Fasteeth®

Studies were also carried out to ensure that the denture adhesive, Fasteeth®, used to glue the coverglass to the polycarbonate cover of the flow cell had no negative effect on the growth of bacteria. These studies were conducted in the same manner as those for ciprofloxacin and the Hoechst stain.

Hoechst Stain Effects on *P. aeruginosa* Adhesion

Tests were performed to determine whether the Hoechst stain interferes with the normal adherence of the cells to a surface. The flow cell was assembled without a polymer sample and an untreated cell suspension was run through the cell for four hours. Since the base of the flow cell is a clear polycarbonate, light microscopy could be used to monitor cell adhesion using video recording. Experiments were also carried out using stained cells, still in the staining liquid, and stained cells that had been washed and resuspended in "clean" medium.

The possibility of ciprofloxacin and Hoechst interfering with each other was also investigated. Plate counts were used as in the susceptibility studies. Microscope slides were also made and any change in staining ability was noted.

Tubing

All of the tubing used was FDA approved MasterFlex (Cole Palmer) for use with either pharmaceuticals or food. Tubing used included: Pharmed®, size 13,14, and 16; Norprene® Food, size 13 and 14; and Tygon® Food, size 14. Before each use it was sterilized by autoclaving at 121°C for 15 minutes.

Biomaterial Fabrication

BP Control Polymer

A poly (ether) urethane (Biospan™)/ poly ethylene glycol (PEG) film (BP) was prepared for use as a control. The method of fabrication was as follows: (1) 20 ml of deionized water was used to dissolve 2 g of PEG (Polyscience), (2) the resulting solution was lyophilized, and the powder was sieved to obtain 90 µm or smaller particles, (3) this powder was mixed with a 24% Biospan™ solution (Polymer Technology Group Inc.), (4) the polymer solution was degassed, transferred to a Teflon mold (Chemware), and incubated at 60°C for one day, and finally (5) the film was dried completely in a vacuum chamber.

Test Polymers

The test polymers were manufactured by the same procedure, except the ciprofloxacin containing films also had an equal amount of ciprofloxacin added in step (1). In addition, the test polymers were coated with either triethylene glycol dimethyl ether (triglyme) or triglyme plus n-butylmethacrylate (BMA) by glow discharge plasma deposition (GDPD).

Note: All test materials were fabricated at the University of Washington by Connie Kwok.

RESULTS AND DISCUSSION

Pseudomonas aeruginosa Growth Experiments

Batch Studies

Growth Rate. Studies were conducted to determine the growth rate of *P. aeruginosa* under experimental conditions **Table 3** (Appendix A). The specific growth rate, μ , was found to be 0.467 hr^{-1} .

Experiment	specific growth rate (hr^{-1})
1	0.480
2	0.502
3	0.444
4	0.4436
Average	0.467 ± 0.025

Table 3. Specific growth rate for *P. aeruginosa* grown at room temperature with 500ppm glucose, fully aerated.

Susceptibility Studies. In addition, susceptibility studies were done on ciprofloxacin, Hoechst 33342, and Fasteeth® (Appendices B,C, and D). The ciprofloxacin tests confirmed the published MIC^{90} of $1 \mu\text{g/ml}$. The Hoechst stain (33342) were shown not to inhibit growth at $10 \mu\text{g/ml}$. **Table 4** summarizes the results of the Hoechst 33342 susceptibility tests. Results from the Fasteeth® susceptibility studies show no negative effects on *P. aeruginosa*.

Hoechst 33342 concentration ($\mu\text{g/ml}$)	Growth inhibited?
0	No
5	No
10	No
50	Yes

Table 4. Summary of Hoechst 33342 studies

Flow Cell Experimental Protocol

The concentration of resuspended bacteria used to challenge the test polymers was roughly 5×10^8 cells/ml. The flow rate to the parallel plate flow cell was maintained at approximately 1.0 ml/min for the duration of each experiment. Samples were taken for total counts and viable counts from both the system influent and the effluent at $t = 0, 2, 4$, and 6 hours for every experiment. Only effluent samples were collected after $t = 6$ hours. Effluent samples were also used to determine if any ciprofloxacin eluted from the test polymers containing the antibiotic. All the experiments were carried out at room temperature in the medium previously described.

Flow Cell Experiments

The ability of a polymer to affect bacterial adherence and colonization was examined in flow cell experiments run on four different formulations of a poly (ether) urethane (PEU) base material. The control material (BP), was a PEU base matrix (Biospan™) containing poly (ethylene glycol) (PEG) as a pore forming agent.

The materials tested included: (1) **triglyme**, BP coated with triethylene glycol dimethyl ether (triglyme), (2) **ciprotriglyme**, BP made up of equal parts PEG and ciprofloxacin, coated with triglyme, (3) **BMA**, BP coated with a layer of n-butylmethacrylate (BMA) underneath the triglyme coating, and (4) **ciproBMA**, BP made up of equal parts PEG and ciprofloxacin, coated with BMA followed by triglyme (**Table 5**). The triglyme coating was used to control the release rates of the model antibiotic, ciprofloxacin.

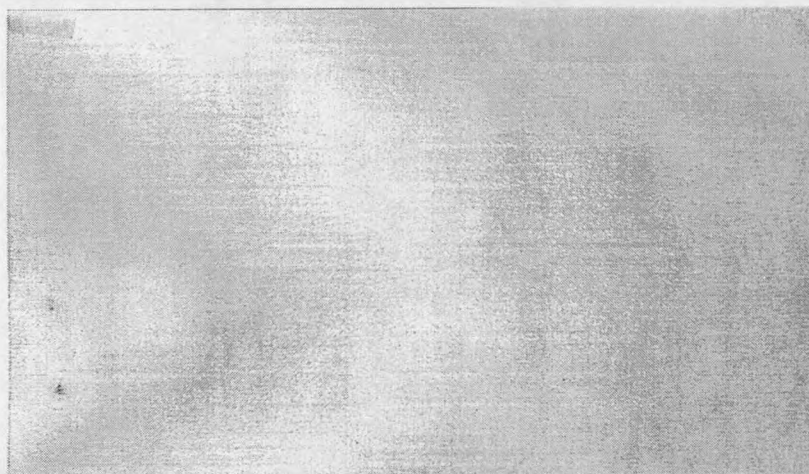
Polymer	Formulation
BP/Control	Biospan TM / PEG (poly ethylene glycol)
triglyme	Triethylene glycol dimethyl ether coated BP
ciprotriglyme	BP made with equal parts PEG and ciprofloxacin, coated with triglyme
BMA	BP coated with n-butylmethacrylate followed by a coating of triglyme
CiproBMA	BP made with equal parts PEG and ciprofloxacin., coated with BMA followed by a coating of triglyme

Table 5 . Description of polymers used in flow cell experiments.

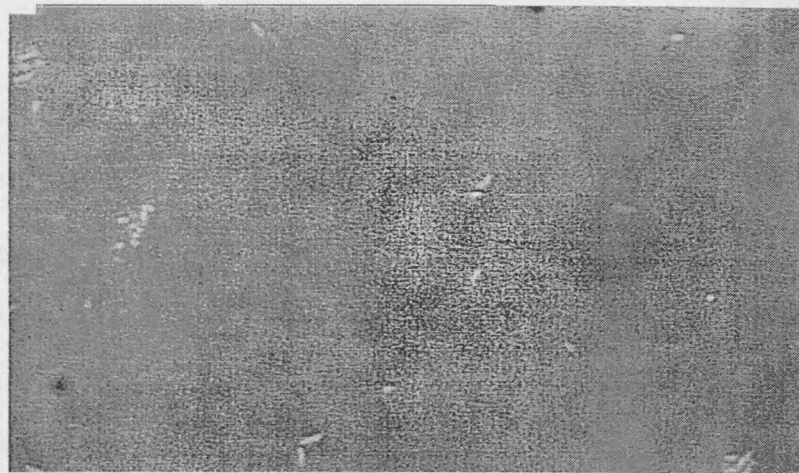
Three samples of each polymer were tested. A good representation of the events at the polymer surface is provided by image analysis (IA) as it is obtained from pictures of the stained cells on the surface. **Figures 6 and 7** illustrate the attachment and growth of *P. aeruginosa* on the control polymer over the course of an experiment. **Figure 8** displays the differences in cell density on three different polymers after 24 hours in the flow cell. A significant decrease in bacterial adhesion and colonization was evident when the test polymers were compared to the BP polymer at the end of the experiments and over the entire run using pictures taken for IA (**figures 6, 7, and 8**).

The graphs generated from the image analysis data (**figures 9, 10, and 11**) were obtained from a single run for each polymer. Each experiment was, however, replicated three times. The error bars were obtained because at least three different fields of view were taken for each time point from which the average cell density and the standard deviation were calculated (Appendix E).

Figure 6. Biospan/Control time course of cell adhesion.



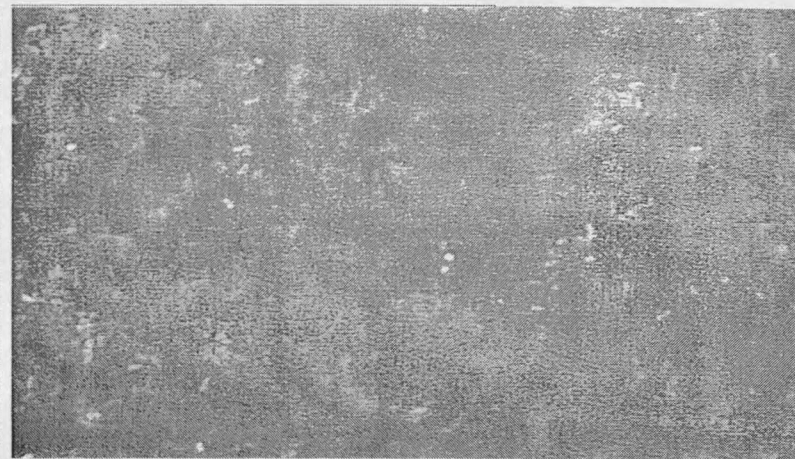
t=0 hours



t=2 hours



t=4 hours

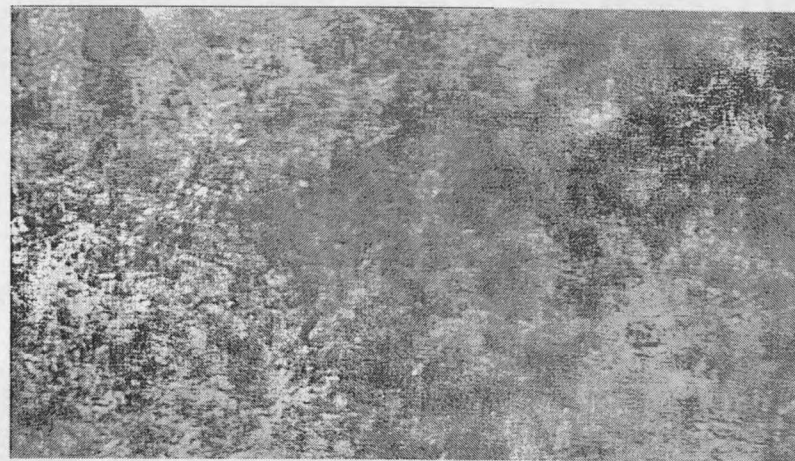


t=6 hours

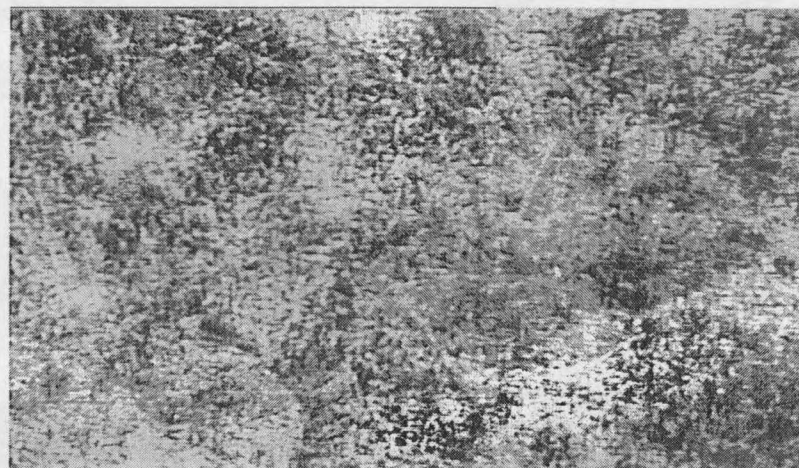
Figure 7. Control/Biospan polymer bacterial colonization.



t=16 hours



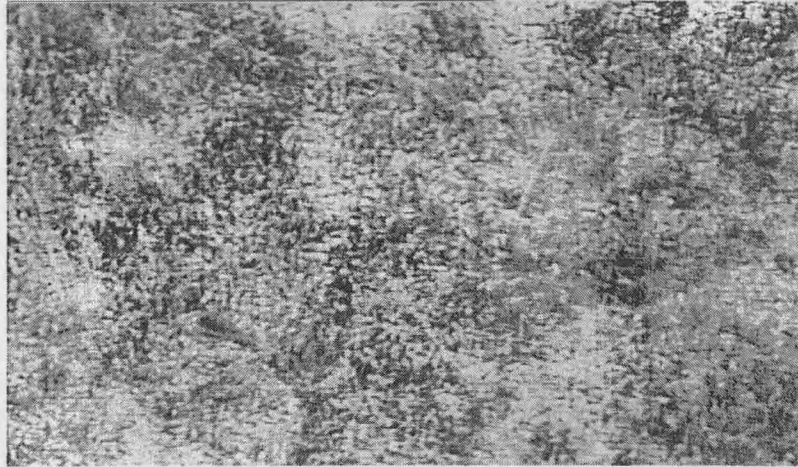
t=20 hours



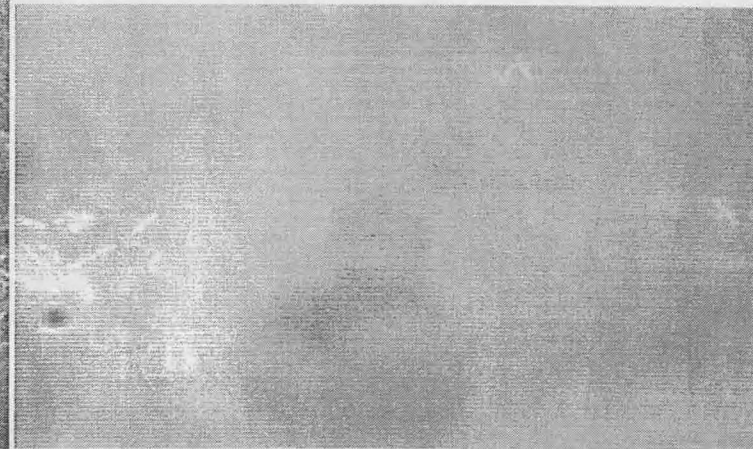
t=24 hours

Figure 8. Comparison of the extent of colonization of different polymers at $t=24$ hours.

Control/Biospan



triglyme



Cipro-triglyme

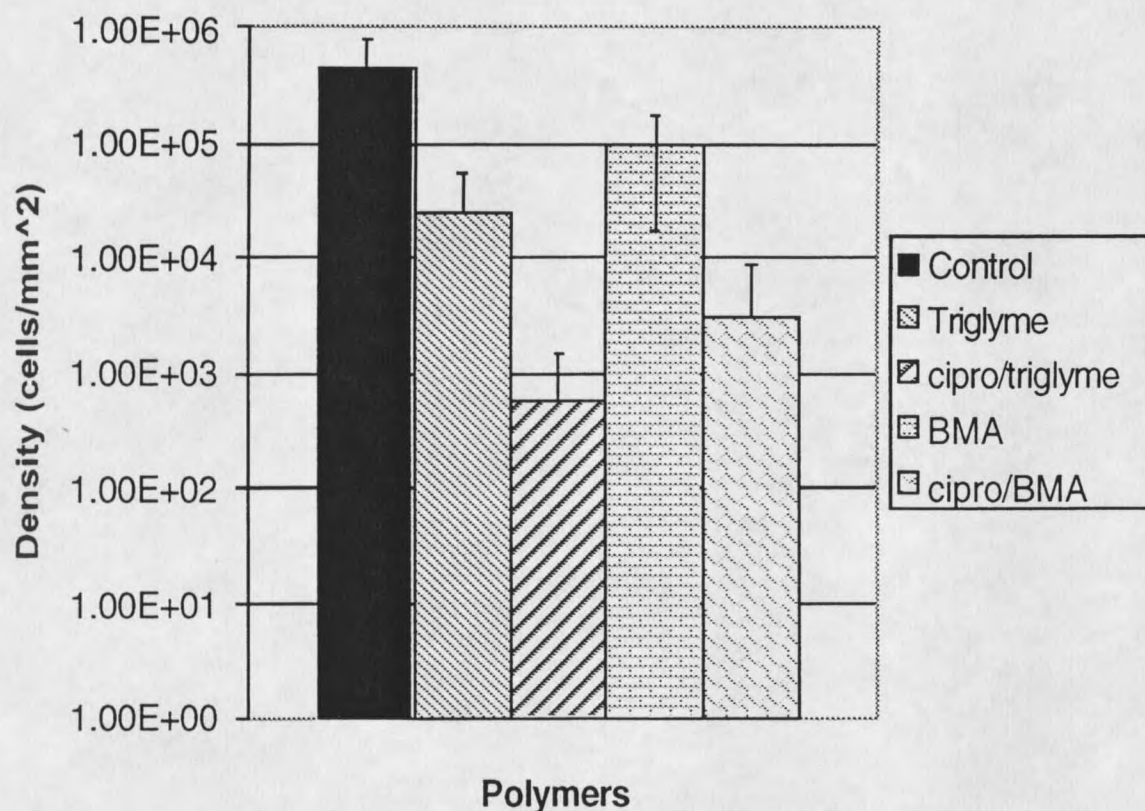


Figure 9. Direct counts of cell density on the polymer surface after 24 hours. (L-R) Control, triglyme, ciprotriglyme, BMA, ciproBMA

Figure 9 clearly shows that the coatings of triglyme and BMA + triglyme reduce bacterial colonization by at least half when compared to the BP control. It can also be seen from **figure 9** that the addition of ciprofloxacin to the polymer formulation decreases bacterial colonization by at least two orders of magnitude. Note that the y-axis on **figure 9** is a logarithmic scale.

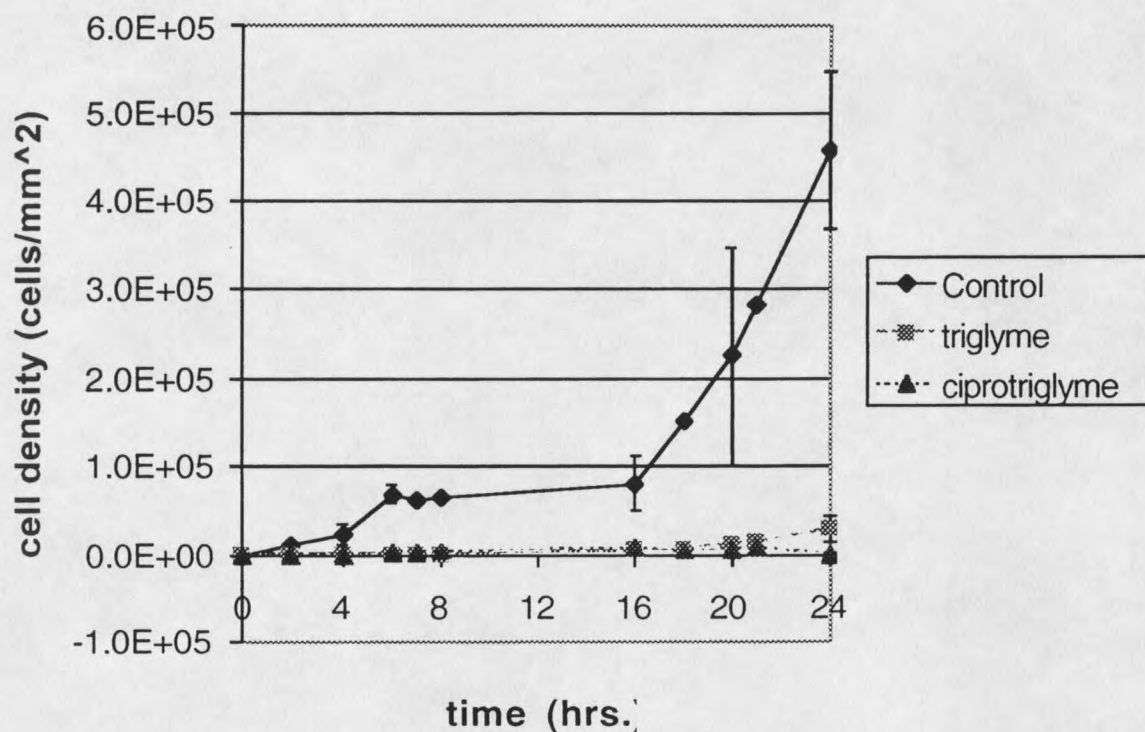


Figure10. Time course cell colonization curve of control, triglyme, ciprotriglyme obtained from image analysis.

Figure 10 shows that bacterial attachment and colonization to the control polymer is much greater than to either the triglyme or ciprotriglyme polymer. Analysis of the data in Appendix E will show the same trend for BMA and ciproBMA. At $t = 6$ hours the feed was switched from a medium containing $\sim 5 \times 10^8$ cells/ml with no glucose to a sterile feed of complete medium plus 500 ppm glucose. Therefore, any increase in cell density can be attributed to growth. Slightly more growth can be seen on the triglyme than on the ciprotriglyme over the course of the experiment, however neither of these polymers exhibits the growth found on the control polymer.

Figure 11 reveals that bacteria are attaching to the triglyme and ciprotiglyme polymers, but not to the same extent as to the BP control polymer. The bacteria attach to the control polymer at a rate of 1.14×10^4 cells/mm²/hr while the rates of attachment to the triglyme and the ciprotiglyme are significantly lower, 66 cells/mm²/hr and 537 cells/mm²/hr respectively. While the attachment rate is greater on the ciprotiglyme than on the triglyme. The rate of growth is greater on the triglyme than on the ciprotiglyme. It can

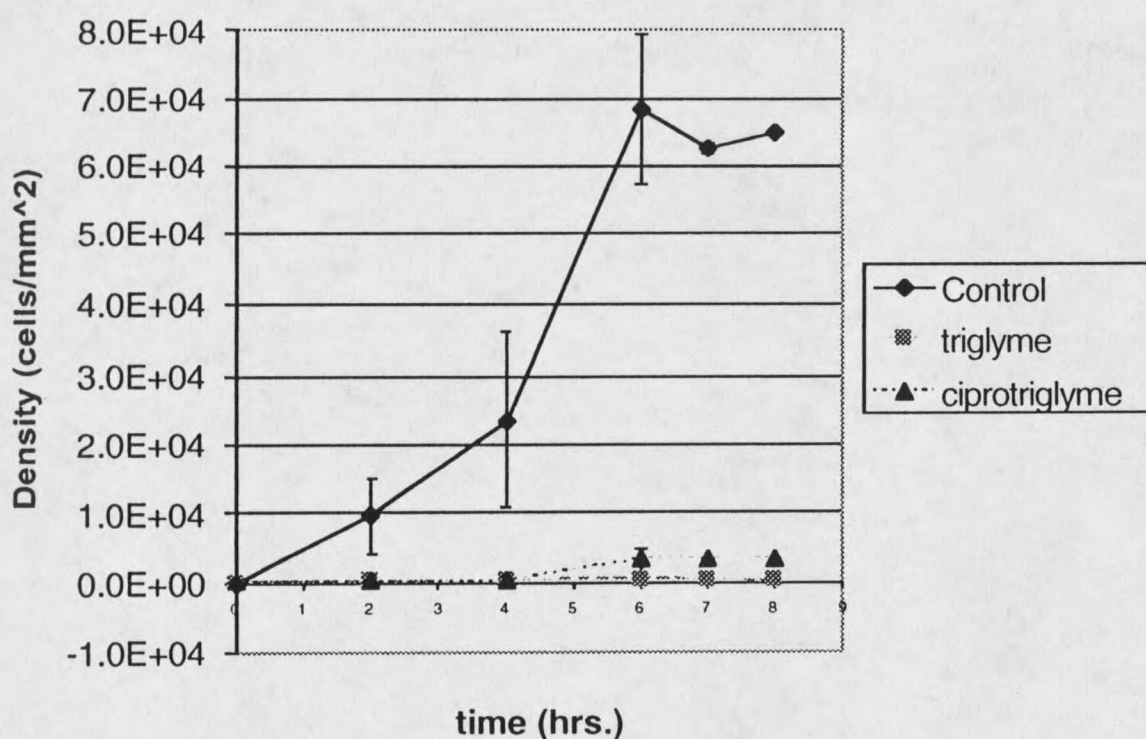


Figure 11. Attachment rate differences are evident within the first 6 hours of the flow cell experiments. The control polymer demonstrates a much more rapid attachment rate than either triglyme or ciprotiglyme.

be seen from **figure 10** that the control polymer exhibits an exponential rate of growth from t=16-24 hours, that is significantly higher than either triglyme or ciprotiglyme. What

is not readily seen is the fact that triglyme exhibits a faster growth rate than the ciprotriglyme, 1.81×10^3 cells/mm²/hr versus 27 cells/mm²/hr.

Total counts of the effluent found in Appendix F back up the image analysis results (figures 12,13). **Figure 12** shows that for the first six hours $\sim 5 \times 10^8$ cells/ml are being pumped through the flow cell. Once the supply of cells is cut off at $t=6$ hours cells can still be seen in the effluent, implying that growth of new cells is taking place in the flow cell system.

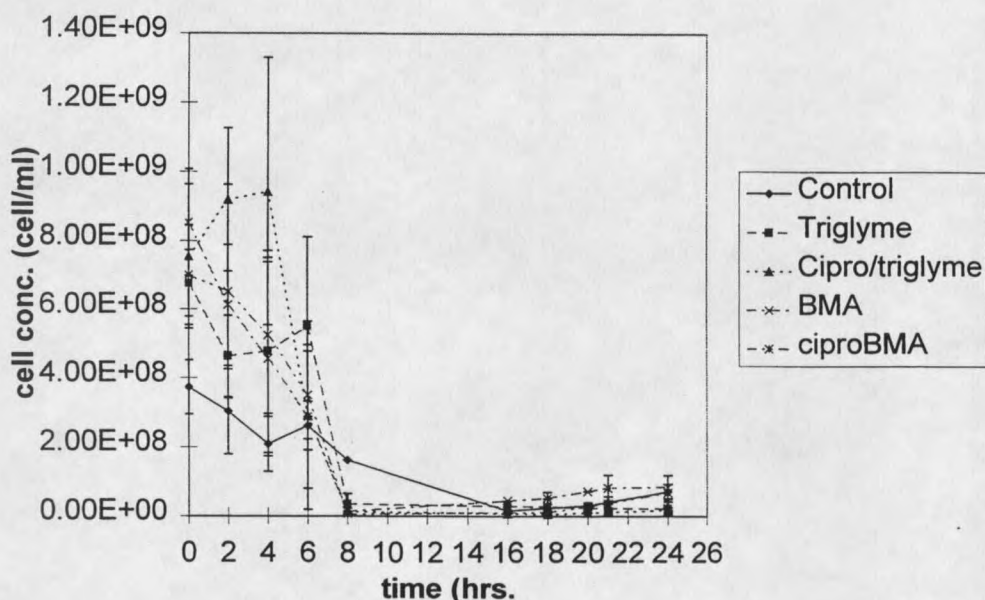


Figure 12. Total counts made with Acridine Orange. From $t=0-6$ hours, a feed composed of a bacterial suspension in medium without a carbon source was pumped through the flow cell, after $t=6$ hours the feed was switched to sterile complete medium.

Figure 13 obtained from total cell counts displays the same trend with regard to the amount of cell density on the polymer surface after 24 hours as **figure 9** which was made from direct count data. The control polymer exhibits the highest cell density after 24 hours. The effluent data does not demonstrate as much of a difference between polymers as the image analysis data, which may be explained by the growth and sloughing of bacteria in the effluent tubing. It is to be expected that the samples collected from the

effluent would have higher cell counts since there is more surface area available for cell growth.

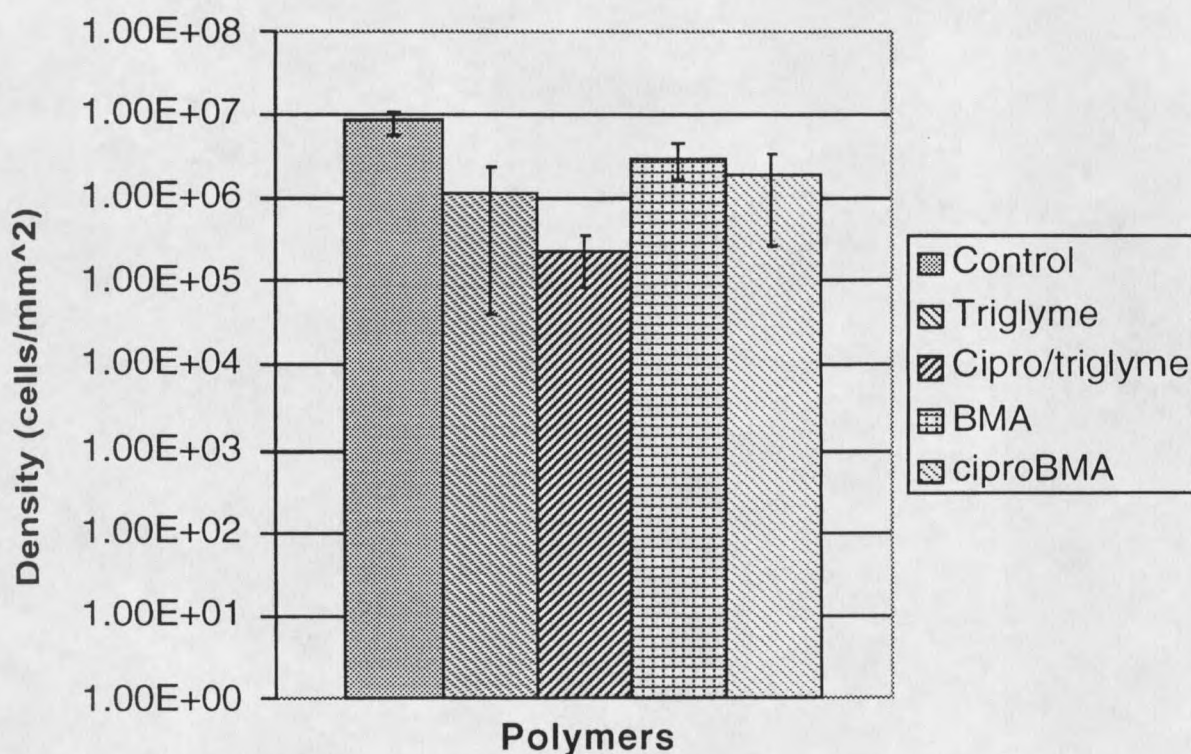


Figure 13. Calculated cell density on the “test” polymers using total cell count (acridine orange) data. Note that it follows the same trend as the image analysis data.

Figure 14 shows that viable cells are being pumped through the flow cell, while **figure 15** demonstrates that the concentration of ciprofloxacin at the polymer surface is high enough to have a noticeable effect on the density of bacteria on the polymer. (Appendix G - Plate Count Data)

Effluent viability data from t = 0-24 hours

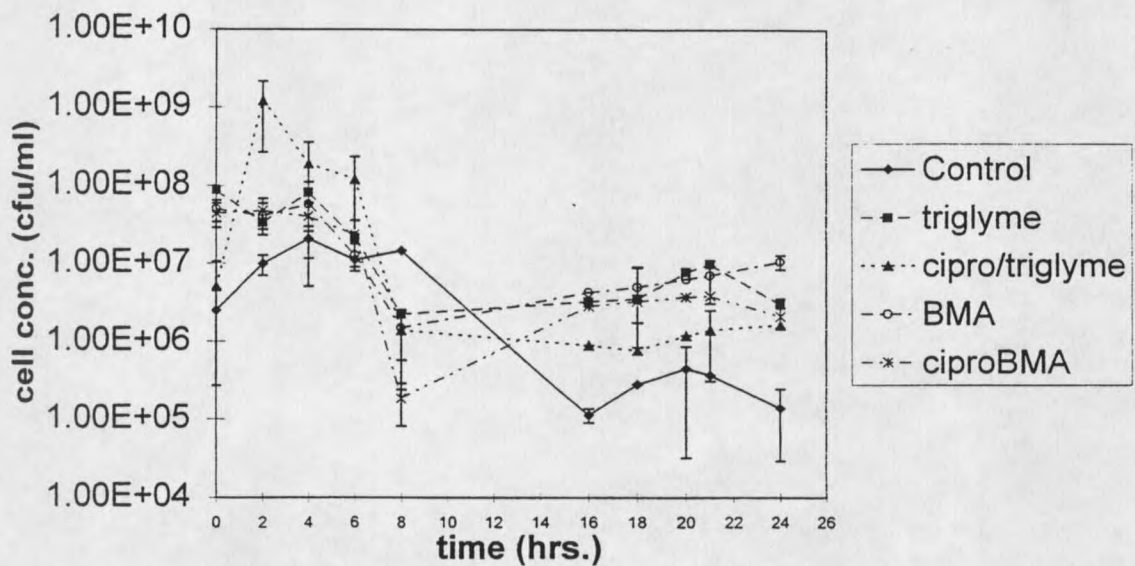


Figure 14. Plate count data from effluent samples taken from the polymer flow cells during the course of the experiments shows that viable cells are going through the flow cell.

As stated previously, total counts and viable counts are not considered to be as accurate as direct counts. The effect of the extra surface area available for cell adhesion and growth provided by the tubing was not factored into the results. Subsequent sloughing and/or entrapment of cells in this region of the flow cell setup could affect the cell concentration data collected. **Figure 16** demonstrates the variation obtained from the

concentration data collected. **Figure 16** demonstrates the variation obtained from the

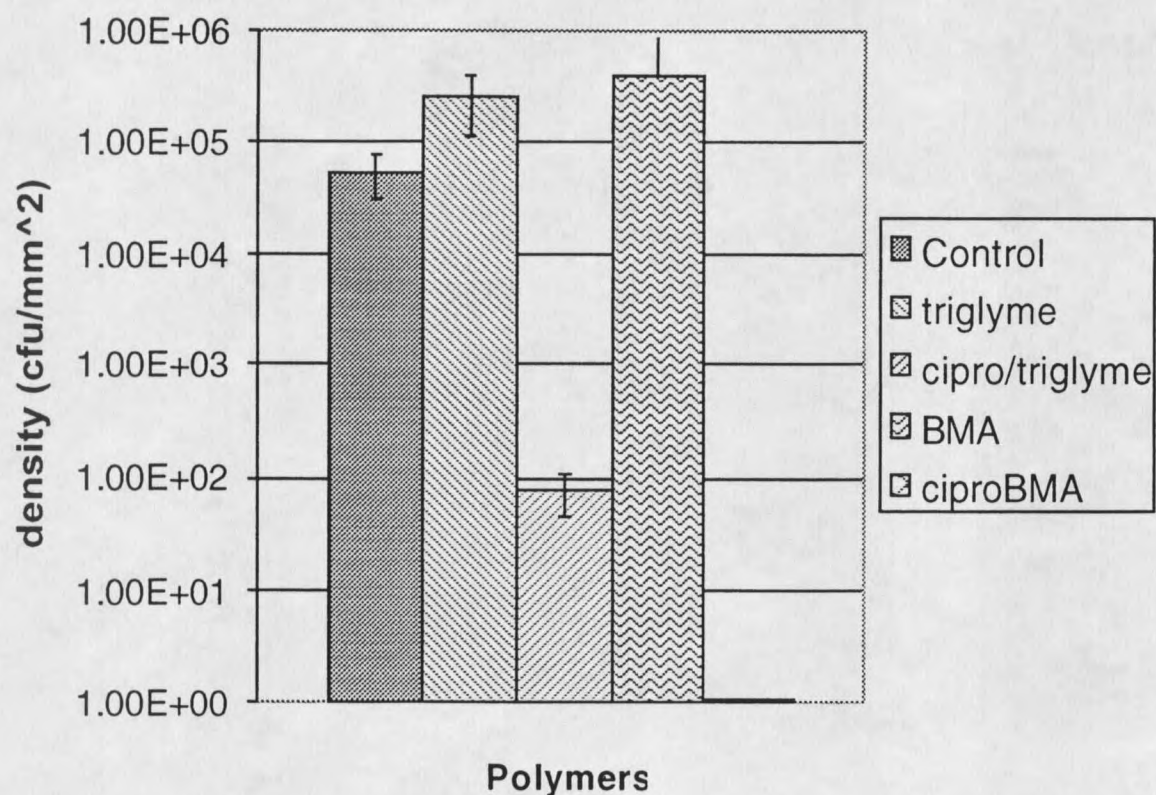


Figure 15. Cell densities on polymers calculated from plate count data. No colonies were found on any of the plates made for the ciproBMA polymer. (L-R) Control, triglyme, ciprotriglyme, BMA, ciproBMA.

same polymer using different techniques. As stated previously the results obtained from IA are considered to be the most accurate since they are taken from the polymer surface and not the effluent.

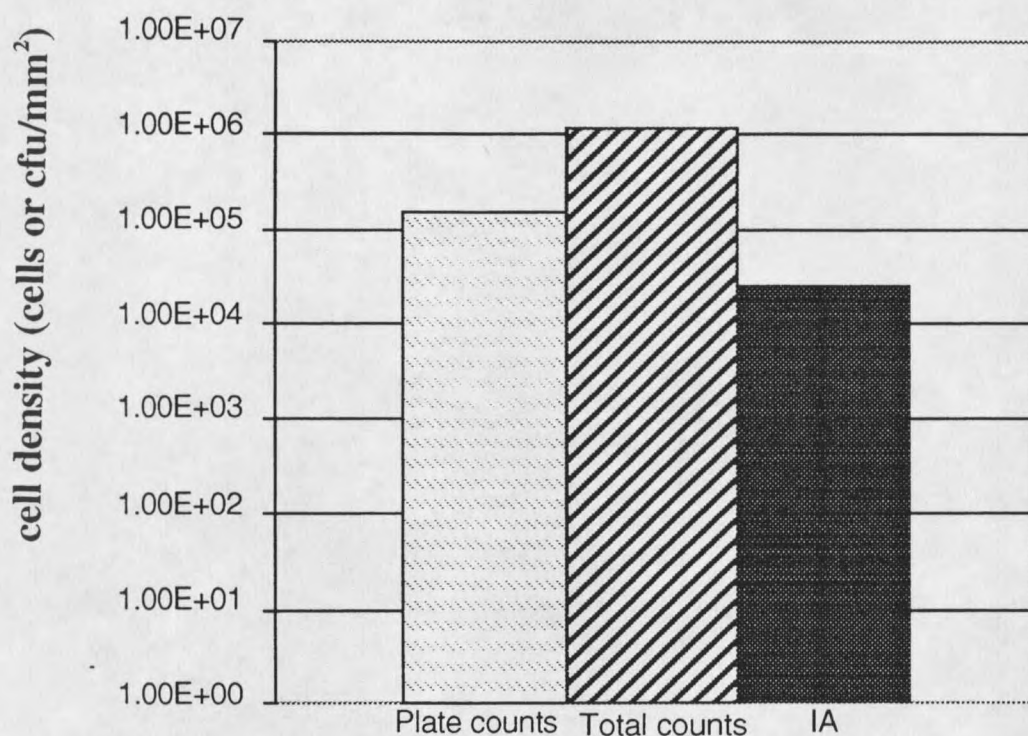


Figure 16 demonstrates the variations in bacterial density on the triglyme polymer after twenty four hours using image analysis (IA), acridine orange staining of the effluent, and plate counts of the effluent.

Ciprofloxacin release

The extra surface area could also factor into the amount of ciprofloxacin detected in the effluent (**table 6**) of the polymers incorporating ciprofloxacin. In one millileter of effluent roughly 3 ug of ciprofloxacin should be detected . It should be noted that all of the polymers containing ciprofloxacin still retained significant amounts of ciprofloxacin. This is to be expected given that the initial loading of ciprofloxacin was 0.4g. Additionally,

an earlier study found that these polymers continue to elute ciprofloxacin for up to 128 hours in a well stirred flask (Kwok, C.S., et al, 1997). The erratic release of ciprofloxacin seen in **table 6** may be due to: the interaction of the antibiotic with cells growing in the effluent tubing, ciprofloxacin degradation resulting

Experiment	Time (hours)	Cipro. conc. ($\mu\text{g/ml}$)	# of points above detection
Cipro triglyme	0	35 ± 21	2
	2	17	1
	4	5	1
	6	4	1
	8	ND	0
	18	ND	0
	21	18	1
	24	5 ± 5	2
CiproBMA	0	6 ± 5	3
	2	1	1
	4	ND	0
	6	ND	0
	8	29	1
	18	2	1
	21	ND	0
	24	ND	0

Table 6. Ciprofloxacin concentrations in effluent. ND = not detectable

from light exposure, pulsating flow, unforeseen release behavior, or damage to polymer coating. Appendix H expands on the mathematical theory behind the controlled-release of a drug from a one-dimensional slab under flow conditions.

Overall the flow cells experiments showed that ciprofloxacin could successfully be incorporated into Biospan™/PEG polymers and still maintain its efficacy. In addition, they demonstrated that cell adhesion and colonization of opaque materials could be monitored continuously for up to 24 hours under flow conditions. The results involving the triglyme and BMA coatings were also encouraging.

SUMMARY

The research conducted for this study involved exposing planktonic *Pseudomonas aeruginosa* to 10 µg/ml of the DNA stain Hoechst 33342 for 3 hours. The cell suspension was then washed and resuspended in fresh complete medium minus glucose, the carbon source. Every two hours, for six hours, a fresh batch of resuspended cells was pumped over a "test" polymer in a flow cell being monitored microscopically for bacterial attachment. After six hours, the flow was switch from the cell suspension to complete medium plus glucose in order to observe cell adhesion and growth over the 24 hour run. Pictures were taken periodically over the course of the experiment and examined by image analysis to detect changes in bacteria cell density on the surface of the "test" polymer. The results obtained from image analysis were for the most part confirmed by total and viable cell counts performed on effluent samples. Within the limitations and scope of this work, the results show that:

- 1) Both the BMA coating and the triglyme coating can cut bacterial adhesion and colonization in half.
- 2) The addition of the ciprofloxacin to the polymer matrix decreases bacterial adhesion and colonization by at least two orders of magnitude when compared with the control.
- 3) Hoechst 33342 can be used at 10ug/ml without interfering with *P. aeruginosa* growth patterns.
- 4) Hoechst 33342 enables bacterial adhesion and biofilm formation on an opaque surface to be monitored in a flow cell system for up to 24 hours.

The results of this research indicate that it would be worthwhile to pursue development of the "test" polymers for use as biomedical materials. Further investigation into how well image analysis correlates with actual bacterial cell adhesion and colonization

is also suggested from these results. It would be interesting to run a set of experiments on a substratum comparing image analysis and destructive sampling, in which the flow cell is broken down and the substratum is removed at every time point. In addition, a better indication of the long-term performance of these materials could be gained by conducting tests over a longer period of time, a week or more. Likewise, the exposure of the material to proteins before and/or during bacterial challenge would provide more realistic test conditions. Dealings with industry have shown that the types of adhesion and colonization experiments conducted in this study are among the initial steps in gaining approval from the FDA.

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APPENDICES

APPENDIX A

Growth Rate Experiments

Growth Curves (500 ppm Glucose) Room Temperature					
time	count	conc. (cells/ml)	average	std. dev.	Specific growth rate
0	28	1.59E+06	7.11E+06	6.87E+06	0.480466905
	52	2.95E+06			
	296	1.68E+07			
1	40	2.27E+06	7.85E+06	4.08E+06	
	210	1.19E+07			
	165	9.36E+06			
2	28	1.59E+06	2.04E+06	7.66E+05	
	25	1.42E+06			
	55	3.12E+06			
3	281	1.59E+07	1.38E+07	1.56E+06	
	216	1.23E+07			
	233	1.32E+07			
4					
5	98	5.56E+07	5.50E+07	2.12E+06	
	101	5.73E+07			
	92	5.22E+07			
6	177	1.00E+08	7.36E+07	2.06E+07	
	89	5.05E+07			
	123	6.98E+07			
7	290	1.65E+08	1.22E+08	3.18E+07	
	198	1.12E+08			
	156	8.85E+07			
8	56	3.18E+08	2.10E+08	8.20E+07	
	21	1.19E+08			
	34	1.93E+08			
9	114	6.47E+08	5.22E+08	7.79E+07	
	82	4.65E+08			
	93	5.28E+08			
	79	4.48E+08			
10	141	8.00E+08	7.49E+08	9.30E+07	
	109	6.19E+08			
	146	8.28E+08	--		
11	282	1.60E+09	1.15E+09	2.84E+08	
	206	1.17E+09			
	172	9.76E+08			
	150	8.51E+08			
12	200	1.13E+09	1.10E+09	3.29E+07	
	186	1.06E+09			
	195	1.11E+09			
13	76	2.16E+09	2.77E+09	1.07E+09	
	75	2.13E+09			
	163	4.62E+09			
	76	2.16E+09			
14	127	3.60E+09	3.40E+09	3.56E+08	
	102	2.89E+09			
	130	3.69E+09			

15	273	7.75E+09	8.17E+09	3.18E+08	
	300	8.51E+09			
	291	8.26E+09			
16	287	1.63E+10	1.55E+10	7.65E+08	
	278	1.58E+10			
	255	1.45E+10			
17	295	1.67E+10	1.45E+10	1.91E+09	
	213	1.21E+10			
	260	1.48E+10			
18	313	1.78E+10	1.63E+10	1.20E+09	
	261	1.48E+10			
	286	1.62E+10			

time	count	Conc. (cells/ml)	average	std. dev.	Specific growth rate
0	20	1.13E+06	2.71E+06	1.91E+06	0.50174225
	109	6.19E+06			
	57	3.23E+06			
	17	9.65E+05			
	36	2.04E+06			
1					
2					
3	121	6.87E+06	7.46E+06	7.67E+05	
	117	6.64E+06			
	137	7.77E+06			
	151	8.57E+06			
4					
5					
6	160	9.08E+06	1.28E+07	3.51E+06	
	309	1.75E+07			
	210	1.19E+07			
7	35	1.99E+07			
	28	1.59E+07			
	38	2.16E+07			
8					
9	78	4.43E+08	4.85E+08	2.68E+07	
	87	4.94E+08			
	91	5.16E+08			
	86	4.88E+08			
10					
11					
12	69	3.92E+08	4.00E+08	1.47E+07	
	73	4.14E+08			
	67	3.80E+08			
	73	4.14E+08			
13	107	6.07E+08	6.49E+08	5.49E+07	
	128	7.26E+08			
	108	6.13E+08			
14	64	1.82E+09	1.31E+09	2.92E+08	
	40	1.13E+09			
	39	1.11E+09			
	42	1.19E+09			
15	64	1.82E+09	1.82E+09	4.01E+07	
	66	1.87E+09			
	64	1.82E+09			
	62	1.76E+09			
16					
17	121	3.43E+09	3.62E+09	1.42E+08	
	129	3.66E+09			
	133	3.77E+09			
18	364	2.07E+10	2.27E+10	1.46E+09	

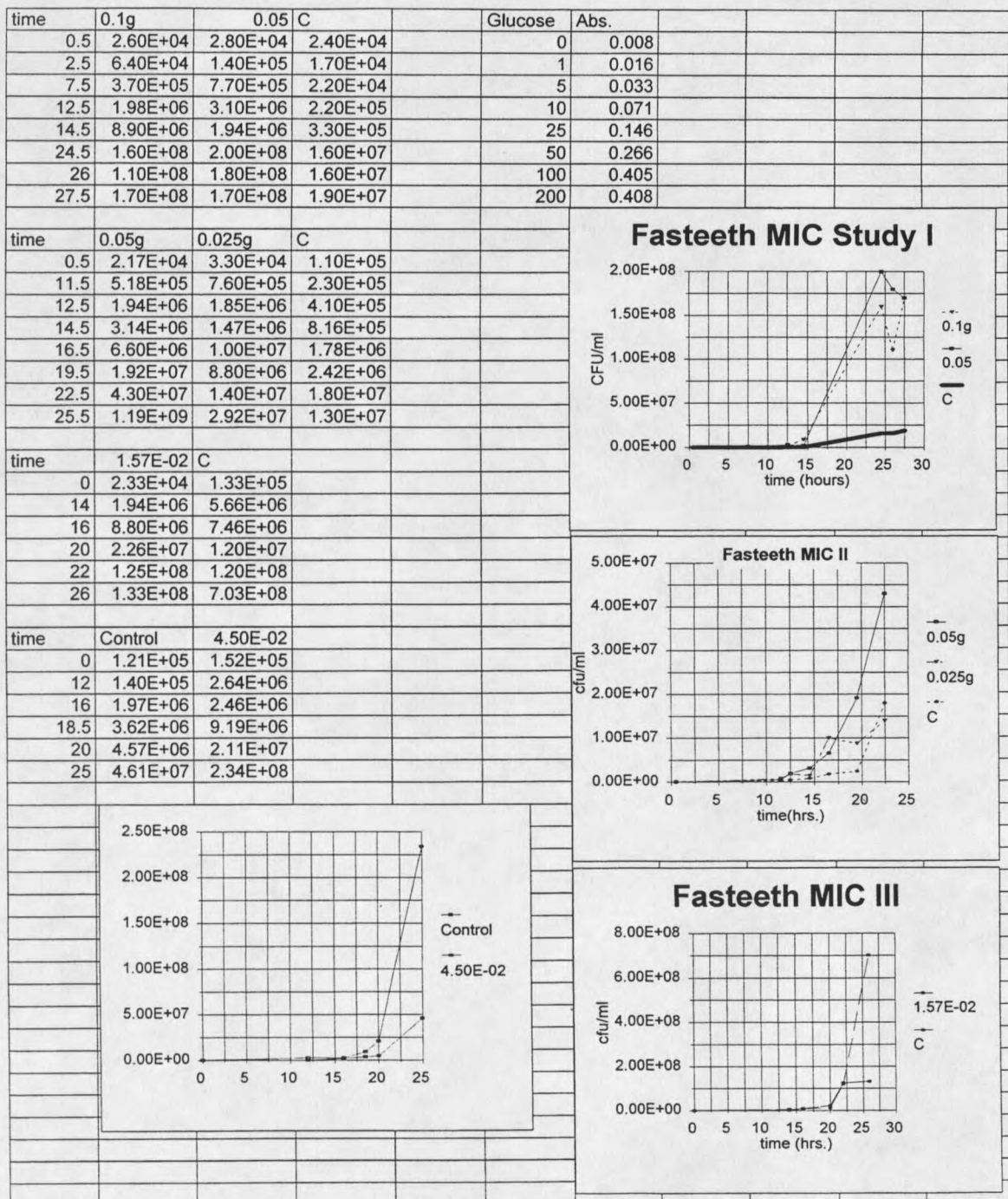
time	count	Conc. (cells/ml)	average	std. dev.	Specific growth rate
0	23	5.22E+06	6.39E+06	1.14E+06	0.443703052
	27	6.13E+06			
	37	8.40E+06			
	30	6.81E+06			
	22	4.99E+06			
	30	6.81E+06			
11	119	1.35E+10	1.19E+10	1.65E+09	
	119	1.35E+10			
	90	1.02E+10			
	90	1.02E+10			
12	45	1.02E+10	9.34E+09	2.31E+09	
	26	5.90E+09			
	32	7.26E+09			
	38	8.63E+09			
	54	1.23E+10			
	52	1.18E+10			
13	49	1.11E+10	1.26E+10	1.31E+09	
	48	1.09E+10			
	59	1.34E+10			
	61	1.38E+10			
	54	1.23E+10			
	63	1.43E+10			
14	135	3.06E+10	3.02E+10	2.50E+09	
	128	2.91E+10			
	150	3.40E+10			
	120	2.72E+10			
15	167	3.79E+10	3.68E+10	1.66E+09	
	168	3.81E+10			
	152	3.45E+10	--		
16	239	5.42E+10	4.95E+10	6.19E+09	
	201	4.56E+10			
	250	5.67E+10			
	183	4.15E+10			
17	57	6.47E+10	7.26E+10	9.60E+09	
	77	8.74E+10			
	56	6.36E+10			
	66	7.49E+10			
18	86	9.76E+10	1.02E+11	8.04E+09	
	82	9.31E+10			
	101	1.15E+11			
	90	1.02E+11			
19	121	1.37E+11	1.21E+11	1.82E+10	
	124	1.41E+11			

	92	1.04E+11			
	89	1.01E+11			
20	89	1.01E+11	1.07E+11	8.30E+09	
	96	1.09E+11			
	105	1.19E+11			
	86	9.76E+10			
21	101	1.15E+11	1.24E+11	1.04E+10	
	122	1.38E+11			
	114	1.29E+11			
	100	1.13E+11			
22	67	7.60E+10	1.11E+11	2.16E+10	
	115	1.31E+11			
	97	1.10E+11			
	112	1.27E+11			

[illegible]

APPENDIX B

FASTEETH® SUSCEPTIBILITY

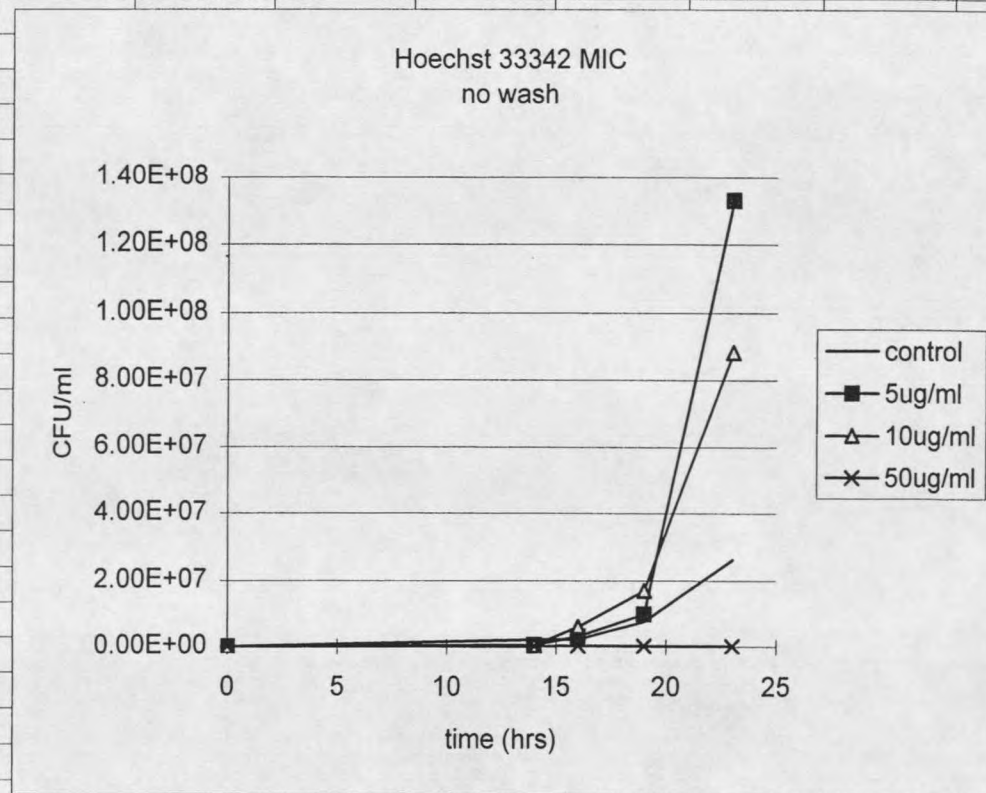


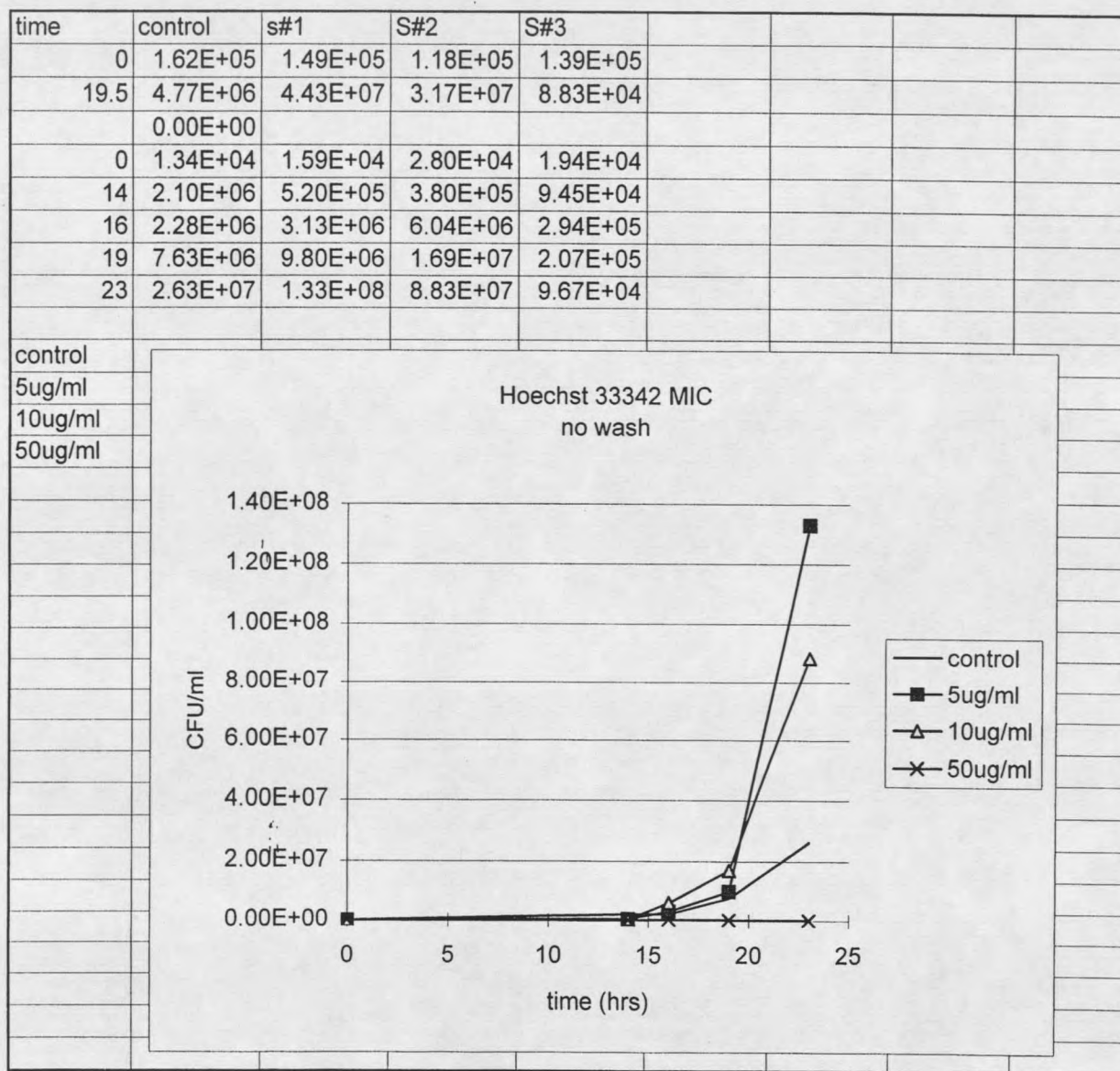
APPENDIX C

Hoechst 33342

time	control	s#1	S#2	S#3				
0	1.62E+05	1.49E+05	1.18E+05	1.39E+05				
19.5	4.77E+06	4.43E+07	3.17E+07	8.83E+04				
	0.00E+00							
0	1.34E+04	1.59E+04	2.80E+04	1.94E+04				
14	2.10E+06	5.20E+05	3.80E+05	9.45E+04				
16	2.28E+06	3.13E+06	6.04E+06	2.94E+05				
19	7.63E+06	9.80E+06	1.69E+07	2.07E+05				
23	2.63E+07	1.33E+08	8.83E+07	9.67E+04				

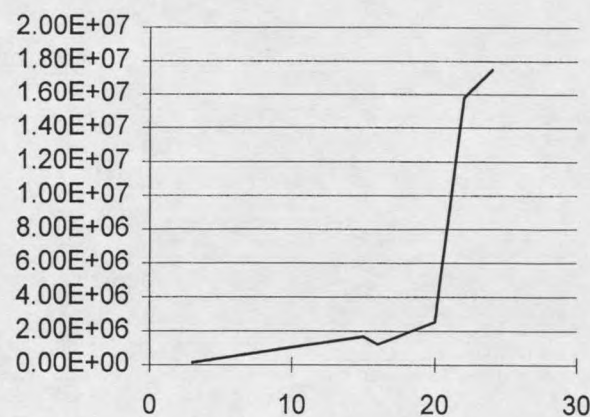
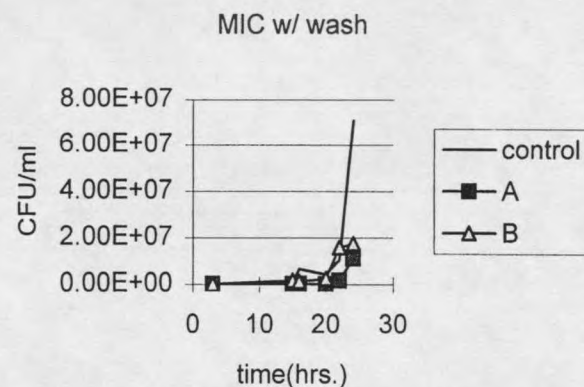
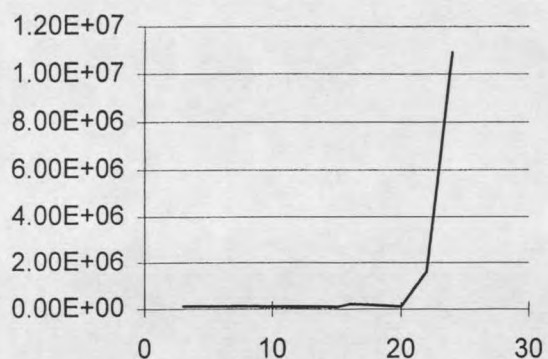
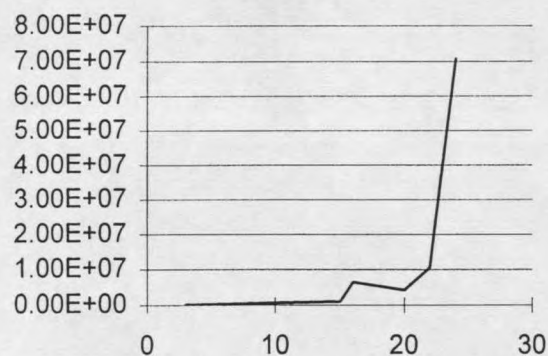
control
5ug/ml
10ug/ml
50ug/ml





Hoescht MIC - incubate in stain for 3 hrs. then wash

time	control	A	B
3	1.29E+05	1.29E+05	1.39E+05
15	1.04E+06	1.00E+05	1.69E+06
16	6.45E+06	2.27E+05	1.22E+06
20	4.23E+06	1.13E+05	2.54E+06
22	1.06E+07	1.60E+06	1.58E+07
24	7.07E+07	1.09E+07	1.75E+07



APPENDIX D

Ciprofloxacin

Ciprofloxacin MIC study Plate counts						
time	count (1ug/ml)	cfu/ml	average	std. dev.	count (1ug/ml)	cfu/ml
0	96	9.60E+04	9.62E+04	5.30E+03	21	2.10E+04
	96	9.60E+04			28	2.80E+04
	105	1.05E+05			22	2.20E+04
	10	1.00E+05				
	9	9.00E+04				
	9	9.00E+04				
15.5	67	6.70E+04	8.65E+04	2.66E+04	3	3.00E+03
	85	8.50E+04			2	2.00E+03
	77	7.70E+04			2	2.00E+03
	5	5.00E+04				
	11	1.10E+05				
	13	1.30E+05				
17	76	7.60E+04	6.87E+04	2.20E+04	20	2.00E+04
	65	6.50E+04			27	2.70E+04
	41	4.10E+04			20	2.00E+04
	11	1.10E+05				
	5	5.00E+04				
	7	7.00E+04				
20	31	3.10E+04	2.30E+04	1.28E+04	13	1.30E+04
	33	3.30E+04			0	0.00E+00
	5	5.00E+03			0	0.00E+00
22	0	ND			0	
	0				0	
	0				0	
24	0	ND			0	
	0				0	
	0				0	
time	1 ug/ml	std. dev.	0.1 ug/ml	std dev	control	std dev
0	7.20E+04	3.45E+04	5.66E+04	2.63E+04	5.93E+04	1.70E+03
15.5	5.84E+04	4.52E+04	5.10E+06	1.68E+06	9.03E+06	4.19E+05
17	5.32E+04	2.83E+04	3.80E+06	2.21E+06	1.53E+07	9.63E+05
20	1.37E+04	1.37E+04	6.70E+06	3.81E+06	9.63E+07	7.41E+06
22	0	0	2.54E+07	5.87E+06	3.77E+07	4.50E+06
24	0	0	9.20E+06	4.40E+06	3.77E+07	4.50E+06

average	std. dev.	count (0.1 ug/ml)	cfu/ml	average	std. dev.	count (0.1 ug/ml)
2.37E+04	3.09E+03	75	7.50E+04	7.83E+04	3.40E+03	20
		83	8.30E+04			20
		77	7.70E+04			14
		8	8.00E+04			
		8	8.00E+04			
		6	6.00E+04			
2.33E+03	4.71E+02	66	6.60E+06			36
		77	7.70E+06			30
		56	5.60E+06			41
2.23E+04	3.30E+03	56	5.60E+06	5.87E+06	6.02E+05	151
		53	5.30E+06			171
		67	6.70E+06			155
						17
						13
						14
4.33E+03	6.13E+03	TNTC				119
		TNTC				53
		TNTC				29
		215	2.15E+07	2.32E+07	3.62E+06	253
		189	1.89E+07			256
		247	2.47E+07			235
		19	1.90E+07			21
		27	2.70E+07			28
		28	2.80E+07			42
		Bad plate				60
		Bad plate				110
		Bad plate				17
						5
			--		4.1E	7

[illegible]

APPENDIX F

Flow Cell Experiments: Image Analysis

IMAGE ANALYSIS

Images of the polymers were taken using a CCD camera. They were then analyzed using Image Pro Plus. The analysis of each image was automatically saved as an Excel file, generating 48 Excel sheets per experiment. Since a hard copy of this information would be too bulking to include in the Appendices a disk with all of the data will be kept with a copy of this thesis at the Chemical Engineering Department and at the Center for Biofilm Engineering.

The spreadsheet are labeled with the time at which the image was taken, the field of view (eg. A, b, c, etc.), parameters measured (eg. Area, ave. dia., width, length). For every field of view taken, two spreadsheets were generated. One consisted of the parameters measured, and the other gave the dimensions of the field of view (length x width).

At the beginning of each experiment an image of a micrometer was taken in order to calibrate all of the measurements for the experiment. Cell numbers were calculated by summing the area values of one field of view and dividing by 0.75 for a high end value or 5.0 for a low end value. These values were than averaged to get the average values and standard deviations. The above values were obtained from **Bergey's Manual of Determinative Microbiology** which gave the width of *P. aeruginosa* at 0.5-1.0 μ m and the length as 1.5-5.0 μ m. This method for calculating cell numbers was found to be accurate, as actual cell numbers could be counted for the early time points. They fell within the range calculated by the above method.

The files are listed as:

Control

Triglyme

Ciprotriglyme

BMA

CiproBMA

The graphs found in the thesis text were made from the above files and then converted into data which was easier to handle. The files from which the graphs were made are:

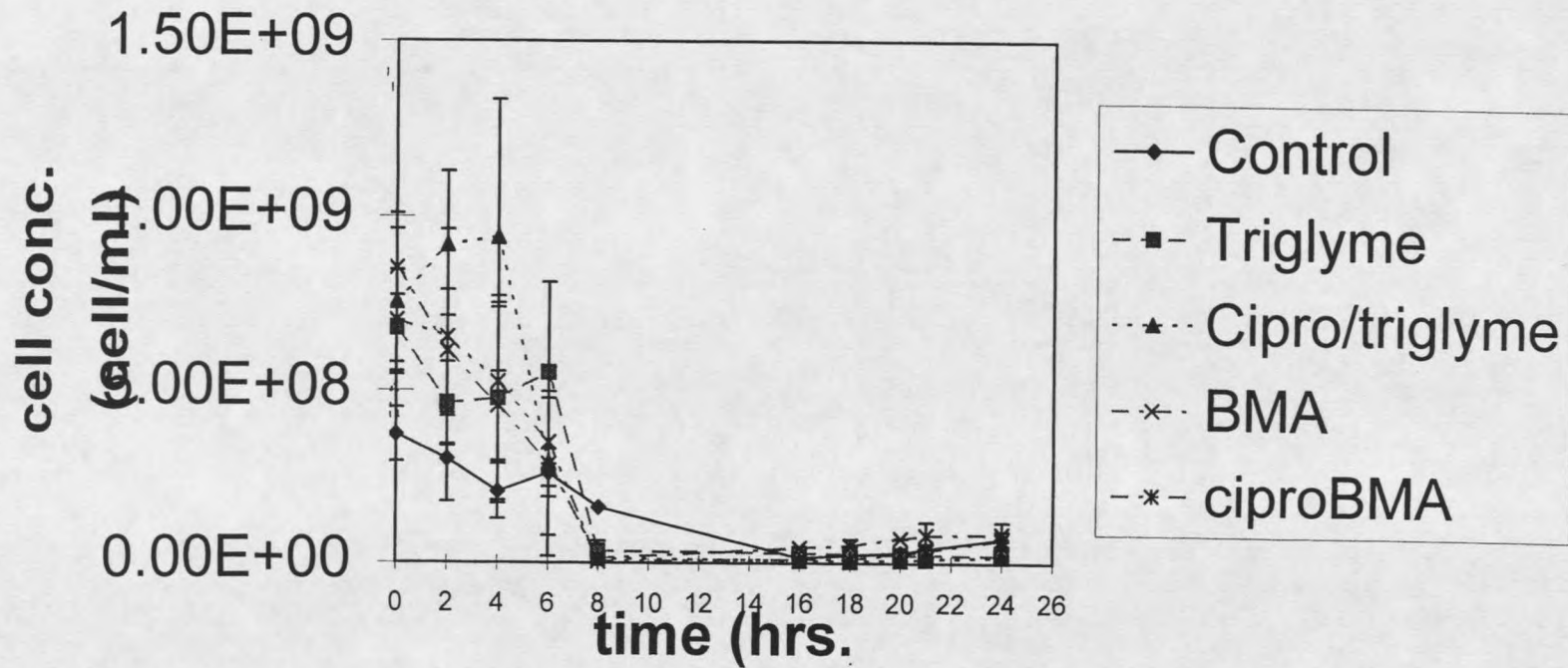
Endpoint

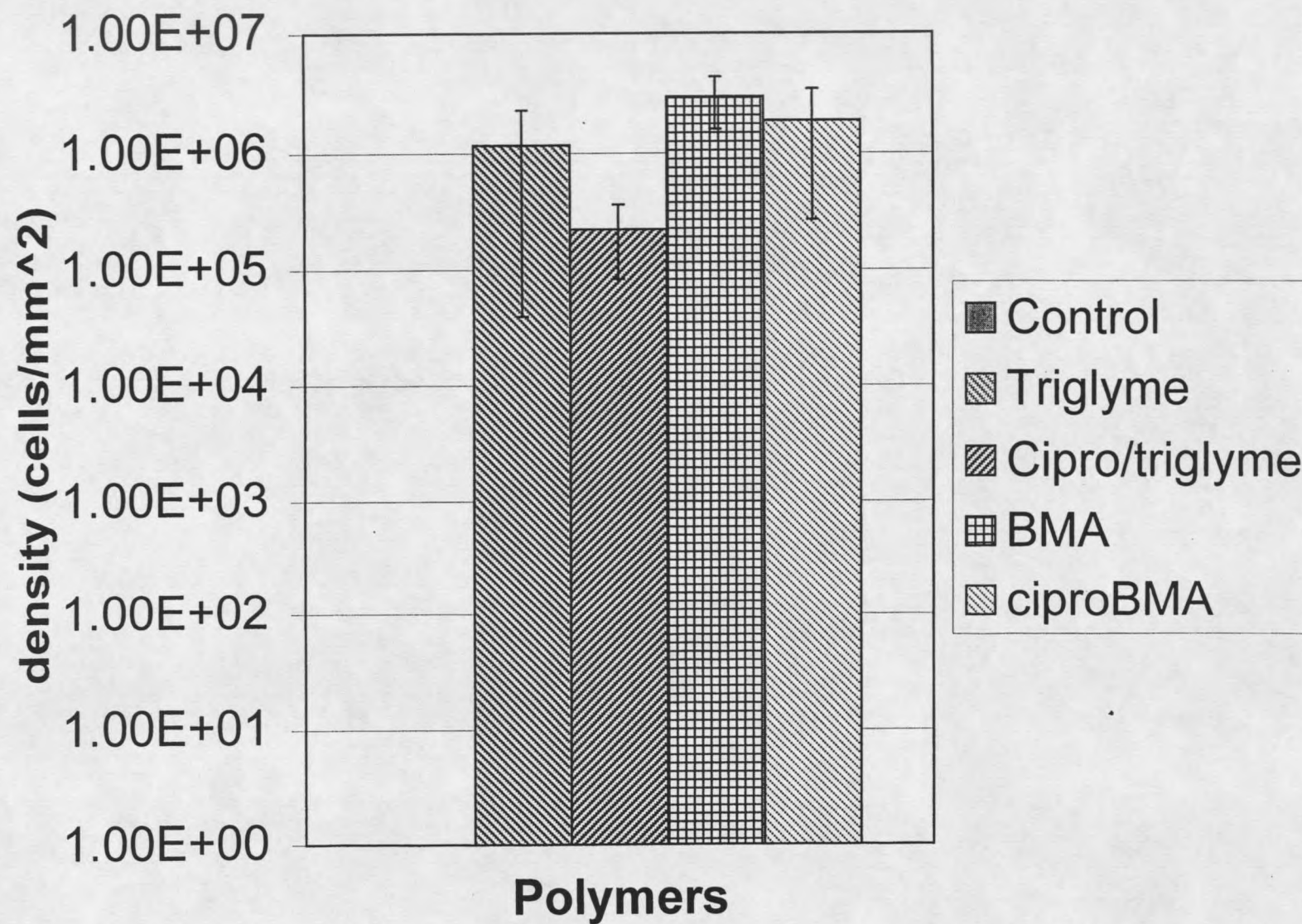
Curves

APPENDIX F

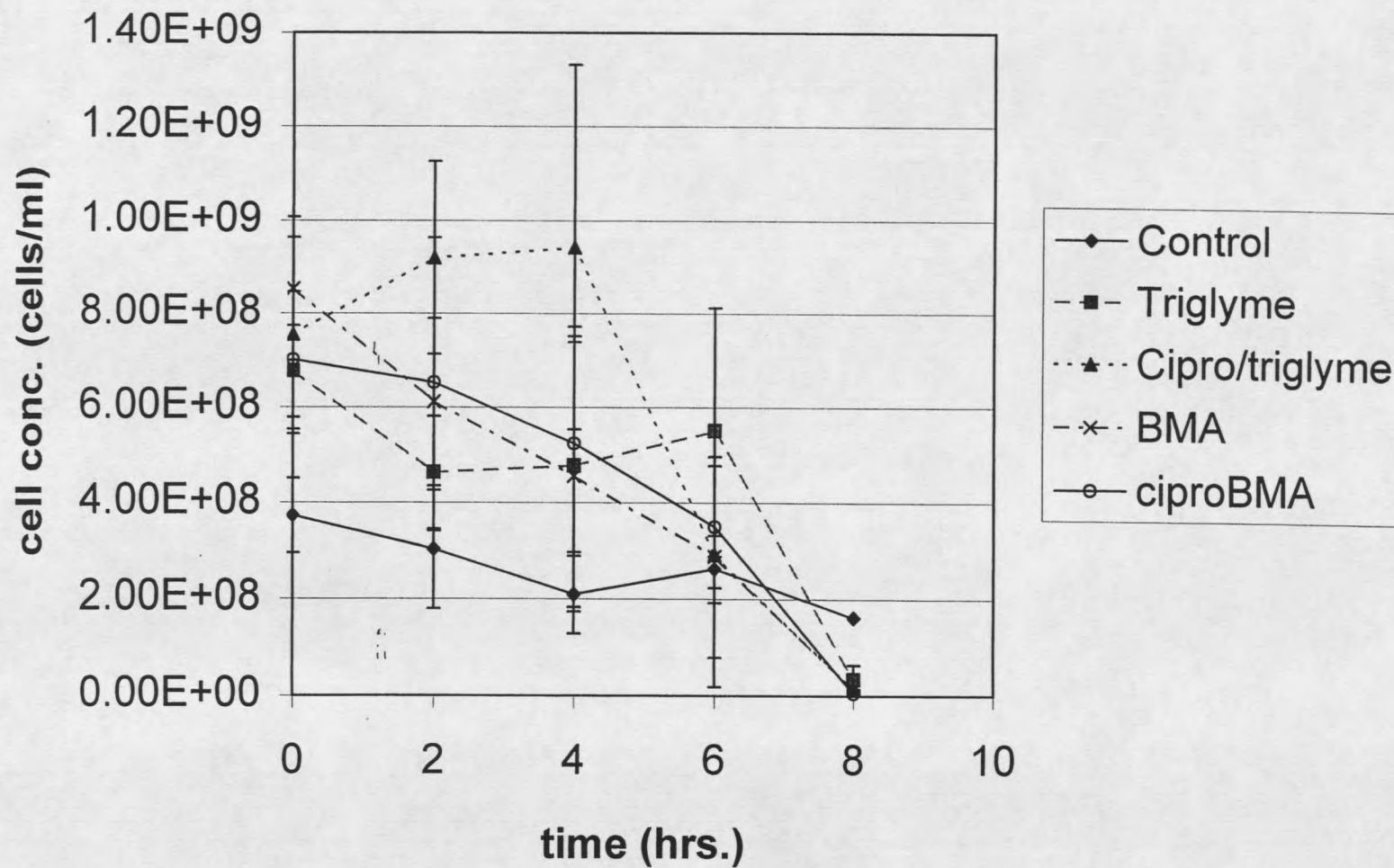
Flow Cell Experiments: Total Counts/AO

Effluent counts over a 24 hour time period - Comparison





Effluent data t=0-8, Total counts



[illegible]

		area (mm ²)					
		725.78	7.14E+02	4.41E+01			
		655.03					
		761.02					
Polymer							
count	conc.						
33	1.50E+08	2.01E+08	6.60E+07				
50	2.27E+08						
41	1.86E+08						
47	2.13E+08						
455	1.03E+08						
430	9.76E+07						
450	1.02E+08						
62	2.81E+08						
54	2.45E+08						
59	2.68E+08						
48	2.18E+08						
65	2.95E+08						
51	2.32E+08						
density							
8.45E+06	2.77E+06						

time (out)	cell count	cells/ml	average	std. Dev.	time (out)	average	std. Dev.
0	65	2.95E+08	3.73E+08	7.80E+07	0	3.73E+08	7.80E+07
	74	3.36E+08			2	3.03E+08	1.23E+08
	111	5.04E+08			4	2.09E+08	8.00E+07
	77	3.50E+08			6	2.62E+08	6.98E+07
	109	4.95E+08			8	1.62E+08	0
	88	3.99E+08			16	1.59E+07	1.16E+06
	53	2.41E+08			18	2.32E+07	0
	89	4.04E+08			20	3.04E+07	9.01E+06
	73	3.31E+08			21	4.07E+07	0
	82	3.72E+08			24	7.14E+07	2.13E+07
2	101	4.58E+08	3.03E+08	1.23E+08			
	103	4.68E+08			Control		
	101	4.58E+08					
	30	1.36E+08			0	4.91E+08	1.34E+08
	35	1.59E+08			2	7.30E+08	1.52E+08
	44	2.00E+08			4	6.78E+08	1.71E+08
	60	2.72E+08			6	0	0
	68	3.09E+08			8	0	0
	58	2.63E+08			16	0	0
4	30	1.36E+08	2.09E+08	8.00E+07	18	0	0
	35	1.59E+08			20	0	0
	28	1.27E+08			21	0	0
	44	2.00E+08			24	0	0
	19	8.63E+07					
	44	2.00E+08					
	69	3.13E+08					
	72	3.27E+08			time	in-out	
	66	3.00E+08			0	0.00E+00	
	54	2.45E+08			2	0.00E+00	
6	39	1.77E+08	2.62E+08	6.98E+07	4	-2.62E+08	
	60	2.72E+08			6	2.26E+08	
	56	2.54E+08			8	1.62E+08	
	67	3.04E+08			16	1.59E+07	
	38	1.73E+08			18	2.32E+07	
	46	2.09E+08			20	3.04E+07	
	44	2.00E+08			21	4.07E+07	
	72	3.27E+08			24	7.14E+07	
	88	3.99E+08					
	67	3.04E+08					
16	62	1.41E+07	1.59E+07	1.16E+06			
	69	1.57E+07					
	66	1.50E+07					
	76	1.73E+07					
	69	1.57E+07					
	70	1.59E+07					
	73	1.66E+07					

	81	1.84E+07					
	68	1.54E+07					
	67	1.52E+07					
20	161	3.65E+07	3.04E+07	9.01E+06			
	158	3.59E+07					
	196	4.45E+07					
	98	2.22E+07					
	91	2.07E+07					
	100	2.27E+07					
24	206	4.68E+07	7.14E+07	2.13E+07			
	121	2.75E+07					
	219	4.97E+07					
	217	4.93E+07					
	360	8.17E+07					
	270	6.13E+07					
	420	9.53E+07					
	280	6.36E+07					
	430	9.76E+07					
	390	8.85E+07					
	320	7.26E+07					
	360	8.17E+07					
	430	9.76E+07					
	380	8.63E+07					

Triglyme time (in)	AO counts count	cells/ml	average	std. Dev.			
0	77	3.50E+08	5.17E+08	2.24E+08			
	83	3.77E+08					
	78	3.54E+08					
	67	3.04E+08					
	88	3.99E+08					
	115	5.22E+08					
	96	4.36E+08					
	88	3.99E+08					
	83	3.77E+08					
	195	8.85E+08					
	208	9.44E+08					
	188	8.53E+08					
2	99	4.49E+08	7.65E+08	2.51E+08			
	120	5.45E+08					
	102	4.63E+08					
	130	5.90E+08					
	138	6.26E+08					
	136	6.17E+08					
	133	6.04E+08					
	232	1.05E+09					
	265	1.20E+09					
	260	1.18E+09					
	197	8.94E+08					
	190	8.63E+08					
	190	8.63E+08					
4	238	1.08E+09	7.08E+08	3.07E+08			
	225	1.02E+09					
	215	9.76E+08					
	100	4.54E+08					
	64	2.91E+08					
	99	4.49E+08					
	124	5.63E+08					
	98	4.45E+08					
	194	8.81E+08					
	188	8.53E+08					
	151	6.85E+08					
	176	7.99E+08					
6	0	0	0	0			
	0	0					
	0	0					
	0	0					
	0	0					
	0	0					
	0	0					

[illegible]

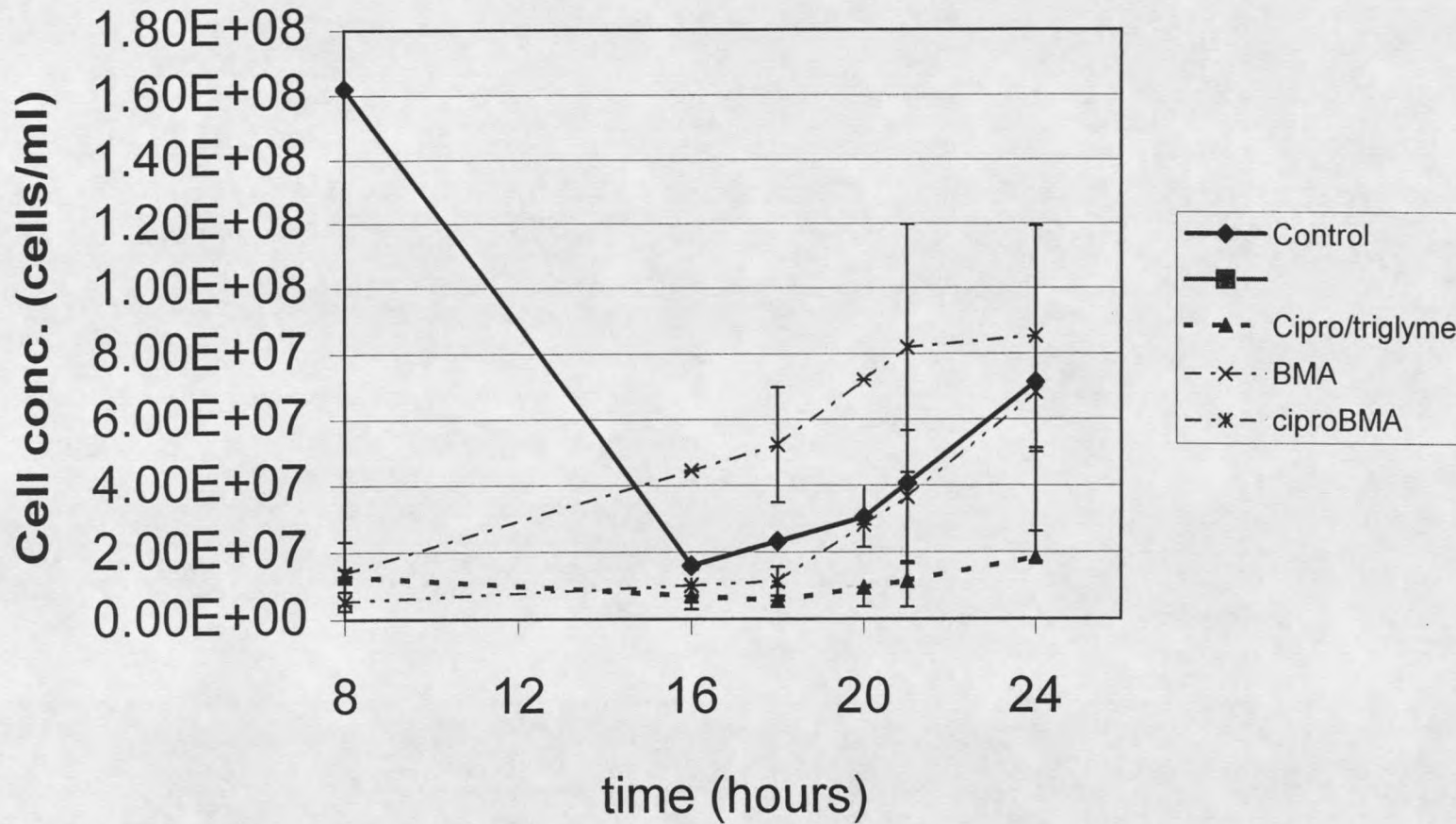
time (out)	average	std. Dev.		time	in-out		
0	6.76E+08	9.70E+07		0	5.00E+08		
2	4.63E+08	1.18E+08		2	3.02E+08		
4	4.77E+08	2.94E+08		4	2.31E+08		
6	5.50E+08	2.62E+08		6	5.50E+08		
8	3.49E+07	3.02E+07		8	3.49E+07		
16	2.84E+07	0		16	2.84E+07		
18	2.68E+07	2.38E+07		18	2.68E+07		
20	2.41E+07	0		20	2.41E+07		
21	2.28E+07	1.16E+07		21	2.28E+07		
24	2.23E+07	1.96E+07		24	2.23E+07		
triglyme							
time (in)							
0	5.17E+08	2.24E+08					
2	7.65E+08	2.51E+08					
4	7.08E+08	3.07E+08					
6	0	0					
8	0	0					
16	0	0					
18	0	0					
20	0	0					
21	0	0					
24	0	0					

time (out)	count	cells/ml	average	std. Dev.
0	130	5.90E+08	6.78E+08	9.70E+07
	136	6.17E+08		
	157	7.13E+08		
	150	6.81E+08		
	123	5.58E+08		
	148	6.72E+08		
	128	5.81E+08		
	140	6.36E+08		
	172	7.81E+08		
	152	6.90E+08		
	205	9.31E+08		
	152	6.90E+08		
2	150	6.81E+08	4.63E+08	1.18E+08
	67	3.04E+08		
	101	4.58E+08		
	100	4.54E+08		
	63	2.86E+08		
	96	4.36E+08		
	99	4.49E+08		
	100	4.54E+08		
	110	4.99E+08		
	71	3.22E+08		
	130	5.90E+08		
	136	6.17E+08		
	157	7.13E+08		
	150	6.81E+08		
4	28	1.27E+08	4.77E+08	2.94E+08
	18	8.17E+07		
	38	1.73E+08		
	23	1.04E+08		
	26	1.18E+08		
	127	5.77E+08		
	134	6.08E+08		
	115	5.22E+08		
	128	5.81E+08		
	150	6.81E+08		
	120	5.45E+08		
	205	9.31E+08		
	145	6.58E+08		
	215	9.76E+08		
6	47	2.13E+08	5.50E+08	2.62E+08
	49	2.22E+08		
	49	2.22E+08		
	59	2.68E+08		
	58	2.63E+08		
	164	7.44E+08		

	219	9.94E+08		
	180	8.17E+08		
	175	7.94E+08		
	191	8.67E+08		
	127	5.77E+08		
	134	6.08E+08		
	115	5.22E+08		
	128	5.81E+08		
8	16	7.26E+07	3.49E+07	3.02E+07
	10	4.54E+07		
	18	8.17E+07		
	20	9.08E+07		
	16	7.26E+07		
	2	9.08E+06		
	4	1.82E+07		
	4	1.82E+07		
	5	2.27E+07		
	4	1.82E+07		
18	9	2.04E+07	2.68E+07	2.38E+07
	1	2.27E+06		
	7	1.59E+07		
	0	0.00E+00		
	0	0.00E+00		
	99	4.49E+07		
	142	6.45E+07		
	128	5.81E+07		
	108	4.90E+07		
	165	7.49E+07		
	27	1.23E+07		
	30	1.36E+07		
	33	1.50E+07		
	37	1.68E+07		
	30	1.36E+07		
21	8	1.82E+07	2.28E+07	1.16E+07
	20	4.54E+07		
	10	2.27E+07		
	12	2.72E+07		
	19	4.31E+07		
	27	1.23E+07		
	30	1.36E+07		
	33	1.50E+07		
	37	1.68E+07		
	30	1.36E+07		
24	25	1.13E+07	2.23E+07	1.96E+07
	11	4.99E+06		
	22	9.99E+06		
	23	1.04E+07		
	16	7.26E+06		

[illegible]

Acridine Orange effluent counts t= 8-24 hours



[illegible]

[illegible]

time (out)	average	std. Dev.					
0	7.54E+08	2.10E+08					
2	9.19E+08	2.07E+08					
4	9.42E+08	3.88E+08					
6	2.92E+08	2.73E+08					
8	1.28E+07	2.16E+06					
16	7.05E+06	0					
18	5.61E+06	4.06E+06					
20	9.47E+06	0					
21	1.14E+07	5.73E+06					
24	1.84E+07	7.74E+06					
Cipro/triglyme							
time (in)							
0	9.32E+08	3.29E+08					
2	9.85E+08	2.02E+08					
4	9.54E+08	4.81E+08					
6	0	0					
8	0	0					
16	0	0					
18	0	0					
20	0	0					
21	0	0					
24	0	0					

time (out)	count	cells/ml	average	std. Dev.
0	120	5.45E+08	7.54E+08	2.10E+08
	130	5.90E+08		
	153	6.95E+08		
	156	7.08E+08		
	52	4.72E+08		
	51	4.63E+08		
	89	8.08E+08		
	118	1.07E+09		
	81	7.35E+08		
	111	1.01E+09		
	96	8.72E+08		
	119	1.08E+09		
2	92	8.35E+08	9.19E+08	2.07E+08
	78	7.08E+08		
	69	6.26E+08		
	83	7.54E+08		
	90	8.17E+08		
	103	9.35E+08		
	135	1.23E+09		
	143	1.30E+09		
	134	1.22E+09		
	117	1.06E+09		
	191	8.67E+08		
	167	7.58E+08		
	186	8.44E+08		
4	186	8.44E+08	9.42E+08	3.88E+08
	241	1.09E+09		
	289	1.31E+09		
	243	1.10E+09		
	159	1.44E+09		
	177	1.61E+09		
	31	2.81E+08		
	20	1.82E+08		
	102	9.26E+08		
	103	9.35E+08		
	80	7.26E+08		
	99	8.99E+08		
	99	8.99E+08		
6	92	8.35E+08	2.92E+08	2.73E+08
	65	5.90E+08		
	82	7.44E+08		
	65	5.90E+08		
	54	4.90E+08		
	21	1.91E+08		
	26	2.36E+08		
	17	1.54E+08		

	25	2.27E+08		
	20	1.82E+08		
	133	3.02E+07		
	113	2.56E+07		
	93	2.11E+07		
	114	2.59E+07		
	134	3.04E+07		
8	68	1.54E+07	1.28E+07	2.16E+06
	52	1.18E+07		
	44	9.99E+06		
	75	1.70E+07		
	48	1.09E+07		
	57	1.29E+07		
	66	1.50E+07		
	50	1.13E+07		
	52	1.18E+07		
	51	1.16E+07		
18	44	9.99E+06	5.61E+06	4.06E+06
	57	1.29E+07		
	34	7.72E+06		
	37	8.40E+06		
	35	7.94E+06		
	8	1.82E+06		
	6	1.36E+06		
	5	1.13E+06		
	8	1.82E+06		
	13	2.95E+06		
21	71	1.61E+07	1.14E+07	5.73E+06
	94	2.13E+07		
	79	1.79E+07		
	77	1.75E+07		
	29	6.58E+06		
	31	7.04E+06		
	28	6.36E+06		
	25	5.67E+06		
	30	6.81E+06		
	61	1.38E+07		
	61	1.38E+07		
	52	1.18E+07		
	64	1.45E+07		
	3	6.81E+05		
24	35	7.94E+06	1.84E+07	7.74E+06
	45	1.02E+07		
	56	1.27E+07		
	38	8.63E+06		
	53	1.20E+07		
	182	2.07E+07		
	205	2.33E+07		

4.1

[illegible]

[illegible]

time(out)	count	conc. (cells/ml)	average	std. dev.
0	83	7.54E+08	8.53E+08	1.55E+08
	78	7.08E+08		
	86	7.81E+08		
	93	8.44E+08		
	81	7.35E+08		
	82	7.44E+08		
	77	6.99E+08		
	92	8.35E+08		
	121	1.10E+09		
	116	1.05E+09		
	125	1.13E+09		
2	92	8.35E+08	6.12E+08	1.77E+08
	76	6.90E+08		
	109	9.90E+08		
	92	8.35E+08		
	60	5.45E+08		
	55	4.99E+08		
	63	5.72E+08		
	72	6.54E+08		
	47	4.27E+08		
	43	3.90E+08		
	44	3.99E+08		
	67	6.08E+08		
	56	5.08E+08		
4	47	4.27E+08	4.56E+08	2.82E+08
	52	4.72E+08		
	59	5.36E+08		
	60	5.45E+08		
	36	3.27E+08		
	97	8.81E+08		
	30	2.72E+08		
	50	4.54E+08		
	115	1.04E+09		
	104	9.44E+08		
	19	1.73E+08		
	14	1.27E+08		
	28	2.54E+08		
	22	2.00E+08		
	21	1.91E+08		
6	16	1.45E+08	2.88E+08	2.01E+08
	15	1.36E+08		
	13	1.18E+08		
	21	1.91E+08		
	16	1.45E+08		
	55	4.99E+08		
	40	3.63E+08		
	45	4.09E+08		

	85	7.72E+08		
	73	6.63E+08		
	20	1.82E+08		
	20	1.82E+08		
	20	1.82E+08		
	20	1.82E+08		
	17	1.54E+08		
8	6	1.36E+07	1.37E+07	9.34E+06
	0	0.00E+00		
	5	1.13E+07		
	4	9.08E+06		
	1	2.27E+06		
	27	2.45E+07		
	28	2.54E+07		
	26	2.36E+07		
18	77	6.99E+07	5.23E+07	1.75E+07
	73	6.63E+07		
	83	7.54E+07		
	73	6.63E+07		
	72	6.54E+07		
	68	6.17E+07		
	17	3.86E+07		
	19	4.31E+07		
	15	3.40E+07		
	12	2.72E+07		
	12	2.72E+07		
21	310	7.04E+07	8.18E+07	3.79E+07
	300	6.81E+07		
	140	3.18E+07		
	230	5.22E+07		
	210	4.77E+07		
	285	1.29E+08		
	290	1.32E+08		
	340	1.54E+08	--	
	250	1.13E+08		
	123	5.58E+07		
	136	6.17E+07		
	144	6.54E+07		
24	107	4.86E+07	8.53E+07	3.40E+07
	108	4.90E+07		
	112	5.08E+07		
	270	1.23E+08		
	250	1.13E+08		
	320	1.45E+08		
	260	1.18E+08		
	148	6.72E+07		
	153	6.95E+07		
	150	6.81E+07		

[illegible]

[illegible]

time (in)	ave.	std. dev.					
0	6.73E+08	1.90E+08					
2	8.97E+08	6.10E+08					
4	7.26E+08	1.28E+08					
6	0	0					
time (out)							
0	7.00E+08	1.46E+08					
2	6.52E+08	3.11E+08					
4	5.24E+08	2.27E+08					
6	3.50E+08	1.27E+08					
8	5.45E+06	2.86E+06					
16	1.01E+07	0					
18	1.13E+07	4.30E+06					
20	2.81E+07	0					
21	3.65E+07	2.01E+07					
24	6.82E+07	5.10E+07					

	time (out)	count	conc.	average	std. dev.
	0	71	6.45E+08	7E+08	1.46E+08
		71	6.45E+08		
		76	6.9E+08		
		99	8.99E+08		
		103	9.35E+08		
		84	7.63E+08		
		91	8.26E+08		
		65	5.9E+08		
		60	5.45E+08		
		51	4.63E+08		
	2	30	2.72E+08	6.52E+08	3.11E+08
		42	3.81E+08		
		24	2.18E+08		
		31	2.81E+08		
		23	2.09E+08		
		130	1.18E+09		
		66	5.99E+08		
		66	5.99E+08		
		90	8.17E+08		
		97	8.81E+08		
		67	6.08E+08		
		88	7.99E+08		
		113	1.03E+09		
		104	9.44E+08		
		107	9.71E+08		
	4	83	7.54E+08	5.24E+08	2.27E+08
		72	6.54E+08		
		84	7.63E+08		
		90	8.17E+08		
		66	5.99E+08		
		57	5.18E+08		
		89	8.08E+08		
		67	6.08E+08		
		30	2.72E+08		
		20	1.82E+08		
		34	3.09E+08		
		28	2.54E+08		
		30	2.72E+08		
	6	39	3.54E+08	3.5E+08	1.23E+08
		46	4.18E+08		
		54	4.9E+08		
		59	5.36E+08		
		52	4.72E+08		
		18	1.63E+08		

		30	2.72E+08		
		25	2.27E+08		
		35	3.18E+08		
		48	4.36E+08		
		52	4.72E+08		
		40	3.63E+08		
		28	2.54E+08		
		14	1.27E+08		
	8	17	3.86E+06	5.45E+06	2.86E+06
		11	2.50E+06		
		12	2.72E+06		
		23	1.04E+07		
		9	4.09E+06		
		16	7.26E+06		
		20	9.08E+06		
		8	3.63E+06		
	18	35	1.59E+07	1.13E+07	4.30E+06
		37	1.68E+07		
		36	1.63E+07		
		34	7.72E+06		
		27	6.13E+06		
		39	8.85E+06		
		54	1.23E+07		
		28	6.36E+06		
	21	300	6.81E+07	3.65E+07	2.01E+07
		275	6.24E+07		
		300	6.81E+07		
		255	5.79E+07		
		275	6.24E+07		
		85	1.93E+07		
		67	1.52E+07		
		79	1.79E+07		
		70	1.59E+07		
		93	2.11E+07		
		70	3.18E+07		
		61	2.77E+07		
		50	2.27E+07		
		48	2.18E+07		
		78	3.54E+07		
	24	42	1.91E+07	6.82E+07	5.10E+07
		57	2.59E+07		
		70	3.18E+07		
		67	3.04E+07		
		49	2.22E+07		
		270	1.23E+08		
		305	1.38E+08		
		385	1.75E+08		
		270	1.23E+08		

[illegible]

APPENDIX G

Flow Cell Experiments:
Viable Counts/Plate Counts

Plate counts were conducted by placing ten 10 microliter drops of properly diluted effluent and influent on plate count agar plates and incubating overnight at room temperature before counting. Several serial dilutions were made for each sample to ensure that countable plates, 3 to 300 colonies, were obtained. Only viable cells would develop into colonies on the plates, therefore the counts performed in this manner are classified as viable counts or plate counts.

Control	Plate count				
time (in)	count	conc. (cells/ml)	average	std. dev.	
0	159	1.59E+07	9.36E+06	6.28E+06	
	193	1.93E+07			
	188	1.88E+07			
	68	6.80E+06			
	64	6.40E+06			
	58	5.80E+06			
	32	3.20E+06			
	48	4.80E+06			
	32	3.20E+06			
2	298	2.98E+07	3.74E+07	8.52E+06	
	289	2.89E+07			
	329	3.29E+07			
	54	5.40E+07			
	39	3.90E+07			
	40	4.00E+07			
4	79	7.90E+06	3.30E+07	3.28E+07	
	111	1.11E+07			
	98	9.80E+06			
	92	9.20E+07			
	86	8.60E+07			
	23	2.30E+07			
	21	2.10E+07			
	13	1.30E+07			
6	0				
time (in)	ave. conc.	std. dev.	time (out)	ave. conc.	std. dev.
0	9.36E+06	6.28E+06	0	2.49E+06	2.22E+06
2	3.74E+07	8.52E+06	2	9.93E+06	2.93E+06
4	3.30E+07	3.28E+07	4	2.08E+07	9.22E+06
6	0	0	6	1.11E+07	0
			8	1.47E+07	0
			16	1.13E+05	2.35E+04
			18	2.81E+05	0
			20	4.49E+05	4.17E+05
			21	3.71E+05	0
			24	1.38E+05	1.09E+05
	Polymer	1.30E+06			

	area	average	std. dev.		
	725.78	713.9433333	44.07229		
	655.03				
	761.02				
	density				
	5.37E+04	5.48E+04	3474.333		
	5.95E+04				
	5.12E+04				

time(out)	count	conc. (cells/ml)	average	std. dev.
0	49	4.90E+06	2.49E+06	2.22E+06
	50	5.00E+06		
	67	6.70E+06		
	7	7.00E+05		
	6	6.00E+05		
	9	9.00E+05		
	17	1.70E+06		
	8	8.00E+05		
	11	1.10E+06		
	125	1.25E+07	9.93E+06	2.93E+06
	122	1.22E+07		
2	119	1.19E+07		
	9	9.00E+06		
	10	1.00E+07		
	4	4.00E+06		
	30	3.00E+07	2.08E+07	9.22E+06
	33	3.30E+07		
	31	3.10E+07		
	104	1.04E+07		
	94	9.40E+06		
	106	1.06E+07		
	21	2.10E+07		
4	21	2.10E+07		
	13	1.30E+07		
	6 no data			
	71	7.10E+04	1.13E+05	2.35E+04
	105	1.05E+05		
	120	1.20E+05		
	15	1.50E+05		
	11	1.10E+05		
	12	1.20E+05		
	24	2.40E+04	4.49E+05	4.17E+05
20	26	2.60E+04		
	53	5.30E+04		
	77	7.70E+05		
	89	8.90E+05		
	93	9.30E+05		
	17	1.70E+05	1.38E+05	1.09E+05
	26	2.60E+05		
	29	2.90E+05		

	26	2.60E+04		
	59	5.90E+04		
	20	2.00E+04		
Polymer	10	1.00E+06	1.30E+06	5.51E+05
	13	1.30E+06		
	13	1.30E+06		
	58	5.80E+05		
	56	5.60E+05		
	119	1.19E+06		
	171	1.71E+06		
	161	1.61E+06		
	244	2.44E+06		

triglyme	Plate counts			
time (in)	count	conc. (cells/ml)	average	std. dev.
0	167	1.67E+08	1.08E+08	7.00E+07
	184	1.84E+08		
	182	1.82E+08		
	31	3.10E+07		
	37	3.70E+07		
	47	4.70E+07		
2	86	8.60E+07	5.22E+07	3.34E+07
	78	7.80E+07		
	74	7.40E+07		
	120	1.20E+07		
	112	1.12E+07		
4	159	1.59E+08	5.62E+07	4.89E+07
	116	1.16E+08		
	145	1.45E+07		
	244	2.44E+07		
	224	2.24E+07		
	42	4.20E+07		
	32	3.20E+07		
	39	3.90E+07		
6	0			
	0			
	0			
	time (in)	ave. conc.	std. dev.	
	0	1.08E+08	7.00E+07	
	2	5.22E+07	3.34E+07	
	4	5.62E+07	4.89E+07	
	6	0	0	
	Polymer			density
	7.82E+06	4.41E+06		2.48E+05
				2.48E+05
	area	Average		2.83E+05
	945.58	907.2333333		
	945.88			
	830.24			
time (out)	average	std. dev.		

0	8.82E+07	7.14E+07		
2	3.36E+07	1.25E+07		
4	8.21E+07	1.39E+07		
6	2.14E+07	4.39E+07		
8	2.23E+06	1.25E+05		
16	3.26E+06	0		
18	3.52E+06	2.46E+06		
20	7.73E+06	0		
21	9.84E+06	1.05E+07		
24	2.99E+06	1.01E+06		
	Polymer	50	5.00E+06	7.82E+06
		61	6.10E+06	
		62	6.20E+06	
		108	1.08E+07	
		47	4.70E+06	
		15	1.50E+07	
		16	1.60E+07	
		13	1.30E+07	
		331	3.31E+06	
		281	2.81E+06	
		301	3.01E+06	
		97	9.70E+06	
		60	6.00E+06	

	time (out)	count	conc. (cells/ml)	average	std. dev.
	0	167	1.67E+08	8.82E+07	7.14E+07
		136	1.36E+08		
		173	1.73E+08		
		176	1.76E+07		
		186	1.86E+07		
		168	1.68E+07		
	2	51	5.10E+07	3.36E+07	1.25E+07
		47	4.70E+07		
		46	4.60E+07		
		40	4.00E+07		
		52	5.20E+07		
		34	3.40E+07		
		177	1.77E+07		
		188	1.88E+07		
		213	2.13E+07		
		24	2.40E+07		
		25	2.50E+07		
		26	2.60E+07		
	4	77	7.70E+07	8.21E+07	1.39E+07
		97	9.70E+07		
		104	1.04E+08		
		60	6.00E+07		
		67	6.70E+07		
		91	9.10E+07		
		78	7.80E+07		
		83	8.30E+07		
	6	48	4.80E+06	2.14E+07	4.39E+07
		34	3.40E+06		
		35	3.50E+06		
		30	3.00E+06		
ave.		28	2.80E+06		
2.60E+05		34	3.40E+06		
std.dev.		129	1.29E+08		
16266.57	8	22	2.20E+06	2.23E+06	1.25E+05
		24	2.40E+06		
		21	2.10E+06		
	18	153	1.53E+06	3.52E+06	2.46E+06
		225	2.25E+06		
		198	1.98E+06		

		22	2.20E+06		
		15	1.50E+06		
		16	1.60E+06		
		62	6.20E+06		
		60	6.00E+06		
		84	8.40E+06		
	21	179	1.79E+06	9.84E+06	1.05E+07
		275	2.75E+06		
		247	2.47E+06		
		22	2.20E+07		
		3	3.00E+06		
		27	2.70E+07		
4.41E+06	24	254	2.54E+06	2.99E+06	1.01E+06
		240	2.40E+06		
		264	2.64E+06		
		30	3.00E+06		
		37	3.70E+06		
		58	5.80E+06		
		20	2.00E+06		
		31	3.10E+06		
		16	1.60E+06		
		279	2.79E+06		
		338	3.38E+06		
		297	2.97E+06		

cipro/triglyme	Plate counts					
time (in)	count	conc. (cfu/ml)	average	std, dev,		time (out)
0	15	1.50E+08	6.54E+08	3.07E+08		0
	13	1.30E+08				
	43	4.30E+08				
	89	8.90E+08				
	87	8.70E+08				
	82	8.20E+08				
	84	8.40E+08				
	84	8.40E+08				
	92	9.20E+08				
2	96	9.60E+08	1.03E+09	4.8E+08		
	81	8.10E+08				
	116	1.16E+09				
	164	1.64E+09				
	169	1.69E+09				2
	184	1.84E+09				
	54	5.40E+08				
	97	9.70E+08				
	128	1.28E+09				
	3	3.00E+08				
	5	5.00E+08				
	7	7.00E+08				
4	55	5.50E+08	9.18E+08	3.63E+08		
	31	3.10E+08				
	90	9.00E+08				
	14	1.40E+09				
	10	1.00E+09				4
	4	4.00E+08				
	90	9.00E+08				
	99	9.90E+08				
	115	1.15E+09				
	100	1.00E+09				
	80	8.00E+08				
	162	1.62E+09				
6	0	0				
						6
time (Out)	average	std. dev.				
0	5.06E+06	5.53E+06				
2	1.21E+09	9.44E+08				
4	1.92E+08	1.66E+08				
6	1.21E+08	1.12E+08				
8	1.40E+06	1.16E+06				
16	9.00E+05	0				
18	7.74E+05	9.80E+05				
20	1.20E+06	0				
21	1.41E+06	1.10E+06				

[illegible]

count	conc. (cfu/ml)	average	std, dev,
23	2.30E+04	5.06E+06	5.53E+06
21	2.10E+04		
20	2.00E+04		
114	1.14E+07		
103	1.03E+07		
120	1.20E+07		
10	1.00E+07		
13	1.30E+07		
9	9.00E+06		
0	0.00E+00		
0	0.00E+00		
0	0.00E+00		
0	0.00E+00		
87	8.70E+08	1.21E+09	9.44E+08
92	9.20E+08		
190	1.90E+09		
102	1.02E+09		
75	7.50E+08		
96	9.60E+08		
72	7.20E+08		
91	9.10E+08		
167	1.67E+09		
6	6.00E+08		
2	2.00E+08		
40	4.00E+09		
57	5.70E+08	1.92E+08	1.66E+08
25	2.50E+08		
36	3.60E+08		
128	1.28E+08		
93	9.30E+07		
168	1.68E+08		
56	5.60E+07		
48	4.80E+07		
55	5.50E+07		
48	4.80E+07	1.21E+08	1.13E+08
51	5.10E+07		
90	9.00E+07		
149	1.49E+08		
180	1.80E+08		
162	1.62E+08		
26	2.60E+08		
12	1.20E+08		
39	3.90E+08		
70	7.00E+05		
39	3.90E+05		
65	6.50E+05		

3	3.00E+05	1.40E+06	1.16E+06
15	1.50E+06		
45	4.50E+06		
89	8.90E+05		
127	1.27E+06		
127	1.27E+06		
10	1.00E+06		
14	1.40E+06		
5	5.00E+05		
8	8.00E+05	7.74E+05	9.18E+05
19	1.90E+06		
29	2.90E+06		
199	1.99E+05		
225	2.25E+05		
213	2.13E+05		
23	2.30E+05		
25	2.50E+05		
25	2.50E+05		
36	3.60E+05	1.41E+06	1.10E+06
92	9.20E+05		
94	9.40E+05		
53	5.30E+05		
82	8.20E+05		
67	6.70E+05		
8	8.00E+05		
8	8.00E+05		
10	1.00E+06		
169	1.69E+06		
142	1.42E+06		
24	2.40E+06		
45	4.50E+06		
29	2.90E+06		
25	2.50E+04	1.64E+06	1.90E+06
29	2.90E+04		
52	5.20E+04		
6	6.00E+04		
8	8.00E+04		
1	1.00E+04		
139	1.39E+06		
10	1.00E+06		
38	3.80E+06		
62	6.20E+06		
32	3.20E+06		
21	2.10E+06		
34	3.40E+06		

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Polymer						
count	conc.	average	std. dev.			
27	2.70E+06	1.17E+07	13381334			
127	1.27E+07					
32	3.20E+07					
43	4.30E+07					
39	3.90E+06					
40	4.00E+06					
36	3.60E+06		density			
41	4.10E+06		4.04E+05	3.91E+05		
59	5.90E+06		3.85E+05			
54	5.40E+06		3.84E+05			
area	average	std. dev.				
870.61	900.566667	21.21723566				
914.06						
917.03						

time (out)	count	conc. (cfu/ml)	average	std. dev.
0	40	4.00E+07	4.99E+07	1.54E+07
	35	3.50E+07		
	50	5.00E+07		
	81	8.10E+07		
	70	7.00E+07		
	57	5.70E+07		
	408	4.08E+07		
	386	3.86E+07		
	367	3.67E+07		
2	37	3.70E+07	4.13E+07	1.79E+07
	25	2.50E+07		
	36	3.60E+07		
	65	6.50E+07		
	77	7.70E+07		
	34	3.40E+07		
	25	2.50E+07		
	31	3.10E+07		
4	46	4.60E+07	5.77E+07	5.26E+07
	33	3.30E+07		
	32	3.20E+07		
	158	1.58E+08		
	92	9.20E+07		
	130	1.30E+08		
	13	1.30E+07		
	9	9.00E+06		
	6	6.00E+06		
6	92	9.20E+06	1.29E+07	4.96E+06
	72	7.20E+06		
	88	8.80E+06		
	9	9.00E+06		
	7	7.00E+06		
	7	7.00E+06	--	
	169	1.69E+07		
	192	1.92E+07		
	191	1.91E+07		
	20	2.00E+07		
	14	1.40E+07		
	24	2.40E+07		
	101	1.01E+07		
	119	1.19E+07		
	149	1.49E+07		
	10	1.00E+07		
	12	1.20E+07		
	11	1.10E+07		
8	46	4.60E+05	1.49E+06	9.12E+05
	13	1.30E+05		

	13	1.30E+05		
	191	1.91E+06		
	217	2.17E+06		
	244	2.44E+06		
	18	1.80E+06		
	24	2.40E+06		
	20	2.00E+06		
18	46	4.60E+06	4.98E+06	4.14E+06
	54	5.40E+06		
	46	4.60E+06		
	3	3.00E+06		
	4	4.00E+06		
	18	1.80E+07		
	38	3.80E+06		
	55	5.50E+06		
	52	5.20E+06		
	22	2.20E+06		
	13	1.30E+06		
	22	2.20E+06		
21	26	2.60E+06	7.04E+06	3.94E+06
	31	3.10E+06		
	44	4.40E+06		
	3	3.00E+06		
	3	3.00E+06		
	3	3.00E+06		
	93	9.30E+06		
	99	9.90E+06		
	106	1.06E+07		
	16	1.60E+07		
	8	8.00E+06		
	7	7.00E+06		
	62	6.20E+06		
	71	7.10E+06		
	75	7.50E+06		
	8	8.00E+06		
	15	1.50E+07		
	3	3.00E+06		
24	90	9.00E+06	1.06E+07	2.25E+06
	89	8.90E+06		
	90	9.00E+06		
	13	1.30E+07		
	13	1.30E+07		
	11	1.10E+07		
	111	1.11E+07		
	97	9.70E+06		
	107	1.07E+07		
	14	1.40E+07		
	13	1.30E+07		

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time (out)	count	conc. (cfu/ml)	average	std. dev.		1.5
0	31	3.10E+07	4.27E+07	1.53E+07		
	28	2.80E+07				
	37	3.70E+07				
	49	4.90E+07				
	49	4.90E+07				
	49	4.90E+07				
	79	7.90E+07				
	33	3.30E+07				
2	29	2.90E+07	4.78E+07	2.03E+07		
	25	2.50E+07				
	17	1.70E+07				
	67	6.70E+07				
	81	8.10E+07				
	68	6.80E+07				
	45	4.50E+07				
	48	4.80E+07				
	50	5.00E+07				
4	52	5.20E+07	4.04E+07	1.98E+07		
	50	5.00E+07				
	56	5.60E+07				
	55	5.50E+07				
	72	7.20E+07				
	147	1.47E+07				
	143	1.43E+07				
	28	2.80E+07				
	22	2.20E+07				
6	24	2.40E+07	2.36E+07	1.25E+07		
	49	4.90E+07				
	138	1.38E+07				
	126	1.26E+07				
	150	1.50E+07				
	12	1.20E+07				
	16	1.60E+07				
	13	1.30E+07				
	42	4.20E+07				
	34	3.40E+07				
	28	2.80E+07				
8	11	1.10E+05	1.85E+05	1.04E+05		
	24	2.40E+05				
	32	3.20E+05				
	80	8.00E+04				
	117	1.17E+05				
	91	9.10E+04				
	34	3.40E+05				
18	56	5.60E+04	3.28E+06	5.57E+06		
	60	6.00E+04				

	57	5.70E+04			
	6	6.00E+04			
	4	4.00E+04			
	9	9.00E+04			
	82	8.20E+05			
	81	8.10E+05			
	85	8.50E+05			
	15	1.50E+06			
	11	1.10E+06			
	7	7.00E+05			
	152	1.52E+07			
	137	1.37E+07			
	142	1.42E+07			
21	183	1.83E+07	3.86E+06	5.28E+06	
	111	1.11E+07			
	126	1.26E+07			
	186	1.86E+06			
	159	1.59E+06			
	148	1.48E+06			
	10	1.00E+06			
	16	1.60E+06			
	15	1.50E+06			
	66	6.60E+05			
	84	8.40E+05			
	61	6.10E+05			
	22	2.20E+06			
	10	1.00E+06			
	15	1.50E+06			
24	22	2.20E+06	2.10E+06	5.80E+05	
	24	2.40E+06			
	131	1.31E+06			
	167	1.67E+06			
	141	1.41E+06			
	20	2.00E+06			
	28	2.80E+06			
	30	3.00E+06			

APPENDIX H
Mathematical Theory

MATHEMATICAL THEORY

Kwok (1997) used Basmadjian and Sefton to determine that the drug concentration immediately next to the polymer in a tube was:

$$C_s / (N_{kill} * r_o / D) = A * [(x/r_o) / Re * Sc]^{1/3} \quad \text{where } (x/r_o * Re * Sc) < 10^{-4} - 10^{-3}$$

C_s – surface concentration ($\mu\text{g}/\text{cm}^3$)

N_{kill} – minimum killing release rate ($\mu\text{g}/\text{cm}^2 \cdot \text{s}$)

R_o – effective hydraulic radius (cm)

D – diffusivity of ciprofloxacin in fluid (cm^2/s)

A – constant for geometry

x – axial distance from the entrance (cm)

Re – Reynold's number

Sc – Schmidt number

Laminar flow with constant velocity, v , was used estimate N_{kill} as $5.7 \times 10^{-3} \mu\text{g}/\text{cm}^2 \cdot \text{s}$ using the equation above.

The ciprofloxacin impregnated polymers were assumed to behave as if dissolution into a falling film was occurring, allowing Fick's second law of diffusion to be modified as follows.

Fick's second law of diffusion:

$$Mc_i / Mt = D_{ip} * M^2 c_i / Mx^2$$

does not adequately model the controlled-release of ciprofloxacin as it does not take into consideration the fluid flow over the polymer and results in a $t^{-1/2}$ release rate. A better

equation would be:

$$v_z(x) * Mc_i/Mz = D_{ip} * M^2c_i/Mx^2$$

where fluid flow is assumed to be occur in the z direction and diffusion is assumed to be in the x direction. The following boundary conditions should be sufficient for the situation encountered in the flow cell over the first 24 hours:

- 1) $c_A = 0$ at $z = 0$
- 2) $c_A = 0$ at $y = \infty$
- 3) $c_A = c_{A0}$ at $y = 0$

The solution, therefore, is:

$$C_A/C_{A0} = \text{erfc} [y/(4*D_z/v_{\max})]$$

APPENDIX I

Polymer Surface Area Measurements

POLYMER	Date of experiment	Surface area (mm ²)
Control	6/9/97	725.78
	6/28/97	655.03
	7/26/97	761.02
Triglyme	8/8/97	945.58
	8/10/97	945.88
	8/22/97	830.26
Ciprotriglyme	8/16/97	906.49
	8/24/97	973.97
	8/29/97	943.52
BMA	8/31/97	870.61
	9/7/97	914.06
	9/21/97	917.03
CiproBMA	9/5/97	881.81
	9/14/97	876.06
	9/19/97	852.82

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