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PRINCIPAL INVESTIGATOR: Roy D. Bloebaum Ph.D.

CONTRACTING ORGANIZATION: Western Institute for Biomedical Research
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Salt Lake City, UT 84148

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14. ABSTRACT

The goal of this research program is to develop safe and efficacious osseointegrated implants that can be implanted into our warrior amputees who have limited residual limb length, extensive heterotopic ossification or difficulty with socket suspension type systems, leaving them with a non-functional limb or limbs. The new technology is to assure skin attachment at the percutaneous site, where the implant exits the skin, to prevent infection. Strategies explored in this research are the use of porous coating biomaterials that will allow immediate skeletal and subdermal skin attachment to facilitate a single stage operation for eventual rapid patient rehabilitation. The safe and efficacious introduction of the osseointegrated implant technology for our deserving warriors is the overall goal of our program.

15. SUBJECT TERMS

Osseointegration; Amputees; Implant Design

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INTRODUCTION

The goal of this research program is to develop safe and efficacious osseointegrated implants that can be implanted into our warrior amputees who have limited residual limb length, extensive heterotopic ossification or difficulty with socket suspension type systems, leaving them with a non-functional limb or limbs. The new technology is to assure skin attachment at the percutaneous site, where the implant exits the skin, to prevent infection. Strategies explored in this research are the use of porous coating biomaterials that will allow immediate skeletal and subdermal skin attachment to facilitate a single stage operation for eventual rapid patient rehabilitation. The immediate subdermal attachment to the porous coating is hypothesized to prevent infection.

BODY

The Tasks and Milestones for Year 2 are on target and we are making excellent progress despite many challenges as previously discussed in Year 1, mainly the loss of manufacturing commitments and capabilities.

The two main focuses of our study for Year 2 consist of sheep design, manufacturing of implant and surgical trials on Time 0 specimens, histology and mechanical testing of implants. The second focus of Year 2 was human morphometric studies on variations due to ethnicity, gender and age; designing and manufacturing of human implants, and confirmation of fit/fill.

Sheep Model

Goal: Design and manufacture surgical-grade, titanium implants, begin surgical trials in the sheep model focusing on infection prevention, histological analyses and mechanical stability of the implant.

Manufacturing

As mentioned in the previous report, the Zimmer Corporation failed in its commitment to provide tantalum porous coated implants for the sheep and human models. Although Mr. Marc Richelsoph committed his new company to support our effort, start-up difficulties made us realize that we would not be able to meet our deadlines if we had to wait for Intelligent Designs Inc. to be completely capable of supporting our efforts. He worked diligently with our staff to create an initial design that would be efficacious, yet due to time, budget and other complications he was not able to complete the design and manufacturing of the implant.

We immediately began a nation-wide search for commercial partners to support our program. We found a local orthopaedic firm, Medicine Lodge, Logan, UT, that committed to manufacturing the implants for $200,000 dollars. Medicine Lodge, Logan, UT, took the initial design from Mr. Richelsoph and modified it to the specifications needed to begin manufacturing. We found a new titanium porous coating, manufactured by Thortex, Portland, OR, that had the same "Velcro" properties as Zimmer's tantalum product. Thortex committed to porous coating the implants with their revolutionary K2 titanium porous coating at no cost to the project. We were off and running with a $200,000 short fall.

Dr. Bloebaum has captured 60% of the funds needed to pay for the implants by increasing efficiency within the program and without compromising the PRMRP budget. Medicine Lodge has been patient with the reimbursement schedule.

Three implant sizes (1, 2 and 3) have been manufactured along with broaches (sizes 0, 1, 1+, 2, 2+ and 3). Two sets of broaches were required so that we could perform five surgeries over a five-day period.

The first surgery was performed on May 27, 2008 and a second surgery on May 29, 2008. The Time 0 sheep were mechanically tested and histology was performed on the tissue.

Two mechanical tests were performed using Instron 8500 (Instron, Canton, Mass), tensile test and 4-Point bend; the results showed that failure occurred at 2452N/mm for the tensile test. Initial data showed that on the 4-pt bend test the bone cracked at -4018.9 N and the implant failed and bent at -3173.5N. The results of the mechanical testing had given us the confidence to do live surgery.

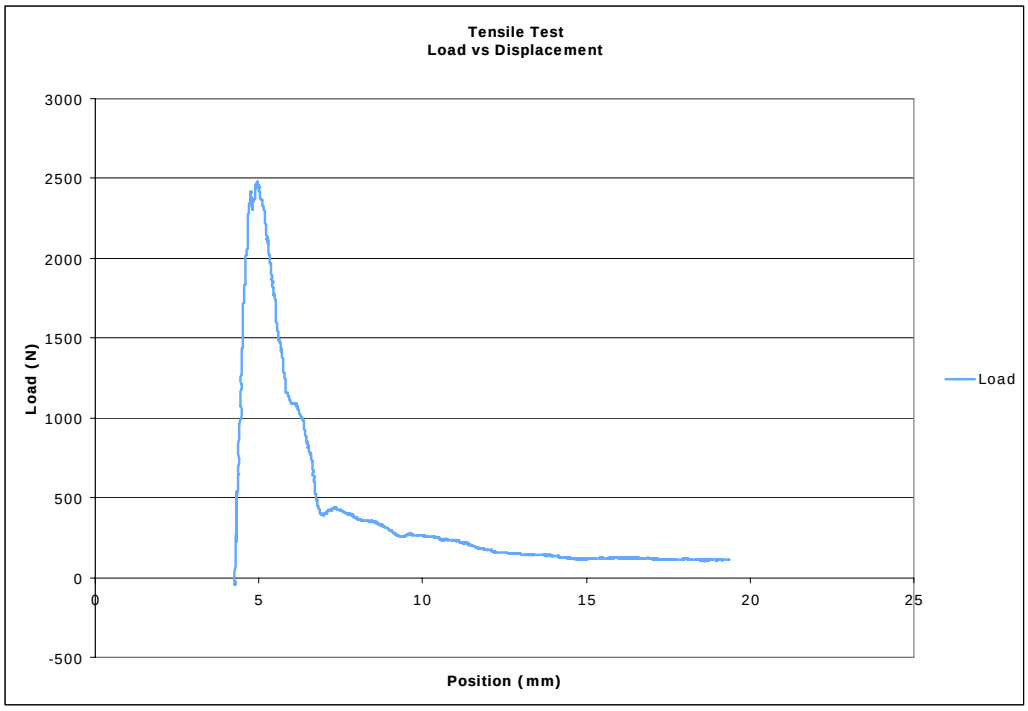

Figure 1: Force-displacement data from tensile testing of "Time-0" animal. Nearly 2500N were required to pull the implant out of the medullary canal following mock surgery.

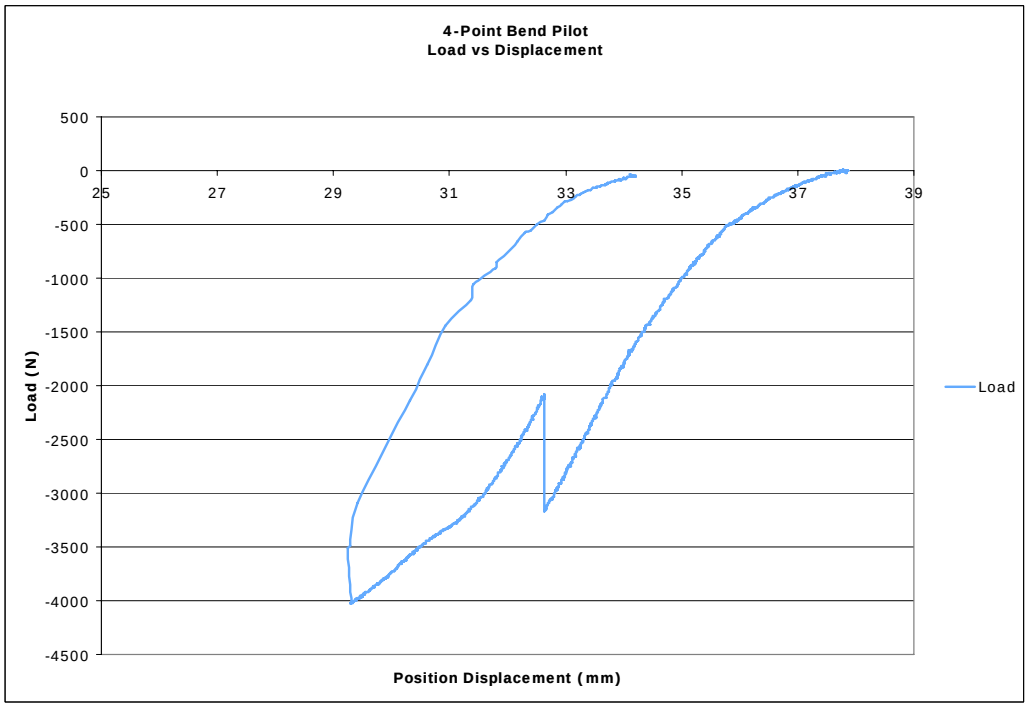

Figure 2: Force-displacement data for 4 point bending test of "Time-0" animal. Nearly 4000N were required to break the bone/implant complex following mock surgery.

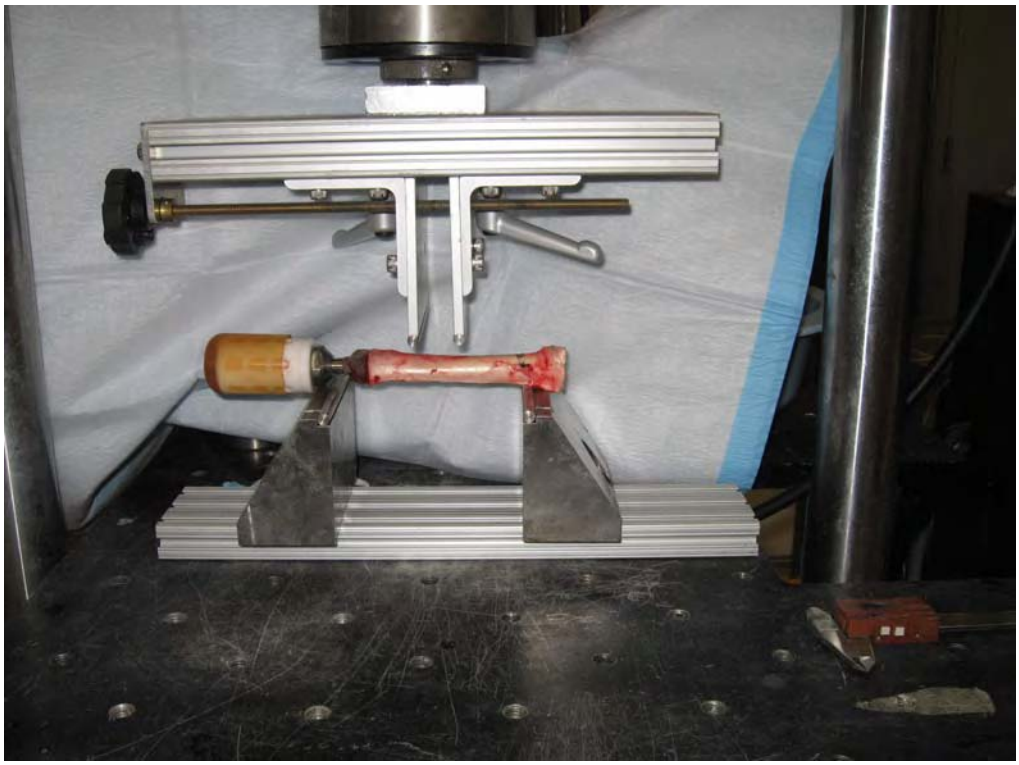

Figure 3: Setup of mechanical 4-pt bend

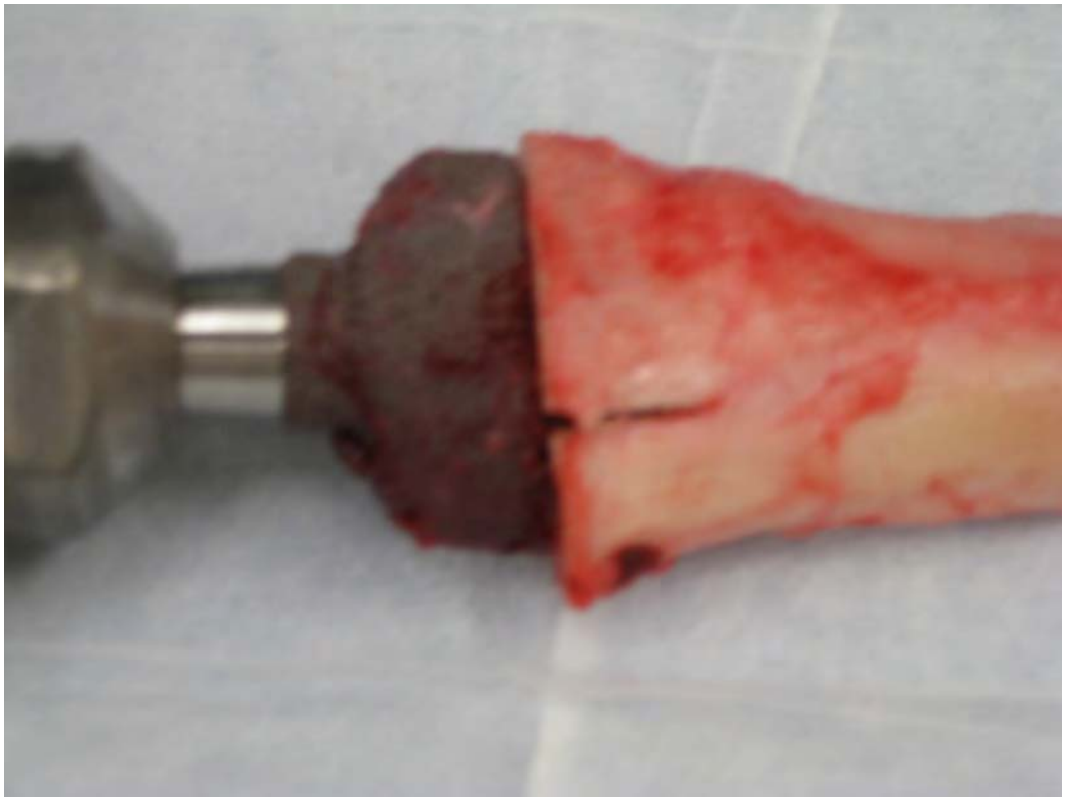

Figure 4: Break in bone due to mechanical test failure at 4018.9N

Figure 5: 4-pt Bend mechanical test performed on implant, point at which the implant is visible.

IACUC approved animal studies, gait and force plate analysis, are progressing on or ahead of schedule. To date, pre-operative vertical force measurements have been recorded from a total of 20 animals -- four animals have reached the 3 month time-point. Preoperatively, the subjects' forelimbs were loaded on an average of 35% more than the hind limbs. Postoperatively, this difference decreased to 22% for the 1 month, and has remained constant at both the 2 and the 3 month time points. The surgically altered limb's peak force per body weight was approximately 82% of the pre-operation conditions for the first two months following surgery and about 85% for the third month, all deemed significantly different (Figure 6).

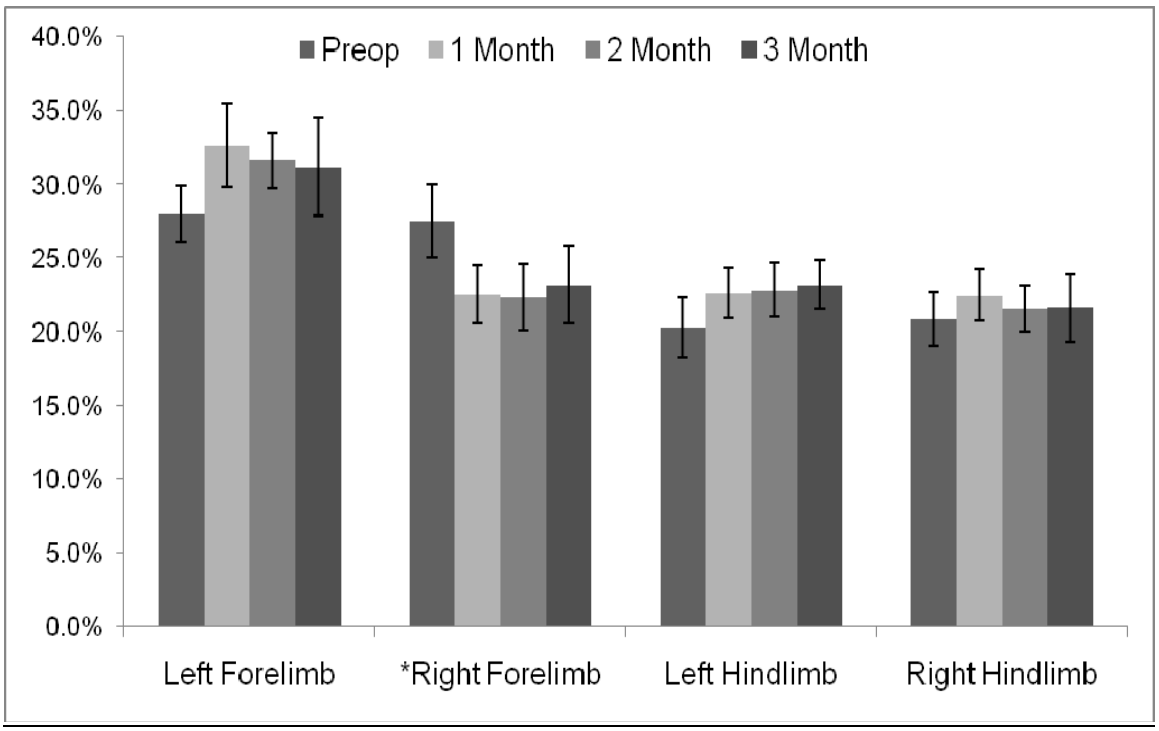

Figure 6

Prosthetic limb loading can be compared to preoperative limb loading by measuring limb forces before and after amputation. If done over a few months, this can not only give an understanding of prosthetic limb loading, but also rehabilitation timelines which can be applied later in a human model. Data demonstrated that the subjects were compensating for the surgically altered right forelimb by applying a higher load to the left forelimb. Further data compiled over the next several months from these subjects and others will give more of an understanding of prosthetic limb loading.

To date, of the 35 sheep operated on, 3 had loose implants 1-2 weeks postoperative and had to be sacrificed early. The early sacrifices were not unexpected since our sizing studies in Year l suggested the best we could accomplish without having possibly 5 sizes of implants and ballooning our implant costs to $300,000 was an 80% chance of an appropriate fit and fill of the implant into the bone. Preoperative radiographic screening and animal selection has improved, which has reduced our losses due to early fixation failure to less than 10% currently. Our goal was to design and manufacture surgical implants and to begin our Time 0 testing on sheep. We have accomplished this goal and feel encouraged that we are taking the proper steps to create the most efficacious osseointegrated percutaneous implant for our wounded soldiers and amputees.

Human

Goals: Evaluate variation in human morphometry due to ethnicity, gender, and age; design and manufacturing of human implants and confirmation of fit/fill.

In order to better understand the sizing requirements and to address the question as to whether custom implants in above-knee patients with amputations would require expensive custom type implants, a morphometric study was conducted on human male and female cadaveric femurs. Morphometric variations of the periosteal surface of long bones have been identified with changing age, gender and ethnicity [1]. However, until the recent interest in the percutaneous osseointegrated implants, which use the intramedullary canal for fixation, little interest and no data is available to determine how implant designs will be influenced by demographic differences of the amputees as well as variations along the length of the bone. This would be critical information for establishing implant design parameters that are required to assure endosteal attachment of these percutaneous osseointegrated implants.

The goal of this study is to determine the endosteal canal morphometric variations existing in the male and female human femur to examine the requirements for future translational design work on percutaneous osseointegrated implant for patients with above-knee amputations. Data from this study could contribute to understanding the potential cost of implant production and sizing requirements for patients with above-knee amputations.

Results thus far indicate that the AP and the ML diameters of the medullary canal vary greatly with not only anatomical locations, but also by the gender of the femur donor. Anteroposteriorly (Figures ____), the female intramedullary canals measured approximately 15% less than their male counterparts. For both genders, there was nearly a 100% increase in the AP diameters from the superior (65%) location to the most distal (35%) location.

Mediolaterally (Figures 7&8), the female intramedullary canals measured approximately 10% less than their male counterparts. For both genders, there was nearly a 50% increase in the AP diameters from the superior (65%) location to the most distal (35%) location.

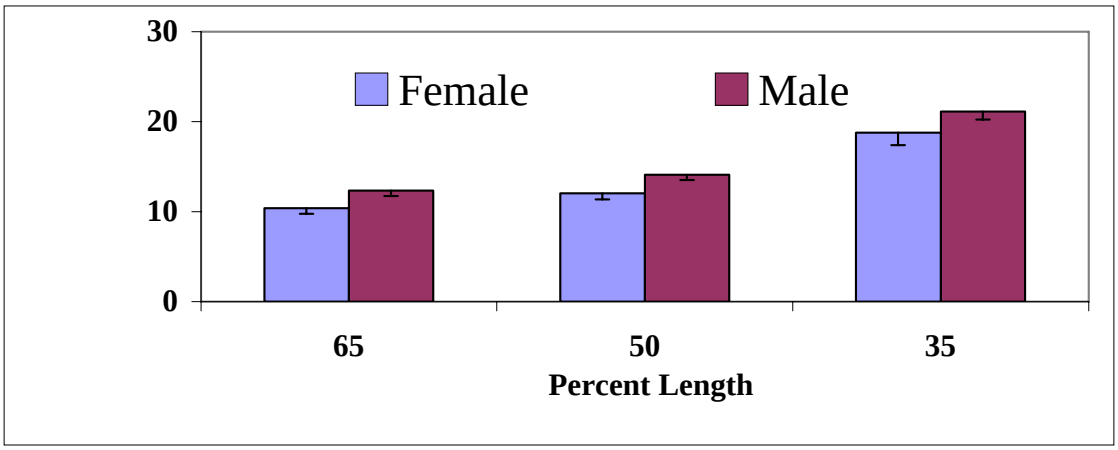

Figure 7: Anteroposterior intramedullary diameters of male and female femora measured at 65% (most superior) to 35% (most distal).

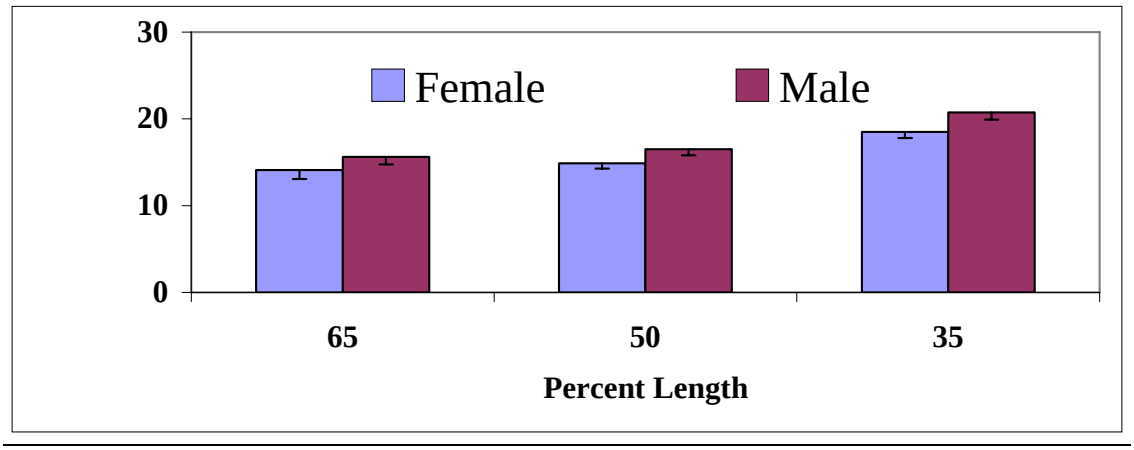

Figure 8: Mediolateral intramedullary diameters of male and female femora measured at 65% (most superior) to 35% (most distal).

To date a total of 184 femurs have been scanned, reconstructed using MIMICS (Materialise USA), periosteal and endeosteal analysis has been performed using custom written code with Matlab (Mathworks, USA). The femurs can be broken down as follows: Caucasians comprise our largest numbers with males totaling 35 pairs and female 14 pairs and ages from 16-60. There are 26 pairs of African American males, 8 pairs of females with ages ranging from 15-70. We were able to obtain 10 Hispanic male pairs, ages 18-44, and do not at this time have any females from this ethnicity. We believe that due to religious and cultural beliefs, Hispanics are less likely to donate their bodies to science. Initial results show that the intramedullary canal of Hispanic males are smaller than the mean population, while there are some African American males that have larger than normal canal diameters. Based on the data, there is no real difference in canal diameter between African American and Caucasian female. Further studies will be needed to determine if a variation occurs between ages and Hispanic females would need to be included in the study if available.

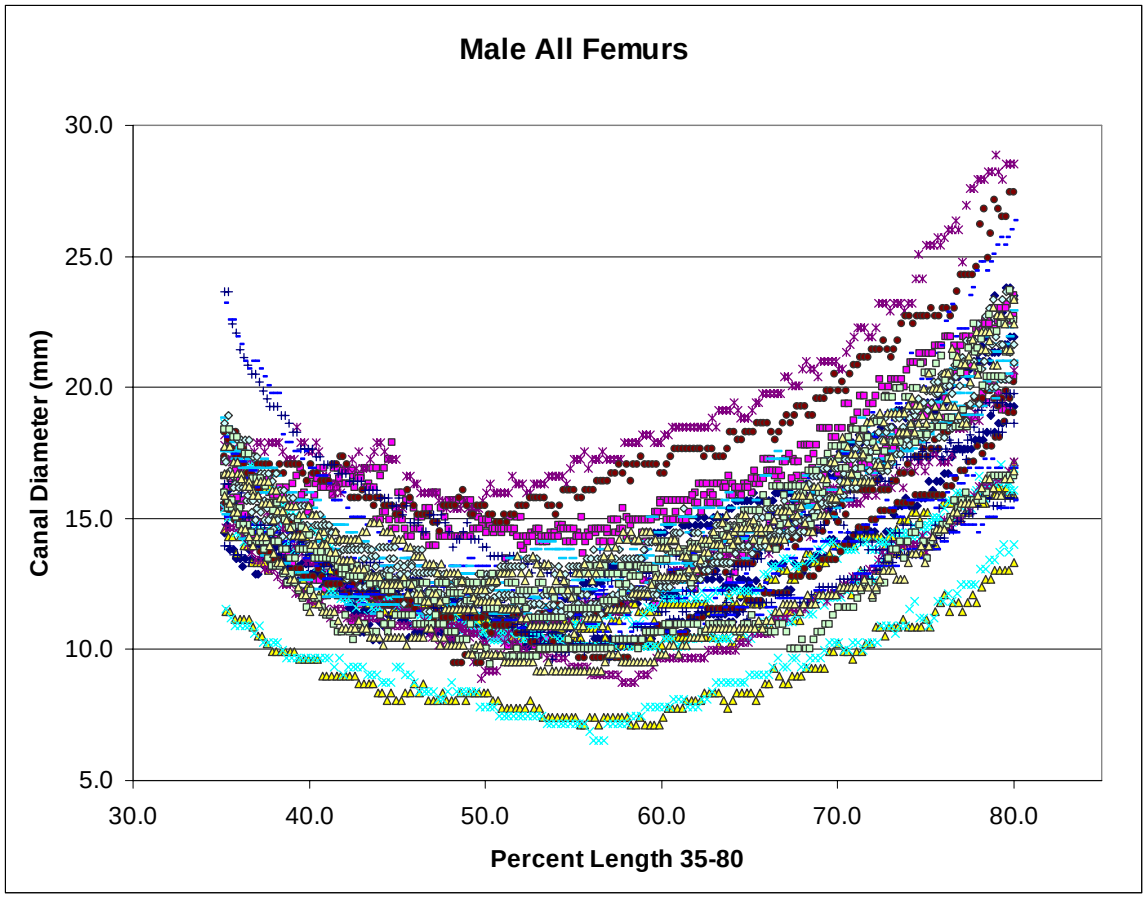

Figure 9

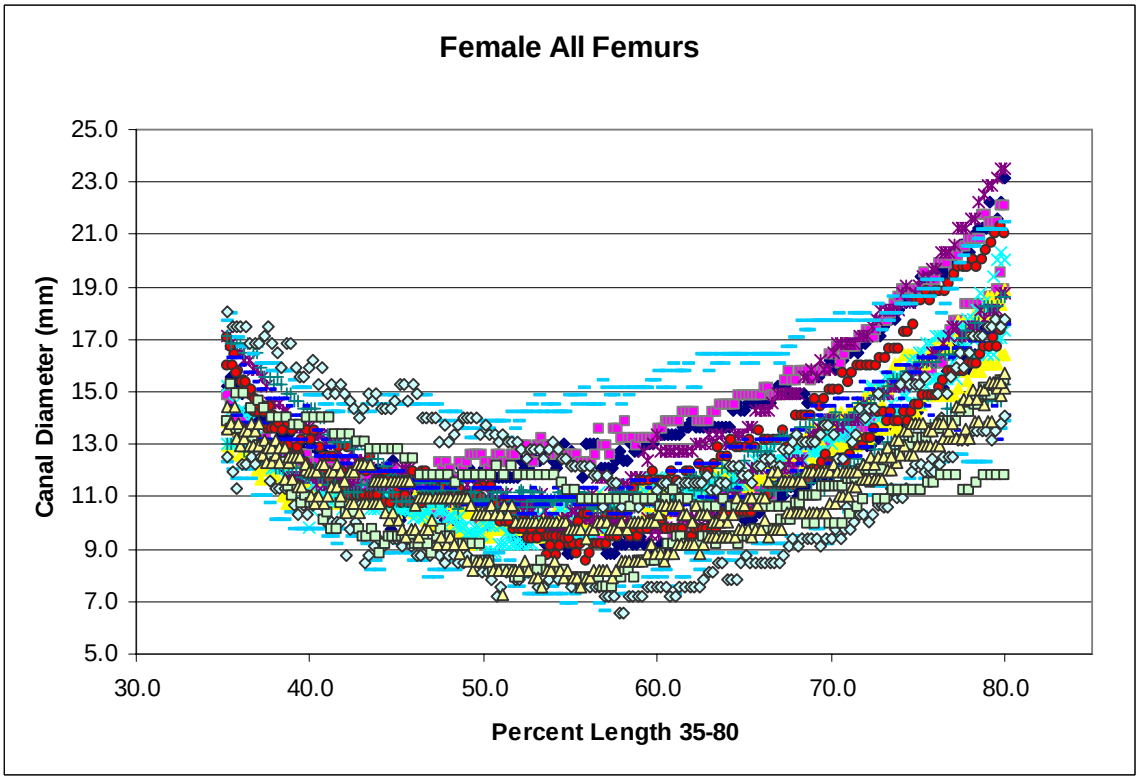

Figure 10

We are currently working on designing an implant based on results of endosteal data obtained. Looking at the graphs above, one can see that there is a distinct difference between male and female canals. There are other factors that our going into the implant design. Once a design is complete and has been sent to the manufacturers, a virtual implant will be implanted into the femur at the correct amputation site and a fit and fill analysis will be performed. Along with a virtual fit and fill, the Orthopedic Research Lab will be printing rapid prototypes to test in the cadaveric tissue prior to titanium implants being implanted and the subsequently mechanically tested for tensile and torsional tests. Further investigations are being conducted using the data from this study to perform virtual implantation procedures to better understand implant sizing and fit and fill requirements for patients with above-knee amputations.

Therefore, to date, we are back on schedule for accomplishing the Milestones in Task 2 as outlined below.

Task 2 (Year 2)

Milestone a.	Manufacture implants	(Complete)
Milestone b.	Implant Time 0 sheep	(Complete)
Milestone c.	Biomechanically test Time 0 sheep	(Complete)
Milestone d.	Manufacture human cadaver femur implants	(In Progress)
***Milestone** f.*	Biomechanically test initial implant attachment in human cadaveric femurs	(In Progress)
***Milestone** g.*	Establish mechanical criteria for "safety release mechanism for the human cadaveric human femur	(In Progress)
Milestone h.	Manufacture safety release mechanisms for cadaveric femurs	(Patent Filed, (Prototype Tested)
Milestone i.	Validate safety release mechanism in the cadaveric femur	(In Progress)

In summary, IACUC approved animal studies, gait and force plate analysis are progressing on or ahead of schedule despite corporate challenges and the administrative burdens of reporting to three different institutions.

KEY RESEARCH ACCOMPLISHMENTS & REPORTABLE OUTCOMES

Two abstracts have been accepted to the prestigious peer reviewed Orthopaedic Research Society Meeting in Las Vegas, February 22-25, 2009.

The first one, "Development of a single stage surgical model for percutaneous osseointegrated implants for amputees" (Bloebaum RD, Beck JP, Olsen R, Norlund L, Bachus KN: 55th Annual Meeting of the Orthopaedic Research Society. Las Vegas, NV, 2009, vol 34, p 2255, see Appendix) demonstrates the proof of concept that a single stage operative procedure can be performed on a large animal, allowing for immediate weight bearing and the prevention of periprosthetic infection for up to 9 months. In our previous sheep studies (Emily Perry, et al., see Appendix), the percutaneous sites became infected within 3-6 weeks. Of the 35 animals which have postoperative periods 8-36 weeks, none have demonstrated signs of infection despite observing extensive exoprosthetic and limb contamination from feces and urine in their large outdoor shelters.

These results have allowed us to test a new hypothesis that the intraoperative attachment of the skin to the K2 titanium coating is immobilizing the skin at the periprosthetic regions, preventing infection. To test this hypothesis, we will be implanting three sheep in the 3, 6, 9 and 12 month periods without the porous coating at the subdermal soft tissue region to confirm that the infection signal was present. This protocol change has been approved by all three IACUCs (DOD, Frontier Biomedical and VA Medical Center). This advancement has

demonstrated that the established sheep model has the ability to provide translational scientific information prior to introducing the osseointegrated technology into our warrior and Veteran population.

The second related ORS abstract (Shelton TJ, Bloebaum RD, Bachus KN: Vertical forces of percutaneous, osseointegrated implants of an ambulating ovine amputation model: 55th Annual Meeting of the Orthopaedic Research Society. Las Vegas, NV, 2009, vol 34, p 1986, see Appendix) details the advances in gait and force plate (Tekscan HR MAT) analysis that can document the forces on all four limbs pre- and postoperatively. Although the abstract, submitted in July 08, only showed an n=4 animals, as of this writing, 25 sheep have been tested.

The early data showed that at the 3 week period, an 18% reduction in load occurred postoperatively in the implant limb. The data showed that the unimplanted limb was compensating, although this was not apparent on gait analysis. Longer term data at the 3, 6, 9 and 12 month periods will determine if normal loads will return over the course of the study.

Additional Funding and Studies which Parallel the Program

The VARR&D has awarded funding ($150,000) for one year to begin the development of a pig model to test the ability of different porous coating types to assure skin immobilization and viability. The pig was chosen since it has a skin structure similar to human skin. This model should provide essential translational data for the human implant design; animals are being implanted at this time.

The University of Utah recently awarded $35,000 to our lab to develop an "intelligent" osseointegrated implant. The design is in the provisional patent process. The implant has been designed to create an electrical field that will have two functions:

1. Prevent infection and biofilm formation on the implant surface (see Costerton, et al. 1994 in Appendix).
2. Stimulate early bone attachment and treat the osteopenia associated with socket technology and non-physiological loading of the bone. If the milestones are met by February 09, another $35,000 will be awarded. We are on target at this time.

CONCLUSIONS

The work is progressing on schedule despite financial and bureaucratic challenges. The establishment of a translational research animal model is a major advance in the field of limb amputation research. Although patients are being implanted with osseointegrated devices in Europe, there remains serious infection and implant design issues (see Dr. Beck Trip Report, Appendix 5) that suggests the human model is experimental with limited ability to test hypotheses. There is no doubt that the human studies in Sweden and Germany are concerning at this time.

To date, two patents are in progress: one for the safety release device and the other for an "intelligent" implant design. As the research continues, we hope at the end of the PRMRP funding period in 2010 that we will have a clear experimental vision and data that will show us the pathway for the safe and efficacious introduction of this exciting technology by no later than 2015. We currently are organizing a team of experts to study the pathway for human clinical trials, FRB approvals and FDA requirements to achieve our goals of human studies by 2015.

REFERENCES

See appendices for referenced materiel.

APPENDICES

1. Bloebaum RD, Beck JP, Olsen R, Norlund L, Bachus KN: Development of a single stage surgical model for percutaneous osseointegrated implants for amputees. 55th Annual Meeting of the Orthopaedic Research Society. Las Vegas, NV, 2009, vol 34, p 2255.

2. Shelton TJ, Bloebaum RD, Bachus KN: Vertical forces of percutaneous, osseointegrated implants of an ambulating ovine amputation model. 55th Annual Meeting of the Orthopaedic Research Society. Las Vegas, NV, 2009, vol 34, p 1986.

3. Perry EL, Beck JP, Williams DL, Bloebaum RD: Assessing periimplant tissue infection prevention in a percutaneous model. Submitted to JBMR-B 12/2/08.

4. Costerton JW, Ellis B, Lam K, Johnson F, Khoury AE: Mechanism of electrical enhancement of efficacy of antibiotics in killing biofilm bacteria. Antimicrobial Agents Chemother 38(12):2803-2809, 1994.

5. Dr. Beck trip report and Appendices.

SUPPORTING DATA

See Figures 1, 2, 6, 7, 8, 9 and 10.

Development of a Single Stage Surgical Model for Percutaneous Osseointegrated Implants for Amputees

Journal:	*ORS 55th Annual Meeting*
Abstract ID:	ORS2009-2259
Presentation Type:	Either Poster or Podium
Categories:	Arthroplasty-Implant Fixation, Infection and Inflammation-Orthopaedic Infection, Trauma-Clinical Trauma Research

Development of a Single Stage Surgical Model for Percutaneous Osseointegrated Implants for Amputees

+[1,2] Bloebaum R D; [1,2]Beck J P; [4]Olsen R; [4]Norlund L; [3,2,1] Bachus K N

[1] Bone & Joint Research Laboratory, DVA Salt Lake City Health Care System, Salt Lake City, [2]Department of Orthopaedics, University of Utah School of Medicine, [3]Orthopaedic Research Laboratory, University of Utah Orthopaedic Center, Salt Lake City, UT, [4]Frontier BioMedical, Logan, Utah

Senior Roy.Bloebaum@hsc.utah.edu

INTRODUCTION:

Limb amputation is a devastating event resulting in associated loss of everyday function and a massive change in the quality of life. To date, socket technology is the standard of care to attach, or dock, the exoprosthetic device to the residual amputated limb in an attempt to impart partial restoration of use and function. However, sockets are not without shortcomings: they can overload and irritate adjacent soft tissues, can cause disuse osteoporosis of the residual limb, can be difficult to properly fit due to weight fluctuations and muscle atrophy, and difficult if not impossible to use with short residual limbs. More reliable and secure limb attachment would facilitate implementation of new technologies that expand prosthetic function and user capabilities. It is for these patients that a new approach to limb attachment and functional integration is needed.

Percutaneous osseointegrated implants, as a docking technology for exoprosthetic limbs, are being considered around the world as alternatives to sockets. The European experience with skeletally fixed percutaneous exoprosthesis attachment has shown significant increases in mobility, activity levels, and gait performance among amputees enjoying this technology when compared to their previous conventional socket technologies. While these achievements are encouraging, the European technique requires two-staged surgical procedures, sometimes up to 18-months apart, to place the osseointegrated anchoring implant. The second stage places the percutaneous attachment to the implant. Infections of the soft tissues neighboring the percutaneous pylon are reported and occasional deep infection of the implant requires removal. These limitations remain sources of critical concern.

The overall goal of our research team is to develop an infection-free percutaneous osseointegrated implant system, using a one-stage operative protocol, which can be clinically introduced into the amputee population based on translational research. In order to achieve this goal, the first phase of the program was to develop a weight bearing animal model that would confirm the potential for the single stage procedure. The criteria required for our model were that it should have similar bone healing and remodeling rates as human bone, have a weight range similar to humans and have a bone structure that would limit the number of implant sizes required.

METHODS:

CT images of 12 metacarpal III bones, retrieved from sheep carcasses, were taken with a GE High Speed CTI single slice helical scanner. One millimeter thick slices were taken every millimeter along the entire length of each bone at a pitch of 1 (1x1x1), MAS=100, and kV=100, and a FOV (field of view) =16. Using MIMICs (Materialize, USA), 3D reconstructions of the metacarpals were obtained and the entire metacarpal reconstructed. Specifically, the intramedullary canal was reconstructed and 3D renderings of 3 sizes of stylized implants were created. Carcass soft tissue dissection and skin flap blood supply studies were done before beginning pilot survival study surgeries on sheep intended for the single-stage operation.

The titanium implants were designed in house and manufactured by Medicine Lodge, Inc. (Logan, UT). The porous coated titanium, on the grit-blasted surface of the implant, was applied and donated by Thortex Corporation (Portland, OR) (Figure 1). The porous coating was characterized by SEM/BSE analysis (Figure 1) and demonstrated a 52.1± 16.5% pore size with a highly irregular and roughened surface. This allowed the coating to immediately interlock with the bone and undersurface of the skin at the time of implantation.

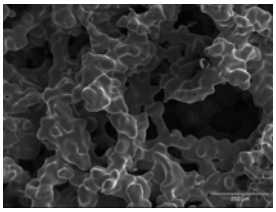

Figure 1: SEM image of titanium porous coating showing the irregular texture and porous openings.

Ten survivor (Columbus) female sheep were selected for this study (1,2). The metacarpal III bone was specifically selected from cadaveric studies examining initial implant sizing, biomechanical attachment strength, and the ability to allow for only three implant sizes.

After establishing an anterior skin flap, the right forelimb was transected at the distal metaphyseal flare of the third metacarpal. The metacarpal was fitted with a primary percutaneous, intramedullary osseointegrated prosthesis and the animal allowed to recover and immediately bear weight. As described, the intramedullary implant is made of grit-blasted titanium alloy with a porous coating on the distal end to allow the residual skin to adhere (Figure 2). A titanium Morse Taper protrudes from the distal surface extending through the skin flap. It is met with a titanium adapter which connects to the exoprosthesis (Figure 2). The exoprosthesis is made of a Delrin core surrounded by a 70A durometer polyurethane. The operative site was examined daily for periprosthetic and systemic signs of infection, and cultures were taken, as required, with dressing changes. Limb weight bearing gait forces were measured in five sheep before and after implant surgery.

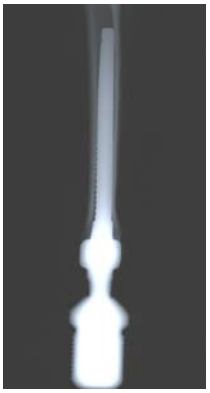

Figure 2: Postoperative x-ray of implanted sheep bone.

RESULTS:

Of the ten pilot sheep, one sheep was sacrificed due to an undersized implant that lacked proper fit and fill. Of the remaining nine animals, all remained infection-free, and load bearing for up to 3 months (with an average of 6 weeks) as of the time of this submission.

Of the nine animals, none showed gait abnormalities or soft tissue signs of infection based upon culture studies and clinical observations. Marsupialization of the skin, at the porous coating–Morse Taper-skin interface, a common problem with percutaneous devices, was absent. These data were supporting evidence that the implant design and porous coating met our objectives of assuring initial skin attachment and immobilization and thus prevented early infection.

DISCUSSION:

Our sheep weight-bearing model should become a platform to provide important translational information prior to the introduction of osseointegrated implant technology into the US health care system. Without such a model (one that allows biomechanical, material evaluation, skin integration, osseointegration and microbiological animal studies), it appears premature to introduce this technology into patient care based only on the current world literature and the European clinical experience.

There are several anecdotal reports in the popular press concerning the introduction of this technology in canines, but methodical, long-term data collection has not been done. The level of amputation and the limb involved is also variable. Long term post-operative x-rays, microbiological and infection documentation, and data on skin and bone changes are needed. Our model will allow carefully controlled clinical follow-up studies, based on power analysis, and the further development of hypotheses testing.

REFERENCES:

1. Willie B, et al., J Biomed Mater Res A 69A(3):567-576, 2004.
2. Bloebaum RD, et al. J Biomed Mater Res A 81A(2):505-514, 2007.

ACKNOWLEDGMENTS:

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From: onbehalfof@scholarone.com on behalf of heflin@ors.org
Sent: Mon 11/10/08 4:43 PM
To: Roy Bloebaum
Cc: null@scholarone.com
Subject: ORS 55th Annual Meeting Abstract Notification (Abstract ID ORS2009-2259)

10-Nov-2008

Dear Dr. Roy Bloebaum:

Congratulations! Your abstract, entitled "Development of a Single Stage Surgical Model for Percutaneous Osseointegrated Implants for Amputees" has been accepted for the ORS 55th Annual Meeting, February 22-25, 2009 in Las Vegas, Nevada. The program is still being finalized and an e-mail will be sent on Monday, November 17 with details on the presentation type: a podium, short talk poster, NIRA finalist or general poster format.

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The DEADLINE TO WITHDRAW your abstract is Monday, November 24, 2008.

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Thank you for submission, and congratulations on its acceptance.

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Brian Johnstone, PhD
ORS Program Committee Chair

BJ/mjh

From: heflin@ors.org [mailto:heflin@ors.org]
Sent: Tue 11/18/08 12:02 PM
To: Roy Bloebaum
Subject: ORS 55th Annual Meeting Poster Notification

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Your abstract has been chosen as a poster presentation at the 55th Annual Meeting of the Orthopaedic Research Society, February 22-25, 2009, Las Vegas, Nevada. Details are listed below.

FINAL POSTER #: 2255
SESSION TITLE: Poster Session 63 Arthroplasty - Implant Fixation
POSTER TITLE: ORS2009-2259. Development of a Single Stage Surgical Model for Percutaneous Osseointegrated Implants for Amputees
AUTHORS: Roy Bloebaum(1, 2)
INSTITUTIONS: 1. Research Service, DVA SLC Health Care System, Salt Lake City, UT, USA. 2. Orthopaedics, University of Utah School of Medicine, Salt Lake City, UT, USA.

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* Poster set-up is Saturday, February 21, 1:00 pm - 5:00 pm
* Presenters must attend the three (3) posters sessions and be available to answer questions.

Sunday, February 22, 4:30 pm - 6:30 pm
Even numbered posters - 4:30 - 5:30
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Program Committee Chair

BJ/mjh

Vertical Forces of Percutaneous, Osseointegrated Implants of an Ambulating Ovine Amputation Model

Journal:	*ORS 55th Annual Meeting*
Abstract ID:	ORS2009-2761
Presentation Type:	Poster Preferred
Categories:	Gait and Kinematics-Gait, Kinematics, Kinesiology, Trauma-Clinical Trauma Research, Arthroplasty-Implant Fixation

Vertical Forces of Percutaneous, Osseointegrated Implants of an Ambulating Ovine Amputation Model

[1,3]Shelton, T J; [2]Bloebaum, R D; +[1,2,3]Bachus, K N
+[1]University of Utah Orthopaedic Research Laboratory, Salt Lake City, UT,
[2]Bone & Joint Research Lab, DVA SLC Health Care System, Salt Lake City, UT, [3]University of Utah Department of Bioengineering, Salt Lake City, UT
kent.bachus@hsc.utah.edu

INTRODUCTION:

With recent ongoing military actions in Afghanistan and Iraq, many soldiers are coming home with amputated arms or legs or both. This, along with other factors, has led to new prosthetics being developed world wide. One such advancement is the development of percutaneous, osseointegrated prosthesis. To facilitate this development, an amputated sheep model is being used, and in order to characterize the effectiveness of this device, limb forces must be measured. It is the aim of this study to compare prosthetic limb loading to pre-surgery limb loading in an ovine model.

METHODS:

Subjects:

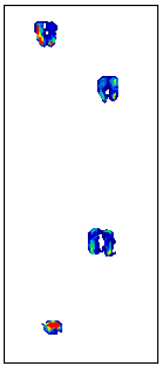
Four ovine subjects' limb forces were measured pre-operation and post-operation. Subjects' right forelimb was amputated and the primary metacarpal was fitted with a percutaneous, osseointegrated prosthesis at the time of surgery and then allowed to recover. The intramedullary implant is made of a titanium alloy with a porous coating on the distal end to allow the residual skin to adhere. A titanium Morse Taper protrudes off the distal surface through the skin and is met with a titanium adapter which connects to the exoprosthesis. The exoprosthesis is made of a Delrin core surrounded by a 70 A durometer polyurethane.

Forces were measured one week before surgery and three weeks post-operatively. For each trial, the subject was allowed to walk across a pressure walkway at least five times. Each subject also stood on the pressure mat for at least three seconds in order to determine the subjects' body weight. It is estimated that twenty more animals will undergo this same procedure within the next six months. Forces will also be measured 4 weeks, 2 months and 3 months post-operatively and as needed to give a better understanding of rehabilitation timelines and loading of the prosthesis over time. This experiment has been approved by the local institutional animal care and use committees.

Equipment:

A Tekscan HR Mat (Tekscan, Boston Massachusetts) consisting of three sensors was used. This mat has a sensing area 442 mm in width and 1463 mm in length and a spatial resolution of 3.9 sensel per sq-cm, while only being 6 mm thick so as to not disrupt the subjects normal gait behavior. This mat measured the vertical forces applied to the mat from each of the four limbs (Figure 1). The length of the mat allowed for at least one measurement per limb per pass, allowing for a better understanding of load bearing in a gait cycle. The mat was covered with a 3 mm thick outdoor carpet to protect the sensors and to prevent the subject from slipping. However, this did not affect the resolution of the HR Mat and calibration was done with the carpet on, minimizing error from the shielding of the carpet.

Calibration was performed following manufacture directions. This was achieved by an approximately 100 kg individual (roughly that of a sheep) standing on one foot on the sensor, to minimize the area to match more that of a sheep. Each sensor was calibrated individually on each day of testing to improve accuracy.



Figures 1 & 2: Tekscan image with all of subject's limbs on mat (left). Image of subject walking on HR Mat in the elliptical enclosure (right).

The HR mat was placed on the ground in a long section of a steel elliptical walkway. The walkway is four meters in width and seven meters in length and allows the subjects to ambulate freely (Figure 2).

A digital video camera was used for each trial and synched the video with the Tekscan video to ensure proper analysis of limbs. The HR Mat was set to record at 30 Hz to match that of the video to allow better synchronization.

Data Analysis:

Each video was analyzed and each hoof strike was analyzed individually in order to determine the peak force per limb when the subject walked across the mat. The data was normalized by dividing the peak force by the body weight of a subject, determined by summing the average force applied to each limb during a three second time frame in which the subject was standing on the mat. The forelimb versus hindlimb data were determined as well as the peak vertical force as a percent body weight was determined for the right forelimb. Data was then recorded in excel and data is presented in means and 95% confidence intervals. Statistical significance was determined using a student t-test.

RESULTS:

Preoperatively, the subjects' forelimbs were loaded an average of 36% more than the hindlimbs. Postoperatively, the difference between forelimb and hindlimbs was about 25%; however, with a 95% confidence interval applied, this difference is not statistically significant. The surgically altered limb's peak force per body weight decreased by nearly 18% three weeks following operation, deemed statistically significant based upon a 95% confidence interval (Figure 3).

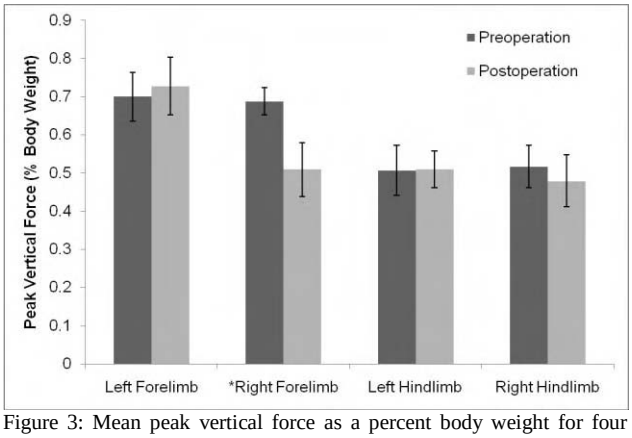


Figure 3: Mean peak vertical force as a percent body weight for four subjects' four limbs preoperatively and three weeks following operation with 95% confidence intervals applied. The surgery was performed on the right forelimb.

DISCUSSION:

Prosthetics limb loading can be compared to preoperative limb loading by measuring limb forces before and after amputation. If done over a few months, this can not only give an understanding of prosthetic limb loading, but also rehabilitation timelines which can be applied later in a human model. The forelimbs are loaded an average of 36% more preoperatively and 25% postoperatively. The surgically altered forelimb was loaded approximately 18% less three weeks after operation. Data demonstrates that the subject was compensating for the surgically altered right forelimb by applying a higher load to the left forelimb. Further data compiled over the next several months from these subjects and others will give more of an understanding of prosthetic limb loading.

From: onbehalfof@scholarone.com on behalf of heflin@ors.org
Sent: Mon 11/10/2008 6:51 PM
To: Trevor Shelton
Cc: null@scholarone.com
Subject: ORS 55th Annual Meeting Abstract Notification (Abstract ID ORS2009-2761)
10-Nov-2008

Dear Mr. Trevor Shelton:

Congratulations! Your abstract, entitled "Vertical Forces of Percutaneous, Osseointegrated Implants of an Ambulating Ovine Amputation Model" has been accepted for the ORS 55th Annual Meeting, February 22-25, 2009 in Las Vegas, Nevada. The program is still being finalized and an e-mail will be sent on Monday, November 17 with details on the presentation type: a podium, short talk poster, NIRA finalist or general poster format.

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SESSION TITLE: Poster Session 57 Gait and Kinematics, Kinseology
POSTER TITLE: ORS2009-2761. Vertical Forces of Percutaneous, Osseointegrated Implants of an Ambulating Ovine Amputation Model
AUTHORS: Trevor Shelton(1); Kent Bachus(2); Roy Bloebaum(3, 4)
INSTITUTIONS: 1. Bioengineering, University of Utah Orthopaedic Research Laboratory, Sale Lake City, UT, USA. 2. Orthopaedics, University of Utah, Salt Lake City, UT, USA. 3. Research Service, DVA SLC Health Care System, Salt Lake City, UT, USA. 4. Orthopaedics, University of Utah School of Medicine, Salt Lake City, UT, USA.

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Brian Johnstone, PhD
Program Committee Chair

BJ/mjh

Assessing Periimplant Tissue Infection Prevention

in a Percutaneous Model

Emily L. Perry[1,2]; James P. Beck[1,2]; Dustin L. Williams[3]; Roy D. Bloebaum[1,2]

1. Bone and Joint Research Lab, Veterans Affairs Health Care System, Salt Lake City, UT.
2. Orthopaedics, University of Utah, Salt Lake City, UT.
3. ARUP Institute for Clinical and Experimental Pathology, Associated Regional and University Pathologists (ARUP) Laboratories, Salt Lake City, UT.

Reprint requests sent to:
Roy D. Bloebaum, Ph.D.
Bone and Joint Research Lab (151F)
Department of Veteran Affairs
Salt Lake City Health Care Systems
500 Foothill Boulevard
Salt Lake City, Utah 84148-9998

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ABSTRACT

Background:

Infection remains the main challenge to percutaneous, intramedullary osseointegrated implant technology. The purpose of this investigation was to determine if a broad spectrum Ceragenin™ (CSA - 13) antimicrobial could prevent pin track infections in a percutaneous tibial pin site in a sheep model.

Methods:

In 20 sheep a smooth titanium alloy pin was percutaneously inserted through the medial skin and both cortices of the proximal tibia. In ten sheep the pin/skin interface was treated with a CSA-13-embedded foam pad. Ten sheep served as controls receiving an untreated pad. At the end of 24 weeks or if they presented with clinical signs of infection, euthanasia followed. Histological stains were processed from soft tissue and bone, and bacterial cultures were taken from tissue, bone and blood. In addition to clinical signs, sheep were considered infected if at least one tissue culture and/or histologically stained sample was positive.

Results:

Compared to the controls, CSA-13 did not prevent pin track infection ($p=0.88$). Large gaps around the pin indicated a lack of skin-pin adhesion.

Conclusions:

In this application, CSA-13 was not effective in preventing pin track infections. This study suggests that maintaining skin attachment at the implant surface of osseointegrated implants is essential and antimicrobial treatments should be considered a secondary barrier of infection prevention.

Key words/phrases: osseointegration, percutaneous, infection, antimicrobial, ceregenin

BACKGROUND AND INTRODUCTION

Sixty-four percent of combatants injured in World War I survived their injuries. In contrast, 72% of those injured in the Vietnam War survived. Today, approximately 87% of those injured in Operation Iraqi Freedom are surviving.[1,2] Of those wounded in the Iraq war, 2% have one or multiple amputations compared with 3% in Vietnam and 1% in WWI.[1] Advanced treatment strategies are now saving lives that would have been lost in previous wars.[2] Yet, although survival rates have increased, new challenges in the aftercare and treatment of warriors exist. This is especially apparent in those with high level, multiple limb amputations. Conventional socket prostheses are extremely difficult to fit on a short residual limb. Complex docking mechanisms are required and sockets impinge on the proximal joint (pelvis or shoulder girdle) when the artificial limb is moved.[3] Other problems associated with socket technology include heat and sweating in the prosthetic socket, sores/chafing, skin breakdown, infection and skin lesions.[4,5]

Eliminating the socket, by directly attaching the artificial limb to the residual bone, increases the range of motion of the proximal joint, permits comfortable sitting, and even allows osseoperception (sensory input through the prosthesis).[6,7] Osseointegration technology, in human volunteers was first introduced by Dr. Rickard Brånemark of Gothenberg, Sweden. This proof of concept was found to increase the quality of life of amputees, especially those with short residual limbs.[4,7,8]

Infection was the most common complication encountered by Brånemark in a 3-year follow-up reporting the results of the first 16 transfemoral patients to receive the percutaneous implant.[9] Infection was also a significant problem in the United Kingdom's experience using the Branemark method. After 1 year, 2 of the 11 patients (11%) had

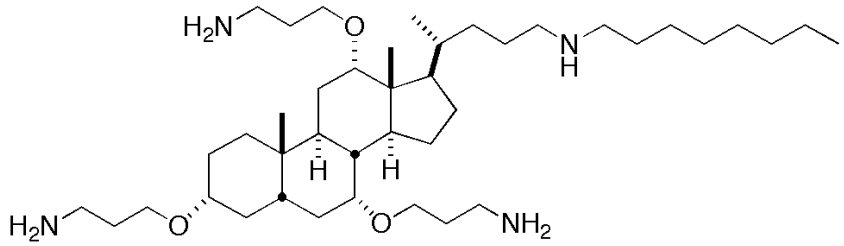
their abutment and internal fixture removed because of infection.[10] Infection can lead to life-altering consequences including chronic infection, bone resorption leading to fracture or implant loosening, and possible eventual higher reamputation of the residual limb.[9-11] Thus, the development of an effective percutaneous infection prevention strategy is crucial to safely implement the percutaneous osseointegrated implant technology in a growing amputation patient population.

Playing a key role in the development of infection is *Staphylococcus aureus*, which has been found to be a common pathogen involved in biomaterial, device-related, and/or pin track infections.[12,13] Compounding the problem, *S. aureus* can also tolerate high saline levels,[14,15] which leaves suspect the Brånemark method of infection prevention which relies upon mechanically flossing the prosthesis/skin interface with cotton cloth moistened with saline.[16] It seems reasonable that exploring a method employing an antimicrobial other than saline would be advisable.

Importantly, novel therapy developments must address newly-emerging strains of bacteria displaying antibiotic resistance. For example, antibacterial peptides that have been isolated from diverse organisms,[17,18] display broad-spectrum antibacterial activity and rapid killing times. These peptides are potential candidates as interface antimicrobials and are less likely to induce the formation of resistant strains of bacteria. One of the first antibacterial peptides discovered, Pexiganan, has been studied *in vitro* and in a transcutaneous bone/pin rabbit model and found to have bactericidal properties.[19] However, Pexiganan partly illustrates the limitations of using naturally derived antibacterial peptides. First, because of their relatively large size (> 20 amino acids), manufacturing antibacterial peptides can be technically difficult and costly.[20] Second,

many of these peptides are degraded quickly in the presence of proteases.[21] Because of these limitations, focus has shifted from the production of these peptides to developing mimics of these naturally effective cationic antimicrobials.

One such mimic has been developed by the synthesis of steroids with amine groups. This novel series of antimicrobial compounds, termed Ceragenins™, is based on derivatives of bile acids with covalently attached amines (Figure 1).[22,23] CSA-13 is the most potent of the Ceragenin compounds tested to date[22] and has been shown *in vitro* to effectively kill both Gram-negative and Gram-positive bacteria.[24] Although Ceragenins™ have been found to have weak hemolytic activity,[22] they display broad-spectrum bactericidal activity,[25] which gives them significant potential to be used as a therapy of infection surrounding percutaneous implants.



CSA-13

Figure 1: CSA-13 structure displaying steroid backbone and attached amine groups giving the structure its characteristic amphipathic morphology.

To test the efficacy of CSA-13 as an infection prevention strategy in a percutaneous implant, a model was needed that would provide a challenging environment

mimicking a hypothetically contaminated osseointegrated implant in an active warrior amputee. It further needed to mimic the abundant soft tissue motion observed clinically in the distal ends of residual limbs observed in our local veteran amputees. A positive infection signal was also needed to show positive indications of infection in the control animals to duplicate the type of infections currently seen in the osseointegrated amputees[9] and to provide a challenge for the CSA-13 to act as the primary barrier to infection.

In a previously tested rabbit model of osseointegration, the implant was inserted into the tibia near the ankle joint. Only 1 in 10 of the test rabbits experienced an exit-site infection.[26] This low infection rate was improved in the Bone and Joint Research Laboratory rabbit model,[19] but the rabbit's behavior became another variable and was a detriment which led to the use of a more compliant sheep model. A sheep model was chosen because of previously successful studies on bone and fracture healing using this animal.[27,28] The proximal end of the tibia was kept as the surgical implantation site in the chosen sheep model to allow for maximal soft tissue motion around the implant site.

Hypothesis Tested and Rationale

The sheep animal model and more particularly the proximal medial tibial site of surgical implantation were chosen because of the more predictable behavior found in sheep as well as the presence of soft tissue motion allowing testing of the chosen antimicrobial as a primary barrier to infection. This animal model allowed testing of the following hypothesis: A cationic steroidal antimicrobial (CSA-13), as a medical device, will prevent pin track infections at a mobile, soft tissue, percutaneous implant site in a

sheep model. CSA-13 was chosen to act as a primary barrier to pin track infection at a percutaneous implant site with surrounding highly mobile soft tissue.

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MATERIALS AND METHODS

Implant Design

Implants were machined from titanium alloy (Ti-6Al-4V), 95 mm in length, with a 5 mm maximum diameter, and manufactured by the University of Utah School of Medicine Machine Shop. The implants protruded from the skin only on the medial side and had a 10 mm long, 1 mm deep notch cut into the metal 5 mm away from the exposed portion of the implant (Figure 2). At the opposite end a 28 mm length of threading was located 5 mm from the tip. Implants were passivated using nitric acid, and autoclaved in separate autoclave pouches for sterilization.

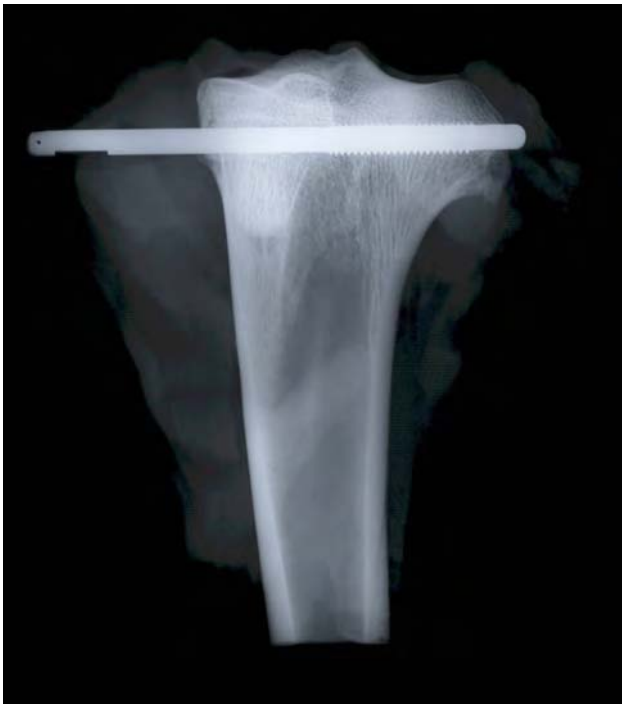

Figure 2: Anterior-posterior contact radiograph of pin placement in sheep tibia. Notice the medial tibial entry point at the coronal midline, approximately one cm distal to the knee joint line.

Study Design

Animal protocols and amendments were reviewed and approved by the Salt Lake City, Utah, Veterans Affairs Institutional Animal Care and Use Committee (IACUC) and the University of Utah IACUC in accordance with NIH guidelines.

Approximately 25 mm outer diameter, 4 mm inner diameter, 2-5 mm thick polyurethane foam pads (Rynel, Inc., Wiscasset, ME, USA) were prepared as a vehicle for the cationic steroidal antimicrobial (CSA-13)-polyurethane polymer conjugate (Cerashield™, Ceragenix Pharmaceuticals, Inc., Denver, CO). The polyurethane used to

conjugate with the CSA-13 had a high acid number (19) (AST Products, Inc., Billerica, MA) in contrast with the polyurethane foam pads used in both groups which were not acidic. These pads had a central circular hole that allowed the pad to fit tightly around the shaft and cover the skin-implant interface. Twenty nonpregnant adult Rambouillet ewe sheep, 2.5 – 6 years old, 75-90 kg, were randomized into two groups. Ten sheep received the CSA-13-polymer conjugate treated polyurethane foam pads. Ten control baseline sheep received autoclaved polyurethane foam pads without the CSA-13-polymer conjugate. CSA-polyurethane polymer coated pads and the control pads remained at the implant site for the length of the study. The pads were changed at least weekly or more frequently in the first days after surgery if purulent discharge, blood and/or exudate were present.

Surgery

The animals were fasted for approximately 12 hours prior to surgery and given an oral bolus of Tetracycline (0.5 gram-1.5 gram 12 hours preoperatively and 0.5 gram – 1 gram 1 hour before surgery) to reduce bacterial activity in the rumen. To determine baseline skin, nose, and throat flora, swabs were obtained from these locations and cultured using standard microbiological procedures. Animals were initially anesthetized with an intravenous injection of diazepam (0.1-0.5 mg/kg) and an IM injection of ketamine hydrochloride (4.4-7.5 mg/kg to effect). They were intubated and maintained under anesthesia with isoflurane (0.5-5% to effect) in oxygen. A rumen tube was placed as needed to control regurgitation. Lactated Ringer's solution was administered via the IV catheter throughout the procedure at a rate of approximately 15 ml/kg/hr.

The hind leg and area over the sacrum and the dorsal aspect of the neck was close-shaved. A 50 μg Fentanyl patch was placed on the neck and the area over the sacrum was prepped with betadine scrub, alcohol and betadine solution. Morphine (0.1 mg/kg not to exceed 10 mg total dose) was given through an epidural catheter to provide post operative analgesia. Anesthesia monitoring included: respiratory rate, tidal volume, end tidal CO2, heart rate, and oxygen saturation. Preparation of the surgical site included scrubbing with Betadine soap followed by a 70% alcohol wipe repeated three times with a final spray of Betadine solution.

Because the skin at the level of the sheep's knee was highly mobile, the site of the medial skin wound was determined to allow the skin tension on the implant, once inserted to be equal when the limb was in full flexion or full extension and under minimal tension when the limb was in a normal standing at rest position. An appropriate medial tibial entry point at the mid-coronal position and approximately one cm distal to the knee joint line was determined (Figure 2). A 2.5 mm guide wire was then driven across the tibia and pierced the lateral cortex in the coronal plane. Care was taken to avoid the *digital extensor foramen* which would have produced a tenodesis. Accurate implant placement was verified with a mobile C-Arm image intensifier (Series 9800TM Mobile C-Arm, 1k x 1k Mobile Workstation, OEC Medical Systems, Inc., Salt Lake City, UT).

A #15 blade scalpel was then used to enlarge the medial incision to accept a 5-mm cannulated reamer which reamed across both cortices. During reaming the skin surrounding the incision was secured using a drill sleeve. The implant was then drilled across both cortices with the lateral blunt end of the implant extending only 3 or 4 mm beyond the bone and buried in the lateral soft tissues. The medial end of the implant

protruded approximately 1.5 – 3 cm outside the skin and allowed for attachment of the control and antimicrobial pads.

Depending on the group to which the sheep was randomly assigned, CSA-polyurethane polymer coated or uncoated control, sterile blank polyurethane foam pads were placed at the medial implant-skin interface. Two CSA-13-polyurethane conjugate or two control pads stacked on top of each other were placed with one pad being in direct contact with the skin-implant interface (Figure 3). The initial removal torque was measured using a torque indicator (Dillon QuantrolTM AFTI Advanced Force/Torque Indicator, Meldrom Scale Co., UT). The torque indicator was set to measure data in a counterclockwise fashion. A silicon/Teflon washer was placed on top of the pads and the entire construct secured with a modified Jurgen ball. Because the ball was flush with the tip of the implant, when the set screw was tightened onto the flat surface on the pin, the apposing flank of the sheep's abdomen was protected from abrasion (Figure 3).

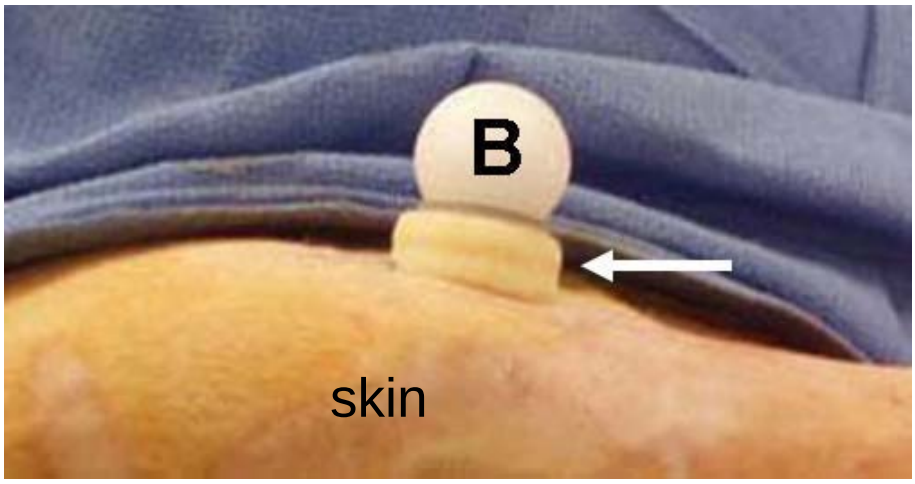

Figure 3: Completion of surgical procedure. Placement of pads (arrow), washer, and modified Jurgen ball (B) for maintaining pad contact at the skin-implant interface.

Anesthesia was discontinued and the animal was recovered. The animal was placed in its cage after it was determined that it was breathing on its own. Recovery was monitored until the animal was able to stand unassisted. Feed was returned at this time. Flunixin (1.1-2.2 mg/kg) and Ceftifur (Excenel) (1.1 – 2.2 mg/kg) were administered daily IM for 48-96 hours postoperatively as prophylactic antibiotics.

Pad Exchange Protocol

During pad exchange, the modified Jurgen ball and silicon/Teflon washer were removed and wiped with isopropyl alcohol. To reduce the risk of contamination, when a new pad was placed at the skin-implant interface, the implant was also wiped with Betadine followed by alcohol. Care was taken not to allow the Betadine or alcohol to contact the wound site, avoiding an additional variable of antimicrobial treatment. Pads were aseptically removed and a bacterial culture swab was taken of the wound site. The pads and the swabs were cultured on Columbia blood agar (Hardy Diagnostics, Santa Maria, CA) and left overnight at 37˚ C for observation the following day. The wound site was gently irrigated with 20-30 ml of sterile saline and dabbed with sterile gauze which removed varying quantities of dried exudates. Two new pads (CSA-13-polymer conjugate coated or untreated, depending on the group) were placed over the implant and lightly pressed against the wound site and secured with a clean silicon/Teflon ring and Jurgen ball.

Euthanasia Criteria

The study team observed the sheep daily for general health and signs of infection. Euthanasia was performed when the implant site had Grade II clinical signs of infection as described by Checketts et al.:[29] These are: 1. Redness of skin, 2. Discharge from the implant site, and 3. Pain and tenderness in soft tissues. Animals were also euthanized if they showed Grade I[29] clinical signs of infection demonstrated by slight redness around the implant with slight discharge with one or more of the following: 1. Appetite suppression, 2. Limited water consumption, 3. Lethargy, 4. Distress/limping, and/or 5. Pain and tenderness at the implant site.

If the animal had one or more of the previous and/or unforeseen complications with the model design, such as implant loosening, the animal was also euthanized.

Microbiologic Cultures

Immediately prior to euthanasia the sheep was calmed by an intravenous injection of 5 ml of Ketamine. Betadine and alcohol were applied liberally at the jugular vein site. Approximately 5 cc of blood were drawn aseptically and transferred to an aerobic blood culture bottle (BD BACTEC™ Plus Aerobic/F Medium, 50/sp, catalog # 442192, Franklin Lakes, NJ). Euthanasia was then performed by an IV injection of Beuthanasia D at approximately 1 mL per 10 lbs of body weight. The pads, either control or CSA-13, were removed from the implant site and a swab for culture was taken. A swab distal to the implant site was likewise taken to determine if normal flora bacteria were

contributors to the infection. The implant site and surrounding areas were then clipped and sterilized using Betadine/alcohol.

A 7 mm biopsy punch was used to take a tissue sample 5 mm away from the implant insertion site (to keep the tissue-implant interface intact for light microscope analysis and histology) and through sterile skin. This allowed evaluation of the tissues for infection deep into the implant insertion site. Tissue was placed in Fastidious Broth (Hardy Diagnostics, catalog #K31, item #15923, Santa Maria, CA). The pin was wiped clean with alcohol and an extraction torque measurement was obtained using the torque indicator. A bone marrow sample was obtained for culture after aseptically removing the lower half of the tibia and swabbing through the medullary canal nearest to the implant insertion site. The limb was disarticulated at the knee joint and placed in formalin for further histological processing. Pads and swabs were cultured on Columbia blood agar overnight at 37˚ C whereas the blood culture bottle was incubated for 48 hours and subsequently plated on Columbia blood agar overnight at 37˚ C. The tissue sample in broth was incubated overnight at 37˚ C then streaked onto Columbia blood agar and incubated overnight at 37˚ C. The presence or absence of bacteria was observed qualitatively indicating a positive or negative infection. Isolates were permsaved for future identification.

Imaging

Gross photos were taken of all implant-containing specimens. Radiographs were then taken at 70 kV for 90 seconds on AGFA Scopix CR5B Electronic Imaging Film (AGFA HealthCare, Branchburg, NJ), using the Faxitron Cabinet X-Ray System Model

43855A (Faxitron X-RAY LLC, Wheeling, IL), and AGFA CP 1000 X-ray Film Processor (AGFA HealthCare, Branchburg, NJ) (Figure 2).

Histology

Tissue samples were obtained using a scalpel from an area directly superior and directly inferior to the implant site. These samples were dehydrated using a Vacuum Infiltration Processor (Tissue Tek Vacuum Infiltration Process, Miles Scientific, Elkhart, IN) and embedded in paraffin (Surgipath Medical Industries, Inc., Richmond, IL) using the Histocentre 2 embedding center (Thermo Shandon, UK). They were then sliced to 5 μm using a Reichert-Jung (Leica) 2050 Microtome (Leica Microsystems Inc., Bannockburn, IL) with Accu-Edge® Low Profile Blades (Sakura Finetek U.S.A., Inc., Torrance, CA). At least three slices were obtained from each sample. The slices were then placed on slides and cover slipped for staining.

Bone samples were obtained by using an 8 mm outside diameter (6 mm inside diameter) screw extracting bit in conjunction with a United Heavy Duty Drill Press Model No. 810 (United by New Corp, Las Vegas, NV). Two samples were taken on the superior side on the medial and lateral aspects and one sample was taken on the inferior side.

The bone samples were decalcified, dehydrated, and embedded in paraffin. They were then cut to 5 μm widths using a microtome and cover slipped for staining. Stains performed on both bone and tissue samples were the Brown-Brenn modified gram stain, Peroidic Acid-Schiff (PAS), and Hematoxylin and Eosin.[19] The Brown-Brenn stain was used to detect the presence of bacteria. The periodic acid-Schiff stain was used to detect

the presence of fungus and the H&E stain was used for observing inflammation and fibrosis. The H&E stain was performed using a Microm DS 50 Slide Stainer (Richard-Allan Scientific, Kalamazoo, MI). The Brown-Brenn stain and PAS stain were performed at ARUP Laboratories (Salt Lake City, UT). All histological analyses were performed by a board-certified pathologist at ARUP, while blinded to the study groups.

Methyl Methacrylate Embedment and Contact Radiography

Specimens containing implants were cut superior and inferior to the implant approximately 2-5 cm near the implant using a water saw (Marmed Inc., Cleveland, OH). These cut specimens were then dehydrated using a Vacuum Infiltration Processor (Tissue Tek Vacuum Infiltration Process, Miles Scientific, Elkhart, IN), and embedded in methyl methacrylate (MMA) using modified standard procedures.[30] Individual specimens were then cut with an industrial vertical band saw (Model 20, Rockwell International, Pittsburg, PA) to remove excess MMA and reduce the size of the specimen for grinding.

Specimens were then ground on an 8-inch Buelher Polimet 1 Polisher (Buehler Ltd, Lake Bluff, IL). These sections (now between 3-5 mm thick) were then radiographed at 70 kV for 35 seconds on AGFA Scopix CR5B Electronic Imaging Film (AGFA HealthCare, Branchburg, NJ), using the Faxitron Cabinet X-Ray System Model 43855A (Faxitron X-RAY LLC, Wheeling, IL), and AGFA CP 1000 X-ray Film Processor (AGFA HealthCare, Branchburg, NJ).

Scanning Electron Microscope Imaging and Analysis

Three untreated control specimens and four CSA-13 treated specimens were ground on the 8 inch grinding wheel to obtain a mirrored finish. These polished specimens were then sputter coated with carbon for 15 seconds using a carbon coater (Model Number 208, Cressington Scientific Instruments Ltd., Watford, England). The carbon coated specimens were individually placed in a JSM-6100 Scanning Electron Microscope (SEM) (JOEL USA, Inc., Peabody, MA) equipped with a backscattered electron detector (Tetra, Oxford Instruments Ltd, Buckinghamshire, UK) and attached image capture software (Noran System Six, Thermo Scientific, Madison, WI). SEM settings were set at the following: voltage: 20kV, working distance: 15 mm, probe current: -0.9 nA. The probe current was measured with a SM-16100 probe current detector (JOEL USA, Inc., Peabody, MA) attached to an external picoammeter (Keithley Instruments, Cleveland, OH). Fine alterations in probe current were made frequently throughout image acquisition. To obtain images of a larger portion of the pin-implant interface, probe current was changed to approximately -3.0 nA and working distance was altered to 34 mm at 12x magnification or 39 mm at 10x magnification.

Microscope Analysis

Six 3-5 mm sections (two untreated control and four CSA-13 treated) were glued to plastic slides and ground to 50-70 μm thick specimens. These specimens were then stained with Sanderson's Rapid Bone Stain using an Acid Fuchsin counterstain (Surgipath Medical Industries, Inc., Richmond, IL) and examined under light microscope (Nikon Eclipse E600, Nikon, Japan) with associated camera (Optronics, Goleta, CA) and

image capture and processing software (Optronics MagnaFIRE™ SP version 1.0x5, Optronics, Goleta, California).

Appositional Bone Index Measurements

Appositional Bone Index (ABI) measurements[31] were taken to quantify the percent of bone in contact with the implant at euthanasia using Image Pro Plus software (Media Cybernetics, Inc., Bethesda, MD). Measurements of the areas of radiolucencies were also taken and divided by the length of possible bone-implant contact to provide an average width of radiolucency. These average widths of radiolucency were compared between the untreated control and CSA-13 treated groups also using the Image Pro Plus software.

Statistics

A log-rank test for equality of survivor functions was used to compare infection rates between the two study groups. A p-value of ≤ 0.05 was considered statistically significant. Sheep were classified as infected if they demonstrated two or all three of the following: 1) Clinical signs greater than a Grade I infection as demonstrated by slight redness around the implant with slight discharge.[29] 2) Positive culture results for blood, soft tissue, and/or bone samples taken at euthanasia, and/or 3) Positive bone and/or soft tissue histology results of samples processed after euthanasia. Kaplan-Meier survivorship curves were used to display these time to infection rates. The Fisher's exact test was used to compare clinical implant loosening and the Wilcoxon-Mann-Whitney test was used to compare the Appositional Bone Index (ABI) between the groups. A

student t test was used to compare the average widths of radiolucency between the groups as well as initial fixation torque. An analysis of covariance (ANCOVA) was used to compare final fixation torque.

RESULTS

Clinical Observations

The model was observed to have highly aggressive soft tissue motion. The muscle and skin motion of the medial side of the tibia was observed to tear the skin surface and expose the underlying torn proximal ends of the *fibularis tertius* and *extensor digitorum longus* muscles. This led to gaps in the skin tissue ranging from approximately 15 mm to 30 mm around the implant-skin interface. These findings indicated the absence of skin-implant interface contact (Figure 4).

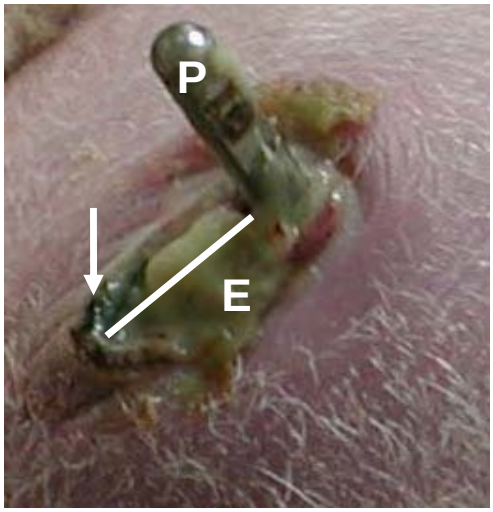

Figure 4: Skin gap at pin (P) site created by movement of muscle and skin. Notice the skin boundary (arrow) and exudates (E) surrounding the pin/skin interface. The size of the gap is approximately 24 mm (solid white line).

The wound site was observed to expand approximately 1-7 mm beyond the diameter of the pads in 10% (1 of 10) of CSA-13 treated sheep and 30% (3 of 10) of untreated control sheep. Clinical signs of infection, in addition to other signs of distress and model complications observed in the groups, included high rates of redness, discharge, necrotic tissue, limping, the pad not covering the wound site, and swelling. These indicators of infection were found in both the CSA-13 treated sheep and the untreated control sheep.

Infection Rates

Eighty-five percent (17 out of 20) of the sheep had bacteria cultured in the blood, bone, and/or soft tissue samples taken at euthanasia. Of these 17, nine were CSA-13 treated and 8 were untreated controls. The CSA-13 treated group had one positive blood culture, nine positive soft tissue cultures, and zero positive bone cultures. The untreated controls had two positive blood cultures, eight positive soft tissue cultures, and three positive bone cultures.

Eighty-five percent (17 out of 20) of the sheep had positive histology results showing small, gram positive rods indicative of infection. From these positive results, eight CSA-13 treated and nine untreated controls had positive histological results in soft-tissue specimens taken adjacent to the implant sites. One CSA-13 treated and two

untreated controls had positive bone histological results from samples removed either proximal or distal to the implant.

Upon combining the results of culture, histology, and clinical signs of infection, it was determined that, at euthanasia, 95% (19 of 20) of the sheep were infected. Five percent of the sheep (1 of 20, CSA-13 treated) were euthanized due to clinical signs of infection but were not classified as infected because of negative culture and histology results.

The infection data demonstrated that, when compared to the untreated control pads, the CSA-13 did not prevent pin track infection ($p=0.88$ with euthanasia as endpoint, Figure 5).

All sheep were euthanized between 8 and 40 days, after surgical implantation, due to clinical signs of infection or animal distress. All sacrifices occurred prior to the intended 6-month endpoint.

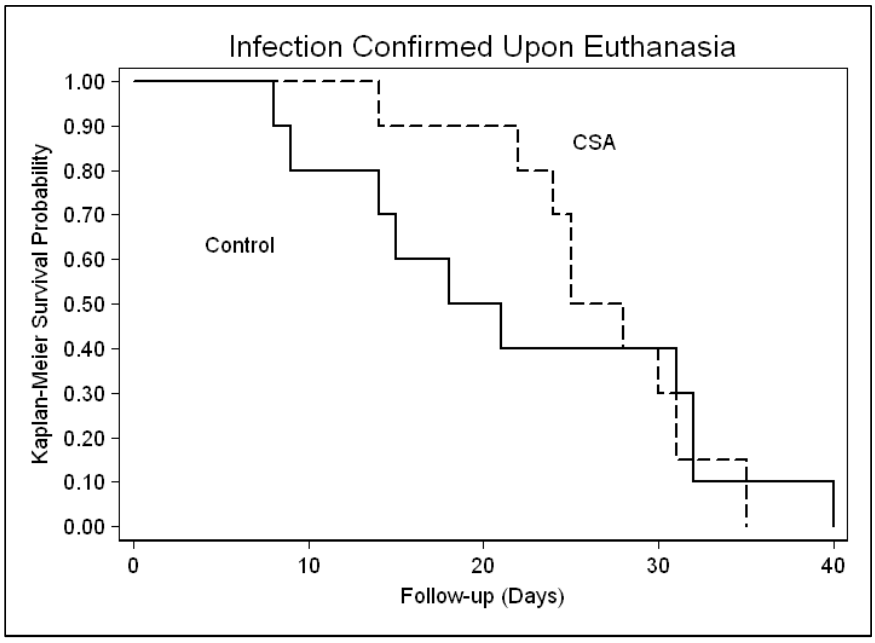

Figure 5: Kaplan-Meier curve showing that CSA-13 treated pads did not significantly prevent infection compared to untreated control pads (p=0.88).

Clinical Loosening

Large gaps in the skin around the implant indicated a lack of skin-implant contact and extreme soft tissue mobility. Movement of traversed muscle and contact with the apposing flank may have caused loading on the implant and micromotion leading to increased infection rates and implant loosening. The CSA-13 treated group had a higher rate of clinical implant loosening when compared with untreated controls (Fisher's exact test p=0.005). Nine of 10 sheep treated with CSA-13 had clinically loose pins at the time of sacrifice. In contrast, 2 of 10 untreated control sheep had clinically loose pins at time of sacrifice.

Radiographic Loosening

In addition to clinical implant loosening, radiographs confirmed implant loosening. It was found that the CSA-13 treated group [median (IQR), 1.4 (0, 11.2)] had a higher percentage of radiographic lucency and a lower ABI than the untreated control group [8.6 (0, 41.7)]. However, the variation in percent bone contact between the CSA-13 treated sheep and the untreated controls was not statistically significant (Wilcoxon-Mann-Whitney test, $p = 0.53$). This radiographic analysis suggested that both groups showed clinically unacceptable radiographic lucency, indicative of early implant loosening (Figure 6).

The average width of radiographic lucency was compared between the two groups and not found to be statistically significant (cranial side radiographic lucency widths, $p=0.57$; caudal side radiographic lucency widths, $p=0.09$). The average radiographic lucency widths from the control group were 1.00 mm (cranial side) and 1.11 mm (caudal side). The average radiographic lucency widths for the CSA-13 treated group were 0.93 mm (cranial side) and 0.87 mm (caudal side). Fibrous tissue was visually observed within these gaps between bone and tissue.

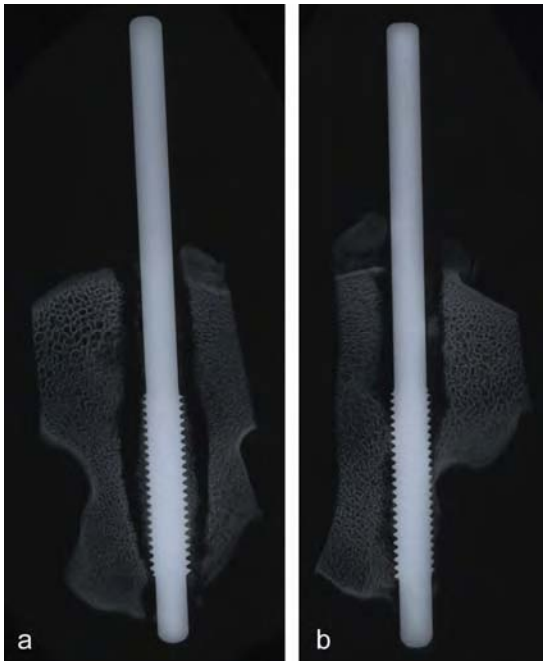

Figure 6: a) Untreated control animal, euthanized for infection after 32 days. b) CSA-treated animal, euthanized for infection after 30 days. Both a and b showed positive histology results for bone infection and had a limited percentage of bone contact. The radiographs clearly demonstrate that there was extensive radiographic loosening in both groups.

Histological Review

Implant-bone interfaces as well as the area of bone immediately surrounding the interface site were histologically examined. Even after 25 days *in situ*, it was found that bone fragments did not incorporate with the host bone tissue in either the CSA-13 treated group or the untreated control groups at the bone-implant interface. The lack of healing response was in contrast to woven bone formation and remodeling, which were occurring approximately 1-4 mm away from the implant site (Figure 7).

Further histological examination of the pin-implant interface revealed viable osteocytes within microns of the interface which suggests that the bone was viable but that remodeling at the interface was arrested (Figure 8). These viable osteocytes are evidence that the lack of bone remodeling at the interface was not due to necrosis of the bone caused by possible bone overheating during implantation.

Fibroblasts and fibrous tissue were observed at the pin-implant interface which demonstrated a foreign body response to the implant (Figure 9).

A lack of bone healing response at the implant-bone interface as well as the formation of woven bone millimeters away from this interface was also found using a scanning electron microscope (SEM) in backscatter electron imaging mode (Figure 10).

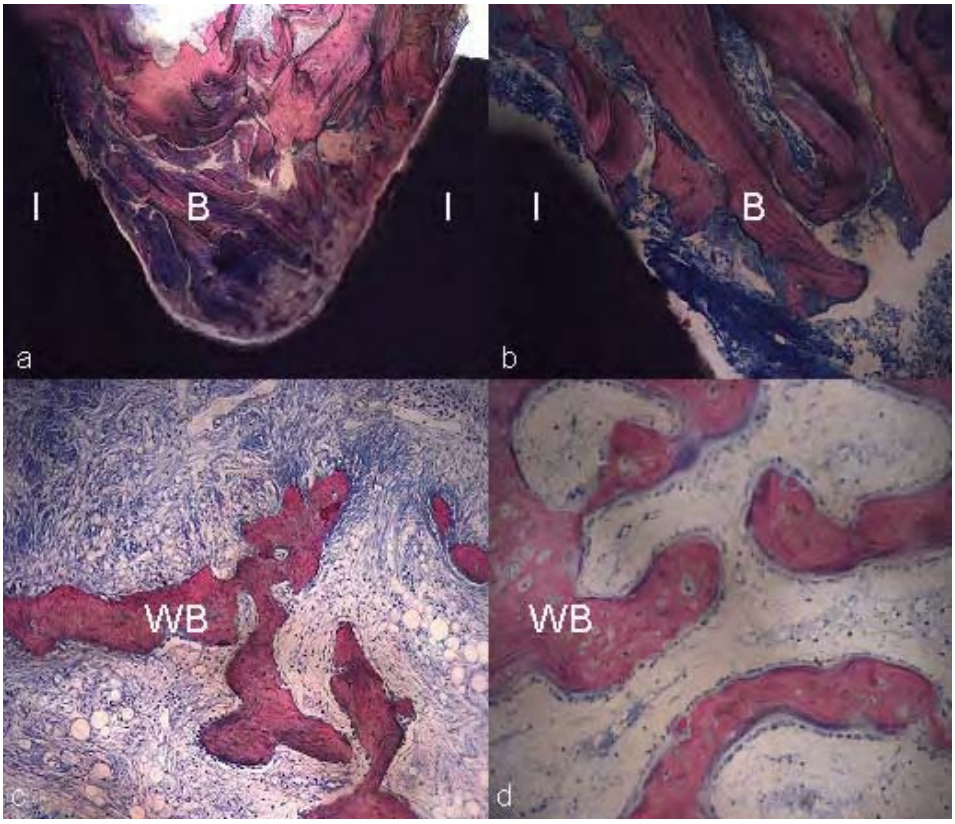

Figure 7: Demonstration of lack of healing response at bone-implant interface with healing response approximately 1-4 millimeters away from interface. a) Untreated control, 32 days: Bone(B)-implant(I) interface showing unincorporated bone fragments without remodeling activity. b) CSA-13 treated, 25 days: Bone(B)-implant(I) interface with bone fragments demonstrating no healing or remodeling activity. c) Untreated control, 32 days: Woven bone (WB) formation identified by osteoblast lining around the perimeter of the bone. d) CSA-13 treated, 25 days: Woven bone (WB) being created with osteoid secretion from osteoblasts lining the bone. Slides stained using Sanderson's Rapid Bone Stain. a, b, d original, uncropped images taken at magnification of 200x. c

original image taken at magnification of 100x. c and d taken approximately 1-4 mm away from bone-implant interface.

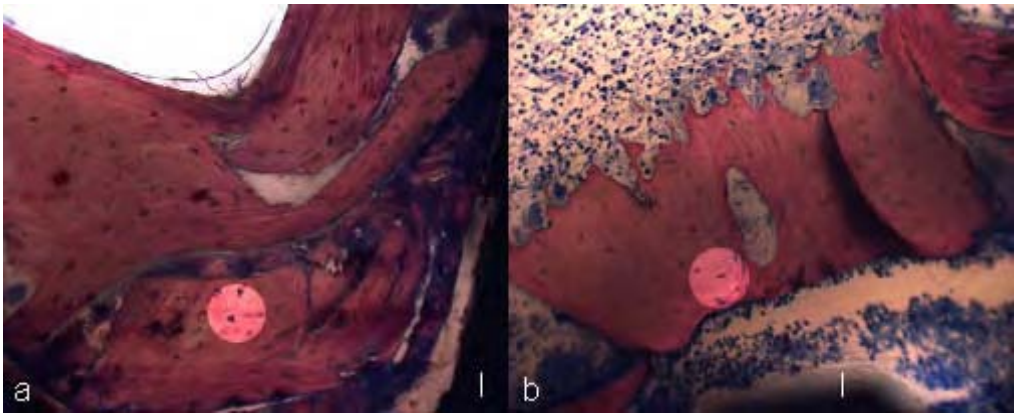

Figure 8: Viable osteocytes in bone located within microns of the implant in both untreated control and CSA-13 treated specimens. a) Untreated control, 8 days: Viable osteocytes (example within circle) in bone within microns of the implant (I) demonstrating healthy bone at interface near the time of surgical implantation. b) CSA-13 treated, 25 days: Viable osteocytes (example within circle) also found within microns of the implant demonstrating that the bone remained healthy. These viable osteocytes demonstrate that the bone near the implant was not excessively heated and that no bone necrosis occurred during the surgical procedure. Slides stained using Sanderson's Rapid Bone Stain. Original images taken at magnification of 200x.

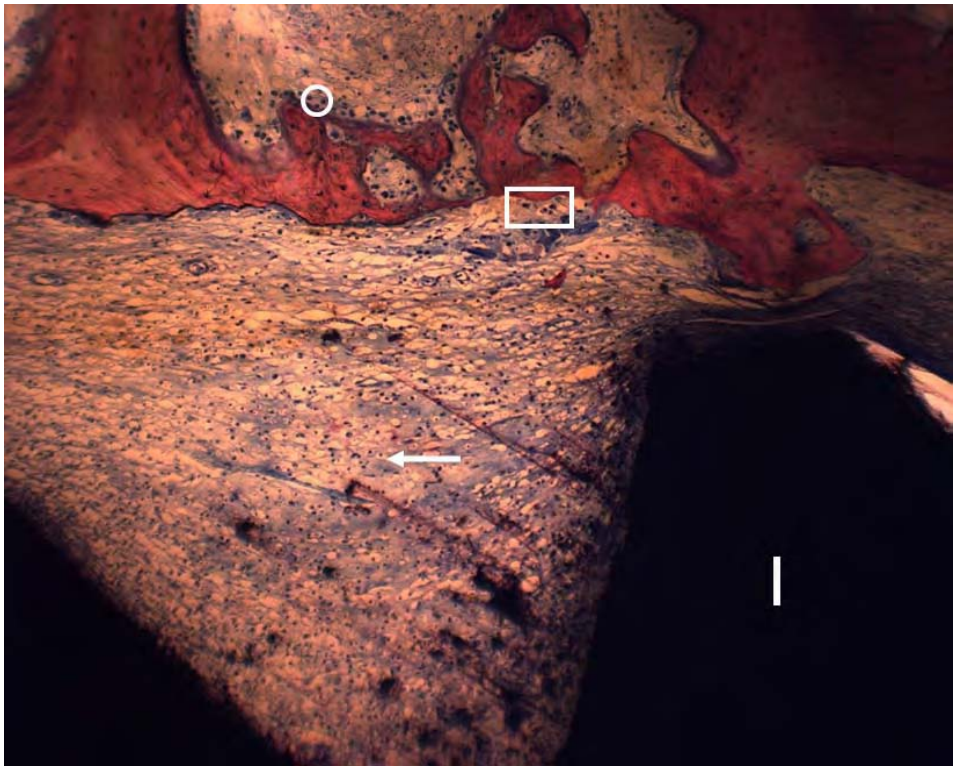

Figure 9: CSA-13 treated, 30 days: Demonstration of fibroblast formation (arrow) of fibrous tissue in foreign body response surrounding implant (I). Osteoblasts (examples within circle) and osteoclasts (examples within rectangle) activity also found within one millimeter of the implant but not directly at the interface. Slides stained using Sanderson's Rapid Bone Stain. Original image taken at magnification of 100x.

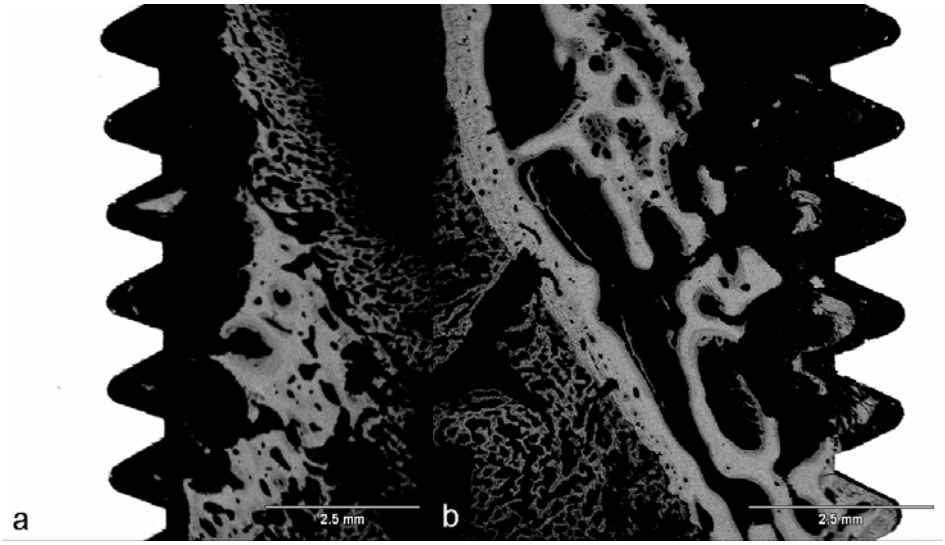

Figure 10: Backscatter electron images of bone tissue showing lack of bone healing and/or lack of ongrowth on implant surface. a) CSA-13 treated, 30 days: showing lack of bone contact at bone-implant interface and woven bone formation approximately 2 - 4 mm away from the interface. b) Untreated control, 40 days: showing bone-implant interface with bone fragments with no healing or remodeling activity and woven bone formation approximately 2.5 – 4 mm away.

Torque

The time zero torque values measured for the CSA-13 treated group had a mean of 0.60 ± 0.47 N-m and a median of 0.43 N-m. The time zero torque values measured for the untreated control group had a mean of 1.29 ± 0.60 N-m and a median of 1.37 N-m. There was a statistically significant difference found in torque at time zero between the groups (p=0.02, student t test) showing that the untreated control group had a tighter initial fixation immediately following surgery. Controlling for this baseline difference,

using an analysis of covariance, there was not a significant difference found in endpoint torque between the groups ($p=0.80$).

DISCUSSION

Goals of Study

The first goal of the study was to develop an animal model that would accurately represent the mobile soft tissue conditions at the distal end of the residual limb of active warrior amputees. These mobile soft tissue conditions were intended to be unlike the Pendegrass et al. goat model.[32] The second goal was to examine the use of a broad-spectrum antimicrobial as a primary barrier to infection rather than skin immobilization followed by a mechanical saline flossing treatment used in the Brånemark patient model.[8] A third goal was to develop an animal model that would have a strong infection signal, contrasting with the Gerritsen rabbit model which only had a 1 in 10 infection rate[26] in the control animals. This would provide an infection signal that would possibly model an active amputee with retained residual limb distal soft tissue.

The first goal was achieved as a highly mobile soft tissue/implant interface was observed around the percutaneous implant at the proximal end and medial region of the tibia.

The second goal was not achieved, but the results support the idea that antimicrobials could only be used as secondary rather than as primary barriers to infection surrounding osseointegrated implants. This leads to a need for further exploration of antimicrobial usage as a secondary barrier to infection. The goal of infection prevention using an antimicrobial as a primary barrier appeared to have been significantly hindered by the challenging animal model. The lack of ability to prevent infection without skin immobilization supports the conclusion that soft-tissue motion around the implant, although similar to that seen at the distal end of residual limbs in

amputees, must be controlled and/or eliminated. Control of implant/soft tissue motion must be included as a major operative and implant design component of the infection-prevention strategy before osseointegrated implant technology is introduced as a standard of care.

The third goal was achieved as 10 of 10 untreated control sheep were infected prior to the 6-month predetermined endpoint of the study. This demonstrated that a strong infection signal was present in this model.

Limitations

CSA-13 was not effective in preventing pin track infections along percutaneous implants in this study's sheep model. Nevertheless, because of other factors involved, such as excess amounts of skin and soft tissue motion at the foam pad-implant site interface, this result does not conclusively exclude CSA-13 as a preventative barrier to pin track infections if a stable skin-implant interface could be established. These limitations include the following.

The untreated control group had higher time zero torque values and thus stronger initial fixation. This stronger initial fixation could have been a key factor in the delay of clinical pin loosening observed in the untreated control group when compared with the observed clinical loosening in the CSA-13 treated group.

CSA-13 itself could have caused hemolysis along the implant track. CSA-13 is known to be active on biological membranes and this accounts for some of its bactericidal properties.[22,25] This increased amount of hemolysis and possible lysis of

other cell types may have contributed to possible earlier tissue fibrous capsule formation along the length of the implant that limited bone healing and osseointegration.

Another limitation discovered was that the initial implantation technique allowed for a 0.5 mm circular gap in the bone on the medial aspect. This is believed to be due to the implant threads which were machined with a 0.5 mm difference in height with respect to the shaft of the implant. Because this study's threading was limited to a combination of cancellous bone and cortical fixation on the lateral aspect of the bone (approximate unthreaded portion of pin in tibia: 15-30 mm, length of threading in each pin: 28 mm), 15-30 mm of bone was not in apposition to the implant surface, but allowed for a 0.5 mm gap at the medial cortical bone region (Figure 2). This gap could have allowed fluid transport and mechanical loosening caused by micromotion of the implant when the soft tissue loads were applied during ambulation of the sheep. Pressure was also applied to the implant site by the apposing flank when the sheep was resting in both a standing and lying down position. Thus, the resulting micromotion of the implant may have also contributed to initial bone resorption, fibrous tissue formation, and implant loosening.[33]

A condition observed *in vivo* that did not exist *in vitro* was exudate leaving the fresh implantation site. In the first few days following surgery the exudate included blood and serous discharge but was followed by necrotic tissue and pus as infection developed. This exudate soaked into the CSA-13 treated pad. The exudate then hardened and formed a crust. This may have precluded the CSA-13 from diffusing into the wound site. The known effective delivery method of CSA-13, previously determined by *in vitro* testing, required a fluid medium for proper elution. Thus, effective delivery of the antimicrobial may have been compromised and its efficacy not accurately

represented. In addition, the motion of the soft tissues in the proximal ends of the *fibularis tertius* and *extensor digitorum longus* muscles at the implant site pressed and withdrew against the skin-pad interface. This may have also led to prevention of constant and complete contact between the delivery device and the skin-implant interface.

These delivery device limitations, probably compounded by the rigorous *in vivo* model, suggest that CSA-13 should not be discounted as an effective bactericidal treatment until tested in a less rigorous *in vivo* model. Such experimentation could allow for a more complete understanding of CSA-13's infection prevention qualities in osseointegrated implants.

Although the soft tissue motion observed in this sheep model appeared to be a limitation that prevented the type of excellent skin attachment observed in the Pendegrass et al. goat model,[32] this motion mimicked the conditions observed at the distal ends of residual limbs of amputees, the intended benefactors of this research. Thus, the soft tissue motion in this model reinforced the conclusion that a skin barrier to potential infection is an essential factor in implementing osseointegration technology in residual limbs of amputees.

Conclusions

The data suggests that implant-skin attachment is essential before antimicrobials can be determined efficacious. Moreover, aggressive soft tissue motion and animal behavior may result in an over-challenging environment. These results ultimately reinforce the importance of limiting soft tissue motion around percutaneous devices in order to prevent infection and micromotion which may have led to implant loosening.

This conclusion is in agreement with that made by Pendegrass et al.[32] as well as Branemark et al., as shown in human studies.[34] Ultimately, following dermal attachment to the implants and limiting soft tissue motion around osseointegrated implants, antimicrobials may be more effectively used as secondary barriers to prevent infection.

Future Studies

Possible future studies extending from this animal model include testing for percutaneous implant dermal attachment in a less-rigorous animal model. Following this dermal attachment success, CSA-13 should be examined in an alternative delivery system, as well as other antimicrobials, as secondary barriers to infection surrounding osseointegrated implants. Such studies may indicate the potential antimicrobial effects on the skin seal around percutaneous implants as well as intact human skin. For example, a one stage osseointegration procedure could be examined and multiple implant designs could be compared including microscopic porous coated, threaded, and macroscopic porous coated designs.

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REFERENCES

1. American war and military operations casualties: Lists and statistics: Congressional Research Service; 2007 June 29. Report nr Order Code RL32492.
2. Gawande A. Casualties of war - Military care for the wounded from Iraq and Afghanistan. N. Engl. J. Med. 2004;351(24):2471-75.
3. Miguelez JM, Miquelez MD. The MicroFrame: The Next Generation of Interface Design for Glenohumeral Disarticulation and Associated Levels of Limb Deficiency. JPO 2003;15(2):66.
4. Hagberg K, Branemark R. Consequences of non-vascular trans-femoral amputation: a survey of quality of life, prosthetic use and problems. Prosthet Orthot Int 2001;25(3):186-94.
5. DesGroseilliers JP, DesJardins JP, Germain JP, Krol AL. Dermatologic problems in amputees. Can Med Assoc J 1978;118(5):535-7.
6. Hagberg K, Haggstrom E, Uden M, Branemark R. Socket versus bone-anchored trans-femoral prostheses: hip range of motion and sitting comfort. Prosthetics and Orthotics International 2005;29(2):153-63.
7. Branemark R, Branemark PI, Rydevik B, Myers RR. Osseointegration in skeletal reconstruction and rehabilitation: a review. J Rehabil Res Dev 2001;38(2):175-81.
8. Brånemark P-I, Chien S, Gröndahl H-G, Robinson K, editors. The Osseointegration Book. From Calvarium to Calcaneus. Berlin, Germany: Quintessenz Verlags-GmbH; 2005.
9. Michael J. ICAP Highlight: Osseointegration. 2000.
10. Sullivan J, Uden M, Robinson KP, Sooriakumaran S. Rehabilitation of the trans-femoral amputee with an osseointegrated prosthesis: the United Kingdom experience. Prosthet Orthot Int 2003;27(2):114-20.
11. Smith TJ, Galm A, Chatterjee S, Wells R, Pedersen S, Parizi AM, Goodship AE, Blunn GW. Modulation of the soft tissue reactions to percutaneous orthopaedic implants. J Orthop Res 2006;24(7):1377-83.
12. Maderazo EG, Judson S, Pusternak H. Late infections of total joint prostheses: A review and recommendations for prevention. Clin Orthop 1988;229:131-142.
13. Mahan J, Seligson D, Henry SL, Hynes P, Dobbins J. Factors in pin tract infections. Orthopedics 1991;14(3):305-8.
14. Sharp SE, Searcy C. Comparison of mannitol salt agar and blood agar plates for identification and susceptibility testing of Staphylococcus aureus in specimens from cystic fibrosis patients. J Clin Microbiol 2006;44(12):4545-6.
15. Becton DaC. Mannitol Salt Agar. Technical Center/Inserts/Mannitol_Salt_Agar. Volume 2008. Franklin Lakes, NJ. p Intended use of Mannitol Salt Agar.
16. Branemark R. Osseointegration. In: Workshop WRAMCaDoVAAHP, editor. Salt Lake City, Utah; 2003.
17. Matsuzaki K, Sugishita K, Harada M, Fujii N, Miyajima K. Interactions of an antimicrobial peptide, magainin 2, with outer and inner membranes of Gram-negative bacteria. Biochim Biophys Acta 1997;1327(1):119-30.
18. Matsuzaki K. Why and how are peptide-lipid interactions utilized for self-defense? Magainins and tachyplesins as archetypes. Biochim Biophys Acta 1999;1462(1-2):1-10.

19. Rosenbaum Chou TG, Bloebaum RD. Examining Pexiganan Effects on Pin Track Infection in a Transcutaneous Implant Model. 2008; San Francisco, CA.
20. van 't Hof W, Veerman EC, Helmerhorst EJ, Amerongen AV. Antimicrobial peptides: properties and applicability. Biol Chem 2001;382(4):597-619.
21. Savage PB. Design, synthesis and characterization of cationic peptide and steroid antibiotics. Eur J Org Chem 2002:759-768.
22. Epand RF, Savage PB, Epand RM. Bacterial lipid composition and the antimicrobial efficacy of cationic steroid compounds (Ceragenins). Biochim Biophys Acta 2007;1768(10):2500-9.
23. Bucki R, Sostarecz AG, Byfield FJ, Savage PB, Janmey PA. Resistance of the antibacterial agent ceragenin CSA-13 to inactivation by DNA or F-actin and its activity in cystic fibrosis sputum. J Antimicrob Chemother 2007;60(3):535-45.
24. Savage PB, Nielsen J, Lai X-Z, Feng Y, Crook M, Li Y, Gard N, Linford MR, Genberg C. Antibacterial properties of cationic steroid antibiotics. 2007. p 1-12.
25. Savage PB, Li C, Taotafa U, Ding B, Guan Q. Antibacterial properties of cationic steroid antibiotics. FEMS Microbiol Lett 2002;217(1):1-7.
26. Gerritsen M, Lutterman JA, Jansen JA. Wound healing around bone-anchored percutaneous devices in experimental diabetes mellitus. J Biomed Mater Res 2000;53(6):702-9.
27. Augat P, Merk J, Wolf S, Claes L. Mechanical stimulation by external application of cyclic tensile strains does not effectively enhance bone healing. J Orthop Trauma 2001;15(1):54-60.
28. Claes L, Augat P, Suger G, Wilke HJ. Influence of size and stability of the osteotomy gap on the success of fracture healing. J Orthop Res 1997;15(4):577-84.
29. Checketts RG, MacEachern AG, Otterburn M. Pin track infection and the principles of pin site care. In: Giovanni De Bastiani AGAaAG, editor. Orthofix External Fixation in Trauma and Orthopaedics. Great Britain: Springer-Verlag London Limited; 2000. p 97-103.
30. Sanderson C, Kitabayashi LR. Parallel experience of two different laboratories with the initiator perkadox 16 for polymerization of methylmethacrylates. J. Histotechnol. 1994;17(4):343-348.
31. Bloebaum RD, Rhodes DM, Rubman MH, Hofmann AA. Bilateral tibial components of different cementless designs and materials: Microradiographic, backscattered imaging, and histologic analysis. Clin. Orthop. 1991;268:179-187.
32. Pendegrass CJ, Goodship AE, Blunn GW. Development of a soft tissue seal around bone-anchored transcutaneous amputation prostheses. Biomaterials 2006;27(23):4183-91.
33. Green SA. Complications of external skeletal fixation. Clin Orthop Relat Res 1983;180:109-16.
34. Branemark PI, Albrektsson T. Titanium implants permanently penetrating human skin. Scand J Plast Reconstr Surg 1982;16(1):17-21.

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Mechanism of Electrical Enhancement of Efficacy of Antibiotics in Killing Biofilm Bacteria

J. WILLIAM COSTERTON,[1]* BRIAN ELLIS,[1] KAN LAM,[1] FRANK JOHNSON,[2] AND ANTOINE E. KHOURY[3]

Center for Biofilm Engineering Montana State University, Bozeman, Montana,[1] and Institute of Medical Engineering, University of Ottawa, Ottawa,[2] and Hospital for Sick Children, University of Toronto, Toronto,[3] Canada

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The bioelectric effect, in which electric fields are used to enhance the efficacy of biocides and antibiotics in killing biofilm bacteria, has been shown to reduce the very high concentrations of these antibacterial agents needed to kill biofilm bacteria to levels very close to those needed to kill planktonic (floating) bacteria of the same species. In this report, we show that biofilm bacteria are readily killed by an antibiotic on all areas of the active electrodes and on the surfaces of conductive elements that lie within the electric field but do not themselves function as electrodes. Considerations of electrode geometry indicate that very low (<100 μA/cm²) current densities may be effective in this electrical enhancement of antibiotic efficacy against biofilm bacteria, and flow experiments indicate that this bioelectric effect does not appear to depend entirely on the possible local electrochemical generation of antibacterial molecules or ions. These data are expected to facilitate the use of the bioelectric effect in the prevention and treatment of device-related bacterial infections that are caused by bacteria that grow in biofilms and thereby frustrate antibiotic chemotherapy.

Work in many laboratories (16, 17, 32), including our own (3, 12, 33), has clearly established that biofilm bacteria are resistant to antibiotics and biocides at levels 500 to 5,000 times higher than those needed to kill planktonic cells of the same species. The mechanism of this inherent resistance of glycocalyx-enclosed biofilm bacteria to antimicrobial agents is not conclusively established but appears to depend on both diffusion limitation (25) and physiological properties associated with low growth rates (8, 9, 16, 17) in biofilm populations. Direct examination of the surfaces of medical devices that have become the foci of device-related bacterial infections shows that these pathogens grow in well-developed adherent biofilms (12), and clinical experience (21) indicates that these chronic infections are highly refractory to antibiotic therapy. Consequently, device-related bacterial infections are aggressively treated with combinations of antibiotics (2, 27), but in many cases, the biofilm-colonized device must still be removed to facilitate the resolution of these infections (21, 37).

An increasing number of laboratories have begun to examine the effects of electric fields and current densities on biological systems (1, 5, 15, 19, 28, 31, 34, 35, 38, 41), mainly because of interest in the electroporation and electrofusion processes that are very useful in genetic research (31). This body of work has shown that electric fields and currents can be used for electroporation and electrofusion (31), electroosmosis, iontophoresis (6, 13–15), and the electroinsertion of specific proteins (30). During this work, it has been noted that electric fields and currents can influence the organization of biological membranes (10, 28, 31, 35, 40, 42) and membrane analogs (1, 18), metabolic and developmental processes within both prokaryotic and eukaryotic cells (19, 24, 34, 38, 42), and possibly even the shape of the cell (36), cell behavior (41), and the dimensions of the bacterial glycocalyx (4). Most of these studies have used high-intensity fields and currents, in the kilovolt-per-centimeter range, but a significant number (5, 13, 15, 24, 36) have also focused on the effects of low-intensity fields and currents on biological systems for which significant effects have been documented, especially embryonic systems (34).

We have reported that low-intensity electric fields (field strength of 1.5 to 20 V/cm and current densities of 15 μA/cm² to 2.1 mA/cm²) can completely override the inherent resistance of biofilm bacteria to biocides (7) and antibiotics (26). This bioelectric effect reduces the concentrations of these antibacterial agents needed to kill biofilm bacteria to 1.5 to 4.0 times those needed to kill planktonic cells of the same species. The present study was undertaken to examine the mechanism of this bioelectric effect, with the working hypothesis that the electric field aids the penetration of the antibacterial agents through the biofilm by a form of electrophoresis that may be assisted by the electrochemical generation of agents that enhance the efficacy of these agents.

MATERIALS AND METHODS

Bacterial strains. A strain (UR-21) of *Pseudomonas aeruginosa* obtained from a patient with a chronic urinary tract infection was used in these experiments because of the facility with which it formed thick biofilms. Aliquots were stored at −70°C, on brain heart infusion agar, and fresh aliquots were used to start each experiment. The MIC for this strain was 1.0 mg/liter.

Flow cell. The development of a three-electrode flow cell (Fig. 1) allowed us to extend our study to ascertain if the bioelectric effect was applicable to all electrode surfaces and to inert nonconductive (or conductive) materials placed between the electrodes. The two exterior stainless steel (type 316) electrodes (E1 and E3) were connected together to act as the anode with E2 (type 316) as the cathode for 64 s, and then the current was reversed so that E1 and E3 acted as the cathode while E2 became the anode. Both stainless steel (type 316) inserts (I1 and I2) were placed between the electrodes in the electrical field and treated the same way as the electrodes

* Corresponding author. Mailing address: Center for Biofilm Engineering, Montana State University, Bozeman, MT 59717-0398. Phone: (406) 994-1960. Fax: (406) 994-6098.

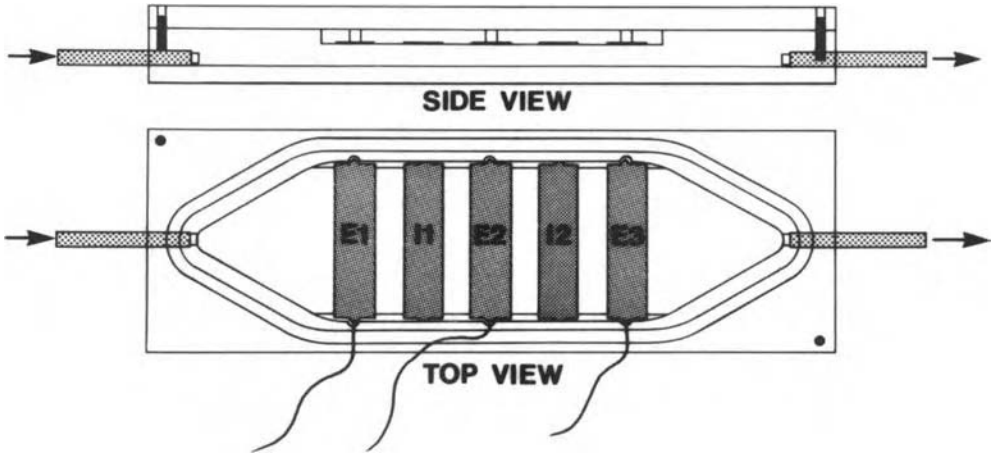

FIG. 1. Diagrammatic representation of the in vitro flow cell device showing the design of the Perspex flow chamber and the five type 316 stainless steel elements that compose the electrodes and the inserts. E1 and E3 are both connected to one pole of the power source and constitute one electrode, while E2 is connected to the opposite pole and constitutes the other electrode. I1 and I2 are not connected to the power source, and they constitute inserts within the system.

regarding microscopy and viable cell counts. The electrodes were connected to a direct current (DC) generator whose voltage output was adjustable up to 10 V, and the current was variable up to 50 mA. The current polarity was alternated every 64 s to help prevent the accretion of ions on the stainless steel surfaces.

Biofilm generation. Adherent, glycocalyx-enclosed biofilm populations were generated on the type 316 stainless steel (0.08% C, 2.00% Mn, 0.045% P, 0.03% S, 1.00% Si, 16.00 to 18.00% Cr, 10.00 to 14.00% Ni, 2.00 to 3.00% Mo; remaining percentage was Fe) elements of the flow cell. The flow cell experiments involving *P. aeruginosa* utilized the chemically defined simple salts medium M-56 [30.6 mM Na_2HPO_4, 19.8 mM KH_2PO_4, 7.57 mM $(NH_4)_2SO_4$, 10.0 mg of $MgSO_4 \cdot 7H_2O$ per liter, 1.00 mg of $Ca(NO_3)_2 \cdot 4H_2O$ per liter, 1.84 mg of $FeSO_4 \cdot 7H_2O$ per liter], which was supplemented with glucose (0.9% [vol/vol]) and L-leucine (0.066 g/liter). The resistivity of this medium was 3.86 mS/cm. Two liters of this medium was inoculated (2% [vol/vol]) with a culture of strain UR-21 which had been grown in M-56 supplemented with 1% brain heart infusion broth for 16 h in an orbital shaker (125 rpm at 37°C), and the medium was pumped through the flow cell at a rate of 60 ml/h with a peristaltic pump (Cole-Parmer, Chicago, Ill.). After 24 h of colonization, the flow was stopped and the culture fluid was replaced (time = 0) with fresh sterile medium with or without tobramycin sulfate (Sigma Chemical Co.) at a concentration of 5.0 mg/liter, because the planktonic MIC for the urinary tract infection isolate used in these studies was 1.0 mg/liter. The flow was then reestablished at a rate of 60 ml/h.

Sampling protocol. After 24 or 48 h of exposure to sterile medium with or without tobramycin and with or without the application of the DC electric field, the flow cell was dismantled. The electrodes and inserts were cut in half and the halves were processed separately for scanning electron microscopy and viable cell counts. For scanning electron microscopy, the electrodes were fixed in 5% glutaraldehyde (in 0.1 M cacodylate buffer, pH 7.0) overnight at 4°C, washed five times in cacodylate buffer, and air dried. The samples were coated with Au-Pd in a sputter coater and viewed with a Hitachi S450 electron microscope. For the determination of viable-cell counts, the biofilm on the other half of each electrode and insert was dispersed into 5 ml of phosphate-buffered saline (PBS) by aseptic scraping and by the application of low-power sonic energy (model 2200; Branson Ultrasonics Corporation) as outlined in previous publications (2, 3, 33, 39). Each suspension was vortexed and serially diluted in PBS prior to being plated in duplicate for the counting of CFUs. The brain heart infusion agar plates were incubated for 18 h at 37°C prior to counting.

RESULTS

The degree of biofilm formation by cells of *P. aeruginosa* on the stainless steel elements of the flow cell was seen to be much higher (Fig. 2) than that produced by the same organisms in our previous experiments (7, 26) using stainless steel studs in the modified Robbins device, and the number of sessile organisms increased still further in untreated control preparations (Fig. 2) during these 48-h experiments. Electrical treatment alone for 24 h produced a 500-fold decrease in the number of viable cells on the stainless steel elements of the flow cell, including the electrically passive inserts, but the number of viable cells in these biofilms returned to pretreatment levels during a further 24-h exposure to the electric field. Antibiotic treatment alone produced a gradual 100-fold decrease in the number of viable cells in the biofilms on the stainless steel elements of the flow cell, but 5×10^5 cells per cm^2 remained alive following this 48-h exposure. Treatment of these established *P. aeruginosa* biofilms with 5.0 times the MIC of tobramycin in the presence of the electric field produced an almost complete kill ($<10^2$ viable cells per cm^2) of these sessile cells in 48 h (Fig. 2). This level of kill of biofilm bacteria was found on the surfaces of all five steel elements (Fig. 1) of the flow cell (three electrodes and two electrically unconnected inserts). The field strength of this electric field was calculated to be 5 V/cm, and the current was measured at ±15 mA at the time of polarity reversal and at 6.0 to 6.7 mA in the stable period between alternations, giving a calculated average current density of 1.7 mA/cm^2 in this geometrically complex system. Because the biofilm was recovered from all areas of the stainless steel elements of these flow cells, for analysis for viable organisms, we can state that virtually no biofilm bacteria remained alive after 48 h of exposure to an antibiotic within this electric field.

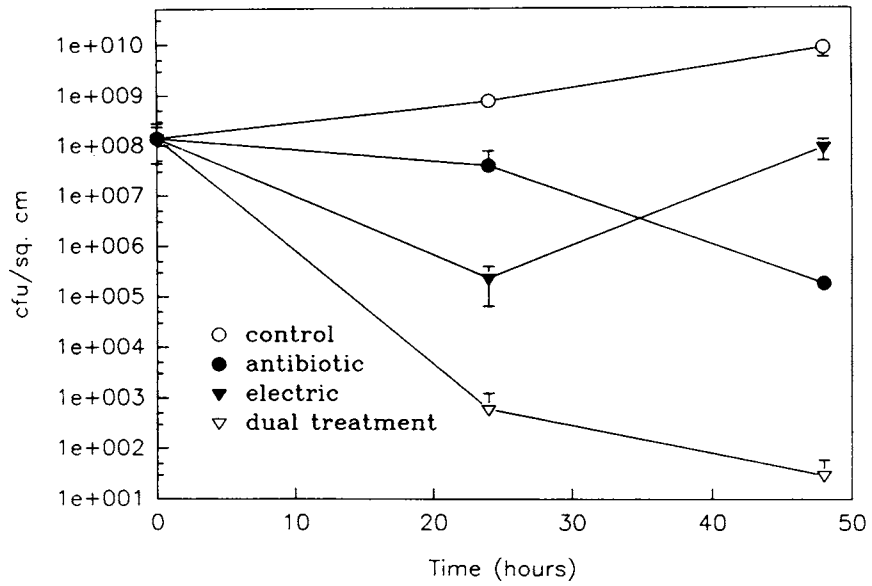

FIG. 2. Graphic representation of the average CFU of viable biofilm cells of *P. aeruginosa* per square centimeter on all five steel elements of the flow device in untreated-control experiments and following exposure to 5.0 times the MIC of tobramycin in the presence and absence of the electric field. Virtually all of the biofilm cells were killed by 5.0 times the MIC of tobramycin in the presence of the electric field, while significant numbers of viable cells remained following exposure to the antibiotic alone or to the electric current alone and the sessile population increased in the absence of any treatment.

The portions of the outer electrodes (Fig. 1) that did not face the center electrode would experience only very low current densities, and the actual values of current densities over the surfaces of these electrodes is very difficult to estimate. For this reason, the average current density was estimated by dividing the total current by the total area. The average current density at the central electrode will necessarily be twice that of the outer electrodes. It is of interest to calculate that the current density at the outer electrodes will diminish towards the extreme edges and will reach values of <100 $\mu A/cm^2$ in these regions that, nonetheless, experienced almost total killing of biofilm bacteria.

We examined the *P. aeruginosa* biofilms on the various steel elements of the flow cell (by scanning electron microscopy) before and after treatments to promote killing of these sessile organisms by the bioelectric effect. Following 24 h of colonization to produce biofilms with a cell density of 10^8 CFU/cm^2, and before any experimental protocols were initiated, all steel surfaces were covered by thick biofilms of slime-enclosed bacterial cells (Fig. 3A). After 48 h of treatment with the electric field alone, the biofilm in Fig. 3B, which still contained large numbers of viable bacteria (Fig. 2), was structurally intact and structurally similar to the untreated biofilm seen in Fig. 3A. Biofilms that had been treated for 48 h with the antibiotic alone also contained large numbers of viable bacteria and resembled the untreated biofilm in all structural parameters (Fig. 3C). However, treatment with tobramycin at 5.0 mg/liter (5.0 times the MIC) in the presence of the electric field killed virtually all of the biofilm bacteria on all five steel elements of the flow cell (Fig. 2), and examination of the electrode surfaces showed that the biofilm had been at least partially removed (Fig. 3D, area I) on the edges of the electrodes nearest the electrode of opposite polarity. Residual biofilm (Fig. 3D, area II) and intact bacterial cells (Fig. 3E) were clearly seen in areas of the electrodes and in areas of the inserts that were not subjected to maximum field strengths by close juxtaposition with the opposite electrode, but the cells remaining on the denuded areas were severely distorted and obviously cavitated (Fig. 3F).

We have noted that biofilm bacteria are not killed by the application of an electric field alone (7, 26) (Fig. 2) and that biofilm bacteria on electrically passive inserts within electric fields (Fig. 1) are killed by low concentrations of an antibiotic, which suggests that the penetration of the biofilm by the antibiotic is enhanced by an electric field that is not, in itself, damaging to biofilm bacteria. However, we felt that we should still examine the possibility that these sessile cells were being killed by the combined effect of the antibiotic and a molecule or ion (perhaps chlorine, peroxide, or superoxide) that is generated electrochemically at the electrode surface. To examine this possibility we undertook the series of experiments outlined in Table 1. Flow cells were colonized to produce biofilms on all five elements, as in the experiments whose results are summarized in Fig. 2, but the surviving bacteria were quantitated on each element individually in order to detect possible downstream effects caused by ion generation. In the untreated-control experiments no statistically significant differences in the numbers of viable biofilm bacteria were seen among the five elements (three electrodes and two inserts). Similarly, there were no significant differences among the elements when the flow cell was treated with the antibiotic alone (5.0 times the MIC of tobramycin), but the numbers on all elements were significantly different from the numbers in the untreated-control experiment, and a general 1-log reduction in the number of viable biofilm bacteria was recorded. When the electrodes of the flow cell were energized in the absence of the antibiotic, the number of surviving biofilm bacteria on the inserts was not significantly different from the number on the five elements of the untreated control, and the number on the energized electrodes was reduced less than 1 order of magnitude. We should note that any ions that had been electrochemically generated in this electric treatment had

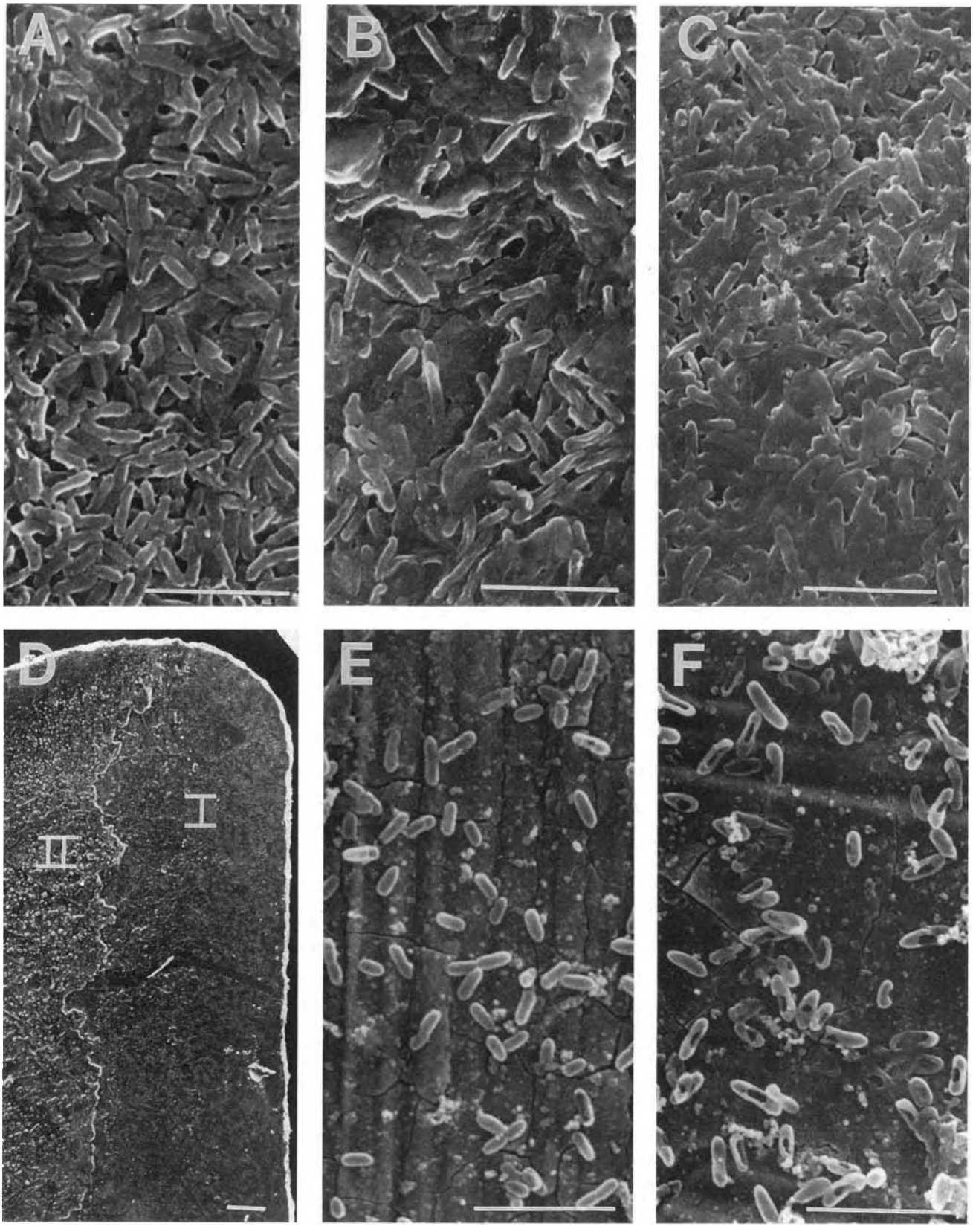
A
B
C
D
I
II
E
F

TABLE 1. Killing of biofilm bacteria on individual elements of the flow cell by various treatments[a]

Treatment	Avg CFU/cm^2 on element:				
	E1	I1	E2	I2	E3
None (control)	5.22×10^8	3.45×10^8	2.35×10^9	3.95×10^8	4.18×10^8
Antibiotic	1.96×10^7	2.29×10^7	2.35×10^7	1.51×10^7	3.74×10^7
Electric	8.07×10^7	2.17×10^8	6.54×10^7	2.25×10^8	3.02×10^7
Electric + antibiotic	1.65×10^2	1.55×10^3	6.71×10^2	8.00×10^2	5.90×10^2
Downstream antibiotic	1.76×10^6	6.76×10^5	1.16×10^6	1.28×10^6	2.19×10^6

[a] All elements were treated for 48 h, and all experiments were repeated four times except the downstream test, which was repeated five times. Statistics were done on a Macintosh computer by using the SuperANOVA program for one-way analysis of variance and multiple comparison of means by the Tukey-Kramer test ($\alpha = 0.05$). The analysis was performed on the log transformed data (i.e., CFU per square centimeter = log (CFU per square centimeter + 1).

very little effect on the viability of biofilm bacteria anywhere in the flow cell in the absence of the antibiotic. When the biofilm bacteria in the flow cell were exposed to 5 μg of tobramycin per ml in the presence of the DC field, the numbers of surviving biofilm bacteria were reduced to the same extent on both the electrodes and the inserts; these reductions were highly significant as determined by comparison of the numbers with those after any other treatment (control, antibiotic alone, or electrical field alone). We conducted downstream-antibiotic experiments in which a separate five-element flow cell was attached by tubing downstream from a flow cell being treated with an antibiotic in the presence of a DC field. We speculated that any electrically generated antibacterial ions would be carried to the downstream flow cell, with the antibiotic, and would enhance the killing of biofilm cells in the downstream flow cell. The data concerning the survival of biofilm bacteria in this downstream flow cell (Table 1) show a significant but minor (1-log) reduction compared with data on exposure to the antibiotic alone (Table 1), and we conclude that the effects of any electrochemically generated species were minor.

Next we reasoned that any electrochemically generated ions that might enhance the efficacy of an antibiotic against biofilm bacteria would be carried downstream within a single flow cell, so the upstream side of E1 (Fig. 1) would accumulate only very low concentrations. The downstream elements (I2 and E3) would, by the same process, be exposed to higher concentrations of this putative ion. The data concerning the killing of biofilm bacteria in electric and electric-antibiotic treatments in Table 1 indicate no asymmetry in the killing of these sessile bacteria on the upstream and downstream elements of the flow cell. To examine this hypothesis still further, we conducted an experiment to examine the extent of killing of biofilm bacteria on individual elements of a flow cell in which only electrodes E1 and E2 were connected to the electrical generator. Element E3 must be considered outside the DC field. In this experiment, the flow cells were colonized for 24 h and then treated for 24 h with 5 μg of tobramycin per ml, with only the first two electrodes connected to the power supply (5 V, 64-s reversal of polarity). The biofilm kill data clearly indicate that these adherent organisms are killed more rapidly on elements E1 and E2 and significantly more slowly on elements I1 and I2 in this 24-h period. Elements E1, I1, E2, I2, and E3 had 4.92×10^4, 2.24×10^5, 3.87×10^4, 2.44×10^5, and 3.43×10^6 CFU/cm^2, respectively; data are the averages of five experiments whose results were analyzed by the SuperANOVA program as for Table 1. There is no significant difference between the first four elements in the group; however, E3, which was electrically passive in this experiment, had a number of adherent organisms significantly different from that of any other single element within the group. These data indicate that any electrochemically generated ions have only a minor effect on the enhancement of the killing of biofilm bacteria by antibiotics within a DC field.

DISCUSSION

Relative to biofilm bacteria, planktonic bacteria can be exquisitely susceptible to biocides and antibiotics, if they lack resistance mechanisms and contain the appropriate target molecules, because they float or swim in the bulk fluid and are readily accessed by these antibacterial agents. Biofilm bacteria are often found to be involved in industrial problems (39) or in medical infections (21, 33) that have resisted clearance by biocides or antibiotics, and many reports (8, 16, 17, 21, 33, 39) have documented their inherent resistance to these agents, even at concentrations 500 to 5,000 times those needed to kill planktonic cells. This inherent resistance of biofilm bacteria to antibiotics is now widely accepted (12, 21) as the basis for the remarkably refractory response of device-related and other chronic bacterial infections to antibiotic chemotherapy. A vigorous debate concerning the mechanism of this inherent resistance of biofilm bacteria to antibiotics currently rages, and it now seems clear that the resistance is a function of both altered growth rate and physiology (8, 9, 16, 17, 32) and of diffusion barriers provided by the biofilm matrix (12, 21, 25).

Against the background of this inherent resistance of biofilm bacteria to antibacterial agents, it is significant that we have recently shown that the efficacy of biocides (7) and antibiotics (26) in killing biofilm bacteria can be radically enhanced if these agents are used within a low-intensity DC electric field.

FIG. 3. Scanning electron micrographs of the surfaces of various biofilm-colonized steel elements of the flow cell illustrated in Fig. 1. (A) Surface of the biofilm in an untreated device at 0 h (i.e., after 24 h of colonization), when this biofilm contained large numbers of living bacteria (Fig. 2). (B and C) Surfaces of the biofilms on electrode (E2) surfaces when the devices had been treated with the electric field alone (B) or with the antibiotic (tobramycin) alone (C); both biofilms contained substantial numbers of viable bacteria. (D) Surface of an electrode (E1) from a device that had been treated with tobramycin (5.0 times the MIC) in the presence of the electric field; we noted the partial removal of the biofilm from the edge of this electrode (area I) nearest to electrode E2 and its retention in the area (area II) more distant from the electrode of opposite polarity (E2). There were no living bacteria on the surface of this electrode, but visually intact bacterial cells were seen (E) in area II, while severely disrupted and cavitated cells were seen (F) on the denuded surface of area I. Bars = 5 μm.

This bioelectric effect has been shown to operate in the killing of biofilm cells of several species of gram-negative and gram-positive bacteria, and of fungi, by several different chemical classes of biocides and antibiotics (7, 26). Because only a DC electric field produces the bioelectric effect, and because the electric field does not by itself kill biofilm bacteria, we have evolved a working hypothesis that this effect depends largely on electrophoretic forces that allow the antimicrobial agents to overcome diffusion barriers that would otherwise limit their access to their targets within bacterial and fungal cells. Similar DC currents have been used clinically to drive chemotherapeutic molecules into solid tumors (29) and antibiotic molecules into the inner ear (11) and other tissues (5).

However, there are many ways in which physical forces affect biochemical processes in biological systems. Bacterial cells depend, as do all living cells, on physical phenomena such as membrane potentials (10) for their basic metabolic activity, and it is reasonable to expect that delicate cellular electrical equilibria may be disturbed by the imposition of an external electric field. It has been shown that external fields can affect the α-helix content (28, 42) and orientation (10) of membrane proteins in eukaryotic cells and the electrophoretic mobilities of bacterial membrane proteins (10, 20). Electric fields can even be used to effect the electroinsertion of specific proteins into the membranes of living cells (30). These molecular perturbations of important membrane components may affect the organization of membranes (10, 28, 31, 35, 40, 42), and we expect that these structural changes would influence the permeability of membranes vis-à-vis antibiotics and biocides (22, 23). Profound membrane perturbations, caused by very intense electric fields, are routinely used in electroporation to facilitate exogenous-DNA exchange in genetic experiments (31).

Davis and his colleagues (13–15) have reported that planktonic cells of *E. coli*, *P. aeruginosa*, *Proteus mirabilis*, and *Candida albicans* can be killed by electric fields and current densities similar to ours but without the use of antibiotics. Davis et al. (13, 15) attributed the killing of these planktonic cells to iontophoresis, in which the accretion of metal ions on or in the bacterial cell could be responsible for this bactericidal effect, or the effect could be caused by the electrical generation of chloride species (14) with biocidal properties. The low-strength electric fields used in the present study did not, by themselves, kill biofilm bacteria, and we suggest that ion binding by the exopolysaccharide matrix of the biofilm (12) may protect these sessile cells from iontophoretic killing.

In the present study of the bioelectric effect, we have set out to examine the extent to which electrochemical effects at the electrode surface contribute to the enhanced efficacy of antibacterial agents. Any putative electrochemical mechanism must only contribute to the bioelectric effect because the electric fields used in these experiments do not, in themselves, kill biofilm bacteria. To examine this phenomenon in a rational manner, we designed the flow cell illustrated in Fig. 1 so that the stainless steel electrodes (E1, E2, and E3) would constitute a symmetrical field and the electrically passive steel inserts (I1 and I2) would be placed symmetrically within that field. Our data clearly show that biofilm bacteria are killed on all steel surfaces within the flow cell in the presence of an antibiotic, including those bacteria that were not on electrodes and those that were adherent to the outside edges of the outer electrodes that would be subjected to only very low current densities. Biofilm cells were seen to be cavitated, and biofilms were partially removed on the inside edges of electrodes facing the oppositely charged electrodes, where the current density would be highest, while no obvious cell damage or biofilm disturbance was seen in areas that would be subjected to lower current densities. However, virtually all biofilm bacteria were dead on all of the steel surfaces of the flow cell after 48 h of exposure to 5.0 times the MIC of tobramycin within the electric field. Because very low levels of current density were effective, and because nonelectrode surfaces were affected, we feel that electrochemical effects were not of paramount importance in the bioelectric effect in this system. If an electrochemically generated molecule or ion were an essential contributor to the bioelectric effect, one would expect that it might be carried some distance downstream in an actively flowing system. Our flow experiments show that the bioelectric effect is seen on the element (I2) immediately downstream of an active electrode in an asymmetrically electrified flow cell but that it is seen only to a minor extent on the next element (E3) downstream or in a separate downstream flow cell connected to the electrically active flow cell. In light of these data, electrically assisted electrophoresis remains our preferred explanation of the bioelectric effect with some suggestion of an electrochemically generated agent that enhances killing by antibiotics.

In our plans to use the bioelectric effect to prevent and to treat device-related bacterial infections, we are encouraged by these observations that relatively weak electric fields enhance the efficacy of antibiotics in killing biofilm bacteria even on surfaces that are not, in themselves, electrodes. We are further encouraged by evidence that, at the field strengths used in this study, the effect is not largely dependent on potentially damaging electrochemically generated ionic or molecular species.

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REFERENCES

1. **Amory, D. E., and J. E. Dufey.** 1985. Model for the electrolytic environment and electrostatic properties of biomembranes. J. Bioenerg. Biomembr. **17**:151–174.
2. **Anwar, H., and J. W. Costerton.** 1990. Enhanced activity of combination of tobramycin and piperacillin for eradication of sessile biofilm cells of *Pseudomonas aeruginosa*. Antimicrob. Agents Chemother. **34**:1666–1671.
3. **Anwar, H., M. K. Dasgupta, and J. W. Costerton.** 1990. Testing the susceptibility of bacteria in biofilms to antibacterial agents. Antimicrob. Agents Chemother. **34**:2043–2046.
4. **Applegate, D. H., and J. D. Bryers.** 1990. Effects of carbon and oxygen limitations and calcium concentrations on biofilm removal processes. Biotechnol. Bioeng. **37**:17–25.
5. **Belehradek, J., S. Orlowski, L. H. Ramirez, G. Pron, B. Poddevin, and C. M. Mir.** 1994. Electropermeabilization of cells in tissues assessed by the qualitative and quantitative electroloading of bleomycin. Biochim. Biophys. Acta **1190**:155–163.
6. **Berger, T. J., J. A. Spadaro, S. E. Chapin, and R. O. Becker.** 1976. Electrically generated silver ions: quantitative effects on bacterial and mammalian cells. Antimicrob. Agents Chemother. **9**:357–358.
7. **Blenkinsopp, S. A., A. E. Khoury, and J. W. Costerton.** 1992. Electrical enhancement of biocide efficacy against *Pseudomonas aeruginosa* biofilms. Appl. Environ. Microbiol. **58**:3770–3773.
8. **Brown, M. R. W., D. G. Allison, and P. Gilbert.** 1988. Resistance of bacterial biofilms to antibiotics: a growth-related effect? J. Antimicrob. Chemother. **22**:777–783.
9. **Brown, M. R. W., P. J. Collier, and P. Gilbert.** 1990. Influence of growth rate on susceptibility to antimicrobial agents: modification of the cell envelope and batch and continuous culture studies. Antimicrob. Agents Chemother. **34**:1623–1628.
10. **Cevc, G.** 1990. Membrane electrostatics. Biochim. Biophys. Acta **1031**:311–381.
11. **Chang, I. W., Y. B. Cho, and S. C. Lim.** 1989. Iontophoretic application of cefotiam hydrochloride into inner ear through round window membrane. Chonnam J. Med. Sci. **2**:141–144.

12. **Costerton, J. W., K.-J. Cheng, G. G. Geesey, T. I. Ladd, J. C. Nickel, M. Dasgupta, and T. J. Marrie.** 1987. Bacterial biofilms in nature and disease. Annu. Rev. Microbiol. **41:**435–464.
13. **Davis, C. P., N. Wagle, M. D. Anderson, and M. M. Warren.** 1991. Bacterial and fungal killing by iontophoresis with long-lived electrodes. Antimicrob. Agents Chemother. **35:**2131–2134.
14. **Davis, C. P., N. Wagle, M. D. Anderson, and M. M. Warren.** 1992. Iontophoresis generates an antimicrobial effect that remains after iontophoresis ceases. Antimicrob. Agents Chemother. **36:**2552–2555.
15. **Davis, C. P., S. Weinberg, M. D. Anderson, G. M. Rao, and M. M. Warren.** 1989. Effects of microamperage, medium, and bacterial concentration on iontophoretic killing of bacteria in fluid. Antimicrob. Agents Chemother. **33:**442–447.
16. **Eng, R. H. K., F. T. Padberg, S. M. Smith, E. N. Tan, and C. E. Cherubin.** 1991. Bactericidal effects of antibiotics on slowly growing and nongrowing bacteria. Antimicrob. Agents Chemother. **35:**1824–1828.
17. **Gilbert, P., M. R. W. Brown, and P. J. Collier.** 1990. Influence of growth rate on susceptibility to antimicrobial agents: biofilms, cell cycle, dormancy, and stringent response. Antimicrob. Agents Chemother. **34:**1865–1868.
18. **Glaser, R. W., S. L. Leikin, L. V. Chernomordik, V. F. Pastushenko, and A. J. Sokirko.** 1988. Reversible electrical breakdown of lipid bilayers: formation and evolution of pores. Biochim. Biophys. Acta **940:**275–287.
19. **Goodman, R., C. A. L. Bassett, and A. S. Henderson.** 1983. Pulsing electromagnetic fields induce cellular transcription. Science **220:**1283–1285.
20. **Gowrishankar, T. R., R. C. Lee, P. K. Patel, and D. E. Golan.** 1991. Cell shape dependent rectification of surface receptor transport in sinusoidal a.c. electric fields, p. 32. Abstr. 11th Annu. Meet. Bioelectric Repair and Growth Society 1991.
21. **Gristina, A. G., J. J. Dobbins, B. Giammara, J. C. Lewis, and W. C. DeVries.** 1988. Biomaterial centered sepsis and the total artificial heart: microbial adhesion vs. tissue integration. JAMA **259:**870–877.
22. **Hancock, R. E. W.** 1984. Alterations in outer membrane permeability. Annu. Rev. Microbiol. **38:**237–264.
23. **Hancock, R. E. W.** 1991. Bacterial outer membranes: evolving concepts. ASM News **57:**175–182.
24. **Harrington, D. B., R. Meyer, Jr., and R. M. Klein.** 1975. Effects of small amounts of electric current at the cellular level. Ann. N. Y. Acad. Sci. **247:**300–305.
25. **Hoyle, B. D., J. Alcantara, and J. W. Costerton.** 1992. *Pseudomonas aeruginosa* biofilm as a diffusion barrier to piperacillin. Antimicrob. Agents Chemother. **36:**2054–2056.
26. **Khoury, A. E., K. Lam, B. D. Ellis, and J. W. Costerton.** 1992. Prevention and control of bacterial infections associated with medical devices. Am. Soc. Artif. Intern. Org. J. **38:**M174–M178.
27. **Levy, S. B., J. P. Burke, and C. K. Wallace (ed.).** 1987. Antibiotic use and antibiotic resistance worldwide. Report of a study sponsored by the Fogarty International Center of the National Institutes of Health, 1983–1986. Rev. Infect. Dis. **9:**S231–S316.
28. **McLaughlin, S.** 1989. The electrostatic properties of membranes. Annu. Rev. Biophys. Biophys. Chem. **18:**113–136.
29. **Miklavcic, D., G. Sersa, S. Novakovic, and S. Rebersek.** 1990. Tumor bioelectric potential and its possible exploitation for tumor growth retardation. J. Bioelectr. **9(2):**133–150.
30. **Mouneimne, Y., P.-F. Tosi, R. Barhoumi, and C. Nicolau.** 1991. Electroinsertion of xeno proteins in red blood cell membranes yields a long lived protein carrier in circulation. Biochim. Biophys. Acta **1066:**83–89.
31. **Neumann, E., A. E. Sowers, and C. A. Jordan (ed.).** 1989. Electroporation and electrofusion in cell biology. Plenum Press, New York.
32. **Nichols, W. W., M. J. Evans, M. P. E. Slack, and H. L. Walmsley.** 1989. The penetration of antibiotics into aggregates of mucoid and non-mucoid *Pseudomonas aeruginosa*. J. Gen. Microbiol. **135:**1291–1303.
33. **Nickel, J. C., I. Ruseska, J. B. Wright, and J. W. Costerton.** 1985. Tobramycin resistance of *Pseudomonas aeruginosa* cells growing as a biofilm on urinary catheter material. Antimicrob. Agents Chemother. **27:**619–624.
34. **Nuccitelli, R.** 1990. Vibrating probe for studies of ion transport, p. 273–310. *In* J. K. Foskett and S. Grinstein (ed.), Noninvasive techniques in cell biology. Wiley-Liss, New York.
35. **Pethica, B. A., and D. G. Hall.** 1982. Electric field effects on membranes. J. Colloid Interface Sci. **85:**41–51.
36. **Rajnicek, A. M., C. D. McCaig, and N. A. R. Gow.** 1994. Electric fields induce curved growth of *Enterobacter cloacae*, *Escherichia coli*, and *Bacillus subtilis* cells: implications for mechanisms of galvanotropism and bacterial growth. J. Bacteriol. **176:**702–713.
37. **Root, R. K., D. D. Trunkey, and M. A. Sande (ed.).** 1987. New surgical and medical approaches in infectious diseases. Churchill Livingstone, New York.
38. **Rowley, B. A., J. M. McKenna, and G. R. Chase.** 1975. The influence of electrical current on an infecting microorganism in wounds. Ann. N. Y. Acad. Sci. **247:**543–551.
39. **Ruseska, I., J. Robbins, J. W. Costerton, and E. S. Lashen.** 1982. Biocide testing against corrosion-causing oil field bacteria helps control plugging. Oil Gas J. **8:**253–264.
40. **Sabelnikov, A. G., E. S. Cymbalyuk, G. Gongadze, and V. L. Borovyagin.** 1991. *Escherichia coli* membranes during electrotransformation: an electron microscope study. Biochim. Biophys. Acta **1066:**22–28.
41. **Shi, W., M. J. Lentz, and J. Adler.** 1993. Behavioral responses of *Escherichia coli* to changes in temperature caused by electric shock. J. Bacteriol. **175:**5785–5790.
42. **Tsong, T. Y.** 1990. Electrical modulation of membrane proteins: enforced conformational oscillations and biological energy. Annu. Rev. Biophys. Biophys. Chem. **19:**83–106.

Trip Report

Bone Anchored Amputation Prostheses
3rd and 4th of September 2008

Centre of Orthopaedic Osseointegration
Sahlgrenska University Hospital
Gothenburg, Sweden

History of Osseointegration: Rickard Branemark

Osseointegration (OI) is defined as the intimate attachment of bone to a Titanium (Ti) implant surface without fibrous ingrowth or foreign body reaction. Branemark feels that this attachment is at the nanometer surface level and demonstrated the direct attachment of a stem cell to a Ti surface with scanning electron microscopy (SEM).

He postulates that motion of an implant, in bone, results in inflammation and fibrous tissue formation at the interface. Thus, he feels that too early weight bearing ambulation will produce motion at the implant/bone interface and result in failure of attachment with formation of a fibrous tissue barrier. This theory is exemplified in his 6 month clinical interval, after first stage fixture implantation, before any external prosthesis is attached to the bone and patient weight bearing begun. His paradigm is directly translated from his father's pioneering work on dental implants. He does not deny the possibility that almost immediate weight bearing, on a securely implanted prosthetic attachment, may allow OI and thus lessen the rehabilitation time but he emphasized that his system works and he does not want to diverge from a successful technique.

He emphasized taking care not to disrupt normal biological functions. ("Don't kick around biology.") To avoid generating heat, during reaming of the medullary canal, all reaming was done by hand and interrupted periodically to allow cooling of the reamer and the bone. Power tools were rigorously avoided both in preparing the canal and in inserting the primary "fixture". In dental and amputation applications disuse results in osteoporosis and atrophy and resultant loss of boney tissue. In spite of this he felt that "biology must heal into biomaterial" and that by delaying force applied to the interface he can achieve 100% as opposed to 85% success of OI. ("Wait for biology to react in both osteopenic and good bone".)

He mentioned evaluation of the attachment by resonance frequency analysis and the PhD Thesis by L. Sennerby (investigator) in this area. The bone anchored hearing aid (BAHA) is another application of OI and allows bone transmission of sound. BAHA works much like the phenomenon of "osseoperception," with transcutaneous bone anchored prostheses, that allows the patient to perceive irregularities on the surface beneath the prosthetic foot.

Overview of the Surgical Procedure for the Upper and Lower Extremity: Orjan Berlin

Patient selection criteria are based on those that would allow good wound healing and also includes psychological testing. An attempt is made to find those patients who are likely to be compliant and those with reasonable expectations and a full awareness of the attendant complications. Bone is evaluated for osteopenia and osteoporosis. Metabolic bone disease patients, i.e. renal osteodystrophy etc., would be excluded. Patients with diabetes mellitus, those on corticosteroids and those post chemotherapy as well as smokers are considered for exclusion.

There are three components of the Branemark OI device. The "fixture" is the component that is implanted into the medullary canal during the first stage procedure. It is 8cm long and 16mm to 20mm in diameter increasing in 0.5mm increments. The fixture is completely circumferentially threaded. It is made of pure Ti. The appropriate size is determined with X-Ray and CT imaging. It is placed in the diaphyseal bone 2cm deep to the end of the bone. "Funnel shaped" anatomy must be avoided, at either end of the long bone, therefore metaphyseal flare regions are excluded from levels suitable for implantation (although not so rigorously at the proximal bone ends). In the femur, this requires distal resection 18 cm up from the knee joint line. This 18cm also allows room for the external prosthesis resulting in parallel and level knee joint axes. In the process however, this technique can eliminate a bone transaction level completely distal to the adductor magnus insertion with a resultant loss of thigh adduction power. Some soft tissue revision can be done during Stage I but most skin revision and myodesis and tenodesis are reserved for the Stage II procedure. After the skin has healed from Stage I the patient is allowed to wear the conventional socket prosthesis for six weeks until the Stage II procedure.

At the Stage II operation the "abutment" is placed. It also is made of pure Ti and is screwed into the fixture with a Ti alloy screw. This configuration allows the abutment, in the event of a sudden catastrophic overload, to break before the shaft of the bone or the "fixture." The "Rotasafe" is a second overload protection device that joins the external prosthesis to the intramedullary stem. It works, as the name implies, in rotation and not in laterally applied stress and strain moments. All patients, at some point, break their "abutments" but they are easily replaced, without anesthesia and in an outpatient setting.

Soft tissue revision, including removing any redundant skin and subcutaneous fat, is done during the second procedure. Myodesis and tenodesis are done and the skin is attached to the bone end. This involves scraping the skin down to dermis as in preparing a "full thickness" skin graft. A 3 cm diameter circle of skin is prepared and the skin is sutured to the bone. No muscle is left over the end of the bone thus myodesis involves attaching the muscle to the periosteal cortical surface. After wound closure the skin is held against the end of the bone with a pressure dressing ("hockey puck") as well as the deep sutures.

Deep vacuum wound drains are placed. The "hockey puck" dressing is left on for 3 to 4 days before changing and the patient is kept in the hospital for 10 days or until the skin is seen to be attached to the bone end.

All bone work is done, as mentioned, without power and a jig is clamped to the shaft of the bone to allow finding and maintaining the center of the canal thus assuring an equal amount of bone to be reamed circumferentially. This jig is also used to screw in the fixture. It does not accommodate the anterior femoral bow. Dr Branemark has not yet developed "bone taps" but is thinking about doing so.

Rehabilitation for upper and lower extremity
Prosthetics, Physiotherapy and Occupational Therapy:

Kirsten Hagberg:

The lower extremity rehabilitation regimen involves very gradual and protected increases in weight bearing and activity. It has been developed to be easily followed by the patients, is carefully individualized and must be able to be done at home. Weight bearing is begun on a short prosthesis, 4 to 6 weeks following the Stage II procedure. Twenty kilograms of weight bearing is allowed twice daily for 30 minutes and is born on a bathroom scale to measure the precise amount of weight applied. Activity is progressed based upon the Visual Analogue Pain Score (VAS) 0=no pain to 10=unbearable pain. Pain experienced, during increasing weight bearing, is graded and a VAS of 0 through 3 is safe, 3 to 5 is acceptable; above 5 requires rest. Weight is increased by 10 Kg/week until full weight bearing is achieved. Exercises on all four extremities as well as TheraBand resistive muscle strengthening (extension, abduction and adduction) for motors across proximal joints are included in the program. In addition active and passive range of motion of proximal joints is done on a mat each day. If pain >5 occurs the protocol is dropped to half speed. Under normal conditions rehabilitation is completed in 6 months following Stage II.

The half speed protocol takes 10 to 12 months after Stage II. Later training can include cycling, rowing on an erg machine and walking with crutches. All patients with high bilateral above knee amputations, except one, have required crutches to ambulate for the rest of their lives. See Appendix #1

Eva Haggstrom:

Heterotopic bone is not disadvantageous as it stabilizes the skin and soft tissues to the bone. The quality of bone is much softer in the upper extremity. Rehabilitation is much quicker in the upper because it is not weight bearing.

Many different external prostheses have been and can be used. The C-leg seems the most commonly used prosthesis. Rehabilitation is begun with a short prosthesis and this can also be used later for near ground activities such as gardening.

Soft tissue support is accomplished with a plastic cap on the abutment when in bed and with a "donut" between the abutment and the external prosthesis when ambulating.

Exoprostheses also have torsion adapters and shock absorbers built into the devices. These cushion the loading and prevent breakage in torsion. The Rotasafe attaches directly to the abutment with an "Allen screw" mechanism. When training begins it is adjusted to release when 13 to 14 newton/meters of torsional force are applied. When fully osseointegrated it may be adjusted to release at 30 newton/meters. The Rotasafe releases in clicks and rotates 20 degrees per click. With a strong continual force it may rotate through 360 degrees and cause a fall.

Cosmetic covers can be used and a pad with a dressing is used when swimming

See Appendix #2.

Stewe Jonsson:

Standard thumb amputation prostheses act as a post to give opposition but do not allow thumb positioning as in OI thumbs. OI gives a new suspension platform. After S1 procedure, when there is no edema, a standard cosmetic prosthesis may be worn. After S2 in thumb and forearm prostheses a cosmetic or lightweight myoelectric prosthesis is used. When the OI has fully matured they progress to a myoelectric hand.

BE amputations require fixtures in both the radius and the ulna and custom prostheses must be constructed to accommodate for the varying degrees of, and the convergence of, the axes of rotation of the fixtures. A "PUCK" is made that allows a constant distance between the fixtures and the exoprosthesis is attached to this plastic disc. This system allows quick donning and doffing and is strong and readily kept clean.

At the transhumeral level, after the S2 procedure, weight bearing and **weight traction** devices are added to the abutment. Distal caps and soft tissue supports are also used. (This is further explained in greater detail in the next section.)

Care is taken to maintain motion in the proximal joints.

Their series includes about 30 upper limb cases. The levels include finger/thumb, partial hand, transradial (both short and long) and transhumeral also both short and long. The causes of loss of limb include, trauma, tumor and congenital dysmelia. Upper extremity exoprostheses include cosmetic, myoelectric, multifunctional, body powered and hybrid types thus illustrating the versatility of OI attachment. See Appendix #3.

Kerstin Caine-Winterberger:

After the S1 transhumeral procedure, gradual range of motion is begun immediately with internal and external rotation of the shoulder at 3 weeks. Shoulder girdle strengthening begins at 6 weeks and resistive exercises, with Theraband, are begun at 8 weeks.

After the S2 procedure, with abutment placement, the skin is allowed to heal onto the bone and only gentle range of motion exercises are done during this period. At about 3 to 4 weeks post S2, a short training prosthesis is applied. This device allows traction weights to be added beginning with 100 grams and adding 100 grams each week. Axial loading of 5 kg on a bathroom scale is begun at this time, twice daily, and this increases at a rate of 1 kg/week. The VAS pain scores are used as guides to regulate the speed of the individualized rehabilitation.

At 6 weeks post S2, floor exercises are begun 3 to 4 times per week.

At 12 weeks post S2, a cosmetic prosthesis can be applied but the traction weights must be increased until the weight of the proposed final prosthesis can be achieved without incurring pain. Care is taken to avoid rotational overloads. The final functioning prosthesis is implemented at various times based upon individual judgment.

See Appendix #4.

Current Osseointegration Research:

Basic Research- Peter Thomsen:

Rehabilitation proceeds slowly because it is impossible to accurately determine the progress of osseointegration. Thus, the tendency is to err on the conservative side of increasing weight bearing in an attempt to avoid loosening and failure.

They are investigating resonance frequency analysis of OI in amputations. This technique has been used in dental applications. There are spin-off companies doing this in Goteborg, Sweden.

He reiterated that the soft tissue seal is the key to avoiding infection.

In the process of osseointegration, motion produces cellular signals to the undifferentiated mesenchymal cells. These cellular signals influence differentiation and the formation of collagen rather than bone. He described this process in a Ti window placed in the tibia of a rabbit. He feels that the Ti implant is fixed directly to bone without intermediate cartilage because there is no motion and that motion is the enemy. My question, is perhaps force without motion the friend?

His vision for the future in this field includes:

1) An intelligent screw that gives off signals to the bone.
2) Individual fitting of intramedullay fixtures.
3) Rapid prototyping to build the actual prostheses, not just a plastic prototype.
4) Using not only inert materials but bioactive materials.
5) Using combinations of materials, cells and biological signals. This involves moving from implants alone to implants+cells+growth factors

Using biomaterials such as an ultra-thin coating of hafnium and determining tissue response to hafnium at the nano level with cellular attachment. He quoted a work done by Mohammedi et al, Tissue response to hafnium, J Mater Sci Mater Med 2001;12:603-611

Microfabrication: An array of laser-ablated Ti (Ti6A14V) causes a change in the phenotype of cells. He relates a "biohelix" which is basically a screw with a laser ablated structure at the bottom of the grooves of the screw helix that allows nano attachment. Also he has a laser treated Ti surface that shows surface roughness at both the micro (10 to the minus 6) and the nano (10 to the minus 9) scales and that these scale differences produce different cellular responses.

He can also produce nanocrystalline apatite that apparently changes the cellular response based upon the nanostructure.

He is using focused ion beam microscopy that shows the first intact metal/bone interface, eliminating artifact.

He is trying to modulate biological response with growth factors and cytokines, tissue engineering and stem cell and autologous cell therapy. He is interested in trying various combinations of growth factors, cytokines, extracellular matrix components and endothelial cells.

He feels they have the main center for osseointegration in the world and he is interested in collaboration both within Sweden and apparently outside of Sweden.

Through the Chalmers Technology Institute, part of the University of Goteborg, he has many basic science folks…physicists, chemists, engineers etc.

Bjorn Rydevik:

He described the Sahlgrenska along with Chalmers both funded by the founders of the Swedish East India Company. They have 80 orthopaedists and 25 residents. They are doing basic science, molecular cell biology and have the largest THR registry in the world. They are using radiostereoanalysis to determine hip component position.

They are collaborating with U Cal SD, U Cal SF, U Miami, Dartmouth and Mass General. They will hold SICOT 8/30 to 9/5/2010. There will be a special conference on osseointegration (COO) next spring in San Francisco.

Kerstin Hageberg: The OPRA Study

The OPRA study is a prospective clinical study, the acronym meaning, Osseointegrated Prosthesis for the Rehabilitation of Amputees. It began in 1999, with assessments of trans-femoral amputees beginning prior to the S1 procedure and until 2 years following S2. There were 51 patients in the study and 55 implants (4 treated bilaterally) at inclusion. The first 18 patients to reach the two year benchmark have been isolated and analyzed. The remainder will be reported on in 2010.

The graphic results of the 18 patient portion of the study can be seen in the handout.

Objective measurements are X-Rays, registration of complications, hip range of motion, energy cost (PCI) during gait, gait analysis and vibrometric analysis. Subjective measurements are General and Specific Health Related Quality of Life (HRQL)

In summary the prosthetic use score, mobility score and global score are all significantly improved while the problem score is decreased. "Individuals treated with the OI prostheses report significant improvements in both general and condition specific HRQL at two years following the surgery. For one of the 18 patients the treatment was a failure due to loosening of the implant."

OPRA STUDY TODAY

The larger OPRA study at the time of the meeting: thirty six patients have passed the 2-year follow-up. 3 implants have been removed. The plan is to continue following the patients at 3, 5, 7, 10 and 15 years.

This is basically incomplete information without enough detail. If you do well you do well if you don't you are in the 7/39 infection rate. (See infections below and See Appendix #5)

Lars Hageberg: Infections.

His handout summarizes his findings and should be read.

Basically, they did a cross-sectional study of bacterial colonization, infection frequency and antibiotic treatment on transhumeral, transfemoral, forearm and tibial amputees during a 6 month period and then repeated the study on the same cohort 2-3 years later. Patients were rated as definite, possible, probable implant infection, local soft tissue infection or no infection with or without bacterial colonization at the interface. There were 39 patients with 45 implants with a mean implant time of 56 months (range 3-132) at the beginning of the study. At the initial evaluation there were 3 implant infections and at follow-up 8. Five initially had local infection and 11 had local infection at 3 years. Initially 24 had superficial colonization and 13 at follow up. Seven had no initial infection and negative culture and 4 were negative at follow up. Two patients initially had draining fistulae and these continued to drain but they also continued to use the prosthesis and this was considered a satisfactory situation. The most common microbe was S aureus but coagulase negative Staphylococci, Sterptococcus A, B, G, Enterococcus, gram negative rods and alfa Streptococcus were also cultured. Eleven patients had 2 bacterial species cultured and one had 3 species. One half of the patients had secretions whether or not they cultured bacteria or were infected. As best I can determine they had between 7.5% and 20% clinical infection rate, depending upon the time of evaluation.. Final 7/39= 17%

Their conclusion was there were "few severe infectious complications"..associated with the technique. Those who had poor osseointegration from the beginning, with motion, were most likely to become infected. They have not removed any good OI implants for infection. Their infection control strategy involves flossing with clean water or with saline twice daily and brushing their teeth twice each day.

The antibiotics used to treat the infections, seen at last visit, were based upon culture results and included:

1) Chronic Clindamycin and Rifampicin for S aureus and coag-Staph
2) Six months of Ciprofloxacin for E. coli and coag-Staph
3) Revision and Ticarcillin for S aureus and Group Strep
4) Long term Clindamycin for coag-Staph
5) Long term Clindamycin for Group B Strep and Proteus
6) No treatment for S aureus
7) No treatment chronic fistula
8) No treatment chronic fistula.

When infections occurred for the first time 18 months from the operation they were considered to be of hematogenous origin. In 1995 they had to do one reamputation to control infection. (See Appendix #6)

Orjan Berlin: Surgical Indications

First evaluated by the team based upon clinical information, X-Ray and CT and indications:

1) Problems with socket technology.
2) Full grown.
3) Normal anatomy.
4) <70 years old.
5) <100kg.
6) No diabetes mellitus or arteriosclerotic peripheral vascular disease.
7) No skin diseases.
8) No corticosteroid or chemotherapy.
9) Not pregnant.
10) No drugs- NSAID's ASA bisphosphonates.
11) May take Ca++, Vitamin C, Vitamin D
12) Compliant

They must all agree that the patient is compliant, has reasonable expectations and is aware of the risks of mechanical loosening, infection, metal fatigue of the fixture and the abutment.

Improvement can be expected in sitting, all day prosthetic use, no sweating and increased hip motion. No improvement is expected in phantom pain and discarding crutches (in bilateral amputations)

After all this the patient is sent home to contemplate the whole process for 2 weeks and then a decision is made.

Rickard Branemark: Problem Solving

1) Abutment Change: All abutments break sooner or later. The fixture is intentionally stronger than the abutment. The system is modular and the abutment can be changed in 10 minutes, as an outpatient and without anesthesia. It is not done in an OR but clean, aseptic conditions are required
2) Skin Healing Problems: Sometimes full flap necrosis can occur. If it does wait for demarcation and then do a split thickness skin graft.
3) Short Stumps: The proximal canal is metaphyseal rather than diaphyseal. The fixture, with time, will become surrounded with bone. Slow formation of bone is achieved with controlled loading. There is a 50% success rate with short stumps.
4) Fractures: Base of neck femoral fractures can be treated with a DHS.
5) Superficial Infections: C&S, Antibiotics as appropriate but start with Fluoroxacillin 750mg TID X10 days. If recurrent consider restabilization of the soft tissue.
6) Deep Distal Infection: (Well osseointegrated) Pull out the abutment, remove/excise the fistula, close in layers and replace the abutment." The osseointegration protects the fixture from infection."
7) Deep Infection: Stable implant- antibiotics by mouth for 1 year. If unstable implant do revision surgery. Remove the fixture, clean the cavity (I&D) place antibiotic beads or cement and put on antibiotics (IV,PO). Wait 6 months and place a new fixture and begin process again. He would also reculture with the above and maybe do bacterial DNA, PCR etc.
8) Implant Fracture: Re-amputation or carefully core out the remaining implant and replace with a larger one.
9) Functional MRI's of the brain, after OI, show changes that seem to indicate that OI helps "phantom pain".

GAIT LAB

They have an 8 camera gait lab built in their "Cold War" bomb shelter. They have 2 force plates synchronized with cameras. They use 20 markers on the body and 3 on the Rotasafe. They can track 20 markers.

Study of transfemoral OI: Three days pre-op, 18 months 2, 3,4,5,7 years. Results; N=14 show increase in hip extension, decrease in pelvic tilt and pelvic obliquity and a move toward more normal gait,

Upper extremity: Stewe Jonsson

1) It is very important to ventilate the interface site to prevent moisture and infection.
2) Tighten the abutment screw.

He supposed there was no improvement in "phantom pain" post OI but the 3 UE patients that we talked to said they were improved.

For transfemoral tighten the abutment to 12 N/M if good bone quality; if not good use 8 N/M. Must check up each time and tighten the abutment.

A German orthopaedist, Dietrich Schulte-Bockholt removed an ESKA femoral stem, Ti coated, that required a special chisel and was a tough operation.

INTEGRA makes the Rotasafe, implants and the surgical equipment to place the Branemark implants.

Evaluation of Sensitivity:

The "psychophysical"sensation = osseoperception. Apparently there are increased neuropeptides (calcitonin gene related peptide) in OI patients. There is measurable cortical activation on sensory stimulation of an OI thumb. In one study with 21 OI patients vs. 43 conventional socket prostheses, in transfemoral amputees, there were more significant changes but "no conclusive results' although they seemed to perceive high frequency vibrations and OI had a lower threshold to perception..

REHABILITATION SUMMARIES:1990 to 2008: They have done 100 lower extremity patients with 106 limbs.

1) 61% males 39% females
2) 67% trauma, 21% tumor, 12% others
3) Mean age 32 years old, (10 years to 63 years old)
4) Mean time from amputation to OI surgery is 11.5 years.
5) 4 patients have died.

Apparently analyzed group 72 patients/ 78 implants, not sure of mean follow up time but 94% use the prostheses, 4 don't because of severe "phantom pain" 1 with osteomyelitis and over all summary is that they have improved walking habits, become more physically fit with time and have decreased energy costs.

UPPER EXTREMITY: 1990 to 2008 32 patients with 43 implants and 4 non-users

1) Thumb: 6 patients, 6 implants with one non-user because of infection.
2) Trans radial: 10 patients with 20 implants. 1 overload accident with fracture. (1993 to 1998)
3) Trans humeral: 15 patients, 15 implants with one non-user because of shoulder problems DJD.
4) Partial hand 1 patient, 2 implants with 1 non-user not OI related.

MEDICAL ECONOMICS:

They did a cost analysis of OI vs socket technology. Both the costs and the consequences of the process were evaluated. Comparing the cost/unit of effectiveness it is a good value for the money.

Improved sitting comfort, hip range of motion, walking habits, general prosthesis use and time each day of prosthesis use. There is less energy consumption and improved health related quality of life. (SF36 was used in the OPRA study.)

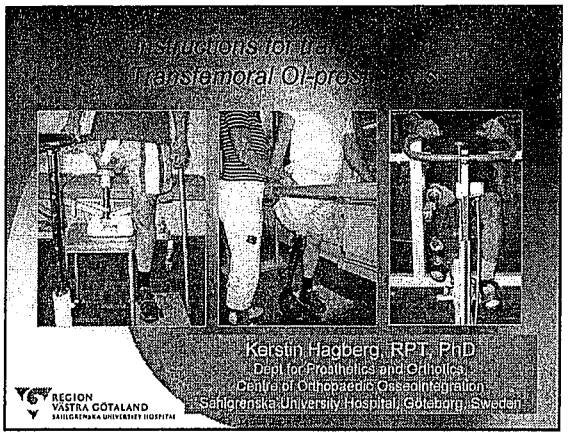
In Sweden the costs were evaluated from a societal perspective- all costs are paid by taxes. Direct costs = all resources. Indirect costs = the initial hospitalization, out patient follow up and expenses for the surgeon, indirect loss as a consequence of disease.

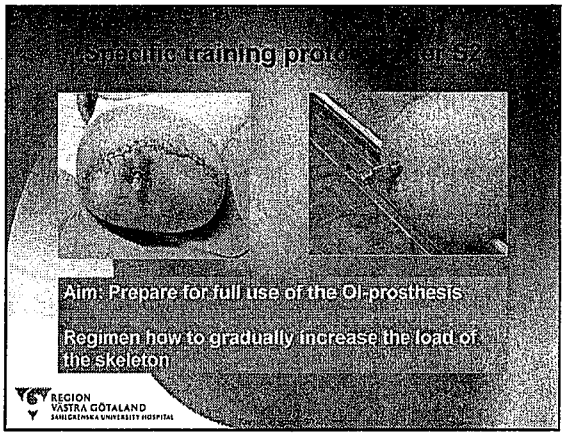
Cost of OI minus cost of socket technology, 132 euros/ year more for OI than socket. The bottom line is it is more expensive but it is worth it.

Meeting of The Orthopaedic Society for Osseointegration , UCSF, San Francisco, CA., May 1-2, 2009, Richard O'Donnell Same meeting in Gothenburg August 30-31, 2010

James Peter Beck MD
Orthopaedic Surgeon
Adjunct Assistant Professor
The University of Utah
Department of Orthopaedic Surgery
Bone and Joint Research Lab,
VAH Salt Lake City, Utah

APPENDIX 1


Instructions for Hand
Transfemoral OI-prosthesis
Kerstin Hagberg, RPT, PhD
Dept for Prosthetics and Orthotics
Centre for Orthopaedic Osseointegration
Sahlgrenska University Hospital, Göteborg, Sweden
REGION VÄSTRA GÖTALAND
SAHLGRENSKA UNIVERSITY HOSPITAL


Specific training protocol for S2
Aim: Prepare for full use of the OI-prosthesis
Regimen how to gradually increase the load of the skeleton
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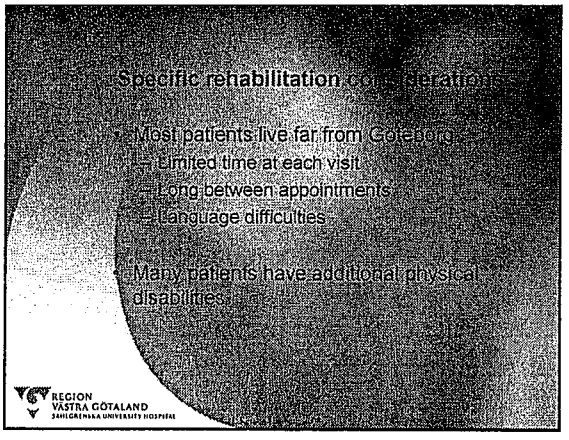
Specific rehabilitation considerations
Most patients live far from Göteborg
Limited time at each visit
Long between appointments
Language difficulties
Many patients have additional physical disabilities
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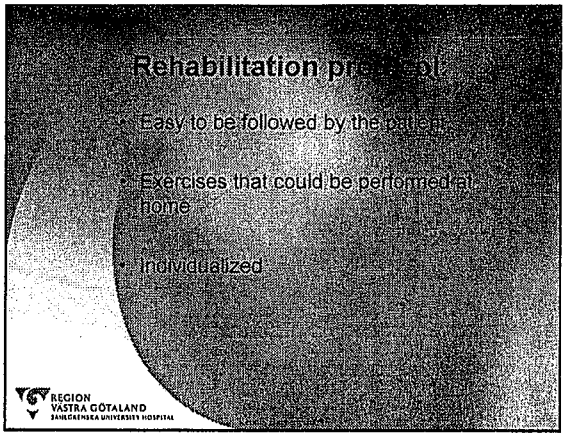
Rehabilitation protocol
Easy to be followed by the patient
Exercises that could be performed at home
Individualized
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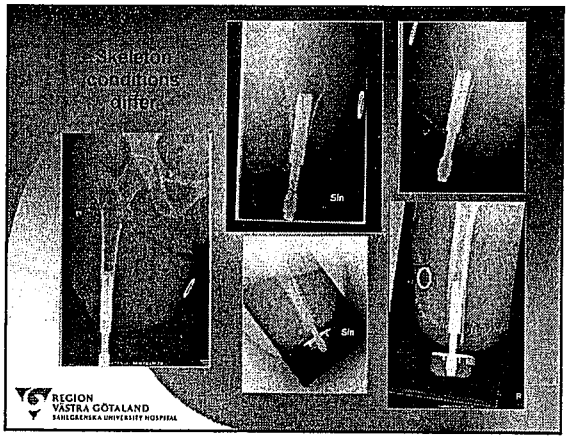
Skeleton conditions differ
Sin
Sin
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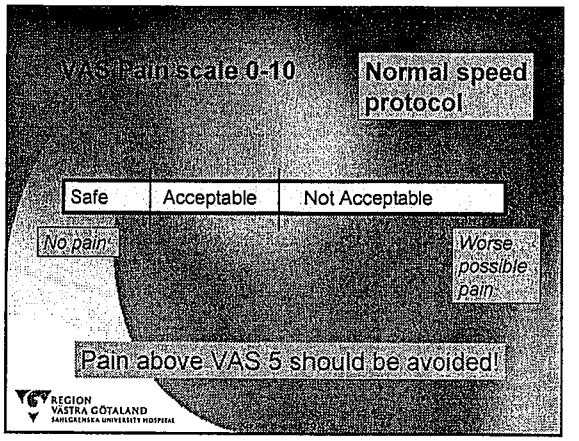
SAHLGRENSKA UNIVERSITY HOSPITAL


VAS Pain scale 0-10
Normal speed protocol
Safe
Acceptable
Not Acceptable
No pain
Worse possible pain
Pain above VAS 5 should be avoided!
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VAS Pain scale

Half speed protocol

Not acceptable

No pain

Worse possible pain

Pain above VAS 3-4 should be avoided!

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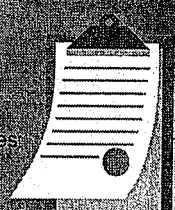
Rehabilitation prot

- Normal speed protocol
  ~ 6 months
- Half speed protocol
  ~ 10 – 12 months

Short training prostheses
Long CI-prostheses



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Short training prostheses

Axial weight-bearing and weight

- Starts 4-6 weeks after S2-
  Weight-bearing: 20 kg
- Bathroom scale
- Two times 30 min/day
- Increase weight-bearing
  10 kg/week

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Additional tra

to increase hip range of motio
and for loading the skeleton in differ

Exercise program on the mat

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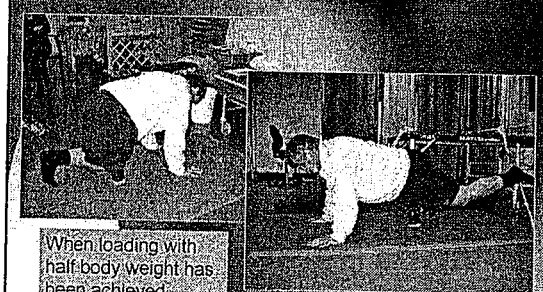
Crawling and exercises



When loading with half body weight has been achieved

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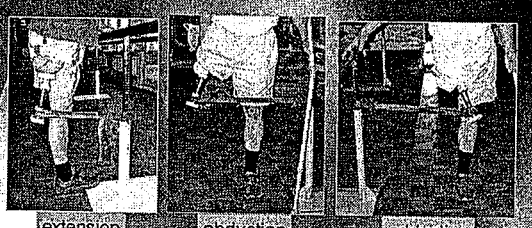
Elastic band resist



extension abduction adduction

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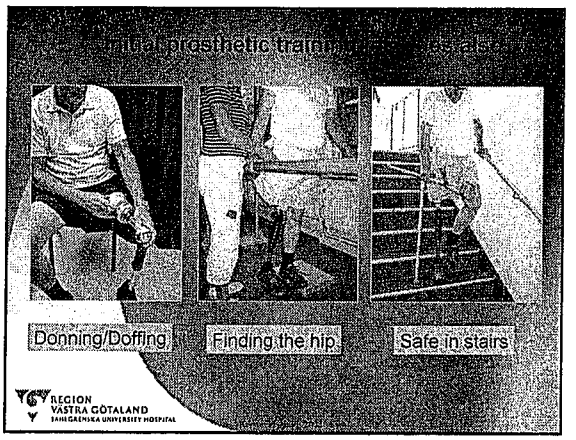
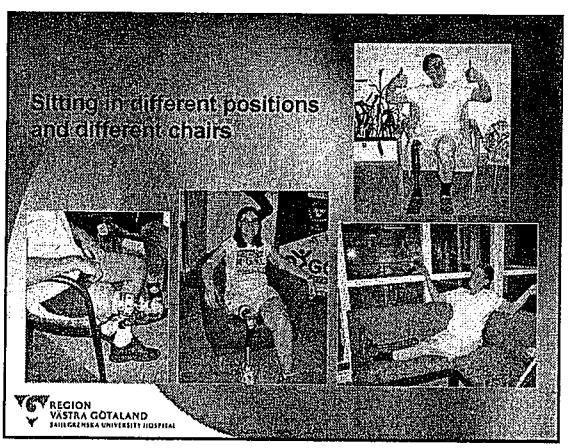
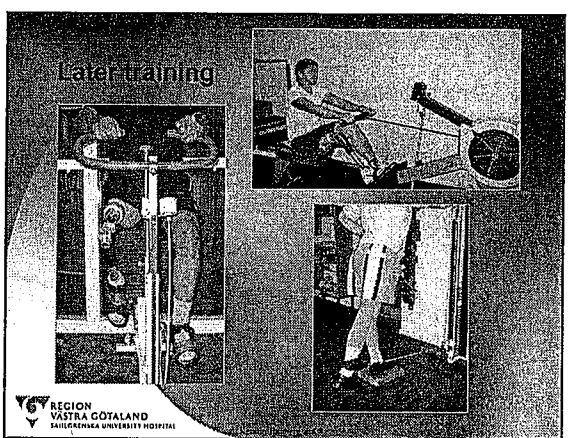
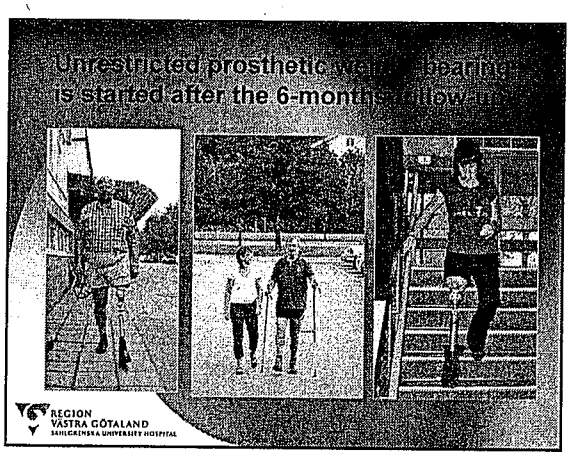
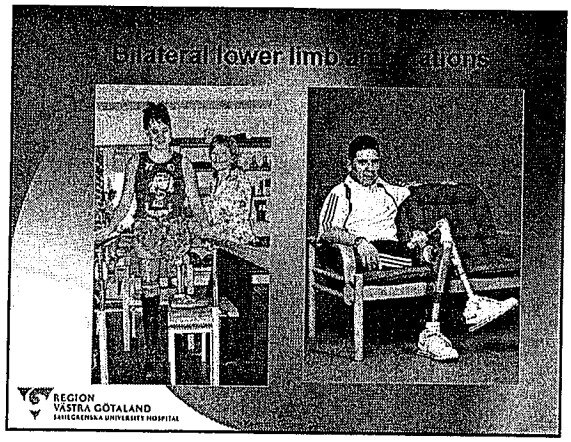


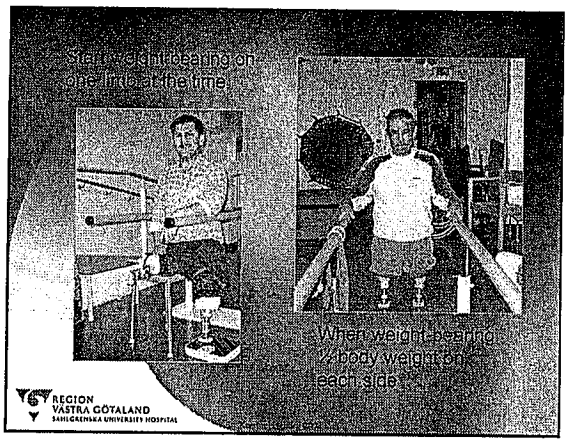


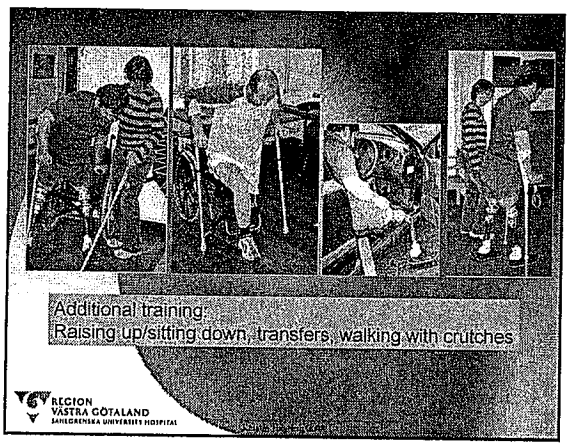


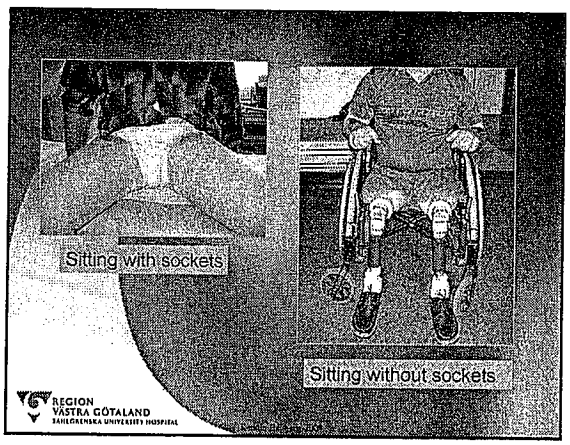
CONTINUE CYCLING 6 MONTHS
? SII IF POOR QUALITY OF BONE



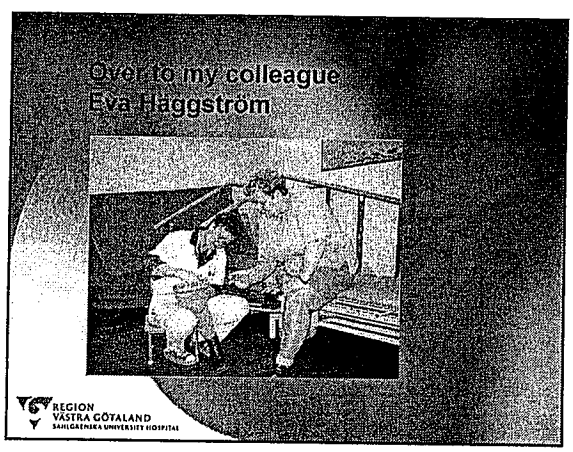








MOST PATIENTS WITH BILATERAL HIGH FEMORAL AMPUTATIONS REQUIRE CRUTCHES AN 4POINT GAIT.



Schematic schedule for rehabilitation after the second surgery (S2) at treatment for bone anchored transfemoral prostheses.

No increase of the training is to be done faster than the normal speed program. No loading or exercises that cause pain above VAS 5 for normal speed program and VAS 3-4 for half speed program should be performed. If so – slow down the progress of the program!

Week After S2	Normal Speed program No pain above VAS 5	Half speed program No pain above VAS 3-4
1-2	Immobilized	Immobilized
3-4	Gentle exercises	Gentle exercises
4-6	**Start training with short prosthesis:** o Load 20 kg, axial loading and gentle weight-shifting, avoid all rotation while loading, increase 10 kg/week, load 2 x 30 minutes daily o Exercise program with short prosthesis	**Start training with short prosthesis:** o Load 15 kg: axial loading and gentle weight-shifting, avoid all rotation while loading, increase 5 kg/week, load 2 x 30 minutes daily o Exercise program without wearing short prosthesis
7-8	Exercise program increased: o Add 1 kg weight on short prosthesis o Crawling with small steps on all fours *	Exercise program increased: o Add the short prosthesis when performing the program
9-10	Exercise program increased: o Increase to 2 kg on short prosthesis if ok o Add resistance by light or medium elastic band on short prosthesis o Exercises on all fours *	Exercise program increased: o Add 0.5 kg weight on short prosthesis
11-13	**Start training with OI-prosthesis** o Start in parallel bars o Get used to donn and doff and wear the OI-prosthesis o Standing with no aid o Walking with 2 crutches and load ~20 kg o Sitting in chairs with different heights o Use the prosthesis at most 2 x 1 hour a day, only indoors o No exercises with short prosthesis	Exercise program increased: o Increase to 1 kg on short prosthesis if ok o Crawling with small steps on all fours * o Add resistance by light elastic band on short prosthesis
14-16	Gradually increase time of prosthetic use and activity, all walking with 2 crutches; o Load gradually more on the prosthesis when walking o Walking on stairs o Walking outdoors on level ground o Sitting/driving car o Exercise program with short prosthesis	Exercise program increased: o Exercises on all fours * o Increase resistance of elastic band if ok
16-24	Gradually increase time of prosthetic use and activity, all walking with 2 crutches; o Prosthetic use all day o Walking on slopes and on uneven ground o Add bicycling on exercise bike o Start training steps with less support; sideways, walking with a stick etc.	**Start training with OI-prosthesis** o Follow instructions for normal speed program when starting to use the OI-prosthesis, but with slower progress
At 24 weeks	**6 months follow-up with x-ray** Decision on regularly walking without support	**6 months follow-up with x-ray** Decision on how to increase prosthetic use and activity

* Crawling and exercises on all fours should not be started until loading with half body-weight is achieved.

APPENDIX # 2

Bone anchored amputation prosthesis using the method of Brånemark osseointegration

18 years of experience
Lower extremity

Eva Häggström.
CPO Orthopaedic Engineer

Dept of Prosthetics and Orthotics
Sahlgrenska University Hospital
Göteborg Sweden

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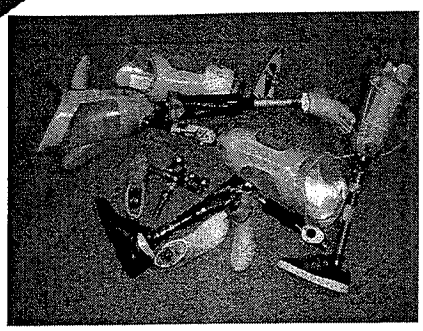

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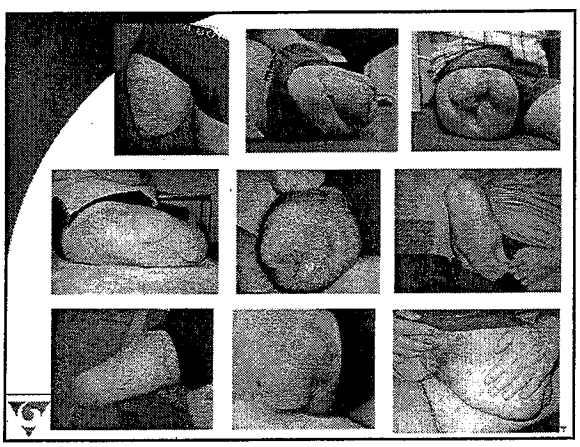

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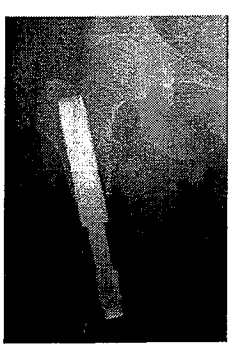
Treatment :

Op. Stage 1 (S1)
Socket prostheses
Op. Stage 2 (S2)

- **Rehabilitation with short training prosthesis**
- **Rehabilitation with long OI-prosthesis**



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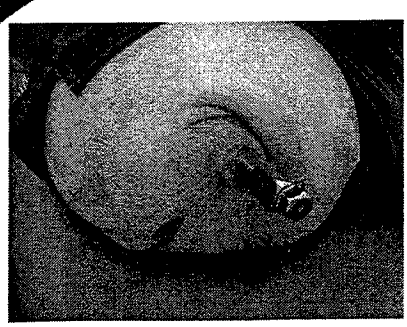

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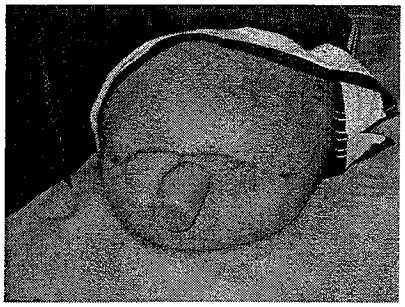
Distal Cup



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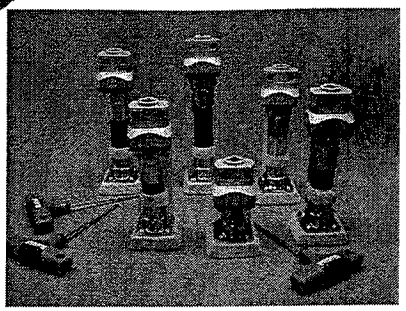

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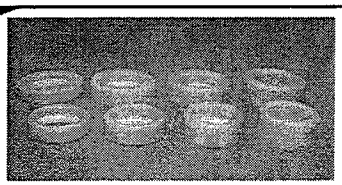
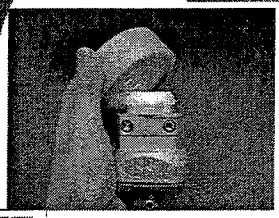
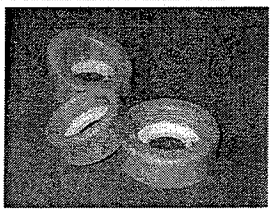
MULTIPLE SIZES -
THIS DEVICE STABILIZES SKIN AND MINIMIZES INFECTION

Soft Tissue Support







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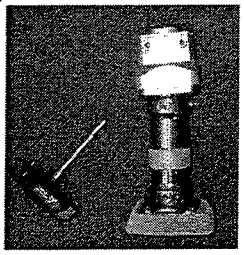

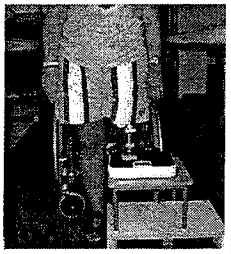

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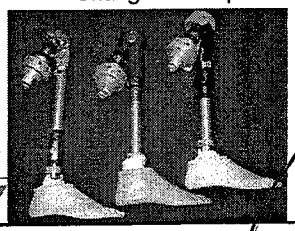
- Partial loading, two crutches during 3 months
- Additional adjustments
- Change of components



- Torsion adapter
- Shock absorber

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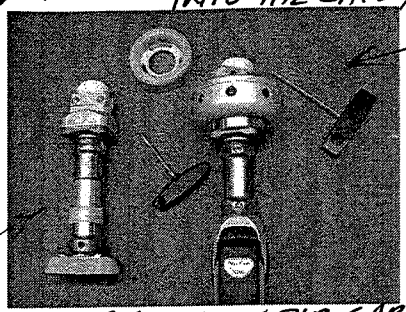
ROTOSAFE : TOO MUCH ROTATION GOES INTO THE SAFETY MODE



WORKS GREAT IN THE GARDEN

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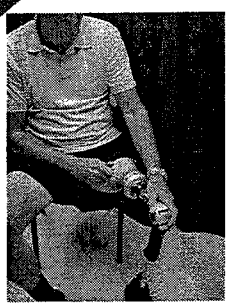
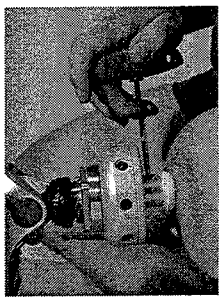
CUSHIONS LOADING

NEED TORSION ADAPTER TO REDUCE DANGEROUS LOADING





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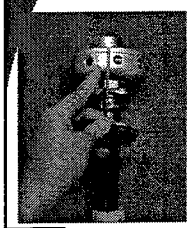
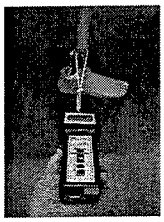
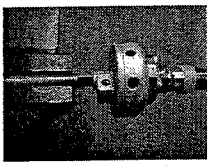
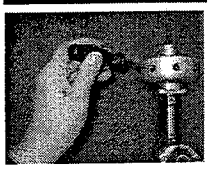
Setting of Connection Rotasafe









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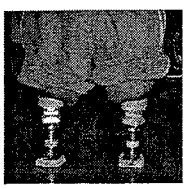
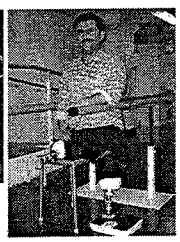
ROTATIONAL MARKERS

BEGIN WITH 13 TO 14 NEWTON/METERS

30 N/M WHEN FULLY OSSEOINTEGRATED

Bilateral amputee´s





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USES ONE CRUTCH AT HOME BUT 2 WHEN OUTSIDE.

Cosmetic Cover

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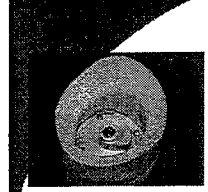

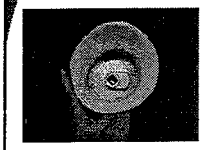

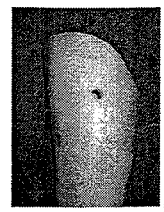

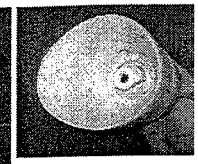

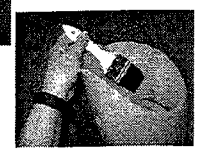

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Bath and Swimming

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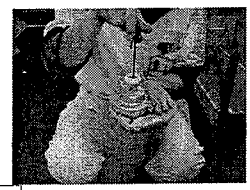

Patient Information

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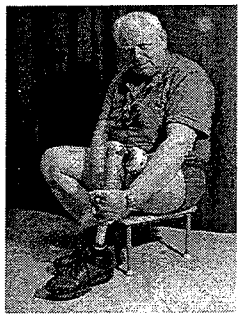
IMPORTANT IMPORTANTE VIKTIGT



TEAMWORK

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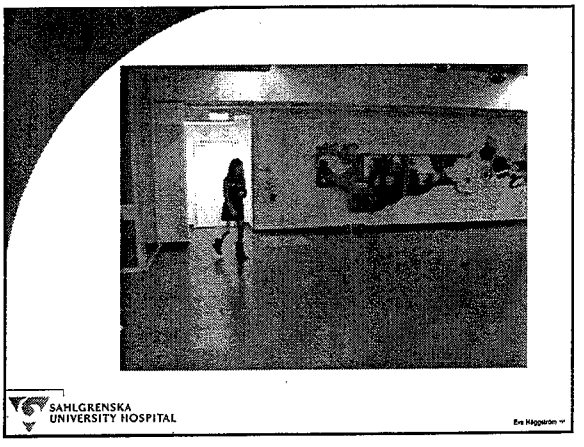


ROTOSAFE RELEASES IN CLICKS -
20 N/M PER CLICK - MAY ROTATE
DEGREES
360° MAY CAUSE A FALL

Bone anchored amputation prosthesis. September 3-4 2008

Bone anchored prostheses, osseointegration upper extremity.

Stewe Jönsson
CPO Orthopaedic engineer
Department of Prosthetics and Orthotics
Sahlgrenska University Hospital
Göteborg Sweden

REGION VÄSTRA GÖTALAND
SAHLGRENSKA UNIVERSITY HOSPITAL

Ordinary socket and harness suspension can cause tissue problems, discomfort non stable and non reliable suspension.
Sometimes because/together with a short stump

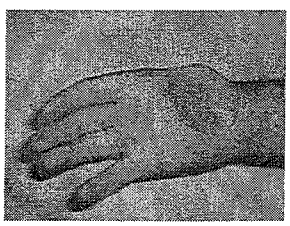
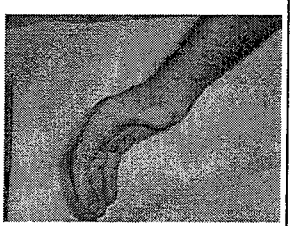


The main reason for osseointegration is to solve those problems

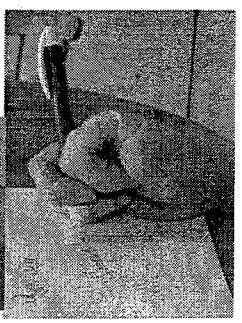
---

Thumb finger level

Aesthetic problems.

Functional problems.

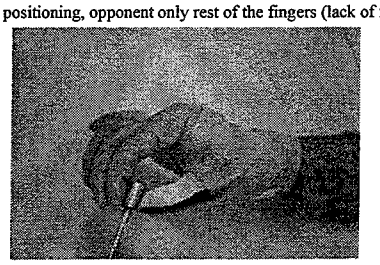
Options

- Toe transfer.
- Index finger to thumb / pollicisation
- Prosthesis

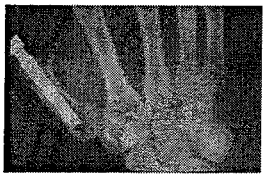
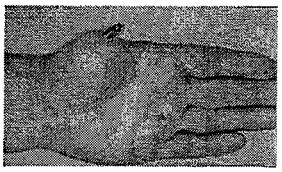
Ordinary thumb prosthesis

Working type, HD silicone. Varying grade of appearance. Always cover parts of the hand in aim of suspension (lack of appearance).

No thumb positioning, opponent only rest of the fingers (lack of function).



Osseointegration results in a new suspension platform.

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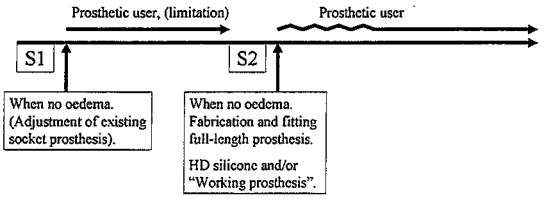
Prosthetic procedure thumb/finger.



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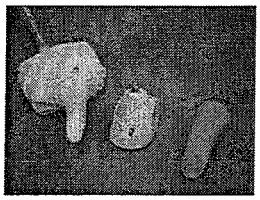
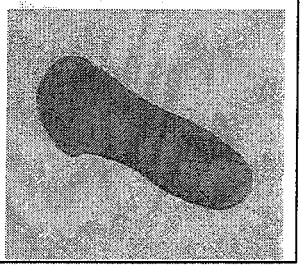
Individual impression Mold-making CM prosthesis

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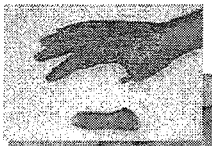
Appearance



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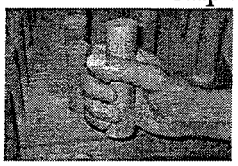
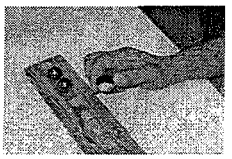
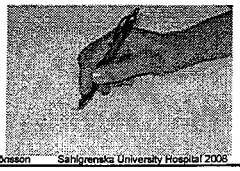
Grip function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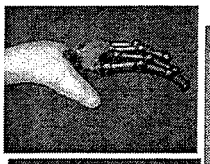
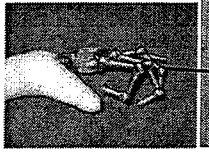
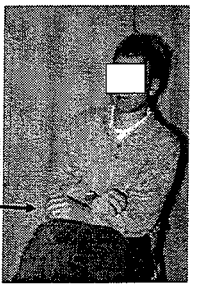
Partial hand solution

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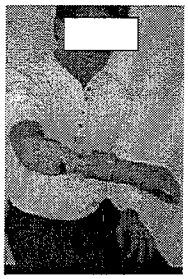
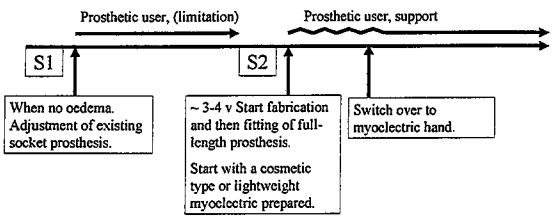
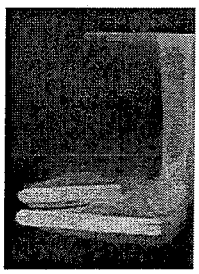
The first transradial case 1990

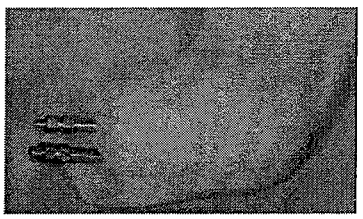
The first transradial patient was implanted -90. Provided with a osseointegrated prosthesis, myoelectric type -91.



Prosthetic procedure transradial level.





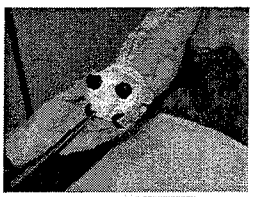


Fixtures in both radius and ulnae.
The abutment follows the skeleton anatomical direction.
Results in an individual abutment situation. NEED OF INDIVIDUAL IMPRESSION

Analysis of the abutment position/direction, over an elbow flexion and extension cycle.

Impression of the abutment in an optimal position.

Direct control instrument, for prosthesis length and alignment.

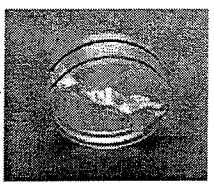


Fabrication of the attachment interface "PUCK"

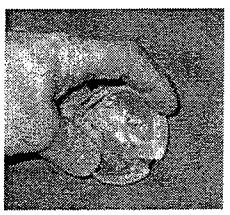
Abutment situation

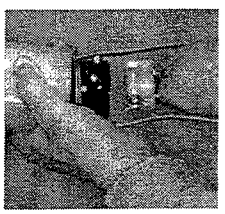
Puck

Copying



The "puck" system

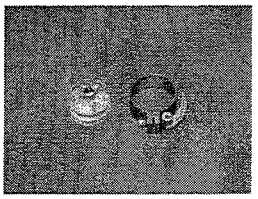


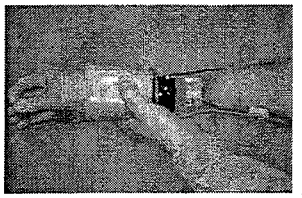


Handle individual abutment situation
Easy do adapt
Easy to keep clean "hygienic"
Long durability

Attachment device.
Two sizes 40 and 50 mm

Easy and quick
donning and doffing





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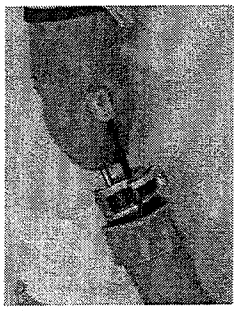
- Quick connector. Attachment device
- Flexible bars as electrode holder
- Temperature insulation
- Shock absorber
- (Fail-safe)



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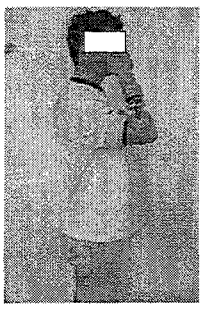
Full freedom of movement in the elbow joint



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Osseointegrated bilateral 1992-3

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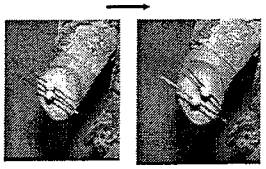
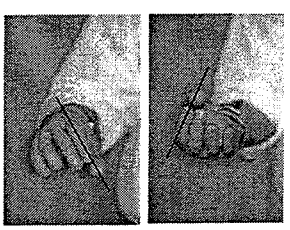
Transradial level. Pronation / supination

Movement contains of



Range depends of stumplength



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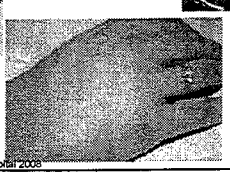
Long transradial, HD CM aesthetic type.



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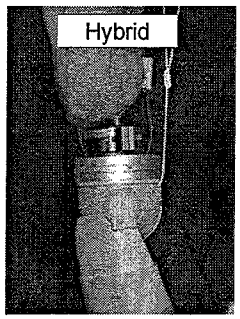
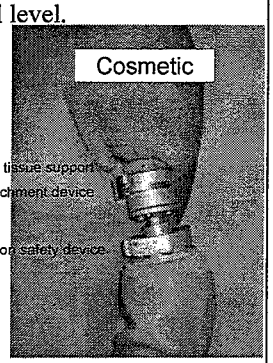
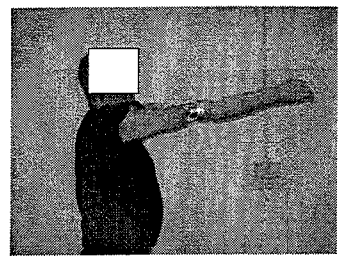
Close up transhumeral level.

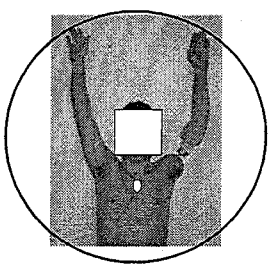




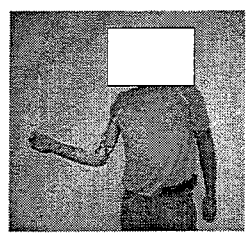
Stable fixation

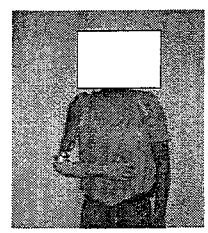


Range of motion

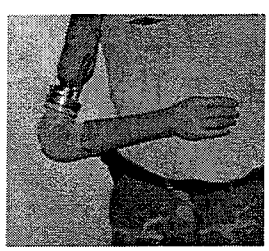


Free shoulder rotation





Multifunctional
Electrical elbow, hand and wrist rotator



Conclusion, upper extremity

Levels
- Finger.
- Partial hand.
- Transradial Short /long.
- Transhumeral Short /long.

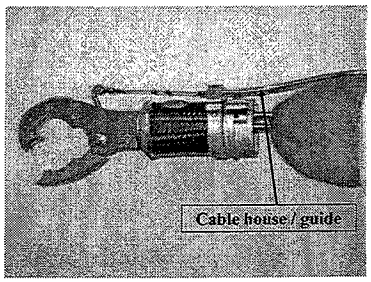
Cases
- Trauma.
- Tumour.
- Cong, (dysmelia).

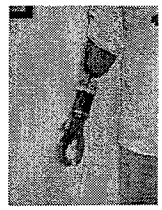
Not for growing persons, yet.

Prosthetic types
- Cosmetic.
- Myoelectric.
- Multifunctional
- Body powered.
- Hybrid.

Today, around 30 upper limb cases.

Body powered





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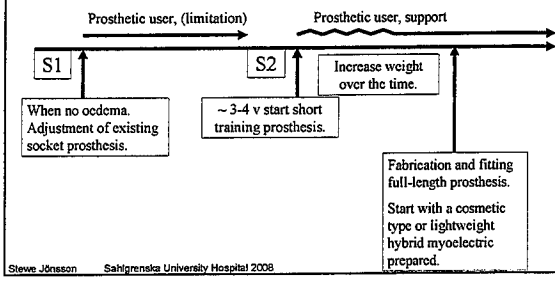
Prosthetic procedure transhumeral level.



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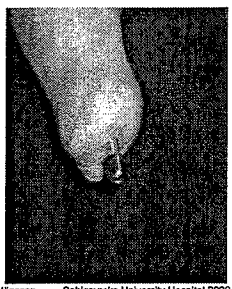
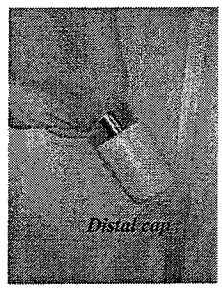
> Transhumeral level <





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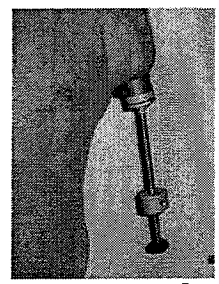
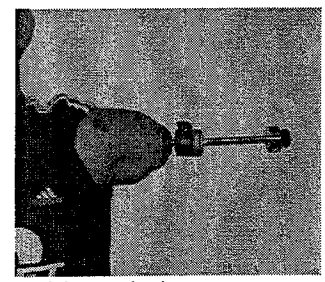
Initial training prosthesis





Increase weight over the time

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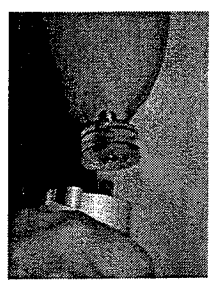
Attachment device, easy don/doffing



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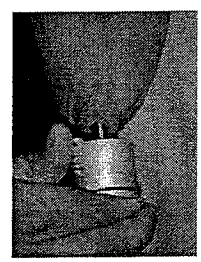
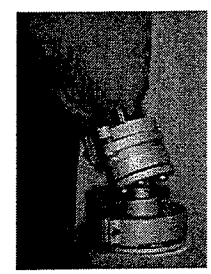
Reliable and stable fixation





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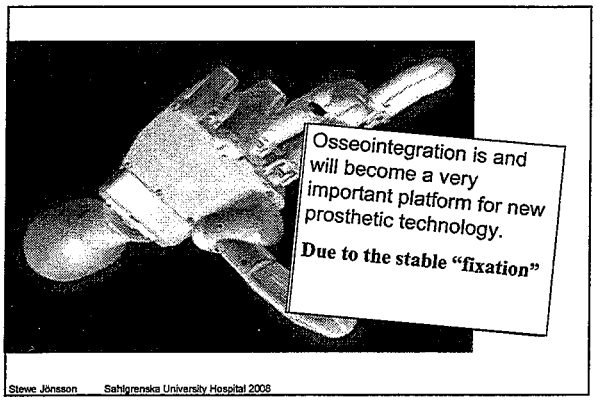
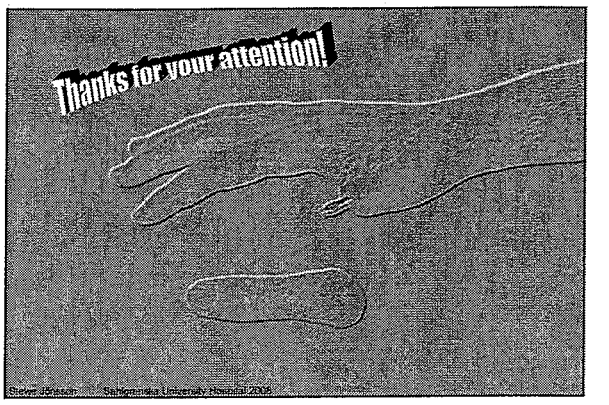




DASH "DISABILITY ARM SHOULDER HAND

APPENDIX # 4

Rehabilitation protocol after second surgery (Stage 2) for transhumeral bone anchored prostheses.

No increase of training is to be done faster than normal speed. No loading exercises that could cause pain above VAS 5 for normal speed. If so, slow down to lighter weight(s)

Weeks after S2	Normal speed. No pain above VAS 4
0-1	Gentle exercises
2-4	Increased shouldermotion
3-4	Start training with short training prosthesis • Apply weights on the training prosthesis. Start with 100 grams. Wear the prosthesis daily, but no risky activities. Add 100 grams per week. • Axial weight-loading using scales twice daily. Start off with 5 kg. Add 1 kg/week • Myo-training with electrodes, when electric prosthesis is prescribed.
6	• Exercises on the floor with short prosthesis for arms, shoulders and back 3-4 times /week
Approx. 12	• Provide patient with a cosmetic or lightweight prosthesis without grip-function, when the pat without problems reaches the calculated weight of the full-length prosthesis. • Gentle exercises with prosthesis. Avoid rotation overload forces with the prosthesis. • Light bilateral ADL-activities.
	• The pat can be fitted with a functional heavier prosthesis with grip-function, when the surgeon indicates. • Functional prosthetic grip-training with bilateral activities.

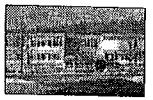
Some patients have individualised time schedules and weight-loading.

REHABILITATION OF BONE ANCHORED PROSTHESES OF UPPER EXTREMITY



Kerstin Caine-Winterberger

Reg. Occupational Therapist
Upper Limb Prosthetic Centre
Dept. of Prosthetics and Orthotics
Sahlgrenska University Hospital
Gothenburg, Sweden

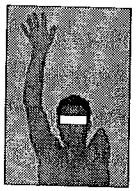
TRANSHUMERAL AMPUTATION

STAGE I

Pre/post-operative instructions:

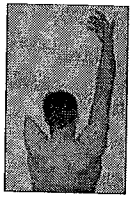
- ROM in the shoulder:

flexion



extension

abduction

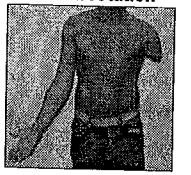


3 weeks post-operative

ROM in the shoulder:

Internal rotation

external rotation



6 weeks post-operatively

Strengthening exercises of the shoulder, back, chest and upper arm-muscles

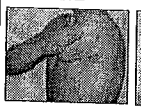
Flexion/extension with back-rotation

Retraction of the scapula with external rotation

Flexion/extension

EXERCISES WITH RESISTENCE

Flexion



Extension

Adduction

Abduction

8 weeks post-operative

Loading exercises
with elastic-bands
or therabands

STAGE II

Post-operative instruction

- Gentle exercises

3 weeks post.operative

- Internal/external rotation of the shoulder

6 weeks

- Full range of motion

3 weeks post-operative

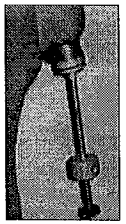
Weight loading by application of weights on short prosthesis

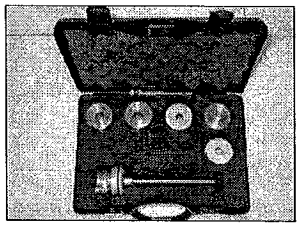
Start with 100 grams depending on:

- •Skeleton quality
- •Indication of pain in stump muscles or bone.

Increase weight weekly

SHORT TRAINING PROSTHESIS

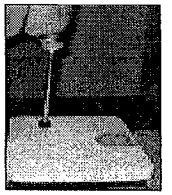




VAS = VISUAL ANALOGE SCALE . Max. 4

3 - 6 weeks post-operative

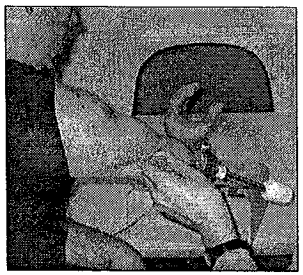
Loading exercises in axial direction with short prosthesis using a pair of scales



Start with 5 kg. Slow increase of weight each week depending on:

- Skeleton quality
- Indication of pain in stump muscles or bone.

EMG-detection and training

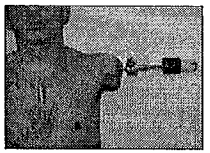


Triceps for opening the hand

Biceps for closing the hand

Start 3 weeks post-operative

TRAINING OF R.O.M. AND STRENGTH WITH SHORT PROSTHESIS AND APPLIED WEIGHTS

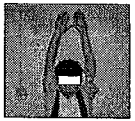


6-8 weeks postop.

PROVISION OF LIGHTWEIGHT PROSTHESIS

R.O.M. TRAINING WITH COSMETIC PROSTHESIS

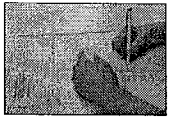
Start off with gentle exercises. Increase ROM over time

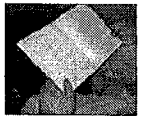




TRAINING WITH LIGHT-WEIGHT PROSTHESIS

Light ADL-activities

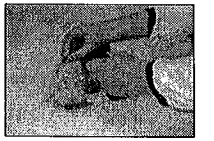


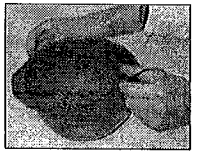


Holding a paper whilst writing

Holding a magazine or book

Holding bread whilst slicing





Holding a bowl whilst stirring

FINAL PROSTHESIS

Individual:

Depending when the patient reaches the weight of the final prosthesis by loading and weight-training.

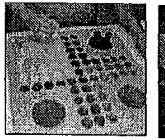
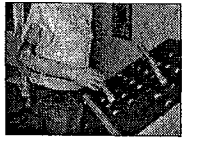
TRAINING WITH PROSTHESIS

- Don/doff prosthesis


- Positioning of elbow and forearm/wrist


TRAINING WITH MYO-ELECTRIC PROSTHESIS

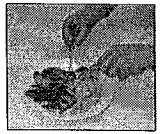
Opening/closing of prosthetic grip

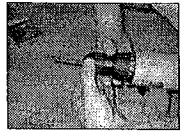
ACTIVITIES OF DAILY LIVING







Carry with the prosthesis

Bilateral activities

TRANSRADIAL AMPUTATION

STAGE I

Pre/post-operative instructions:

- ROM in shoulder and elbow:

3 weeks postoperatively:

Pronation

Supination

6-8 weeks post-operatively

Exercises with weights

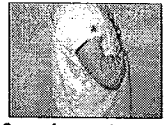
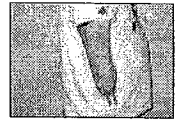
Biceps


Triceps

Application of previous prosthesis

STAGE II

Post-operatively

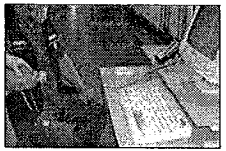



3 weeks post-operative

Pronation

Supination

EMG detection and training



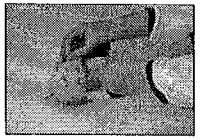
Extensors for opening the hand

Flexors for closing the hand

Approx. 4-5 weeks after operation.

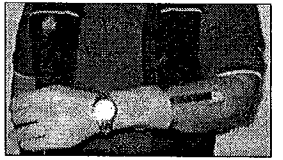
Starts off with a light weight prosthesis.

For transradial amputation no short training-prosthesis is used.



The prosthesis is used in light resisted ADL-activities.

A myo-electric prosthesis supplied within 3 months postoperatively



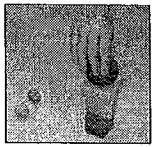
Application of final prosthesis depends on:

- Skeleton quality
- Indication of pain in stump muscles or bone.

Prosthetic training

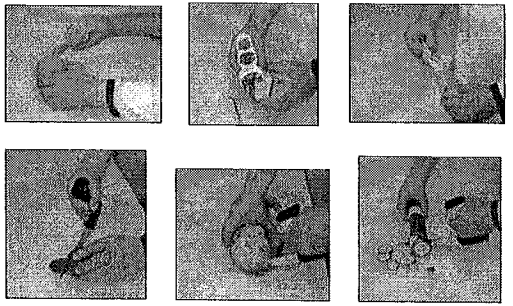
Opening/closing of prosthetic grip

Picking up objects

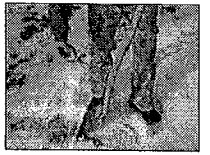


Playing games

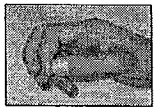
Bilateral ADL-activities: peeling and cutting



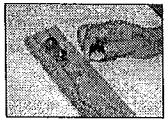
CLEANING AND GARDENING



THUMB-AMPUTATION
with osseointegration in 2 stages

TRAINING

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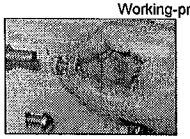
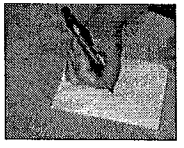
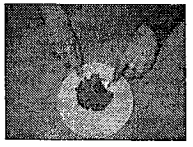
ADL-ACTIVITIES

Working-prosthesis



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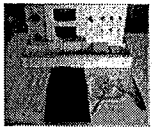
EVALUATION UPPER LIMB

Pre/post-operative assessment:

- Questionnaire
- DASH
- VAS
- Goniometer

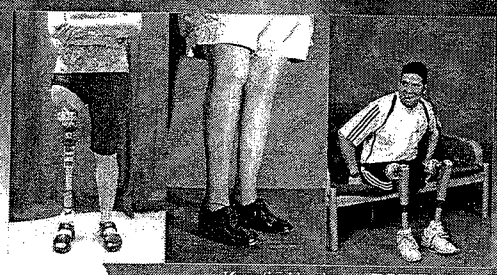

- Sollerman´s grip-function test *
- Jamar for full hand grip-strenght *
- B&L for pinch-grip *

* thumb-level


Kerstin Caine-Winterberger, 2008

APPENDIX #5

Introduction to the OPRA study



Kerstin Hagberg, RPT, PhD
Dept for Prosthetics and Orthotics,
Centre of Orthopaedic Osseointegration
Sahlgrenska University Hospital, Göteborg, Sweden

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OPRA study

Osseointegrated Prosthesis for the Rehabilitation of Amputees

- Prospective clinical investigation - started 1999
- Assessments performed prior to S1 and until 2 year following S2
- Inclusions finished December 2007
- Includes 51 patients with 55 implants (4 treated bilaterally)

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OPRA study

Osseointegrated Prosthesis for the Rehabilitation of Amputees

Inclusion criteria

TF-amputation
< 70 yrs of age
Problems with conventional socket prosthesis
Normal and mature skeletal anatomy

Exclusion criteria

Severe vascular disease
Other medical disease or medication that could negatively affect the treatment
Pregnancy
Weight > ~100 kg

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Details the 51 patients at inclusion

Gender (n)	27 male, 24 female
Mean age (min-max)	44 years (19-64)
Mean age at amputation (min-max)	32 years (15-63)
Mean years Since amputation (min-max)	12 years (10 mths-42 yrs)
Amputation cause (n)	38 trauma 12 tumour 2 arterial embolus 3 infection
Prosthetic user (n)	41 used socket prosthesis 10 did not use socket prosthesis

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More details…

- Example of other disabilities:
  - Contralateral lower limb amputation (6 TFA, 1 TTA)
  - Contralateral knee and/or foot impairment
  - Paralyze of arm
  - Severe back pain
- ~ 50% Sweden, ~ 50% Norway or Spain

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Assessments

Objective measurements

X-rays
Registration of complications
Hip range of motion
Energy Cost (PCI)
Gait analysis
Vibrometric analysis

Subjective measurements

General and Specific Health Related Quality of Life (HRQL)

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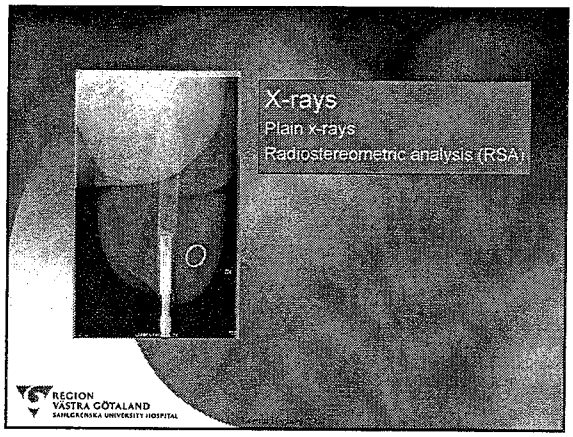
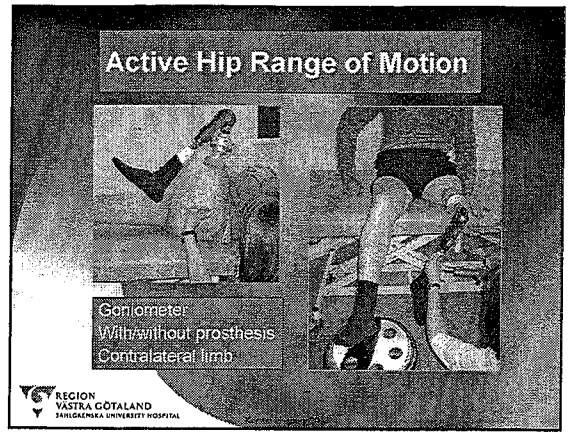
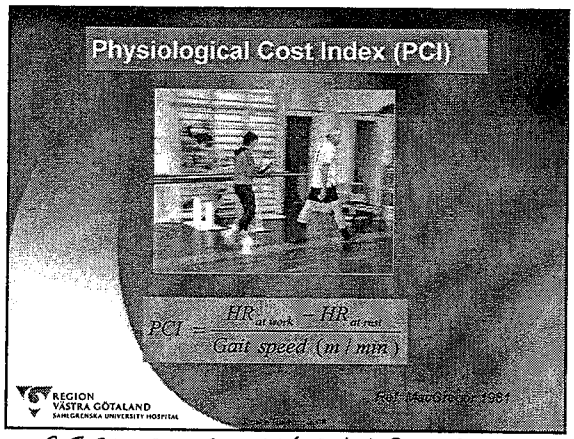
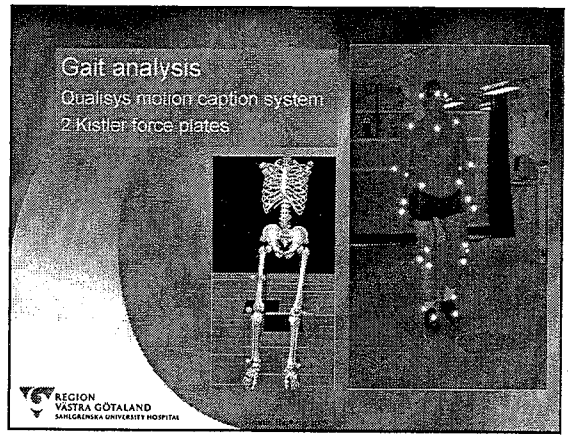
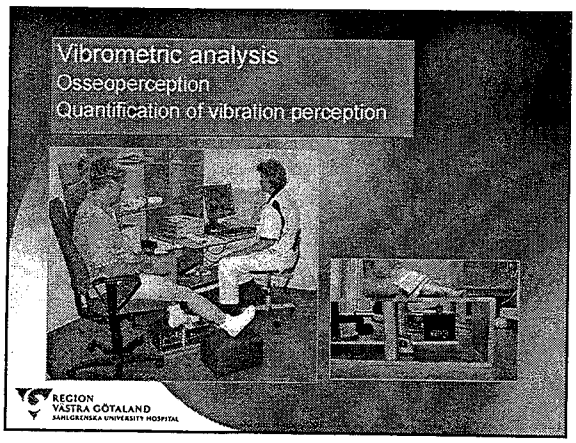


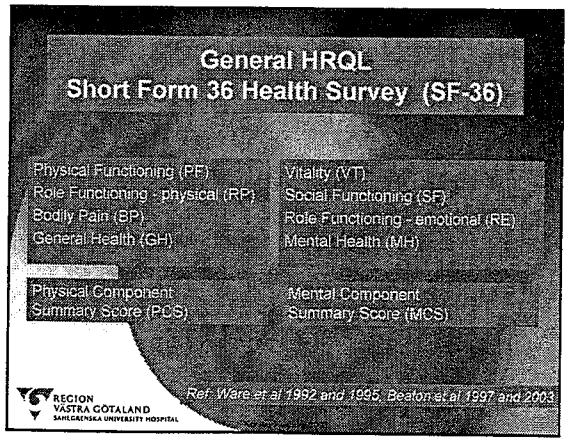




REFERENCE MACGREGOR 1981







Specific HRQL Questionnaire for Persons with a Transfemoral Amputation (Q-TFA)

A targeted self-report outcome measure developed for non-elderly TF-amputees using socket or OI-prostheses

1. Prosthetic Use Score (0-100)
2. Prosthetic Mobility Score (0-100)
3. Problem Score (100-0)
4. Global Health Score (0-100)

Adequate measurement properties
Ref: Hagberg K, Branemark R, Hagg O. (2004) -IRRD

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First study with prospective result

Aim

To report the prospective outcome at 2-year follow-up on the general and specific HRQL for the first 18 consecutive included patients in the OPRA-study

Ref: Hagberg, Branemark et al 2008, Prosthetics Orthotics Int 32, 1 29-41

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BILATERAL HIGH THIGH AMPUTEES DIDN'T USE SOCKET PROSTHESES

Patients

- n=18 (8 male, 10 female)
- Mean age at inclusion: 45 yrs (22 - 62)
- 16 unilateral TFA, 2 bilateral TFA
- Cause of amputation:
- 12 trauma, 5 tumour 1 arterial embolus
- Mean time since amp: 15 yrs (10 months – 33 yrs)
- 15/18 prosthetic users at inclusion

BILATERALS DIDN'T USE IN THIS GROUP

Ref: Hagberg, Branemark et al 2008, Prosthetics Orthotics Int. 32, 1, 29-41

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HAGBERG, BRANEMARK 2008 PROSTHETIC ORTHOTICS INT 32 1-29-41

RESULTS at 2-year follow-up

17/18 Prosthetic use - unrestricted weight-bearing
1/18 Did not use the OI-prosthesis

SF-36

↑ Physical Function	34 → 57	p=0.001
↑ Role Functioning -physical	38 → 65	p=0.004
↑ Bodily Pain	57 → 71	p=0.046
↑ Physical Component Score	31 → 42	p=0.001

Q-TFA
All four scores were statistically significantly improved

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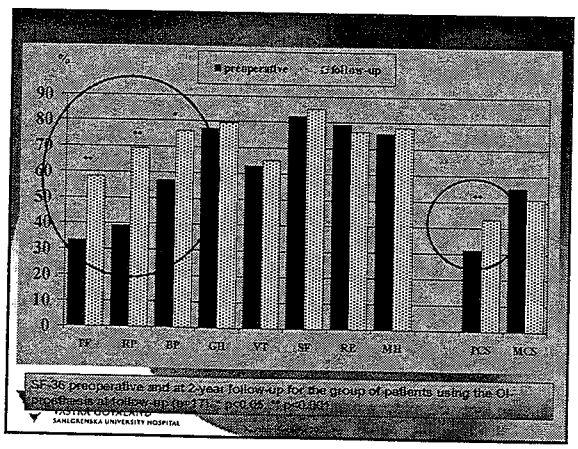

SF-36 preoperative and at 2-year follow-up for the group of patients using the OI-prosthesis at follow-up (n=17). * p<0.05 ** p<0.001

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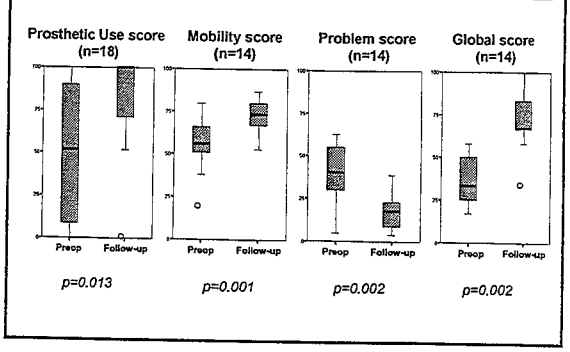
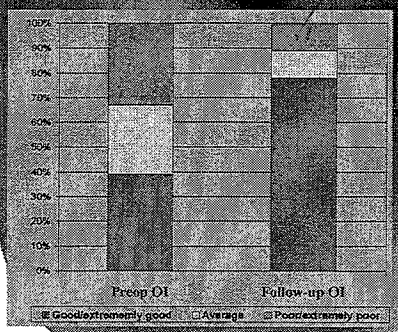
Q-TFA
Questionnaire for persons with a Transfemoral Amputation



LOOSENING

Overall situation (n=18)



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Conclusion

Individuals treated with OI-prostheses report significant improvements in both general and condition-specific HRQL at two years following the surgery.

For one of the 18 patients the treatment was a failure due to loosening of the implant.

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OPRA study today

- 36 implants have past the 2-year follow-up
- 3 implants has been removed
- Final outcome will be reported during year 2010
- Continue to follow the patients
  3, 5, 7, 10, 15 yrs

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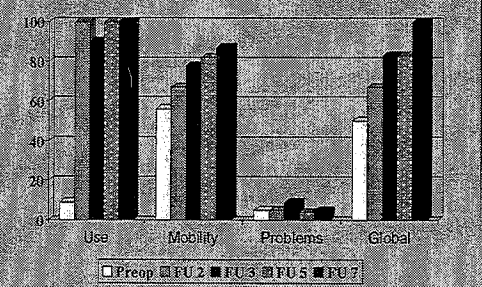
Individual profile of Q-TFA

Male, born 1975, TFA due to trauma 1995, treated with OI-prosthesis 1999



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APPENDIX #6

Osseointegrated titanium implants for prostheses attachment in arm and leg amputees - bacterial colonization at the skin implant interface and infectious complications.

Sahlgrenska University Hospital, SE 416 85 Göteborg, Sweden. lars.hagberg@medfak.gu.se,

Background : Osseointegrated titanium implants entails a direct contact between the fixture and the bone tissue, assuring a stable attachment for prostheses. Previously, the use of metal implants has commonly failed, mostly due to infectious complications.

Methods:A cross-sectional study of bacterial colonization, infection frequency and antibiotic treatment in arm and leg amputees with osseointegrated titanium implants during a 6-month period. A clinical examination was performed and a questionnaire was answered. Bacterial cultures were taken from the skin implant interface. A second assessment of the cohort was performed 2-3 years later. Based on clinical, radiological and bacteriological findings patients were classified as definite, possible, probable implant infection, local soft tissue infection or no infection with or without bacterial colonization around the skin penetrating area.

Results: 39 patients with 45 titanium implants (33 femoral, 1 tibial, 3 humeral, 4 ulnar, 4 radial) with a mean implant time of 56 months (range 3-132) were included. No patient refused inclusion. Two femoral implant patients had chronic fistulas as signs of deep implant related infection. They were not treated with antibiotics and used their prosthesis daily. The condition was unchanged during the observation period. One patient with a successful re-implantation received long-term flucloxacillin due to a previous implant related osteitis. This patient was re-classified as non-infected during the follow-up period. Five patients had been treated with short-term oral antibiotics during the previous 6 months before inclusion due to local infection at the skin penetrating area. Fourteen patients had secretion from the skin implant interface and in 10 patients it was discoloured. Cultures were negative in 8 patients, showed 1 bacterial species in 19, two various bacterial species in 11, and three species in one patient. *Staphylococcus aureus* was the most common finding - 16 patients (17 implants). *Coagulase negative staphylococci* were cultured from 10, *Streptococcus group A, B or G* from 10, *Enterococcus sp* from 3, *alfa-streptococcus* from one and gram negative rods from 4.

At follow up 2-3 years later an additional 5 patients had evidence of implant related infection. Two of these had poor primary fixture osseointegration as predisposing factor, two had a mild distal infection involving bone and soft tissue, one a classical osteomyelitis at mid fixture level. Only one implant was extracted during the observation period. Eleven patients had a history of local infection around the skin penetrating area during the past 6 months before follow-up. Out of these 8 had been prescribed short oral antibiotic treatments (10 days). *Staphylococcus aureus* was still the most common bacterial finding at the skin implant interface - 16 patients (41 %).

Conclusions: In spite of frequent colonization around the skin implant interface with potentially virulent bacteria such as *Staphylococcus aureus,* and bacteria associated to biomedical devices infections such as *Coagulase negative staphylococci*, the titanium implant system for bone-anchored prosthesis in persons with an upper or lower limb amputation cause few severe infectious complications.

Selected References

Branemark, R., Branemark, P. I., Rydevik, B. and Myers, R. R. (2001). Osseointegration in skeletal reconstruction and rehabilitation: a review. *J Rehabil Res Dev* **38**(2): 175-81.

Branemark, R. and Thomsen, P. (1997). Biomechanical and morphological studies on osseointegration in immunological arthritis in rabbits.

Brånemark, R. (1996). A biomechanical Study of Osseointegration. In-vivo measurments in rat, rabbit, dog and man. Dept of Orthopaedics, Inst of Surgical Sciences and Inst of Anatomy and Cell Biology, University of Gothenburg, Thesis

Frossard, L., Stevenson, N., Smeathers, J., et al. (2008). Monitoring of the load regime applied on the osseointegrated fixation of a trans-femoral amputee: a tool for evidence-based practice. *Prosthet Orthot Int* **32**(1): 68-78.

Hagberg, K. (2006). Transfemoral Amputation, Quality of Life and Prosthetic Function. Studies focusing on individuals with amputation due to reasons other than peripheral vascular disease, with socket and osseointegrated prostheses. Department of Orthopaedics, Institute of Clinical Sciences, The Sahlgrenska Academy, Göteborg University, Thesis, http://hdl.handle.net/2077/726

Hagberg, K. and Branemark, R. (2001). Consequences of non-vascular trans-femoral amputation: a survey of quality of life, prosthetic use and problems. *Prosthet Orthot Int* **25**(3): 186-94.

Hagberg, K., Branemark, R., Gunterberg, B. and Rydevik, B. (2008). Osseointegrated trans-femoral amputation prostheses: prospective results of general and condition-specific quality of life in 18 patients at 2-year follow-up. *Prosthet Orthot Int* **32**(1): 29-41.

Hagberg, K., Brånemark, R. and Hägg, O. (2004). Questionnaire for Persons with a Transfemoral Amputation (Q-TFA): Initial validity and reliability of a new outcome measure. *J Rehabil Res Dev* **41**(5): 695-706.

Hagberg, K., Haggstrom, E. and Branemark, R. (2007). Physiological cost index (PCI) and walking performance in individuals with transfemoral prostheses compared to healthy controls. *Disabil Rehabil* **29**(8): 643-9.

Hagberg, K., Haggstrom, E., Uden, M. and Branemark, R. (2005). Socket versus bone-anchored trans-femoral prostheses: hip range of motion and sitting comfort. *Prosthet Orthot Int* **29**(2): 153-63.

Hagberg, K., Häggström, E., Jönsson, S. and Brånemark, R. (2008). Osseoperception and Osseointegrated Prosthetics Limbs. Psychoprosthetics. Gallagher, P., Desmond, D. M. and MacLachlan, M. London, Springer-Verlag: 131-140.

Lee, W. C., Frossard, L. A., Hagberg, K., et al. (2007). Kinetics of transfemoral amputees with osseointegrated fixation performing common activities of daily living. *Clin Biomech (Bristol, Avon)*.

Palmquist, A. (2008). On a novel technique for preparation and analysis of the implant surface and its interface to bone. Institute of Clinical Sciences, Sahlgrenska Academy, University of Gothenburg. Thesis, http://hdl.handle.net/2077/10315

Palmquist, A., Jarmar, T., Emanuelsson, L., et al. (2008). Forearm bone-anchored amputation prosthesis: a case study on the osseointegration. *Acta Orthop* **79**(1): 78-85.

Sullivan, J., Uden, M., Robinson, K. P. and Sooriakumaran, S. (2003). Rehabilitation of the transfemoral amputee with an osseointegrated prosthesis: the United Kingdom experience. *Prosthet Orthot Int* **27**: 114-120.