出國報告（出國類別：進修）

應用組織工程治療重大骨缺損之轉譯研究

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摘要

發展組織工程爲現代醫學的重大課題之一，也是生物技術產業的明日之星。其中，轉譯研究是連結基礎研究與臨床應用的橋樑，重要性不可言喻。完整的轉譯研究需要建立一個穩定可靠的動物實驗模型與齊備的最終分析技術，如此便能廣泛運用再各種生醫材料或細胞治療的研究上，達到事半功倍的效果。此次赴美國密西根大學骨科實驗室進修，參與的研究計劃分別爲：研究結合週邊注射幹細胞與機械力刺激治療大鼠股骨幹重大骨缺損的效果、研究去礦化骨基質(DBM)與幹細胞及機械力刺激治療大鼠股骨幹重大骨缺損的效果。計畫內容完整涵蓋了動物實驗、幹細胞培育、製備去礦化骨基質、組織學、微電腦斷層測試、生物力學測試及相關分子生物學技術。初步研究成果發表於 2008 American Orthopaedic Research Society。

關鍵字：組織工程，重大骨缺損，幹細胞，去礦化骨基質

目次

本文

一、研究目的

過去的五十年是骨科發展史上輝煌的一頁。藉由生物力學與材料科學的進步，受傷的肢體，在各種內、外固定器的幫助下，可以快速的恢復功能，而磨損不堪的關節可以接受人工材料的替換而延續功能，人們的生活品質獲得了實質的改善。然而，仍然有許多問題沒有解決，其中一項重要的議題是骨骼的重大缺損。這些缺損來自於創傷，來自於腫瘤、感染甚至是先天發育上的缺陷；目前的解決方案是：壹、利用自體骨移植，例如說腓骨、肋骨、腸骨骨移植等，但缺點是數量有限，而且可能產生捐贈處的併發症。貳、利用牽引生骨術(distraction osteogenesis)，使自體骨循牽引方向生成，但是手續繁複，而且病患必須忍受沉重外固定器的不便，與常伴隨而來的肢體僵硬。叁、利用異體骨移植，但它的癒合率不及自體骨移植，且有感染之虞，更何況在臺灣，骨骼捐贈的意願顯然不及其他器官。肆、利用生醫材料，例如生物陶瓷，包括了珊瑚質(hydroxyl appetite)、磷酸鈣、硫酸鈣等。然而它的問題在於是否能夠有效的與自體骨整合，進一步成爲真正的骨骼。綜觀上述的四種方法，都各有其優點和限制。

生醫材料在數量上沒有限制，透過構型設計可以提供結構強度；而幹細胞具有不斷複製及受引導分化的特性，可以成爲造骨細胞來製造骨骼；其他諸如 BMP(bone morphogenic protein) 等可以刺激骨生成的因子都可以成爲支持骨生成的來源。結合了生醫材料與細胞的組織工程學就成了解決重大骨缺損最有潛力的方案，也是骨科未來最有發展的領域。

轉譯研究是連結基礎研究與臨床應用的橋樑，重要性不可言喻。完整的轉譯研究需要建立一個穩定可靠的動物實驗模型與齊備的最終分析技術，如此便能廣泛運用再各種生醫材料或細胞治療的研究上，達到事半功倍的效果。

二、過程

密西根大學(University of Michigan)位於密西根州的 Ann Arbor，位處美國中西部，四季分明，每年雪季約有四至六個月。Ann Arbor 是一個典型的大學城，人口約 12 萬人，治安良好，生活費及基本物價較其他大都會區來得低。密西根大學的醫學院名列全美前十大，其骨科部骨科實驗室於骨科生物力學基礎研究亦享有盛名。目前實驗室主持人是 Professor Steve Golstein，專長即爲生物力學。實驗室所在的 Biomedical Science Research Building (BSRB) 擁有完整的動物實驗中心，設施及門禁管制比照醫院開刀房，並設有專職照護人員及獸醫師。骨科實驗室除了專用開刀房外，有自己的 mechanical shop 可自行製造所需工具及載具或固定器，擁有 MicroCT、DEXA、Faxitron、MTS 等。實驗室設有骨科組織學處理單位，有專職技工兩名，可處理各種脫鈣及未脫鈣之組織切片、染色，也可處理帶金屬組織之切片。實驗室也設有分子生物研究室，可進行細胞培養及其他各種分子生物學實驗；設備相當完善。

本人於 96 年 6 月底抵達 Ann Arbor,向國際中心報到並完成各式身分證件申請及保險辦理及宿舍安頓所需的手續後,於 7 月 1 日展開實驗室研究工作。研究計劃分別爲：研究結合週邊注射幹細胞與機械力刺激治療大鼠股骨幹重大骨缺損的效果、研究去礦化骨基質(DBM)與幹細胞及機械力刺激治療大鼠股骨幹重大骨缺損的效果。兩項計畫都需要使用大鼠動物模型，由於手術原爲本人之熟練技術，動物手術並無困難；然根據其大學規定，仍然需接受各種指定之動物操作學習講習方能接觸動物，可見其嚴

謹。第一項計畫所測試之治療方式爲靜脈注射幹細胞然後施以電腦控制之機械刺激，其先驅實驗在本人加入前不久已經開始；本人加入後，除了負責動物手術及電腦控制之機械刺激之外，也負責顯微電腦斷層掃描及組織學部分的分析。該項計畫爲求嚴謹，所使用之有效動物數目高達 140 隻以上，連同執行實驗到最終分析工作量十分龐大；所幸該實驗室不但硬體設備齊全，更有許多技術人員可以諮詢及幫忙，獲益良多。此外，原本實驗設計中打算利用以螢光顯微鏡追蹤綠螢光幹細胞，然因組織自發螢光及其他問題無法有效辨別；經本人建議及克服相關問題，成功以免疫組織化學分析在未脫鈣之塑料包埋切片中顯示綠螢光細胞的分佈，不僅對研究計畫提供實質貢獻，本人也在過程當中有所收穫。經過團隊的努力合作，相關研究成果發表於 2008 American Orthopaedic Research Society 以及 Midwest Tissue Engineering Conference。第二項計畫利用去礦化骨基質(DBM)攜帶幹細胞，直接植入股骨缺損部位。由於先前的合作經驗，實驗室賦予本人建立製造去礦化骨基質(DBM)之任務；本人除了完成該項任務，也建立了第二項計畫中實驗細部技術(例如去礦化骨基質與幹細胞、甘油的比例，以確保細胞活性和手術植入時之可操作性)和其先驅實驗。不過由於計畫龐大耗時，而本人進修期限屆滿，無法繼續完成；該計畫目前轉由他人接手繼續完成。

三、心得

發展組織工程中，轉譯研究是連結基礎研究與臨床應用的橋樑；完整的轉譯研究需要建立一個穩定可靠的動物實驗模型與齊備的最終分析技術，如此便能廣泛運用再各種生醫材料或細胞治療的研究上，達到事半功倍的效果。此外，轉譯研究也是臨床醫師最適合發揮的領域。事實上，許多的研究技術榮陽團隊都能勝任有餘，然而比較缺乏縱向與橫向的資源整合；如果可以加以整合，相信一定可以將研究能量放大。

四、建議事項

1. 希望骨科部可以整合多人意見，共同發展一或數種共通動物實驗模型，並與其他研究團隊整合出完整最終分析之資源，如此一來便可與相關各領域之研究合作，提高研究之量與能。
2. 密西根大學聘請曾擔任或現職於各大生物醫學期刊雜誌編輯顧問之語文系教授開辦論文寫作課程；重點針對各種投稿策略、寫作技巧授課解惑，也分析各種實戰經驗。該語文中心已經受邀與日本大學舉行類似課程多次，建議本院教研部可以考慮延攬類似師資進行定期或不定期之短情課程。
3. 希望可以根據進修者計畫及意願，將進修時間延長至兩年。

五、附錄

研究計劃

Title

Effect of combined controlled mechanical loading and systemic mesenchymal stem cells on fracture healing

Introduction

Fracture healing is a complex process involving numerous cell types, and a variety of spatially and temporally related regulators. Many different factors influence the healing process. Among these, mechanical forces have been shown to play an important role in the extent and character of the repair process. While prior studies have investigated the effect of physical forces on cell differentiation, biofactor expression, and mechanical competence of repair, the mechanosensory and response mechanisms are poorly understood. This study examines the temporal effects of a controlled mechanical environment placed across a fracture gap at different stages of the repair process. Specifically, this study was designed to investigate the timing of mechanical load and its effects on systemic mesenchymal stem cell (MSC) homing and local cell behavior during fracture repair.

Methods

Sixty-two, 6-month-old, male, Sprague-Dawley rats underwent a 2mm segmental osteotomy in the mid-diaphysis of each femur. Briefly, after a 1cm exposure and elevation of the soft tissues, four 0.062-inch diameter threaded pins were placed through predrilled holes made in the diaphysis using a specialized guide. A two-piece external fixator with locking plate was then affixed to the pins. An osteotomy was created with an oscillating saw, and the surrounding tissues were then closed.

MSCs were harvested from 2- to 4-month-old green fluorescent protein (GFP) transgenic rats and cultured in growth medium containing 10% fetal bovine serum (Hyclone) at 37°C. Non-adherent cells were removed after 24 hours and the culture medium was changed three times per week. After 12-14 days, the cells were released from the cell culture plate with 0.25% trypsin for 5 minutes and replated at a density of 700,000 cells per 10cm culture dish. After reaching confluence, the process was repeated and these cells were considered second passage cells. After 24 hours in standard growth medium, the medium was removed and replaced with a serum free defined medium consisting of a 60%/40% mixture of DMEM/MCDB1 (Gibco/Sigma) containing 1% antibioitic/antimycotic (Gibco), 1% LA-BSA (Sigma), 0.01% PDGF-β (Cell Signaling), 0.001% bFGF (Cell Signaling), and 0.05% insulin (Sigma). In preparation for cell injection, these second passage cells

were released with trypsin and resuspended in 1 ml PBS at a concentration of 1 million cells per ml. Systemic injections of these cells were performed via the tail vein immediately prior to mechanical loading.

Axial mechanical stimulation was performed with a linear precision table and servo controlled stepper motor. The system provides controlled axial motion with displacement monitored by an LVDT. The rats were placed in a sling so that the fixator could be properly aligned, and the locking plate was removed once secured in the loading device. Mechanical stimulation occurred for five consecutive days beginning at 0, 3, 10, or 24 days post-operative (groups A through D respectively) at a magnitude of ±8% strain and a rate of .313 Hz for 510 loading cycles. Rats were euthanized 10, 24, or 48 days post-operative.

Immediately after sacrifice, both femora were excised and surrounding soft tissue was removed without disturbing the callus around the fracture site. A temporary fixator was then placed adjacent to the existing fixator to facilitate the removal of the original fixator and pins. Bones were then scanned via *ex-vivo* μCT (GE Health Systems) at a voxel size of 18 microns. A region of interest was created encompassing the 2mm osteotomy site after subtracting the cortical bone from the region. After scanning, specimens were embedded in PMMA, cut into 5μm thick sections, mounted, and then either left unstained to look for GFP activity or stained using toluidine blue or safranin-O and fast green.

Eight additional animals were analyzed for progenior cell migration using single photon emission computed tomography (SPECT). For injection into these animals, the MSCs were respuspended in PBS at a concentration of 1 million cells per ml. $Indium^{111}$ was added to the cell suspension and allowed to diffuse into the cells for 30 minutes. The suspension was then centrifuged and any free floating $indium^{111}$ was removed from the supernatant. Cells were injected prior to loading as above, and the animals were scanned and loaded using SPECT for three consecutive days.

Ratios between loaded and unloaded limbs analyzed via μCT were calculated for all outcome variables with unity indicating no change within groups, and a student's t-test was used to determine differences in the means within groups. A Kruskal-Wallis test was used to determine differences in the means between groups. All experimental procedures were approved by the University Committee on Use and Care of Animals.

Results

Data from the early time points indicate a trend toward an initial inhibition in callus formation due to load. As healing progresses, the loaded limbs not only recover the lost

callus size, but exceed their unloaded controls as can be seen from the data at day 48 (Figure 1). In the groups loaded 0 or 3 days post-surgery (groups A and B), the BMD ratio remained unchanged throughout the healing process, indicating that even though the callus volume varied over time, the proportion of mineralized tissue within the fracture callus was equal within these groups. However, for the groups loaded 10 and 24 days post-surgery (groups C and D), there was an increase in BMD by day 48 indicating that not only did these groups form a larger callus by this time point, but that this callus also formed more mineral. The tissue mineral density across all groups and time points remained the same (data not shown) indicating that load did not influence the timing of mineralization.

SPECT images from animals injected and loaded 0 or 3 days post-surgery showed little sign of activity at either fracture site with most of the activity concentrated in the lungs and liver. Animals that were loaded 10 or 24 days post-op showed movement of the MSC out of the lungs and into the fracture sites, with some of the images suggesting a preferential homing to the loaded fracture site (Figure 2).

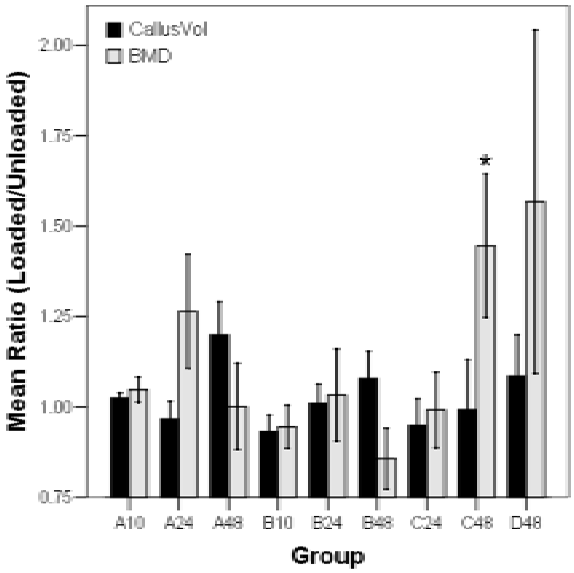


Figure 1: The ratio of the callus volume between the loaded and unloaded limb shows an initial decrease in callus size due to load, but at later time points load seems to increase callus size. BMD is unchanged in groups A and B and increases in groups C and D indicatiing that loading during the later stages of fracture healing may promote a higher proportion of mineral. (* indicates significance within group, $p<.05$)

Figure 2: This SPECT image from a rat injected and loaded 24 days post-op shows a preferencial homing of the radiolabelled MSC to the loaded fracture site. There was no MSC activity found in the limbs of animals loaded 0 and 3 days post-op, while there was

measurable activity in the limbs of 10 and 24 day animals.

Discussion

It appears that load initially inhibits callus formation, but by day 48 not only has the callus size recovered, but it has also increased. It also appears that a delay in loading creates a callus with a higher proportion of mineral by day 48, as indicated by results from groups loaded 10 and 24 days post-operatively. With the exception for the BMD value for the C48 group, none of the values reached significance. This was due to the high variability between animals, which will be accounted for as more animals are added to the study.

The SPECT images show that we are able to measure MSC migration to the fracture sites, and it appears that this migration is affected by the local mechanical environment, though it is too early to tell if this is the case. The scans from time points early in the fracture healing process did not show any migration to the osteomies indicating that the initial hematoma may not be an environment conducive to MSC migration. In addition to more SPECT scanning, specimens from rats injected with the GFP MSCs will be processed for histology and analysed through flouresceent microscopy for the presence of exogenous MSC activity.

Preparation of Demineralized Bone Matrix

(Developed by 蘇宇平)

Retrieval of femurs of rat

In the OR

- Standard aseptic setup in OR. Put the rats on Haloxane anesthesis
- The diaphyses of the femurs, tibias and humerus of adult Sprague-Dawley rats are retrieved via lateral longitudinal incisions. Remove the attached soft tissue. Immerse the bones with NS in a sterilized 300ml beaker.
- Euthanize the rats right after surgery

In Dissection Room

- Aseptic maneuver through the whole procedure
- Set up an aseptic area with sterilized blue drape.
- Clean medullary canal. Cut cortices into 1~3mm segments by bone cutter. Wash with normal saline.
- Bones will be stored at 4℃ temporally or -80℃ permanently if needed.

Defatting

- Transfer the cortical bone pieces to 1:1 chloroform-methanol, 1:100 solid to liquid phase for 4 hours at 25℃.
- Wash the bones 3 times with copious amounts of distilled water (change water for 2 hours)

Drying and storage

- Immerse with absolute ethanol for 1 hour
- Change to Ethyl ether for 0.5 hour
- Dry at 37℃ overnight and stored

Homogenization

- Place the bone pieces (Size should be less than 3mm) into an aseptic round bottomed, white plastic tube.
- Attach the sterilized generator shaft to the body of homogenizer in aseptic manner.
- Put the head of homogenizer into the plastic tube. Carefully turn the speed up to 8 (on old homogenizer) and last for 20, 10 and 10 seconds consecutively. Particle size had been measured 50~450 μm.

Demineralization

Place and weight the particles on a plastic dish. Take Faxitron (25 kv, 4 sec, 4X)

0.6 N HCL (100ml : 1gm), 24 hours at 2℃

Dump HCL. Particles are neutralized with Na Phosphate dibasic buffer (300ml:1gm)

Wash with copious amounts of water (change water for 2 hours)

Absolute ethanol 1 hour, ethyl ether 0.5 hour

Dry at 37℃ overnight

Take Faxitron (25 kv, 4 sec, 4X)

How to make 0.6N HCl

Concentrate HCL (36.5-38%) = 10~10.4 N = 10N (HCL=36.45)

10 V1=0.6V2, V2=100V1/6

	Concentrate HCL	Add H2O to
100ml 0.6N HCL	6ml	100ml
1000ml 0.6N HCL	60ml	1000ml

Using IHC to track GFP(+) MSC on PMMA-embedding slide

Developed by 蘇宇平

IHC protocol for Green Fluororecent Protein in rat plastic slide

Deplasticising, Rehydration, Decalcification (all at room temperature)

1. Deacrylize plastic slides with 1:1 mixture of xylene and chloroform for 30 minutes.
2. Couple dips in Xylene, rehydrate the slides through 100% EtOH 2min, 95% EtOH 2min, 70% EtOH 2min and then couple dips in distilled water(dH2O).
3. Decalcify the slides with 8% Formic acid for 10 min. Dip slides in dH2O jar for 30 seconds with one change of dH2O.
4. Drain off the fluid, wipe margins with Kimwipes

For proteinase K digestion

1) Prepare TE buffer (50 mM Trise base, 1 mM EDTA , pH8.0)
2) Prepare proteinase K stock solution (25X, 10mg/ml)
   Weight out protinase K 10 mg dissolved in 1 ml of TE buffer, aliquot and store at -20 ℃
3) Prepare proteinase K working solution with TE buffer (1X, 400ug/ml).
   40ul 25X Stock solution + 960ul TE buffer
4) Cover sections with Proteinase K working solution and incubate for 20 minutes at 37 °C in humidified chamber.
5) Allow sections to cool at room temperature for 10 minutes.
6) Drain the fluid off the slides with dH2O, then dip in dH2O jar for 5 min with 1 change of dH2O

Quenching, Blocking, Primary antibody incubation

1. Put the slides in the wet box
2. Quench endogenous peroxidase by a 10:1 mixture (be freshly made) of methanol and 30% H2O2 for 30 min, drain off the fluid, followed by 2 washes with dH2O.
3. Wash and cover the sections with 0.1%TPBS twice, 5 min of each
4. Apply blocking solution (10% of goat normal serum, Vector, in 0.02% TPBS containing 1.5%BSA) for 30 min @RT
5. Gently drain off the excessive blocking serum then, without any washing, wipe margins with Kimwipes. Apply primary antibody solution (1:1000 Rabbit anti-rat GFP, Chemicon, diluted with 0.02% TPBS containing 1.5%BSA) overnight @4 ℃

6. Drain off the fluid, gently wash twice and cover the sections with PBS for 5 min. Drain off the fluid again and wipe margins with Kimwipes.
7. Apply secondary antibody (1:500 biotinylated goat anti-rabbit IgG(H+L), Vector, diluted with 0.02% TPBS containing 1.5%BSA) for 45 min @RT
8. Wash twice and cover the sections with PBS for 5 min.
9. Prepare Vectastain Elite ABC reagent (in 15 min before use)
    i. Add exactly 1 drop of reagent A to 5 ml of 1XPBS, mix
    ii. Add exactly 1 drop of reagent B to the above mixed reagent
10. Drain off the fluid and wipe margins with Kimwipes, then, incubate sections with ABC reagent for 30 min @RT
11. Wash twice and cover slides with PBS for 5 min.
12. Prepare Vector Nova Red substrate solution (in 5 min before use) with kit
    i. Add 3 drops of Reagent 1 to 5ml dH2O (not ddH2O), mix well
    ii. Add 2 drops of Reagent 2, mix well
    iii. Add 2 drops of Reagent 3, mix well
    iv. Add 2 drops of Hydrogen Peroxide solution, mix well
13. Incubate Nova Red solution until the desired stain intensity develop (5-15 min)
14. Wash sections in dH2O for 5 min.
15. Counterstain (if needed), Rinse with water
16. Dehydrate through serial alcohol: 75%EtOH 2min, 95% EtOH 2min, 100% EtOH 5min, xylene 15 min, then mount with coverslip.

Effect of MSC-impregnated DBM on healing of femoral critical bone defect.

This pilot study will be conducted to testify the readiness of the local delivered MSC used in the part II of the DOD project and to provide a preliminary observation of their healing effects on rat femurs with a 2mm or 5mm defect. No mechanical loading will be applied on any group of rats.

H1: *DBM, although with osteoconductivity, cannot heal a 5mm bone defect.*

A 5mm bone defect will be created on both sides of femurs of rats. DBM will be added into either side randomly.

	Experiment side	Control side
Group A (n=5)	DBM	-

H2: *DBM, combined with glycerol, act as an ideal carrier of MSC.*

A 5mm bone defect will be created on both sides of femurs of 5 adult SD rats. The defect will be designated randomly into either experimental or control side.

	Experiment side	Control side
Group B (n=5)	DBM+Glycerol+MSC/PBS	MSC/PBS

H3: *DBM-MSC-composite, while presents without mechanical load, obtains higher healing rate on 2mm defects than 5 mm defects.*

On group 1, a 5mm bone defect will be created on both sides of femurs of rats. DBM-MSC-composite will be added into either side of femurs randomly.

On group 2, a 2mm bone defect will be created instead of a 5mm one. The other conditions are the same with group 1.

	Experiment side	Control side
Group C (n=5): 5mm def.	DBM+Glycerol+MSC/PBS	-
Group D (n=5): 2mm def.	DBM+Glycerol+MSC/PBS	-

Analysis

All the animals will be euthanized 42 days after surgery. The final analysis includes radiography, microCT, torsional test and histology.

Using a 4-pins external fixator to create a 5mm critical femoral bone defect on SD rats

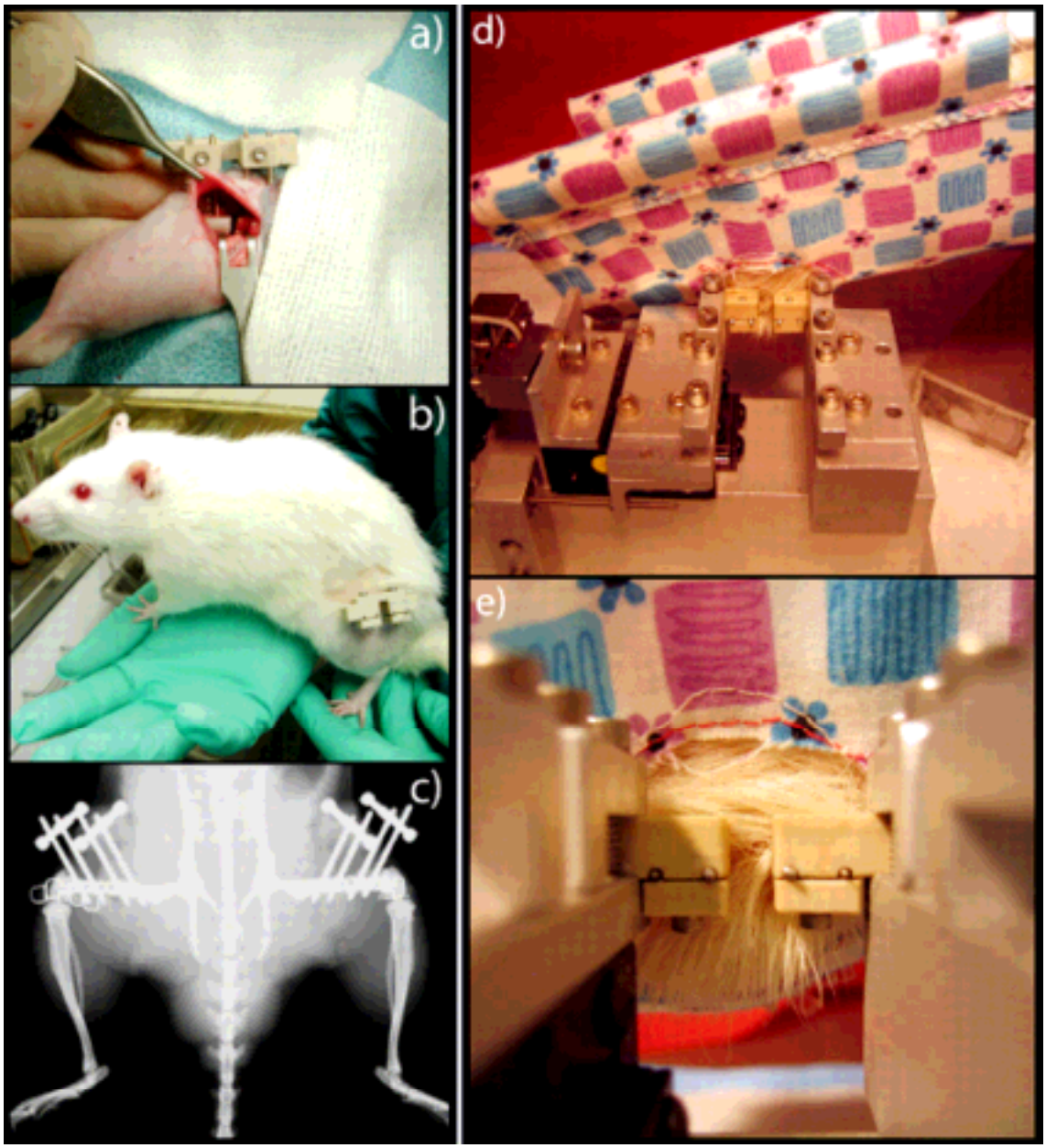
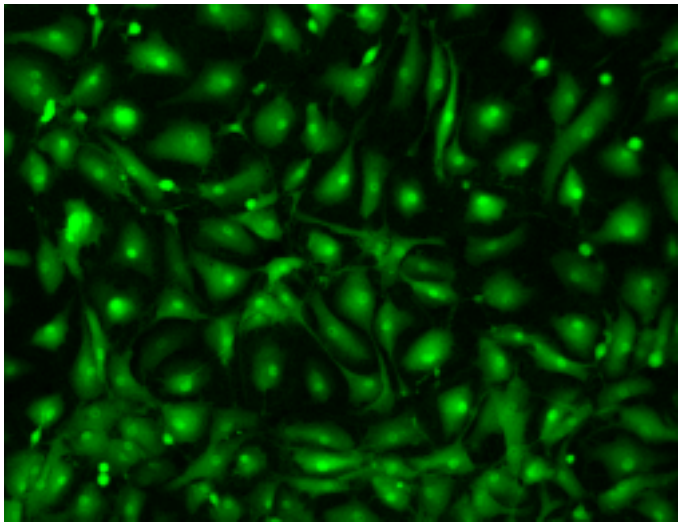
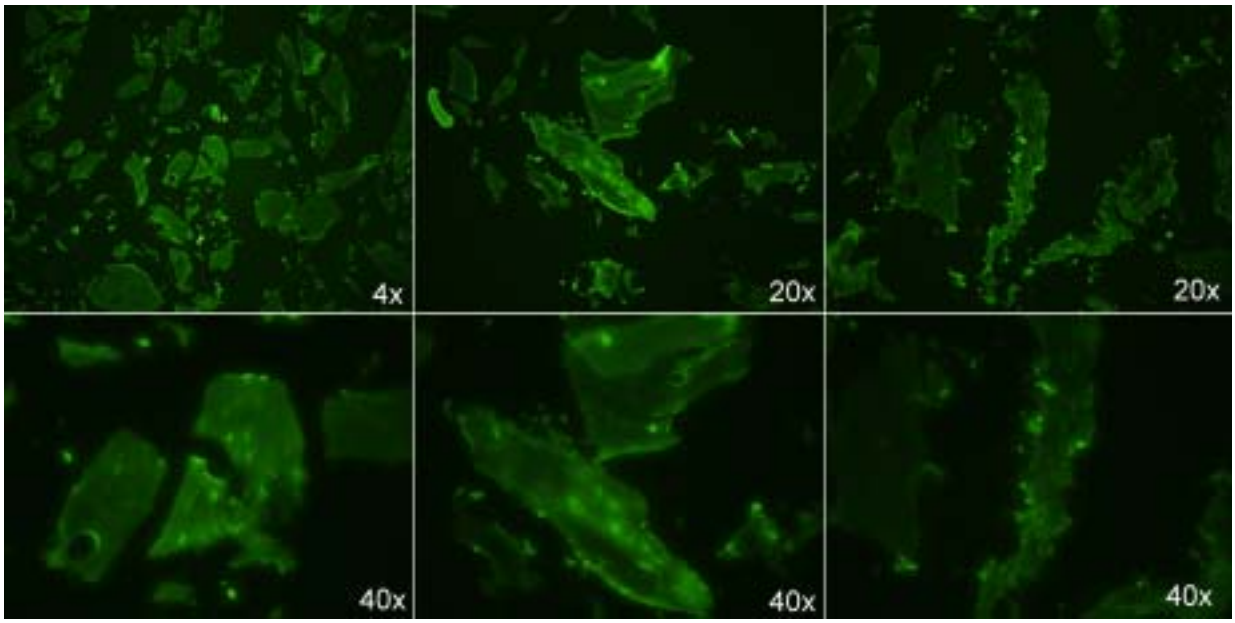


Figure 2 - 1: Overview of surgery and fixture for axial displacement. A two-millimeter segmental osteotomy was made at the mid-diaphysis of each femur (a). The fractures were stabilized using four threaded pins and a two-piece fixator that is locked in a rigid configuration for normal ambulation (b, c). During axial stimulation, the rat is anesthetized, placed in a sling, and the fixator aligned with the fixture clamp (d). The close-up view of the fixator shows the two unlocked halves during stimulation (e).

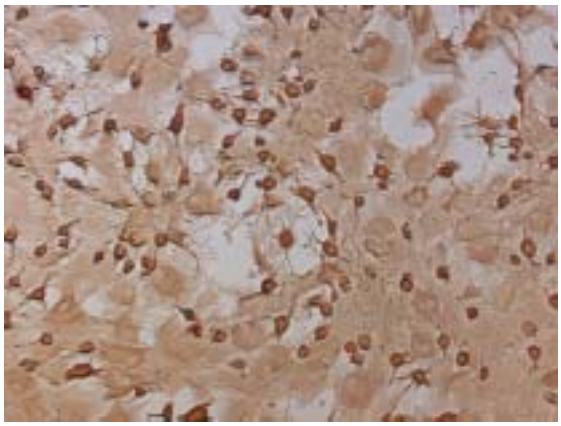
MSC harvest and culture (P2)

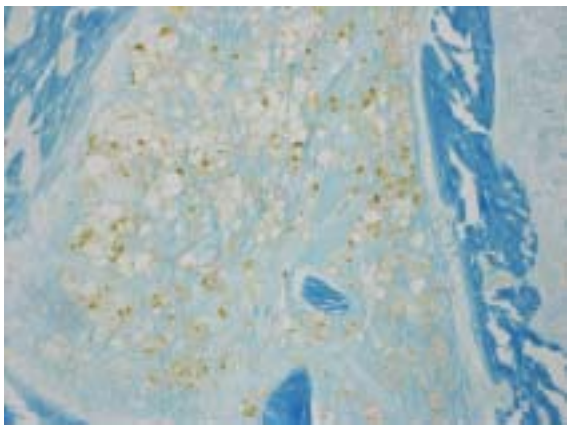


Fluorescent Scope of In Vitro Culture of MSC with DBM(Day 14)



IHC (SP-DAB) for GFP(+) MSC





Poster Exhibition at 2008 American Orthopaedic Research Society

Influence of Controlled Mechanical Stimulation and Donor Mesenchymal Progenitor Cells on Fracture Healing

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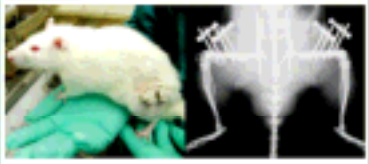
Introduction

Fracture healing is a complex process involving numerous cell types, and a variety of spatially and temporally related regulators. Mechanical forces have been shown to play an important role in the extent and character of the repair process. While prior studies have investigated the effect of physical forces on cell differentiation, biofactor expression, and mechanical competence of repair, the mechanosensory and response mechanisms are poorly understood. The purpose of this study was to evaluate the temporal effect of a controlled mechanical environment on fracture repair. Specifically, this study was designed to investigate the timing of mechanical load and its influence on systemic mesenchymal stem cell (MSC) homing and local cell behavior during fracture repair.

Materials



- 110 Sprague-Dawley rats
  - 2 mm, bilateral osteotomy
- Axial mechanical stimulation
  - 5 consecutive days
  - 0, 3, 10, or 24 days post-op
  - ±8% ε, 0.3 Hz, 510 cycles
- GFP+ mesenchymal stem cells
  - 1x10^6 cells via the tail vein
  - Injected on first loading day
- Micro-CT scanning
  - 18 μm voxels
- Histology
  - Toluidine blue and basic fuchsin
  - Safranin-O and fast green
- Torsion testing
  - Rate of 0.5 deg/sec
- Planer gamma imaging
  - Short term MSC tracking

Acknowledgements

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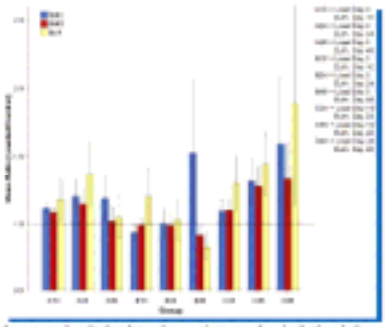
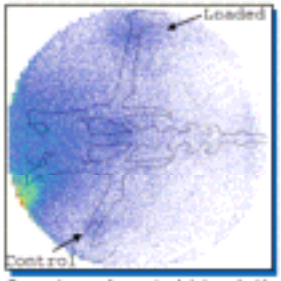
Results



In groups loaded at later time points, mechanical stimulation increased mineralization measures as detected by μCT. Due to high variability in the healing process no statistical differences were detected within groups. When data is pooled by time of euthanasia, significant differences are detected for bone volume, tissue mineral content, and bone volume fraction at day 24 and bone mineral content at day 48 ($p<.05$, data not shown). Tissue mineral density remained unchanged in all groups (data not shown). The torsion results were inconclusive, but they seem to indicate decreases in torsional properties except for Group D48 (data not shown). Error bars represent ±1 SE.

From a qualitative histologic analysis, it appears that loading accelerates the fracture healing process. At 24 days, the loaded limbs show an increase in chondrogenesis. At 48 days, the stimulated defects have more cortical bridging and a limited amount of remaining cartilage indicating more advanced endochondral ossification.



Gamma images from animals injected with donor MSC and loaded 24 days post-op showed evidence of migration of the MSCs into the fracture sites, with the appearance of preferential homing to the loaded fracture site. Animals that were loaded 0, 3, or 10 days post-op showed some migration of cells into the defect areas, but there was no evidence of preferential homing. In all groups, the many of the cells remained in the lungs and spleen throughout the scanning period.

Conclusions

Load has a definitive effect on the fracture healing process. Measures of mineralization as determined through μCT are increased, especially in groups loaded in later stages of healing. Histology shows accelerated healing through the presence of more cartilage early in the healing process, and more bony bridging and endochondral bone formation at late healing stages. The torsion testing also showed trends of increased torsional mechanical stability in the late loading group. Gamma imaging shows the preferential migration of MSC in those animals as well. All of this seems to indicate that loading later in the healing process may provide an advantage in fracture repair.

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