การใช้ Autologous Stem Cells ที่ได้มาจากข้อเท้าและเท้า ในการผ่าตัดเพื่อปรับโครงสร้างของรยางค์ขา

นายแดเนียล ลี

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรดุษฎีบัณฑิต
สาขาวิชาชีวเวชศาสตร์ (สหสาขาวิชา)
บัณฑิตวิทยาลัย จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2554

AUTOLOGOUS FOOT AND ANKLE STEM CELL APPLICATIONS FOR LOWER EXTREMITY RECONSTRUCTION SURGERY

Mr. Daniel Lee

A Dissertation Submitted in Partial Fulfillment of the Requirements

for the Degree of Doctor of Philosophy Program in Biomedical Sciences

(Interdisciplinary Program)

Graduate School

Chulalongkorn University

Academic Year 2011

Thesis Title	AUTOLOGOUS FOOT AND ANKLE STEM CELL APPLICATIONS FOR LOWER EXTREMITY RECONSTRUCTION SURGERY
By	Mr. Daniel Lee
Field of Study	Biomedical Sciences
Thesis Advisor	Associate Professor Prawit Janwantanakul, Ph.D.
Thesis Co-advisor	Assistant Professor Praneet Pensri, Ph.D.

Accepted by the Graduate School, Chulalongkorn University in Partial Fulfillment of the Requirements for the Doctoral Degree

…… Dean of the Graduate School

(Associate Professor Pornpote Piumsomboon, Ph.D.)

THESIS COMMITTEE

…….. Chairman

(Professor Apiwat Mutirangura, Ph.D., M.D.)

……..……. Thesis Advisor

(Associate Professor Prawit Janwantanakul, Ph.D.)

…….. Thesis Co-advisor

(Assistant Professor Praneet Pensri, Ph.D.)

…….. Examiner

(Associate Professor Sittisak Honsawek, Ph.D., M.D.)

…….. Examiner

(Assistant Professor Tewin Tencomnao, Ph.D.)

…….. External Examiner

(Professor Thep Himathongkam, M.D.)

แดเนียล ลี: การใช้ Autologous Stem Cells ที่ได้มาจากข้อเท้าและเท้าในการผ่าตัดเพื่อปรับโครงสร้างของรยางค์ขา. (AUTOLOGOUS FOOT AND ANKLE STEM CELL APPLICATIONS FOR LOWER EXTREMITY RECONSTRUCTION SURGERY) อ. ที่ปรึกษาวิทยานิพนธ์หลัก: รศ. ดร. ประวิตร เจนวรรธนะกุล, อ. ที่ปรึกษาวิทยานิพนธ์ร่วม: ผศ. ดร. ปราณีต เพ็ญศรี, 48 หน้า.

งานวิจัยนี้มีวัตถุประสงค์เพื่อศึกษาวิธีการใหม่ในการประยุกต์ใช้ Stem Cells ในการผ่าตัดเพื่อปรับโครงสร้างของรยางค์ขา ซึ่งจากการทบทวนวรรณกรรมพบว่า มีการศึกษาการใช้วิธีการนี้น้อยมาก โดยเฉพาะในการผ่าตัดซ่อมแซมรยางค์ขา และอุปกรณ์ยึดตรึงภายนอก อาจจำเป็นสำหรับการประยุกต์ใช้ stem cells เพื่อการผ่าตัดซ่อมแซมรยางค์ขา การศึกษานี้เป็นการทบทวนวรรณกรรมในเชิงลึกเกี่ยวกับการประยุกต์ใช้ stem cells เพื่อการผ่าตัดซ่อมแซมรยางค์ขา

สาขาวิชา ชีวเวชศาสตร์ ลายมือชื่อนิสิต .....

ปีการศึกษา 2554 ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์หลัก.....

ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์ร่วม.....

5287858120 : MAJOR BIOMEDICAL SCIENCES

KEYWORDS : STEM CELL / LOWER EXTREMITY / SURGERY / RECONSTRUCTION

DANIEL LEE: AUTOLGOUS FOOT AND ANKLE STEM CELL APPLICATIONS FOR LOWER EXTREMITY RECONSTRUCTION SURGERY. ADVISOR: ASSOC. PROF. PRAWIT JANWANTANAKUL, Ph.D., CO-ADVISOR: ASST. PROF. PRANEET PENSRI, Ph.D. 48 pp.

The objectives of this study were to examine the novel applications of stem cells for lower extremity reconstructive surgeries. This innovative modern new approach has little to scarce findings in literature. Even the available scarce results that have been shown on stem cells lack evidence in the surgical treatment for lower extremity reconstruction surgery. A comparative review reveals that an external fixation device may be needed to determine if stem cells may be of benefit for lower extremity reconstruction surgery. A comprehensive review of past literature is provided with extensive background in the stem cell applications for lower extremity reconstruction surgery.

Field of Study : Biomedical Sciences Student's Signature .....

Academic Year : 2011 Advisor's Signature .....

Co-advisor's Signature .....

ACKNOWLEDGEMENTS

I, Prof. Daniel Lee, DPM, FACFAS, would like to deeply express my gratitude and appreciation to my advisor Prof. Dr. Prawit Janwantanakul. He was always kind and instrumental in guiding me through a very complex process into the PhD Degree.

Also, I would like to express my deepest gratitude and appreciation to Prof. Dr. Praneet Pensri and Prof. Dr. Apiwat Mutirangura for their patience, support and kindness in allowing me to fulfill one of my dreams in completing my PhD Degree with Chulalongkorn University.

I feel forever appreciative to the Committee and the Inter-Department Program of BioMedical Sciences, Faculty of Graduate School, Chulalongkorn University, and for funding from Dr. Praneet, the Chulalongkorn University Centenary Academic Development Project – 15, in allowing me to be part of this World-Class program.

This thesis is dedicated to the people of Thailand and the Chulalongkorn University. Since my first love with Thailand in 2005, and the introduction to the passion of people of Thailand through Prof. Thep, I have been passionately inspired to contribute and support in thanksgiving. It is my earnest hope that this thesis is just the beginning of a long and prosperous relationship for the progress and health of the people of Thailand and the Chulalongkorn University.

CONTENTS

PAGE

PAGE

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AOFAS	=	American orthopaedic foot and ankle surgeons
BMP	=	Bone morphogenic protein
BSP	=	Bone sialoprotein
CD	=	Cluster of differentiation
CT	=	Computer tomography
Exfix	=	External fixation
HLA	=	Human leukocyte antigen
HSC	=	Hematopoietic Stem Cells
Infix	=	Internal fixation
MRI	=	Magnetic resonance image
MSC	=	Mesenchymal stem cells
VEGF	=	Vascular endothelial growth factor

CHAPTER I

INTRODUCTION

1.1 Background & Rationale

Lower extremity deformity due to diabetes is a high risk factor for limb loss (1). Limb reconstruction alternatives of this condition are limited and challenging, with some controversy in literature as to which fixation guidance (2-8).

Lower extremity deformity, joint and muscular instability, and ulcerations are constant factors in diabetic Charcot neuro-arthropathy when dealing with limb salvage or reconstruction situations. When non-operative treatments have reached its limitations, surgery is then indicated. The unstable and malaligned lower extremities with or without ulceration are a major clinical challenge and undertaking. This requires extensive soft tissue release and bony resection to realign the lower extremity. This type of deformity is a complex disorder, which due to the lack of number of treatments and availability of adequate surgical technology, lacks evidence-based, universally agreed upon treatment protocols (9).

Lower extremity amputation rates among the diabetic population keep rising. While amputation is an option, the International Diabetes Federation estimates that the life expectancy after major lower extremity amputation is approximately 40 percent with a five-year survival. The goals for limb salvage should be not only to prevent amputation but also to establish a long-lasting stable, ambulatory limb with a plantigrade foot.

Adult mesenchymal stem cells (MSC) are powerful orthopaedic tool for bone regeneration due to their ability to differentiate into osteoblasts (10-13). An MSC-containing matrix can provide all three

essential bone growth properties for successful bone remodeling and repair: osteoinductive, osteoconductive and osteogenic.

Early animal studies have shown promise and hypo-immunogenic response (14-16). In an athymic rat model for bone formation, L4-5 posterolateral spinal fusions revealed osteoblastic lining areas of new woven bone formation in 8 weeks. In a canine mid-femoral diaphyseal segmental defect, both the autologous and allogenic MSCs revealed healing equivalency, and no cellular immune response at 16 weeks. In a baboon fibular osteoperiosteal defects, within 12 weeks of MSC implantation, revealed various degrees of mineralization. Fluorescently labeled cells were found within the areas of newly forming bone and not in the host marrow spaces or cortical resected segment margins. Bone formation was also noted in an ectopic site using human MSCs in a rat model, revealing osteogenic activity. MSCs were noted to be differentiating directly into osteoblastic to form bone, with no evidence of endochondral osteogenesis and induced (osteoinduction) host cells noted to differentiate along an osteoblastic lineage expressing for BMP2, BPM6, BSP and VEGF.
Current ability to bring MSC containing products is based on the ability and evolution on what is known about the MSC. Because MSCs do not have Class II surface antigens and other co-stimulatory molecules it can be implanted between individuals with HLA or other matching. Class II surface antigens and other co-stimulatory molecules are required for a host to mount a T-Cell reaction to foreign cells.

The source of most MSC today is organ donor marrow. Bone marrow has two types stem cells: HSCs and MSC. The majority of cells in marrow are HSCs (Hematopoietic Stem Cells). HSCs are >99% nucleated cells, are immunogenic, and require HLA and ABO matching for allogenic transplant and are CD 45+. The HSCs are not immune privileged and must be depleted from the marrow prior to implantation. The cells are identified by being positive for surface marker CD (cluster of differentiation) 45. MSCs are present in 1/500,000 nucleated cells, are immune privileged, lack Class II antigens, are cytokine producers; BMP-2&6, VEGF, others, and are CD 105+, CD 166+. MSCs, thus, are in the minority and are identified by CD markers CD 105 and CD 166. MSCs are CD 45

negative. No single marker or set of markers is unique to the MSC but CD105 and CD166 are commonly accepted. MSCs, as cytokine factories, provide 2 important physiologic properties: Anti-inflammatory and immune-modulatory cytokine production can regulate the immune system and suppress an immune reaction; and produce BMPs for osteoinduction. MSC take advantage of the following properties due to the expression and/or secretion of various cytokines to diminish or prevent scar formation, to stimulate angiogenesis, to differentiate into different connective tissue phenotypes depending on the local environment. It is understood that MSCs are multipotential. When provided by the right cues from the environment in which they are placed they can form tissues of mesodermal origin such as bone, cartilage etc. The nature of local cues is poorly understood.

1.2 Lower Extremity Reconstruction Surgery

Lower extremity surgical intervention: Diabetic foot and related complications offer numerous challenges from the foot/ankle and wound perspective. Clinical biomechanics plays a key role in the development of ulcers and the persistence of wounds. Muscle imbalance and neuropathy once developed into the diabetic patient, become a hindrance in the healing potential of the diabetic foot. Standardized procedures and evaluation methods will be introduces in the evaluation of each patient with a diabetic foot ulcer. A comprehensive podiatric and orthopaedic evaluation will ensure proper understanding of the foot/ankle mechanics leading to development of ulcers in the diabetic patient. All wounds are different as are their corresponding foot mechanics. Proper diagnosis and assessment are key in the successful treatment of the diabetic foot.

Amputation is always an option only. As such, there are many ways to salvage the lower extremity. Saving the lower extremity will keep the patient alive and functional and prevent another amputation and ultimately their lives. Functional foot and ankle surgery, and minimally invasive amputations are key in the survival of the patient and limb. This will help guide each key future physician and surgeon to the proper and most efficient evaluation, examination, diagnosis of the foot/ankle leading to diabetic foot ulcers, its best mechanisms to prevention and long-term success.

1.3 The Diabetes Problem

Diabetes is rapidly becoming the number one disease globally, soon to surpass HIV in numbers. Diabetes is associated with various problems resulting in morbidity and mortality, one of the most significant being lower extremity amputation. The International Diabetes Federation has estimated that approximately one amputation occurs somewhere in the world every 30 seconds, as the result of diabetes related complications. The majority of amputations world wide, are on diabetic patients, with the predominant medical cause leading to amputation being the diabetic foot ulcers. Diabetic foot ulcers (DFU) are better described as ulcers resulting from prolonged pressure and/or neuropathy on the insensate diabetic foot, although other factors including impaired vascular status, injury and infection may also contribute to the final outcome. When amputation occurs personal, social and financial burdens are incurred. In countries with limited resources and limited employment, amputation may place an individual in a position where they are no longer able to work, be productive or financially or physically sustain themselves. Creating centers for diagnosis, treatment and prevention of the diabetic foot complications would significantly impact the lives of the individual while helping society the country as a whole. The barriers to attaining the aforementioned goal are lack of structured diabetic foot treatment centers, limited physician education materials, unavailable organized country specific protocols, patient education materials which are culture and lifestyle specific, and finally, the small number of individuals willing to dedicate their career and time to the establishment of country specific centers globally.

The current figures in the United States are staggering. The unprecedented increased rate of diabetes growth and diabetes related diagnoses in the United States are cause for alarm and crisis. It is estimated that there are approximately 24 million people suffering from diabetes in the US with a projection to increase to 50 million by 2040 (17, 18). The financial cost was directly related to diabetic foot ulcers and amputations in 2007 were $31 billion (19). All associated healthcare costs are alarmingly high, as data shows the total annual cost of diabetes treatment in 2002 (including direct and indirect costs) was estimated at $132 billion, or one out of every 10 healthcare dollars spent in the

United States (20). Other studies have suggested that diabetes-related amputations cost approximately three billion dollars per year (approximately $40,000 per amputation procedure) (21). With the rise of diabetes diagnoses, there is also an expected rise in the number of amputees. In latest study from Brazil, almost 46,300 (8500-80,900) limb amputations and 12,400 (2300-21,700) deaths occur as a result of diabetic foot disease each year (22). The annual cost associated with these hospital admissions is estimated to be almost $US 264 million ($US 51 to 461 million). The estimated annual cost for patients with amputation is nearly $US 128 million ($US 24.5 to 222.3 million).

1.4 The Approach

The approach to "Stem Cell Applications for Lower Extremity Reconstruction Surgery" for this doctorate proposal is to address concerns:

1.4.1 Determine if any past clinical trails and/or case studies demonstrate any efficacy.

1.4.2 Determine if a randomized trial will help demonstrate efficacy.

1.4.3 Provide healthcare providers with a template for a clinical protocol by actually conducting a clinical trial.

1.4.4 Create a comparative study with a non-treated control and different fixation methods.

1.4.5 Provide physician and health care provider with further information on stem cell use in stem cell applications in lower extremity reconstruction surgery.

1.5 The Proposal

The proposal for this PhD program is to illustrate and describe in 2 (two) manuscripts/papers on the topic of stem cell applications for lower extremity limb salvage surgery.

These manuscripts/papers will be submitted for publication and for fulfillment of the requirement for the PhD program.

1.5.1 Establish a retrospective review of patients treated in the past with stem cell applications for lower extremity limb salvage surgery and without.

1.5.2 Establish a retrospective review of patients treated in the past with stem cell applications for lower extremity reconstruction surgery with and without external fixation.

1.5.3 Extensively review past literature on the topic.

1.5.4 Program Description

The rise of diabetes and its complications, leading to preventable limb amputations and deaths, dictates a high demand for proper evaluation, assessment and diagnosis of the diabetic foot. The diabetic foot treatment subspecialty profession is a branch of the medical subspecialties devoted to the prevention, diagnosis and treatment of foot and ankle disorders, diseases and injuries.

1.6 Research Questions

1.6.1 Do past studies and literature support the use of stem cells in the surgical treatment?

1.6.2 Would the use of external fixation in the lower extremity establish a suggested benefit to the use of stem cells in the treatment of lower extremity reconstruction surgery?

1.6.3 Does an extensive review of past literature support the study thesis?

1.7 Objectives of the study

1.7.1 To review past patients treated with stem cells for lower extremity reconstruction surgery.

1.7.2 To identify benefit of external fixation with the use of stem cells for the surgical treatment.

1.7.3 To conduct an extensive literature review of currently available data on the stem cell applications for lower extremity reconstruction surgery.

1.8 Hypotheses of the study

1.8.1 Scarce results have been shown on stem cells in the surgical treatment for lower extremity reconstruction surgery.

1.8.2 An external fixation device is needed to determine if stem cells may be of benefit for lower extremity reconstruction surgery.

1.8.3 A comprehensive review of past literature is needed to provide clinicians with extensive background in the stem cell applications for lower extremity reconstruction surgery.

1.9 Conceptual framework

1.9.1 Conduct a brief retrospective review of use of stem cells applications for lower extremity reconstruction surgery.

1.9.2 Conduct comparison between stem cell use and without to determine if stem cells may be of benefit for lower extremity reconstruction surgery.

1.9.3 Conduct comparison between stem cell use and without use of external fixation to determine if this may be of benefit for lower extremity reconstruction surgery.

1.9.4 Design research plan

1.9.5 Collect data on retrospective studies and literature.

1.9.6 Collect data

1.9.7 Analyze data

1.9.8 Draft manuscript

1.9.9 Submit manuscript for publication to Journal

1.10 Research design

Comprehensive review of published literature and protocols on stem cell applications for lower extremity reconstruction surgery.

1.11 Research Methodology

1.11.1 Comprehensive review of published literature and protocols on worldwide treatment with stem cell applications for lower extremity reconstruction surgery.

1.11.2 Retrospective review of patients with lower extremity reconstruction surgery with stem cells.

1.11.3 Conduct an evaluation of external fixation benefit when using stem cells for the lower extremity reconstruction surgery. Materials and Methods: Case comparison between stem cell use and not used in single site: Bilateral: right and left foot on same patient 12 week follow up, One foot with internal fixation and other with external fixation, Bone healing in 12-week follow up, Time to clinical healing, Time to radiographic healing, Complications per fixation type and location of joints. Charts were reviewed from initial visit to final treatment and progress note. All statistics, previous treatments, co-morbidities, surgeries, wound treatments and results were recorded. Complete clinical and radiographic assessments were performed. Standard surgery procedures were followed. Bone surface preparation was performed via denuding of non-viable and articular tissues then subchondral fenestration until good viable bleeding bone level was achieved. The matrix was introduced into prepared sites and appropriate fixation was utilized. Clinical healing time (CHT) was defined as initial protected weight-bearing tolerance with little to no pain, no signs of inflammation, and full incision healing. Radiographic healing time (RHT) was defined as initial signs of bone consolidation with trabeculation and obliteration of the surgical site.

Nonunion was defined as > 6 months of no radiographic changes with or without hardware complication. Risk factors include diabetes, tobacco use, and chronic renal disease. Risks and benefits of surgery discussed with all patients undergoing surgery. Patients were not enrolled in any other study at the time of surgery and treatment wit BMA. Patients were not receiving any other experimental device.

Investigational Review Board permission obtained to review charts. Patient is already scheduled for a surgery prior to screening and enrollment. Patient willing and able to sign a voluntary informed consent. Patient must be able and willing to attend follow up visits and study examinations. Patient willing to undergo various fixation options. Inclusion: Diabetes, Charcot neuroarthropathy, Failure in non-operative treatment, Between ages of 18 and 60, ABI between 0.7 and 1.2 or Tc PO2 > 30 mmHg at the ankle or a Doppler waveform consistent with biphasic or triphasic flow. Exclusion: Patients with bone infection / osteomyelitis, Systemic bone tumor / process / Cancer, Severe anemia, ABI < 0.6, Serum albumin < 2.5

Renal failure with creatinine > 2.5, Malignancy, Pregancy.

1.11.4 Size Sampling:

n = 2 . {s/d}2 . {ta,u + t2b,u}2

Where:

n = required sample size

s = population standard deviation

d = difference desired to detect

a = desired significance level

u = degrees of freedom

b = desired type II error rate.

ta,u and t2b,u are the t statistics for the selected a, b, and u

Sample size was calculated as minimum of 8.4 per group, thus we rounded up to 10 per group to account for any technical difficulties. Thus, the total sample size is 80, which represents 8 possible types x 10 samples/type. Right foot with internal and external fixation (with and without stem cells), and Left foot with internal and external fixation (with and without stem cells). We estimate that we will have more than 80 patients to be statistically powered for this study. In fact, we estimate at least this amount per anatomical part or per surgery type which can take to 5-10 years to complete.

1.11.5 Information of Surgery:

1.11.5.1 Internal Fixation: Accomplished with larger incisions, applying internal screws and plates and requires large incision/soft tissue healing

1.11.5.2 External Fixation: Accomplished with no incisions, thin wires, no screws/plates, and requires minimal or no incision/soft tissue healing

1.12 Mission Statement

To determine the effect, if any, of stem cell applications for lower extremity reconstruction surgery.

1.13 Vision

To determine if stem cell applications is of value in the lower extremity reconstruction surgery.

1.14 Benefit of the study

To provide foundation findings to one of the most intriguing techniques of our times, using stem cells for the healing for our patients suffering with diabetes and lower extremity reconstructive surgery for limb salvage.

CHAPTER II

LITERATURE REVIEW

2.1 Introduction: Analyses of Need

A precursory review of the literature indicates minimal data is available on stem cell applications for lower extremity limb salvage surgery. A need exists to further investigate this area of potential benefit in the surgical treatment for limb salvage.

2.2 Adult mesenchymal stem cells (MSC)

These are newly discovered techniques tools in the surgery. They have studied in labs and non-human studies, and considered to be powerful orthopaedic tool for the bone regeneration due to their multiple abilities to differentiate into cells generating osteoblasts. Thus, they can provide all three essential bone growth properties for successful bone remodeling and repair: osteoinductive, osteoconductive and osteogenic.

2.3 Preliminary studies

In many non-human studies, the basic science of MSCs were discovered. The first animal studies have shown promise and hypo-immunogenic response. In the simplest models, an athymic rat model for bone formation in spine surgery for L4-5 posterolateral spinal fusions revealed osteoblastic lining areas of new woven bone formation in merely 8 weeks. Advancing to higher animals, a canine mid-femoral diaphyseal segmental defect, both the autologous and allogenic MSCs revealed healing equivalency, and no cellular immune response at the 16-week period. Furthermore, in another higher animal study, a baboon, the study looked into the fibular osteoperiosteal defects, where within 12

weeks of MSC implantation, revealed various degrees of mineralization in the bone defects. Fluorescently labeled cells were found within the areas of newly forming bone and not in the host marrow spaces or cortical resected segment margins. To study the ability for self-regeneration, another study was performed to observe for bone formation in an ectopic site using human MSCs in a rat model, revealing then the osteogenic activity. MSCs were here noted to be differentiating directly into osteoblastic to form bone, without any evidence of endochondral osteogenesis and osteoinduction host cells noted to differentiate along an osteoblastic lineage expressing for BMP2, BPM6, BSP and VEGF. In basic science, it is now know that MSCs do not have Class II surface antigens and other co-stimulatory molecules. Thus, as such, the Class II surface antigens and other co-stimulatory molecules are required for a host to mount a T-Cell reaction to foreign cells. Currently, the common source of most MSC today is organ donor marrow. As we know, the bone marrow has two types stem cells: HSCs and MSC. The majority of cells in marrow are HSCs (Hematopoietic Stem Cells). The HSCs are >99% nucleated cells, are immunogenic, and require HLA and ABO matching for allogenic transplant and are CD 45+. Thus, these HSCs are not immune privileged and must be depleted from the marrow prior to implantation. The cells are identified by being positive for surface marker CD (cluster of differentiation) 45. MSCs are present in 1/500,000 nucleated cells, are immune privileged, lack Class II antigens, are cytokine producers; BMP-2&6, VEGF, others, and are CD 105+, CD 166+. MSCs, thus, are in the minority and are identified by CD markers CD 105 and CD 166. MSCs are CD 45 negative. No single marker or set of markers are unique to the MSC but CD105 and CD166 are commonly accepted. MSCs, as cytokine factories, provide 2 important physiologic properties: Anti-inflammatory and immune-modulatory cytokine production can regulate the immune system and suppress an immune reaction; and produce BMPs for osteoinduction. MSC take advantage of the following properties due to the expression and/or secretion of various cytokines to diminish or prevent scar formation, to stimulate angiogenesis, to differentiate into different connective tissue phenotypes depending on the local environment. It is understood that MSCs are multipotential. When provided by the right cues from the environment in which they are placed they can form tissues of mesodermal origin such as bone, cartilage etc. The nature of local cues is poorly understood.

CHAPTER III

MATERIALS AND METHODS

3.1 This was retrospective observation review of 11 patients. In this cohort, there were 7 patients who underwent diabetic Charcot neuro-arthropathy foot and ankle reconstructive surgery with stem-cell bone for bone regeneration and remodeling and repair compared to 4 who did not have this type of bone tissue.

3.2 The postoperative follow up consisted on both parameters of clinical and radiographic healing times, AOFAS scores, and complications. The AOFAS scores pre and postoperative were measured. This study was approved by the Institutional Review Board at our institution. In surgery, all surgical sites consisting of bone surface preparation was performed via manual and power instrumentation of denuding of non-viable bone and tissue and articular tissues then subchondral fenestration until good viable bleeding bone level was achieved.

3.3 The stem cell bone was then introduced into prepared sites and fixation was utilized. Clinical Healing Time (CHT) was defined as initial protected weight-bearing tolerance with little to no pain, and no signs of inflammation and full incision healing. Radiographic Healing Time (RHT) was defined as initial sign of bone consolidation with trabeculation and obliteration of surgical site. Nonunion was defined as >6months of no radiographic changes with/without hardware complication. Risk Factors include diabetes, tobacco, and chronic renal disease.

3.4 A comprehensive review of past literature was performed needed to provide clinicians with extensive background in the stem cell applications for lower extremity reconstruction surgery.

CHAPTER IV

RESULTS

4.1 In the graft group, there were 7 patients (n=7). The mean age was 53 years old (25-70) with a mean follow up of 11.6 months (6-17). The mean preop/postop AOFAS were 49 and 85.

4.2 In the non-graft group, there were 4 patients (n=4), The mean age was 53 years old (24-76) with a mean follow up of 11.5 months (6-17).

4.3 The mean preop/postop AOFAS were 49 and 85. Ankle, hindfoot, and midfoot were addressed, including, ankle/subtalar/midfoot non-unions/mal-unions/deformity correction. (Table 1) The mean clinical healing time and radiographic healing times for the non- graft vs. graft groups were 4.9 vs. 6.7, ($p<0.123$) and 6.4 vs. 9.2 ($p<0.024$). There were non-unions and/or delayed unions in each group. There were no inflammatory, no immunogenic rejection symptoms in the graft group noted. For complications, in the graft group, there were hardware/external fixation failure (5); non-union (1), delayed union (2) in the ones with co-morbidities of tobacco and renal disease. In the control group, there were nonunions (2), in the ones with co-morbidities of tobacco and renal disease.

4.4 Due to the lack of extensive credible material on the treatment with stem cells for lower extremity reconstruction surgery, the result cannot be determined until clinical comparative data is completed and supported by available evidence.

4.5 Based on the information provide and the study parameters, we expect the results to reveal: Improved bone healing, Improved bone regeneration, Improved clinical outcome in the external fixation group than the internal fixation group, No difference between right and left foot

4.6 Statistical analysis

All descriptive and inferential statistical analyses will be performed using the SPSS Statistical, Versions 15 and 17 (Chicago, IL). When appropriate, continuously distributed numerical outcome data will be analyzed using Student's Independent Groups T-Tests and One and Two Factor Analysis of Variance. Categorical outcome variables will be analyzed using Person's $\chi 2$ Test of Association. All results will be considered statistically significant when $p<=.05$. When appropriate, post hoc testing will be performed on the numerical data using the Bonferroni correction method for holding the experiment wise alpha=.05.

CHAPTER V

DISCUSSION

5.1 Historical perspective

Historically, it was thought that MSCs were developmentally restricted to specific cell lineages. Now, it is known to undergo transdifferentiation by which one committed cell type is reprogrammed into a cell of another lineage. These MSC are thought to be multipotential cells capable of giving rise to tissues of mesenchymal origin, including bone, cartilage, fat, tendon and muscle. Thus, making promising candidates for cell-based tissue engineering for the repair of lost or damaged tissues.

5.2 Clinical observation

Clinically, it appears that both groups heal similarly. Of note, the decrease in inflammatory signs in the graft group is observed, but difficult to make any objective assessments at this point with our current measuring tools. The improvement in radiographic healing time between the groups may indicate increased mineralization and bone trabeculation in the surgical site of the bone graft group. It is unknown the number of MSCs require to obtain fusion. In a study done on tibial non-unions, the author noted that if there were 1500 MSCs/cc the non-unions would heal, but would not heal with lesser concentrations. This study demonstrates that minimum number of autologous MSCs injected into tibial non-union site required for healing.

5.3 Cellular healing rationale

Cellular health is directly related to donor and processing of the tissue. It is unclear if this would affect our patient population. As our patients, due to diabetes, already possess this as a co-morbidity.

It is our hope that the graft tissue has more viable healthy cells than what is found in the surgical site. It is our hope that during the procurement and processing of tissue, all three-bone growth properties of MSCs are preserved: osteogenic (MSCs and osteoprogenitors), osteoinductive (cellular BMP +/- partial demineralized cortical bone), and osteoconductive (cancellous bone matrix). Human MSCs does decline with age. MSCs showed diminishing numbers of MSCs in marrow with age.

5.4 Cellular aging

Aging of MSCs is related to cellular health. The current understanding in healing of the elderly and cellular aging prompted concerns regarding the age of cells used for cell based therapy. There are several factors associated with cellular aging: changes in quantity, changes in quality, differentiation/regeneration capacity, changed mobilization capacity, and poor bone/tissue.

5.5 Lack of data in literature

Currently, there is lack of data in our literature. Attempt to demonstrate that cell numbers required not yet known to achieve healing of a critical sized bone defect. Furthermore, there are still various limitations: anecdotal accounts, retrospective studies, preliminary clinical outcomes, heterogeneous patient population, multiple and different procedures, fixations, lack of comparison to autografts, synthetic, other biologic grafts. Future studies should focus on clinical and advanced imaging studies (i.e.; CT/MRI/Nuclear medicine) to assess the properties of these viable stem cells undergoing bone regeneration/repair procedures.

5.6 Amputation is always an option only

As such, there are many ways to salvage the lower extremity. Saving the lower extremity will keep the patient alive and functional and prevent another amputation and ultimately their lives. Functional foot and ankle surgery, and minimally invasive amputations are key in the survival of the patient and

limb. MSC take advantage of the following properties due to the expression and/or secretion of various cytokines to diminish or prevent scar formation, to stimulate angiogenesis, to differentiate into different connective tissue phenotypes depending on the local environment.

5.7 What is then known about stem cells

It is understood that MSCs are multipotential. When provided by the right cues from the environment in which they are placed they can form tissues of mesodermal origin such as bone, cartilage etc. The nature of local cues is poorly understood. Although there is strongly suggestive evidence that mesenchymal stem cells assist in wound healing, there is a paucity of validated clinical research with adequate number of subjects and well designed clinical studies to prove the efficacy of this wound therapy utilizing MSCs. Large level 1 studies are needed to determine the value of MSCs in wound therapy.

5.8 Diabetic Charcot foot problem

Charcot foot deformity leading to amputation and complications costs over U$100,000 per person. Over 20% of diabetic patients are affected per year in the US. Additionally, diabetes is not the only condition leading to Charcot. Diabetic shoes and palliative care does not help in call cases mechanical and functional abnormalities lead to this problem. Lower extremity amputation rates among the diabetic population keeps rising. While amputation is an option, the International Diabetes Federation estimates that the life expectancy after major lower extremity amputation is approximately 40 percent with a five-year survival. The goals for limb salvage should be not only to prevent amputation but also to establish a long-lasting stable, ambulatory limb with a plantigrade foot. Potential Applications of Stem Cells: No risk of transmitting infections diseases that occurs with allogeneic products. Both hematopoietic and mesenchymal stem cells are available. Stem cells consist of inflammatory cell progenitors, mesenchymal stem cells and multipotent stem cells. Stem cells and its progenitor cells have significant immunological properties and regenerative potential.

5.9 A Comparative Case of Stem Cell Application

Diabetic Charcot deformity correction / repair for lower extremity limb reconstruction is chosen. This is common in my practice and hospital. Demographics and presentation is more consistent. This is much more useful to the world and for surgeons dealing with this devastating condition for lower extremity reconstruction surgery. Stem cells have plasticity and ability to differentiate into various tissue types including bone and skin cells. Stem cells can participate in cellular activity contributing to tissue regeneration and repair. Studies are limited in their application and use in the bone and wounds, particularly the diabetic patient.

5.10 Advantage of Stem Cells

They may transdifferentiate into local cell types, may differentiate into fibroblasts, may expedite the repair process, may secrete growth factors and cytokines, and may expedite bone healing and consolidation. Bone healing is responding to applications of stem cells. There is lack of literature in this topic as it is novel. The study was useful in providing information on tolerability of stem cells. The study supports future large scales studies to support definitive efficacy and validation. The study supports a foundation for future research.

5.11 Strengths and Limitations of this study and suggestions for further study

There are clear obstacle and strategies to solve these projects. These studies we would like to present through corresponding papers for publication. They are extensive and difficult to analyze and to achieve a meaningful outcome.

5.12 Sample size

This was calculated as minimum of 8.4 per group, thus we rounded up to 10 per group to account for any technical difficulties. Thus, the total sample size is 80, which represents 8 possible types x 10 samples/type.

Right foot with internal and external fixation (with and without stem cells), and

Left foot with internal and external fixation (with and without stem cells)

We estimate that we will have more than 80 patients to be statistically powered for this study. In fact, we estimate at least this amount per anatomical part or per surgery type, which can take to 5-10 years to complete.

5.13 There are other clinical barriers for Stem Cell Application

Operative intervention, Careful patient preparation, Poor native/host bone condition, High levels of matrix metalloproteases, Poor control of underlying disease state such as arterial disease, venous return and diabetes, Poor patient nutrition / health status, Immunosuppression in select patients

CHAPTER VI

CONCLUSION

6.1 The findings of all present studies and corresponding papers will provide fundamental information, which will be essential for planning reconstruction surgery. At this time, there is known no reproducible benefit.

6.2 There is sufficient pre-clinical and animal data to move forward with this application. There is enough cases demonstrating safety and potential for use.

6.3 The pre-clinical animal, in-vitro and preliminary clinical data described above provide evidence that MSC application appears to be safe, and has potential effectiveness as graft, but it is still inconclusive and needs further validating studies.

6.4 Complex non-union and mal-union reconstructive surgeries create challenging environment for bone growth and healing; especially in the diabetic Charcot lower extremity surgery.

6.5 The ability to utilize MSCs to differentiate into the type of specialized cells is promising, Further long-term and randomized studies are needed to delineate specifics indications and applications.

REFERENCES

(1) Zgonis, T., Roukis, T.S., and Lamm, B.M. Charcot foot and ankle reconstruction: current thinking and surgical approaches. *Clin Podiatr Med Surg* 24, 3 (Jul 2007) : 505-17.

(2) Fabrin, J., Larsen, K., and Holstein, P.E. Arthrodesis with external fixation in the unstable or misaligned Charcot ankle in patients with diabetes mellitus. *Int J Low Extrem Wounds* 6, 2 (Jun 2007) : 102-7.

(3) Kusakabe, N., Takagi, M., and Tsuzuki, N. Tibio-talo-calcaneal fusion with a free vascularized fibular graft in comminuted open fractures of the talus and the calcaneus. *J Orthop Trauma* 6, 3 (1992) : 386-90.

(4) Pelton, K., Hofer, J.K., and Thordarson, D.B. Tibiotalocalcaneal arthrodesis using a dynamically locked retrograde intramedullary nail. *Foot Ankle Int* 27, 10 (Oct 2006) : 759-63.

(5) Caravaggi, C., Cimmino, M., Caruso, S., and Dalla Noce S. Intramedullary compressive nail fixation for the treatment of severe Charcot deformity of the ankle and rear foot. *J Foot Ankle Surg* 45, 1 (Jan-Feb 2006) : 20-4.

(6) Pinzur, M.S., and Noonan, T. Ankle arthrodesis with a retrograde femoral nail for Charcot ankle arthropathy. *Foot Ankle Int* 26, 7 (Jul 2005) : 545-9.

(7) Zarutsky, E., Rush, S.M., and Schuberth, J.M. The use of circular wire external fixation in the treatment of salvage ankle arthrodesis. *J Foot Ankle Surg* 44, 1 (Jan-Feb 2005) : 22-31.

(8) Mendicino, R.W., et al. Tibiotalocalcaneal arthrodesis with retrograde intramedullary nailing. *J Foot Ankle Surg* 43, 2 (Mar-Apr 2004) : 82-6.

(9) Burns, P.R., and Wukich, D.K. Surgical reconstruction of the Charcot rearfoot and ankle. *Clin Podiatr Med Surg* 25, 1 (Jan 2008) : 95-120.

(10) Jaiswal, N., Haynesworth, S.E., Caplan, A.I., and Bruder, S.P. Osteogenic differentiation of purified, culture- expanded human mesenchymal stem cells in vitro. *J Cell Biochem* 64, 2 (Feb 1997) : 295-312.

(11) Bruder, S.P., Fink, D.J., and Caplan, A.I. Mesenchymal stem cells in bone development, bone repair, and skeletal regeneration therapy. *J Cell Biochem* 56, 3 (Nov 1994) : 283-94.

(12) Livingston, T.L., et al. Mesenchymal stem cells combined with biphasic calcium phosphate ceramics promote bone regeneration. *J Mater Sci Mater Med* 14, 3 (Mar 2003) : 211-8.

(13) Rush, S.M., Hamilton, G.A., and Ackerson, L.M. Mesenchymal stem cell allograft in revision foot and ankle surgery: a clinical and radiographic analysis. *J Foot Ankle Surg* 48, 2 (Mar-Apr 2009) : 163-9.

(14) Le Blanc, K., Tammik, C., Rosendahl, K., Zetterberg, E., and Ringden, O. HLA expression and immunologic properties of differentiated and undifferentiated mesenchymal stem cells. *Exp Hematol* 31, 10 (Oct 2003) : 890-6.

(15) Arinzeh, T.L., et al. Allogeneic mesenchymal stem cells regenerate bone in a critical-sized canine segmental defect. *J Bone Joint Surg Am* 85-A, 10 (Oct 2003) : 1927-35.

(16) Bruder, S.P., Jaiswal, N., Ricalton, N.S., Mosca, J.D., Kraus, K.H., and Kadiyala, S. Mesenchymal stem cells in osteobiology and applied bone regeneration. *Clin Orthop Relat Res* 355 Suppl (Oct 1998) : S247-56.

(17) Narayan, K.M., Boyle, J.P., Geiss, L.S., Saaddine, J.B., and Thompson, T.J. Impact of recent increase in incidence on future diabetes burden: U.S., 2005-2050. *Diabetes Care* 29, 9 (Sep 2006) : 2114-6.

(18) National diabetes fact sheet: national estimates on diabetes. Centers for Disease Control and Prevention Web- site. Available at: http://www.cdc.gov/diabetes/pubs/pdf /ndfs_2007.pdf. Accessed February 3, 2010.

(19) Rogers, L.C., Lavery, L.A., and Armstrong, D.G. The right to bear legs--an amendment to healthcare: how preventing amputations can save billions for the US Health-care System. *J Am Podiatr Med Assoc* 98, 2 (Mar-Apr 2008) : 166-8.

(20) Paul Hogan, et al. "Economic Costs of Diabetes in the U.S. in 2002," *Diabetes Care* 26 (2003) : 917-32.

(21) Arran Shearer, et al, "Predicted Costs and Outcomes from Reduced Vibration Detection in People with Diabetes in the U.S.," Diabetes Care 26 (2003): 2305-10; and Adam Gordois, et al, "The Health Care Costs of Diabetic Peripheral Neuropathy in the U.S.," Diabetes Care 26 (2003): 1790-95.

(22) Rezende, K.F., et al. Predicted annual costs for inpatients with diabetes and foot ulcers in a developing country-a simulation of the current situation in Brazil. *Diabet Med* 27, 1 (Jan 2010) : 109-12.

APPENDICES

APPENDIX A

Paper #1

Title: Stem Cell Applications in Diabetic Charcot Foot and Ankle Reconstructive Surgery

Journal Published: WOUNDS 2010; 22:226–229.

APPENDIX B

Paper #2

Title: Comprehensive review of the clinical application of autologous mesenchymal stem cells in the treatment of chronic wounds and diabetic bone healing

Journal Published: International Wound Journal, (*in press*).

APPENDIX C

Paper #3

Title: Limb salvage surgery and wound treatment in the establishment of globally standardized diabetes, amputation, and limb salvage centers to address lower extremity morbidity and mortality in Thailand

Journal Published: Journal of the American College of Certified Wound Specialists, 2010; 2: 32-36.

APPENDIX D

Clinical scoring system

TABLE 1
Ankle-Hindfoot Scale (100 Points Total)

Pain (40 points)	
None	40
Mild, occasional	30
Moderate, daily	20
Severe, almost always present	0
Function (50 points)	
Activity limitations, support requirement	
No limitations, no support	10
No limitation of daily activities, limitation of recreational activities, no support	7
Limited daily and recreational activities, cane	4
Severe limitation of daily and recreational activities, walker, crutches, wheelchair, brace	0
Maximum walking distance, blocks	
Greater than 6	5
4–6	4
1–3	2
Less than 1	0
Walking surfaces	
No difficulty on any surface	5
Some difficulty on uneven terrain, stairs, inclines, ladders	3
Severe difficulty on uneven terrain, stairs, inclines, ladders	0
Gait abnormality	
None, slight	8
Obvious	4
Marked	0
Sagittal motion (flexion plus extension)	
Normal or mild restriction (30° or more)	8
Moderate restriction (15°–29°)	4
Severe restriction (less than 15°)	0
Hindfoot motion (inversion plus eversion)	
Normal or mild restriction (75%–100% normal)	6
Moderate restriction (25%–74% normal)	3
Marked restriction (less than 25% normal)	0
Ankle-hindfoot stability (anteroposterior, varus-valgus)	
Stable	8
Definitely unstable	0
Alignment (10 points)	
Good, plantigrade foot, ankle-hindfoot well aligned	10
Fair, plantigrade foot, some degree of ankle-hindfoot malalignment observed, no symptoms	5
Poor, nonplantigrade foot, severe malalignment, symptoms	0

TABLE 2
Midfoot Scale (100 Points Total)

Pain (40 points)	
None	40
Mild, occasional	30
Moderate, daily	20
Severe, almost always present	0
Function (45 points)	
Activity limitations, support	
No limitations, no support	10
No limitation of daily activities, limitation of recreational activities, no support	7
Limited daily and recreational activities, cane	4
Severe limitation of daily and recreational activities, walker, crutches, wheelchair	0
Footwear requirements	
Fashionable, conventional shoes, no insert required	5
Comfort footwear, shoe insert	3
Modified shoes or brace	0
Maximum walking distance, blocks	
Greater than 6	10
4–6	7
1–3	4
Less than 1	0
Walking surfaces	
No difficulty on any surface	10
Some difficulty on uneven terrain, stairs, inclines, ladders	5
Severe difficulty on uneven terrain, stairs, inclines, ladders	0
Gait abnormality	
None, slight	10
Obvious	5
Marked	0
Alignment, 15 points	
Good, plantigrade foot, midfoot well aligned	15
Fair, plantigrade foot, some degree of midfoot malalignment observed, no symptoms	8
Poor, nonplantigrade foot, severe malalignment, symptoms	0

APPENDIX E

Table 1

GRAFT	N	F/U	Age	Pre-AOFAS	Post-AOFAS	CHT	RHT	Exfix/Infix
Charcot	7	11.6 (6-17)	53	49	85	4.9	6.4	22 vs 5

CONTROL	N	F/U	Age	Pre-AOFAS	Post-AOFAS	CHT	RHT	Exfix/Infix
Charcot	4	11.5 (6-17)	53	49	85	6.7	9.2	14 vs 3

p<.04 p<.001 p<.001

APPENDIX F

Figure 1 Intraoperative view of stem cell bone graft

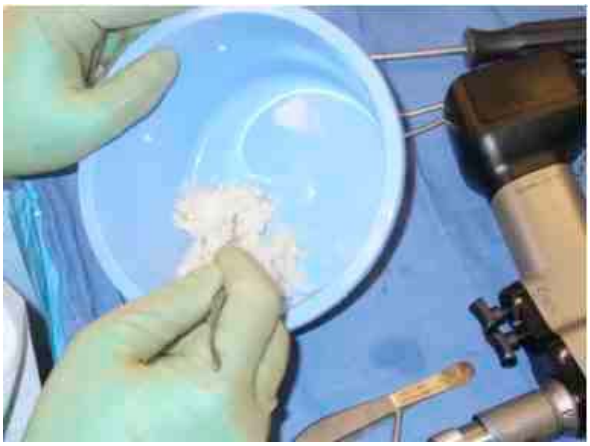


Figure 2 Intraoperative view of stem cell bone graft application into surgical site

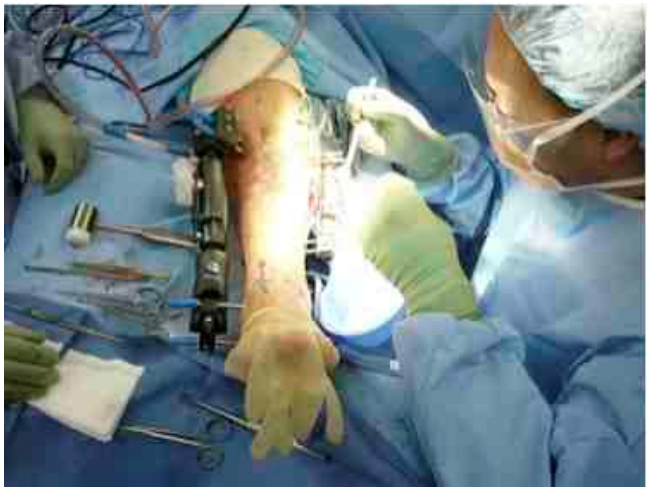


Figure 3 Intraoperative clinical view of external fixation

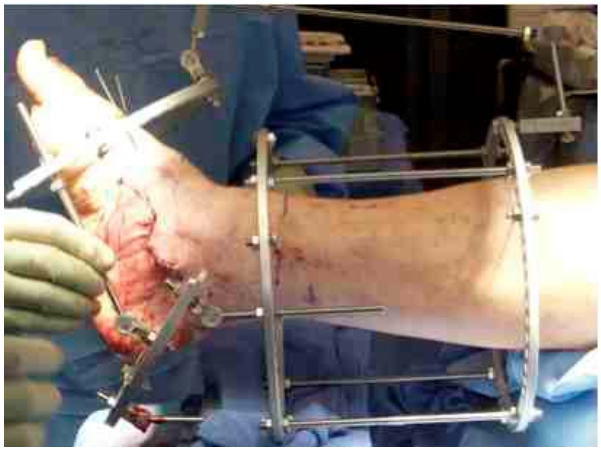
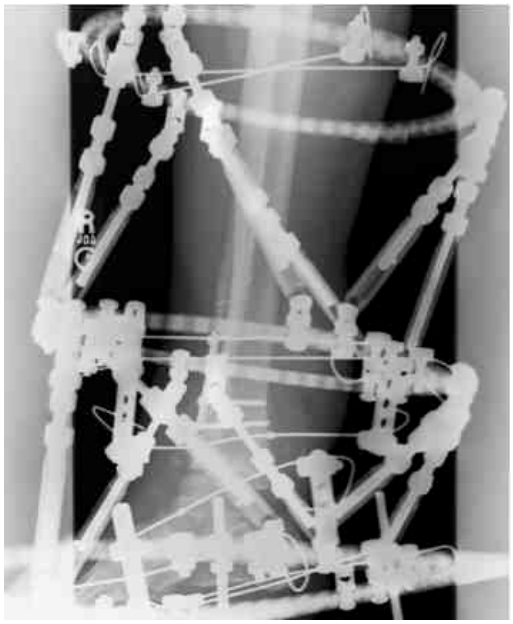


Figure 4 Postoperative imaging view of external fixation



APPENDIX G

PROTOCOL

Section: Protocol

1. Research Question: What is your research question?

   What is the optimal level of bone healing with autologous stem cell in diabetic Charcot reconstructive surgery?

2. Objectives: List your measurable study objectives.

   Predictor variable(s): AOFAS scores, Clinical/Radiographic healing times
   Outcome variable(s): Serun vitamin D level, PTH, TSH, Rate of fracture healing, non-unions, need for prolonged casting, immobilization, continuous pain, need for subsequent surgeries, Duration of treatment

   We aim to determine how autologous stem cells are associated with bone healing outcomes in diabetic charcot reconstructive surgery.

3. Significance:
   A. Summarize the background of and rationale for the proposed study; include existing relevant knowledge discussed in the literature.

   Autologous foot and ankle stem cells are readily available in Charcot surgeries. Their effects in bone healing in the light of Charcot surgery are unknown and little literature exists. Lower extremity reconstruction for the treatment of diabetic Charcot remains controversial in our literature due to lack of publications despite continuous use and evolution of these treatments methods. The application of internal fixation, consisting of screws and plates, has been widely used. However, this does not always lend itself for an appropriate correction or repair of deformity or malalignment of the bones and joints. The size and dimensions of the type of screws utilized have been in controversy, as well. The use of external fixation in general has allowed minimal incisional approach, soft tissue preservation and fracture/bone correction. Early weight bearing during the bone healing phase has allowed quicker rehabilitation with many of our patients, as compared to internal fixation and prolonged cast immobilization.

   External fixation has been described in the ancient Egyptian documents and old European documents in the treatment of fractures and injuries of the extremity. In 1950's, Dr. Ilizarov, a Russian Surgeon, described the use of pins, wires and rings as to build an external fixator for the treatment of trauma, fractures, dislocations, and

deformities.

Lately, many external fixation systems have been developed in the US. With the advances in medicine and surgery, we are able to provide more low risk and sophisticated treatment options that have not been previously available. It is still unknown how to best produce a fusion site, deformity correction or fracture care with external fixation method. Understanding the use of various available external fixators and their characteristics will allow us to minimize number of surgeries, minimize attempts for bone union, minimize non-unions and mal-unions, and thus allow a shorter recovery and return to our patients' normal quality of life.

The Northern California KP region and our South Sacramento members happen to live geographically above the 32nd parallel. We are inherently deprived Vitamin D metabolism for much part of the year.. Due to the zenith angle caused by the Earth's tilted axis, areas above the 32nd parallel receive no solar UVB rays from October to April. These are the rays that metabolize Vitamin D in our skin and without this exposure most persons in Northern California are under producing Vitamin D.

Low vitamin D levels can affect bone healing and metabolism (stress fracture, bone non-unions, poor bone healing, delayed union). To compound this effect, popular dermatological precautions in the last 20-30 years of avoiding sunlight also have the effect of further reducing serum vitamin D production in the skin.

The current best evidence suggests a lack of data on this important topic. (Michael F Holick, Tai C Chen, Zhiren Lu, Edward Sauter, Vitamin D and Skin Physiology: A D-Lightful Story. JOURNAL OF BONE AND MINERAL RESEARCH, Vol 22, Sup 2, 2007.

The ability to assess patients for optimum bone healing and metabolism is still in question due to lack of a suitable reliable biomarker and multiple other uncontrolled variables. There is however data to suggest trends: vitamin D serum levels <20 leading to nonunion, mean levels of 33 in fracture patients on supplementation over the course of 365 days post fracture, mean levels of 29 all patients post-fracture, and noted decreased serum levels (mean 25 to 21) during fracture healing, intake of 800 IU increased bone mass density after 6 weeks post-op, 1.0 microgram/day decreased osteopenia and drops in calcium and phosphate, 10-20 ng./Kg. weight/day decreased risk of bone disease). Currently, these studies rely on multiple labs (serum levels, bone density scans, radiography and bone biopsies), which are not standardized nor provide predictive information.

1: Fragomen AT, Meyers KN, Davis N, Shu H, Wright T, Rozbruch SR. A biomechanical comparison of micromotion after ankle fusion using 2 fixation techniques: intramedullary arthrodesis nail or Ilizarov external fixator. Foot Ankle Int. 2008 Mar;29(3):334-41.

Section: Protocol

2: Eylon S, Porat S, Bor N, Leibner ED. Outcome of Ilizarov ankle arthrodesis. Foot Ankle Int. 2007 Aug;28(8):873-9.

3: Salem KH, Kinzl L, Schmelz A. Ankle arthrodesis using Ilizarov ring fixators: a review of 22 cases. Foot Ankle Int. 2006 Oct;27(10):764-70.

4: McGarvey WC, Burris MW, Clanton TO, Melissinos EG. Calcaneal fractures: indirect reduction and external fixation. Foot Ankle Int. 2006 Jul;27(7):494-9.

5: Elomrani NF, Kasis AG, Tis JE, Saleh M. Outcome after foot and ankle deformity correction using circular external fixation. Foot Ankle Int. 2005 Dec;26(12):1027-32.

6: Emara KM, Allam MF. Management of calcaneal fracture using the Ilizarov technique. Clin Orthop Relat Res. 2005 Oct;439:215-20.

7: Roukis TS, Zgonis T. Postoperative shoe modifications for weightbearing with the Ilizarov External Fixation System. J Foot Ankle Surg. 2004 Nov-Dec;43(6):433-5.

8: Antoci V, Voor MJ, Seligson D, Roberts CS. Biomechanics of external fixation of distal tibial extra-articular fractures: is spanning the ankle with a foot plate desirable? J Orthop Trauma. 2004 Nov-Dec;18(10):665-73.

9: Burns JK, Sullivan R. Correction of severe residual clubfoot deformity in adolescents with the Ilizarov technique. Foot Ankle Clin. 2004 Sep;9(3):571-82.

10: Misson JR, Anderson JG, Bohay DR, Weinfeld SB. External fixation techniques for foot and ankle fusions. Foot Ankle Clin. 2004 Sep;9(3):529-39.

11: Zgonis T, Jolly GP, Blume P. External fixation use in arthrodesis of the foot and ankle. Clin Podiatr Med Surg. 2004 Jan;21(1):1-15.

12: Seibert FJ, Fankhauser F, Elliott B, Stockenhuber N, Peicha G. External fixation in trauma of the foot and ankle. Clin Podiatr Med Surg. 2003 Jan;20(1):159-80.

13: Beals TC. Applications of ring fixators in complex foot and ankle trauma. Orthop Clin North Am. 2001 Jan;32(1):205-14.

14: Shawen SB, McHale KA, Temple HT. Correction of ankle valgus deformity secondary to multiple hereditary osteochondral exostoses with Ilizarov. Foot Ankle Int. 2000 Dec;21(12):1019-22.

15: Aktuğlu K, Ozsoy MH, Yensel U. Treatment of displaced pylon fractures with circular external fixators of Ilizarov. Foot Ankle Int. 1998 Apr;19(4):208-16.

16: Stasikelis PJ, Calhoun JH, Ledbetter BR, Anger DM, Mader JT. Treatment of

infected pilon nonunions with small pin fixators. Foot Ankle. 1993 Sep;14(7):373-9.

Additional references:
Holick MF 2003 Vitamin D: A millennium perspective. J Cell Biochem 88:296–307.
Holick MF, Tian XQ, Allen M 1995 Evolutionary importance for the membrane enhancement of the production of vitamin D3 in the skin of poikilothermic animals. Proc Natl Acad Sci USA 92:3124–3126.

Tian XQ, Chen TC, Matsuoka LY, Wortsman J, Holick MF 1993 Kinetic and thermodynamic studies of the conversation of previtamin D3 to vitamin D3 in human skin. J Biol Chem 268:14888–14892.

Clemens TL, Henderson SL, Adams JS, Holick MF 1982 Increased skin pigment reduces the capacity of skin to synthesis vitamin D3. Lancet i:74–76.

Matsuoka LY, Ide L, Wortsman J, MacLaughlin J, Holick MF 1987 Sunscreens suppress cutaneous vitamin D3 synthesis. J Clin Endocrinol Metab 64:1165–1168.

Webb AR, Kline L, Holick MF 1988 Influence of season and latitude on the cutaneous synthesis of vitamin D3: Exposure to winter sunlight in Boston and Edmonton will not promote vitamin D3 synthesis in human skin. J Clin Endocrinol Metab 67:373–378.

Holick MF 2004 Vitamin D: Importance in the prevention of cancers, type 1 diabetes, heart disease, and osteoporosis. Am J Clin Nutr 79:362–371)

B. Why is this study important to Kaiser Permanente and/or the community?

The study may lead to better clinical outcomes and reduced healthcare costs. Proof of an association between stem cells and Charcot bone healing will give the opportunity to help patients at risk.

4. Methods:

A. Describe the study design, the protocols to be followed, and the methods to be used to accomplish the objectives listed in #2 above.

We propose a retrospective chart review of orthopedic patients to determine how autologous stem cells with internal or external fixation are associated with bone healing. Our study population will consist of orthopedic patients including all ages, races, and backgrounds seen in the Kaiser Permanente South Sacramento Orthopaedics Dept. for treatment of diabetic charcot (ankle, foot) from October 2009-December 2011. We will include only patients who have a diabetes with Charcot deformity. We will collect data on patient demographics, vitamin D levels, clinical

Section: Protocol

outcomes, and healthcare cost analysis. All data will be entered into an electronic data collection instrument. Data will be described using simple statistics. Comparisons will be made between recorded fixation types and outcome indicators of bone healing.

B. Identify and explain any experimental procedures, investigational and non-formulary medications or devices, and clearly distinguish how these differ from KPNC routine care. If the study involves an investigational drug, device, or biologic, state who will administer or dispense the drug, device, or biologic to the participant.

This is a purely observational study with no experimental interventions.

C. What is the rationale for the number of participants selected for the study, including sample size/power calculations, and the statistical method(s) by which the data will be analyzed?

We propose a convenience sample of orthopedics patients seen at Kaiser Permanente South Sacramento. We believe that we will find ample numbers of eligible patients to gather an adequate sample size. We plan to use this study as a pilot project in order to devise a future prospective cohort study.

Sample size was determined based on variability of levels and types of bone presented in the preliminary studies in the literature, which was about 50%, and looking for a difference in level changes of 50-60%. Study power was selected to be 80%. Using the equation:

$$n = 2 \cdot \{s / d\}^2 \cdot \{t_{a,u} + t_{2b,u}\}^2$$

where n = required sample size

s = population standard deviation

d = difference desired to detect

a = desired significance level

u = degrees of freedom

b = desired type II error rate.

Section: Protocol

taµand t2bu are the t statistics for the selected ąb and u

Sample size was calculated as minimum of 8.4 per group, thus we rounded up to 10 per group to account for any technical difficulties. Thus, the estimated total sample size is 80, which represents Sample size was calculated as minimum of 8.4 per group, thus we rounded up to 10 per group to account for any technical difficulties. Thus, the total sample size is 80, which represents 8 possible types x 10 samples/type: Right foot with internal and external fixation (with and without stem cells), and Left foot with internal and external fixation (with and without stem cells) x 10 samples/level. We estimate that we will need more than 80 patients to be statistically powered for this study. In fact, we estimate at least this amount per anatomical part or per surgery type due to our large group of co-investigators involved in this study.

D. Describe the sequence of the components of the study, including the expected duration of each component and timeline for the study.

We plan to obtain IRB approval in January 2012. We will obtain medical record numbers for potentially eligible patients in December 2011. We will then begin chart review in January 2012 and continue through May 2012. We will perform data analysis in June and July 2012.

5. Confidentiality: Describe how you will assure protection of non-electronic study data and other study documents containing participant information (e.g., screening logs, cassette tapes, hardcopies of medical records, consent forms, letters) collected during the course of this study. Note: Protection of electronic study data is examined in section F. Locked office/cabinet, only accessible by investigator, and destroyed.

6. Participant Recruitment:

A. How will you assure equitable selection of participants/records (i.e., no group will be arbitrarily excluded from participation and no group will bear the burden of increased risk)?
B. Will participants be contacted and recruited into the study? [ ]

Section: Protocol

Yes [X] No
If No, skip to question 7.

If Yes, check all method(s) of contact with participants

[ ] Referral from TPMG physicians or KP providers or employees

[ ] Investigators' own patient panels

[ ] In-person at KPNC

[ ] In-person elsewhere

[ ] Telephone calls – submit script

[ ] Letters/post-cards to participants' homes – submit letter/post-card

[ ] Survey/questionnaire(s) mailed to participants' homes – submit survey

[ ] Other internal KPNC methods (e.g., flyers, prior KP studies) – submit material(s)

[ ] Other external methods (e.g., newspapers, Internet, community

Section: Protocol

presentations) – submit material(s)

[ ] Other:

C. Describe in detail the process for recruiting participants.
D. Will participants be compensated?
[ ] Yes [ ] No
If Yes, specify how much and when they will be compensated.

7. Vulnerable Populations

NOTE: Special attention is required for minors and pregnant women per federal regulations.

Does the study protocol allow for the inclusion of any vulnerable populations (participants and/or their medical records)?

[X] Yes, the study may include vulnerable populations.
If Yes, check the study populations that may be included as study participants:

[X] Minors (under age 18)	[X] Pregnant women
[X] Diminished cognitive capacity	[X] Developmentally disabled
[ ] Terminally ill	[X] KP employees / TPMG physicians
[X] Non-English speakers	[X] Educationally or economically disadvantaged
[X] Undocumented immigrants	[ ] Other:

If Yes, describe the additional safeguards that will be used to protect the rights and welfare of the population(s) identified:

This is a data-only chart review study.

Section: Protocol

[ ] No, the study **does not allow** for the inclusion of any vulnerable populations, including minors or pregnant women, at any time.

8. Informed Consent: *Informed consent is the process by which an individual learns about a study and its potential benefits and risks to determine if he/she wishes to participate. Typically, a research participant's decision to participate in research is documented by a signature on a consent form. The informed consent process continues until the study is complete. Note: Surgical consent is a separate and different process.*

Check all that apply. Note: If more than one box checked, indicate for which study component the consenting process or waiver is intended

[ ] I plan to obtain written informed consent – **complete box A**

[ ] I plan to obtain informed consent, but I am requesting a waiver of the requirement for participants to sign a written consent form for this study – **complete box B**

[X] I am requesting a waiver of informed consent for this study – **complete box C**

Box A: Obtaining written informed consent:

A1) Describe the informed consent process.

A2) Specify the organizational affiliation, position, training and licensure of the person(s) who will obtain consent.

Box B: Requesting waiver of the requirement for participants to sign a written consent form:

B1) Check one and describe how your study meets this criteria.

[ ] The research involves no more than minimal risk to participants and involves no procedures for which signed consent is normally required outside of the research context. Describe.

OR

[ ] The signed consent document would be the only record linking the participants to the research, and the principal risk to participants would be potential harm resulting from a breach of confidentiality. Describe.

B2) Describe the informed consent process.

B3) Specify the organizational affiliation, position, training and licensure of the person(s) who will obtain consent. If not applicable, explain.

Section: Protocol

Box C: Requesting waiver of informed consent:

C1) Describe how the research involves no more than minimal risk to the participants:

This is a retrospective, data-only chart review study. The only potential risk would be a breech of patient confidentiality. All patient lists will be stored on the KPNC server behind the firewall. Once data is abstracted, each case will be assigned a unique identifier and otherwise de-identified. The key containing patient identifying information and unique identifiers will be stored separately. This system should ensure that any breech of PHI is extremely unlikely.

C2) Describe how the waiver will not adversely affect the rights and welfare of the participants:

This is a data-only study with minimal risk. We will take steps to ensure there is no breech of patient confidentiality.

C3) Describe how the research cannot be practicably carried out without the waiver:

Obtaining consent would require contacting each patient which is not practical.

9. Will you provide additional pertinent information to study participants after they have participated in the research? [ ] Yes [X] No, not appropriate

If Yes, describe:

10. Risks and Benefits:

A. Describe potential and anticipated (including frequency and severity) physical, psychological, social, and economic risks to research participants. Note: Privacy risks are examined in detail in section F. If you believe that none of these risks exist for this study, describe why.

This is a data-only study with minimal risk. The only potential risk would be breech of confidentiality. We will take measures to ensure that no breech of confidentiality occurs (as described).

B. How have the study's risks been minimized by the study design and methods?

As stated above, all patient lists will be stored on the KPNC server behind the firewall. Once data is abstracted, each case will be assigned a unique identifier and otherwise de-identified. The key containing patient identifying information and unique identifiers will be stored separately. This system should ensure that any breech of PHI is extremely unlikely.

Section: Protocol

C. How will participant safety be monitored and assured?

The primary investigator will closely supervise all data collection and take responsibility for all data management. Any potential breech of confidentiality will be reported to the IRB.

D. What are the benefits of the study to participants and to society?

There are no direct benefits to participating patients. Research that will allow us to improve care of bone healing will benefit orthopedic and other patients clinical outcomes.

E. How are potential risks to participants justified by potential benefits?

N/A

F. Does this study have a Data and Safety Monitoring Board? [ ] Yes

11. Genetic Testing:
    A. Will genetic testing be part of this study? [ ] Yes
       If No, skip to question 12.
    B. Identify the procedures/tests and the organizational affiliation of those who will be conducting them.
    C. Will the results of any genetic tests be given to study participants, their family members, or their providers? [ ] Yes
    D. Explain which genetic test results will be communicated and how.
    E. Describe the storage and disposal protocol for the genetic material.

12. Specimen (e.g., blood, tissue) Collection and Storage:
    A. Will specimens be collected as part of this study? [ ] Yes
       If No, skip to question 13.
    B. Identify the specimens and the procedures/tests to be performed.
    C. Will specimens be stored as part of this study? [ ] Yes
       If specimens will be stored, describe the storage location, how specimens will be stored (where, how long, under whose control) and how they will be disposed of.
       Will specimens be banked for future research? [ ] Yes
       If Yes, describe the purpose.
    D. Will any specimens be sent outside the organization? [ ] Yes

Section: Protocol

If Yes, describe the specimen, associated identifying information, and recipient:

E. Will the results of tests done on any specimens be given to study participants, their family members, or their providers? [ ] Yes

If Yes, explain which test results will be communicated, and when and how they will be communicated.

13. Radiation Exposure: *See SOP KP-201: KP Research Use of Radiation Procedures, Radioactive Drugs, Radiopaque Contrast Agents, and Radiopharmaceuticals*

A. As part of this study, will any research participant be exposed to more ionizing radiation than occurs in the course of standard care? [ ] Yes

If No, skip to question 14.

If Yes, your research protocol will require review by a radiation safety committee,

complete B and C below.

B. Provide details.

C. List the facility in which the radiation will occur.

14. Gene Therapy / Gene Transfer / rDNA / Biohazardous Agents:

Does the study involve any of the above? [ ] Yes

If Yes, describe.

15. Risks to KP:

Is there anything about the nature of this study which, if revealed to the public, could put KP at risk or competitive disadvantage? [ ] Yes

If Yes, describe in detail.

16. Research Study Sites:

A. List all KPNC facilities in which study activities will take place:

Note: You will be asked to obtain approval signatures for each of these facilities in section I.

SSC

B. Will non-KPNC institutions be participating in this study [ ] Yes

(other KP regions, universities, contract research organizations [CROs],

other healthcare organizations, etc.)?

If Yes, list the institutions and describe their study roles.

BIOGRAPHY

Prof. Dr. Daniel Lee is Director of Residency Program, at the Trauma Regional Medical Center in the Department of Orthopaedic Surgery / Podiatric Surgery at the Kaiser Permanente South Sacramento Medical Center. He is committee head for Podiatric Surgery for the Graduate Medical Education and Professor, Drexel University, School of Medicine, and Western University College of Podiatric Medicine and Biomechanics. He is active in the leadership as fellow of the American College of Foot and Ankle Surgeons, and US-Board-Certified in Foot and Ankle Surgery. He is active as national and international speaker in foot and ankle surgery, diabetic foot, lower extremity reconstruction, biomechanics, sports medicine, limb salvage and revision surgery, and journal reviewer for multiple journals. He graduated with two bachelors degrees in Biochemistry and Spanish Literature, obtained his medical doctorate degree from medical school with scholarship at the California School of Podiatric Medicine, and subsequently completed 3-years of Internship and Residency at the University of Southern California Medical Center. He then completed three fellowships in advanced training in Biomedical Sciences at the prestigious National Institute of Health in Bethesda, Maryland; and in orthopaedic surgery at the famous Schulthess Klinic in Switzerland and at the Russian Ilizarov Scientific Center in Siberia, Russia. He has studied for Doctorate Degree PhD in Biomedical Sciences Program, Graduate School, Chulalongkorn University