Polyester Sutures with Antimicrobial Absorbable Coatings

By

Amanda D. Jones

A Thesis Submitted to the Faculty of Graduate Studies
in partial fulfillment of the requirements for the degree of

Master of Science

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THE UNIVERSITY OF MANITOBA

FACULTY OF GRADUATE STUDIES

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ABSTRACT

Infection at a surgical incision due to sutures is a prevalent problem. Such infections are premised on the fact that foreign materials, such as sutures, can help potentiate infection by harbouring bacteria. In fact the clinical use of braided sutures is avoided due to the belief that bacteria can collect within the interstices of the braided structure. This thesis studied whether covering a braided suture with an absorbable coating containing an antibiotic would result in bacteriostatic activity to treat suture infections prophylactically. Tests were also conducted to determine how the coating would affect the sutures with regards to handling and mechanical characteristics important for good knot strength and a secure suture wound.

Each sample of polyester suture braid was coated with one of two different antibiotics, clindamycin and moxifloxacin, at two loading dosages. The sutures were then tested *in vitro* for rate of antibiotic release and inhibitory activity against *Staphylococcus aureus*. After exposure to a buffer solution for eight time periods from 0 to 7 days, the rate of antibiotic release was measured using UV-spectroscopy and the inhibitory activity of each treated suture was measured using zone of inhibition tests. In addition, handling and mechanical properties of the uncoated braided suture, coated braided suture (no antibiotic) and a monofilament suture of comparable diameter were measured. The handling properties tested were flexural rigidity and tissue drag and the mechanical properties tested were tensile strength and knot pull strength.

The results from these tests demonstrated that incorporation of an antibiotic within an absorbable coating provided effective bacteriostatic properties within an absorbable coating for two days without greatly compromising the initial handling and mechanical characteristics of the suture in comparison to the uncoated braided and monofilament sutures tested.

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CHAPTER 1

INTRODUCTION

It has been estimated that between two to four million hospital patients in the United States (US) suffer from infections each year (Speller & Humphreys, 1998). Research shows that device and suture infections account for 45% of all nosocomial infections acquired during hospital stays (Hayes, Soule, & LaRocco, 1987). Further research shows that the majority of nosocomial surgical wound infections (60-80%) occur at the incision (Hayes, Soule, & LaRocco, 1987). Moreover, of all infections, the most frequently isolated organism from infected surgical wounds is *Staphylococcus aureus* (Hayes, Soule, & LaRocco, 1987).

The human cost of suture-related infections is enormous. Suture associated infections affect well over one million patients each year. Almost equally as important as the human cost of infection is the monetary costs associated with infections. In the US it is estimated that hospital acquired infections result in patients having to remain in the hospital an average of an extra four days. This translates into an approximate $2,100 additional hospital fee per patient (Speller & Humphreys, 1998). Thus, in the US alone, 2.5 billion dollars in additional health care costs are incurred each year as a result of this type of suture material complications.

The presence of a suture in a wound has been shown to significantly increase the likelihood of infection to surrounding tissue. This serves to exacerbate other surgical complications such as wound disruption and a chronic inflammatory response in patients (Chu & Williams, 1984). Limiting the infection rate of a suture is particularly important

in vascular surgery because infections in the cardiovascular system often result in loss of life or limb (Scher, Bernstein & Jones, 1985).

A suture is defined as a thread that either adjoins adjacent cut tissues or compresses blood vessels to stop bleeding (Mezzarese et al., 1997; Batra et al., 1992). Sutures can be made of natural or synthetic materials (both absorbable and nonabsorbable) in monofilament or braided form. Coatings are sometimes applied to suture materials to enhance certain properties, such as surface smoothness, reduce friction and improved biocompatibility.

Braided suture materials provide surgeons with enhanced handling properties compared to monofilament structures. For example, braided sutures offer superior flexibility compared to monofilament sutures. Braided sutures also offer ease of manipulation. This is vital for the surgeon to be able to tie a secure knot. However, concern over the introduction of bacteria within the interstices of a braided suture material often precludes its use in situations of high risk such as in cardiovascular procedures (Haluck et al., 1990). Other uses for sutures with antimicrobial properties would include inguinal hernia repair, bowel surgery, other surgeries involving deep tissue healing, or for patients who are immunocompromised. Further concerns over the use of braided sutures include, the trauma associated with the roughness of the braids, which may adversely effect healing of the wound.

But this concern is not isolated to braided materials alone. The mere presence of a foreign material in a wounded area incites the host's natural defense mechanisms. The most common form of host defense caused by the foreign material is an inflammatory response, which markedly increases its susceptibility to infection. This fact makes all

forms of surgical devices vulnerable to infection. The above fact was cogently demonstrated by Elek and Conon's adherence study to suture materials in 1957. In this study Elek and Conon showed that 7.5×10^6 viable staphylococci were required to induce infection intradermally while only 300 of the same bacteria were required to elicit a similar infection in the presence of a silk suture (Chu & Williams, 1984).

Implanted polymer materials have also been shown to have a propensity to attract macrophages. Normally essential to the host's natural inflammatory response, the prominent presence of these cells in the vicinity of a biomaterial may present the potential for destruction and damage of surrounding tissue. While some inflammatory response is expected and is a natural part of the process of integrating an implant into a body, there is a fine balance between the production of healthy growth promoting factors and destructive proteolytic factors. Researchers believe that this balance is controlled by macrophages (Laurencin & Elgendy, 1994). Macrophages become activated and release a large number of secretory products when they adhere to polymer materials. Such secretions include various enzymes and growth factors that can greatly affect tissue-polymer reactions.

Naturally absorbable suture materials, such as catgut, are often used as surgical sutures. However, synthetic absorbable sutures are more advantageous because the absorption rate is predictable and reproducible. Synthetic absorbable sutures break down by hydrolysis, whereas, gut material degrades via enzymes and phagocytosis, which results in a less consistent and predictable degradation process than its synthetic counterpart (Stone, 1988). As a result, a more biocompatible tissue reaction occurs in absorbable braided sutures than with catgut sutures (Rodeheaver, Powell, Thacker, &

Edlich, 1987). This makes synthetic absorbable materials more suitable as surgical sutures because high predictability is important for wound healing. Furthermore, the more consistent absorption rate of synthetic absorbable polymer materials permits the possibility of controlled drug delivery to a localized area.

Furthermore, since catgut sutures have lower tenacity compared to absorbable braided sutures, rapid strength loss will be especially noticeable if the surrounding wound or tissue becomes infected (Stone, 1988). Thus, a synthetic absorbable braided suture is more likely to retain its strength than a naturally absorbable material.

Problem Statement

Despite aseptic operating procedures and new systemic drug therapies, the wound infection rate remains quite high at an average of 8% (Chu, von Fraunhofer & Greisler, 1996). Currently, in high-risk surgical infections such as cardiovascular procedures, the use of braided sutures is prohibited because of its perceived high potential for infection compared to monofilament sutures and its tendency to cause high surface friction due to the physical structure of the braid. However, this perception precludes surgeons from benefiting from the noted advantages that braided sutures possess over monofilament sutures, such as good handling properties essential for sound knot security. Thus, the demand for alternative suture materials for use in high-risk surgeries remains.

Objectives

To resolve this problem prophylactically, this thesis will examine the efficacy of coating a braided suture with an absorbable coating made of an epsilon caprolactone-co-

glycolide copolymer in which an active antibiotic has been incorporated. This recently developed biodegradable suture coating technology by Poly-Med Inc. (Pendleton, SC, USA) allows for the potential development of a controlled drug delivery system. As the coating degrades at a predictable rate, it may be able to provide enhanced drug delivery to the wound site. The two antibiotics selected for this study (clindamycin and moxifloxacin) have been shown to be highly effective against *Staphylococcus aureus*, the most widely pathogenic organism found in suture infections. A polyester braided suture will be used as the base material for the degradable antimicrobial coating.

This thesis further posits that the study and use of these materials will provide an effective alternative to the current materials that are available. It will focus particularly on surgical situations where the high possibility of infection is a deterrent, such as in cardiovascular surgical procedures, inguinal hernia repair, bowel surgery, other surgeries involving deep tissue healing, or cases with immunocompromised patients.

There are five main objectives of this study. These are:

1) To compare the level of antimicrobial activity (measured by zones of inhibition against *Staphylococcus aureus*) during the period of coating absorption *in vitro* for two types of antibiotic-coated braided suture materials (clindamycin and moxifloxacin) and two loading dosages of antibiotic (10x MIC and 100x MIC), as well as coated braided suture material (no antibiotic) and uncoated braided controls.

2) To determine the level of adherence of *Staphylococcus aureus* to the antibiotic-coated suture materials, coated braided suture material, uncoated braided suture, and monofilament suture of equal diameter.

3) To compare the level of antibiotic release (measured by UV-spectroscopy) during the period of coating absorption *in vitro* for two types of antibiotic-coated braided suture materials (clindamycin and moxifloxacin) and two levels of loading antibiotic (10x MIC and 100x MIC), as well as the coated braided suture material (no antibiotic) and uncoated braided material controls.

4) To compare the differences in initial handling properties caused by application of coating by way of flexural rigidity and tissue drag of the coated braided, uncoated braided, and monofilament sutures.

5) To compare the differences between the initial mechanical properties by way of tensile strength and knot pull strength of the following suture materials: coated braided suture, uncoated braided suture, and monofilament suture.

Null Hypotheses

The thesis was divided into four major experiments, Experiment 1: Antimicrobial Activity (H_{01}), Experiment 2: Microbial Adhesion (H_{02}), Experiment 3: Coating Absorption (H_{03}), and Experiment 4: Handling and Mechanical Properties (H_{04} & H_{05}).

H_{01}: There is no significant difference in the diameter of the zones of inhibition against *Staphylococcus aureus* between antibiotic-coated suture materials, coated braided, or uncoated braided, after each period of coating absorption in buffer.

H_{02}: There is no difference in the level of adherence of *Staphylococcus aureus* to each of the antibiotic-coated suture materials, coated suture materials, uncoated braided suture, and monofilament suture of equal diameter.

H_{03}: There is no significant difference in the rate of antibiotic release between the antibiotic-coated suture materials, coated braided, or uncoated braided, after each period of coating absorption in buffer.

H_{04}: There is no significant difference in initial handling properties of flexural rigidity or tissue drag between the coated braided and uncoated braided sutures, and the monofilament sutures.

H_{05}: There is no significant difference in the initial mechanical properties by way of tensile strength and knot pull strength of the coated, uncoated braided, and monofilament suture materials.

Definitions

1. Absorbable coating – a coating that breaks down and is capable of being absorbed by animal tissue.
2. Antimicrobial activity – will be measured by the zone of inhibition and absorption of the coating materials over various buffer exposure periods. In other words, the "activity" will be indicated by the degree of inhibition noted by the size of the zone and rate of release of antibacterial agent, determined by the concentration gradient of the coating and drug in their respective buffers.

3. Antibacterial agent – any chemical compound that is capable of either killing bacteria (bactericide) or disrupting the metabolism of an organism (bacteriostat).
4. Bactericidal – an agent that kills microbial growth.
5. Bacteriostatic – an agent that inhibits microbial growth.
6. Biocompatibility – describes the cell-mediated interactions between an implanted biomaterial (eg. polymer) and adjacent biological tissues, and in general is considered a surface phenomenon (Laurencin & Elgendy, 1994. p.28).
7. Biodegradable (coating) – a coating that can be hydrolyzed or converted by the body into naturally occurring (biocompatible) products that are then readily excreted.
8. Biomaterial – a natural or synthetic material that is placed in contact with living tissue and/or biological fluids (Laurencin & Elgendy, 1994. p.28).
9. Coefficient of Friction – "...[t]he quotient obtained by dividing the force necessary to move one body over another at a constant speed by the weight of the body" ("Coefficient", 2001, para. 1). A different coefficient of friction must be calculated for each different pair of surfaces in contact.
10. Flexural Rigidity– "a measure of stiffness, where two equal and opposite forces are acting along parallel lines on either end of a strip of unit width bent into unit curvature in the absence of any tension" (ASTM D 1388, 2001, p.1).
11. Knot security – the tensile force applied from within the loop (the patient's side of the knot) required to break the knot apart (measured in Newtons).

12. Level of adherence – will be determined by the number of microorganisms adhering to the suture materials as viewed and qualitatively measured from the scanning electron microscope (SEM) images.

13. Macrophage – "…a large mononuclear phagocytic cell, present in blood, lymph, and other tissues… They phagocytose [engulf] and destroy pathogens [i.e. bacteria]; some macrophages also activate B cells and T cells" (Prescott, Harley & Klein, 1999. p.G17).

14. Zone of Inhibition – "clear area of no growth of microorganism, cultured onto the surface of an agar growth medium, in proximity to the borders of a specimen placed in direct contact with the agar surface" (AATCC, 1994).

Limitations

Guilford Mills Incorporated (Fuquay-Varina, NC, USA) supplied the spun polyester multifilament partially oriented yarn (POY) samples. The yarn was drawn, heat set and braided into sutures at the College of Textiles, North Carolina State University (Raleigh, NC, USA). Poly-Med Inc. (Pendleton, SC, USA) coated the sutures. Therefore, the type of sampling was determined by the availability of suture material. In addition, the coating process may have been affected due to limited quantities of both the braided suture and the drugs.

Delimitations

Only one manufacturer of polyester yarns, one braider and one finisher to coat the sutures was used. Therefore, if a different process, technique, or supplier were to be used,

the data might vary. Furthermore, only two drugs (clindamycin and moxifloxacin) were used as the antibiotic agents for these coatings, and only one type of knot strength test (knot pull strength) was performed. Hence, the results may not necessarily apply to other types of antibiotic drugs or other types of knot strength tests.

Only one strain of one type of organism was used to test the antimicrobial efficacy of the coated suture materials. A laboratory strain of *Staphylococcus aureus* was chosen as the test organism because it was the standard test strain indicated in the standard disk diffusion method by the National Committee for Clinical and Laboratory Standards (NCCLS), a consensus organization. Therefore, the results were limited to this bacterium alone. Other tests on different types of bacteria, most notably clinical isolates, would need to be performed to confirm the efficacy of these sutures.

CHAPTER 2

LITERATURE REVIEW

Bacterial-Foreign Body Interactions

In 1957, Elek and Conen demonstrated that the number of bacteria needed to establish an infection could be reduced 10,000-fold with the use of a silk suture. Their groundbreaking study showed that the body could cope with tissue injury, even when faced with a large inoculum of bacteria. However they noticed that when a foreign body, such as a suture enters the body it enables the bacteria to subvert host natural defense mechanisms against infection. It is the presence of foreign materials like sutures in the host that cause increased inflammatory responses. Once this occurs there is a high likelihood of infection (Katz, Izhar, & Mirelman, 1981).

Sutures act as a vehicle for mechanical transport of bacteria into surgical wounds. In effect, sutures act as a conduit for bacteria, which otherwise would not gain entry past the host's first line defense mechanisms. Even with the use of sterile techniques, bacteria either on the surgeon's hand or the patient's own skin can sometimes contaminate the wound. For example, coagulase negative *Staphylococcus epidermidis* (a common hand organism) is commonly considered a leading pathogen because of its prominent role in facilitating device related infections.

According to Altemeier and Culbertson in 1970, there were only three factors that contributed to wound infection. These were: the number of bacteria contaminating the wound, the virulence of that bacteria and the host's resistance (Chu & Williams, 1984). However, with the advent of biofilm infections on medical devices, the complexity of such infections has increased enormously.

Some bacteria are capable of growing a sticky sugar coating or glycocalyx. Bacteria of the same species or others that do not produce the capsule can harbour in glycocalyx. In this way, some organisms that are commonly low virulence organisms have become common pathogens due to the production of glycocalyx. In addition, bacteria that may not produce a capsule on the skin may produce one in the body, leading to higher virulence and pathogenesis. Bacteria form a biofilm (a complex network of microorganisms within a glycocalyx) on surfaces, which is often undetectable by routine clinical tests for months after surgery. Furthermore, even small numbers of bacteria can survive in the glycocalyx network or in the interstices of the implant weeks or months after surgery before becoming clinically obvious (Greco, 1991) and once a biofilm has developed, the bacteria within it become immune to antibiotic treatment and ultimately leads to an incurable infection or removal of the device. Bacterial strains that have been known to produce this glycocalyx and develop biofilms on medical devices include *Pseudomonas aeruginosa*, *Streptococcus viridans* and *Staphylococcus aureus* (Chaudhary & Simmons, 1991).

Once a biofilm has been established, bacterial adhesion is thought to be irreversible (Glaser, Leikin, Chernomordik, Pastushenko, & Sokirko, 1987). However, if the biofilm is not given a chance to develop by eradicating bacteria soon after contamination, for example by local prophylactic treatment, then serious infections and device removal could be prevented. It has been demonstrated that bacterial attachment can be reversible (Chu & Williams, 1984). Chu and Williams (1984) observed a "zig-zag" absorption pattern when various suture materials were exposed to two different organisms over time. They found that rather than a linear pattern of increasing or

decreasing organisms, there was a tendency on the ten different suture materials studied for the bacterial counts to fluctuate up and down over time.

This pattern of absorption, explained first by Marshall (1971), represents a dynamic balance between electrical repulsion energies at changing electrolyte concentrations within the body's environment and van der Waals attractive forces between molecules in close proximity. Further evidence that the adherence profiles are dynamic came from the more extreme profile of *Escherichia coli (E. coli)* to that of *Staphylococcus aureus (S. aureus)* against all the materials studied. In addition to the differences between their cell wall structures, *E. coli* is a motile bacterium, whereas, *S. aureus* is not. This accounts for *E. coli*'s greater capability for movement.

In his seminal research study, Chu & Williams (1984) examined the primary factors affecting suture infections. Among these were the surface characteristics of the microorganisms as well as the physical and chemical properties of the materials. In this study the researchers established that bacteria with very different surface characteristics, namely, *S. aureus* and *E. coli*, each uniquely contributed to the degree of adherence to implanted materials. *S. aureus* represented a Gram-positive organism, which consists of a cell wall structure with a single rigid peptidoglycan layer. In contrast, the representative Gram-negative organism, *E. coli,* has a multilayered cell wall with additional layers of lipopolysaccharide and protein.

In addition to the degree of motility, which affected the extremity of their fluctuating adherence patterns, the surface characteristics of the bacteria were found to have an impact on their adherence to the different types of suture surfaces. Chu & Williams challenged ten different suture materials against the two organisms by using

radiolabelled bacteria. The materials tested included absorbable, synthetic, natural, monofilament, braided and both coated and uncoated suture materials.

Three centimetre segments of each suture were placed in phosphate buffered saline for 20, 60, 160 and 180 minutes. After rinsing to remove nonadherent radiolabeled bacteria, the sutures were placed in sodium hydroxide and shaken for one hour to remove adherent bacteria. Thereafter the scintillant fluid was added to determine the radioactivity, measured as counts per minute in a liquid scintillation counter.

An adherence index against the suture that showed the least bacterial adherence after 20 minutes of immersion was also calculated. The synthetic absorbable monofilament suture material, the polydioxanone suture (PDS), showed the least adherence of both types of bacteria after 20 minutes and was used as the point of reference to compare all other suture materials in the study.

Based on previous studies, it was believed that the reason why the PDS suture had attracted the fewest bacteria was due to its monofilament structure. This is because monofilament sutures have less surface area than do braided sutures. However, it was posited that this could not be the only reason for the PDS sutures attracting lower bacterial levels. In their examination, the researchers found that after 20 minutes the braided nylon sutures (Surgilon) had a higher index with *E.coli* than monofilament nylon sutures (Ethilon). However, after longer periods of contact, the difference became too small to be significant. In contrast, Surgilon had half of the *S. aureus* of Ethilon at 20 and 180 minutes of contact. This suggested that the surface characteristics of the organism had a greater influence on adhesion than the physical structure of the suture.

Furthermore, while a braided structure does not necessarily mean that the suture is more likely to adhere bacteria, chemical properties of a polymer also elicit or detract bacterial adherence. For example, while the nylon-braided suture adhered fewer *S. aureus*, the braided polyglycolic acid suture (Dexon) showed increases in bacterial adherence to both species over time. The greater bacterial adherence to PGA than to PDS thus was not due to its absorbability or physical structure, but to its more hydrophobic chemical structure, which consists of fewer water affinitive ester groups than PDS.

Suture Adherence Studies

Several studies have dealt specifically with the adherence of bacteria to suture materials (McGeehan, Hunt, Chaudhuri, & Rutter, 1980; Katz et al., 1981; Chu & Williams, 1984; Scher, Bernstein, & Jones, 1985; Ananthakrishnan, Rao, & Shivam, 1992). Table 1 summarizes the adherence results given in these studies. Since all of these adherence studies compared materials that were expected to adhere to bacteria, they showed relatively high numbers of bacterial adherence (i.e. magnitude of $10^{6\text{-}7}$ bacteria). The studies did not intend to address how infections could be inhibited, but to assess which suture materials were more likely to cause infection than the others.

Similar to the research findings delineated in the study by Chu & Williams (1984), the above researchers were able to isolate some of the key physical and chemical differences between suture materials associated with adhesion. Specifically that a more hydrophobic suture, such as polyglycolic acid, is more likely to adhere bacteria than a suture with more hydrophillic ester groups such as polydioxanone (PDS). These research findings also showed a lack of evidence for the case against braided materials, which have long

been characterized as more naturally capable of harbouring bacteria within its interstices (Alexander, Kaplan & Altemeir, 1967; Katz et al., 1981). However, if we look at the results of such studies, all the suture materials adhered large numbers of bacteria in the range of 10^{6-7} bacteria/ml (Katz et al., 1981; Scher et al., 1985). Therefore, even though the Dexon (polyglycolic acid braided absorbable sutures) adhered the most bacteria, there was relatively little difference (one order of magnitude) compared to the other sutures studied (braided silk, monofilament polypropylene, chromic catgut, braided nylon polyamide 66) in terms of their ability to promote infections.

Chu & Williams (1984) demonstrated that while braided nylon material had initially appeared to have a greater affinity to attract bacteria compared to the same material in monofilament form, the differences in bacterial adherence proved to be insignificant over time. For example, they found that after 20 minutes with an inoculum of *E.coli*, braided nylon sutures (Surgilon) had an increased adherence index with *E.coli* than (Ethilon) monofilament nylon sutures (Chu & Williams, 1984). However, after longer time periods, the differences between the two different configurations of the nylon suture were negligible. On the other hand, Surgilon adhered half the *S. aureus* of Ethicon at 20 and 180 minutes of contact with these bacteria. This once again suggests that the surface properties of the bacteria are equally as important as the physical nature of the suture. Furthermore, braided sutures may not be as prone to causing infection as is commonly perceived and may offer an effective alternative to monofilament sutures.

Chu & Williams' study also showed that the coatings of the braided suture materials play a role in determining adherence. (1984). Siliconized braided polyester

Table 1: Bacterial Adherence Studies to Suture Materials

Citation	Suture Type	Brand/ (Manufacturer)	Adherence to Organism	Results
(Katz, Izhar, & Mirelman, 1981)	1) catgut monofilament (absorbable)	(Ethicon)	size 3-0 sutures to *S. aureus* (radiolabelled) for 20 minutes exposure (cfu/cm)	1) 0.5 X 10^7 cfu/cm
	2) polyglycolic acid (PGA) (absorbable)	Dexon (Davis & Geck)		2) 1.7 X 10^7 cfu/cm
	3) silk (nonabsorbable)			3) 0.8 X 10^7 cfu/cm
	4) Nurolon, braided polyamide 6,6 (nonabsorbable)	(Ethicon)		4) 2.5 X 10^6 cfu/cm
	5) Nurolon, monofilament polyamide 6,6 (nonabsorbable)	(Ethicon)		5) 2.4 X 10^6 cfu/cm
	6) Silicone-treated braided polyester	Ti-cron (Davis & Geck)		6) 0.8 X 10^6 cfu/cm
(Scher, Bernstein, & Jones, 1985)	1) polypropylene	Prolene (Ethicon)	size 4-0 sutures exposed to *S. aureus* (radiolabelled) for 4 hours (mean counts/min/cm)	1) 0.01 X 10^6 counts/min/cm
	2) silicone-treated braided polyester	Ti-cron (Davis & Geck)		2) 2.61 X 10^6 counts/min/cm
	3) Teflon-treated braided polyester	(Tevdek-Deknatel)		3) 3.85 X 10^6 counts/min/cm
(Chu & Williams, 1984)	1) catgut (all absorbable materials)		size 2-0 sutures exposed to a) *S.aureus* and b) *E.coli* for 20, 60, 120, and 180 minutes averages given in (bacteria/ml)	1a) 4.9 X 10^5 cfu/cm 1b) 7.6 X 10^5 cfu/cm
	2) braided PGA	Dexon		2a) 8.8 X 10^5 cfu/cm 2b) 8.0 X 10^3 cfu/cm
	3) braided polyglycolidelactide	Vycryl		3a) 5.5 X 10^5 cfu/cm 3b) 8.1 X 10^3 cfu/cm
	4) polydioxanone (PDS) monofilament (absorbable)			4a) 0.8 X 10^5 cfu/cm 4b) 6.1 X 10^3 cfu/cm
(Ananthakrishnan, Sambasiva, & Shivam, 1992)	1) cotton (nonabsorbable)		size 2-0 sutures exposed to a) S.aureus and b) E.coli for 20, 60, 120, & 180 hours (averages in cfu/ml are given)	1a) 3.1 X 10^2 cfu/cm 1b) 2.1 X 10^2 cfu/cm
	2) silk (nonabsorbable)			2a) 4.1 X 10^2 cfu/cm 2b) 3.9 X 10^2 cfu/cm

sutures (Ti-cron) had the highest initial adherence index among the three polyester sutures, but its adherence index decreased drastically over time. At 120 and 180 minutes, Ti-cron's level of adherence dropped slightly below that of the PDS reference suture. In comparison, the adherence profile of the uncoated braided polyester was similar to that of the PDS reference suture. The results imply that the coating does have a positive effect on reducing bacterial adherence.

While there is a lack of agreement in the literature as to which materials have the highest tendency to promote infection, it is generally accepted that sutures do attract bacteria and it is a problem that needs to be addressed. Research demonstrates that bacterial surface properties of potentially contaminating bacteria as well as the chemical and physical properties of the suture materials must be assessed when implanting a surgical suture. However, having conclusively demonstrated the innate propensity of materials to cause infections there is a noted dearth of research on antimicrobial suture materials. One of the purposes of this thesis is to examine and conduct a more innovative study of antimicrobial suture materials with the future goal of reducing the rate of surgical infections.

Antimicrobial Suture Materials

Despite the fact that bacterial adherence and infection rates remain a problem, few researchers have published studies addressing the prevention of such infections locally. To help alleviate this problem, a group of Russian researchers have studied antibiotic-bound suture materials (Aleksandrov, Volenko, Vasina & Sidorova, 1991; Iushkov et al., 1991; Klimenkov, Smolianskaia, Iskenderov, Gavrilova & Petrova, 1992). These Russian

researchers have studied ionically bound antibiotics to polyacrylamide suture materials. The antibiotics include gentamycin, and a combination of diocidine and quinocidine. The most comparable results are outlined in Table 2.

To treat the incision wound site prophylactically with their gentamycin-bonded suture materials Iushkov and his colleagues used a five to ten day period as a target time because it is the period when infection is greatest and prophylactic antibiotics are usually given systematically to the patient (Iushkov et al., 1992). They embedded the suture threads with 7% gentamycin by weight of the suture.

The sutures were tested against both aerobic (*S. aureus, E.coli, Streptococcus pyogenes, Streptococcus viridans, Proteus mirabilis, and Salmonella species*) and anaerobic bacteria (*Bacteroides fragilis* and *Clostridium perfringens*). However, the latter results were not given. Bacterial counts from implanted sutures in dogs were assessed on three different agar environments and the growth/number of colonies (mixed) was recorded. After one to five days of implantation, the sutures were removed and sample fragments were placed in agar and incubated. Total growth or abundant growth was found on all the plates from the untreated sutures throughout each day of study, whereas, the 7% gentamycin-treated suture threads showed no growth on any plates on days 1 & 3, and from sparse to moderate growth on the various plates on days 2, 4, & 5.

Since the researchers did not thoroughly describe the method, it is difficult to make conclusions based on their microbial assessment. However, inhibition of bacterial growth was not demonstrated, particularly since moderate bacterial growth was shown on several plates. Also, it is questionable that the suture results against the anaerobic

Table 2: Bacterial Adherence Studies to Antibacterial Sutures

Citation	Suture Type	Manufacturer	Adhesion to Organism	Results
(Aleksandrov, Volenko, Vasina, & Sidorva, 1991)	1) AG (5% gentamicin) 2) ADX (7.5% 1:1 diocidine/ quinocidine) 3) G-2 (5% gentamicin)* 4) DX-2 (7% diocidine/ quinocidine)* *unspecified technique used to apply antibiotics in these samples	Capromed Capromed Capromed Capromed	10^6 cfu/ml bed of a) *E.coli* or b) *S.aureus* zone of inhibitions of 2 cm sutures given in mm	1a) 16.1 mm 1b) 26.1 mm 2a) 21.2 mm 2b) 20.7 mm 3a) 33.7 mm 3b) 29.3 mm 4a) 30.8 mm 4b) 27.1 mm
(Klimenkov, Smomolianskaia, Iskendevor, & Gavrilova, 1992)	1) control (at time zero) polycaproamide suture (size 5-0) with 11.1% gentamicin 2) 11.1% gentamicin-treated (size 5-0) polycaproamide sutures 3) control polycaproamide suture (size 0) with 11.3% gentamicin 4) 11.3% gentamicin-treated (size 0-0) caproamide sutures		1.5 cm suture fragments exposed to isotonic buffer solution - aliquots were then exposed to a lawn of *B. subtillis* - treated samples were tested over an 11 day period (averages in mm are given)	1) 19 mm 2) 16.3 mm (average over 11 days) 3) 29.0 mm 4) 25.2 mm (average over 11 days)

organisms were not provided and may imply that the sutures were even less successful at inhibiting growth against anaerobic organisms than they achieved against the aerobic organisms.

Aleksandrov and colleagues (1992) studied a comparison between gentamycin, and diocidine and quinocidine-treated polycaproamide suture materials. Two separate adhesion techniques were used to bind the antibiotics to sutures however, they failed to provide specifics on the techniques used. Their reported test results showed that the technique used to coat the G-2 (5% gentamycin) and DX-2 (7% diocidine and quinocidine) samples resulted in larger zones of inhibition and longer lasting zones than their counterparts, AG (5% gentamycin) and ADX (7.5% diocidine and quinocidine).

In their study, the "coating" technique was successful at slowing the rate of reduction of the zone of inhibition diameters left in incubation over one week compared to the "uncoated" counterparts. It was further shown that G-2 and DX-2 sutures reduced to less than ten percent of their initial zone after seven days, whereas, ADX and AG lost their zones after 3 days and 4 days, respectively. These results show that the antibiotic used is bacteriostatic. More simply put, the antibiotic inhibits bacteria for a short period of time before the bacteria grows back. Despite its apparent success, a killing effect was not achieved and eradication of bacterial infection was not proven.

The only information regarding the bonding of the gentamicin to the polycaproamide suture in Klimenkov, Smolianskaia, Iskenderov, Gavrilova and Petrova's study (1992) is that the antibiotic (gentamycin) was bound ionically and unevenly to the suture. During the course of their *in vitro* experiment in sodium chloride, the release of gentamycin (reported as the zone of inhibition of the suture on the agar) varied between

13-19.5 mm for the size 5-0 material and 17-31 mm for the size 0 suture. The researchers claimed that the differences between the results were not meaningful because there was an unequal spreading of the antibiotic compound over the whole length of the suture. Therefore, the technique of antibiotic application did not prove to be uniform.

The lack of uniformity of the antibiotic application did not preclude the successful inhibition of *Bacillus subtillis (B. subtillis)* in this case, but may result in poor reproducibility and predictability of future results. Moreover, *B. subtillis* was the only organism studied. The limited scope of this study means that more extensive research will have to be undertaken to prove that this gentamycin-treated suture material will be useful against other important organisms involved in suture infections (i.e. *S. aureus*). Also, their study did not mention if the suture was monofilament or multifilament or if the antibiotic application deterred or aided in any of the important surface or handling properties desirable of suture materials.

Antimicrobial Biotextiles

In contrast to the scant published research on antimicrobial suture materials, the techniques used in the creation of antimicrobial biotextiles materials, such as vascular prostheses and wound dressings is well documented. Methods that have been used to apply antimicrobial properties to textiles and other polymer materials are: textile dying technology (Phaneuf et al., 1993), freeze drying (Khang, Choi, Cho, & Rhee, 2000), water infusion (White et al., 1984; Haverich, Hirt, Karck, Siclari, & Wahlig, 1992; Göeau-Brisonnière et al., 1994; Wachol-Drewek, Pfeiffer, & Scholl, 1996; Cormio et al., 1997; Stemberger et al., 2000), organic solvent infusion (Bervenisty et al., 1988),

surfactant-binding (Kajicek, Dvorak & Chvapil, 1969; Harvey, Ralph & Greco, 1981; Harvey, Alcid & Greco, 1982; Greco et al., 1985; Chevru et al., 1991; Raad et al., 1998), and nanocrystalline applications of silver (Wright, Lam & Burrell, 1998; Darouiche, 1999; Tabola, Chinn, Moore, & Darouiche, 2000).

The surfactant-binding systems achieved longer release rates compared to dipping in antibiotic solution. This technique works by first adhering (via organic solvent infusion) an insoluble surfactant onto a polyester or polypropylene prosthesis. The long chains alkyl groups of the surfactant generate a positive charge on the prosthesis to which a subsequent adhesion of a usually negatively charged drug can bind ionically. This process binds the drug significantly better than if the surfactant was not present because it produces a chemical bond between the drug and the material. However, the major problem with this method is that drugs bound to the surface of a material in this manner are loosely held with dissociation constants of about $10^{-3} - 10^{-4}$ mol/L (Greco, Trooskin, Donetz, & Harvey, 1985). Moreover, the stoichiometry of maximal binding is found to be much lower than one drug per surfactant molecule (Greco et al., 1985). Therefore, only some surfactant molecules are involved in drug binding which constrains the overall inhibitory effect of the coating.

Furthermore, the rate of dissociation of the drug can be relatively slow. In some cases it takes days to dissociate. As a result, the drug used will not have the opportunity to enter a bacterial cell and ultimately debilitate the metabolism of the bacterium until it has dissociated from the surfactant molecule bound to the textile. Despite its long dissociation, this kind of response is not a disadvantage in the case of vascular grafts because patients in this case are often subject to infection by other conduits (i.e.

cathethers etc.) several days after surgery. However, a suture is often susceptible to contamination at the time it is implanted. Thus, the infection is not only localized, but it needs to have enough drugs to inhibit bacterial growth within the critical postoperative period, which according to Victorian Postgraduate Medical Foundation's "Antibiotic Guidelines" is in the first 6-8 hours after surgery (Ananthakrishnan, Rao, & Shivam, 1992). Therefore, a surfactant-binding system would not deliver an efficient local prophylactic treatment for prevention of suture-related infections.

Other options include coatings or polymers that degrade and, as they hydrolyse, release an embedded drug. The studies by Khang (2000) and Stemberger (2000) noted above involved such systems through their use of emulsion freeze-drying and water-immersion techniques.

In their study, Khang and his colleagues impregnated gentamycin sulfate into PLLA (poly(L-lactide)) tissue scaffolds. The gentamycin released from the PLLA was determined by high- pressure liquid chromatography (HPLC). Release of gentamycin sulfate was found to increase with increasing load concentrations. The scaffolds also showed an increased in wetting (hydrophilicity) due to the surface characteristics of gentamicin sulfate. This is due to the fact that one end of the gentamycin antibiotic is more hydrophobic than the other. The sulfate end gives off a negative charge making it more hydrophilic. The result of binding the drug to the PLLA translated into a positive effect on cell growth and adhesion than the polymer surface alone. The gentamycin with the highest loading (20 w/v%) showed the highest release rate for the first two days and then a more slowly increasing release rate for the following five days. It is difficult to

determine how effective the materials were because a bacterial adhesion experiment was not conducted.

In a similar study Stemberger and his colleagues (2000) embedded antibiotics, gentamicin and ofloxacin as well as anti-thrombin drugs into solvent-casted poly(D,L)lactide stents (Boehringer, Ingelheim). In their study the adhesion study results were quite poor. The *Staphylococcus epidermidis* adhesion studies showed that the anti-thrombin drugs gave the best reductions, which was only 32% less than the coated-stents without drugs. Therefore, the polylactic acid stents, water-infused with either antibiotics or anti-thrombin drugs, were unsuccessful at reducing bacterial adhesion. This may be explained by the combination of techniques and drug used.

One of the main problems with current application techniques to biotextiles and degradable polymers like PLLA and PDLA (above) is the ability to adhere or embed sufficient antibiotic within the polymer system, and in the latter case, to do so without degrading the polymer. For example, solvent and water infusion systems can cause the polymer to swell and cause the drug to become entrapped in the bulk polymer (Greco, 1991). Not only may this modify the physical properties of the absorbable polymer, but it may also result in longer diffusion rates and sacrifice the effectiveness of the drug. Clearly a controlled and reproducible process that involves minimal swelling would best achieve the application of a drug into such a polymer system.

Antimicrobial Materials: Study Protocols

Phaneuf and colleagues (1993) incorporated antimicrobial properties into a Dacron fabric using a textile dying technique, the pad/heat dry method. The researchers

quantified the antibiotic in the fabric by UV-spectrometry based on the peak wavelength at which the antibiotic absorbs light. Microbiologic studies were then carried out to test the maximum inhibitory and maximum bactericidal concentrations (MIC and MBC, respectively) of ciprofloxacin against *S. aureus* (ATCC 29213) in trypticase soy broth (t-soy broth). The antibiotic solutions in t-soy broth were observed for bacterial growth (indicated by turbidity) after 24 hours in an incubator. The broth solutions were then backplated on t-soy agar plates and examined after 24 hours to determine the MBC.

The ciprofloxacin-treated Dacron samples (1 cm^2) were then tested against various concentrations of *S.aureus* (10^7, 10^6, 10^5, 10^4 bacteria/ml) to determine the initial adherent bacteria on the starting samples. Sterile treated Dacron materials were incubated in bacteria solutions for 24 hours at 37°C and the solutions were backplated. The Dacron pieces were then transferred to a sterile test tube containing 30 ml PBS and sonicated in an ice bath for 10 minutes at 60 Hertz. After sonication 100 μl of the sonicate was also backplated onto an agar plate. Colony forming units (CFUs) were counted on the agar plates after 24 hours incubation. From the backplate counts, the MIC, and MBC of the treated segments were determined. These studies showed that the MIC and MBC of ciprofloxacin against *S.aureus* (29213) were 0.9 μg/ml and 2.5 μg/ml, respectively and a 1 cm^2 antibiotic-pad/heat-treated Dacron fabric inhibited 10^7 cfu/ml and killed 10^6 cfu/ml.

A pharmacokinetic profile was also determined by washing the fabrics at up to 336 hours then sterilized and placed on a bed of *S.aureus* streaked onto agar plates in order to determine the zone of inhibition. The purpose of such a study is to determine the antibiotic release over a period of washings. The results of this test revealed a rapid

release of the antibiotic after 48 hours of washing of antibiotic-treated fabrics versus a slow release over 336 hours of the antibiotic-pad/heat-treated fabrics.

Such zone of inhibition tests could be adapted to testing suture materials and may be a good way to identify the inhibitory concentration of antibiotic-coated suture material. However, as an alternative to the parallel streak method, Vercaigne and Zhanel (2000) used a zone diffusion study that involves forming a lawn of bacteria on the plates of agar, resulting in a more uniform zone of inhibition. This test may be much more conducive to determining the absorbance and inhibitory activity profile of the suture materials of study.

In this study, central venous catheters were locked with various antibiotics (cefazolin, vancomycin, ceftazidime and gentamycin) and heparin. After exposure in an incubator, the catheters and the antibiotic solutions were semi-quantitatively assessed for microbial susceptibility against clinical isolates using a modified agar dilution procedure. A bed of bacteria (0.5 Mcfarland standard or 1 X 10^8 bacteria/ml) of each isolate was swabbed onto Mueller-Hinton agar plates to create a lawn of bacteria. Next, a 10 μl sample of each test solution –antibiotic solution (positive control), heparin solution (negative control), and test sample of antibiotic-heparin solution –were directly pipetted onto the agar plates. The plates were incubated for 18-24 hours at 35°C. After incubation, each test sample was measured and compared to the controls. This semiquantitative method of assessing inhibitory activity of bacteria can be easily adapted to a coated suture study.

Mechanical Characteristics of Sutures: The Surgical Knot

A surgeon affixes a suture by creating a loop around a fixed perimeter and tying a knot. The knot is composed of a number of throws. The throw or wrap refers to the wrapping or looping of two strands of a thread around each other. After the knot is tied, the ends remaining are cut. The length between the knot and the cut suture ends are referred to as the knot ears.

A knot is referred to as "square" when the ear of a two throw knot exits on the same loop side of the knot with the two throws parallel to each other (Rodeheaver, Thacker, & Edlich, 1981). A single throw is made by wrapping the two strands around each other to result in a 360° angle of rotation around the other strand. Alternatively, a double throw will result in a 720° angle.

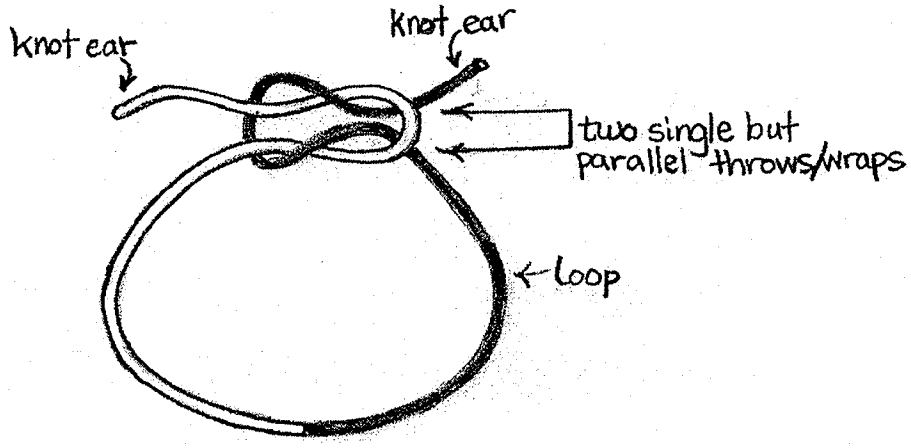


Figure 1: Diagram of a Square Knot

In comparison, the knot is considered a granny knot if the right ear and loop exit or cross at different sides of the knot.

Tera and Aberg developed a universal language of knot identification in 1976 (Rodeheaver et al., 1981). An Arabic number indicates the number of wraps involved in each throw and either a cross (X) or a parallel symbol (=) is used to signify the relationship between each throw. A cross throw is equivalent to the granny knot (1X1) where the knot ear is on the opposite side of the initiating side of the loop. Whereas, the parallel symbol represents the square knot as described above, designated 1=1.

There are several aspects of the suture that play a role in its surgical performance. These include: biocompatibility, handling properties, as well as the physical aspects, such as minimization of tissue drag, high initial strength and knot strength (Bezwada *et al.*, 1995). A secure suture knot is vital to achieve good tissue juxtaposition and successful healing. Ideally, a knot requires as few turns as possible because the knot is the weakest component of any suture loop (Chu, 1983). In effect, the stiffer the suture, the lower the knot strength. This is due to the fact that it becomes more difficult to manipulate and form a knot the stiffer it is. Moreover, once it is formed, the knot is more likely to slide open. The higher the coefficient of friction, the better the knot security but it also increases the possibility of tissue drag and in turn tissue injury or scarring due to suturing.

Textile scientists often emphasize the tensile strength of a linear strand of suture material, which can be tested using a standard test method (ASTM D2256). However, the most surgically significant test of tenacity for the surgeon is when the suture is knotted (Stone, 1988). The strongest linear strand is "useless unless a stable knot can be placed" (Stone, 1988. p.713). To remain stable in its knotted form, a suture must be resistant to slippage and be capable of maintaining the strength of the knotted loop.

Researchers have found that coated braided sutures could overcome knot slippage only when a (1=1=1=1) knot construction consisting of four throws was used (Li, 2000; Rodeheaver et al., 1983). In order to ensure that the knot breaks open and fails during the test, as well as to prevent the braided suture knot from sliding, a double knot or two continuous square knots (1=1=1=1) should be tied. One method of measuring the strength of a knot is the knot pull strength test, a standard method developed by the United States Pharmacopeia (USP). USP is the institution that sets many of the standards for suture performance in North America.

An important initial handling property of a suture material is the degree of flexibility. For example, a more rigid or stiffer suture reduces the ease of handling for the surgeon as well as the security of the knot. The degree of stiffness or flexibility of a suture material has been tested according to the ASTM D1388-96 standardized heart loop test (Hong et al., 1998). For this test, the two ends of the specimen are clamped together to form what is referred to as the heart shaped loop. The loop length from the suture ends to the base of the loop is measured. This measurement is then used to derive the bending length, in accordance with the theoretical relationship provided in the method and a measure of flexural rigidity is determined using the equation provided in the method.

The resistance to passage or tissue drag of a suture material through tissue is another important property. Tissue drag determines the ease with which the suture can pass through tissue and is measured in terms of the resistance or the force required to pull or drag the suture through a given tissue or tissue simulant. A high tissue drag indicates that the material is likely to cause damage to the surrounding tissues. Currently, there is no USP standardized method to test the tissue drag of suture materials, however, in house

methods have been developed to attempt to simulate tissue drag (Hong, 1995; Gupta, 1985) and have been used to show that coated sutures have a lower coefficient of friction than uncoated sutures (Gupta, 1985). In a tissue simulant test for tissue drag the suture is passed through the tissue simulant and the force required to pull the specimen through the simulant is recorded. The force measured is directly related to the resistance that the suture material creates as it moves through the tissue simulant and is unique for that material.

Other researchers, such as Rodeveaver and colleagues, have tested sutures for tissue drag in a similar fashion *in vivo*, using surgically prepared paravertebral rabbit skin (Rodeveaver et al., 1983). Rodeheaver and colleagues also demonstrated that this tissue drag force is correlated to in vitro coefficient of friction values, determined by rubbing two sutures together under a weight. In this study, three sutures were compared: Dexon S, an uncoated braided polyglycolic acid suture (Davis & Geck, Wayne, N.J.); a new version of Dexon S, a coated (Poloxamer 188) braided polyglycolic acid suture; and a coated Vycril (polyglactin 910) braided suture (Ethicon, Inc., Somerville, N.J.). The results showed that coated suture materials reduce coefficient of friction values, however, these tests did not necessarily correlate to tissue drag as one might expect. For example, the coefficient of friction coated PGA suture was significantly lower than the uncoated PGA suture but did not differ significantly from the coated Vycril suture. However, the tissue drag measurements showed that the uncoated PGA samples required an average of 4.97±0.54 N, the coated PGA samples produced an average tissue drag force of 0.61±0.14 N, but in comparison the coated Vycril sutures produced a significantly higher a mean tissue drag of 3.04±0.31 N.

Another interesting finding in this braided suture study was that these tissue drag values correlated with the stiffness values of each suture –the coated Vycril was associated with the greatest rigidity, then the uncoated PGA, then the coated PGA. The coated Vycril suture was also associated with the highest resistance to straightening (measured by the load required to straighten each material caused by the memory due to packaging). The load required to straighten the coated PGA was greater than the load required the uncoated PGA as might be expected, however, these values did not correlate to drag at all. Therefore, there seems to be no hard and fast rules between drag, coefficient of friction or rigidity.

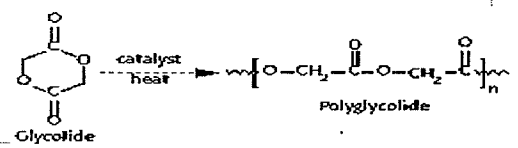
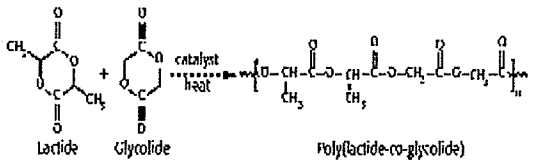
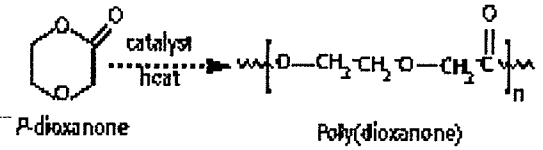
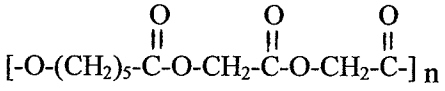
Absorbable and Nonabsorbable Suture Materials

Shinohara, Matsuo and Kikuchi (1996) compared nonabsorbable monofilament nylon and absorbable nylon suture materials (either polydioxanone or polyglyconate) to determine the rate of stitch abscesses (swollen tissue with accumulating pus due to infection) for cleft lip repair in infants. Cleft lip repair is a difficult procedure because very thin tissues are sutured. Thus, if an abscess occurs it necessitates removal of the stitches (Shinohara et al., 1996). Polydioxanone and polyglyconate were used in this study because they are the most widely used absorbable suture materials (See Table 3, pg 33, for "Commonly Used Absorbable Sutures").

In this clinical study, an abscess rate of 14% in the control group (nonabsorbable nylon sutures), whereas, no abscesses occurred in the test group (absorbable polydioxanone or polyglyconate sutures). This led the researchers to conclude that absorbable suture materials can help reduce the rate of tissue abscesses. Absorbable

materials are less likely to cause abscesses because there is very little degeneration resulting from the inflammatory cells at the wound site. This is because absorbable materials are broken down by hydrolysis, which causes a minimal tissue reaction in comparison to nonabsorbable materials, which degrade mainly by phagocytosis.

Table 3: Commonly Used Absorbable Sutures

Polyglycolic acid	Dexon® (braided)	
Polylactide-co-glycolide	Vicryl® (braided)	
Polydioxanone (PDS)	PDS(monofilament)	
Poly-ε-caprolactone-co-glycolide	Monocryl® (monofilament)	

The literature presents conflicting results when it comes to bacterial adherence onto absorbable suture materials. When polyglycolic acid (PGA) sutures were first introduced, Edlich and colleagues implied that the material might be bactericidal or

bacteriostatic because they found a reduction in bacterial adherence to PGA over time (Edlich in Scher et al., 1985). McGeehan and colleagues (1980) further supported this possibility when they discovered that PGA braided absorbable sutures (Dexon) produced the least number of infections in guinea pigs compared to braided silk, monofilament polypropylene, chromic catgut, and braided nylon (polyamide 66).

In contrast, researchers have also shown that Dexon adhered more bacteria than the other materials studied, which included chromic catgut (absorbable), Nurolon braided polyamide 66 (nonabsorbable), Nylon (polyamide 66) monofilament, braided silk (nonabsorbable), and Ti-cron silicone coated braided polyester sutures (Katz et al., 1981). However, the results were such that all the sutures had high adhesion rates in the range of 10^6-10^7 bacteria/cm.

In Chu & Williams' (1984) study on bacterial adherence to suture materials, PGA had a much higher incidence of infection than the polydioxanone (PDS) reference suture (which overall, adhered the least of either bacteria tested). PDS is also an absorbable material. The researchers proposed that the reason for the difference between the two absorbable materials was chemical in nature. This conclusion was based on the fact that PGA is more hydrophobic than PDS.

Bezwada and colleagues (1995) reported that a copolymer (25/75 mol%) of epsilon-caprolactone and glycolide (Monocryl) produced no bacterial infections in mice nor did the suture interfere with tissue defence to bacterial contamination. While no bacterial adhesion studies were performed, the researchers tested the Monocryl sutures in rats until they degraded (119 days for the size 2-0 suture and 91 days for the 6-0 suture).

At six periodic intervals, tissue reactions were recorded on a scale of no reaction (0) to extensive reaction (56+). Tissue reactions were viewed from excised cross-sections of the implanted sutures and mainly included the attraction of macrophages and fibroblasts, fewer lymphocytes and plasma cells and the odd polymorphonuclear leucocyte and giant cell. Monocryl sutures scored from zero to 20 (averaging 11.8 and 6.7, for the size 2-0 and 6-0 sutures). Monocryl also competed very well with other sutures tested (PDS II, chromic gut and Vyryl) of the same USP size (2-0). In terms of straight tensile strength (16.14 lb) and knot-pull strength (8.11 lb) it ranked the best. It produced the second longest percent elongation compared to the second generation PDS suture (39% compared to 51%, respectively). Monocryl also ranked superior in handling characteristics like stiffness and tissue drag.

Suture Coatings: Surface Characteristics and Biocompatibility

To alleviate the friction caused by the texture of braided sutures, surface coatings are often applied. Furthermore, while much of the literature mentions that infection is a limiting factor with braided suture materials, there is inadequate research on efforts to alleviate these concerns (Bezwada et al., 1995). Moreover, making the coating absorbable offers the opportunity of incorporating compounds such as antibiotics into the braided suture product.

While synthetic absorbable braided sutures offer a greater degree of biocompatibility, strength and more efficient handling properties (such as improved surface smoothness, lower tissue drag and superior knot security) than catgut sutures, surgeons remain reluctant to use braided synthetic absorbable sutures (Rodeheaver et al.,

1987). It is also known that handling characteristics of braided synthetic sutures improve with the use of absorbable coatings (Redeheaver et al., 1983). Moreover, synthetic absorbable braided filaments have been proven to be superior in strength retention and tissue reactivity. They tend to produce fewer post surgical complications and more positive effects on wound healing when compared to catgut sutures (Rodeheaver et al., 1983).

Suture coatings provide enhanced biocompatibility, which helps reduce healing time. In addition, the coating decreases the friction of the braided yarn, thus facilitating less abrasion and scarring due to implantation of the suture (Bezwada et al., 1995). A suture coated with an epsilon caprolactone and glycolide copolymer (Monocryl) has proven not to interfere with tissue defense and not to attract bacteria (Mazzarese et al., 1997; Bezwada et al., 1995).

After an examination of the various studies it can be concluded that the combination of strong tensile properties of a braided suture material with reduced surface friction offered by an absorbable coating such as epsilon caprolactone and polyglycolide, coupled with the reduced concern for infection, offers a natural progression within suture research to the present materials of study.

Coating and Antibiotic Properties

A patented coating process (US Patents 5,773,563) of an epsilon-caprolactone and glycolide copolymer (See Table 3 for chemical formula) has been used to coat suture materials. This patent is a continuation of US Patent 5,522,842. Epsilon-caprolactone is the major component of this polymer coating because of its low melting, low glass

transition temperature, and ability to enhance the surface properties of coated multifilament sutures (Shalaby, 1998). These patented epsilon-caprolactone polymers are comprised of mainly nitrogenous polyester with the addition of glycolate sequences as minor components within the chain.

Each chain of the polymer terminates with a carboxyl acid end group linked to an amine molecule. Basic amine functional groups are linked to the ester chain ionically or covalently to induce catalyzed hydrolysis. These polymers have accelerated absorption profiles compared to a polyester chain without the glycolide sequences, which make them especially useful as absorbable coatings on multifilament sutures and other medical devices. Other inventions using epsilon-caprolactone as a coating for multifilament surgical sutures contain this as a major co-polymer with the remaining co-polymer being composed of glycolide and glycolic acid (Shalaby, 1998). This invention differs from other proposed techniques through its ability to significantly improve the bioabsorption capabilities of epsilon-caprolactone based polymers.

The challenge in the production of such a coating is to incorporate sufficient amounts of glycolide to achieve good absorbability without compromising its crystallinity and melting properties. For example, if too much glycolide is incorporated, the coating will become amorphous or liquid near room temperature (Shalaby, 1998). This would result in an inappropriate coating for sutures since the surgeon needs to handle the sutures at room temperatures. Moreover, if too much glycolic acid is used in the polymerization process, it will prevent sufficiently long polymer chains from forming, thereby, limiting the lengthy chains required to achieve a low friction surface. A low friction surface is advantageous because it enables a suture to glide through a tissue.

A high friction surface tends to cause abscesses. Therefore, desirable surface properties may be sacrificed if just the right amount of glycolic acid is not used.

The patented coating is a careful combination of amine functionalities, epsilon-caprolactone, and glycolide to balance the ester sequences. The ester sequences can also be derived from lactide _-dioxanone or one or more of the corresponding acidic forms of these polymers (Shalaby, 1998). The basic amine functionalities are unique to this patented coating and they improve the absorbability by speeding up the rate of hydrolysis (Shalaby, 1998).

Incorporating 1-10% amine functionality has been found to substantially increase absorption without compromising crystallinity or melting point. The exact combination of the components during polymerization of the coating will depend on the parameters outlined in this thesis. The desired absorption rate *in vitro* for the thesis will be 7 days.

Coating Process

According to the patent, the epsilon-caprolactone-co-glycolide coating is applied to the braided suture at a low viscosity melt between 70-100°C (Shalaby, 1998). Excess coating is removed by passing the suture through a pad of non-woven fabric like polypropylene. The coating application requires a 1-15% organic solvent solution (i.e. toluene, acetone, etc.) at room temperature. The solvent then evaporates by air-drying at room temperature (or between 25-75°C). Afterwards, the coated suture may then be heated to insure an even distribution of the coating.

The percent add-on of the coating is varied between 1-10% depending on the suture size. At this level of coating the suture handling properties and knot tie down

characteristics are enhanced without compromising other properties like knot strength and knot security.

Coating Parameters for the Base Polymer and Antibiotics

A) Base Polymer

The coating application does not depend on the base polymer material except that the base material must have a melting point above 70-100°C to undergo the coating process without degeneration (since this is the temperature in which the coating is applied). The base polymer is a braided polyester (polyethelene terephthalate) that has a melting point of about 265°C. See Figure 2 for a "Chemical Structure of Polyethylene Terephthalate".

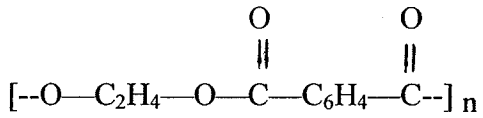


Figure 2: Chemical Structure of Polyethylene Terephthalate

B) Antibiotics

The antibiotics must have a melting point above 100°C if they are to be incorporated with the polymer melt. The melting points of clindamycin hydrochloride and moxifloxacin are about 141°C and 210°C, respectively. In addition to having a high melting point, these antibiotics also have small molecular weights (425.0 and 437.9 g/mol, respectively). The chemical structures of each antibiotic are shown in Figures 3 & 4 below. Their small size allows them to integrate evenly into the coating and to degrade easily at the time of hydrolysis. Both drugs are also soluble in water and ethanol.

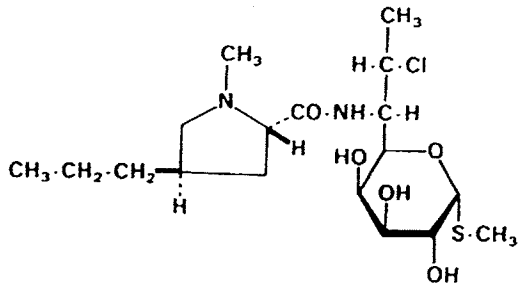


Figure 3: Chemical Structure of Clindamycin (Moffat, 1986)

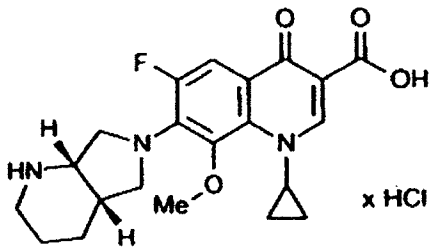


Figure 4: Chemical Structure of Moxifloxacin (Zhanel et al., 1999)

Antibiotic Parameters for Antimicrobial Inhibition

Disturbing trends in antimicrobial resistance estimate that "[o]ver 90 percent of strains of *S. aureus* demonstrate resistance to penicillin and related antimicrobial agents" (Hooton & Levy, 2001. p.6). Since *S. aureus* accounts for most infections that occur at the incision and resistant species of *S. aureus* are becoming more prevalent, the treatment of such organisms should be considered in the development of a local prophylactic suture coating. For this reason, one of the new fluoroquinolone antibiotics, moxifloxacin, with tested inhibition against resistant *S. aureus* (at $\geq$16 μg/ml) will be used. Such organisms are also highly sensitive to moxifloxacin, with a minimum inhibitory concentration against 90% (MIC_{90}) of methicillin sensitive *S. aureus* tested of 0.12 μg/ml, and an

MIC_{90} of 0.06 μg/ml to susceptible S. aureus (Zhanel et al., 1999). Moxifloxacin has a broad range antimicrobial activity against other aerobic bacteria including both sensitive and resistant *S. epidermidis* and *S. pneumoniae*, Gram-negative bacteria such as *E. coli*, as well as anaeobes like *Bacteroides fragilis*.

Moxifloxacin must enter the bacteria and accumulate intracellularly to kill bacterial cells. Inside the cell, the drug targets two sites: DNA gyrase (this enzyme is essential to DNA replication by introducing the negative twists into the structure of the DNA) and topoisomerase IV, which assists with the segregation of replicating chromosomes or plasmids (Zhanel et al., 1999).

Moxifloxacin will be tested alongside another antibiotic coated suture to ensure internal validity of the coating method. The second drug chosen is clindamycin, an older but common agent for treatment against skin and soft tissue infections, abdominal infections and gynecologic infections. Clindamycin is also a broad-spectrum antibiotic including aerobic, and anaerobic bacteria. Both penicillinase and non-penicillinase producing strains of *S. aureus* are sensitive to this drug, as is *S. epidermidis* and most *Streptococci*.

Clindamycin inhibits protein synthesis in bacterial cells. The site of binding is the 50S subunit of the ribosome. When clindamycin binds, it inhihits amino acids from binding to those ribosomes on the RNA chain and prevents protein synthesis. Clindamycin is also a drug of interest because it has been found to limit adhesion of microorganisms (including *S. aureus*) at subinhibitory concentrations of the drug (Khardori et al., 1991; Gismondo et al., 1990; Doran & Rissing, 1983). A drug that limits adhesion in this way may be of interest for incorporation into a biomaterial surface such

as a suture material because biomaterials tend to attract cell adhesion and make the surrounding tissue more prone to infection.

It has been shown that subinhibitory concentrations of clindamycin not only limit *Staphylococcus* adhesion via limitation of fibronectin using a phagocytosis assay (Doran & Rissing, 1983), but a study also showed limited *Staphlococcus epidermidis* growth on surgical nets after exposure to subinhibitory levels of clindamycin (Gismondo et al., 1990). The result of this exposure was viewed under a scanning electron microscope. Although a reduction was apparent, organisms were still visible on the surgical nets but fewer than the nets that were exposed to the same organism that had not been grown with sub-inhibitory levels of clindamycin.

Observations

At the end of the comprehensive literature review several observations are worthy of note. These are:

1) Suture materials are known to subvert host defense mechanisms.
2) The likelihood of infection, especially within hospitals is increasingly problematic with resistant organisms.
3) The critical post-operative time for infection is 6-8 hours after surgery and prophylactic treatments are usually 5-10 days after surgery.
4) Adherence depends on the surface characteristics of the bacterium as well as the chemical and surface qualities of the suture.
5) Coatings like poly-epsilon-caprolactone-co-glycolide have been shown to enhance biocompatibility by inducing a low inflammatory response.

6) The patented polymer coating process by Poly-Med Inc. will provide custom-made antimicrobial bioabsorbable coated suture materials made to degrade and provide a local prophylactic effect.

7) The percent add-on of the coating by weight of the suture material shall be between 1-10% to optimize suture handling properties and knot security. These properties are important to a suture's physical and mechanical performance as well as its biocompatibility.

8) The coating process should provide a predictable and reliable degradation process.

9) Bacterial adhesion studies compare the relative adherence of different materials to bacteria but there is a great void of research on ways of combating these high adherence statistics to suture materials.

10) Many antimicrobial biotextile studies use absorption rate studies (i.e. zone of inhibition tests) to determine the relation of release of compound to inhibitory activity.

11) *S. aureus* is the major pathogen involved in suture wound sepsis.

12) To prevent the braided suture knot from sliding, a double knot or two continuous square knots (1=1=1=1) should be tied.

13) There are standard methods for testing tensile properties and elongation and knot pull strength of yarns and these tests have been previously applied to the testing of suture materials.

14) The flexural rigidity of a suture material can be tested according to the ASTM D1388-96 standardized heart loop test.

CHAPTER 3

SUTURE MATERIALS

The following description pertains to sutures in all of the experiments. Size 0 braided 100% polyethylene terephthalate (PET) suture yarns were provided by the College of Textiles at North Carolina State University (Raleigh, NC, USA). The 9 tex (80 denier)/ 34 filament, partially oriented yarn (POY) feedstock (Guilford Mills Inc., Fuquay-Varina, NC) with dull surface finish and regular tenacity, was drawn and plied by a draw-winding process at the College of Textiles to make a 21 tex (190 denier)/ 102 filament yarn. This yarn was then braided into a 10-end hollow circular suture braid. These braids were then sent to Poly-Med, Inc. (Pendleton, SC, USA) to undergo the patented epsilon-caprolactone-co-glycolide coating process. There, two antibiotics (clindamycin and moxifloxacin) were impregnated in two different loading dosages of 10X and 100X the MIC of each antibiotic into the epsilon caprolactone-co-glycolide suture coating.

Monofilament Maxon sutures were provided by AutoSuture, Inc. (Montreal, QC). These green, USP size 0 (3.5 metric) monofilament sutures were made of completely resorbable poly(glycolide-co-trimethylene carbonate) polymers. The chemical structure is predominantly glycolide similar to the Dexon suture as shown in Table 3, however, the glycolide segments are joined by a trimethylene carbonate group. The Maxon suture is 33% by weight trimethylene carbonate and 67% glycolide (Chu, von Fraunhofer, & Griesler, 1997). An image of the three main suture types (braided coated, braided uncoated and monofilament sutures was provided in Figure 5.

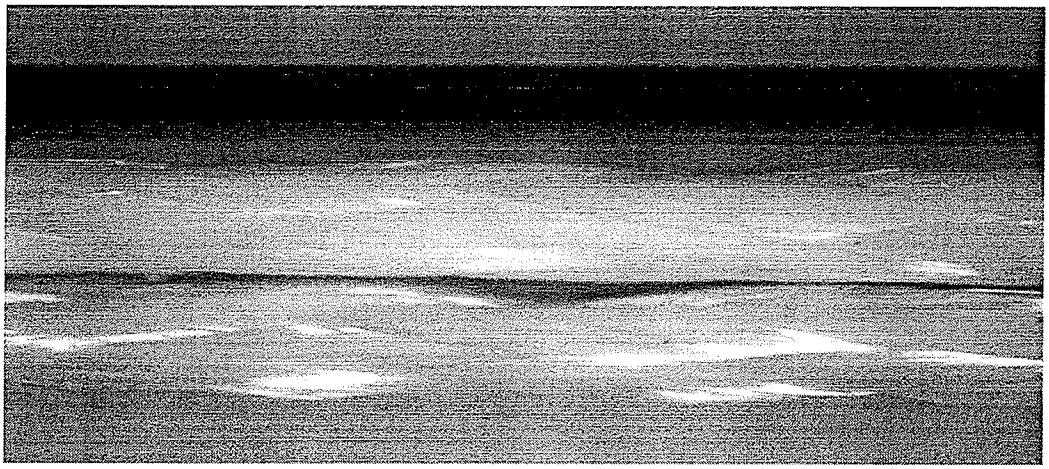


Figure 5: Suture Materials

SUTURE DIAMETER

Method

The suture diameter for each sample was measured using an Armes 202 Compressometer (Waltham, MA, USA). The presser foot had a diameter of 25.4 mm and weighed 91.54 g. The thickness of the antibiotic coated braided, coated braided (no antibiotic), uncoated braided and monofilament sutures (N=7) were measured by the Compressometer to the nearest thousandth of an inch and the mean and standard deviation were converted to millimetres.

Results

The mean suture diameters of the antibiotic sutures (0.85% clindamycin, 8.5% clindamycin, 0.1% moxifloxacin and 1.0% moxifloxacin) were 0.446±0.035 mm, 0.552±0.035 mm, 0.508± 0.025 mm, and 0.475±0.073 mm, respectively. The diameters

of the coated (no antibiotic) braided, uncoated braided and monofilament sutures were 0.497±0.046 mm, 0.537±0.054 mm, 0.432±0.029 mm, respectively. These results were summarized in Table 4, "Suture Diameters".

Table 4: Suture Diameters

(N=7)	Clinda 0.85%	Clinda 8.5%	Moxi 0.1%	Moxi 1.0%	Coated	Uncoated	Monofilament
1	17.0	20.0	21.0	16.0	19.0	25.0	18.0
2	19.0	23.0	20.0	23.0	18.0	19.0	18.0
3	20.0	21.0	21.0	17.0	18.0	20.0	16.0
4	17.0	22.0	20.0	21.0	22.0	23.0	15.0
5	17.0	23.0	20.0	15.0	18.0	20.0	17.0
6	16.0	20.0	20.0	19.0	22.0	21.0	18.0
7	17.0	23.0	18.0	20.0	20.0	20.0	17.0
Mean (10^{-3}in)	17.6	21.7	20.0	18.7	19.6	21.1	17.0
Std Dev.	1.4	1.4	1.0	2.9	1.8	2.1	1.2
Mean (mm) =	0.446	0.552	0.508	0.475	0.497	0.537	0.432
Std Dev.	0.035	0.035	0.025	0.073	0.046	0.054	0.029
C.V.%	7.9	6.4	5.0	15.3	9.3	10.0	6.8

Discussion

The variations in suture diameter amongst the antibiotic coated sutures may have been a factor of the coating process. Each suture coating was mixed separately with the coating and might have resulted in variability amongst the coated suture diameters. Variability within each suture coating is a factor of solution viscosity, temperature, humidity and the amount of coating material and length of suture the coater has to work with. The quantities of the drugs provided may have been insufficient for the coater to achieve an even coating, in addition to the limited lengths of braided suture.

The slightly reduced diameter of the coated suture compared to the uncoated and monofilament sutures may be an effect of the coating by compacting the braided

structure. This may be further demonstrated by the reduced standard deviation (±0.046 mm) of the coated sample compared to the uncoated braided sample (±0.054 mm).

CHAPTER 4

METHODS, RESULTS & DISCUSSION

Experiment 1: Antimicrobial Activity

VARIABLES

Independent Variables

1) Absorption exposure time

The six exposure time-periods included: time zero, day 1 and each day thereafter until day 5. At time zero, the dry suture was placed on the bed of bacteria and the buffer alone was spotted onto the standardized disk as a control.

2) Type of suture

The following samples and their corresponding adsorbed coating samples and two sets of negative controls (uncoated and coated sutures without antibiotic, with and without bacteria) in buffer were applied (N=6) to the agar surface at the different absorption time periods:

List of Samples I

1) Braided suture coated with clindamycin 0.85%
2) Braided suture coated with clindamycin 8.5%
3) Braided suture coated with moxifloxacin 0.1%
4) Braided suture coated with moxifloxacin 1.0%
5) Uncoated braided suture and bacteria
6) Coated braided suture (no antibiotic) and bacteria
7) Uncoated braided suture with no bacteria
8) Coated braided suture without antibiotic and no bacteria

Dependent Variables

Rates of Inhibition: The sutures were exposed to a 0.1M phosphate buffered saline solution (pH 7.4) at 35°C (to simulate body temperature) for six time periods from

time zero to five days. Buffers were changed daily and replaced with fresh buffer, representing "sink" conditions, to represent a complete change over of new fluids. This modified zone diffusion test, adapted from Vercaigne & Zhanel (2000), measured the bacteriostatic or inhibitory effect created by the clear zones caused by the treated suture materials after each buffer exposure time period. Following the exposure times to buffer, a 10 µl aliquot of the adsorbed coating in buffer and the suture itself were exposed to a Mueller Hinton agar plate pre-swabbed with ***S. aureus***. Each test sample was incubated for 24-hours at 35°C so the organisms could grow into a lawn of bacteria.

Thereafter, the diameter of the zone of inhibition of each suture and buffer sample was measured (in millimetres) at three points along each 1 cm suture length (below the one knot end, at mid-length, and above the other end of the knot), and zones around the

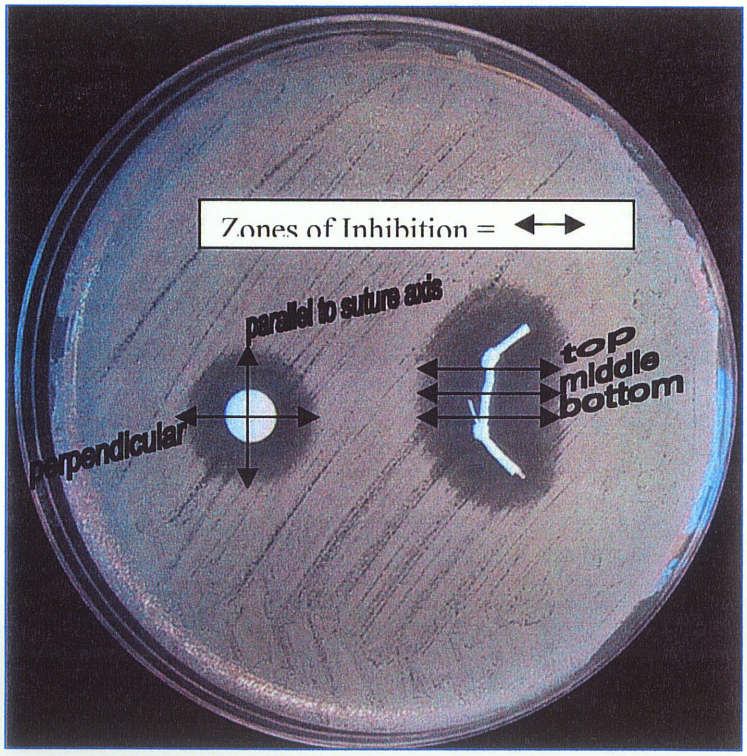


Figure 6: Diagram of Zone of Inhibition/Diameter Measurements

discs were measured at two points (both horizontally and vertically to the suture adjacent to the disc). See Figure 6, "Diagram of the Zone of Inhibition/Diameter Measurements".

METHOD

Zones of Inhibition

For this semi-quantitative modified zone diffusion procedure, six suture specimens were placed in buffer (separately) then exposed to *S.aureus* for each of the samples noted in List of Samples I, on p. 49. A 3.3 cm length of each suture specimen was tied with 0.5 cm knot ears at each end to prevent fraying. The remaining straight length of suture was the portion (1 cm) of the specimen used to measure the diameter of each zone of inhibition. Each suture specimen and a 10 _l aliquot of buffer adsorption solution were "spotted" onto the same agar plate, pre-swabbed with a 0.5 McFarland inoculum concentration of the test organism (*S. aureus*). To ensure as little variation between "spots" as possible, the buffer was spotted onto a standardized 0.5 cm disk, ensuring as uniform circumference as possible to measure the zones of inhibition.

Calibration and X MIC

In addition, a X MIC/cm value for each treated suture was estimated from the zones of a test of known concentrations of each drug spotted on a lawn of bacteria.

Test Organism

According to the National Committee for Clinical and Laboratory Standards (NCCLS), the test organism recommended for the agar diffusion assays, like the modified zone diffusion study outlined above, was American Type Culture Collection (ATCC)

strain *S. aureus* (ATCC 25923), whereas, the organism recommended for antimicrobial susceptibility tests was *S. aureus* (ATCC 29213) (NCCLS, 2000 a; NCCLS, 2000 b).

Organisms were prepared from a skim milk stock, which was plated onto Meuller-Hinton (MH) agar overnight. An isolated colony from this 24-hour plate was then plated onto another MH agar plate for 24-hours. Isolated colonies from the second agar plate were prepared with equivalent turbidity to the 0.5 McFarland standard (barium sulfate suspension solution) prepared according to the NCCLS guidelines (NCCLS, 2000 a; NCCLS, 2000 b). Comparison to this standard results in an inoculum concentration of 1×10^8 cfu/ml. In general, this turbidity resulted in consistent bacterial counts of 1×10^8 cfu/ml (±50 cfu/ml) for both ATCC strains of *S. aureus*.

Within 15 minutes after standardization of the inoculum suspension, a sterile cotton swab was used to apply the inoculum to the plate. The swab was rotated several times in the inoculum then pressed on the inside wall of the tube to remove excess suspension. The suspension was then plated onto Mueller Hinton agar plates using the swabbing procedure outlined in the NCCLS guidelines.

Minimum inhibitory concentration (MIC) tests were also conducted to determine whether or not the antibiotics were within range of the literature MIC found against the *S. aureus* strains used. MIC tests of each antibiotic in buffer solutions were conducted according to the NCCLS, "Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically; Approved Standard –Fifth Edition" macrodilution (tube) broth method (NCCLS, 2000 a).

Statistical Analysis

T-tests were conducted on the mean absorbance measurements of the lower and higher loading dosages of the suture materials. Significance was determined to be $p<0.05$.

RESULTS

Zones of Inhibition

Figures 7-9 show digital images of some sample plates of the 0.1% and 1.0% moxifloxacin coated sutures during the first three time periods. Figures 10-12 show the 0.85% and 8.5% clindamycin coated sutures during these three time periods. It is clear from these images, that the clindamycin coated suture zones of inhibition were larger than the moxifloxacin coated suture zones of inhibition. As shown in Figure 8, after 24 hours exposure to buffer the 0.1% samples of the moxifloxacin coated sutures did not demonstrate inhibitory zones; however, the 1.0% still showed small zones. It is interesting to note that microcolonies were found around the periphery of the 1.0% moxifloxacin coated suture clear zone on day 1. These microcolonies were likely a result of resistant strains to the *S. aureus* organism used. Figure 9 demonstrated that by Day 2 there were no zones for either moxifloxacin coated suture.

Figures 8-10 demonstrate the gradual release of the clindamycin coated sutures. Zones of inhibition were observed for the buffer and sutures at both loadings up to 24 hours after buffer exposure. After two days of buffer exposure, no zone of inhibition was found on the 0.85% clindamycin coated sutures (Figure 12) and by day 3 no zones were found on either of the clindamycin coated sutures. There were no microcolonies found in the clear zones of the clindamycin coated sutures.

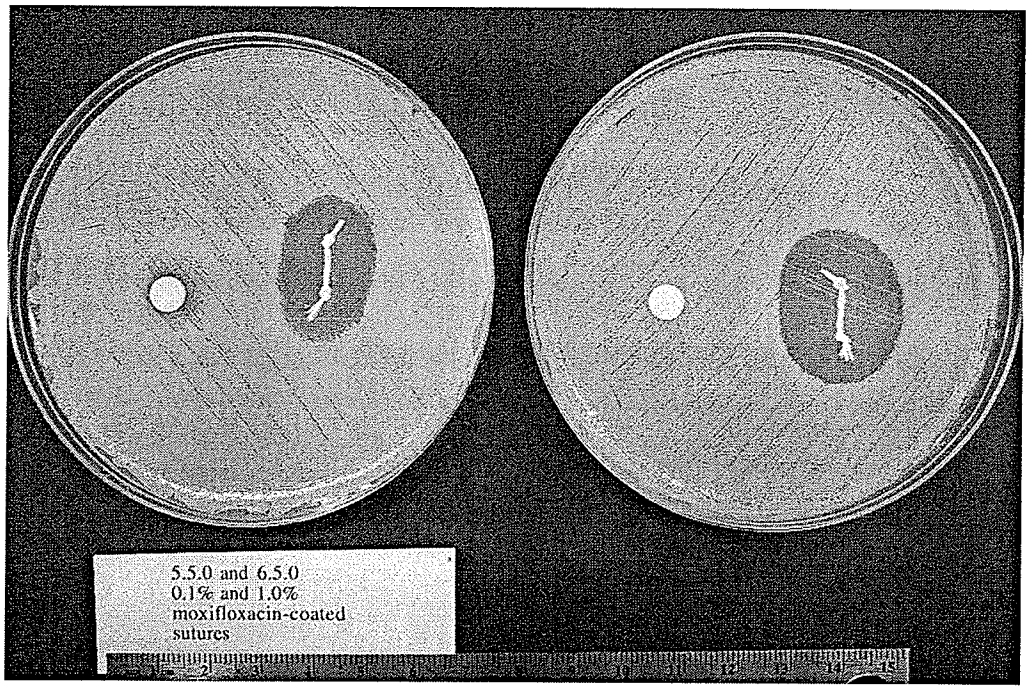


Figure 7: 0.1% and 1.0% moxifloxacin coated sutures at time zero

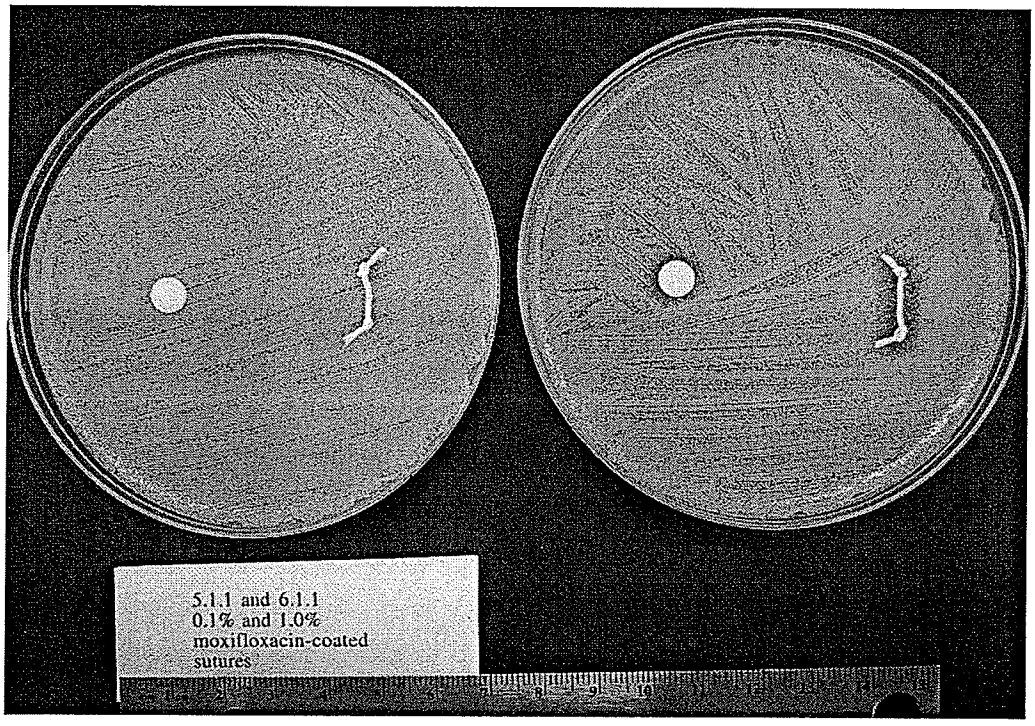


Figure 8: 0.1% and 1.0% moxifloxacin coated sutures after 1 day of buffer exposure

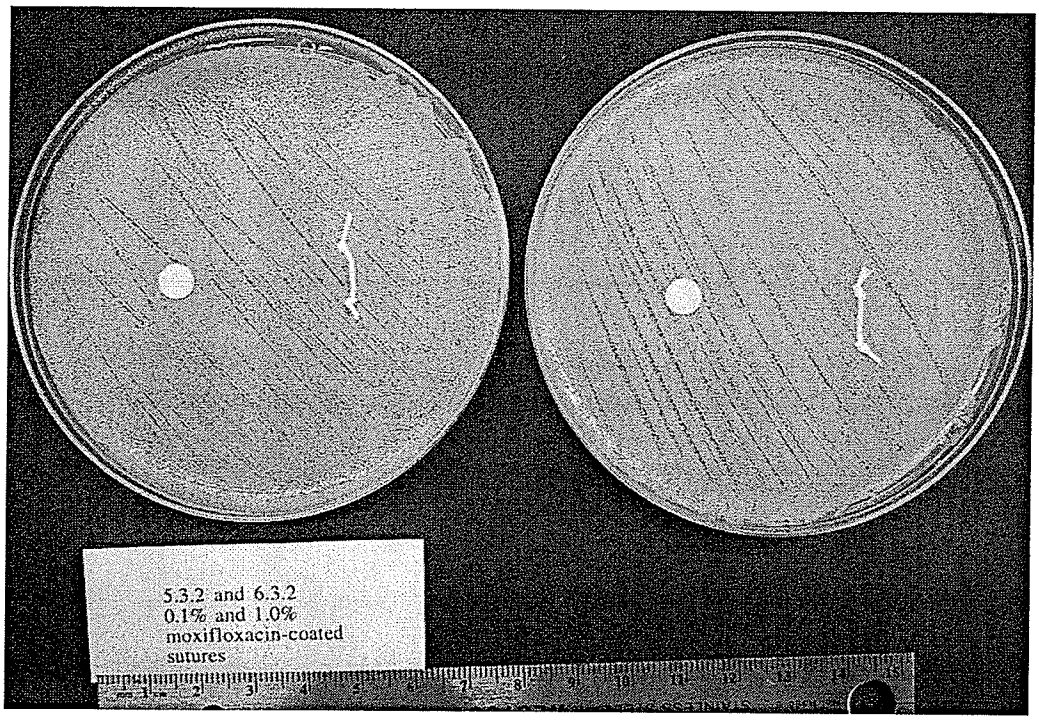


Figure 9: 0.1% and 1.0% moxifloxacin coated sutures after 2 days of exposure to buffer

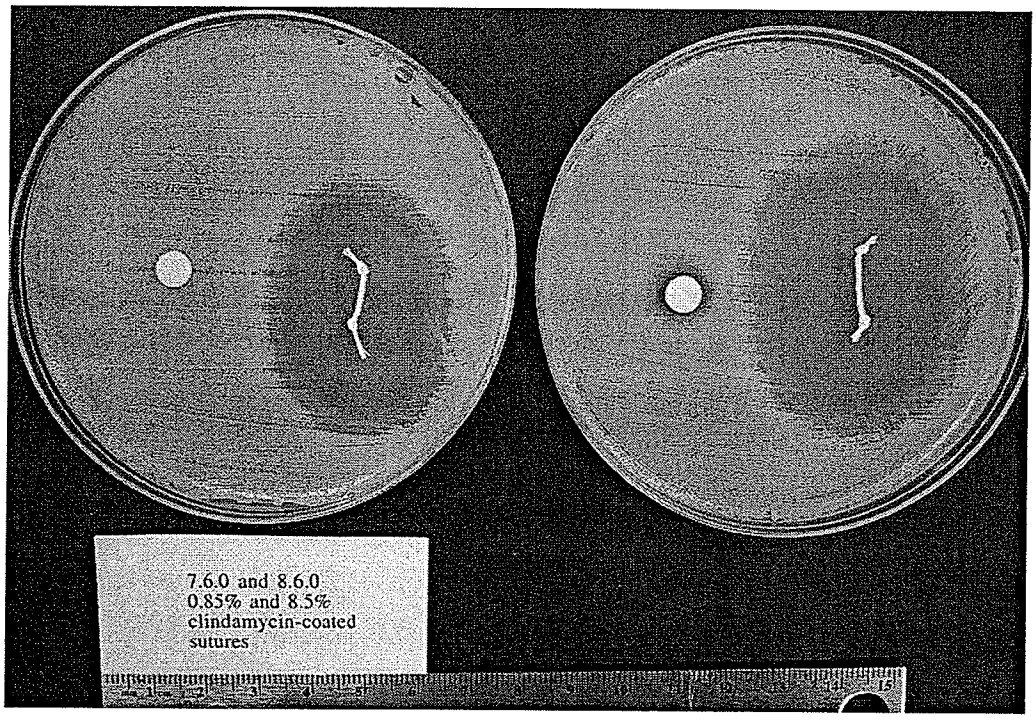


Figure 10: 0.85% and 8.5% clindamcyin coated sutures at time zero

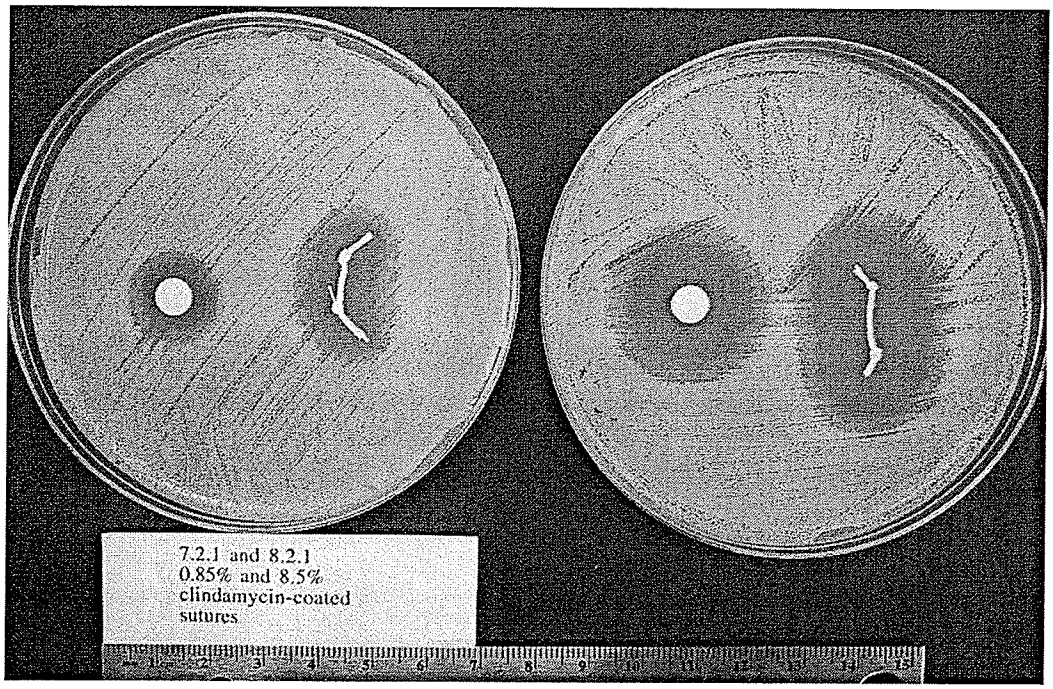


Figure 11: 0.85% and 8.5% clindamycin coated sutures after 1 day of buffer exposure

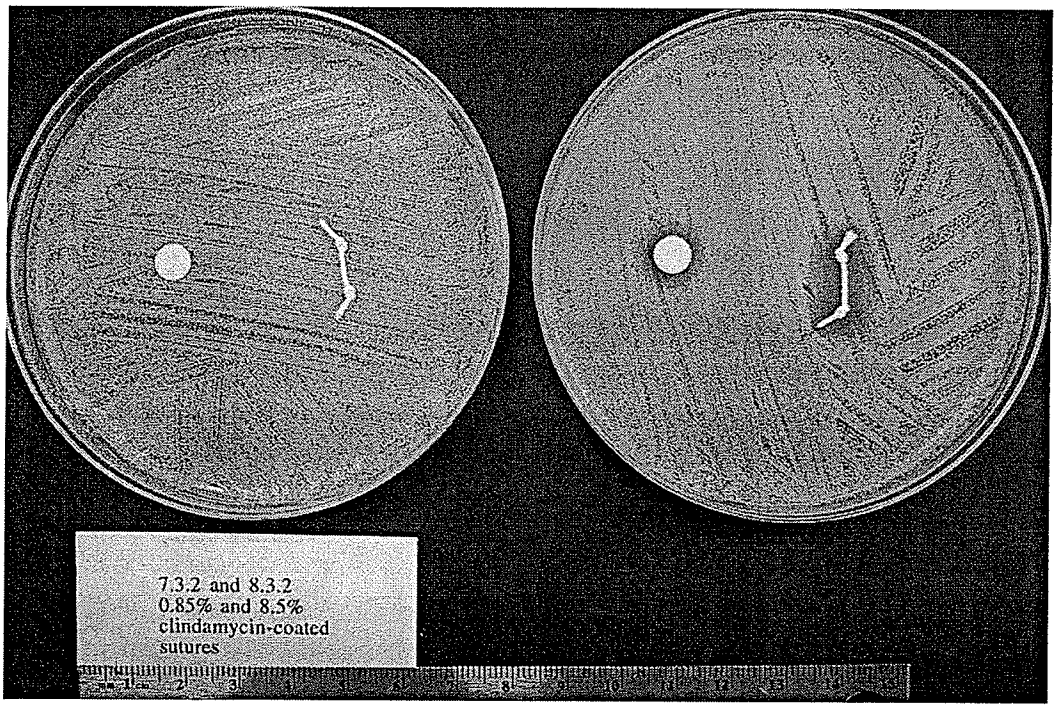


Figure 12: 0.85% and 8.5% clindamycin coated sutures after 2 days of exposure to buffer

The difference in zones between the moxifloxacin and clindamycin coated sutures was the result of at least two factors, the concentration gradient of each drug and the solubility of the drug. For example, clindamycin was imbedded into the coatings at a loading dosage 8.5 X greater than the moxifloxacin coatings. In addition the solubility of clindamycin in water was higher than that of moxifloxacin (since moxifloxacin required a 0.1N NaOH solution to dissolve completely in water whereas, clindamycin did not). Since the agar was an aqueous based medium, the solubility as well as the concentration gradient would have likely affected the size of the zones that resulted for each antibiotic coated suture material.

The means of the suture zones and buffer zones of inhibition of each sample at each time period were calculated. These results are found in Table 5 and Figure 13 and the results of the buffer zones of inhibition were provided in Table 6 and Figure 14. The results in Figure 13 show that the zones of inhibition were greater for the larger loading dosages of each antibiotic and the most concentrated adsorption of the sutures occurred at the point of contact at time zero then released over a two day period in the case of both moxifloxacin coated sutures and lower loading of clindamycin, and three days in the case of the higher loading of the clindamycin-coated sutures.

Similarly, the PBS solutions with the adsorbed suture coatings released the antibiotics in the 0.85% clindamycin and 1.0% moxifloxacin sutures within a two-day period and the 8.5% clindamycin coated sutures released its active agent within three days (Figure 14). However, no zones were observed for the buffer samples with either adsorbed coated (no antibiotic) or the 0.1% moxifloxacin coated sutures throughout the five day period.

Table 5: Summary of Suture Zones of Inhibition (mm)

Sample	time 0	day 1	day 2	day 3	day 4	day 5
Inoculated Samples:						
Braided Uncoated						
mean (N=6)	0	0	0	0	0	0
Std dev	0	0	0	0	0	0
Braided Coated						
mean (N=6)	0	0	0	0	0	0
Std dev	0	0	0	0	0	0
0.1% moxifloxacin						
mean (N=6)	14.0	0.0	0	0	0	0
Std dev	8.2	0.0	0	0	0	0
1.0% moxifloxacin						
mean (N=6)	20.2	7.5	0	0	0	0
Std dev	4.0	1.1	0	0	0	0
0.85% clindamycin						
mean (N=6)	32.0	15.7	0	0	0	0
Std dev	2.1	1.1	0	0	0	0
8.5% clindamycin						
mean (N=6)	41.5	26.8	8.9	0	0	0
Std dev	1.1	0.5	1.3	0	0	0
Inoculum Counts	**X 10EX8 cfu/ml**					
mean (N=14)	1.05	0.74	0.42	0.27	0.13	1.09
Std dev	0.3	0.16	0.13	0.07	0.07	0.17
Control Samples:						
Braided Uncoated						
mean (N=6)	0	0	0	0	0	0
Std dev	0	0	0	0	0	0
Braided Coated						
mean (N=6)	0	0	0	0	0	0
Std dev	0	0	0	0	0	0

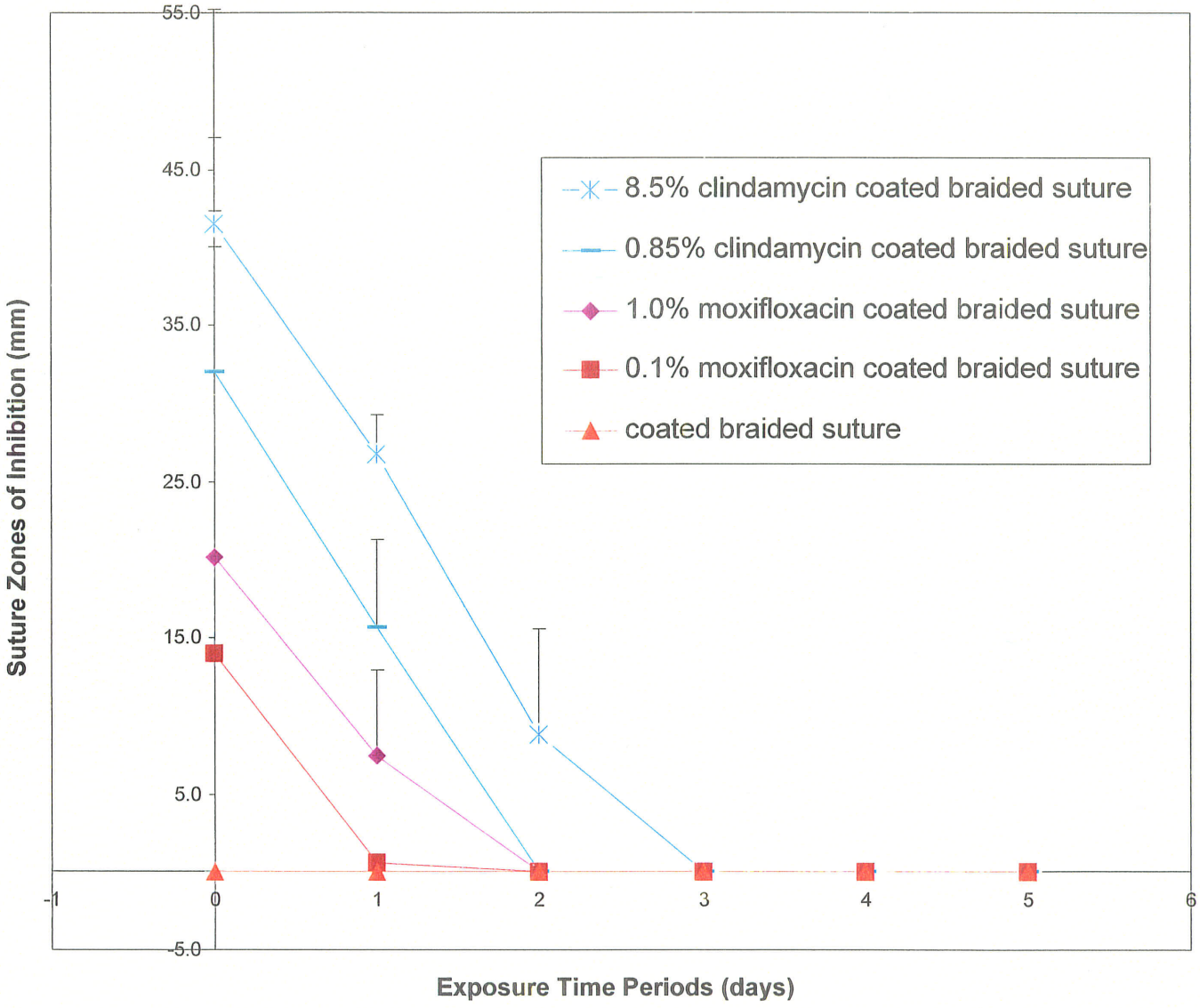


Figure 13: Zones of Inhibition of Clindamycin and Moxifloxacin Coated Sutures Exposed to PBS with Daily Buffer Changes

Table 6: Summary of Buffer Zones of Inhibition (mm)

Sample	time 0	day 1	day 2	day 3	day 4	day 5
Inoculated Samples:						
Braided Uncoated						
mean (N=6)	0	0	0	0	0	0
Std dev	0	0	0	0	0	0
Braided Coated						
mean (N=6)	0	0	0	0	0	0
Std dev	0	0	0	0	0	0
0.1% moxifloxacin						
mean (N=6)	0	0	0	0	0	0
Std dev	0	0	0	0	0	0
1.0% moxifloxacin						
mean (N=6)	0	8.7	0	0	0	0
Std dev	0	1.1	0	0	0	0
0.85% clindamycin						
mean (N=6)	0	15.5	0	0	0	0
Std dev	0	0.7	0	0	0	0
8.5% clindamycin						
mean (N=6)	0	27.5	10.1	0	0	0
Std dev	0	0.4	1.0	0	0	0
Inoculum Counts	**X 10EX8 cfu/ml**					
mean (N=14)	1.05	0.74	0.42	0.27	0.13	1.09
Std dev	0.3	0.16	0.13	0.07	0.07	0.17
Control Samples:						
Braided Uncoated						
mean (N=6)	0	0	0	0	0	0
Std dev	0	0	0	0	0	0
Braided Coated						
mean (N=6)	0	0	0	0	0	0
Std dev	0	0	0	0	0	0

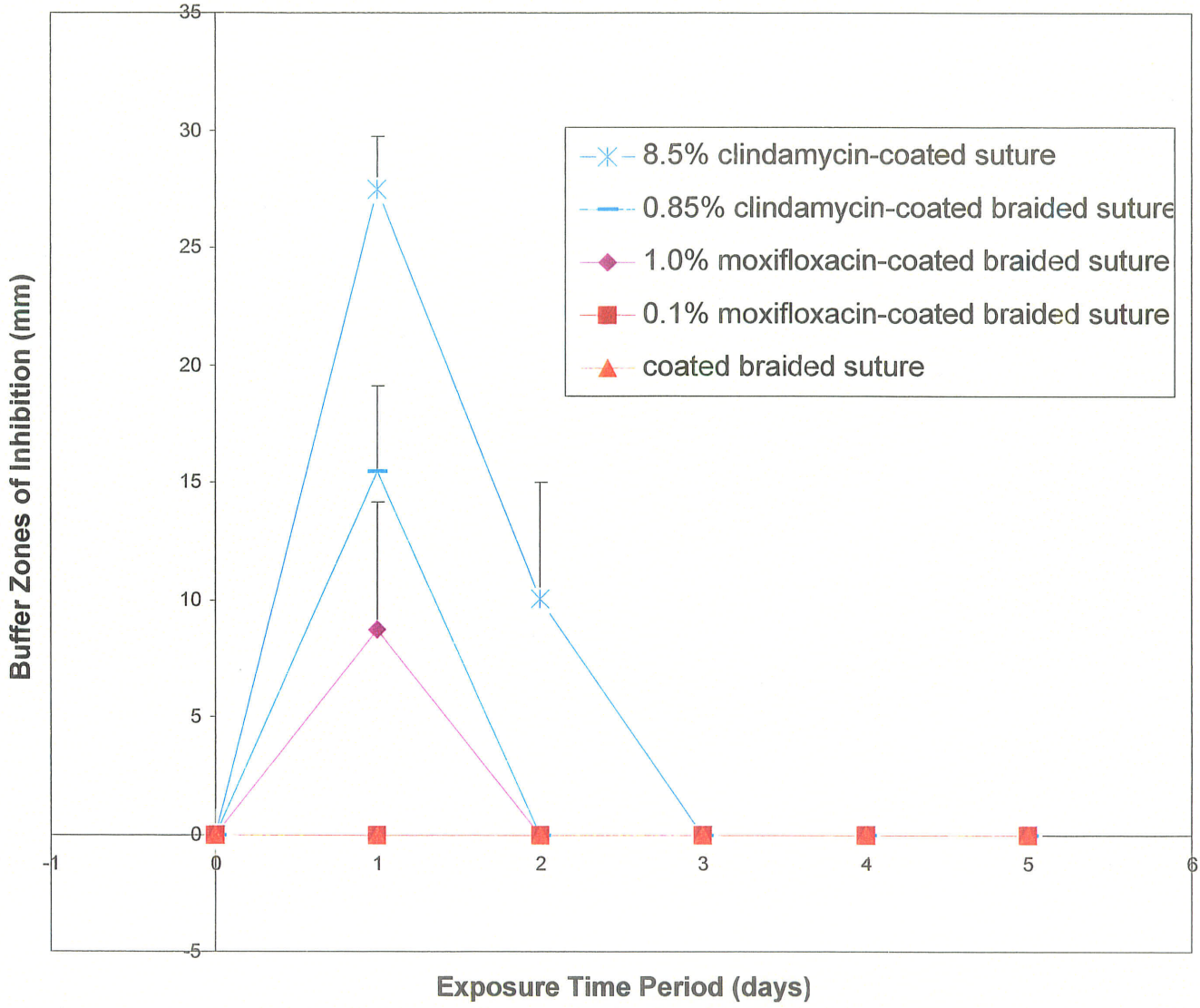


Figure 14: Zones of Inhibition of Buffer from Clindamycin and Moxifloxacin Coated Sutures Exposed to PBS with Daily Buffer Changes

Calibration and X MIC

In addition, the minimum inhibitory concentration (MIC) of moxifloxacin and clindamycin against the test organism was determined to be 0.030µg/ml and 0.488µg/ml, respectively, which are equivalent to the MIC ranges found in the current NCCLS guidelines. These MIC values were compared to the known concentration values/cm derived from the calibration curve. As shown in Figure 15, "Concentration and Zone Diameter Calibration Curve", the release of drug from the coated sutures was represented by an exponential release curve for each suture.

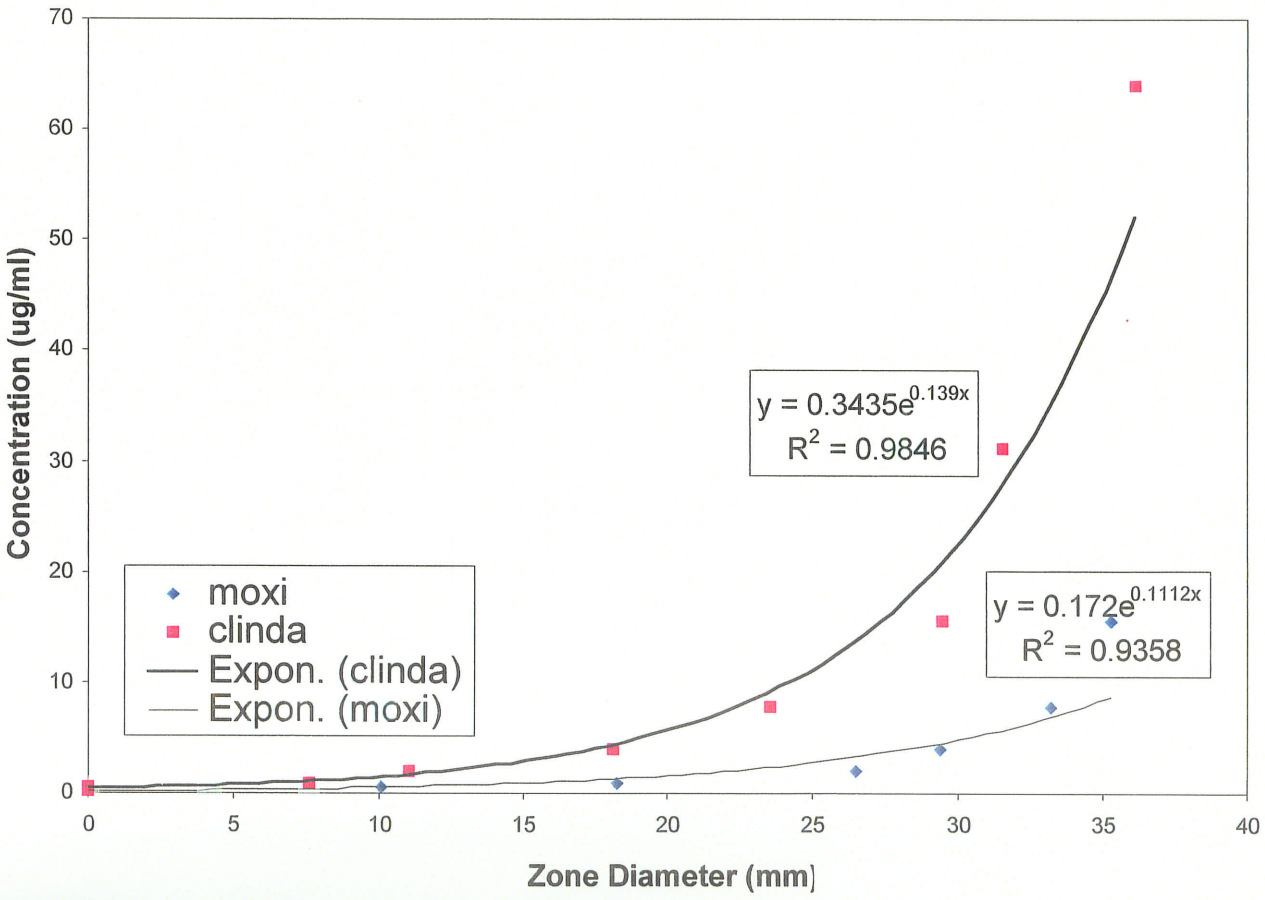


Figure 15: Concentration and Zone Diameter Calibration Curve

The results of the XMIC/cm (Table 7, "Summary of Suture Concentration and XMIC Values" and Table 8: "Summary of Buffer Concentration and XMIC Values") showed that the moxifloxacin actually demonstrated relatively more activity than clindamycin in terms of the MIC.

Sample	time 0 (mm)	Conc. (ug/ml)	day 1 (mm)	Conc. (ug/ml)	day 2 (mm)	Conc. (ug/ml)
0.1% moxifloxacin						
mean (N=6)	14.0	**0.20**	0.0		0.0	
Std dev	8.2	**0.12**	0.0		0.0	
XMIC/cm		**2.03**				
1.0% moxifloxacin						
mean (N=6)	20.2	**0.22**	7.5	**0.19**	0.0	
Std dev	4.0	**0.04**	1.1	**0.03**	0.0	
XMIC/cm		**2.17**		**1.89**		
0.85% clindamycin						
mean (N=6)	32.0	**0.54**	15.7	**0.43**	0.0	
Std dev	2.1	**0.03**	1.1	**0.03**	0.0	
XMIC/cm		**0.33**		**0.27**		
8.5% clindamycin						
mean (N=6)	41.5	**0.61**	26.8	**0.50**	8.9	**0.39**
Std dev	1.1	**0.02**	0.5	**0.01**	1.3	**0.06**
XMIC/cm		**0.38**		**0.31**		**0.24**

Table 7: Summary of Suture Concentration and XMIC Values

Sample	time 0 (mm)	day 1 (mm)	Conc. (ug/ml)	day 2 (mm)	Conc. (ug/ml)
0.1% moxifloxacin					
mean (N=6)	0	0		0	
Std dev	0	0		0	
1.0% moxifloxacin					
mean (N=6)	0	8.7	**0.19**	0	
Std dev	0	1.1	**0.02**	0	
XMIC/cm			**1.91**		
0.85% clindamycin					
mean (N=6)	0	15.5	**0.43**	0	
Std dev	0	0.7	**0.02**	0	
XMIC/cm			**0.26**		
8.5% clindamycin					
mean (N=6)	0	27.5	**0.50**	10.1	**0.40**
Std dev	0	0.4	**0.01**	1.0	**0.04**
XMIC/cm			**0.31**		**0.25**

Table 8: Summary of Buffer Concentration and XMIC Values

Statistical Analysis

T-tests were performed between the higher and lower loading dosages of each drug and among the lower loadings and higher loadings for all the days in which readings were found amongst both suture zones of inhibition and buffer zones of inhibition. The Table with the results for these tests was provided in Appendix A: T-Tests for Suture and Buffer Zones of Inhibition. The p-value was extremely small for most of the values. All values were significantly different ($p<0.05$).

DISCUSSION

H_{01}: *There is no significant difference in the diameter of the zones of inhibition against Staphylococcus aureus between coated braided, uncoated braided, or antibiotic-coated suture materials after each period of coating absorption in buffer.* **Rejected.**

The zone of inhibition tests resulted in significant differences amongst the drugs and the loading dosages. Higher loading resulted in greater zones of inhibition and more prolonged inhibitory effects. While the clindamycin sutures achieved larger zone diameters, the standardized curves demonstrated that the amount of drug released related to lower inhibitory values overall when MIC concentrations were considered. Therefore, moxifloxacin coated sutures indicated greater activity overall when compared to clindamycin coated sutures.

CHAPTER 5

METHODS, RESULTS & DISCUSSION

Experiment 2: Microbial Adhesion

VARIABLES

Independent Variables

The list of samples for this test was provided below in List of Samples III:

List of Samples III:

1) monofilament suture exposed to *S.aureus*
2) uncoated braided suture exposed to *S.aureus*
3) coated braided suture exposed to *S.aureus*
4) 0.85% clindamycin-coated suture exposed to *S.aureus*
5) 8.5% clindamycin-coated suture exposed to *S.aureus*
6) 0.1% moxifloxacin-coated suture exposed to *S.aureus*
7) 1.0% moxifloxacin-coated suture exposed to *S.aureus*
8) monofilament suture (without exposure to organism)
9) uncoated braided suture (without exposure to organism)
10) coated braided suture (without exposure to organism)
11) 0.85% clindamycin-coated suture exposed to S. aureus (no rinses)
12) 0.1% moxifloxacin-coated suture exposed to S.aureus (no rinses)

Dependent Variable

The level of adherence was the dependent variable. Level of adherence was judged according to three categories: few if any adherent bacteria (light adherence), moderate adherence and many adherent bacteria (or heavy adherence). The level of adherence was determined visually from the SEM images by the researcher. The images were also qualitatively compared based on the following characteristics: monofilament adhesion, multifilament adhesion, adhesion on antibiotic-treated sutures, and surface uniformity of the coatings.

METHOD

A 3.3 cm sample of each suture was knotted at each end to form a 1.0 cm linear strand in between the knot ears (prepared as described previously for the microbial tests). All specimens were sterilized by dipping into 70% isopropanol then left to air dry under laminar airflow hood unit. Two specimens from each sample were exposed to *S. aureus* (ATCC 29213). The bacteria were grown overnight from a stock culture and plated out twice before preparing the 0.5 McFarland Standard as per the procedure in Chapter 4: Experiment 1, in MH broth (1 ml of a 0.5 McFarland sample prepared in MH broth was added to 4 ml of MH broth) for 24 hours then rinsed prior to fixation. Two suture samples samples (0.1% moxifloxacin-coated and 0.85% clindamycin-coated sutures) were not rinsed prior to fixation, and three controls for each of the coated braided suture, uncoated braided suture and monofilament suture were not exposed to *S. aureus* and were placed in MH buffer (5 ml). The purpose of this test was to determine the degree at which the organism would adhere to the suture materials after a 24 hour period by viewing under a scanning electron microscope (SEM).

Fixation

After the 24-hour exposure period, each specimen was rinsed individually in two successive dips in 0.9 % sterile saline solution, followed by two successive dips into sterile water. The samples were then directly placed into a fixation medium (4% paraformaldehyde; 4% glutaraldehyde mixture of Karnovsky's Solution), covered, and left for 2 hours at room temperature. The purpose of the Karnovsky's solution was to cross-link the proteins of the bacteria such that their membranes and cell structure could remain in tact throughout the SEM preparation, coating and viewing protocol, involving

exposure to vacuum and therefore, great pressures upon the cells. Following this two-hour period the samples in the fixation medium were placed in the fridge (4°C) for 36 hours.

After the fixation period in glutaraldehyde, the samples were rinsed three times for five minutes each in phosphate buffered saline (0.25 M), followed by a 1% osmium tetroxide post-fixation for one hour. This post-fixation medium cross-links fatty acids on or in the cell (Todd in Aldrich & Todd, 1986). In addition, osmium tetroxide aids in improved contrast due to the fact that it is an electron dense stain (Todd in Aldrich & Todd, 1986). After exposure to osmium tetroxide, the specimens were rinsed three times each for 10 minutes with double distilled water. The final stage of this process involved dehydration of the samples in a series of ethanol rinses (from 30-100% ethanol) for 10 minutes each. The last rinse (100% ethanol) was repeated for 30 minutes to completely dehydrate the samples.

SEM preparation

Each sample was then mounted on an SEM metal stub with double–sided tape. The edges of each specimen were secured by carbon paint to ground the conductive material and to prepare the samples for sputter coating. All samples were sputter-coated with gold and palladium on an Edwards S150B Sputter Coater (Edwards High Vacuum, West Sussex, England). The cold plasma discharge of gold and palladium was used to coat the specimens and made them conductive under the electron beam of the electron-scanning microscope. This plasma coating achieved a thin, all-over coating of gold and palladium by electron-transfer from a gold and palladium disk to the sample via an electric current. The samples were viewed using a Cambridge Stereoscan 120 SEM

(Cambridge Instruments, Montreal, CA). The SEM photomicrographs of the specimens were qualitatively compared for bacterial adhesion between the different suture materials and for the surface properties of the coating.

RESULTS

The SEM photomicrographs are compared below in Figure 16-33.

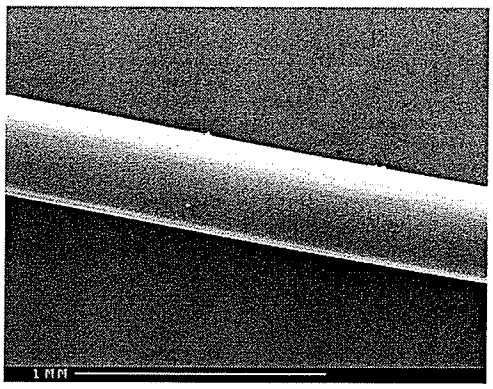


Figure 16: Monofilament Suture (40X)
No organism

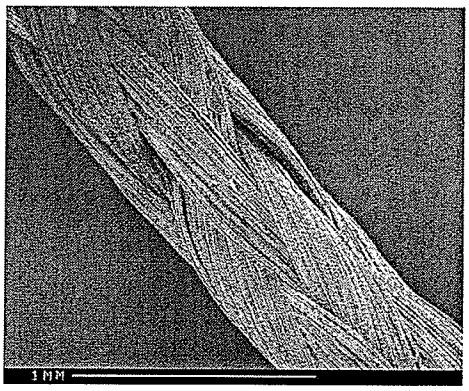


Figure 17: Braided Suture (40X)
No organism

Observations After 24-hr Exposure to *S.aureus*

The samples in the List of Samples III were each placed in duplicate into a test tube, numbered one through twelve corresponding to their respective sample numbers. Each tube also contained the MH broth medium and the inoculum, where applicable. After exposure, control samples in tubes 1-3 (exposed to organism) were cloudy as expected since these were the tubes with untreated sutures exposed to *S. aureus*. Samples in tubes 8-10 were all clear as expected since these were the untreated samples without exposure to *S.aureus* (ATCC 29213). Tubes 4, 5, & 11, which contained moxifloxacin-treated sutures and test organism, were all very clear. Tubes 6,7, & 12, which contained

the clindamycin-treated sutures, were all slightly cloudy but still fairly clear. The tubes containing the treated sutures were clear but in the tubes with the untreated samples the organism was able to reach contact inhibition. This indicated that the treated sutures were able to inhibit organisms after a 24-hour exposure to *S. aureus* (ATCC 29213).

Monofilament Adhesion

The *S. aureus* bacteria adhered lightly-moderately to the polyglyconate monofilament sutures after 24-hours of exposure (Figures 18 & 19). As shown in Figure 19, the *S. aureus* (ATCC 29213) strain grew mainly in small clusters (groups of two or three) on the surface of the monofilament.

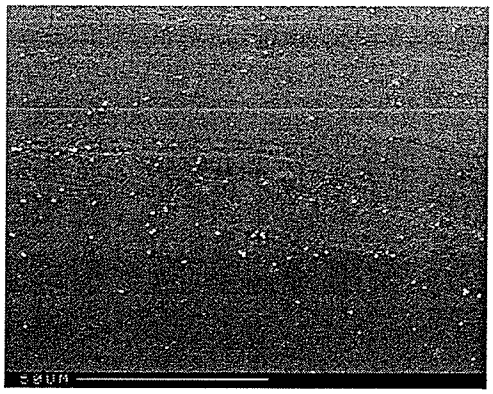


Figure 18: Monofilament with *S.au.* (600X)

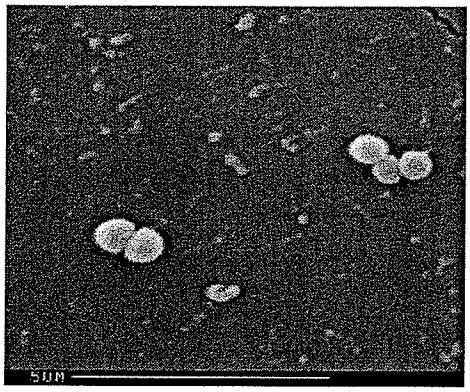


Figure 19: Monofilament with *S.au.* (8,000 X)

Multifilament Adhesion

Figure 20 shows an uncoated sample of multifilament braided suture material compared to Figure 21 of the same sample with *S.aureus*. Note that the bacterial adhesion to this multifilament structure included larger clusters of bacteria and a heavier-moderate bacterial growth compared to the monofilament suture. In general, it appeared that greater numbers of organism adhered to the multifilament compared to the monofilament.

Factors affecting this adhesion may include the larger surface area of the multifilament suture compared to the monofilament suture as well as the chemical structure of the filaments.

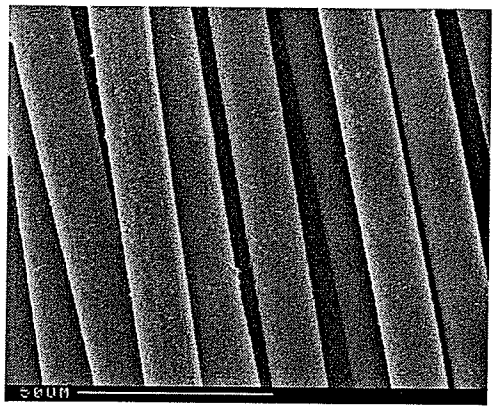


Figure 20: Braided uncoated, no organism (624X)

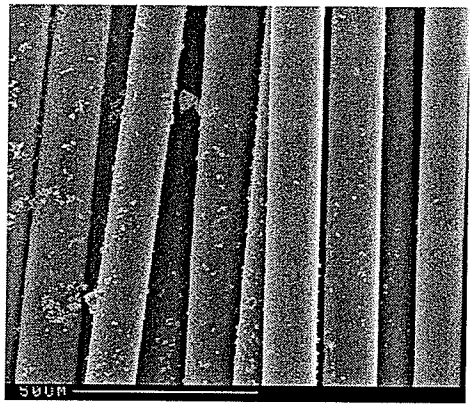


Figure 21: Braided uncoated with *S.au.* (611X)

Adhesion on Antibiotic-Treated Coated Braided Sutures

SEM photomicrographs of the antibiotic-treated coated braided sutures are shown in Figures 22-32. In total four 0.1% moxifloxacin coated sutures and two 1.0% moxifloxacin coated sutures were viewed under the SEM. No adhered bacteria could be found on the 0.1% moxifloxacin coated sutures (Figures 22 & 23) even at 2,550X magnification (Figure 23). It was surprising that few bacteria appeared to be adherent to the 0.1% moxifloxacin sutures as compared to the 1.0% moxifloxacin coated sutures (Figures 27 & 28). Also worth noting, was the difference in surface properties of the 0.1% and 1.0% coatings. For example, the 0.1% moxifloxacin coated surfaces appeared smoother than the 1.0% moxifloxacin coated sutures, which appeared to have some

denser, less uniform areas of the coating. This may have accounted for the higher degree of adhesion to the 1.0% moxifloxacin sutures.

In comparison, the clindamycin-coated sutures (Figures 26-32) showed significantly larger numbers of adherent bacteria than any of the other suture materials. Once again, four of the lower dosage and two sutures with the higher loading sutures were viewed. However, in this case again it was difficult to see a difference in numbers of bacteria adhered, but there were relatively many adhered bacteria (heavy adherence) on both the clindamycin coated sutures. It might be expected that there was no difference between the two loadings of clindamycin since this drug is a concentration-independent drug (usually no greater effect achieved beyond the MIC).

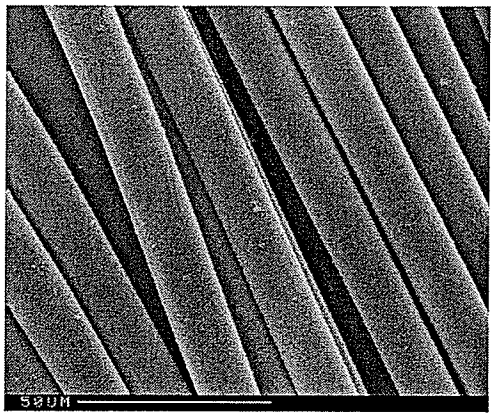


Figure 22: 0.1% moxi with *S.au.* (624X)

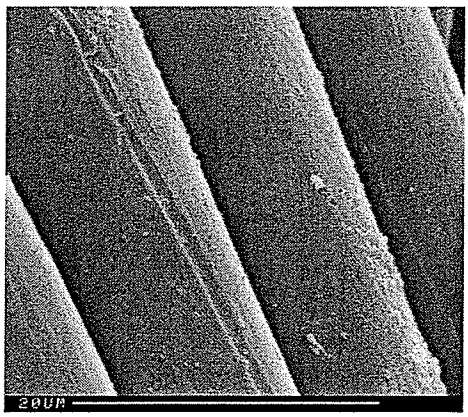


Figure 23: 0.1% moxi with *S.au.* (2,550X)

Furthermore, the bacteria adhered to the clindamycin-coated sutures grew in larger clusters than the bacteria on the moxifloxacin-coated sutures and the monofilament sutures. It was difficult to determine whether or not this effect was a factor of the drug, or the application of coating or both, since so few samples were viewed by SEM. For example, in Figure 26, the filaments that were most noticeably coated where bacteria adhered and also embedded themselves into the coating. This characteristic was also

demonstrated in Figures 31 and 32. The view in Figure 31 showed where the braided structure folds and also where it was more difficult to apply coating, which became apparent as the microscope zoomed out of the same area (Figure 32). In Figure 32 it was apparent that bacteria adhering were particularly abundant in the areas where the coating was also visible. Therefore, the coating may have been a factor in adhesion as well.

The last characteristic explored in the SEM images of the antibiotic-treated coated sutures was the rinsing method. The sutures were rinsed with four rinses after the sutures were exposed to *S.aureus* (ATCC 29213) for 24-hours. In Figure 26, the 0.85% clindamycin suture with rinses showed more bacteria than the same suture without any rinses, Figure 28 There were also no difference viewed between the 0.1% moxifloxacin sutures (all appeared free of bacteria), implying that the rinses had no effect of the numbers of bacteria adhered to the sutures after the 24-hour exposure period.

Coating Application

In Figure 33, a closer view of the variation in the surface texture of the coated multifilament sutures (5-10% by weight of the suture) was provided. The SEM photomicrographs showed that the surface qualities of the coating varied quite broadly from a rough, blotchy application (top, Figure 33 to perfectly smooth and almost invisible (middle, Figure 36) to the folds created in other areas (bottom, Figure 33. No fixation process was applied to the coated braided sutures viewed in Figure 33 accounting for the less soft, stiffer appearance of the specimens that were fixed in Figures 16-32, however, the same tendencies of surface roughness, blotchy character, smoothness, and folding were qualities that can also be seen in Figures 16-32. For example, Figures 25-28 show filaments actually stuck together due to the clumpy nature of the coating.

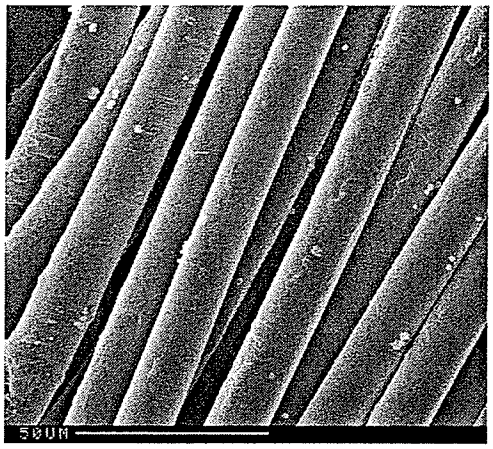


Figure 24: 1.0% moxi with *S.au.* (611X)

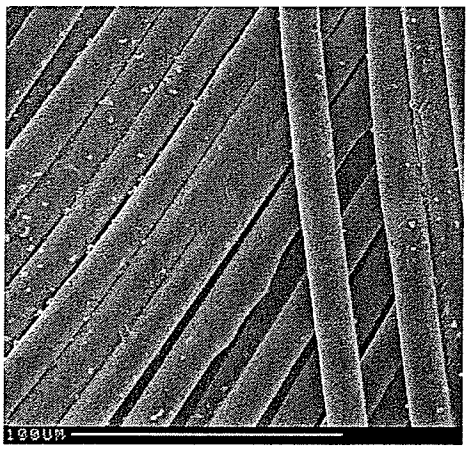


Figure 25: 1.0% moxi with *S.au.* (448X)

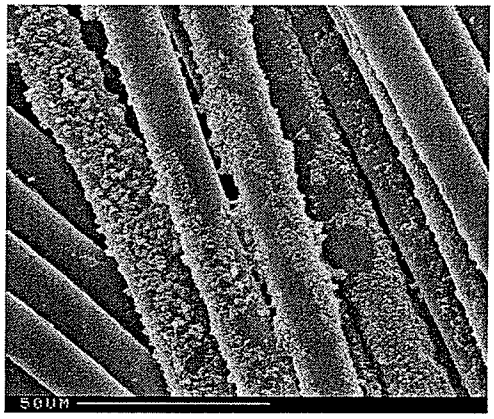


Figure 26: 0.85% clinda with *S.au.* (611X)

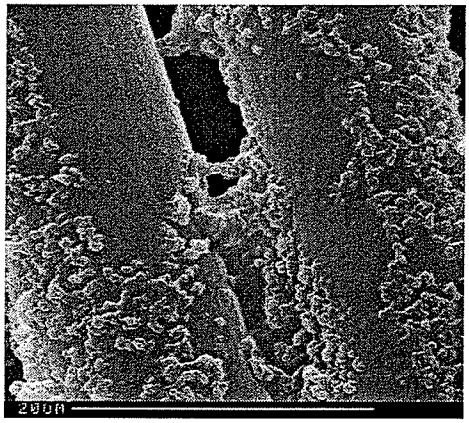


Figure 27: 0.85% clinda with *S.au.* (2,500X)

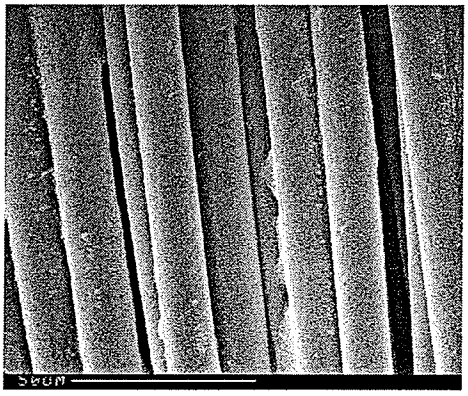


Figure 28: 0.85% clinda with *S.au.* No rinses (611X)

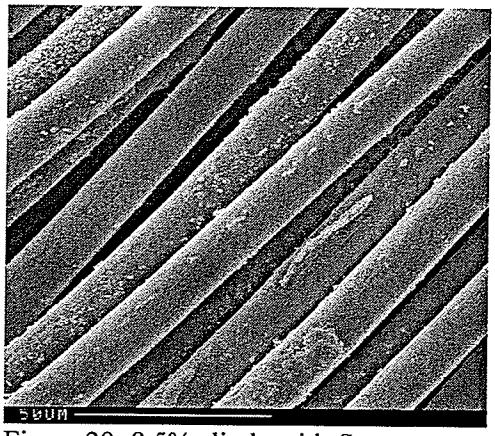


Figure 29: 8.5% clinda with *S.au.* (624X)

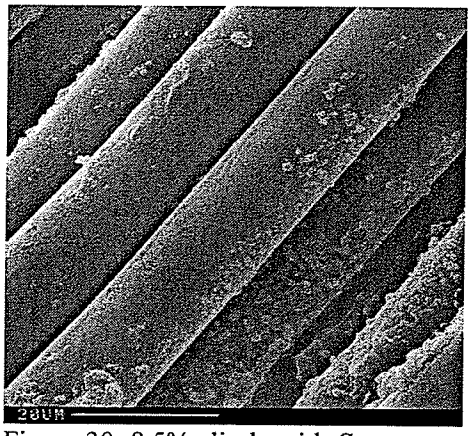


Figure 30: 8.5% clinda with *S.au.* (1,230X)

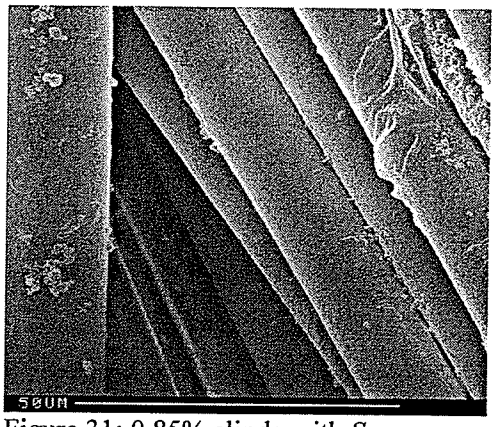


Figure 31: 0.85% clinda with *S.au.* (1,020X)

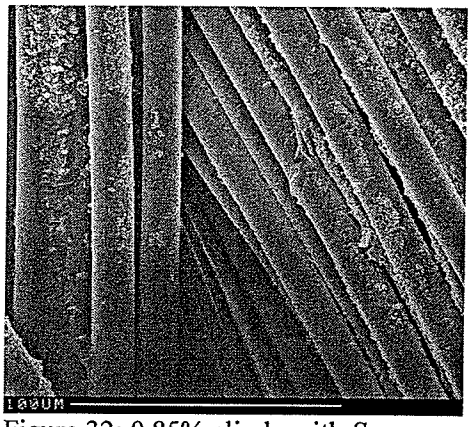
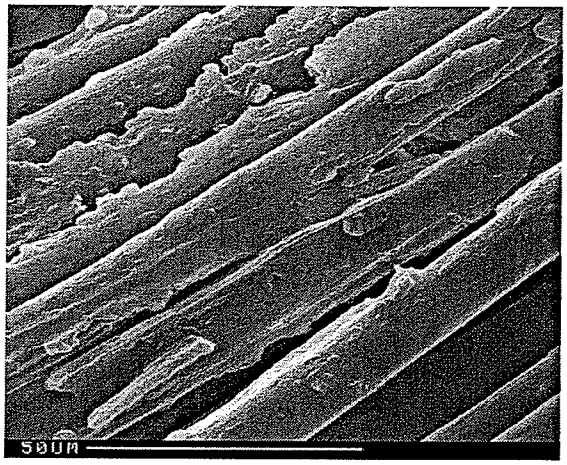
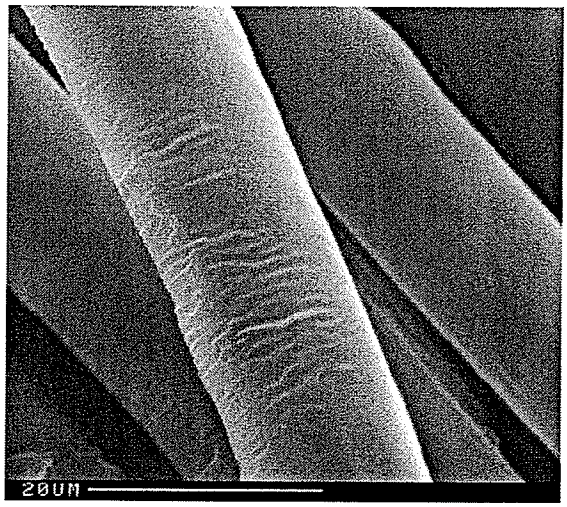


Figure 32: 0.85% clinda with *S.au.* (448X)





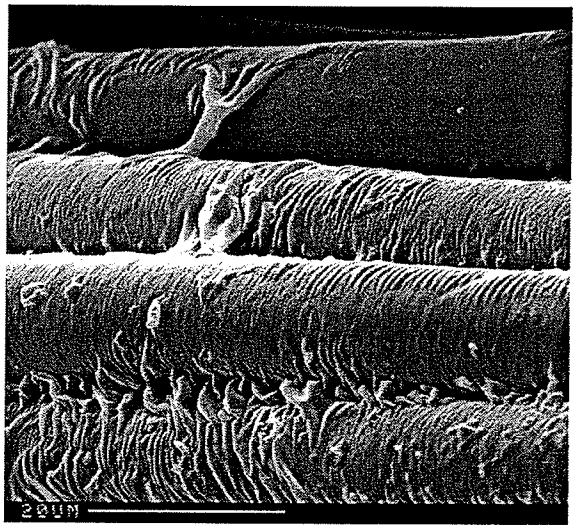


Figure 33: SEM photomicrographs of coated sutures showing variations in the amount and uniformity of the coating

DISCUSSION

H_{02} *There is no difference in the level of adherence of Staphylococcus aureus to each of the antibiotic-coated suture materials, coated suture materials (no antibiotic), uncoated braided suture, and monofilament suture of equal diameter.* **Rejected.**

It was determined from these images that the moxifloxacin-treated sutures were more effective at limiting bacterial adhesion than the clindamycin-treated sutures. The images also indicated that the coating might play a role in attracting microorganisms.

CHAPTER 6

METHODS, RESULTS & DISCUSSION

Experiment 3: Coating Adsorption

VARIABLES

Independent Variables

1) Absorption exposure time

There were eight exposure time periods, time 0, and from 1-7 days. Each day new buffer was replenished.

2) Type of suture coating

A list of these materials is given below List of Samples III.

List of Samples III

1) Braided suture coated with clindamycin 10xMIC
2) Braided suture coated with clindamycin 100xMIC
3) Braided suture coated with moxifloxacin 10xMIC
4) Braided suture coated with moxifloxacin 100xMIC
5) Control braided suture coating without antibiotic
6) Control braided suture without any coating

Dependent Variables

Coating Absorption

1) Level of Antibiotic Release (measured by UV-spectroscopy) and pH measurements. The specimens (N=8) were placed in buffer for the above contact times of the absorption study. At the end of the contact time, the buffer solutions were analyzed using a UV-spectrometer. The buffer solution pH was also analyzed after each time period. A standard calibration curve of each antibiotic in buffer was also determined.

2) Weight Loss Data (measured gravimetrically). The rinsed and dried suture samples were weighed before and after buffer treatment in an attempt to follow the rate of coating adsorption using a Satorius five-decimal weigh balance.

METHODS

For the Coating Adsorption test procedure, each suture material was cut into 60 mm lengths and exposed to 6 ml of buffer solution. The suture ends were knotted to avoid fraying in solution. The test tubes with buffer and sample were placed into a water bath at 35-37°C to simulate body conditions. After each of the eight exposure time periods (time 0, and 1-7 days), the suture was removed from the buffer solution. For the "time zero" measurements, the sutures were exposed to buffer as close to time zero as possible by immediately adding the buffer prior to a five second shake on an automatic shaker.

Absorbance Measures

Of the 6 ml buffer solution, 1.0 ml was tested for absorbance using the Spectronic 3000 UV-spectrometer and the pH was determined using the remaining 5 ml. The UV-absorbance readings of each antibiotic-embedded suture material was carried out at the peak maximum wavelength of each antibiotic. The peak maximum wavelengths of clindamycin and moxifloxacin were determined at 200 nm and 294 nm, respectively.

Calibration and XMIC

A standard calibration curve was devised from known concentrations of each antibiotic. Due to the low wavelength which clindamycin was measured, it was determined that a lower zero reading resulted when a distilled water (adjusted to pH 7.4 using 0.01 N NaOH) was used as the "buffer" as opposed to phosphate buffered saline. This enabled greater sensitivity to clindamycin at lower concentrations. Therefore, the distilled water adjusted to pH 7.4 was used as the "buffer" for both the calibration curve and suture adsorption periods. The moxifloxacin proposed no such difficulties at its peak wavelength at 294 nm. Therefore, the calibration curve and suture exposure periods were all conducted in 0.1 M phosphate buffered saline for the moxifloxacin coated sutures.

It was possible to identify and quantify the antibiotics by using Beer Lambert's law. This law expresses the relationship between absorption (A) and concentration (c), measured in moles per liter:

$$A = \varepsilon b c$$

Where, the two constants are ε, the molar absorptivity in L $cm^{-1}mol^{-1}$, and b is the path length of radiation in centimeters (Skoog, West & Holler, 1992). Therefore, a change in concentration was determined by a change in absorbance. The absorption of antibiotic in each sample of adsorbed buffer was recorded by the UV-spec after the exposure time periods. The average concentration for each suture sample type at the predetermined time periods was then calculated. A calibration curve was prepared of known concentrations of drug. The concentrations of the drug in buffer were then

estimated. These concentration values were also compared to the MIC values of each drug.

pH

The pH of each buffer solution at each time period was also measured using a pH meter. The average pH of each buffer solution was calculated at each time period. After measuring the absorption, the sutures were rinsed five times with distilled water and freeze-dried.

Linear Density

The sutures were then weighed (w_f). The dry weight before (w_i) and after (w_f) each adsorption period were compared. The weight loss will be calculated as a percentage of the original weight of the corresponding specimen:

$$w_{l\%} = (w_i - w_f / w_i) \text{ X } 100\%$$

An average weight loss for each suture type was recorded. Overall changes in linear density (in tex) were also calculated both before and after exposure to buffer, as follows:

Linear density (tex) = sample weight (g) X 1000 /sample length (m)

Due to the fact that a salt residue from the PBS solution may have resulted in an artificially higher weight, care was taken to rinse the suture samples five times with distilled water before freeze drying and weighing. To ensure that a salt residue did not adhere to the coating, a preliminary trial was conducted with the coated and uncoated sutures (without antibiotic) exposed to buffer after various rinses and compared to sutures of the same kind that had not been exposed to PBS.

Statistical Analysis

T-tests were conducted on the mean absorbance measurements of the lower and higher loading dosages of the suture materials. Significance was determined at $p<0.05$.

RESULTS

Absorbance Measures

The UV absorption measurements for clindamycin-coated sutures were illustrated below in Figure 34. The measured UV-absorbance values were normalized with respect to the buffer (i.e. uncoated untreated suture) before plotting against exposure time. In other words, the original absorbance for the braided suture (i.e. the absorbance of the phosphate buffer) was subtracted from all other measurements. The adjustments for these measurements are summarized in Table 9: Clindamycin UV-Absorbance Summary Data. The complete summary of this adsorption study (including absorbance, pH, and linear density measurements) can be found in Appendix B.

The absorbance results for the moxifloxacin-coated sutures were illustrated graphically in Figure 35. As with the Figure 34, the results were normalized with the uncoated braided suture material. The adjustments for these measurements are summarized in Table 10: Moxifloxacin UV-Absorbance Summary Data. The complete summary (absorbance, pH, and linear density measurements) of the moxifloxacin absorption study can be found in Appendix C.

Table 9: Clindamycin UV-Absorbance Summary Data

Average Means (N=8) of Original Measurements								
suture type:	time 0	day 1	day2	day 3	day4	day 5	day 6	day 7
uncoated braided								
mean	0.044	0.113	0.011	0.026	0.012	0.014	0.009	0.009
std deviation	0.012	0.014	0.002	0.002	0.002	0.003	0.001	0.002
coated braided								
mean	0.060	0.151	0.018	0.026	0.015	0.019	0.014	0.015
std deviation	0.011	0.017	0.003	0.003	0.004	0.004	0.003	0.003
0.85% clindamycin-coated								
mean	0.056	0.154	0.025	0.026	0.012	0.027	0.012	0.011
std deviation	0.012	0.005	0.004	0.003	0.002	0.003	0.001	0.002
8.5% clindamycin-coated								
mean	0.189	0.307	0.039	0.026	0.015	0.024	0.013	0.014
std deviation	0.013	0.020	0.008	0.004	0.003	0.004	0.002	0.003
Normalized Data								
suture type:	time 0	day 1	day2	day 3	day4	day 5	day 6	day 7
uncoated braided (zero)	0	0	0	0	0	0	0	0
coated braided								
mean	0.016	0.038	0.007	0.000	0.003	0.005	0.005	0.006
std deviation	0.003	0.004	0.001	0.000	0.001	0.001	0.001	0.001
0.85% clindamycin-coated								
mean	0.012	0.041	0.014	0.000	0.000	0.013	0.003	0.002
std deviation	0.003	0.001	0.002	0.000	0.000	0.001	0.000	0.000

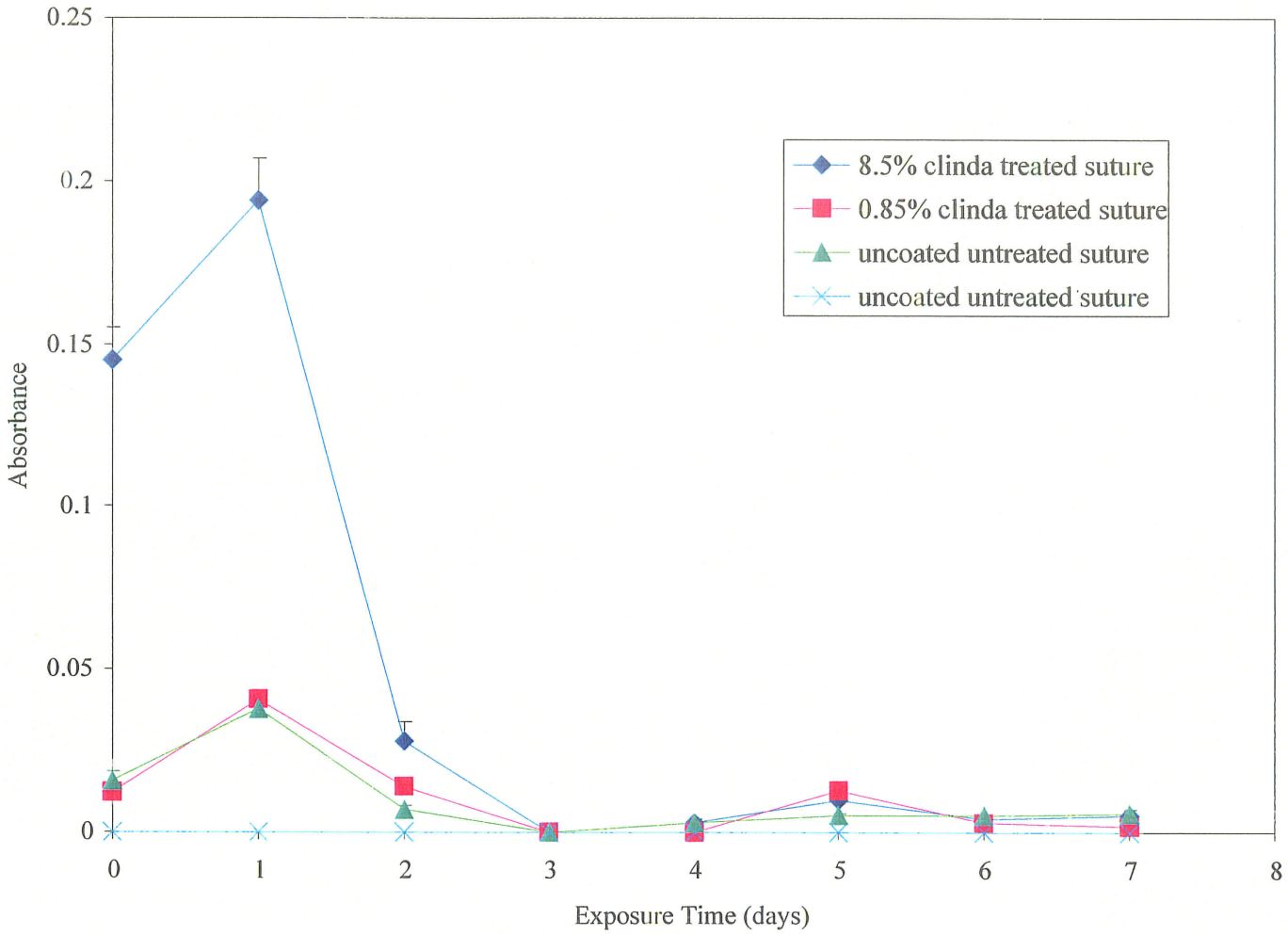


Figure 34: Normalized Absorbance of Clindamycin Coated Sutures Over a Week Under Sink Conditions at 200 nm

Table 10: Moxifloxacin UV-Absorbance Summary Data

Average Means (N=8) of Original Measurements								
suture type:	time 0	day 1	day2	day 3	day 4	day 5	day 6	day 7
uncoated braided								
mean	0.021	0.009	0.012	0.013	0.012	0.012	0.011	0.013
std deviation	0.001	0.004	0.001	0.001	0.001	0.001	0.001	0.001
coated braided								
mean	0.083	0.026	0.044	0.042	0.043	0.052	0.051	0.057
std deviation	0.011	0.003	0.006	0.005	0.015	0.008	0.013	0.006
0.1% moxifloxacin-coated								
mean	0.096	0.021	0.045	0.042	0.035	0.040	0.048	0.056
std deviation	0.010	0.004	0.007	0.008	0.004	0.007	0.010	0.009
1.0% moxifloxacin-coated								
mean	0.116	0.029	0.043	0.035	0.046	0.044	0.045	0.047
std deviation	0.009	0.006	0.007	0.005	0.007	0.005	0.006	0.005
Normalized Data								
suture type:	time 0	day 1	day2	day 3	day4	day 5	day 6	day 7
uncoated braided (zero)	0	0	0	0	0	0	0	0
coated braided								
mean	0.062	0.018	0.032	0.029	0.029	0.040	0.040	0.044
std deviation	0.008	0.002	0.004	0.004	0.010	0.007	0.010	0.004
0.1% moxifloxacin-coated								
mean	0.075	0.013	0.033	0.029	0.023	0.028	0.037	0.043
std deviation	0.008	0.002	0.005	0.006	0.002	0.005	0.007	0.007
1.0% moxifloxacin-coated								
mean	0.095	0.020	0.030	0.022	0.034	0.032	0.034	0.034
std deviation	0.008	0.004	0.005	0.003	0.005	0.004	0.004	0.004

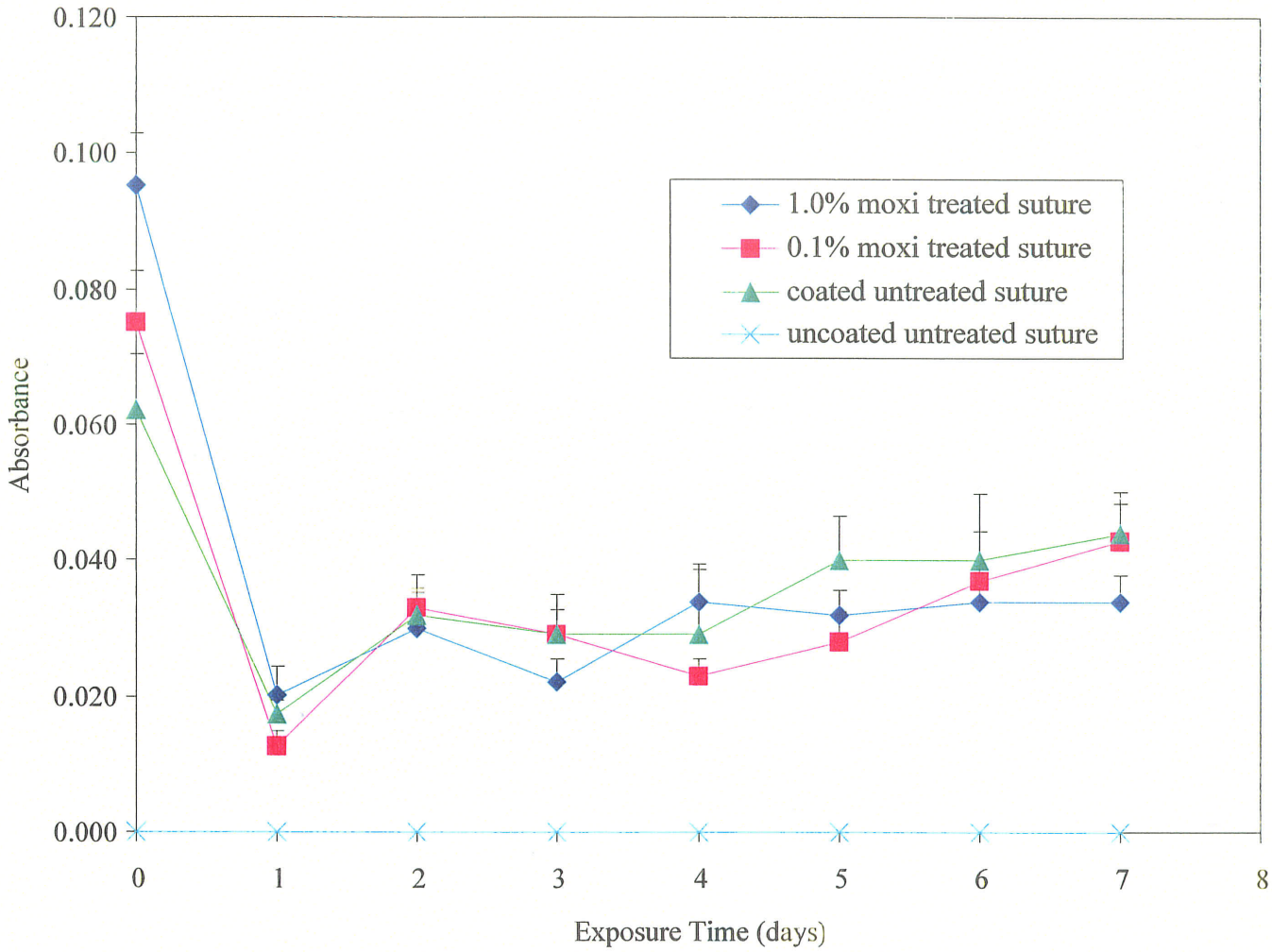


Figure 35: Normalized Absorbance of Moxifloxacin Coated Sutures Over a Week Under Sink Conditions at 294 nm

Figures 34 & 35 demonstrated that a higher loading of each antibiotic generally produced a higher absorbance compared to the sutures with a lower dose of the same antibiotic and a difference could be seen between coated sutures without antibiotic and coated sutures with antibiotic for the first two days. Thereafter, as with the zone diffusion tests, the

antibiotic seemed to be immeasurable. The clindamycin absorbance results suggested a more gradual release of antibiotic than the results for moxifloxacin due to the fact that the absorbance measures increased after a day in buffer compared to moxifloxacin sutures which released most of the coating at time zero. Like in the zone diffusion tests there were marked differences between the two loading dosages for clindamycin, however, less difference was apparent between the lower loading dosage and the coated suture without antibiotic. Differences between the moxifloxacin coated sutures and sutures coated (without drug) were seen only at time zero, thereafter there was little to no difference between measurements.

Calibration and XMIC

Calibration curves were derived for clindamycin and moxifloxacin at their respective peak wavelengths (as shown in Appendices D and E, respectively). Each equation of the line from the graph was used to calculate the concentration values for each 6 cm piece of suture. The absorbance values per specimen (6 cm) for each of the antibiotic treated sutures were adjusted by subtracting the absorbance for the coated suture samples without any antibiotic. Any negative numbers calculated in this adjustment were considered a zero value. The equation of the line for the clindamycin calibration curve was y=0.0194x (see Appendix D) and for the moxifloxacin calibration curve was y=0.0838x (see Appendix E).

Next, these concentration values were converted to "concentration per cm" by dividing the concentration by the suture length, then dividing this number by the MIC to result in a "X MIC/cm" value determined for each antibiotic against the standard strain of *S. aureus.* For clindamycin this MIC value was 0.488 μg/ml and for moxifloxacin the

MIC was found to be 0.030 μg/ml. Table 11 summarizes the Conversions of Absorbance to Concentration and X MIC for Clindamycin Hydrochloride and Table 12 summarizes the Conversions of Absorbance to Concentration and X MIC for Moxifloxacin.

Table 11: Conversions of Absorbance to Concentration and X MIC for Clindamycin Hydrochloride

	time 0	day 1	day 2	day 3	day 4	day 5	day 6	day 7
8.5% clinda Abs/ 6 cm	0.129	0.156	0.021	0.000	0.000	0.005	0.000	0.000
8.5% clinda ug/ml / 6 cm	6.649	8.041	1.082	0.000	0.000	0.258	0.000	0.000
8.5% clinda ug/ml /cm	1.108	1.340	0.180	0.000	0.000	0.043	0.000	0.000
XMIC (per cm)	2.271	2.746	0.370	0.000	0.000	0.088	0.000	0.000
0.85% clinda/ 6 cm	0.000	0.003	0.007	0.000	0.000	0.008	0.000	0.000
0.85% clinda ug/ml / 6 cm	0.000	0.155	0.361	0.000	0.000	0.412	0.000	0.000
0.85% clinda ug/ml /cm	0.000	0.026	0.060	0.000	0.000	0.069	0.000	0.000
XMIC (per cm)	0.000	0.053	0.123	0.000	0.000	0.141	0.000	0.000

Table 12: Conversions of Absorbance to Concentration and X MIC for Moxifloxacin

	time 0	day 1	day 2	day 3	day 4	day 5	day 6	day 7
1.0% moxi Abs/ 6 cm	0.033	0.003	0.000	0.000	0.005	0.000	0.000	0.000
1.0% moxi ug/ml / 6 cm	0.394	0.033	0.000	0.000	0.057	0.000	0.000	0.000
1.0% moxi ug/ml /cm	0.066	0.005	0.000	0.000	0.009	0.000	0.000	0.000
XMIC (per cm)	2.188	0.182	0.000	0.000	0.315	0.000	0.000	0.000
0.1% moxi/ 6 cm	0.013	0.000	0.001	0.000	0.000	0.000	0.000	0.000
0.1% moxi ug/ml / 6 cm	0.155	0.000	0.013	0.000	0.000	0.000	0.000	0.000
0.1% moxi ug/ml /cm	0.026	0.000	0.002	0.000	0.000	0.000	0.000	0.000
XMIC (per cm)	0.862	0.000	0.075	0.000	0.000	0.000	0.000	0.000

As a result of the 8.5% clindamycin suture absorbance measurements, values were recorded at four time periods (time 0, day 1, day 2 and day 5). The 8.5% clindamycin coated sutures absorbed the most overall and achieved about 2.3X the MIC at time zero and 2.7 X the MIC at day 1. At day 2, the absorption drastically dropped to about 0.4X the MIC. At days 3 and 4 the absorbance tests were not successful in recording absorbance values for the antibiotic, however, on day 5 once again readings were successful but drug concentration has drastically dissipated to about 0.1 X the MIC value.

The 0.85% clindamycin-coated sutures do not show a general decrease in concentration over time but rather an increase. However, the readings are so small that the values are nearly negligible (i.e. X 0.05 MIC on day 1, X 0.1 MIC on day 2 and on day 5). Due to the fact that clindamycin was measured at such a low wavelength on the visible spectrum (200 nm), it is possible that even such things as dust and debris in the solutions could have been absorbed and in turn interfered with the absorbance readings for the drug. This possibility, in conjunction with very low concentrations of clindamycin could have obscured these values.

The values for the 1.0% moxifloxacin coated sutures were recorded for three time periods (time 0, day 1, day 4). The 1.0% moxi sutures peaked at time 0 at 2.2 X MIC then drastically seemed to drop at day 1 at about 0.2 X MIC. However, at day 4 a value of 0.3 X MIC was recorded. The 0.1% moxifloxacin sutures showed a similar decrease in concentration over time similar to the 8.5% clindamycin and 1.0% moxifloxacin sutures. At time zero, the 0.1% moxifloxacin sutures peaked but only at about 0.9 X MIC and little moxifloxacin was measured thereafter. The downward trend after time zero also

apparent in the microbial zone diffusion study, however, the zone diffusion test was more reliable at measuring the differences in the absorbed antibiotic over the course of its release.

It was assumed in adjusting the measures for the comparison between absorbance and concentration that the same amount of coating was released from the coated (untreated) specimens as the coated treated specimens. However, this may not be the case. Variations in the add-on of the coating at various sections of the suture could have accounted for some differences in amount of coating absorbed. Another possibility is that the coating interfered with the antibiotic absorption. The low variation (evidenced by the high R^2 values) of the calibration curve measurements for each antibiotic (See Appendices D & E) may imply that the coating or the coating in combination with the drugs has led to some chemical change that made the release of the adsorbed coating immeasurable. It could also be that the coating add-on of the sutures was higher than the add-on of the coated treated sutures (which were all applied in separate batches).

pH

The pH of the absorbed buffers during the clindamycin-coated and moxifloxacin-coated suture adsorbance studies did not deviate greatly, for any of the sutures types, from the target of pH 7.4. The total mean pH for the group of sutures in the clindamycin absorption study, for all the exposure periods, was 7.44±0.11 (see Appendix B), which results in a coefficient of variation of 1.48%. Similarly, the total mean pH for the group of sutures in the moxifloxacin absorption study was 7.39±0.033 (see Appendix C), which results in a coefficient of variation of 0.40%.

Linear Density

The changes in linear density amongst the sutures during the adsorption periods resulted in a general increase in the change in linear density was found with coated sutures in the clindamycin group, however, after day five in the moxifloxacin group the changes dropped unexpectedly. See Figures 36 and 37 below.

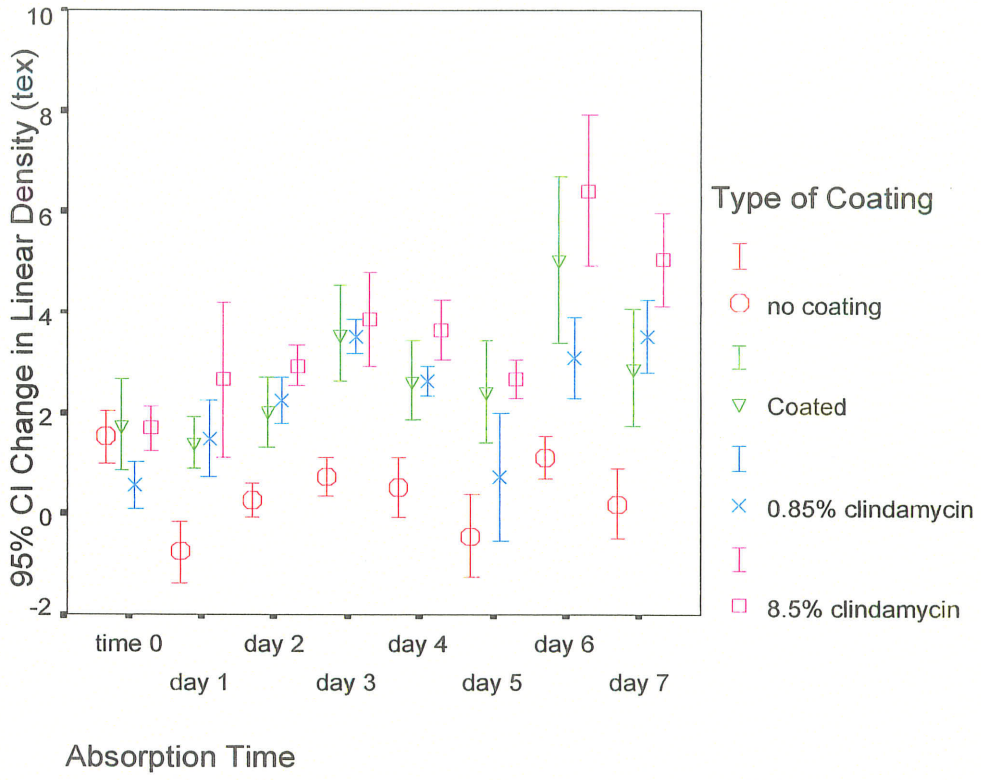


Figure 36: Change in Linear Density over the Absorption Period for Sutures in the Clindamycin Absorbance Study

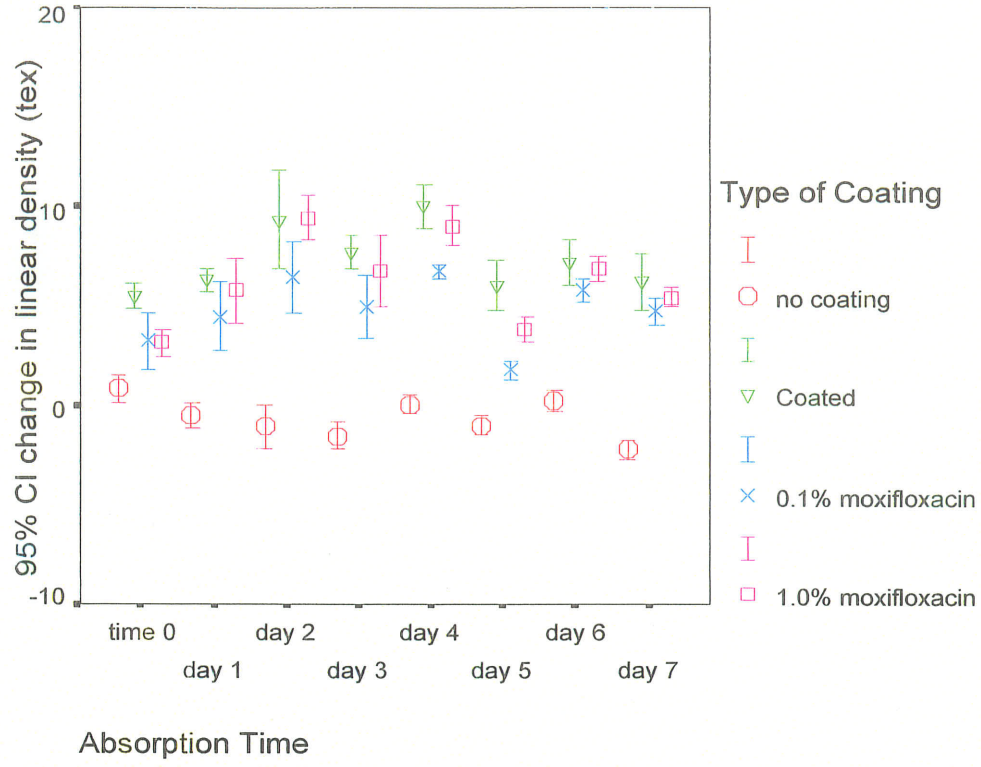


Figure 37: Change in Linear Density over the Absorption Time Period for Sutures in the Moxifloxacin Absorbance Study

Statistical Analysis

The t-tests of the absorbance measurements demonstrated that there were significant differences between the moxifloxacin and clindamycin sutures at the different time periods. These tests were provided in Appendix F.

DISCUSSION

H_{03}: There is no significant difference in the antibiotic release between the different drug loadings of the clindamycin and moxifloxacin coated suture materials. **Rejected.**

Overall, significant differences were found between the loading dosages of the clindamycin and moxifloxacin coated sutures.

CHAPTER 7

METHODS, RESULTS & DISCUSSION

Experiment 4: Handling and Mechanical Properties

VARIABLES

Independent Variables

Suture Samples

The three independent variables for all of the following mechanical characteristics were the following sutures:

1) Uncoated braided
2) Coated braided
3) Monofilament

Dependent Variables

The dependent variables were:

1) Flexural Rigidity
2) Tissue Drag
3) Tensile Strength
4) Knot Pull Strength

Test Conditions

All of the following mechanical tests were conducted under ambient conditions of 21 ($\pm$ 1) $^{\circ}$C temperature and 65 ($\pm$ 2) % relative humidity. The samples were all preconditioned overnight under the same conditions of temperature and relative humidity.

METHODS

Handling Procedures

Flexural Rigidity

The ASTM D1388-96 standard method for the Heart Loop Test was used to measure the flexural rigidity of each suture specimen (ASTM, 2001). In this test, the two ends of the specimen (250 mm) were clamped together to form the heart shaped loop (total circumference of the heart was 150 mm). The loop length from the top edge of the clamps to the base of the loop was measured and used to derive the bending length, in accordance with the standardized table provided in the method. Ten specimens of each suture type were tested.

The following equation, given in ASTM D1388-96, was used to determine the bending stiffness in terms of flexural rigidity:

$$G = Wc^3$$

Where, G is the flexural rigidity (in g·cm), W is the weight per unit area (in g/cm^2), and c is the bending length (in cm).

The bending stiffness (in terms of flexural rigidity) was calculated for each specimen and the average was calculated for the monofilament, coated braided and uncoated braided suture samples.

Tissue Drag

A tissue drag test method was developed in the laboratory using a table-top Instron CRE tester and a 2000 g load cell. An Instron ball burst attachment (outer diameter of 78 mm and inner diameter of 42 mm) was used to clamp the tissue simulant (in this case, a brushed natural suede) at the base of the Instron and pneumatic flat-faced

grips were placed on the upper end of the testing machine. Using a dye cutter, the tissue simulant (0.84±0.04 mm, N=22) was cut into 89 mm diameter circles. This circular piece of tissue simulant was then placed in between the clamps of the ball burst attachment and secured using the metal screws, which were part of the apparatus. The centre point of the circle (44.5 mm) was premarked at the bottom of the sample and each suture was threaded with a needle and passed through the tissue simulant (along the central axis of the Instron). For each new specimen a new tissue simulant was secured onto the metal plate. Ten specimens per sample were tested.

The initial gauge length of the Instron was set to 30 mm and the crosshead speed was set to 100 mm/min. The Instron load cell recorded the force required to pull 150 mm of each suture type through the pigskin in milliNewtons (mN). The peaks were saved and the average force of resistance for each suture type was calculated on the ten highest peak measurements. The highest peaks were chosen because they were indicative of the worst-case scenario of the tissue drag measures for each suture.

Statistical Analysis

The sample means for flexural rigidity and tissue drag force were analyzed using one-way analysis of variance (ANOVA).

METHODS

Mechanical Procedures

Tensile Strength

An MTS Model 1128 constant rate of extension (CRE) universal tester (Eden Prairie, MN, USA) was fitted with pneumatic yarn grips to measure the average tensile

strength and elongation of uncoated braided, coated braided (no antibiotic) and monofilament sutures. Tensile strength and elongation were then measured according to ASTM D2256-90. Tensile strength is a measure of the breaking force or the maximum force used to pull the suture specimen apart and rupture it. The initial gauge length was 150 mm. The crosshead speed of the tester was 90 mm/min and the MTS universal tester was fitted with a 250 N capacity load cell. Eight specimens of each suture type were tested.

Knot Pull Strength

The average knot pull strength was determined for each of the braided uncoated, braided coated (no antibiotic) and monofilament suture materials. The standard USP test method for knot pull strength was used. Samples were tested on an MTS Model 1128 CRE tester (Eden Prairie, MN, USA). The crosshead of the MTS universal tester pulled the knot apart and broke the knot at the peak load. This peak load was recorded by the machine. A double square knot was used to tie the knot for each specimen. Eight specimens of each suture type were tested. The sample means were analyzed using a non-parametric K-independent test statistic (Kruskal-Wallis Test).

Statistical Analysis

The sample means of both the tensile strength tests and the knot pull strength tests were analyzed with a non-parametric K-independent test statistic (Kruskal-Wallis Test).

RESULTS

The descriptive results for the handling properties (flexural rigidity and tissue drag) and the mechanical tests (tensile strength and knot pull strength) were summarized in Table 13.

Table 13: Handling and Mechanical Properties Descriptives

Handling and Mechanical Properties Descriptives					
		N	Mean	Std. Dev	Std. Error
Flexural Rigidity [g*cm]	Monofilament	10	2.88	0.15	0.05
	Braided	10	2.15	0.10	0.03
	Coated Braid	10	2.28	0.17	0.05
	Total	30	2.44		
Tissue Drag [N]	Monofilament	80	10.08	4.49	0.50
	Braided	100	5.17	2.57	0.26
	Coated Braid	80	10.65	3.84	0.43
	Total	260	8.36		
Tensile Strength [N]	Monofilament	9	96.44	8.55	2.85
	Braided	8	70.96	0.66	0.23
	Coated Braid	8	31.79	13.97	4.94
	Total	25	67.60		
Knot Pull Strength [N]	Monofilament	8	34.38	15.37	5.44
	Braided	8	44.80	1.35	0.48
	Coated Braid	8	68.52	0.40	0.14
	Total	24	49.23		

Handling Properties

Flexural Rigidity

The average flexural rigidity of each suture type was summarized in Table 13. According to Table 13, the mean and standard deviations of the monofilament, braided suture (no coating) and coated braided sutures were 2.9±0.1 gcm, 2.2±0.1 gcm, and 2.3±0.2 gcm, respectively. The resulting higher rigidity of the monofilament sutures in comparison to the braided structures was expected. The closeness of the results between the means of the braided sutures implied that the coating added no significant rigidity to

the suture, therefore, the coated suture delivered virtually the same handling property by way of flexibility as did the uncoated braided structure.

Tissue Drag

The average tissue drag results (measured as the force of resistance against pigskin) of each suture type were summarized in Table 13 and were 10.1±4.5 mN, 5.2±2.6 mN, and 10.6±3.8 mN for the monofilament, braided suture (no coating) and coated braided sutures, respectively. The mean resistant force value was twice as high for the monofilament and coated braided sutures than it was for the uncoated braided suture. This may be in part due to the angle at which the suture was pulled through the pigskin, which may have been a factor of the shape of the suture prior to testing. It was observed by the researcher, during testing, that the monofilament and coated braided suture (to a lesser extent) had a memory from the packaging, whereas the braided suture (without coating) did not. As a result, the monofilament sutures in particular held a strongly curved shape and the coated suture held a slightly curved shape as it was pulled through the pigskin. In retrospect, this may have been avoided to some extent if a weight were hung on the end of each suture as it passed through the pigskin.

This test also produced fairly high standard errors of 0.50, 0.26, and 0.43, for monofilament, braided and coated braided sutures, respectively. These variances may be due to the angles the sutures were pulled through the pigskin. Variances in the coated suture measurements implied variances in the coating as well. The braided suture without coating resulted in the lowest, approximately half the variability, of the coated measurements.

Statistical Analysis

It was determined from a Levene's Test for homogeneity of variances that according to the parameters of this test (p must be greater than 0.05 to assume equal variances) only flexural rigidity had a p-value (0.486) significant enough to be used with ANOVA (see Appendix G). The scatterplot for the flexural rigidity values (Figure 38) also demonstrated that the values were fairly linear (r = 0.713) and the histogram (Figure 39) showed that the curve is fairly normal. Since all the suture samples were also independent, the data requirements of independence, normality and equal variances were met for use with ANOVA.

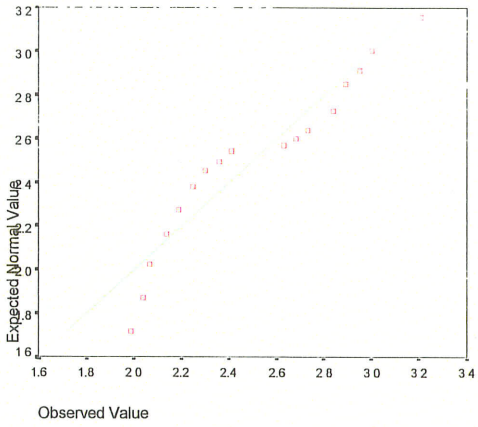


Figure 38: Scatterplot for Flexural Rigidity

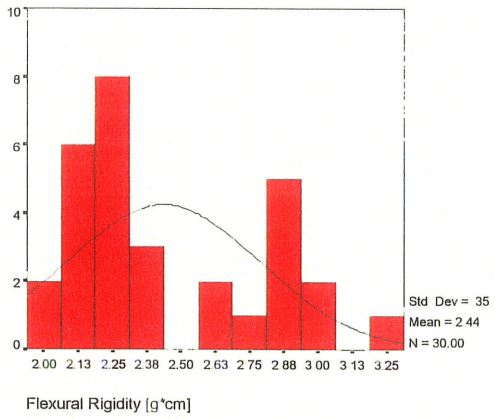


Figure 39: Histogram for Flexural Rigidity

When ANOVA was performed the p-value was negligible (zero to six decimal places see Appendix G). Such a small p-value indicated that the variation in the measurements were not due to chance but to distinct differences between the means of flexural rigidity values between monofilament, braided and braided coated suture materials. For example, the homogeneous groups summary from the one-way ANOVA output (Duncan test) in Table 14 grouped these sutures into their distinctive subgroups.

Table 14: Homogeneous Groups Output (Duncan) for Flexural Rigidity (g*cm)

	N	Subset for alpha = .05	
Suture Material		1	2
Braided	10	2.152	
Coated Braid	10	2.277	
Monofilament	10		2.882
Sig.		0.059832	1

Means for groups in homogeneous subsets are displayed.
Uses Harmonic Mean Sample Size = 10.000.

In Table 14, two homogeneous groups were identified, the first contained the braided (uncoated) and braided coated suture samples, and the second contained the monofilament suture. The two braided sutures did not differ significantly at the 0.05 level of significance, whereas, the monofilament suture material did differ significantly from the braided sutures.

Although the p value for the tissue drag values did not meet the criterion for the Levene's test, analysis of variance was conducted on the data. According to Moore as long as the samples are independent, the distribution is normal and the standard

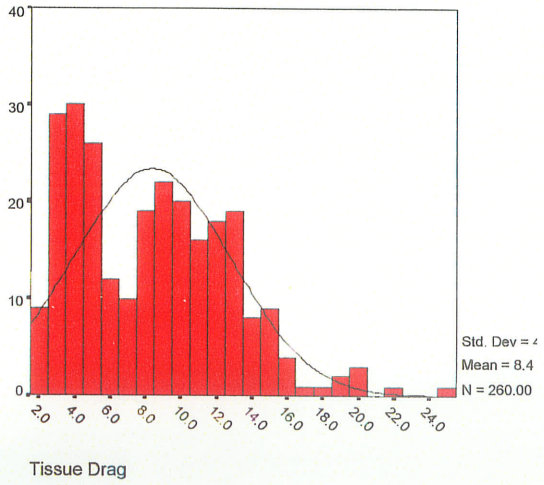


Figure 40: Histogram of Tissue Drag Measures

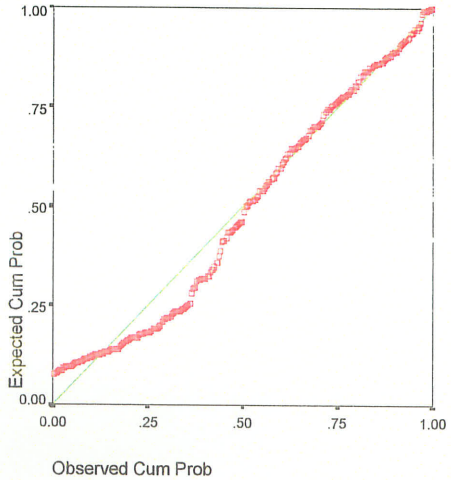


Figure 41: Tissue Drag Normal Probability Plot

deviations of the largest and smallest values are not spread wider than by a factor of two, then "the results of the ANOVA F-test are approximately correct" (Moore, 1995, p.570). In this case, the data were independent, they were fairly normal (as shown in Figure 40) and the largest standard deviation (4.5 mN) was greater than the lowest +standard deviation (2.6 mN) by less than a factor of two. The histogram for the tissue drag measurements (Figure 40) shows that the normal curve was skewed slightly to the left –a product of the variance found in this data, yet the f-Test was still robust. Figure 41: Tissue Drag Normal Probability Plot demonstrated the relationship between measures was linear.

The p-value resulting from the ANOVA f-Test was extremely small (zero to six decimal places), indicative that the variances in the data were not due to chance. The ANOVA output for the F test of the tissue drag measures can be found in Appendix G. Such a small p-value indicated that the variation in the measurements were not due to chance but were due to distinct differences between the means of tissue drag values between the monofilament, braided and braided coated suture materials. For example, the homogeneous groups summary from the one-way ANOVA output (Duncan test) in Table 15, Homogeneous Groups Output (Duncan) for Tissue Drag, identified the sutures by their distinctive subgroups.

Table 15: Homogeneous Groups Output (Duncan) for Tissue Drag (mN)

		Subset for alpha = .05	
Suture Material	N	1	2
braided	100	5.167	
monofilament	80		10.078
coated braided	80		10.646
Sig.		1.000	0.308
Uses Harmonic Mean Sample Size = 85.714			
The group sizes are unequal. The harmonic mean			
of the group sizes is used.			

In Table 15 two homogeneous groups were identified, the first contained the braided (uncoated) suture, and the second contained the coated braided suture and the monofilament suture. The braided uncoated suture differed with the monofilament and the coated sutures for tissue drag, whereas, the monofilament and coated suture materials did not differ significantly from each other.

Figure 42 illustrates this fact, that the monofilament and coated suture had more similar means than the braided suture, but also more similar variations, which are significantly larger than the braided suture.

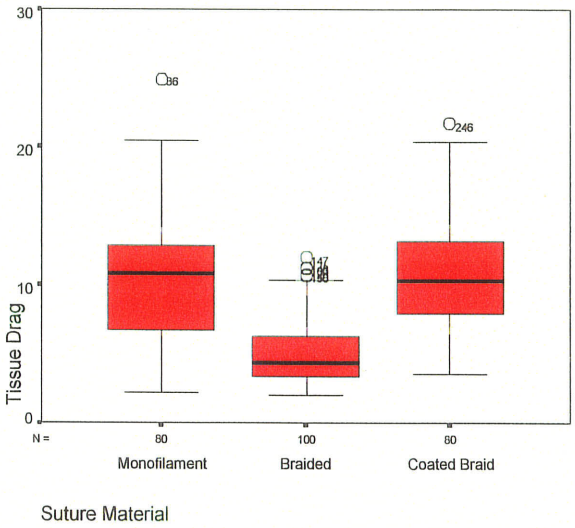


Figure 42: Boxplot of Tissue Drag

Mechanical Properties

Tensile Strength

As listed in Table 13, the mean tensile strength and standard deviation values for monofilament, braided (uncoated) and braided coated sutures were 96.4±8.5 N, 71.0±0.7 N, and 31.8±14.0 N, respectively. While the yarn grips were used to prevent slippage, it was possible that the higher error values for the monofilament and braided coated sutures

were due to some slippage in the grips caused in part by their smoother surfaces. The braided suture without coating experienced a very low error, which may have been a testament to this suture's ability to tie a secure knot.

Knot Pull Strength

As listed in Table 13, the mean knot pull strength and standard deviation values for monofilament, braided (uncoated) and braided coated sutures were 34.4±15.4 N, 44.8±1.3 N, and 68.5±0.4 N, respectively. The knot pull strength tests resulted in the reverse order of force values from coated and monofilament sutures achieved in the tensile strength tests.

The researcher noticed during testing that by far, the monofilament sutures were the most difficult to tie and showed the least knot holding capacity, demonstrated by the lowest knot pull strength values. Furthermore, the high standard error (5.44) found in the monofilament sutures may have been due to the fact that some knots were held better by the monofilament than others. The braided sutures were much easier to knot in comparison to the monofilament structure and were found to hold the knots more securely, as demonstrated by the lower standard errors found for the knot pull strength values of the braided (uncoated) and coated braided sutures of 0.48 and 0.14, respectively.

Statistical Anlaysis

The Kruskal-Wallis nonparametric test for independent means was performed on the mean tensile strength forces and and the knot pull strength force values because variations in standard deviation were greater than a factor of two for the largest and smallest values. For this test procedure, no assumptions about the variances were made.

The results were recorded in the Kruskal-Wallis Output for Tensile and Knot Pull Strength, Table 16.

Table 16: Kruskal-Wallis Output for Tensile and Knot-Pull Strength

Ranks

	Suture Material	N	Mean Rank
Tensile Strength [N]	Monofilament	9	21.00
	Braided	8	12.50
	Coated Braid	8	4.50
	Total	25	
Knot Pull Strength [N]	Monofilament	8	6.13
	Braided	8	10.88
	Coated Braid	8	20.50
	Total	24	

Test Statistics[a,b]

	Tensile Strength [N]	Knot Pull Strength [N]
Chi-Square	21.342	17.172
df	2	2
Asymp. Sig.	.000	.000

a. Kruskal Wallis Test

b. Grouping Variable: Suture Material

In the Kruskal-Wallis test, the scores were ranked without differentiation into groups that are similar, as was the case its parametric counterpart (ANOVA), seen in Tables 12 & 13. The number of cases (N) identifies the distribution of cases across groups (suture materials). The mean rank lists the average rank for each group based on the Kruskal-Wallis ranking in which the closer the groups rank, the less they differ from each other. The Chi-Square statistic showed that the significance of the p-value was extremely small for both properties of tensile strength and knot pull strength, which indicated that the suture materials differ and that these differences are significant.

The results from the Kruskal-Wallis mean ranks showed that the means of the various sutures were quite distinct for these mechanical properties. For example, the monofilament displayed the highest tensile strength, followed by the braided suture and finally the coated braided suture. However, the trend was just as distinct, in the opposite

direction for the knot pull strength values, wherein the coated suture showed the highest values, followed by the braided suture, followed by the monofilament.

DISCUSSION

H_{04}: *There is no significant difference in initial handling properties of flexural rigidity or tissue drag between the coated braided and uncoated braided sutures, and the monofilament sutures.* **Rejected.**

Hypothesis 4 was rejected for flexural rigidity and for tissue drag. Significant differences were found from the analysis of variance at the $p<0.0001$ level among the braided, coated braided and monofilament sutures.

H_{05}: *There is no significant difference in the initial mechanical properties by way of tensile strength and knot pull strength of the following suture materials: coated and uncoated braided sutures, and monofilament suture material.* **Rejected.**

Hypothesis 5 was rejected for the tensile strength and knot pull strength of the braided, coated braided and monofilament sutures. The p-value of the Chi-Square test from the Kruskal-Wallis output was extremely small ($p<0.0001$) for average measures ($N=8$) of both Tensile Strength and Knot Pull Strength measures, indicating that the suture materials differed in these properties significantly.

CHAPTER 8

GENERAL DISCUSSION & RECOMMENDATIONS

This chapter includes a summary of the research study, some general conclusions of the study, and recommendations for future work.

Summary

Suture wound infections are still a concern, despite aseptic operating procedures and new systemic drug therapies. It has been reported that surgeons, such as cardiovascular surgeons, shy away from braided sutures for use in high-risk surgical situations due to the potential for bacteria to harbor within the interstices of a braided structure. The friction of a braided structure compared to a monofilament is another deterrent to use. However, there are advantages to wound closure such as ease of handling and knot tying that are generally advantages for braided sutures, which may lead to a more secure wound and better healing. Few studies have attempted to solve the suture wound infection problem prophylactically and there are currently no commercial antimicrobial sutures marketed. Thus, this study attempted to create an alternative type of braided suture material for use in high-risk surgeries with a coating to improve friction and to act as a vehicle for drug delivery.

There were five main objectives of this study. The first was to determine if the coated sutures with loaded antibiotic produced antimicrobial activity (measured by zones of inhibition against *Staphylococcus aureus*) and to compare these levels during the

period of coating absorption *in vitro.* In order to gather this information, two types of antibiotic-coated braided suture materials (clindamycin and moxifloxacin), and loading dosages of antibiotic (10x MIC and 100x MIC), were formulated and compared to the coated braided suture material (no antibiotic) and uncoated braided material. This type of zone diffusion study had been performed by past researchers who studied sutures covalently bonded to gentamicin and also by researchers studying other biomaterials.

The second purpose was to compare the level of antibiotic release (measured by UV-spectroscopy) during the period of coating absorption *in vitro,* for the four different types of antibiotic-coated braided suture materials mentioned above, as well as coated and uncoated controls. This absorbance method was used to quantify the amount of antibiotic loaded into the suture coating. A similar test method was used previously to test ciprofloxacin heat set polyester fabrics (Phaneuf et al., 1993).

The third objective was to compare the level of adherence of *Staphylococcus aureus* to each of the antibiotic-coated suture materials, braided uncoated suture materials, uncoated braided suture, and monofilament suture of equal diameter. Past studies of clindamycin hydrochloride had shown that at sub-inhibitory concentrations that the drug effected adhesion properties of *S. aureus*. It was thought that for this reason, clindamycin may be an effective drug to load onto a biomaterial surface, which is more prone to adhesion of microorganisms than the surrounding tissue substrate. It was also of interest to the researcher to compare the differences in adhesion of *S.aureus* to clindamycin and moxifloxacin-coated suture materials. Moxifloxacin was of interest as a very potent bactericidal fluoroquinolone.

The fourth objective was to compare the differences in initial suture properties between coated and uncoated braided suture materials and a monofilament, of comparable diameter. Flexural rigidity and tissue drag were the two properties used to compare the braided sutures and monofilament suture material. A standard method was used to assess flexural rigidity and the researcher developed a procedure for testing tissue drag.

Finally, the researcher compared the tensile strength and knot pull strength of the braided uncoated, braided coated and monofilament suture samples. Standard methods were used to assess these mechanical suture properties.

Conclusions

1. **Antimicrobial Activity (Zone Diameter).** The results showed that the antimicrobial activity, as measured by the zones of inhibition, was dependent on the concentration of the drug loading. For example, the higher loading dosages achieved greater zones of inhibition. Furthermore, the statistical analysis showed that there were significant differences amongst the moxifloxacin and clindamycin coated sutures at both loading dosages and between the treated sutures and untreated controls.

The largest zones occurred at the point of contact at time zero then released over a two day period in the case of both moxifloxacin-coated sutures and lower loading of clindamycin, and three days in the case of the higher loading of the clindamycin-coated sutures. Similarly, the zones of the samples of buffer with the adsorbed suture coatings, resulted in a two-day release period for the antibiotics in the 0.85% clindamycin and

1.0% moxifloxacin sutures, and a three day release in the 8.5% clindamycin-coated sutures. However, no zones were measured for the buffer samples with, braided uncoated, coated (no antibiotic) or the 0.1% moxifloxacin-coated sutures throughout the five day period.

These results showed a fairly speedy release of drug from the coating, within the course of two to three days under sink conditions. These results are excellent considering the most crucial prophylactic treatment period is within the first 24 hours. This rate of release would be suitable to inhibit the growth of contaminating *Staphylococcus aureus* and give the host's natural defenses the opportunity to combat the infection.

2. **Microbial Adhesion**. From the SEM images, the moxifloxacin-treated sutures demonstrated less adhesion to bacteria than the clindamycin treated sutures overall since the moxifloxacin sutures resulted in light to moderate adhesion while the clindamycin coated sutures resulted in moderate to heavy adhesion. However, in addition, the 1.0% moxifloxacin coating with more variable surface properties demonstrated more adhesion and the clindamycin sutures, which overall adhered more organisms and were even less uniform than the moxifloxacin coatings. For this reason, it is likely that the surface characteristics of the coating had an effect on the ability of the organisms to adhere.

3. **Absorbance of Drug.** The UV-absorbance measurements results generally concurred with the zone diffusion study results in that the higher loading of each antibiotic generally produced a higher absorbance compared to the sutures with a

lower dose of the same antibiotic. Differences could also be seen between coated sutures without antibiotic and coated sutures with antibiotic for the first two days. Thereafter, as with the zone diffusion tests, the antibiotic appeared completely released. Further more, t-tests showed significant differences amongst the moxifloxacin coated sutures and amongst the clindamycin coated sutures throughout the adsorption of the coatings in buffer.

4. **Flexural Rigidity.** It was determined that the coated braided and uncoated samples had similar flexural rigidity values and that the monofilament was higher than the both the braided (uncoated) and coated braided sutures (by about 1.3 times and 1.2 times, respectively). The analysis of variance also demonstrated that the difference between the means of the three suture types was significant ($p<0.0001$).

5. **Tissue Drag.** The results showed that the monofilament and coated sutures produced a resistant force that was twice as large as the braided suture. The analysis of variance also demonstrated that the means of the force results were significantly difference amongst the three suture types ($p<0.0001$).

6. **Tensile Strength.** The results showed that the monofilament suture had the greatest tensile strength and the coated suture had the least with the monofilament producing approximately 1.7 times more tensile strength than the braided suture and 4.7 times more than the coated braid. Furthermore, the statistical analysis (Kruskal-Wallis

test) demonstrated that the differences between the mean tensile strength values were significantly difference ($p<0.0001$).

7. **Knot Pull Strength.** The results demonstrated the coated braided suture exhibited approximately 2 times greater knot pull strength than the braided suture and about 3 times greater knot pull strength than the monofilament suture. These results according to the Chi-Square test of the Kruskal-Wallis output were also significantly different amongst the various suture types ($p<0.0001$).

GENERAL DISCUSSION

The zone diffusion tests suggest that the incorporation of an antibiotic within an absorbable, coated suture can inhibit bacterial growth on the suture and in the surrounding fluid for a period of upto two days. The UV-spectroscopy results were similar to the zone diffusion results with respect to the rate of drug release, within the first two days. These results also demonstrated in general, drug was released within the first two days. A faster rate of drug release is preferred, particularly within the first 24 hours, since this is the period when prophylactic drug therapy is most essential.

From the SEM studies it appeared as though the moxifloxacin coated sutures were more successful at preventing adhesion than the clindamycin coated sutures. Therefore, moxifloxacin may be more effective at limiting adhesion compared to the clindamycin sutures.

The monofilament suture had greater flexural rigidity than the braided coated and uncoated samples, which implied the braided sutures, had similarly better handling

properties compared to the monofilament sutures. More importantly, it implies that it is possible to coat a braided suture without jeopardize this important handling property, imperative for good knot security.

It was also found that the monofilament and coated braided sutures displayed twice as much tissue drag (resistance force) compared to the braided suture. Greater tissue drag measures may translate into in abscess formation and adverse healing of a suture wound. Therefore, these results imply that the braided material may be less harmful than the monofilament and coated suture material of this study when pulled through tissue.

While the angle of entry through the tissue may have played a role in the results, it is also true that previous tissue drag tests have found that monofilament sutures result in higher tissue drag compared to braided suture materials (Hong, 1995). Therefore, whether or not the angle may be correctable may not be of great importance. If the suture has an innate curvature when used or tested, for example by packaging memory, it may be inevitable that the suture will result in higher tissue drag measures. Furthermore, the results of this study with regards to a similar phenomenon of resistance to straightening was also found in the coated suture materials studied by Rodeaver et al. (Rodeaver et al., 1983), in which the suture with the greatest rigidity was found to also have the greatest tissue drag. The possible causes of higher values for flexural rigidity, which may lead to adverse effects to tissue drag, may include chemical composition, suture construction or the coating material (Rodeheaver et al., 1983).

While the straight tensile strength of the coated sutures were determined to be the weakest compared to the braided uncoated and monofilament sutures, the knot pull

strength was strongest for the coated braided suture. This implies that the coated braided suture will not only tie a more secure knot but it will hold a more secure knot when exposed to higher loads than both the uncoated braided and monofilament sutures. While the coated sutures performed the best in knot pull strength, its tensile strength was the weakest. The importance of the tensile strength would need to be assessed based on what kind of forces would be exerted in the wound area in order to estimate whether or not the coated braided suture would be suitable as a wound closure material.

RECOMMENDATIONS

Due to the nonuniformities of the coating, more tests would have to be conducted to determine if the surface properties of the coating would also have an effect on adhesion of *S. aureus*.

It is also difficult to conclude based on these *in vitro* inhibitory results as to whether the sutures would result in successful results *in vivo*. Therefore, tests would need to be conducted *in vivo* to determine if, for example, the clindamycin dosages would be successful enough at limiting adhesion to prevent infection. This can be tested in a real-life situation, where the inflammatory response is given the opportunity to work.

Of the two types of antibiotic, the moxifloxacin, a bactericidal drug with a siginificantly lower MIC value, did exhibit superior inhibitory performance against *S. aureus*. Due to the lower MIC values, the smaller zones of inhibition may not be a cause for concern since the agar diffusion test is concentration dependent and the concentrations required for clindamycin loading of the sutures were 8.5 times greater. The concern with the use of moxifloxacin for use with these sutures would be the

development of resistance. This is a concern since it was demonstrated that microcolonies developed within the clear zones of the moxifloxacin sutures during this study. A test for resistance could be conducted by taking one of the isolated microcolonies and culturing it overnight then repeating the same zone diffusion study with the microcolonies as the test organism. If there are no zones after 24 hours exposure to Mueller-Hinton broth then the bacteria are resistant.

Other future research could also include varying the polymers chemically to prolong the rate of drug release. In addition, tests could be conducted to determine the degree of the degradation of the each drug in the coating over time. In addition, the two drugs could be combined to determine if a symbiotic effect would result, for a broader spectrum prophylactic dose.

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APPENDICES

Appendix A

T-tests for Suture and Buffer Zones of Inhibition

Micro Results t-tests			
Suture Zones of Inhibition			
sutures	time 0	day 1	day 2
0.1% + 1.0%	0.04954	3.5E-16	ND
0.85% + 8.5%	5.05E-16	6.84E-23	5.66E-16
0.1% + 0.85%	9.99E-12	2.24E-21	ND
1.0% + 8.5%	1.48E-15	5.09E-29	5.66E-16

Micro Results T-tests			
Buffer Zones of Inhibition			
sutures		day 1	day 2
0.1% + 1.0%		ND	ND
0.85% + 8.5%		8.21E-21	9.18E-13
0.1% + 0.85%		ND	ND
1.0% + 8.5%		2.55E-18	9.18E-13

ND = no data (i.e. zero values;
zero values were also obtained for the buffer zones at time zero)

Appendix B

Clindamycin Adsorption Study Summary: Absorbance, pH, and Linear Density Measurements

Abs. Time	Suture Coating		UV Abs.	pH	initial linear density (tex)	final linear density (tex)	change linear density (tex)
time 0	no coating	Mean	0.044	7.55	221.81	220.27	1.54
		Std. Dev	0.012	0.06	4.30	4.48	0.63
	Coated	Mean	0.060	7.50	234.29	232.52	1.77
		Std. Dev	0.011	0.05	4.00	3.29	1.08
	0.85% clindamycin	Mean	0.056	7.50	231.19	230.63	0.56
		Std. Dev	0.012	0.03	1.33	1.11	0.56
	8.5% clindamycin	Mean	0.189	7.41	228.00	226.29	1.71
		Std. Dev	0.013	0.02	1.08	0.87	0.53
day 1	no coating	Mean	0.113	7.27	219.46	220.21	-0.75
		Std. Dev	0.014	0.19	3.26	3.32	0.74
	Coated	Mean	0.151	7.41	235.31	233.90	1.42
		Std. Dev	0.017	0.03	5.02	4.72	0.62
	0.85% clindamycin	Mean	0.154	7.41	231.65	230.15	1.50
		Std. Dev	0.005	0.02	2.17	2.70	0.90
	8.5% clindamycin	Mean	0.307	7.45	200.84	198.15	2.69
		Std. Dev	0.020	0.01	81.16	80.07	1.84
day 2	no coating	Mean	0.011	7.62	217.58	217.31	0.27
		Std. Dev	0.002	0.05	2.86	2.58	0.42
	Coated	Mean	0.018	7.65	232.42	230.38	2.04
		Std. Dev	0.003	0.03	1.75	1.07	0.82
	0.85% clindamycin	Mean	0.025	7.55	229.98	227.71	2.27
		Std. Dev	0.004	0.11	1.28	1.27	0.55
	8.5% clindamycin	Mean	0.039	7.63	227.81	224.85	2.96
		Std. Dev	0.008	0.04	1.29	1.66	0.49
day 3	no coating	Mean	0.026	7.49	218.79	218.04	0.75
		Std. De	0.002	0.04	2.97	3.25	0.46
	Coated	Mean	0.026	7.56	234.96	231.37	3.58
		Std. Dev	0.003	0.05	4.50	3.48	1.13
	0.85% clindamycin	Mean	0.026	7.56	231.67	228.13	3.54
		Std. Dev	0.003	0.05	0.86	0.99	0.41
	8.5% clindamycin	Mean	0.026	7.54	229.60	225.73	3.88
		Std. Dev	0.004	0.07	3.68	2.82	1.10
day 4	no coating	Mean	0.012	7.37	221.79	221.25	0.54
		Std. Dev	0.002	0.06	3.72	3.54	0.72
	Coated	Mean	0.015	7.40	236.25	233.58	2.67
		Std. Dev	0.004	0.04	4.55	3.79	0.95
	0.85% clindamycin	Mean	0.012	7.41	231.23	228.56	2.67
		Std. Dev	0.002	0.04	1.31	1.27	0.36
	8.5% clindamycin	Mean	0.015	7.39	228.81	225.15	3.67
		Std. Dev	0.003	0.02	1.72	1.51	0.72

Appendix B

Abs. Time	Suture Coating		UV Abs.	pH	initial linear density (tex)	final linear density (tex)	change linear density (tex)
day 5	no coating	Mean	0.014	7.40	219.81	220.23	-0.42
		Std. Dev	0.003	0.03	3.77	4.05	0.96
	Coated	Mean	0.019	7.39	237.31	234.88	2.44
		Std. Dev	0.004	0.01	5.49	4.55	1.21
	0.85% clindamycin	Mean	0.027	7.41	230.50	229.75	0.75
		Std. Dev	0.003	0.02	2.34	1.85	1.52
	8.5% clindamycin	Mean	0.024	7.41	228.65	225.96	2.69
		Std. Dev	0.004	0.02	1.76	1.61	0.47
day 6	no coating	Mean	0.009	7.36	220.50	219.38	1.13
		Std. Dev	0.001	0.02	2.17	2.58	0.53
	Coated	Mean	0.014	7.34	238.06	233.00	5.06
		Std. Dev	0.003	0.02	5.34	3.79	1.97
	0.85% clindamycin	Mean	0.012	7.38	231.48	228.37	3.10
		Std. Dev	0.001	0.02	1.86	1.93	0.96
	8.5% clindamycin	Mean	0.013	7.37	230.06	223.65	6.42
		Std. Dev	0.002	0.02	1.42	1.44	1.79
day 7	no coating	Mean	0.009	7.39	218.75	218.54	0.21
		Std. Dev	0.002	0.03	2.73	2.91	0.83
	Coated	Mean	0.015	7.34	234.58	231.67	2.92
		Std. De	0.003	0.03	4.45	3.25	1.39
	0.85% clindamycin	Mean	0.011	7.40	231.81	228.27	3.54
		Std. Dev	0.002	0.03	1.63	1.75	0.87
	8.5% clindamycin	Mean	0.014	7.38	230.23	225.19	5.04
		Std. Dev	0.003	0.05	3.02	2.30	1.11
Total	no coating	Mean	0.030	7.43	219.81	219.40	0.41
		N	59	64	64	64	64
		Std. Dev	0.035	0.13	3.40	3.44	0.96
	Coated	Mean	0.041	7.45	235.40	232.66	2.74
		N	60	64	64	64	64
		Std. Dev	0.046	0.11	4.59	3.71	1.58
	0.85% clindamycin	Mean	0.040	7.45	231.19	228.95	2.24
		N	64	64	64	64	64
		Std. Dev	0.045	0.08	1.68	1.89	1.38
	8.5% clindamycin	Mean	0.079	7.45	225.50	221.87	3.63
		N	63	64	64	64	64
		Std. Dev	0.104	0.09	28.72	28.24	1.77
	Total	Mean	0.048	7.44	227.97	225.72	2.25
		N	246	256	256	256	256
		Std. Dev	0.067	0.11	15.72	15.25	1.86

Appendix C
Moxifloxacin Adsorption Study Summary: Absorbance, pH, and Linear Density Measurements

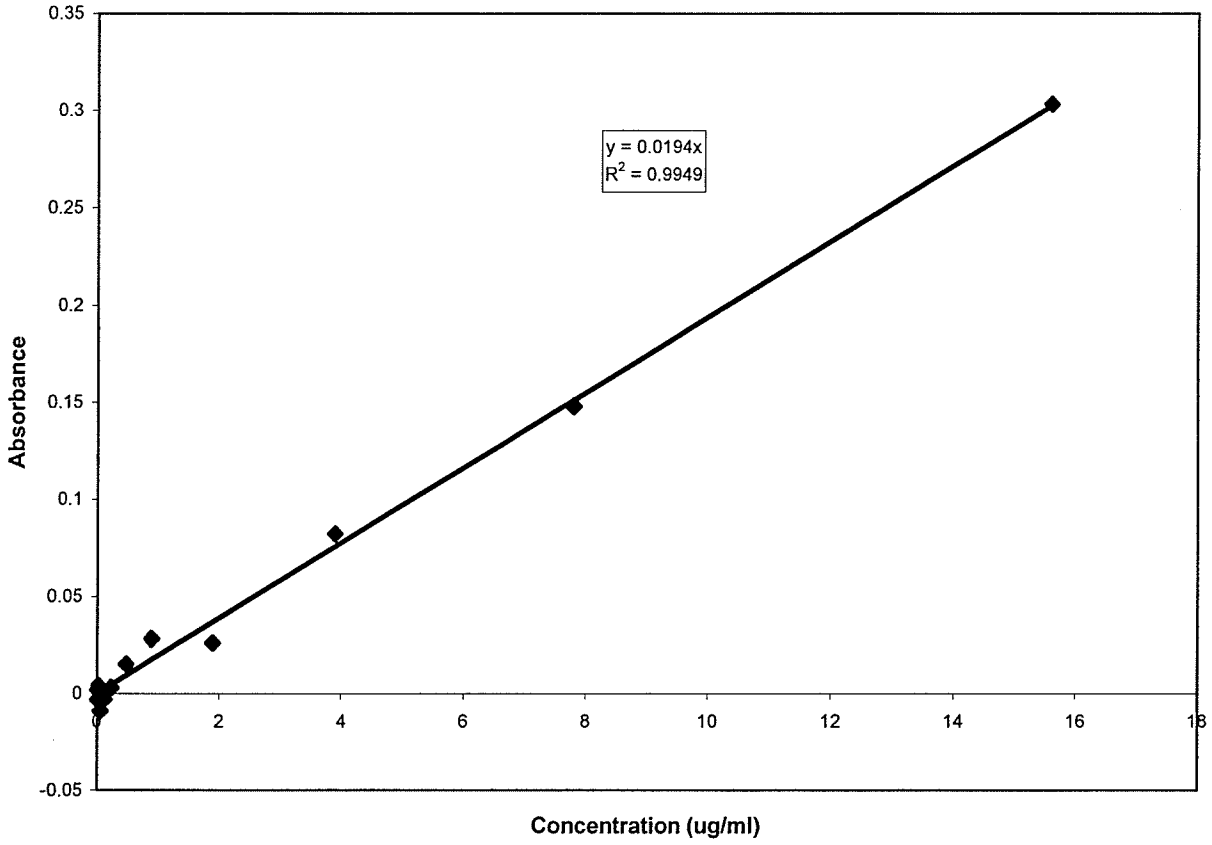
Abs. Time	Suture Coating		UV Abs.	pH	initial linear density (tex)	final linear density (tex)	change linear density (tex)
time 0	no coating	Mean	0.021	7.42	222.42	221.56	0.85
		Std. Dev	0.001	0.01	1.83	2.18	0.83
	Coated	Mean	0.083	7.42	236.23	230.71	5.52
		Std. Dev	0.011	0.01	4.11	3.70	0.73
	0.1% moxifloxacin	Mean	0.096	7.42	235.04	231.77	3.27
		Std. Dev	0.010	0.01	1.55	1.01	1.66
	1.0% moxifloxacin	Mean	0.116	7.43	231.63	228.44	3.19
		Std. Dev	0.009	0.01	1.76	1.73	0.81
day 1	no coating	Mean	0.009	7.36	220.52	220.98	-0.46
		Std. Dev	0.004	0.01	1.66	2.03	0.75
	Coated	Mean	0.026	7.36	241.48	235.15	6.33
		Std. Dev	0.003	0.00	3.02	2.55	0.72
	0.1% moxifloxacin	Mean	0.021	7.36	233.75	229.25	4.50
		Std. Dev	0.004	0.00	2.16	1.85	2.08
	1.0% moxifloxacin	Mean	0.029	7.36	240.08	234.25	5.83
		Std. Dev	0.006	0.00	2.64	1.66	1.97
day 2	no coating	Mean	0.012	7.37	220.73	221.77	-1.04
		Std. Dev	0.001	0.02	1.18	1.92	1.32
	Coated	Mean	0.044	7.36	241.13	231.75	9.38
		Std. Dev	0.006	0.00	3.13	2.53	2.94
	0.1% moxifloxacin	Mean	0.043	7.36	235.38	228.92	6.46
		Std. Dev	0.007	0.01	1.66	2.04	2.17
	1.0% moxifloxacin	Mean	0.045	7.37	239.44	230.00	9.44
		Std. Dev	0.007	0.01	2.76	2.25	1.33
day 3	no coating	Mean	0.013	7.40	221.58	222.95	-1.52
		Std. Dev	0.001	0.01	1.63	1.58	0.77
	Coated	Mean	0.042	7.40	239.63	231.90	7.73
		Std. Dev	0.005	0.00	2.21	2.52	1.02
	0.1% moxifloxacin	Mean	0.042	7.41	234.17	229.17	5.00
		Std. Dev	0.008	0.01	2.49	2.42	1.88
	1.0% moxifloxacin	Mean	0.035	7.40	237.08	230.29	6.79
		Std. Dev	0.005	0.01	1.97	1.36	2.17
day 4	no coating	Mean	0.012	7.42	222.67	222.58	0.08
		Std. Dev	0.001	0.00	1.94	1.90	0.54
	Coated	Mean	0.043	7.43	240.63	230.60	10.02
		Std. Dev	0.015	0.01	3.45	2.89	1.29
	0.1% moxifloxacin	Mean	0.035	7.42	234.02	227.27	6.75
		Std. Dev	0.004	0.01	2.46	2.59	0.40
	1.0% moxifloxacin	Mean	0.046	7.43	238.69	229.65	9.04
		Std. Dev	0.007	0.00	1.91	1.48	1.20

Appendix C

Abs. Time	Suture Coating		UV Abs.	pH	initial linear density (tex)	final linear density (tex)	change linear density (tex)
day 5	no coating	Mean	0.012	7.38	221.73	222.71	-0.98
		Std. Dev	0.001	0.01	1.53	1.73	0.52
	Coated	Mean	0.052	7.38	240.94	234.90	6.04
		Std. De	0.008	0.01	3.93	2.70	1.48
	0.1% moxifloxacin	Mean	0.040	7.37	233.44	231.65	1.79
		Std. Dev	0.007	0.01	1.55	1.76	0.61
	1.0% moxifloxacin	Mean	0.044	7.38	236.96	233.17	3.79
		Std. Dev	0.005	0.01	1.40	0.99	0.76
day 6	no coating	Mean	0.011	7.39	220.96	220.75	0.21
		Std. Dev	0.001	0.01	1.82	2.36	0.61
	Coated	Mean	0.051	7.37	236.94	229.73	7.21
		Std. Dev	0.013	0.01	2.10	1.85	1.42
	0.1% moxifloxacin	Mean	0.048	7.38	232.13	226.31	5.81
		Std. Dev	0.010	0.01	1.79	1.61	0.69
	1.0% moxifloxacin	Mean	0.045	7.37	237.23	230.31	6.92
		Std. Dev	0.006	0.01	1.68	1.73	0.73
day 7	no coating	Mean	0.013	7.41	219.90	222.13	-2.23
		Std. Dev	0.001	0.00	2.02	1.51	0.60
	Coated	Mean	0.057	7.41	240.90	234.67	6.23
		Std. Dev	0.006	0.01	3.41	3.01	1.73
	0.1% moxifloxacin	Mean	0.056	7.40	233.92	229.15	4.77
		Std. Dev	0.009	0.01	1.41	1.69	0.83
	1.0% moxifloxacin	Mean	0.047	7.42	237.62	232.19	5.44
		Std. Dev	0.005	0.01	1.98	1.90	0.56
Total	no coating	Mean	0.013	7.39	221.31	221.91	-0.62
		N	64	64	64	63	63
		Std. Dev	0.004	0.02	1.86	1.97	1.20
	Coated	Mean	0.049	7.39	239.73	232.42	7.31
		N	63	64	64	64	64
		Std. Dev	0.017	0.03	3.60	3.31	2.13
	0.1% moxifloxacin	Mean	0.047	7.39	233.98	229.18	4.79
		N	63	64	64	64	64
		Std. Dev	0.021	0.03	2.04	2.54	2.08
	1.0% moxifloxacin	Mean	0.051	7.39	237.34	231.04	6.31
		N	64	64	64	64	64
		Std. Dev	0.026	0.03	3.11	2.43	2.44
	Total	Mean	0.040	7.39	233.09	228.67	4.47
		N	254	256	256	255	255
		Std. Dev	0.025	0.03	7.62	4.80	3.66

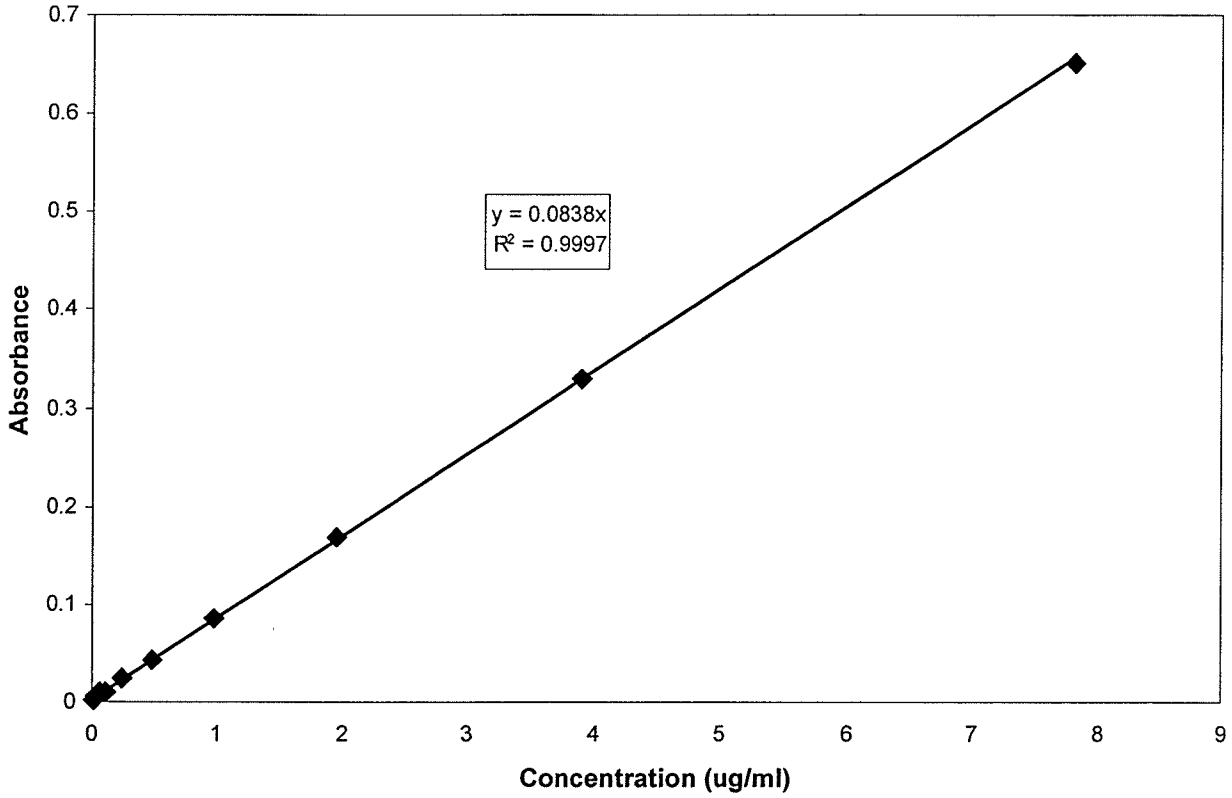
Appendix D

Calibration Curve for Clindamycin Hydrochloride



Appendix E

Calibration Curve for Moxifloxacin



Appendix F
T-tests of the Absorbance Measures for Clindamycin and Moxifloxacin Sutures

Probability results from T-tests for Clindamycin Coated Sutures:

	p-values							
Comparison Between Sutures:	**Time 0**	**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**	**Day 7**
0.85% and 8.5%	0.0000*	0.0000*	0.0003*	0.4787	0.0501	0.0692	0.1572	0.0069*
0.85% and Coated (no drug)	0.2700	0.0000*	0.0002*	0.5000	0.0455*	0.0003*	0.0720	0.0042*
8.5% and Coated (no drug)	0.0000*	0.0000*	0.0000*	0.4787	0.3953	0.0215*	0.2473	0.2996

- * Indicated significant p-values at $p<0.05$ under which the null hypothesis that no differences existed was rejected

Probability results from T-tests for Moxifloxacin Coated Sutures:

	p-values							
Comparison Between Sutures:	**Time 0**	**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**	**Day 7**
0.1% and 1.0%	0.0006*	0.0034*	0.2119	0.0374*	0.0006*	0.1081	0.4077	0.0181*
0.1% and Coated (no drug)	0.0205*	0.0050*	0.2023	0.4721	0.1696	0.0037*	0.3951	0.1215
1.0% and Coated (no drug)	0.0006*	0.1248	0.3665	0.0087*	0.0016*	0.0171*	0.2807	0.0012*

- * Indicated significant p-values at $p<0.05$ under which the null hypothesis that no differences existed was rejected

Appendix G
Statistical Outputs for Handling and Mechanical Properties

Results from Levene's Statistic for Homogeneity of Variances:

Properties	p-values
Knot Pull Strength [N]	0.000174
Tensile Strength [N]	0.008965
Flexural Rigidity [g*cm]	0.485844
Tissue Drag	0.000022

ANOVA
Flexural Rigidity [g*cm]

	Sum of	df	Mean	F	Sig.
Between Groups					
3.048	2	1.524	75.302	0	
Within Groups					
0.547	27	2.02E-02			
Total					
3.595	29				

ANOVA
Tissue Drag (mN)

	Sum of	df	Mean	F	Sig.
Between Groups	1673.4	2	836.679	62.947	0
Within Groups	3416	257	13.292		
Total	5089.3	259			