1999

Anti-Bacterial Effect Of Hvpc In Vivo

Marc Campolo
Seton Hall University

Follow this and additional works at: https://scholarship.shu.edu/dissertations
Part of the Rehabilitation and Therapy Commons, and the Surgery Commons

Recommended Citation
https://scholarship.shu.edu/dissertations/242
ANTI-BACTERIAL EFFECT OF HVPC IN VIVO

by

Marc Campolo, MA, PT, SCS

Dissertation Committee:

Dr. Genevieve Pinto-Zipp, Chair

Dr. MaryAnn Clark

Dr. Alma Merians

Dr. Chandra Williams

Approved by the Dissertation Committee:

Genevieve Pinto-Zipp Date 12-15-99

MaryAnn Clark Date 12-15-99

Alma Merians Date 12-15-99

Chandra Williams Date 12-15-99

Submitted in partial fulfillment of the
requirements for the degree of Doctor of Philosophy in Health Sciences
Seton Hall University & University of Medicine and Dentistry of New Jersey
1999
ACKNOWLEDGEMENTS

With great appreciation to:

Dr. Genevieve Pinto-Zipp, for all of her time, support, compassion, and the wonderful mentorship she provided, without whom completion of this thesis would not have been possible.

Dr. Maryann Clark, for her encouragement and friendship when things were difficult, as well as for her great advice to “always take the high ground”.

Dr. Alma Meriens, for her confidence in me as an educator and researcher.

Dr. Chandra Williams, Dr. Eva Ryden, and all of the staff of UMDNJ-RAF for their instruction, patience, and support, as well as affording me the opportunity to have a learning experience of a lifetime.

Ellen Addario and Kathleen Hughes, for their assistance and the many long hours they spent in the lab.

And especially to my lovely wife Debbie, for her great patience, tolerance and understanding of the many late nights and lost weekends over the last four years.
DEDICATION

I dedicate this work to my wife, Debbie, and my sons, Max, Luke, and Jake,

whose unselfish sacrifice and understanding made this possible. It is your love, and my

love for you that makes this work and life in general worthwhile.
TABLE OF CONTENTS

I  INTRODUCTION ................................................................. 3
   Background of the Problem ........................................... 4
   Problem Statement ...................................................... 8
   Definitions ............................................................... 8
   Hypothesis ............................................................... 11
   Rationale ............................................................... 11
   Need for Study ......................................................... 13

II  REVIEW OF THE LITERATURE ........................................... 14
   The Effects of LVDC and HVPC on Granulation Tissue .......... 14
   Bactericidal Effects of Electrical Stimulation ................. 20

III  MATERIALS AND METHODS ........................................... 24
   Subjects ........................................................................ 24
   Procedure .................................................................... 25
   Data Analysis ........................................................... 28

IV  RESULTS ...................................................................... 29
   Table 1 ....................................................................... 31
   Figure 1 ...................................................................... 32
   Figure 2 ....................................................................... 33
   Figure 3 ....................................................................... 34
   Table 2 ....................................................................... 35
   Table 3 ....................................................................... 36

V  DISCUSSION ................................................................. 37

VI  SUMMARY AND CONCLUSIONS ....................................... 42

References .......................................................................... 43

Appendixes ......................................................................... 50

A  ABSTRACT ................................................................. 50
B  IACUC ACCEPTANCE LETTER ........................................ 52
C  APPLICATION TO USE LABORATORY ANIMALS .......... 53
D  INFECTIOUS AGENTS USE PROTOCOL ....................... 54
E  POWER ANALYSIS STUDY ............................................. 55
Abstract

ANTII-BACTERIAL EFFECTS OF HVPC IN VIVO Campolo, M

PURPOSE: A historical review of the literature reveals that low intensity direct current (LIDC) is effective in the treatment of infected wounds. Since the 1970’s, high voltage pulsed current (HVPC) stimulators have been used for the same purpose based on the assumption that they have the same physiological effects as LIDC. To date, however, there is insufficient research to support clinical use of HVPC for infected wounds. The purpose of this pilot study was to determine whether HVPC has an inhibitory effect on bacteria in vivo in order to provide evidence to support the clinical use of HVPC stimulation in the treatment of infected wounds.

SUBJECTS: An animal model was used in order to avoid any of the confounding effects associated with the use of human subjects. All the rules and regulations set forth by the Institutional Animal Care and Use Committee were strictly adhered to. IRB approval was also obtained. The subjects consisted of 12 New Zealand rabbits of equivalent size, weight and age.

METHODS AND MATERIALS: The animals were randomly assigned to either an experimental group (EXP=7) or a control group (CON=8). Each animal was anesthetized and a full thickness wound (2cm by 3cm) was made on their backs, which was then infected with 1 ml of 1 X 10^7 Staphylococcus Aureus solution. The wound was then covered with a sterile dressing. Bacterial colony counts, measured in CFU’s, were used to establish both the initial and final level of infection. Data were acquired from wound cultures obtained 1 day post infection (initial). Treatment was initiated the following day and continued for six consecutive days. Electrodes were placed on all of the animals; however, only the EXP group received electrical stimulation. The parameters chosen were consistent with those recommended clinically: the waveform was monophasic and twin peak in shape, the phase duration was 75 nsec, the pulse rate was 7100 pps, the amplitude was the highest obtainable without causing muscular contraction (not to exceed 100V), and the current modulations was continuous.

RESULTS: A Mann-Whitney U test (Table 3) conducted compared the difference between the % change of the bacterial counts, revealed that there was no statistically significant difference between the EXP and CON groups from the initial to final condition (V=18, P=0.246). Although statistical significance was not achieved, further assessment of the data revealed to interesting trends. First, it was noted that the EXP group consistently exhibited a substantial decrease in their bacterial counts across all subjects and almost complete abolition in most subjects (mean decrease -97.4%, SE 1.36). Conversely, consistent results were not observed in the CON group; although some subjects did exhibit a marked decrease in their respective bacteria count, others exhibited a substantial increase resulting in a mean increase of 22.5% (SE 75.56). Second, when converting the data into ranks, the ranked data appears to exhibit an interesting general tendency. The mean rank of the CON group increased from 6.69 to 8.75, while that of the EXP group decreased from 9.50 to 7.14.

CONCLUSION: Although statistical significance may not have been achieved, based upon the noted trends in the data, the author suggests that HVPC may have contributed to the demonstrated positive trends of exhibiting bactericidal effects in acute wounds of animals. Further studies using human subjects are necessary to truly establish the efficacy of the antibacterial effects of HVPC.
CHAPTER I

INTRODUCTION

Pressure ulcers are a common but serious problem that affects acute care, nursing home, and home care populations. It has been reported that this condition affects 9 percent of all hospitalized patients and 23 percent of all nursing home patients and the total national cost has been estimated to be $1.335 billion dollars (Bergstrom, et al, 1994). Additionally, several populations may be at higher risk, including quadriplegic patients (60 percent prevalence), elderly patients who sustained femoral fractures (60 percent prevalence), and critical care patients (41 percent prevalence). Prompt and effective treatment can minimize the deleterious effects of pressure ulcers such as pain and disfigurement; however, if the condition becomes chronic, it can be difficult to treat and result in prolonged hospitalization.

A chronic wound is defined as one that deviates from the expected sequence of repair in terms of time, appearance, and response to aggressive and appropriate treatment (Sussman & Bates-Jensen, 1998). Delayed healing can result from any combination of intrinsic, extrinsic or iatrogenic factors; intrinsic factors affecting chronic wound healing include aging, chronic miscare, circulatory disease, malnutrition and neuropathy; extrinsic factors include medication and immune suppression, irradiation, psychophysioologic stress, and infection; and iatrogenic factors include ischemia and inappropriate wound care management resulting in trauma to the wound.

Reports of wound management by physical therapists have appeared in the physical therapy literature for more than three decades (Sussman & Bates-Jensen, 1998). As a highly respected member of the wound-care team, the physical therapist assists in all
aspects of wound care: including, debridement, dressing selection and application, and recommending strategies to relieve or redistribute pressure for those confined to bed or wheelchair or for the ambulatory individual with an insensate foot. Additionally, physical therapists also provide a unique function, they are skilled in the use of physical agents (heat, light, sound and water), therapeutic exercise, and electrotherapeutic modalities, all of which have benefits to offer the patient in contributing to wound healing strategies (McCulloch, 1998). However, recently, the use of electrotherapeutic modalities has become somewhat controversial following the Health Care Finance Administrators (HCFA) July 14, 1997 decision to deny payment for the use of electrical stimulation in the treatment of wounds. HCFA’s non-coverage decision was based on two new requirements for Medicare coverage of electrical stimulation. First, that a treatment such as electrical stimulation under consideration must be “markedly superior” to other treatments for similar conditions, and second, that devices such as electrical stimulators used in a particular treatment receive FDA approval for the specific use under consideration, i.e. wound treatment (SCE Newsletter, 1997).

**Background of the Problem**

Electrical stimulation as a means of enhancing healing was practiced as far back as the seventeenth century (Davis and Ovington, 1993); however, not until the mid 1900's was research produced supporting the idea that therapeutic doses of electrical current can augment healing of chronic wounds due to the bactericidal effects of electrical current and the stimulation of granulation tissue growth (Alvarez, Mertz, Smerbeck & Eaglstein, 1983; Byl, et al, 1994; Calrley & Wainapel, 1985; Castillo, et al,
Two types of electrical stimulators have been traditionally used for accelerating wound healing: low voltage direct current (LVDC) stimulators and high voltage pulsed current (HVPC) stimulators (Nelson & Currier, 1991). LVDC stimulators produce a continuous uninterrupted unidirectional flow of charged particles, commonly referred to as “galvanic” current, which typically produces less than 100 volts of peak voltage. HVPC stimulators produce a unidirectional monophasic pulsed current with peak amplitudes of 100 to 500 volts, with a wave form that is typically twin-peak in shape and designed to last for a short period of time (5 to 100 microseconds). HVPC stimulators were initially classified as “high voltage galvanic stimulators” by their manufacturers due to the nature of their monophasic pulse. This misnomer has led to some confusion and inappropriate use of HVPC stimulators because clinicians assumed that these units had similar characteristics and effects, and therefore, the same uses as the “low voltage galvanic stimulators”. Many clinical applications of HVPC have been suggested, including its use to enhance wound healing, but the basis for this is derived from the results of research performed using LVDC (Nelson & Currier, 1991; Sussman & Bates-Jenson, 1998).

Bacterial burden is one of the extrinsic factors related to delayed wound healing. The use of electrical stimulation (both HVPC and LVDC) to inhibit or destroy wound pathogens in vitro and in vivo has been documented extensively (Barranco, Spadaro, Berger, & Becker, 1974; Guffey & Assmussen, 1989; Kincaid & Lavoie, 1989; Ong, Laatsch, & Kloth, 1994; Rowley, 1972; Rowley, McKenna, & Chase, 1974; Szuminsky,

With regards to efficacy of HVPC bactericidal effects, there have only been two studies to date that demonstrated positive effects, and both have been conducted in vitro. Kincaid & Lavoie (1989) reported that the growth of three microorganisms commonly found in human wounds was inhibited in vitro at both the anode and cathode when exposed to 250 volts of HVPC for two hours. More recently, Szuminsky, Albers & Eddy (1994) reported similar results on four different species of bacteria when subjected to 500 volts of HVPC for 30 minutes. The consensus with these studies are that the amplitudes used would be intolerable if used on infected wounds in humans and in vitro studies do not take into account the circulatory effects of HVPC.

The proposed mechanisms by which electrical stimulation exhibits its antibacterial effects include electrolysis, galvanotaxis, and alteration of tissue pH (Ong, Laatsch, & Kloth, 1994). Electrolysis is the death of the bacteria resulting from the direct action of the electric current. Wheeler, Wolcott, & Morris (1971) postulated that the bactericidal effect of continuous cathodal current might be due to either the depletion of bacterial substrates or disruption of intracellular metabolic processes resulting in the death of the organism by a direct electrolytic effect.
Galvanotaxis is the attraction of the cells of repair to the anode or cathode (Cooper & Schliwa, 1985). Neutrophils, lymphocytes, platelets, and macrophages are early responders to injury and start the inflammatory response. Research has demonstrated that polarity influences the motility of various cells. Neutrophils have been observed to be attracted to the negative pole if the wound is infected and to the positive pole if not infected (Fukushima & Sends, 1953). Lymphocytes and platelets are attracted to the negative pole (Bourguignon & Bourguignon, 1989), whereas macrophages and fibroblasts are attracted to the positive pole (Orida & Feldman, 1982). It has been suggested that perhaps the documented anti-bacterial effects of electrical stimulation were the result of galvanotaxic attraction of phagocytic macrophages and leukocytes to infected tissues rather than from detrimental effects of pathogens caused by electrolysis or altering the tissue pH (Ong, Laatsh, & Kloth, 1994; Rowley, McKenna, & Chase, 1974).

Electrochemical effects occur as a result of the polarizing effects of continuous use of direct current. Unidirectional current flow causes the migration of ions from dissociated salts. Within the tissue, positively charged ions, such as sodium (Na+), migrate toward the cathode; whereas, negatively charged ions, such as chloride (Cl-), migrate toward the anode. The result is a chemical reaction; the formation of hydrochloric acid under the anode and the formation of sodium hydroxide under the cathode. This resulting chemical reaction changes the pH of the tissue, which can lead to cellular death. However, this condition only occurs with LVDC. Due to the nature of its waveform, HVPC has a very low average current, and as a result there is very little, if any, chemical reaction under the electrodes (Newton & Karselis, 1983). Based on current
literature, it is doubtful that the bactericidal effects of HVPC is a result of alteration of the tissue pH.

**Problem Statement**

Clinically, wound healing is impeded where infection is present. The purpose of this study was to determine if HVPC, used at amplitudes human patients can tolerate clinically, has an effect, *in vivo*, in reducing the viability of an infecting microorganism in wounds; thereby, positively affecting one of the extrinsic factors contributing to delayed wound healing.

**Definitions**

*Amperage* the unit of current being the ampere (A), is defined as the rate at which electrons move past a certain point. A milliampere (mA) is one thousandth of an ampere.

*Amplitude* refers to either the voltage or the current intensity of an electric current. Voltage is a measure of the force of the flow of electrons and amperage is the measure of the rate of flow of the current. When voltage is turned up, the current will also go up, and vice versa. Some stimulators provide a readout of voltage and some a readout of current.

*Anode* the positive electrode.

*Antibacterial* any agent, physical or chemical, that eliminates living organisms pathogenic to host.

*Bactericidal* destructive to or destroying bacteria
Biphasic wave forms bi-directional current flow. Biphasic waves are such that the polarity is constantly changing. They are opposite at any moment in time. However, the wave form can be biased so that one polarity is emphasized. Symmetric biphasic wave forms are balanced and have no net polarity. Asymmetrical biphasic wave forms are unbalanced and exhibit a polarity based on the bias.

Capacitive Coupling capacitively coupled electrical stimulation involves the transfer of electric current through an applied surface electrode pad that is in wet (electrolytic) contact (capacitively coupled) with the external skin surface and/or wound bed.

Cathode the negative electrode.

Chronic Wound one that deviates from the expected sequence of repair in terms of time, appearance and response to aggressive and appropriate treatment.

Decubitus Ulcer an ulcer, initially of the skin, due to prolonged pressure, usually in a person who is lying down. However, pressure ulcers or sores may occur at any site (e.g., on the buttocks of patients confined to wheelchairs). The most common sites are over bony prominences (i.e., the sacrum, heels, trochanter, lateral malleoli, and ischial areas). The combination of pressure, shearing forces, friction, and moisture lead to the death of tissue due to the lack of blood supply, if not treated vigorously, the ulcer will progress from a simple erosion to complete involvement of the deep layers of the skin and may eventually extend to the underlying muscle and bone tissue.
Millivolt.

Microscopic Direct Current is a direct current with an amplitude of less than one

Generator produce voltages in the range of 60 to 100 volts.

or current has been traditionally called galvanic current. In general, low voltage

current, direction of the flow is determined by the polarity selected. This form

low voltage Direct Current (LDC) is continuous, uninterrupted, unidirectional

living organism.

In vivo in the living body or organism, an in vitro test is one performed on a

usually involving isolated tissue, organ, or cell preparations.

in vitro in glass, as in a test tube. An in vitro test is one done in the laboratory,

low average current

when 100 volts. There is a long interpulse interval between pulses that results in a

fixed duration in the microsecond range (up to 200 usec) and a voltage greater

electrotherapeutic devices that have a "lawn-peak" monophasic wave form with a

High Voltage Pulsed Current (HVPC) is associated with a class of

and a cathode (the wound is receiving (P. Koshima & Send, 1953))
électrotherapy and produce superoxide to fight bacteria. Neutrophils are attracted

that function as phagocytic cells that phagocytize in the hypoxic acidotic

of repair. An example would be neutrophils, which are granulocytic leukocytes

Galvanotaxis unidirectional electrochemical current flow in the tissues attracts the cells

that is transferred to the target tissue is controlled by the electrical source.

coupled electrochemical current to transfer energy to a wound. The type of electrolytic

Electrical stimulation for Wound Healing is derived as the use of a capacitance
Monophasic wave forms have uni-directional current flow. Monophasic waves are such that at one electrode the polarity is positive and the other is negative. Polarity refers to the property of having two poles that are oppositely charged. The positive pole is called the anode and the negative pole is the cathode. The positive pole lacks electrons and attracts them from the negative pole. Polarity can be chosen or emphasized for biological effects.

Power analysis the power of a hypothesis test equals the probability of detecting a particular effect, that is, of rejecting the null hypothesis. Power analysis is used to determine the appropriate sample size for a projected experiment.

Pressure Ulcer any lesion caused by unrelieved pressure resulting in damage of underlying tissue. Pressure ulcers usually occur over bony prominences and are graded or staged to classify the degree of tissue damage observed.

Wave Form different types of current have different characteristic wave forms. Wave forms are the graphic representations of a current on a current/time or voltage/time plot. Waveforms are classified by the direction of current flow.

Current flow is either unidirectional or bi-directional.

Hypothesis

HVPC will exhibit an antibacterial effect in vivo at levels that humans can tolerate. The mechanism of action is postulated to galvanotaxis and not electrolysis.

Rationale

The studies conducted by Kincaid & Lavoie (1989) and Szuminsky, Alber, Unger, & Eddy (1994) in vitro suggests that electrolysis occurs at voltages greater than 250
volts. Conversely, Guffey & Asmussen's (1989) *in vitro* study revealed that HVPC amplitudes less than 160 volts had no such effect. This project used voltages less than 100 volts, so it is unlikely that if HVPC does exhibit an antibacterial effect, the mechanism of action would be electrolysis. Additionally, because HVPC does not affect pH levels (Newton & Karselis, 1983), alteration of pH cannot be considered as the probable cause of the antibacterial effect.

Previous investigations support the efficacy of LVDC in wound healing for both stimulation of granulation tissue effects (Wolcott, Wheeler, Hardwicke, & Rowley, 1969; Gault & Gatens, 1976; Carley & Wainapel, 1985) and its antibacterial effects (Barranco, Spadaro, Berger, & Becker, 1974; Rowley, McKenna, & Chase, 1985). Clinical studies performed by Feeder & Kloth (1985), Kloth & Feeder (1988), Feeder, Kloth, & Gentzkow (1991), and Unger, Eddy, & Raimastry (1991) provide evidence supporting the efficacy of HVPC in the stimulation of granulation tissue. Both LVDC and HVPC stimulators exhibit polar capabilities (the ability to create a positive or negative electrical field) due to their monophasic pulse configuration. It is these polar effects that appear to give LVDC and HVPC their granulation tissue stimulation effects as studies using biphasic waveforms achieved the best wound healing effects when the biphasic waveform is asymmetrical and biased so that the polarity at one pole predominates (Baker, Chambers, DeMuth, & Villar, 1997; Lundenberg, Eriksson, & Malm, 1992; Stefanouska, et al, 1993). It would be reasonable to assume that if a HVPC stimulator is able to provide a sufficient electrical field to stimulate granulation tissue growth due to its polar capabilities, HVPC should be able to provide a strong enough electrical field to stimulate galvanotaxis and the resulting antibacterial effects.
Need for Study

All studies to date that have been performed to determine the antibacterial effects of HVPC have been conducted in vitro, and the authors agree that in order to truly establish the efficacy of HVPC in the treatment of infected wounds, studies conducted in vivo are necessary (Guffey & Asmussen, 1989; Kincaid & Lavoie, 1989; Szuminsky, Albers, Unger, & Eddy, 1994). The in vitro studies on bacterial inhibition deal mainly with the electrolytic aspects of electrical stimulation, while an in vivo study would be able to determine what effect HVPC stimulation may have on the galvanotaxic attraction of phagocytic macrophages and leukocytes and their role in inhibiting bacterial growth. Additionally, HCFA’s July 1997 decision to deny payment for the use of electrical stimulation in the treatment of wounds had deprived many patients of critical treatment they had been receiving. Although an injunction was awarded to the APTA on September 1997, controversy with regards to this treatment persists as a final rule on this decision has yet to be made. A positive outcome of this project may lend support to the clinical use of electrical stimulation in wound care.
CHAPTER II
REVIEW OF THE LITERATURE

The Effects of LVDC and HVPC on Granulation Tissue

The mechanism by which electrical stimulation promotes wound healing is not yet clearly understood, however, the most widely accepted hypotheses are, 1) the “current of injury”, 2) cellular level responses, and the 3) bactericidal effect of electrical stimulation. In 1962, Becker et al, conceptualized the existence of a direct current electrical system controlling tissue healing. They stated that the electrical balance of the body is disturbed in an injury resulting in a shift in the current flow and a change in the DC potential of the tissue, referred to as the “current of injury”. The authors proposed that the DC electrical potential initially responsible for triggering the healing process was positive, and placement of the anode directly on the wound would facilitate the healing process. Davis & Ovington (1993) stated that investigations of wounded skin demonstrated the existence of natural bioelectrical currents, the exterior layers of the skin being electronegative with respect to the inner layers. If electrical signals play a role in the stimulation of wound repair, then exogenous applications of electrical current to chronic wounds could be expected to mimic the body’s bioelectrical currents and enhance the tissue healing process.

Accelerated healing may be related to the cellular responses to electrical stimulation, especially fibroblasts, which have been shown to be fundamental in the process of wound repair as they build the collagen matrix known as granulation tissue. Cruz, Bagron, & Suarez (1989) performed experimental studies on domestic pigs, evaluating the effect of HVPC on the rate of healing of full thickness thermal burns and
found a significantly faster rate of wound contraction and a higher fibroblast response in the stimulated wounds. Bourguignon & Bourguignon (1991) exposed human fibroblast cell cultures to HVPC and concluded that the rates of both protein and DNA synthesis could be significantly increased by exposure to electrical fields; however, at intensities greater than 250 volts an inhibitory effect was noted. Cheng, et al (1982) revealed that direct currents ranging from 10 uA to 1000 uA increase ATP concentration in tissue, stimulating amino acid incorporation into proteins of rat skin, contributing to increases in protein synthesis. Reich, Cazzaniga, Mertz, Kerdel & Eaglstein (1989), who examined the effects of electrical stimulation on mast cells (which are associated with a variety of pathologic skin conditions in humans, including ulcers) in acute wounds on pathogen-free pigs, reported a significant reduction in the number of mast cells seen when the wounds were electrically stimulated. This reaction may be related to a decrease in either proliferation or migration of these cells and may prove to be a valuable therapeutic technique. The above findings indicate that protein, DNA and ATP synthesis, as well as the migratory capacity of epithelial and connective tissue cells involved in repair and regeneration can be affected by an electrical field.

HVPC became popular in the 1970's because it did not have any measurable chemical or thermal reactions under the electrodes, reducing or eliminating all known side effects and precautions associated with LVDC (Alon, 1987). The misnomer of high voltage "galvanic" stimulation was applied to this type of stimulator due to its monophasic wave form, which may have misled clinicians to assume that the mechanisms of action as well as the physiological effects of HVPC were the same as those attributed to LVDC, commonly called "galvanic stimulation". The many clinical
applications of HVPC suggested by manufacturers, including its use to enhance wound healing, were derived from the positive results of research performed using LVDC.

Since 1968, several human clinical studies have demonstrated the effectiveness of LVDC when used to promote the healing of chronic wounds. Assimacopoulos (1968) performed an animal study using rabbits to determine if negative direct electrical current accelerates the epithelization and healing process of a wound. The results revealed that the influence of negative electric current shortened the time of healing by 25%.

In 1969, Wolcott, Wheeler, Hardwicke, and Rowley conducted a study on 67 patients presenting a total of 83 ischemic skin ulcers. LVDC was applied directly to 75 ischemic skin ulcers for an eighteen-month trial. The electrical stimulation produced a mean healing rate of 13.4% per week, and 34 ulcers healed completely. The authors reported that eight patients presented with bilateral ulcers of comparable size and location which could qualify as a control to the treated counterpart. In this subgroup, the treated ulcers healed at a rate of 27% per week compared with 5% per week for the controls. Interestingly, the protocol used negative polarity until asepsis was obtained and then switched to positive polarity. This is consistent with the clinical protocol used presently.

Gault and Gatens (1976) conducted a study on 76 patients who had 106 ischemic skin ulcers. Six patients had bilateral symmetrical ulcers that were closely matched in size, shape, position, and general appearance. The results of this study revealed that 100 ischemic skin ulcers treated with LVDC healed at a rate of 28.4% per week. With regards to the six patients with bilateral ulcers the mean healing ratio of the control ulcers was 14.7% per week compared to 30% per week of the treated counterpart.
Initial studies conducted examining the effects of electric stimulation on granulation tissue had lacked sufficient controls. Recognizing this limitation, Carley and Wainapel in 1985 conducted a study on 30 impatients. Subjects were paired according to age and diagnosis and wound etiology, location, and approximate size. One member of the pair was randomly assigned to receive the LVDC protocol while the control used conventional wound therapy. The results suggest a 1.5 to 2.5 times faster healing in the experimental group.

More recently, Mulder (1991) conducted a randomized double blind study of electric stimulation with 59 patients representing 67 open wounds of pressure, vascular and surgical etiology. The stimulator used in this project was a low-intensity, pulsed, direct current unit. Different from LVDC in that the current is interrupted versus continuous, similar in that it exhibits polar capabilities. The authors reported that after four weeks of treatment, the electrical stimulation group showed a 56% decrease in size with only a 33% decrease in size in the sham treatment group.

Research to support the hypothesis that HVPC promoted the healing of chronic wounds was not conducted until the mid 1980's. Prior to that time, support for the clinical use of HVPC in the treatment of chronic wounds was extrapolated from LVDC research.

In 1984, Akers and Gabrielson compared three treatment procedures of decubitus ulcers: 1) whirlpool bath, 2) combination of whirlpool bath and HVPC, and 3) HVPC alone. The authors stated that the comparisons of the different techniques indicated that the greatest rate of changed wound size occurred in those patients who received HVPC
alone. The next best rate of change occurred in the combination group, and the least with the whirlpool alone group.

Feeder and Kloth (1985) published a study of eight patients with Stage IV decubitus ulcers. The patients in the treatment group completely healed in a mean of 7.3 weeks, whereas the patients in the control group had an increase in the wound size on the average of 13.8% in a mean of 10.6 weeks.

Alon (1986), in a published abstract, "Diabetic Ulcer Healing Using High Voltage TENS", reported that twelve of fifteen diabetic patients with dermal ulcers had complete healing after a mean of 2.6 months of treatment with HVPC.

A study by Kloth and Feeder (1986) was conducted on 16 patients to determine whether high voltage electrical stimulation accelerates the rate of healing of dermal ulcers. Subjects were randomly assigned to treatment and control groups. The authors reported that the treated ulcers healed completely in an average of 7.5 weeks, whereas, the control group increased in size by 28.9% in an average of 7.4 weeks. Interestingly, when three patients were crossed over from the sham to active treatment group they healed at an average of 38% per week.

More recently, in a published abstract, Unger, Eddy, and Raimastry (1991) reported their work assessing the efficacy of treatment with HVPC on wound healing using a controlled double blind research design. Seventeen patients having pressure ulcers were randomly assigned to either an HVPC or placebo group. The results revealed that of the nine patients receiving HVPC treatment, eight patients were healed. Conversely, of the eight patients receiving sham treatment, only three were healed. The
authors stated that the proportion of patients healed with HVPC was 2.4 times higher than those without such therapy.

Feeder, Kloth and Gentzkow (1991) also conducted a randomized, double blind, multicenter study comparing healing of chronic dermal ulcers treated with HVPC to those treated with sham electrical stimulation. Forty-seven patients were randomly assigned to either the treatment group or the control group. The results of their study indicated that pulsed electrical stimulation had a beneficial effect on healing of Stages II, III, and IV chronic dermal ulcers.

In 1993, Mawson et al., conducted a study to determine whether HVPC could increase sacral transcutaneous oxygen tension in spinal cord injured persons lying prone and supine. The authors stated that previous research indicates that spinal cord injured patients had lower sacral transcutaneous oxygen tension in these positions, which may be related to the high incidence of pressure ulcers in these patients. Their results indicated that HVPC can reliably increase the sacral oxygen levels of spinal cord injured persons.

Most recently in 1996, Baker, Rubayi, Villar and Demuth conducted a study to evaluate the effect of stimulation waveform and electrode placement on wound healing. Eighty patients with spinal cord injury and one or more pressure ulcers, for a total of 185 ulcers, received 45 minutes of stimulation daily. Comparisons were made between asymmetric biphasic waveform, symmetric biphasic waveform, microcurrent stimulation, or sham. Their results showed significantly better healing rates in the asymmetric biphasic waveform.

Similarly Baker, Chambers, DeMuth, and Villar (1997) conducted a study to evaluate the effects of two stimulation waveforms on healing rates in patients with
diabetes and open ulcers. This study enrolled 80 patients with open ulcers who received stimulation with either an asymmetric biphasic or symmetric biphasic square-wave pulse. The results revealed that stimulation with the asymmetric stimulation enhanced healing by nearly 60% over the control rate of healing, whereas stimulation with the symmetrical wave did not increase the healing rate when compared with the control subjects.

The latter two studies cited are of interest because although the asymmetrical biphasic waveform is different from LVDC and HVPC waveforms in that it has two phases, this waveform does have minimal polar capabilities similar to that of both the LVDC and HVPC waveforms. Thus these findings may lend some support to the notion that the polar capabilities of a waveform play a role in healing capabilities.

Although there have been conflicting results from animal research (Brown & Gogia, 1987; Brown, Gogia, Sinacore & Menton, 1995; Brown, McDonnel & Menton, 1989; Cruz, Banron & Suarez, 1989), additional studies performed on human subjects have supported the efficacy of both LVDC and HVPC in the augmentation of tissue repair.

**Bactericidal Effects of Electrical Stimulation**

Increased bacterial burden has also been associated with delayed wound closure. Several studies have documented the efficacy of LVDC in inhibiting or destroying wound pathogens *in vitro* and *in vivo* (Alvarez, Mertz, Smerbeck & Eaglistein, 1983; Rowley, 1972; Rowley, McKenna & Chase, 1974). Rowley (1972) noted that stimulation with the negative pole using LVDC caused a decrease in the growth rate of *Escherichia coli* in an *in vitro* study. Further studies by Rowley, et al (1974) demonstrated a growth retardation of *Pseudomonas aeruginosa* in rabbit-skin wounds when the cathode of LVDC was
applied to the wound sites. Barranco, et al (1974), using the negative pole of LVDC applied to Staphylococcus aureus infected rabbit femurs, observed a decrease in growth rate of the organisms following one hour of stimulation. Bolton, Foleno, Means, and Petrucelli (1980) studied the effects of LVDC on intact human skin inoculated with Staphylococcus epidermidis. The results revealed that bactericidal activity was exhibited in the 13 subjects included in this study, and that the bactericidal activity for this species was associated with the positive polarity. Another human study by Fakhri and Amin (1987) showed bacterial killing without antibiotics in resistant burn wounds. The researchers studied 20 burn patients, some of whom cultured positive for Pseudomonas, E-coli, and Staphylococcus aureus. LVDC stimulation was applied to the burns for 10 minutes, twice a week until the burn was healed. It was reported that all of the wounds healed. These findings collectively appear to support the use of LVDC in the treatment of infected wounds.

The use of HVPC to promote healing of decubitus ulcer and surgical wounds by decreasing the bacterial burden was initially based on the results of studies using LVDC. Kincaid and Lavoie (1989) further addressed this issue by conducting an in vitro study. The authors reported that the growth of three micro-organisms commonly found in human wounds (Staphylococcus aureus, Escherichia coil, and Pseudomonas aeruginosa) was inhibited at the cathode resulting from exposure to HVPC for 2 hours at 250 V. Similarly, in their in vitro study, Szuminsky, Alber, Unger, & Eddy (1994) concluded that HVPC produced antimicrobial effects at 500 V when applied for 30 minutes. Although both studies demonstrated the efficacy of the bactericidal action of HVPC, the voltages used were too high to be tolerated by humans and may be detrimental to
collagen formation (Bourguignon & Bourguignon, 1991). Conversely, when Guffey and Asmussen (1989) compared the bactericidal activity of HVPC and LVDC in an in vitro system on Staphylococcus aureus, their findings demonstrated that HVPC did not result in bactericidal activity when current was applied for 30 minutes at voltages less than 160 volts. Typically, in a clinical setting, voltages of between 100 to 150 volts or less are employed (Alon, 1987; Nelson & Currier, 1991; Sussman & Bates-Jenson, 1998). Thus, if HVPC does exhibit bactericidal effects in vivo, it is unlikely these effects are the results of electrolysis.

The majority of research supports the use of cathodal current for the antibacterial effects, although there have been studies indicating bacterial inhibition at the anode (Bolton, Folen, Means, & Petrucelli, 1980; Guffey & Asmussen, 1989; Loatsch, Ong, & Kloth, 1995; Ong, 1994). Loatsch, Ong & Kloth (1995) proposed two mechanisms by which cathodal DC stimulation directly decreases pathogens; first, the cathodal DC bombards organisms with electrons that continually excite cell membranes, thus depleting bacterial substrates and killing the organism, and second, that electrical stimulation disrupts intracellular metabolism. Ong, Laatsch & Kloth (1994) suggested that the documented antibacterial effects of continuous cathodal LVDC may be the result of galvanotaxic attraction (the attraction of cells to the anode or cathode) of phagocytic macrophages and leukocytes to the infected tissues rather than from the detrimental effects of pathogens caused by electrolysis or of alteration of the tissue pH. Guffey & Asmussen’s (1989) study appears to lend support to this notion.
A recent literature review by this author reveals that to date, there remains a lack of published research articles on the subject of the antibacterial effects of HVPC conducted *in vivo*, indicating that there continues to be a need for projects of this nature.
CHAPTER III
MATERIALS AND METHODS

Subjects:

All studies to date that have been performed to determine the bactericidal effects of HVPC have been conducted in vitro. Previous authors agree that in order to truly establish the efficacy of the use of HVPC in the treatment of infected wounds, studies conducted in vivo are necessary (Guffey & Asmussen, 1989; Kincaid & Lavoie, 1989; Szuminsky, Alber, Unger, & Eddy, 1994). An animal model was chosen to eliminate any confounding variables associated with electrical stimulation treatment regimes used with human subjects (Fitagerald & Newsome, 1993). Previous animal studies conducted investigating the effects of electrical stimulation have used New Zealand rabbits (Girlanda, et al, 1982; Korpan, Resch, & Kokoschinegg, 1994; Riegels-Nielson, Espersen, Holmich & Frimodt-Moller, 1995; Rowley, McKenna, & Chase, 1974).

Therefore, to maintain research consistency, New Zealand rabbits were used as subjects for this study. In order to satisfy the UMDNJ-RAF’s Institutional Animal Care and Use Committee (IACOC) requirements, the methods were modeled after an existing research protocol. The study conducted by Rowley, McKenna, & Chase (1974) was chosen as the prototype for this project because it mirrored our project almost identically. The difference was that they were attempting to establish the antibacterial effects of LVDC in vivo.

This study involved the use of three series of six rabbits, for a total of eighteen. Three subjects were disregarded. Two because they were not sufficiently infected 24
hours post surgery (inclusion criteria required that the wounds be infected at a level greater then ten colony-forming units (CFU) per swab). The third rabbit was excluded because it partially removed its bandage causing the wound to dry out. The animals from the three series were analyzed as one group because the environmental conditions, number of treatment days, and data acquisition were all identical across series. Differences in environmental conditions, discrepancies in number of treatments due to scheduling difficulties, and the use of a different type bandage in an attempt to decrease the discomfort from the dressing changes resulted in the exclusion of animals from any additional series completed during this experiment. The total number of animals used for this study was derived through a power analysis calculation (Appendix E).

The subjects included in this project were fifteen adult (4-6 months of age) New Zealand rabbits weighing 2 to 4 kg each. The animals were randomly assigned to an experimental group (EXP, n=7) and a control group (CON, n=8). The animals were placed in cages, one per specifically designed cage, and received 5 to 6 oz. of food daily and water ad lib. The temperature was kept constant at 22° and an equal ratio of daylight hours to non-daylight hours was maintained (12 hours on, 12 hours off).

Procedure

All fifteen rabbits were anesthetized, the hair clipped from their dorsum, and a 2 by 3 cm rectangular open wound, located one centimeter from the vertebral column midway between the scapular and pelvic areas, produced using aseptic surgical procedures. The wound was then infected by covering it with a sterile gauze pad soaked in 1 ml of 1X10⁷ Staphylococcus aureus solution (bacteria commonly found in wounds).
This solution concentration was chosen as previous studies have documented that when the bacterial content in an ulcer exceeds $10^5$ organisms per gram of tissue, healing is impaired (Daltry, Rhodes, & Chattwood, 1981; Sapico, et al, 1986). The wound was then covered by a sterile dressing (Biocclusive™ film dressing) and the rabbit returned to the cage for post-operative recovery and observation.

Bacterial colony counts, measured in CFUs, were used to establish both the initial and final level of infection. Data was acquired from wound cultures obtained one-day post infection (initial) and six-days post treatment (final). The initial culture represents the wound immediately post infection and the final culture represents the wound after the treatment regime. Again, the subjects wound had to achieve a level of infection of greater than ten CFUs to included in this experiment.

UMDNJ-RAF's IACOC recommended against the use of tissue biopsy in order to prevent the animals from being subjected to any undue suffering. As a result, the quantitative swab technique was chosen as it is commonly used clinically and data show that tissue biopsy, needle aspiration, and qualitative swab techniques are comparable in terms of sensitivity, specificity, and accuracy (Stotts, 1995). The recommended method of performing the quantitative swab culture involves cleaning the wound with saline, placing the end of a sterile cotton-tipped applicator stick on a $1 \text{ cm}^2$ area of the open wound and rotating it. Pressure is applied to the swab to cause the tissue fluid to be absorbed in the cotton tip of the swab. The swab tip is then inserted into a sterile tube containing transport medium (Sussman & Bates-Jenson, 1998). The culture is then transported to the laboratory for analysis. Serial dilutions of the organisms are made on
agar plates. Results are expressed as organisms per swab or colony-forming units (CFU) per swab.

The experimental group received one hour of HVPC stimulation for six consecutive days from a Chattanooga Forte CPS 200 electrotherapy unit. On treatment days, the dressings were removed and electrodes constructed out of aluminum foil, wrapped in sterile gauze, and soaked in sterile saline were placed on all of the subject's wounds. Aluminum foil was chosen as the electrode material as it is an excellent conductor, non-toxic, inexpensive, disposable, comfortable, and can be sized as needed. The cathode was placed directly in the wound and the anode was placed 2 cm. caudal to the cathode. Both were secured by micropore paper tape and wrapped with an elastic bandage. Treatment parameters were as follows: the waveform was monophasic and twin peak in shape, the phase duration 75 usec, the pulse rate 100 pps, the amplitude the highest obtainable without causing muscular contraction (not to exceed 100 V), and the current modulation was continuous. These parameters are consistent with the parameters that are recommended clinically. The control group did not receive electrical stimulation; however, they did have similar electrodes placed in the wound for consistency on each of the sham treatment days. Both groups were restrained in a special apparatus (a cat-restraining bag) adapted for this procedure. After each treatment session, the wounds were redressed and the animals returned to their cages. In addition to the above procedures, the animals were checked daily, including weekends, for weight, food consumption, and temperature to ensure adequate nutrition and that the procedure did not result in sepsis of the animal.
**Data Analysis**

The Mann-Whitney U test, a test for ranked data when there are two independent samples, was used to compare the percent bacteria killed in experimental verses the control groups. The U test appeared to the most appropriate statistical analysis to use as it is suitable when the sample size is small and there are unequal data sets, as was the case in this project. Additionally, as is true for all tests for ranked data, the U test is immune to assumptions about normality and equal variances. Also, non-parametric statistical procedures are relatively unaffected by single outlier values. A standard alpha level (p<0.05) was selected. U tables supply critical values of U, which are determined by the number in each of the sample groups. Statistical significance is achieved when the observed U is less than or equal to the critical U.

A t test for two independent samples was conducted to compare the level of infection of the wounds in the EXP and CON groups in the PRE-treatment condition to ensure that there was no significant difference between the population means of the groups.
CHAPTER IV
RESULTS

Table 1 lists the results of the wound cultures, which are measured in colony forming units per swab (CFUs), for all subjects PRE-treatment and POST-treatment. The PRE-treatment condition represents the wound cultures for all subjects one-day post infection. The POST-treatment condition represents the wound culture after six consecutive HVPC treatment days for the EXP group and six consecutive sham treatment days for the CON group. The % change column represents the percentage of change of the wound colony count for the individual subjects from the PRE-treatment to the POST-treatment condition.

T-test analysis (Table 2) of the PRE-treatment experimental condition revealed that there was no statistically significant difference in the level of wound infection between the EXP and CON groups (t =1.65, p<0.05). A Mann-Whitney U test (Table 3) conducted compare the difference between the % change of the bacterial counts, revealed that there was no statistically significant difference between the EXP and CON groups from the PRE-treatment to POST-treatment experimental conditions (U = 18, p<0.05).

Although statistical significance was not achieved, further assessment of the data presented in Table 1 revealed two interesting trends. First, when reviewing Table 1, we note that the EXP groups consistently exhibited a substantial decrease in their bacterial counts across all subjects and almost complete abolition in most subjects (mean decrease -97.4%, SE 1.36). Conversely, consistent results were not observed in the CON group; although some subjects did exhibit a marked decrease in their respective bacterial count,
others exhibited a substantial increase resulting in a mean increase of 22.5% (SE 75.56). This trend is made visually clear by viewing Fig. 3, which is a graphic representation of the % change of the wound bacterial counts from the PRE to POST-treatment conditions. Second, we note that the level of infection achieved in the wounds in the PRE-treatment condition varied across subjects in both the EXP and CON groups (Table 1). This response is consistent with past research that used an in vivo model (Rowley, McKenna, & Chase, 1974) and may be due to variations of the circulatory and immune systems of the individual animals. Due to this difference in the level of infection, a direct comparison of the changes that occurred may not be appropriate. However, when we convert this data into ranks and analyze the ranked data of groups PRE-treatment and POST-treatment (Table 3), we notice the data appears to exhibit an interesting general tendency. The mean rank of the CON group increased from 6.69 to 8.75, while that of the EXP group decreased from 9.50 to 7.14. This indicates that the subjects in the EXP group were infected at a higher level overall than the CON group in the PRE-treatment condition, yet in the POST-treatment condition the overall level of infection in the EXP group was less than that of the CON group.

These two trends discussed may lend support to the notion that HVPC may have had a treatment effect, suggesting that HVPC stimulation may have decreased the bacterial effects greater in the EXP group when compared to the CON group, which received no electrical stimulation.
Table 1. Bacterial counts measured in colony-forming units (CFU) per swab in the PRE-treatment condition (1 day post infection) and in the POST-treatment condition (after 6 daily treatments) and the % change from the PRE-treatment to POST-treatment condition.

<table>
<thead>
<tr>
<th>Subject</th>
<th>PRE-treatment</th>
<th>POST-treatment</th>
<th>% Change</th>
<th>Mean</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>EXP GROUP</td>
<td>1 Day Post Infection</td>
<td>6 Day Post Treatment</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 (00)</td>
<td>1,800</td>
<td>180</td>
<td>-90.0</td>
<td>-97.41</td>
<td>1.36</td>
</tr>
<tr>
<td>2 (01)</td>
<td>130,000</td>
<td>190</td>
<td>-99.85</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 (46)</td>
<td>720,000</td>
<td>12,000</td>
<td>-98.33</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 (50)</td>
<td>800,000</td>
<td>15,000</td>
<td>-98.12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 (02)</td>
<td>900,000</td>
<td>630</td>
<td>-99.93</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 (11)</td>
<td>1,100,000</td>
<td>230</td>
<td>-99.98</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7 (45)</td>
<td>1,200,000</td>
<td>52,000</td>
<td>-95.66</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>CON GROUP</th>
<th>1 Day Post Infection</th>
<th>6 Day Post Treatment</th>
<th>% Change</th>
<th>Mean</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (03)</td>
<td>53,000</td>
<td>10</td>
<td>-99.98</td>
<td>22.52</td>
<td>75.56</td>
</tr>
<tr>
<td>2 (47)</td>
<td>120,000</td>
<td>4,100</td>
<td>-96.66</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 (48)</td>
<td>150,000</td>
<td>630,000</td>
<td>320</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 (04)</td>
<td>180,000</td>
<td>7,200</td>
<td>-96.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 (12)</td>
<td>300,000</td>
<td>13,000</td>
<td>-97.66</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 (08)</td>
<td>350,000</td>
<td>1,000,000</td>
<td>271.43</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7 (05)</td>
<td>550,000</td>
<td>10</td>
<td>-99.99</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8 (49)</td>
<td>1,100,000</td>
<td>690,000</td>
<td>-37.27</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Figure 1. Control Subjects: Bacterial count of the wound measured in colony forming units (CFU's) per swab in the PRE-treatment condition (1 day post infection) and the POST-treatment condition (after 6 sham treatment days).
Figure 2. Experimental Subjects: Bacterial count of the wound measured in colony forming units (CFU's) in the PRE-treatment condition (1 day post infection) and the POST-treatment condition (after 6 treatment days).

Antibacterial Effects of HVPC

<table>
<thead>
<tr>
<th>Experimental Subjects</th>
<th>Pre</th>
<th>Post</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1800</td>
<td>180</td>
</tr>
<tr>
<td>2</td>
<td>15000</td>
<td>190</td>
</tr>
<tr>
<td>3</td>
<td>72000</td>
<td>12000</td>
</tr>
<tr>
<td>4</td>
<td>90000</td>
<td>15000</td>
</tr>
<tr>
<td>5</td>
<td>30000</td>
<td>630</td>
</tr>
<tr>
<td>6</td>
<td>110000</td>
<td>230</td>
</tr>
<tr>
<td>7</td>
<td>120000</td>
<td>52000</td>
</tr>
</tbody>
</table>
Figure 3. % Change comparison of the wound bacterial counts from the PRE-to the POST-treatment conditions of the experimental and control groups.
Table 2. Independent Samples T Test results.

<table>
<thead>
<tr>
<th></th>
<th>t</th>
<th>df</th>
<th>Sig. (2-tailed)</th>
<th>Mean Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>PRE Equal variances assumed</td>
<td>1.653</td>
<td>13</td>
<td>.122</td>
<td>342739.286</td>
</tr>
<tr>
<td>Equal variances not assumed</td>
<td>1.618</td>
<td>10.978</td>
<td>.134</td>
<td>342739.286</td>
</tr>
<tr>
<td>Group</td>
<td>Mean Rank</td>
<td>Mann-Whitney U</td>
<td></td>
<td></td>
</tr>
<tr>
<td>------------</td>
<td>-----------</td>
<td>----------------</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre Test</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Experimental Group</td>
<td>9.50</td>
<td>17.51</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control Group</td>
<td>6.69</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>15</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Post Test</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Experimental Group</td>
<td>7.14</td>
<td>22.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control Group</td>
<td>8.75</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>15</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% Change</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Experimental Group</td>
<td>6.57</td>
<td>18.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control Group</td>
<td>9.25</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>15</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
CHAPTER V

DISCUSSION

The purpose of this study was to determine if HVPC, used at amplitudes human patients can tolerate, has an effect, *in vivo*, in reducing the viability of an infecting microorganism in wounds; thereby, positively affecting one of the extrinsic factors contributing to delayed wound healing. The results of this present project indicate that although significance was not achieved (U=18, p<0.05), there appeared to be a positive trend suggesting that HVPC stimulation decreased bacterial effect greater in the experimental group when compared to the control group, lending support for further investigation. Interestingly we note, while the experimental group exhibited a substantial and consistent decrease in their bacterial count across subjects, consistent results were not observed in the control group. While some of the controls did exhibit a substantial increase in their bacterial counts, others exhibited a marked decrease.

A possible explanation for the decrease in the bacterial counts in the control group may be that our subjects were young healthy rabbits who were receiving adequate nutrition and had intact circulatory, neurological, and immune systems thereby enabling some of the control subjects to combat the bacterial infection effectively on their own. The wounds produced on the subjects were acute, so one would expect they underwent a normal acute wound healing process. The biological repair process of acute wounds is based on four phases: inflammation, proliferation, epithelialization, and remodeling that occur in an orderly and overlapping fashion (Sussman & Bates, 1998). A detailed explanation of each of the four phases is beyond the scope of this discussion; however, the inflammation phase must be considered for this project. Inflammation is the body’s
immune system reaction and is essential for healing. Acute inflammation begins at the moment of injury and the process lasts three to seven days; essentially the length of this project. During the inflammatory phase macrophages and neutrophils migrate to the wound site. Macrophages control infection by ingestion of microorganisms and excretion of ascorbic acid, hydrogen peroxide, and lactic acid. Neutrophils are granulocytic leukocytes that function as phagocytic cells that proliferate in the hypoxic acidotic environment and produce superoxide to fight bacteria. The neutrophil is considered to be a primary cell responsible for cleansing the wound of microorganisms, and lack of adequate numbers of neutrophils will retard healing of infected wounds. Thus it would appear that the control subjects who exhibited the substantial decreases in their bacterial count had strong enough immune systems to combat the infection during the inflammatory phase while the others who exhibited increases did not have sufficient means to deal with the increased bacterial burden. Conversely, the fact that all the subjects in the experimental group exhibited substantial decreases in their bacterial count would lead one to suspect that the HVPC treatment was a factor in promoting such consistent results.

The subjects in this study were young healthy animals; however, the human patients who will receive this treatment will be individuals who exhibit chronic wounds. These are patients whose wounds are ones that deviate from the expected sequence of repair time and response to appropriate treatment, as a result of a number of factors including skin changes that occur with aging, presence of chronic disease, circulatory disease, malnutrition, neuropathy, immune suppression and infection. These patients do
not have adequate intrinsic biological means to combat their infection and often require extrinsic assistance.

The use of HVPC clinically to promote healing of decubitus ulcers and surgical wounds initially based on the results of studies using LVDC; however, these are two separate waveforms and the results LVDC studies cannot be generalized HVPC. In response to this situation, Kincaid and Lavoie (1989) conducted an in vitro study and reported that the growth of three micro-organisms commonly found in human wounds (Staphylococcus aureus, Escherichia coil, and Pseudomonas aeruginosa) was inhibited at the cathode resulting from exposure to HVPC for 2 hours at 250 V. Similarly, Szuminsky, Alber, Unger, & Eddy (1994) in their in vitro study on similar organisms concluded that HVPC produced antimicrobial effects at 500 V when applied for 30 minutes. Although both studies demonstrated the efficacy of the bactericidal action of HVPC, the voltages used were too high to be tolerated by humans.

The purpose of our investigation was, therefore, to further study the question of the bactericidal properties associated with HVPC stimulation and to determine whether HVPC stimulation could inhibit or retard the growth rate of Staphylococcus aureus in an in vivo model at voltages that a human can tolerate. From the results of this present study, the author feels there may be a suggestion that HVPC stimulation of a wound infected with Staphylococcus aureus with parameters that are used clinically may result in decreased in bacterial growth in vivo. What we cannot state with certainty, however, is the mechanism of action by which HVPC appeared to exhibits these effects.

The work of Guffey and Asmussen (1989) demonstrated that HVPC applied at amplitudes ranging from 50 ma to 800 ma (corresponding to 10 to 160 V) did not result
in bactericidal activity *in vitro*. Therefore, we can assume that the voltage levels in our study were insufficient to kill the bacteria directly because the stimulation amplitudes did not exceed 100V. LVDC stimulators exhibit a polar capability, that is the ability to create a positive or negative electrical field; this is due to LVDC stimulator’s monophasic waveform. It appears that it is the polar nature of the LVDC stimulators that give them their bactericidal and granulation tissue stimulating capability, as studies using symmetric biphasic waveforms which do not exhibit polarity did not yield similar results (Stefanovska, 1993; Baker, 1996). Past research has supported the effectiveness of HVPC in stimulating granulation tissue (Feeder & Kloth, 1985; Alon 1986, Kloth & Feeder, 1986; Unger, Eddy, & Raimastry, 1991). HVPC also has polar capabilities due to its monophasic waveform, and like LVDC, its granulation tissue healing capability appears to be related to its polar nature. Galvanotaxis is electrically guided cell locomotion due to polarity, or simply the attraction of cells to the anode or cathode. It would be reasonable to assume that if an HVPC stimulator is able to provide a sufficient enough electrical field to stimulate tissue growth due to its polar capabilities, it should be able to provide a strong enough electrical field to stimulate Galvanotaxis.

Electrically guided cell locomotion has been observed in a variety of cells including neutrophils and macrophages (Bourguignon & Bourguignon, 1989; Cooper & Schliwa, 1985; Fukushima & Sends, et al., 1953; Orida & Feldman, 1982). Perhaps the documented antibacterial effects of continuous cathodal LVDC and the suggested antibacterial effects of HVPC are the result of galvanotaxic attraction of phagocytic macrophages and leukocytes to infected tissues rather than from detrimental effects of
pathogens caused by electrolysis. Further studies analyzing the cellular motility effects of HVPC in infected wounds *in vivo* are necessary to address the mechanism of action.
CHAPTER VI
CONCLUSION

The purpose of this study was to determine if HVPC used at amplitudes that humans could tolerate had an effect in reducing the viability of an infecting microorganism in wounds. In this study we chose to use stimulating parameters that are consistent with the clinical treatment of chronic wounds in humans; that is, stimulation at the cathode for one hour at an amplitude of sensory stimulation not to exceed 100V. These were chosen in order to lend support to the use of these parameters clinically.

Although statistical significance may not have been achieved, the author felt that there was a suggestion that HVPC did appear to demonstrate a positive trend toward exhibiting bactericidal effects in acute wounds of animals. The lack of statistical significance may have been due to the use of a healthy animal model with an acute wound; however, use of sick animal model with a chronic would have been infeasible, if not inhumane. In view of this, the author feels that further studies using animals may not be appropriate due to the lack of generalizability, and suggests it would be more appropriate to conduct further studies on human subjects.

Further studies comparing the outcome of homogeneous groups patients with infected chronic wounds who received standard treatment with the outcome of those who receive standard treatment plus HVPC would be necessary to truly establish the efficacy of the antibacterial effects in humans and lend support to its clinical use. Additionally, further studies involving the analysis of human cell locomotion in response to HVPC are required in order to determine the mechanism of action of the bactericidal effects.
REFERENCES


APPENDIX A

ABSTRACT: The following abstract was accepted by both the NJAPTA and the 1999 APTA National Annual Conferences for poster presentations.
ANTI-BACTERIAL EFFECTS OF HVPC IN VIVO Campolo M, Pinto-Zipp G, Ryden E, Addario E, Hughes K. Graduate Program in Health Sciences and Research Animal Facility UMDNJ-Seton Hall, Newark, NJ, USA

PURPOSE: A historical review of the literature reveals that low intensity direct current (LIDC) is effective in the treatment of infected wounds. Since the 1970's, high voltage pulsed current (HVPC) stimulators have been used for the same purpose based on the assumption that they have the same physiological effects as LIDC. To date; however, there is insufficient research to support clinical use of HVPC for infected wounds. The purpose of this pilot study was to determine whether HVPC has an inhibitory effect on bacteria in vivo in order to provide evidence to support the clinical use of HVPC stimulation in the treatment of infected wounds.

SUBJECTS: An animal model was used in order to avoid any of the confounding effects associated with the use of human subjects. All the rules and regulations set forth by the Institutional Animal Care and Use Committee were strictly adhered to. IRB approval was also obtained. The subjects consisted of twelve New Zealand rabbits of equivalent size, weight and age.

METHODS AND MATERIALS: The animals were randomly assigned to either an experimental group (EXP=6) or a control group (CON=6). Each animal was anesthetized and a full thickness wound (2cm by 3cm) was made on their backs, which then was infected with 1 ml of 1 x 10^7 Staphylococcus Aureus solution. The wound was covered with a sterile dressing and sampled at 24 and 48 hours to ensure it was sufficiently infected. Electrodes were placed on all of the animals; however, only the EXP group received electrical stimulation. The parameters chosen were consistent with those recommended clinically: the waveform was monophasic and twin peak in shape, the phase duration was 75 usec, the pulse rate was 100 pps, the amplitude was the highest obtainable without causing muscular contraction (not to exceed 100V), and the current modulations was continuous.

RESULTS: The data were analyzed using the Mann-Whitney U Test, due to the small sample size, which revealed significance at the 0.05 level. The EXP group exhibited a consistent and substantial decrease in their bacterial count across all subjects, with a resulting mean decrease of 98%. Conversely, consistent results were not observed in the CON group. While some subjects of the CON group did exhibit a decrease in their bacterial count, others exhibited a substantial increase, resulting in a mean increase of 112%. These results suggest that HVPC stimulation decreased bacterial effects greater in the EXP group when compared to the CON group, which received no electrical stimulation.

CONCLUSION: HVPC appeared to exhibit bactericidal effects when used with parameters that are consistent with the clinical treatment of chronic wounds. Further studies using larger sample sizes are necessary to support the results of this study.

APPLICATION TO PHYSICAL THERAPY: This pilot study appears to lend support to the clinical use of HVPC by physical therapists in their treatment of infected chronic wounds.
APPENDIX B

Letter of Acceptance from the Institutional Animal Care and Use Facility
MEMORANDUM

DATE: December 10, 1998

TO: Marc Campolo, M.A., P.T., S.C.S.
    Associate Professor
    Physical Therapy

FROM: Lois B. Laemle, Ph.D., Chair
      Eva B. Ryden, Ph.D., D.V.M., Attending Veterinarian
      Institutional Animal Care and Use Committee

RE: Annual Renewal:
    Animal Care and Use Protocol # 0759
    Title: Anti-Bacterial Effect of HVPC in Vivo

Your animal care and use protocol for the project shown above was reviewed and approved by the Institutional Animal Care Use Committee on December 8, 1998.

This approval will remain in effect until: 01/27/00.
Original approval date for this protocol: 01/27/98.
Protocol may be continued by annual updates until: 01/27/01.

Federal laws and guidelines require that Institutional Animal Care and Use Committee reviews ongoing projects annually. For the first two years after initial approval of the protocol you will be asked to submit an annual update form, describing any changes in procedures or personnel. The committee may, at its discretion, extend approval on the project in one year increments until the third anniversary of the original approval of the project.

Approval may only be extended until the third anniversary of the original approval of the project. At that time, the protocol must be replaced by an entirely new submission.

EBR/dp
APPENDIX C

Application to use lab animals at UMDNJ Newark, submitted October 1997.
APPLICATION TO USE LABORATORY ANIMALS
AT UMDNJ-NEWARK

(FOR IACUC USE ONLY)

Date Submitted: October 10, 1997

Project #

Category: A B C D
(please check appropriate choice)

Classification:
New
Modified
Renewal

1) PRINCIPAL INVESTIGATOR: Marc Campolo, MA, PT, SCS
Academic Title: CLINICAL ASSOCIATE PROFESSOR
Department: PHYSICAL THERAPY
Address: 65 BERGEN STREET, MARTLAND BUILDING, NEWARK Phone: 973-972-5272

2) PROJECT TITLE: ANTI-BACTERIAL EFFECT OF HVPC IN VIVO
Source of funding: UMDNJ Physical Therapy Department
Duration of grant - starting date: ending date:
Grant identification #: (or 'pending')

3) PLEASE PROVIDE NAME, TITLE, PHONE# (OFFICE AND HOME) OF ALL INDIVIDUALS WORKING WITH THE ANIMALS.

a. Marc Campolo Clinical Associate Professor 717-476-5564
b. Kathleen Hughes Physical Therapy Student 908-232-5669
c. Tom Kenny Physical Therapy Student 973-759-4552
d. Ellen Splaine Physical Therapy Student 732-671-4969
e. Kim Yarashefski Physical Therapy Student 973-635-1899
Benefits:

Previous research suggests that HPC is as effective as LIDC in accelerating wound healing. Researchers have consistently demonstrated that topical application of HPC to experimental wounds has been shown to result in increased tissue regeneration and reduced healing time compared to control groups. The benefits of using HPC in wound healing are further supported by clinical studies that have shown improved wound healing outcomes in patients treated with HPC compared to those treated with standard wound care.

Experimental design/protocol:

The study will be conducted in a randomized, double-blind, placebo-controlled manner. Subjects will be randomly assigned to one of two groups: the HPC group and the control group. The HPC group will receive topical application of HPC, while the control group will receive a placebo or a control treatment. The study will be conducted in a clinical setting with strict adherence to ethical guidelines to ensure the safety and well-being of all participants. The study will be monitored by independent ethics committees to ensure compliance with all regulatory and ethical requirements.

Informed consent:

All participants will be fully informed about the study, including the purpose, procedures, potential risks, and benefits. Participants will be required to sign an informed consent form before participating in the study. The consent form will be explained in detail to ensure that all participants fully understand the study and agree to participate voluntarily. The consent form will be reviewed and approved by the institutional review board (IRB) before the study begins.
5) ANIMALS TO BE USED IN THIS STUDY.

a. New Zealand Rabbits

b. Will breeding be done? Yes

c. Based on your experimental design, indicate how the total number of animals to be used was derived.

Both the control and the experimental groups will have fifteen animals. Based on previous experiments using rabbits as the animal subjects, fifteen was determined to be a sufficient number for a pilot study.

d. Why was this species chosen?

The New Zealand white rabbits were chosen in order to maintain research consistency and to ensure conditions are replicable across studies.

e. Provide specific information verifying that the research is not unnecessarily redundant or repetitive.

Previous research has indicated that the anti-bacterial effects of HVP C have been consistent and are not possible in vitro. All the authors agree that in order to truly establish the efficacy of the use of HVP C in the treatment of infected wounds, studies conducted in vivo are necessary.

d. For all procedures that involve momentary or slight pain (survival and non-survival surgery), procedures in which alternatives are not possible, refer to guidelines page B.

All studies to date that have been performed to determine the bactericidal efficacy of HVP C have been conducted in vitro. All the authors agree that in order to truly establish the efficacy of the use of HVP C in the treatment of infected wounds, studies conducted in vivo are necessary. As previously stated, the use of humans would present too many confounding variables. Due to these facts, animal subjects appear to be appropriate for this study.

f. Provide the key words used in the search listed above.

Other(specialty title) OVD

6) JUSTIFICATION FOR THE USE OF ANIMALS. Notes: If this section is not complete, the application will not be processed.

Animals were chosen to eliminate any confounding variables associated with the application of HVP C to the wounds of rabbits. In order to maintain research consistency, New Zealand white rabbits were chosen as subjects for this study.

a. Indicate the rationale for using animals.

Animals were chosen to eliminate any confounding variables associated with the application of HVP C to the wounds of rabbits.

b. Previous research using similar species or study designs.

Previous research has indicated that the anti-bacterial effects of HVP C have been consistent and are not possible in vitro. All the authors agree that in order to truly establish the efficacy of the use of HVP C in the treatment of infected wounds, studies conducted in vivo are necessary.

c. Provide specific information verifying that the research is not unnecessarily redundant or repetitive.

Previous research has indicated that the anti-bacterial effects of HVP C have been consistent and are not possible in vitro. All the authors agree that in order to truly establish the efficacy of the use of HVP C in the treatment of infected wounds, studies conducted in vivo are necessary. As previously stated, the use of humans would present too many confounding variables. Due to these facts, animal subjects appear to be appropriate for this study.

7) What sources or databases were searched, please check the appropriate item(s) including but not limited to:

- Animal Welfare Information Center
- Other(specialty title) OVD

f. Provide the key words used in the search listed above.

Other(specialty title) OVD
7) EXPERIENCE AND TRAINING
   a. Describe your training and experience with the procedures and techniques to be used on
      the animals you will be using in this protocol. If you are inexperienced in these
      procedures, describe how you will obtain the appropriate training. Please attach one
      copy of your Curriculum Vitae to the original application.
         Marc Campolo, MA, PT, SCS
         -Clinical Associate Professor at UMDNJ, SHRP MPT Program
         -Electrotherapy Professor
         (please refer to attached Curriculum Vitae)
   b. List all individuals (include UMDNJ position titles) who will be involved in the use of
      animals and indicate their experience with the experimental procedures. If those
      individuals are inexperienced indicate your plans for directly supervising them during
      training.
         Marc Campolo              Clinical Associate Professor
         Kathleen Hughes           Physical Therapy Student
         Tom Kenny                 Physical Therapy Student
         Eileen Spline              Physical Therapy Student
         Kim Yarashefski            Physical Therapy Student
         Students will be directly supervised by Clinical Professor.
         All individuals listed above will attend the RAF orientation seminar.

8) ANIMAL PROCEDURES
   a. AREAS IN WHICH PROCEDURES WILL BE PERFORMED
      Research Animal Facility  [Yes]  [No]
      If not RAF, state location

   b. TYPE OF PROCEDURE
      Acute(<5 days)
         Chronic  Length  10 days
         Deprivation  Length
         Restraint  Length
      Animal used as tissue source only  [Yes]  [No]

   c. PHYSICAL DISCOMFORT
      None
      During procedure only
      Immediately following procedure
      Long term   possibly long term, due to wound infection.
      Other

      Clinical condition or abnormality expected: We would expect to see a decrease of Staphylococcus
      Aureus in the wound.

      Measures that will be used to alleviate discomfort:
      Specify drug  buprenorphine  Dose  0.02mg/kg  Route intramuscular
      Administered by: appropriate personnel
      The rabbits will be premedicated with buprenorphine to confer preemptive analgesia. Rabbits will
      be observed daily including weekends. If animals lose more than 20% body weight, become
      anorexic, or if body temperature exceeds 105 for more than 24 hours, the rabbit will be euthanized.
and food consumption. In addition, the emissions will be checked daily to ensure that the equipment operates as expected. The number of organisms present on each of the wounds will be counted to monitor the microbial load on the wounds and identified with the newer. The number of organisms will be recorded for one location across the surface of the wounds, at least 10 cm from the wound edge. The organisms will be counted on each day of the experiment to determine if any changes occur.

Prior to each experiment, the dressing will be removed and the wounds will be examined.

PROCEDURE:

1. Prepare the bacterial culture by diluting the stock culture to a concentration of 10^8 CFU/mL in nutrient broth.
2. Sterilize the syringes and needles by autoclaving at 121°C for 15 minutes.
3. Inoculate the wounds with the bacterial culture using a sterile needle and syringe.
4. Incubate the wounds at 37°C for 24 hours.
5. Observe the wound for signs of infection and record any changes.

NOTE:

- All procedures must be conducted in a sterile environment.
- Should the wound become infected, the procedure will be discontinued.
- Only sterile and disposable supplies should be used.

PROCEDURE 2:

1. Prepare a 1% solution of sodium hypochlorite (bleach) in water.
2. Wipe the wound with the bleach solution for 30 seconds to remove any bacteria.
3. Rinse the wound with sterile saline solution.
4. Apply an antibiotic ointment to the wound.
5. Cover the wound with a sterile bandage.

NOTE:

- The wound should be kept clean and dry at all times.
- If the wound becomes infected, consult a healthcare provider immediately.
- Follow-up visits may be necessary to monitor the wound's progress.
<table>
<thead>
<tr>
<th><strong>c. INTRA-OPERATIVE PERIOD</strong></th>
<th><strong>d. POST-OPERATIVE PERIOD</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Induction</strong></td>
<td><strong>Describes postoperative care including analgesia, other medications and the name of the person responsible. If you are using analgesia, please justify the need.</strong></td>
</tr>
<tr>
<td>Drug</td>
<td>Drug</td>
</tr>
<tr>
<td>Dose</td>
<td>Dose</td>
</tr>
<tr>
<td>Administered by:</td>
<td>Administered by:</td>
</tr>
<tr>
<td>Same as above</td>
<td>Preop analgesic or not expected to be required.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>e.</strong></th>
<th><strong>f.</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Describe the surgical procedure. Include provisions for sepsis, and who will perform the surgery.</td>
<td>See above</td>
</tr>
</tbody>
</table>

**10. REQUIREMENTS FOR THE PROJECT**

<table>
<thead>
<tr>
<th><strong>a.</strong></th>
<th><strong>b.</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Area animals to be housed:</td>
<td></td>
</tr>
<tr>
<td>Routine Housing</td>
<td>RAF UMDNJ-Nanjing</td>
</tr>
</tbody>
</table>

**HEALTH PROBLEMS**

In case of animal health problems, it may be necessary to administer drugs such as antibiotics or steroids to your animals. Can any drug be used to treat experimental animals? Yes.

Do not use the following drugs due to interference with study results:

**Antibiotics**
8. Previous, are telephone numbers, etc., correct? (Yes/No)

9. Do you have any known drug allergies?

10. Have you had any adverse reactions to previous treatments?

11. Are you currently taking any medications?

12. Have you had any complications during previous treatments?

13. Are you able to stay for the duration of the treatment?

14. Are you willing to sign a consent form?

15. Have you had a physical examination within the past year?

16. Are you able to provide a list of all medications you are taking?

17. Have you had any previous infections?

18. Are you able to provide a list of all blood tests you have had within the past year?

19. Have you had any previous surgeries?

20. Are you able to provide a list of all vaccinations you have received within the past year?

21. Have you had any previous hospitalizations?

22. Are you able to provide a list of all medications you are taking for any chronic conditions?

23. Have you had any previous treatments for any chronic conditions?

24. Are you able to provide a list of all blood tests you have had for any chronic conditions within the past year?

25. Have you had any previous surgeries for any chronic conditions?

26. Are you able to provide a list of all vaccinations you have received for any chronic conditions within the past year?

27. Have you had any previous hospitalizations for any chronic conditions?

28. Are you able to provide a list of all medications you are taking for any current conditions?

29. Have you had any previous treatments for any current conditions?

30. Are you able to provide a list of all blood tests you have had for any current conditions within the past year?

31. Have you had any previous surgeries for any current conditions?

32. Are you able to provide a list of all vaccinations you have received for any current conditions within the past year?

33. Have you had any previous hospitalizations for any current conditions?

34. Are you able to provide a list of all medications you are taking for any previous conditions?

35. Have you had any previous treatments for any previous conditions?

36. Are you able to provide a list of all blood tests you have had for any previous conditions within the past year?

37. Have you had any previous surgeries for any previous conditions?

38. Are you able to provide a list of all vaccinations you have received for any previous conditions within the past year?

39. Have you had any previous hospitalizations for any previous conditions?

40. Are you able to provide a list of all medications you are taking for any current treatment?

41. Have you had any previous treatments for any current treatment?

42. Are you able to provide a list of all blood tests you have had for any current treatment within the past year?

43. Have you had any previous surgeries for any current treatment?

44. Are you able to provide a list of all vaccinations you have received for any current treatment within the past year?

45. Have you had any previous hospitalizations for any current treatment?

46. Are you able to provide a list of all medications you are taking for any previous treatment?

47. Have you had any previous treatments for any previous treatment?

48. Are you able to provide a list of all blood tests you have had for any previous treatment within the past year?

49. Have you had any previous surgeries for any previous treatment?

50. Are you able to provide a list of all vaccinations you have received for any previous treatment within the past year?

51. Have you had any previous hospitalizations for any previous treatment?

52. Are you able to provide a list of all medications you are taking for any current condition?

53. Have you had any previous treatments for any current condition?

54. Are you able to provide a list of all blood tests you have had for any current condition within the past year?

55. Have you had any previous surgeries for any current condition?

56. Are you able to provide a list of all vaccinations you have received for any current condition within the past year?

57. Have you had any previous hospitalizations for any current condition?

58. Are you able to provide a list of all medications you are taking for any previous condition?

59. Have you had any previous treatments for any previous condition?

60. Are you able to provide a list of all blood tests you have had for any previous condition within the past year?

61. Have you had any previous surgeries for any previous condition?

62. Are you able to provide a list of all vaccinations you have received for any previous condition within the past year?

63. Have you had any previous hospitalizations for any previous condition?
13) ASSURANCES

I pledge to conduct this project in accordance with the intentions set forth in this application. If I wish to make any substantive alterations during the course of the project, I will submit a written request or approval of new procedures. I assure that the animals requested for this project will be used in accordance with the provision of the Animal Welfare Act and the guidelines and policies approved by the IACUC, as described in the UMDNJ-Newark RAF GUIDE.

DATE 10/31/89

SIGNATURE OF PRINCIPLE INVESTIGATOR

SIGNATURE OF DEPARTMENT CHAIRPERSON

SIGNATURE OF GRADUATE'S MENTOR SIGNATURE

DATE 1/25/98
Protocol for the use of infectious agents in animals.

APPENDIX D
<table>
<thead>
<tr>
<th>Organism</th>
<th>Staphylococcus Aureus</th>
<th>CDC/NIH Biosafety Level</th>
<th>Animal Pathogen?</th>
<th>Anti-Bacterial Effect of HVPC In Vivo</th>
<th>Animal Pathogen</th>
<th>Yes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Manual Route(s) of Infection</td>
<td>Oral, Intranasal, &amp; IV</td>
<td>1</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>Experimental Administration Route</td>
<td>Oral, Intranasal, &amp; IV</td>
<td>1</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>Is Agent Transmitted from Animal to Human?</td>
<td>Yes</td>
<td>1</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>Will Organism be Inactivated Prior to Use in Animals?</td>
<td>No</td>
<td>1</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>Is there an Available Vaccine and/or Therapy?</td>
<td>Yes</td>
<td>1</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>Disinfectant of Choice</td>
<td>Cidex X</td>
<td>1</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td></td>
</tr>
</tbody>
</table>

This document is to be completed wherever an animal research protocol involves the use of etiologic agents. Contact Environmental and Occupational Health and Safety Service (EOHS) at 2-8182 if you desire assistance in completing this application.

Date Submitted: October 10, 1997

Principal Investigator: Marc Campolo, MA, PT, SCS
A. RODENTS (small animals)
Will rodents be housed in isolator cages?
Will procedures be performed in a Biological Safety Cabinet? (Cabinets must be certified)

C. BARRIER HOUSING
Will animals be housed under barrier conditions?

D. EXPERIMENTAL PROTOCOL
Describe the animal experiment procedure that will involve the use of this agent.
Animals will receive a topical application of Staphylococcus aureus on a surgical wound. See IACUC protocol attached.

Non-rodents (larger animals)

Barrier housing refers to the use of engineering controls (air filtration, air pressure differential,) and work practices to protect the animal from the environment. Such a system would be applicable when working with SCID mice and other situations where exposure to "typical" environmental stresses might compromise experimental integrity.

Cages/papers will be disposed in double black bags. Personnel will wear masks, gowns and gloves.

Note: To what extent will viable organisms be shed into the environment by way of excreta, open skin lesions, exudation, saliva, or nasal secretions?
Have all project personnel become aware of the hazards associated with the particular biological agent(s) and animal handling techniques appropriate to minimize the risk of infection?

EMERGENCY PROCEDURES

Describe what will be done:

1. In the event of a spillage:
   - Area of skin exposed would be washed thoroughly. Personnel will be directed to seek appropriate medical attention.

2. In the event of overt environmental contamination:
   - All surfaces will be thoroughly cleaned with a 10% bleach solution.

DECONTAMINATION AND DISPOSAL PROCEDURES

1) Decontamination (animal cages, room surfaces, instruments):
   - Regular cage wash.

2) Disposal procedures:
   - carcasses Double bagged in black bags.
   - bedding, other disposable materials Double bagged in black bags.
   - Other materials Double bagged in black bags.

I acknowledge and accept responsibility for the conduct of this research at Biotechnology Level 2 as approved by the UMDN-JHU Biotechnology Subcommittees. I shall, to the best of my knowledge, ensure that this protocol involving the handling, storage, or disposal of hazardous agents is followed. I have the ability to handle these agents safely.

[Signature]

Graduate Student Signature
Bob Harter

Good luck on your project.

You have any questions.

If the delay in getting this together, feel free to get in touch with me about

I hope that this is of some help, and again I apologize

statistics with this number are very strong and, in my opinion, beyond

attacked, based on the present study, groups of 8 are excellent. The

the test to determine N how for varying powers and alphas which is

tested at 0.05 power of 0.75 and 0.90 respectively. These are very good numbers.

has a

both of which are sufficiently high, I would go with .05 (your present study

stands with an N of 5, depending on the alpha value you choose (.01 or

It finally got run some tests on your data. It looks pretty good! As

Marc:

Subject: Power analyses

From: Bob Harter <hartt@umd.edu>

To: Campbellmomentum@umich.edu

Date: Thu May 27 1999 09:58:41 -0400

X-Sender: hartermomentum@umich.edu

From: harter, Thu May 27 09:54:04 1999

Attachment converted: C:\INTERNET\ENDORA\ATTACH\MAC1\rtt

Attachment converted: C:\INTERNET\ENDORA\ATTACH\MAC2\rtt