

How to Use Fluoroscopic Imaging to Prevent Intra-Articular Screw Perforation During Locked Plating of Proximal Humerus Fractures: A Cadaveric Study

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Purpose: Intra-articular screw perforation is a common complication after open reduction and internal fixation (ORIF) of proximal humerus fractures. The purpose of this study was to determine the sensitivity and specificity of intraoperative fluoroscopic images used to evaluate if the tip of a screw is completely located within the bone of the proximal humerus or if it is intra-articular. The authors hypothesized that: (1) a screw that is completely contained within bone would always image as if it is within bone, (2) intra-articular screws can falsely appear on imaging as if they are completely located within bone, and (3) specific fluoroscopic views can be used to reliably evaluate specific locations of the humeral head.

Methods: 22 proximal humeri in fresh-frozen cadavers were instrumented. The articular surface was divided into equal-sized three rows (superior, central, inferior) and three columns (anterior, middle, posterior) so screws could be placed in reproducible locations at the intersections of the rows and columns. The screws in the first 10 specimens were inserted so their tips were located 2mm beneath the articular surface. The next 12 specimens had screws placed so their tips protruded 2 mm past the articular surface into the glenohumeral joint. 27 different C-arm views were obtained of each specimen/screw configuration for a total of 1242 images.

Results: A screw that is located completely within bone always imaged as if it was completely within bone. There were 0 false positives and therefore specificity was 100%. The average sensitivity of the images of the intra-articular screws was 55%, and varied greatly depending on the specific image and the screw tip exit location (range, 0%-100%) (Table 1). The sensitivity for the inferior row of screws was the lowest (39.1%) and was particularly low for the posterior inferior screw exit location (20.7%).

Conclusion: Screws that are completely contained within bone will never image as if they are intra-articular. Unfortunately, screws that are intra-articular, particularly the posterior inferior screw, can image incorrectly and appear as if they are completely located within bone. We recommend the use of seven specific C-arm images (black highlighted boxes in Table 1) since these views had a sensitivity of 100% for 8 of the 9 screws positions and 97% for the posterior inferior screw and required the least C-arm manipulation. This specific fluoroscopic imaging technique could be used to decrease the chances of placing intra-articular screws during ORIF of proximal humerus fractures.

Table 1. Sensitivity of Each Screw Exit Location for Each of the 27 C-Arm Views

Rainbow Position	Cephalic Cant												Caudal Cant															
	Roll Back				Neutral				Roll Over				Neutral/Cant				Roll Back				Neutral				Caudal/Cant			
Screw Exit Point	Arm	Arm	Arm	Arm	Arm	Arm	Arm	Arm	Arm	Arm	Arm	Arm	Arm	Arm	Arm	Arm	Arm	Arm	Arm	Arm	Arm	Arm	Arm	Arm	Arm	Arm	Arm	
Anterior Superior	83	92	75	92	50	17	58	25	8	58	100	92	100	92	42	92	42	25	25	92	100	75	100	92	58	42	67.3	
Anterior Central	25	67	100	75	100	67	100	42	0	0	58	100	50	100	83	100	67	8	0	50	83	58	100	67	100	67	33	63.0
Anterior Inferior	0	8	58	0	67	100	75	100	83	0	33	83	0	75	100	50	100	42	0	58	100	25	100	100	67	92	42	57.7
Middle Superior	75	92	92	75	67	58	50	42	25	100	100	100	100	100	100	92	50	42	42	67	42	75	92	67	100	100	100	75.6
Middle Central	67	42	83	92	100	92	100	92	42	8	8	75	17	92	100	75	100	67	0	0	50	0	33	92	33	92	83	60.5
Middle Inferior	0	0	17	0	25	75	33	92	0	0	17	0	25	92	17	83	100	0	8	50	0	42	100	8	83	92	38.9	
Posterior Superior	83	92	92	83	92	92	83	100	92	100	50	50	92	75	75	83	92	50	17	0	42	17	33	33	58	83	67.9	
Posterior Central	100	42	25	92	67	92	92	100	50	8	0	33	8	58	33	58	100	8	0	8	8	0	25	0	17	75	44.1	
Posterior Inferior	75	0	0	17	0	25	0	58	83	17	0	8	0	0	25	8	25	75	25	0	8	0	0	8	0	17	83	20.7
Average	56	48	60	58	63	69	66	71	58	37	40	58	44	63	75	61	72	62	17	30	52	28	52	68	44	65	70	55.1

IR = internal rotation, NR = neutral rotation, ER = external rotation. All sensitivities are in percentage, views with 100% sensitivity are bolded, and recommended views are highlighted in black.

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Cortical Bone Drilling Induced Heat Production with Common Drill Devices

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Purpose: This study was designed to compare the heat produced during cortical bone drilling for various sizes of three common drill devices—standard twist drills, Kirschner-wires (K-wires), and a comparable cannulated drill. Previous studies have shown a threshold for thermal osteonecrosis to be 47°C. Significant data exist regarding heat production of standard twist drills; however, there is a paucity of data regarding cannulated drills and K-wires, both of which are used in many different sizes for many different situations. It was hypothesized that peak temperature would increase with bit size, with standard drills producing the least amount of heat followed by cannulated drills and lastly K-wires.

Methods: Three standard drill bits (2.0, 2.5, and 3.5 mm), three K-wires (1.25, 1.6, and 2.0 mm), and one cannulated drill bit (2.7 mm) were employed for comparison. Drill bits were driven by a Stryker hand drill secured on a servo-controlled linear actuator to provide a constant advancing speed of 1 mm/sec. The advancing speed was chosen after motion-testing a senior and resident surgeon. Bone samples were prepared from non-embalmed human tibia and moisturized at 37±1°C prior to drilling. To measure temperature, two thermocouples were embedded 2 mm into the cortical bone at distances of 0.5 mm and 1.5 mm from the drill hole margin. At least eight tests were performed for each drilling tool based on an initial power analysis.

Results: The peak temperature was extracted from each trial for comparison (Figures 1a and 1b). Standard twist drill data exhibited a positive trend between bit size and heat production. The bit size effect was shown to be less significant in K-wire drilling with no statistical difference between the sizes tested ($P > 0.05$). Comparing across different tools (Figure 1c), it can be seen that K-wires result in significantly ($P = 0.008$ at 0.5 mm) higher peak temperatures than standard twist drills of the same size ($\Delta T = 48.7 \pm 4.5^\circ\text{C}$ vs. $\Delta T = 35.1 \pm 9.3^\circ\text{C}$). Figure 1d shows that a 2.7-mm cannulated drill produced more than double the temperature rise of a 2.5-mm twist drill ($\Delta T = 66.8 \pm 10.8^\circ\text{C}$ vs. $\Delta T = 33.1 \pm 8.4^\circ\text{C}$).

Conclusion: Standard twist drills were found to be the most effective drilling devices, producing the smallest temperature rise among all bit types. For K-wires, all sizes reached substantial temperatures to cause instant thermal osteonecrosis. With an insignificant change in heat produced as K-wire size was increased, it was concluded that thermal effects should not be a reason for choosing K-wire size and that the largest size needed can be used. The cannulated drill showed significantly higher temperatures when compared with similar sized standard drills, reaching maximal temperatures comparable to those of a K-wire. This should be considered when choosing to use a standard versus cannulated drill.

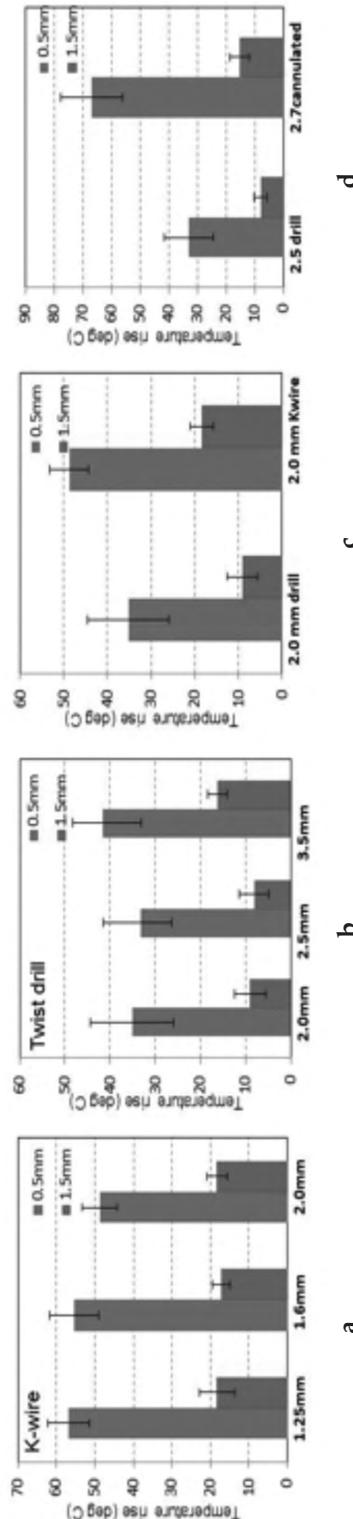


Figure 1. Experimental data with initial temperatures offset to zero.

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Can Views of the Proximal Femur Be Reliably Used to Predict Malrotation After Femoral Nailing? A Cadaveric Validation Study

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Purpose: Malrotation after intramedullary nailing of femoral shaft fractures is a clinical problem that has been reported to occur in 15% to 40% of cases. One technique to evaluate rotation is to compare the amount of lesser trochanter that is visualized on standard AP hip film versus the amount visualized on the contralateral, uninjured side. Although this technique is commonly used, to our knowledge there are no data investigating its validity. The purpose of this study is to determine whether the amount of visualized lesser trochanter can be used as a surrogate for the degree of femoral rotation after fracture fixation. Our hypothesis is that this technique will be able to reliably detect clinically important differences in malrotation.

Methods: Twenty matched cadaveric femur pairs ($n = 40$) were obtained and mounted on a custom jig that allowed rotation along the axis of potential malrotation about a femoral nail. Sequential C-arm fluoroscopic images were taken of the proximal femur at 10° increments of internal and external rotation compared to a true AP of the hip as determined by knee position. The angle of rotation of the femur was measured with a computerized angular sensor affixed to the femoral shaft. The width of lesser trochanter visualized on each image was measured using standard PACS (picture archiving and communication system) clinical software and normalized to the maximum size observed to provide a percentage of trochanter observed for each image. The relationship between percentage of the lesser trochanter observed and angle of femoral rotation was analyzed using a trend line of the data.

Results: Rotation of the proximal femur demonstrates a consistent, linear relationship to the lesser trochanter ($r^2 = 0.87$). This linear relationship indicates that each 10% deviation in lesser trochanteric size corresponds to 7.7° of femoral rotation. The maximal size of the lesser trochanter was seen when the femur was externally rotated to an average of 34°, corresponding to the point when the intertrochanteric ridge begins to be visualized superior to the lesser trochanter. There was little variation in values between the left and right of each pair (paired *t*-test, $P > 0.1$) with the exception of one pair ($P = 0.02$), demonstrating that the contralateral hip is an excellent indicator of rotation.

Conclusion: To our knowledge, this is the first study to attempt to validate the common clinical practice of comparing the amount of lesser trochanter visualized on AP hip films to evaluate femoral rotation after intramedullary nailing. Our data demonstrate that the relationship between angular rotation of the femur and the size of the lesser trochanter is not only highly linear ($r^2 = 0.87$), but that the amount of change is quite sensitive to rotation. Previous authors have argued that clinically significant malrotation is thought to be somewhere in the 15° to 30° range, which corresponds to an easily measured change of 20% to 40% in size of the lesser trochanter. Clinicians can estimate the amount of malrotation using the relationship that roughly 8° of malrotation exists for every 10% difference in normalized size of the lesser trochanter.

See pages 99 - 147 for financial disclosure information.

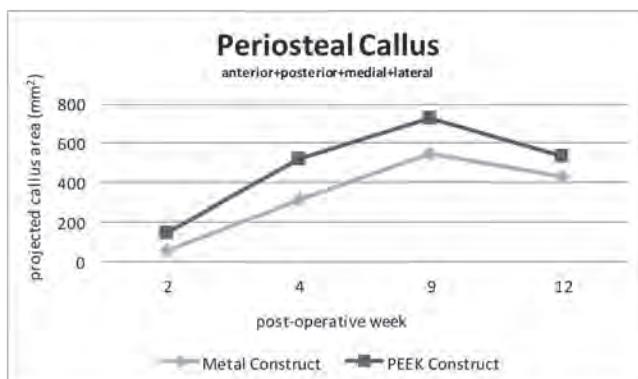
Polyether Ether Ketone (PEEK) Carbon Fiber Composites May Improve Healing of Fractures Stabilized with Intramedullary Nails

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Background/Purpose: Long bone diaphyseal fractures can be treated by using a number of methods. Intramedullary (IM) nailing represents a well-established approach for internal stabilization of bone fractures. Typical IM nail constructs consist of a metallic rod and placement of metallic screws at either end of the nail for stabilization. This study details the outcome of using a new material in the production of IM nail—PEEK-OPTIMA Ultra Reinforced, a carbon fiber-reinforced PEEK (polyetheretherketone) composite. The objective of this study is to compare bone healing of tibial osteotomy repaired with a PEEK carbon fiber composite IM nail to a traditional metallic construct in an established ovine fracture model. The study tested what effect lower modulus PEEK carbon fiber composite implants have on fracture healing in comparison with standard metallic constructs.

Methods: A 3-mm unilateral osteotomy defect was created in the left tibia of 10 sheep. Each animal was either assigned a PEEK or stainless steel (SS) nail for fracture stabilization. All animals were permitted immediate unrestricted weight bearing after surgery. Evaluation of bone remodeling was performed using CT, micro-CT (μ CT), and portable radiography. The PEEK composite and SS IM nails were geometrically identical (10 mm in diameter and 187 mm in length). The material construction of the PEEK composite IM nail provided 59% lower stiffness in 4-point bending when compared to the SS nail. The healing process was monitored via radiography and CT at regular intervals. The animals were sacrificed at week 12; healed tibiae were analyzed by μ CT.

Results: Bridging was observed on radiographs of all animals (5 of 5) implanted with the PEEK construct in contrast to the SS group (3 of 5). Callus formation of weekly radiographs was greater within the PEEK group, especially in the earlier time points: 158% ($P = 0.09$), 67% ($P = 0.08$), and 33% ($P = 0.10$) in weeks 2, 4, and 9, respectively. The callus formation in week 12 was 24% greater for the PEEK group when compared to the SS group ($P = 0.20$).



Conclusion: Improved healing in the form of complete bridging at an earlier time point and greater callus formation was seen in the PEEK nail group compared to the SS group. Potential reasons for the increased healing rate (bridging and callus) within the PEEK group are postulated to be enhanced dynamic loading and reduced stress shielding afforded by the lower modulus of the PEEK nail.

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**BSFF SYMPOSIUM 2:
BONE GRAFT SUBSTITUTION AND AUGMENTATION**

Moderators: *Aaron Nauth, MD*
Peter V. Giannoudis, MD

- | | |
|----------|--|
| 9:35 am | Selecting the Right Bone Graft Substitute for Your Patient
<i>Aaron Nauth, MD</i> |
| 9:45 am | BMPs: Is There Still a Role in 2014?
<i>Peter V. Giannoudis, MD</i> |
| 9:55 am | Bone Marrow Aspirate and Autologous Stem Cells:
Are They Effective?
<i>Joseph M. Lane, MD</i> |
| 10:05 am | Injectable Calcium Phosphates and Sulfates:
When I Use Them and Which One I Use
<i>J. Tracy Watson, MD</i> |
| 10:15 am | Discussion |

NOTES

See pages 99 - 147 for financial disclosure information.

• Montelukast Sodium Enhances Fracture Repair: Is There a Dose Response?

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Purpose: Previous studies have demonstrated that leukotrienes can act as negative regulators of chondrocyte activity, and that selective blockade through the use of leukotriene inhibitors can enhance chondrocyte activity and fracture repair. In this study, we sought to confirm these findings and determine if this effect was dose responsive. We hypothesized that the effect of cysteinyl leukotriene receptor blockade with montelukast sodium would demonstrate dose responsiveness and that increasing doses of the drug would demonstrate improved efficacy.

Methods: 451 animals were enrolled in an IACUC (Institutional Animal Care and Use Committee)-approved study. Animals underwent open retrograde nailing of the right femur followed by midshaft femoral guillotine fracture by standardized weight drop. Animals were divided into four treatment arms and received daily gavage with montelukast sodium at the following doses: control (carrier alone), 0.15 mg/kg once daily, 0.15 mg/kg twice daily, 1.5 mg/kg daily, and 1.5 mg/kg twice daily. Animals were sacrificed at day 7, 10, 14, and 21 post-fracture and underwent analysis by qPCR (quantitative polymerase chain reaction) for gene expression, micro-CT, histology/immunohistochemistry, and mechanical testing.

Results: *Histomorphometry:* We performed histomorphometry on fracture callus sections from a total of 10 histological sections from each dose and time. We are able to demonstrate a clear, albeit small, effect of dose response from low to intermediate group, and a dramatic decline at the highest doses, suggesting potential inhibitory dose effect at the 1.5 mg/kg twice daily levels. *Mechanical Testing (day 21):* These data are largely consistent with our histomorphometry data showing larger callus size in the 1.5 mg/kg daily dosing, and this larger callus translated in this study to a more mechanically robust response by day 21. *Micro-CT:* For the overall bone volume and density, a tight contour around the entire ROI (region of interest) was utilized with no delineation between cortical or trabecular bone. Micro-CT data demonstrate an increased effect at 1.5 mg/kg daily dosing at specific time points, but no difference to control and a decline from 1.5 mg/kg daily was seen with 1.5 mg/kg twice daily dosing. *Gene Expression:* Aggrecan core protein expression levels and others are consistent with data demonstrating an increase to 1.5 mg/kg daily dosing and potential inhibitory dose effect at the highest levels.

Conclusion: Treatment of murine femoral fractures with oral montelukast sodium demonstrated increased callus size and gene expression profiles consistent with enhanced chondrogenesis at early time points, and this effect showed modest increases with escalating drug dosing. However, the highest dose appeared to exhibit potential inhibitory dose effect, with a drop off of nearly every parameter including mechanical testing, micro-CT parameters, and histomorphometry. This has important implications when considering the potential translation of leukotriene blockade for fracture treatments in humans.

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ΔPossible Inhibitory Effect of Bone Marrow-Derived Mesenchymal Stem Cell Application on BMP-2-Mediated Bone Healing in a Critical Size Defect Model

*Motasem I. Refaat, MD; Joel C. Williams, MD; Dominik R. Haudenschild, PhD;
Mark A. Lee, MD;*

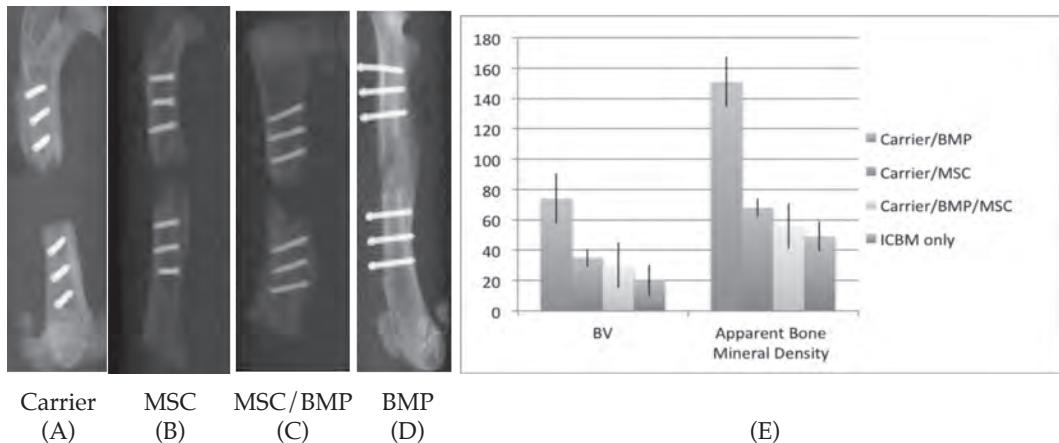
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Purpose: Healing of critical size defects (CSDs) remains a critical clinical challenge in fracture care. Bone morphogenetic proteins (BMPs) are commonly utilized in the setting of defect repair; however, large doses are required with associated costs and complications. Mesenchymal stem cells (MSCs) have been studied as an alternative to BMPs for bone defect repair. Composite constructs utilizing both BMPs plus osteogenic materials are commonly utilized. The purpose of this study was to investigate the relationship of MSCs and BMP response in a reproducible rodent CSD model. Our aim was to determine the efficacy of BMPs, MSCs, and combined application of BMPs plus MSCs deployed via an inert carrier in healing a validated critical size femoral defect model.

Methods: 6-mm diaphyseal CSDs were created in femora of skeletally mature male Fischer 344 rats and stabilized with a radiolucent PEEK (polyetheretherketone) plate and 6 angular stable bicortical titanium screws. MSCs were harvested from the intramedullary canal of a sacrificed Fischer 344 rat and expanded in MSC growth until confluent to 1×10^6 cells (4 passages). Rats were randomly assigned to four treatment groups: carrier alone (ICBM [insoluble collagenous bone matrix]), 2 μg BMP-2 with carrier (positive control), 1×10^6 MSCs with carrier, and 2 μg BMP-2 and 1×10^6 MSCs on carrier. Surveillance radiographs were obtained at 2-week intervals until the end of treatment and scored 0 (no bone formation), 1 (possible union), or 2 (union) by two blinded investigators. All animals were sacrificed at 8 weeks to examine bone formation using radiographs and micro-CT.

Results: All of the 2- μg group demonstrated 100% radiographic union by week 4 (D). None of the rats in the carrier (A) or the MSC group (B) fully united at the time of sacrifice. Rats in the MSC/BMP-2 group also failed to heal (C). Compared to BMP-2 or MSC alone, bone volume (BV) and bone mineral density were both decreased in the MSC/BMP-2 treatments (E). A qualitative analysis was preformed for all groups. Differences in mean values for all groups were tested using the analysis of variance (ANOVA). The analysis was significant for both BV ($P < 0.01$) and bone mineral density ($P < 0.01$) for all groups. Difference in mean values between the BMP-2 group and BMP/cells group were significant using a two-sided *t*-test, BV ($P = 0.014$) and for bone mineral density ($P < 0.01$).

Conclusion: BMP-2 delivered with an inert carrier in our mechanically stable rodent CSD model results in consistent, high-quality bone regenerate. The unmodified MSCs do not reliably heal the critical size defect. The addition of MSCs to the BMP-2 carrier construct demonstrated significantly reduced bone formation and failed to heal. The interplay between BMP-2 and unmodified MSCs merits further study.



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Effects of Reamer-Irrigator-Aspirator Wastewater on Bone Regeneration

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Background/Purpose: The reamer-irrigator-aspirator (RIA) device is capable of obtaining large quantities of autologous bone graft with significantly less donor site morbidity compared to iliac crest bone graft. The reamed femoral contents are aspirated and passed through a filter to separate the desired bone graft from the remaining wastewater (WW). The first aim of this study was to describe a method to concentrate osteogenic growth factors and viable mesenchymal stem cells (MSCs) from RIA WW. The second aim was to examine the effects of WW-derived growth factors on human MSCs *in vitro* as well as in a critical size defect (CSD) mouse calvarium model *in vivo*.

Methods: Twelve male patients scheduled for femoral RIA bone grafting procedures were enrolled. RIA WW and 50 cc of peripheral blood were collected. Peripheral blood was centrifuged to obtain platelet rich plasma (PRP). MSCs were extracted from the WW and the remaining aspirate was concentrated. MSCs were incubated in the presence of PRP or concentrated WW to assess cell proliferation, survival, and mineralization *in vitro*. 5-mm CSDs were made in the calvaria of immunodeficient mice and packed with a collagen sponge alone or a collagen sponge soak-loaded with PRP or WW. Four weeks post-surgery, the calvaria were harvested and examined using micro-CT to determine percent bone ingrowth.

Results: MSCs extracted from RIA WW remain viable after processing and retain multipotency. Concentrated WW yields comparable concentrations of osteogenic growth factors when compared to PRP. Concentrated WW significantly improved MSC proliferation by 4 times and survival by 3 times when compared to MSCs treated with PRP *in vitro* (Figure 1). MSCs treated with WW showed a 500-fold increase in mineralization after 2 weeks when compared to PRP. Significantly higher rates of bone ingrowth were observed in CSDs treated with WW (26%) compared to PRP (20%), $P < 0.01$.

Conclusion: When compared to PRP, concentrated WW was shown to (1) accelerate MSC proliferation, survival, and mineralization *in vitro* by 4, 3, and 500-fold, respectively, and (2) accelerate osteogenesis in a mouse calvarium CSD model *in vivo*.

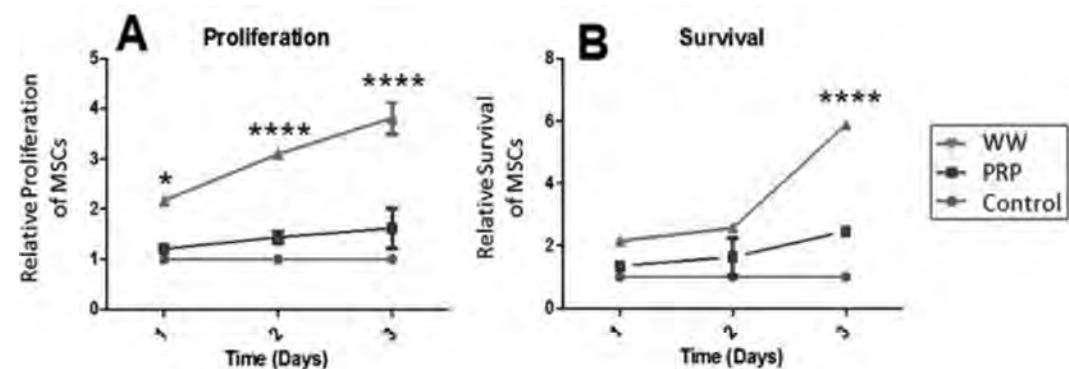


Figure 1. MSCs were incubated 1 to 3 days with normal growth media (Proliferation, A) or nutrient deplete media (Survival, B). For both assays, the media was either left untreated (control) or supplemented with WW or PRP. Significance was calculated between WW and PRP-treated groups.
* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$.

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Is Impaired Fracture Healing in Cigarette Smokers Related to Carbon Monoxide Exposure?

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Purpose: Smoking cigarettes delays fracture healing. Recent evidence has demonstrated that fracture repair can be enhanced by modifying hypoxia signaling during early stages of fracture repair. Additionally, carbon monoxide (CO) exposure *in vitro* has been shown to block hypoxic signaling through the hypoxic inducible factor (HIF) pathway. This led us to hypothesize that the deleterious effect of cigarettes on fracture healing could be due to CO exposure causing inhibition of physiologic hypoxia signaling via the HIF pathway.

Methods: A sealed environmental chamber was fitted with a CO delivery system so that low dose CO could be delivered cyclically, consistent with exposure seen in heavy smokers. Mice were initially treated with 200 ppm CO for 6 hours, alternating with 6 hours of room air, resulting in peak carboxyhemoglobin levels in the 14% to 20% range. After 2 weeks of accommodation, 240 animals underwent closed femoral fracture in an IACUC (Institutional Animal Care and Use Committee)-approved study. Animals were sacrificed at 7, 10, 14, and 21 days after treatment with cyclic CO or room air and fractures explanted for analysis with micro-CT, histology / immunohistochemistry, and qPCR (quantitative polymerase chain reaction) for analysis of chondrogenesis, osteogenesis, and angiogenesis.

Results: Significant changes in fracture repair after cyclic CO exposure were readily apparent and occurred primarily at day 10 post-fracture. Micro-CT data demonstrated significant decreases in BV/TV (bone volume/trabecular volume) parameters ($P = 0.0012$) at day 10 after CO exposure, suggesting significant delays in healing. Furthermore, by day 14 the callus size in CO exposed animals was significantly larger than controls ($P = 0.0017$), suggesting delay in transition from chondrogenesis to osteogenesis. qPCR data were consistent with findings of overall delay in healing by day 10. Expression profiles of angiogenesis genes (Hmox1, Jmjd6, Mmp9, Vegfa, Adora2b) and metabolism genes (Erol, Gys1, Hk2, Ldha, Pfkb3, Pfkl) all showed more than twofold change from control with CO exposure. Markers of chondrogenesis (Sox9, Col2a1, Acan, Col10) were consistently below controls at all time points. Interestingly, osteogenic markers showed variable effects in Runx2, Col1, and Alk phos mRNA expression throughout the time course.

Conclusion: These data have particular relevance when considering pharmacologic treatments that may help overcome the inhibitory effect of cigarette smoke on healing fractures. Clear and consistent decreases in chondrogenesis and delays in fracture repair were seen with cyclic CO exposure at 200 ppm delivered at 6-hour intervals. This implicates CO as a negative regulator of fracture repair at concentrations consistent with that seen in human cigarette smokers. Altered mRNA expression of genes involved in angiogenesis, and decreases in HIF2a expression at early time points, further implicates HIF signaling in this delayed healing.

ΔThe Temporal and Spatial Development of Vascularity in a Healing Displaced Fracture

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Background/Purpose: Underlying vascular disease is an important pathophysiology shared among many comorbid conditions associated with poor fracture healing, such as diabetes, obesity, and age. Determining the temporal and spatial patterns of revascularization following fracture is essential for devising therapeutic strategies to augment this critical reparative process. Seminal studies conducted in the last century have investigated the pattern of vascularity in bone following fracture. The consensus model developed from these studies is of angiogenesis emanating from both the intact intramedullary and periosteal vasculature. Since the plethora of experimental fracture angiography in the early to mid-20th century there has been a paucity of reports describing the pattern of revascularization of a healing fracture. Consequently the classic model of revascularization of a displaced fracture has remained largely unchanged. Overcoming the limitations of animal fracture models performed in the above-described classical studies, we demonstrate for the first time the complete temporal and spatial pattern of revascularization in a displaced/stabilized fracture. These studies were designed specifically to (1) validate the classic model of fracture revascularization of a displaced/stabilized fracture, (2) assess the association between intramedullary and periosteal angiogenesis, and (3) elucidate the expression of vascular endothelial growth factor (VEGF)/VEGF-R (VEGF receptor) in relation to the classic model.

Methods: Midshaft femoral osteotomies ($n = 52$) were fixed with an intramedullary nail. Fracture healing was followed with radiographs, micro-CT, angiography, and histology at 7-42 days post fracture

Results: Representative data of vascularity during fracture repair are presented in Figure 1. Fractures with significant injury to the intramedullary vasculature revascularize initially through the development of a transperiosteal vascular network as a result of increased flow diverted centrifugally resulting from interruption of downstream medullary vascularity. In support of this observation, many enhanced vascular anastomoses developed between the medullary vasculature and the areas of periosteal vascular engorgement. Following the initial phases of fracture revascularization, there exists centrally an avascular cartilaginous matrix predominated by VEGF-A/VEGFR-1 negative cells surrounded by a richly vascular new bone matrix predominated by endothelial cells and osteoblasts expressing high levels of VEGF-A/VEGFR-1 peripherally. Histological data revealed hypertrophic VEGF-A producing chondrocytes in all areas of transition from avascular/soft tissue to vascular hard tissue callus. The chondrocytes continued to hypertrophy and release VEGF-A in a manner that directs the polarized bone formation together; the periosteal vasculature and bone

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eventually unite. Following vascular union our results reveal that bone remodeling follows vascular remodeling in which intramedullary vascularity is re-established.

Conclusion: From these data, in conjunction with classic studies of fracture angiogenesis, we propose a novel model defining the process of bone revascularization. It is our hope that this new model of fracture revascularization of displaced/stabilized fractures will provide insight into the cause of impaired fracture healing, and potential means to restore bone healing.

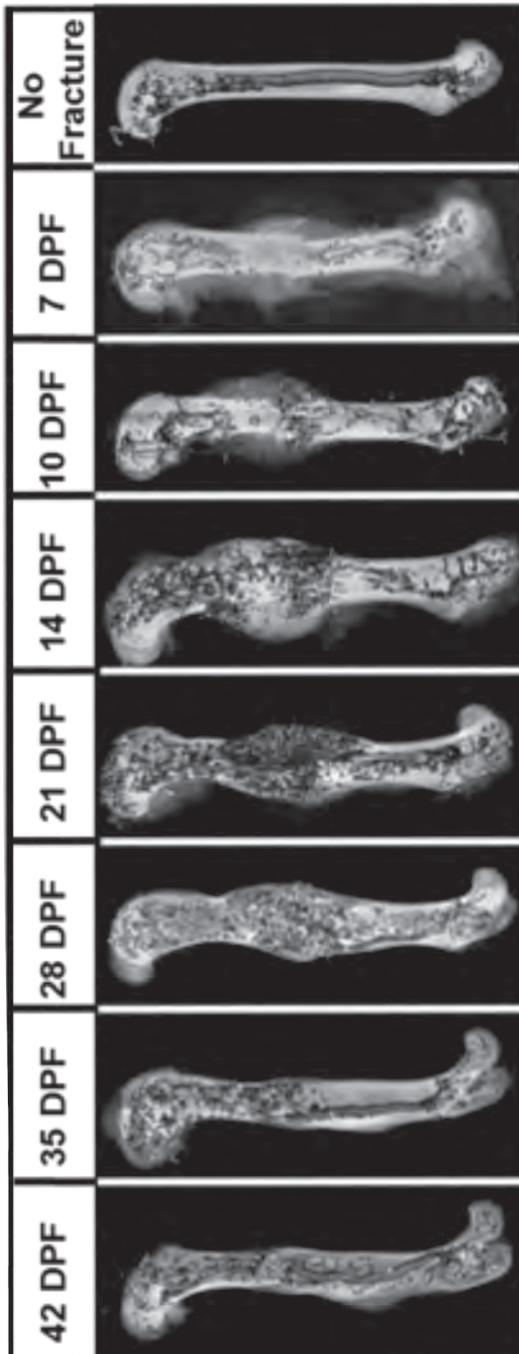


Figure 1.
Angiograph of femur fracture from 0 to 42 days post fracture (DPF). Color denotes vessel size

ΔModulating the Vasculature at a Fracture Through the Therapeutic Application of Placental Stem Cells

Chelsea S. Bahney, PhD¹; Aaron J. Taylor, BS¹; Ali Sadat, DDS¹; Kathryn Tormos, PhD²; Theodore Miclau III, MD¹; Emin Maltepe, MD, MPH²; Ralph S. Marcucio, PhD¹;

¹*Department of Orthopaedic Surgery, University of California, San Francisco, San Francisco, California, USA;*

²*Department of Pediatrics, University of California, San Francisco, San Francisco, California, USA*

Purpose: Blood supply to a fracture is a critical determinant of the rate and extent of healing. Therapies designed to promote bone healing by stimulating angiogenesis have been proposed for a long time, yet to date no effective treatments are available. In this work, we capitalize on a transient process that modifies the vasculature during pregnancy to support the developing fetus. Placental progenitors, trophoblast stem cells (TSCs), promote vasculogenesis in response to fetal hypoxia by physically remodeling the maternal arterioles and secreting angiogenic factors to generate a high-volume, low-pressure fluid exchange. We hypothesize that the therapeutic application of TSCs will promote vascular remodeling and enhance fracture healing.

Methods: All murine studies were approved by the Institutional Animal Care and Use Committee. TSCs were isolated from the day E3.5 mouse blastocyst, transfected with eGFP (enhanced green fluorescent protein) and b-gal (beta-galactosidase) reporter constructs, expanded, and maintained in an undifferentiated state in vitro using published methodology. Nonstable fractures were created in the mid-diaphysis of immunocompromised mice (10-14 weeks, male, SCID Beige mice). Fractures were given and injection of 1×10^6 TSCs in 10 mL of PBS (phosphate-buffered saline), or PBS alone as a control. Fracture healing was evaluated by histology and quantitative stereology 5-28 days post injury. Immunohistochemistry was used to localize the cells following injections, and gene expression arrays were used to determine highly expressed genes from TSC that could benefit fracture repair.

Results: Our data show that TSCs injected to nonstabilized murine fractures engraft into the vasculature (Fig. 1) and enhance the local blood supply (Fig. 2). Furthermore, injection of TSCs increased the volume of the cartilage callus 7 days post fracture, leading to more bone after 14 days of healing (Fig. 3)

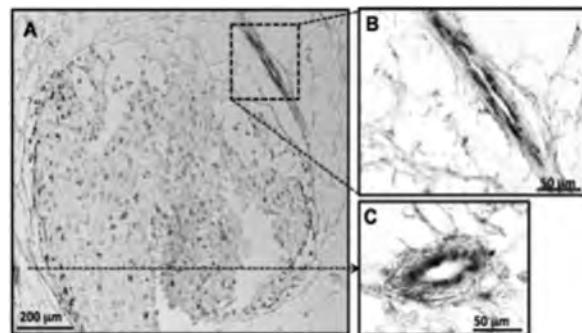


Figure 1. TSC Engraftment and endovascular invasion. (A) Bolus of β -gal labeled TSC located adjacent to fracture. (B-C) Insets of β -gal TSCs intercalated within vascular endothelium.

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Conclusion: To our knowledge, this is the first study evaluating the therapeutic potential of TSCs. Our results have the potential to enhance clinical outcomes in skeletal trauma, where there is often poor vascular perfusion. Importantly, this work may also have a significant impact on the broader function that is often intimately tied to compromised vascularity.

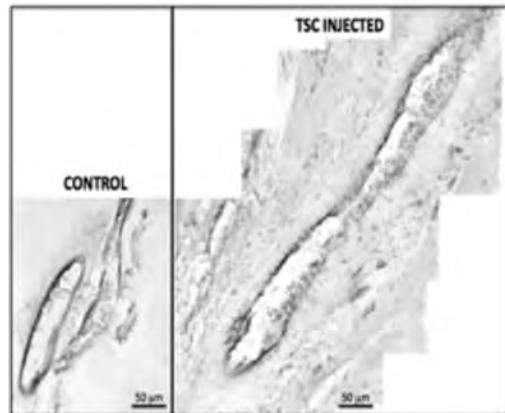
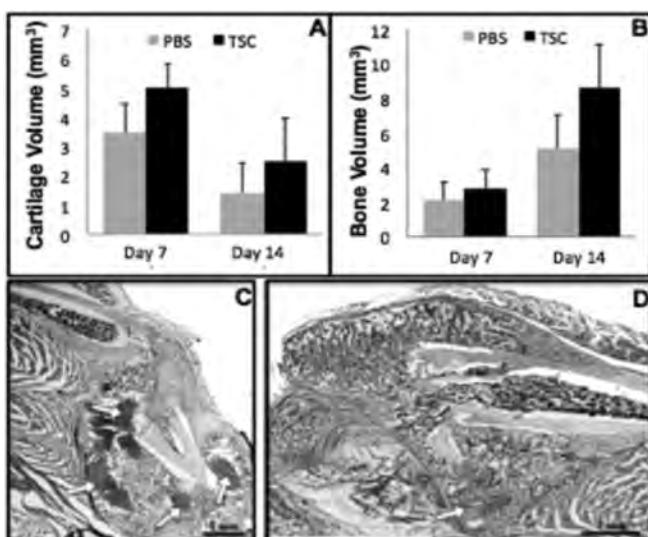


Figure 2. Comparison of average blood vessel diameter near fracture in control versus TSC injected animals shows vasodilation following



TSC injection.

Figure 3. TSC treatment accelerates fracture repair. (A) Cartilage and (B) volume in fracture callus. Safranin-O staining of day 14 fracture (C) control, or (D) TSC treated fractures

Osteogenic, Stem Cell, and Molecular Characterization of the Human Biomembrane ("Induced Membrane") from Trauma Patients

Gabriella Ode, MD; Gretchen Hoelscher, MS; Jane Ingram, BS; Synthia Bethea, BS; James Kellam, MD; Madhav Karunakar, MD; Michael J. Bosse, MD; Helen E. Gruber, PhD; Department of Orthopaedic Surgery, Carolinas HealthCare System, Charlotte, North Carolina, USA

Purpose: The biomembrane (induced membrane) formed around polymethylmethacrylate (PMMA) spacers has great value as reflected in its clinical application in the Masquelet technique. Few studies, however, have evaluated cellular, molecular, or stem-cell features of the human biomembrane. The objective of this study is to evaluate and characterize the human biomembrane in terms of its osteogenic, stem cell, morphologic, and molecular characteristics. We hypothesize that a better understanding of its biologic properties will lead to development of methods that can optimize long-term functional outcomes for traumatic limb salvage / military amputee patients.

Methods: Following IRB approval, biomembrane specimens were obtained from 12 surgeries (11 patients) with complex fractures (mean age 42.7 ± 13.2 years; 3 females, 8 males). Biomembranes from 8 tibias and 2 femurs were processed for routine morphology and molecular analysis or minced and utilized for monolayer cell culture to determination of the presence of stem cell populations. Cells were tested for their ability to differentiate into osteoblasts, chondroblasts and adipose cells using accepted differentiation criteria employing differentiation media (Lonza) and alizarin-red staining of calcified nodules formed by osteoblasts, micromass formation by chondroblasts, and adipocyte formation. The GCOS Affymetrix GeneChip Operating System was used to determine gene expression. Data were normalized and Gen-eSifter™ web-based software used to analyze microarray data. Statistical significance was determined using the Student *t*-test (two-tailed, unpaired, *P* ≤ 0.05 as the significance level).



Table 1. Stem Cell Potency of Human Biomembrane Cells

	Osteogenic differentiation	Chondrogenic differentiation	Adipogenic differentiation
% (#/total)	70% (7/10)	90.9% (10/11)	90% (9/10)

Results: Average duration of the PMMA spacer *in vivo* was 13.5 weeks (range, 6-21). Trabecular bone was present in 33.3% of the biomembrane specimens (Fig. 1, arrow). Biomembrane morphology showed high vascularity and collagen content; all specimens showed positive immunologic presence of bone morphogenetic protein 2 (BMP2) and RUNX2 in the biomembrane stroma. Differentiation of stem cells is shown in Table 1 and osteogenesis (alizarin-red staining of calcified nodules) in Figure 2A. Positive osteogenesis was found in cells from patients with PMMA present for 6-17 weeks (mean, 13.4 weeks). Molecular analyses compared 3 older (mean age, 56.7 years) versus 3 younger patients (mean age, 33.6 years). Biomembranes from older patients showed significant upregulation of aldehyde oxidase

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1 (a producer of hydrogen peroxide/superoxide, $P = 0.03$) and type I collagen ($P = 0.008$), and significant downregulation of matrix metalloproteinase 13 ($P = 0.03$) and tenascin XB (an extracellular matrix protein, $P = 0.01$).

Conclusion: Stem cell differentiation data showed greater variability in pluripotency for osteogenic potential (70%) versus chondrogenic or adipogenic potentials (90.9 and 90%, respectively). Due to the importance and increased use of the Masquelet technique in complicated large bone defects, analysis of data such as these is valuable because it leads to improved understanding of the human biomembrane's osteogenic potential.

**BSFF SYMPOSIUM 3:
THE MANGLED EXTREMITY -
FUNCTIONALITY THROUGH MECHANICS OR BIOLOGICS?**

Moderators: *Edward J. Harvey, MD*
Lisa K. Cannada, MD

12:35 pm	Current Concepts for Infection Management in High MESS Legs <i>Philip Wolinsky, MD</i>
12:45 pm	Heterotopic Ossification in Trauma and Amputations <i>Roman Hayda, MD</i>
12:55 pm	New Concepts in Residual Limb Management and Rehabilitation <i>Lisa K. Cannada, MD</i>
1:05 pm	The Peg Leg or the Six Million Dollar Man- Where Are We? <i>Danielle Melton, MD</i>
1:15 pm	Discussion

NOTES

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Pharmacological Treatment of Compartment Syndrome with Phenylephrine and Dobutamine Was Similar to Fasciotomy

Xuhui Liu, MD; James M. Mok, MD; Heejae Kang, BS; Julie Jin, BS; Alexandar Boehme, BS; Erik N. Hansen, MD; Mark Rollins, MD; Hubert T. Kim, MD; Utku Kandemir, MD;

Department of Orthopaedic Surgery, University of California San Francisco,
San Francisco, California, USA

Purpose: Current treatment for acute extremity symptomatic acute compartment syndrome (CS) is fasciotomy. However, surgical treatment has associated morbidity and may delay the recovery of the patients. The goal of this study is to investigate the feasibility of a novel nonsurgical treatment strategy for acute CS that increases oxygen delivery to the affected extremity by increasing blood pressure in a dog CS model. We hypothesize that pharmacological treatment will raise the blood pressure, improve limb perfusion, and increase tissue oxygenation, thus rescuing muscle from CS.

Methods: CS was induced in the anterolateral compartment on bilateral legs in the animals. Intramuscular tissue oxygenation, compartment pressure, and blood pressure were recorded every 30 seconds. Pharmacological treatment was initiated 1 hour after CS was induced. Infusion of intravenous phenylephrine was titrated as needed to increase the diastolic blood pressure 30 mm Hg above the baseline (creating DP = 0 mm Hg). Intravenous dobutamine was initiated 2 hours later to maintain blood pressure. Six to seven hours after treatment, fasciotomy was performed on one leg of the animals and the skin was closed 1 hour later. In a separate nontreatment control group, CS of equivalent magnitude was induced in 6 animals in which no intervention (pharmacological nor fasciotomy) was performed. Animals were euthanized 2 weeks postoperatively at which point muscle biopsies were performed. Tissue viability was assessed by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay as previously described. This is a validated technique in which the normalized tissue viability index is expressed as a percentage of control (quadriceps muscle).

Results: After induction of CS, pharmacological treatment significantly increased PmO_2 in the anterior compartment muscle. The average PmO_2 in the treatment group was 18.8 ± 4.3 mm Hg (mean \pm standard error [SE]). In contrast, PmO_2 in the non-treated group dropped to 0 mm Hg soon after the CS was induced. Fasciotomy increased the PmO_2 from 18.8 ± 6.7 mm Hg to 35.7 ± 15 mm Hg. Two weeks after surgery, the muscle viability index in pharmacological treated, pharmacological treated plus fasciotomy, and non-treated groups was $128 \pm 15\%$, $94.3 \pm 8.3\%$, and $41.8 \pm 17\%$ (mean \pm SE), respectively. There was no significant difference in viability index between the pharmacological treated and pharmacological -treated plus fasciotomy groups ($P = 0.09$). However, both groups had significantly higher tissue viability compared to the non-treated group ($P < 0.01$).

Conclusion: Our results showed that nonsurgical pharmacological treatment can significantly increase muscle oxygen and viability and may represent an alternative, less morbid treatment for acute CS than fasciotomy. Phenylephrine is often used for trauma patients in the perioperative setting to maintain blood pressure and could serve as initial therapy in patients with possible CS. However, in our study, the effect of phenylephrine decreased over time, and a second line drug (dobutamine) was needed after the first few hours. Keep-

ing the blood pressure at a high level using solely pharmacological agents (phenylephrine / dobutamine combination) yielded similar results as fasciotomy for the treatment of acute CS.

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ΔCarbon Monoxide Releasing Molecule-3 (CORM-3) Diminishes the Oxidative Stress and Leukocyte Migration Across Human Endothelium in an In Vitro Model of Compartment Syndrome

Aurelia Bihari, MS; Gediminis Cepinskas, DVM, PhD; David Sanders, MD;

Abdel-Rahman Lawandy, FRCS;

London Health Sciences Centre, London, Ontario, Canada

Purpose: Acute limb compartment syndrome (CS), a potentially devastating complication of musculoskeletal trauma, results in muscle necrosis and cell death. Oxidative stress due to ischemia and inflammation both appear to contribute to the microvascular dysfunction and parenchymal injury. Recently, carbon monoxide (CO), liberated from the carbon monoxide releasing molecule-3 (CORM-3), has been shown to protect microvascular perfusion and reduce inflammation in a rat model of CS. The purpose of this study was to replicate the CS conditions in vitro, allowing the study of the mechanism(s) of CO protection on the human microvasculature. The ultimate goal is the development of a rational pharmacologic adjunctive treatment for CS, which would reduce the morbidity and disability in patients.

Methods: Human vascular endothelial cells (HUVEC), grown to confluence, were stimulated for 3 hours with either a cytokine / chemokine cocktail representing the serum levels of inflammatory mediators detected in our experimental model of CS ("CS cocktail"), containing tumor necrosis factor alpha (TNF-a), interleukin (IL)-1b, and GRO (1 ng / mL, 100 pg / mL, and 1 ng / mL, respectively), or human serum (40%) isolated from CS patients. Levels of intracellular oxidative stress, measured by the production of reactive oxygen species (ROS) were assessed by oxidation of dihydrorhodamine 123 (DHR-123). Leukocyte migration (transwell inserts) was assessed by quantifying the number of ⁵¹Cr-labeled polymorpho-nuclear cells (PMNs) moving across the HUVEC monolayer in response to the CS cocktail or CS serum stimulation. All experiments were performed in the presence of CORM-3 (100 mM), or its inactive form iCORM-3.

Results: Stimulation of HUVEC with CS cocktail induced a significant increase in the production of ROS, expressed as fluorescence intensity (FI) per mg protein (1118.6 ± 255.6 in CS cocktail versus 600.8 ± 29.2 in control, $P \leq 0.01$), and increased PMN migration across HUVEC ($35.1 \pm 4.9\%$ in CS cocktail vs. $10.0 \pm 2.0\%$ in control, $P \leq 0.05$). CORM-3 treatment completely prevented CS cocktail-induced ROS production (468.3 ± 37.8 vs. 1169.1 ± 155.8 in iCORM-3 group, $P \leq 0.01$), and PMN migration ($12.0 \pm 1.5\%$ vs. $35.0 \pm .9\%$ in iCORM-3 group, $P < 0.05$). In parallel, experiments employing human CS serum stimulation demonstrated that CORM-3 was also very effective in blocking the CS serum-induced ROS production (644.8 ± 114.5 vs. 1059.6 ± 56.3 in iCORM-3 group, $P \leq 0.01$).

Conclusion: Treatment of human vascular endothelial cells with CORM-3 was able to interfere with the intracellular ROS production, and suppressed leukocyte migration across the endothelial barrier. The data indicate that CORM-3 offers potent antioxidant and anti-inflammatory effects, and thus may have a potential therapeutic application to patients at risk of developing CS.

Use of the Reamer/Irrigator/Aspirator During Intramedullary Nailing Decreases Carotid and Cranial Embolic Events

Anna N. Miller, MD¹; Dwight D. Deal, BS¹; James Green, BS²; Tim T. Houle, PhD¹; William R. Brown, PhD¹; Clara R. Thore, PhD¹; David Stump, PhD¹; Lawrence X. Webb, MD³;

¹*Wake Forest School of Medicine, Winston-Salem, North Carolina, USA;*

²*DePuy Synthes, West Chester, Pennsylvania, USA;*

³*Mercer University School of Medicine, Macon, Georgia, USA*

Purpose: Reaming for and placement of intramedullary nails results in bone marrow and fat extravasating into the circulatory system. This may lead to fat emboli syndrome, multiple organ failure, and adult respiratory distress syndrome. Studies show varying results of increased intramedullary pressure and embolic phenomenon with reamed or unreamed nailing. The Reamer/Irrigator/Aspirator (RIA; DePuy Synthes, West Chester, PA) device has been shown to decrease intramedullary pressure during reaming. We hypothesized RIA would reduce the number of micro emboli (ME) detected in the carotid artery and brain compared with both reamed and unreamed nailing.

Methods: A large canine model was used. Each animal underwent either unreamed nailing (UR), reamed nailing (R), or RIA-reamed nailing (RIA) of bilateral femora. During reaming and nailing, the number and size of ME transiting the carotid were recorded by an ultrasonic embolus detector (EDAC; Luna Innovations, Roanoke, VA). The animals remained anesthetized 4 hours, then the brain was harvested for immunostaining (HSP70; hypoxia-inducible factor [HIF]-1 α) and measurement of micro-infarction volumes.

Results: Carotid ME were only detected during the reaming and nailing portions of each procedure. The total ME load passing through the carotid was 0.05 cc (UR), 0.04 cc (R), and 0.01 cc (RIA) (not statistically significant). The number and size of ME of the UR and R group were similar. However, the RIA group had significantly smaller numbers of larger emboli, >200 microns; $P = 0.03$. Pathologic examination of the brain confirmed the presence of particulate emboli (photo center), as well as upregulation of stress-related-proteins, HSP70 and HIF-1 α , detected in all groups.

Conclusion: Further study is required to determine the mechanisms by which ME pass into the arterial system during reaming. RIA decreased ME compared with traditional reamed and unreamed nailing, suggesting intramedullary pressure and heat are important variables. These results may help explain subtle neurobehavioral symptoms commonly seen in patients undergoing intramedullary nailing procedures.

Funding: Provided by NIH R01NS020618-28.

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•**Superoxide Dismutase Mimetic Disrupts Bacterial Biofilms in an Infected Fracture Model**

*Sarah E. Lindsay^{1,2}; James D. Crapo, MD¹; Elizabeth A. Regan, MD, PhD¹;
National Jewish Health, Denver, Colorado, USA;
Stanford University, Palo Alto, California, USA*

Background/Purpose: Implant-associated infections affect more than 50,000 orthopaedic cases a year. *Staphylococcus aureus* is one of the most common pathogens and its ability to persist locally and resist antibiotics is related to its ability to form and maintain biofilm structures. Fixation devices and total joint components provide a surface for bacterial adherence and foster bacterial growth within a 3-dimensional extracellular structure that is phenotypically different from its planktonic (single cell) counterpart. Biofilm associated infections become chronic and difficult to diagnose, leading to fracture nonunions and premature failures of total joints. Reports that bacteria actively modulate their redox environment in a biofilm by downregulating superoxide dismutase (SOD) suggested a novel treatment for biofilms. We postulated that a potent SOD mimetic would interfere with either the establishment or maintenance of the biofilm structure and might improve clinical outcomes. We tested the compound (MnTE-2-PyP) in an in vitro model and a murine infected fracture model with and without antibiotics.

Methods: *In Vitro:* A biofilm-forming subtype of *S. aureus* (ATCC 29213) was used. Biofilm assessment was done using crystal violet assay for extracellular polymeric structure (EPS); *S. aureus* was diluted and plated on sterile 96-well PVC plates. Cultures were grown over 24 hours, treated with drug (30 µM) or PBS (phosphate-buffered saline) at baseline or after 12 hours of growth. Absorbance was read at OD595 using a microplate reader. *Animal Model:* Procedures were approved by the Institutional Animal Care and Use Committee at National Jewish Health. Male C57BL6 mice (20-25 grams) were used. A midshaft femur fracture was created through a lateral incision and then treated with intramedullary fixation using an 8-mm section of 23-gauge needle. 10³ bacteria in 5 µl volume were placed at the fracture site and the soft-tissue envelope was restored with 6-0 Vicryl and skin glue. There were four treatment groups and five mice per group: (1) no drug treatment, (2) SOD mimetic alone (MnTE-2-PyP), (3) cephalexin 250 mg/mL administered in drinking water, and (4) MnTE-2-PyP and cephalexin. The animals were allowed unrestricted activity for 2 weeks. Femurs were harvested at the end of 2 weeks. Bone was dissected free of surrounding muscle, weighed, homogenized, sonicated, then plated for quantitative cultures.

Results: Mn TE-2-PyP disrupted established biofilms (after 12 hours of growth) in vitro at both 15 and 30 µM concentrations. Neither dose prevented the formation of the initial biofilm structure. In the infected fracture model, mice regained full weight bearing within 24 hours when fixation was adequate. Treatment with cephalexin alone reduced the bacterial counts at 2 weeks by 75% compared to no drug treatment, but there were residual bacteria cultured in all of the animals. In the MnTE-2-PyP with cephalexin group, bacterial cultures were zero in all animals at 2 weeks ($P < 0.0001$)

Conclusions: An SOD mimetic drug in combination with standard antibiotic treatment is more effective than antibiotics alone for treating a biofilm associated bone and implant

infection. In vitro work suggests that the drug interferes with maintenance of the biofilm EPS structure, which may allow improved antibiotic penetrance as well as improved immune cell activity.

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•**Rifampin and Minocycline-Containing Coating for Orthopaedic Implants with Potent In Vivo Activity**

*Mark Schallenberger, MS; Todd R. Meyer, PhD;
Bacterin International, Belgrade, Montana, USA*

Purpose: Microbial contamination of implanted devices remains a significant complication in orthopedic medicine. While antimicrobial surface technologies have revolutionized a variety of medical devices, an efficacious antimicrobial coating for orthopaedic devices has remained elusive. To address this challenge we have developed a strongly adherent, biocompatible rifampin- and minocycline-containing coating for orthopaedic implants. Herein, we evaluate the efficacy of coated external fixation pins through several in vitro assays and in an animal model of pin-track infection.

Methods: The in vitro performance of coated Kirschner wires (K-wires) was evaluated by performing repeat zone of inhibition (ZOI) studies and measuring antimicrobial elution kinetics. For the in vivo evaluation of the technology, K-wires with and without the antimicrobial-containing coating were implanted bilaterally into the tibial metaphysis of New Zealand White rabbits. The surrounding soft tissue was surgically closed and the K-wire skin interface was inoculated with a suspension of 1×10^7 colony forming units (cfu) of *Staphylococcus aureus*. After 7 days, the animals ($n = 8$) were euthanized and the severity of infection was evaluated through the enumeration of adherent cfu on the surface of the K-wires. Additionally, pin loosening and local inflammation was evaluated semi-quantitatively. The size of the K-wires and location of placement were chosen to mimic the human clinical use of 5.0-mm half pins. No systemic antibiotics were administered in order to represent a worst-case scenario for microbial virulence.

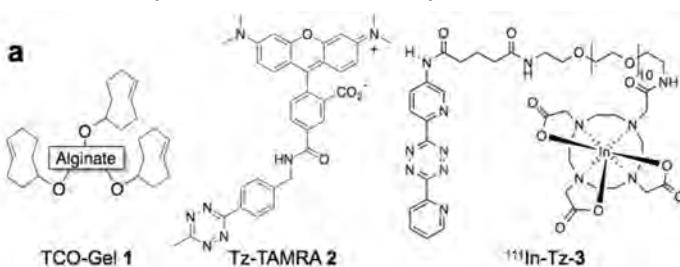
Results: In vitro testing demonstrated that the antimicrobial-containing coating produced sizeable plate-to-plate ZOIs for 42 days and continuously released the antimicrobial agents in quantities above pathogenic MICs (minimum inhibitory concentrations) for at least 70 days. The coating completely inhibited biofilm formation on the surface of the K-wires in vivo (limit of detection = 3.7×10^1 cfu/cm²), while the non-coated K-wires were colonized with $3.0 \times 10^6 \pm 1.5 \times 10^5$ cfu/cm², a 4.9 log reduction ($P < 0.0001$). Clinical microbiology confirmed that the bacteria recovered on the control implants were the strain of *S. aureus* employed in the testing. The coated K-wires also maintained significantly higher anchoring strength ($P = 0.017$) and displayed significantly reduced local tissue inflammation ($P = 0.001$) compared to the controls. Furthermore, animals implanted with coated devices lost significantly less weight during the study (4.9% vs. 12.9%, $P = 0.007$) and were significantly less likely to develop a fever (6.3% vs. 43.8% of study days, $P = 0.017$) than animals implanted with control devices.

Conclusion: As microbial contamination of implants continues to present serious complications in orthopaedic medicine, new technologies are urgently needed to address this challenge. The described rifampin- and minocycline-containing coating has demonstrated excellent in vitro and in vivo activity. These results, coupled with the previously reported biocompatibility and strong coating adhesion, makes this technology an exciting prospect for clinical development.

See pages 99 - 147 for financial disclosure information.

Δ In Vivo Chemistry and Implantable Biomaterial for Targeting Therapeutics**José M. Mejía Oneto, MD, PhD¹; Munish C. Gupta, MD¹; Kent Leach, PhD¹;****Mark A. Lee, MD¹; Maksim Royzen, PhD²;****¹University of California, Davis Medical Center, Sacramento, California, USA;****²University at Albany, State University of New York, Albany, New York, USA**

Purpose: This study is designed to evaluate a novel drug delivery system aimed to optimize local drug concentrations of systemic medications. Prior studies have shown that through in vivo chemistry a systemic molecular payload containing tetrazine (Tz) can be localized to an area previously tagged with an antibody modified with trans-cyclooctenes molecules (TCO). To explore the potential of this method for the treatment of focal orthopaedic infections, we set out to quantify the size of the molecular payload that can be delivered and to establish that a molecular payload could be delivered in vivo to TCO covalently bonded to an alginate gel.

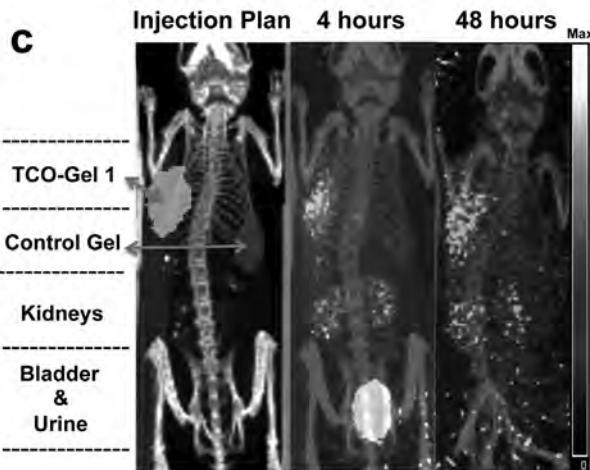
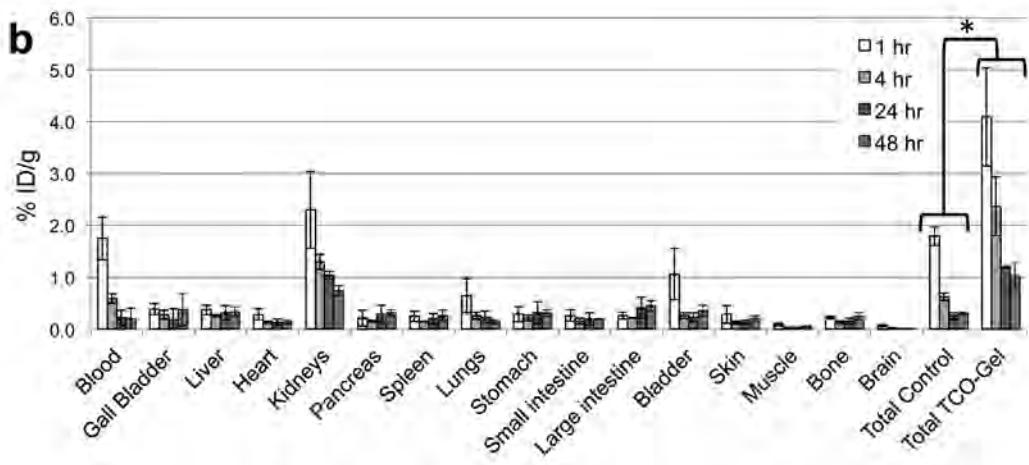


Methods: Investigators synthesized the desired molecules through organic chemistry: a modified alginate (TCO-Gel 1), a fluorescent probe (Tz-TAMRA 2), and a radioactive probe (¹¹¹In-Tz-3) (Figure a). To quantify the maximum amount of a molecular payload that can be delivered to the biomaterial, TCO-Gel 1 was exposed to fluorescent Tz-TAMRA 2 (ex. [excitation] 555 nm, em. [emission] 580 nm), and then the supernatant was removed. Fluorescence images of the supernatant were taken and fluorescence outputs were quantified digitally. Control alginate was treated identically and compared. After Institutional Animal Care and Use Committee approval, in vivo biodistribution studies were carried out by injecting either control or TCO-Gel 1 subcutaneously at each flank area of BALB/c mice. 3 to 4 hours later the subject received a tail vein injection of ¹¹¹In-Tz-3 in normal saline (mean dose 1.63 MBq). Mice (n = 3) were euthanized at 1, 4, 24, and 48 hours. Organs, bodily fluids of interest, and gels were harvested and washed. Radioactivity was measured using a gamma counter, corrected for isotope decay and presented as percent injected dose per gram (%ID/g). A similar approach was used for in vivo imaging studies, except with a larger dose of ¹¹¹In-Tz-2 (38.8 MBq). At 4 and 48 hours, the mouse was anesthetized and imaged with a SPECT (single photon emission computed tomography)/CT imaging station.

Results: Our in vitro studies revealed that a molecular payload of 29.9 nmoles can be delivered per mL of 2.0% (w/v) alginate solution in ddH₂O containing Dulbecco's PBS (phosphate-buffered saline) and calcium sulfate ions as cross-linkers. Our in vivo studies revealed that we can deliver more than 4% ID/g to the subcutaneous space of a murine model at 1 hour compared to < 0.3% ID/g delivered to musculoskeletal areas. The radioactivity level is maintained above 1% ID/g at the TCO-Gel 1 even after 48 hours. The difference between the groups is statistically significant at all time points (Figures b and c).

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Conclusions: We present a simple and modular method to modify a biomaterial with small molecules after in vivo implantation. This approach enables a hydrogel to enhance the spatial location of systemic small molecules through in vivo delivery by an order of magnitude. Further studies are required to assess this methodology with therapeutics molecules that are relevant to orthopaedic challenges.

Sonication Has the Potential to Improve Culture Yield in Patients with Clinical Infection

Hemil Hasmukh Maniar, MD; Kristin McPhillips, MD, MPH; Jove Graham, PhD; Michael Foltzer, MD; Thomas R. Bowen, MD; Daniel S. Horwitz, MD; Geisinger Medical Center, Danville, Pennsylvania, USA

Purpose: The number of patients with orthopaedic infections is rising with increased number of surgeries performed. Most infections involving orthopaedic implants result in the formation of a biofilm on the implant; organisms living in the biofilm are difficult to collect for laboratory analysis because they are adhered to the implant. Sonication dislodges bacteria from metal surfaces using low-frequency ultrasound, allowing for better culture yield. While arthroplasty explants are routinely sonicated, the sonication results of trauma explants are not known.

Methods: In an IRB-accepted retrospective review, all patients who had surgical explantation of an orthopaedic trauma device (plates, screws, nails) from August 2010 to July 2013 were included in the study. External fixators and other implants that intentionally extended through the skin were excluded. A detailed review of the electronic medical record was performed to note the indication for explantation as well as preoperative clinical and laboratory features to diagnose infection. Postoperative results of tissue culture and sonicate fluid were studied. Infected patients without routine cultures were excluded. Patients with intraoperative features of infection were also considered "infected." Clinical evidence of infection was considered the "gold standard."

Results: A total of 146 orthopaedic trauma-related devices (plates = 60, screws = 48, nails = 29, nail screws = 6, other = 3) explanted in the study period were sonicated. 32 of 146 (22%) were from clinically infected patients. 30 of these (94%) had a positive culture and 2 (6%) had a negative culture. In one clinically infected patient with a negative culture, sonication was able to detect presence of a specific organism in low yield. In another patient, with a positive culture, sonication was able to detect an additional organism. Overall, if explanted orthopaedic devices in patients with known clinical infection were to be subjected to sonication only and not cultured, a positive microbiological yield with sonication would be 29/32 (91%) (including counts less than 20 colony forming units) as opposed to 30/32 (94%), a difference not statistically significant (Table 1). Sonication had a high sensitivity 29/32 (91%; 95% confidence interval [CI]: 75%-98%) but low specificity 72/114 (63%; 95% CI: 54%-72%) for clinical infection (Table 2). Some patients without infection did not get routine cultures; however, among those who were cultured, sensitivity and specificity of culture to detect infection were 94% and 88%, respectively

Sonication	Culture		Total
	Positive	Negative	
Positive	28	1	29
Negative	2	1	3
Total	30	2	32

Table 1. Culture Versus Sonication Results in Clinically Infected Patients (N = 32)

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Sonication	Clinical Infection		Total
	Positive	Negative	
Positive	29	42	71
Negative	3	72	75
Trauma	32	114	146

Table 2. Clinical Infection Versus
Sonication Results in All Patients (N = 146)

Conclusion: In clinically suspected cases with infection, combined sonication and culture appears to increase the ability to detect biofilm forming organisms. Appropriate antibiotic therapy specific to those organisms can then be initiated. Further studies with larger sample size would be beneficial.

Pulsatile Lavage of Open Musculoskeletal Wounds Causes Muscle Necrosis and Dystrophic Calcification

Astor Devon Robertson, MBBS¹; Stephen Zhao, BS¹; Thao Nguyen, MD¹; David E. Jaffe, MD¹; Carla Hebert, BS¹; William Lawrence Journey, PhD²; Joseph Stains, PhD¹; Vincent D. Pellegrini Jr, MD³;

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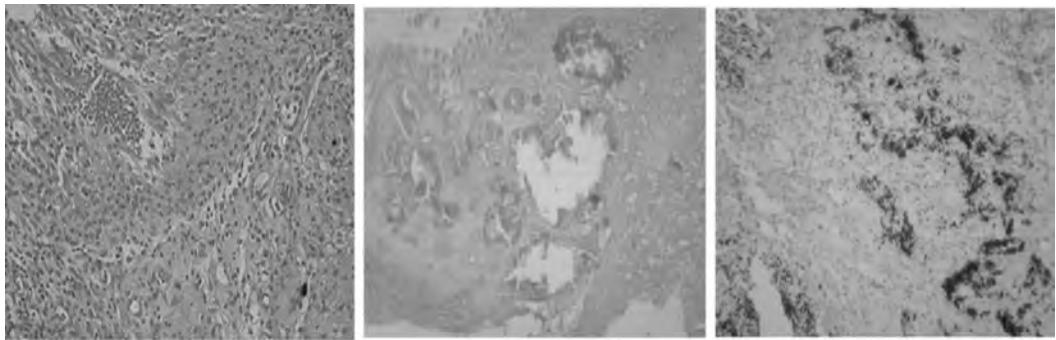
Purpose: Adequate wound irrigation of open musculoskeletal injuries is unanimously regarded as indispensable in the prevention of infection by decreasing bacterial load and other contaminants. While the removal versus the further seeding of debris into host tissue has been the subject of numerous studies, the detrimental effects of irrigation on muscle tissue has hardly been reported. This study aims to assess the relative damage to host muscle by pulsatile versus bulb syringe irrigation.

Methods: 24 Sprague-Dawley rats underwent hindlimb blast amputation via a column of propelled water following detonation of a submerged explosive. All wounds were irrigated with a 40:1 saline to chlorhexidine solution and had primary closure. There were three treatment groups (each n = 12): Group 1 underwent debridement above the zone of injury (ZOI) with through-knee amputation and 250 mL bulb syringe irrigation; Group 2 underwent both pulsatile lavage and through-knee amputation and irrigated with 1 L of solution using pulsatile lavage (Dental Pik) at 15-20 psi. In Group 3, an additional 6 animals were not subjected to the blast procedure but underwent a 3-cm anterolateral left thigh incision down to muscle, and pulsatile lavage of the wound. The animals were followed with serial AP and lateral radiographs monitoring for the appearance and evolution of any soft-tissue radiopacities until euthanasia at 24 weeks. X-ray-guided excisional muscle biopsies on a few representatives from each group were done at 6 weeks and post euthanasia, and prepped for histologic analysis with hematoxylin and eosin (H&E), Alizarin Red, and Von Kossa stains. Both of the latter are special stains for calcium deposits.

Results: All animals treated with bulb syringe irrigation had a benign radiographic course, with no evidence of radiopaque lesions. On the contrary, all animals that were subject to pulsatile lavage at 20 psi developed radiopacities that first appeared at around 10 days postoperatively, increased in density up to around 16 weeks, then showed signs of gradual decrease thereafter. H&E, Alizarin Red, and Von Kossa staining all revealed evidence of tissue damage with an abundance of inflammatory cells, and calcium deposits.

Conclusion: Pulsatile lavage when used in trauma-related musculoskeletal injuries may cause additional insult to viable muscle tissue resulting in muscle necrosis, which can be complicated by dystrophic calcification as evident in our results. Bulb syringe irrigation, while not as effective in the removal of debris, appeared to be the safer irrigation option of the two.

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H&E

Alizarin Red

Von Kossa



Dystrophic calcification is apparent in blast amputation (left) and non-blast (right) limbs following pulsatile lavage.

See pages 99 - 147 for financial disclosure information.

Failure of Indomethacin and Radiation to Prevent Blast-Induced Heterotopic Ossification in an Animal Model

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Robert E. Holmes, MD²; David E. Jaffe, MD¹; Juong G. Rhee, PhD¹;

William Lawrence Fournier, PhD³; Joseph Stains, PhD¹; Vincent D. Pellegrini Jr, MD²;

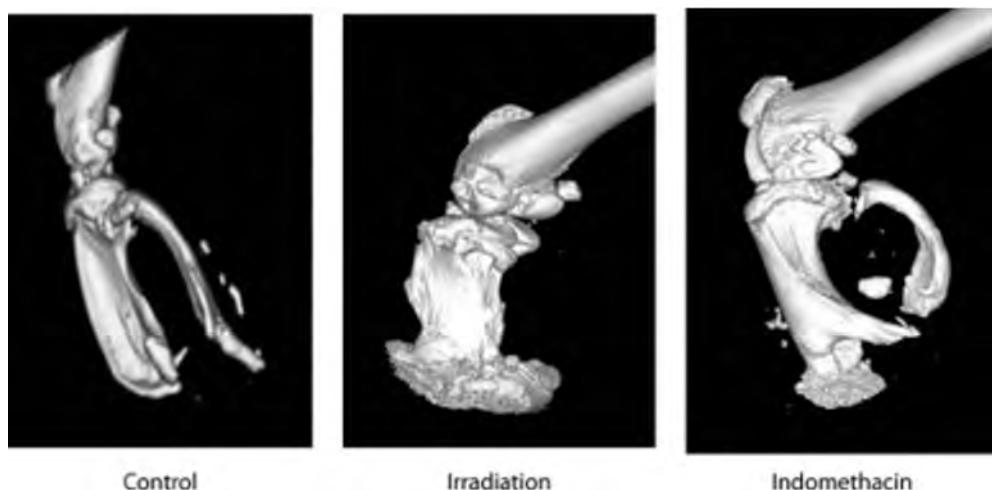
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²Medical University of South Carolina, Charleston, South Carolina, USA;

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Purpose: Heterotopic ossification (HO) in the residual limb has been a common morbidity in soldiers who survive extremity amputation via blast mechanisms during recent war conflicts. While several Level I clinical studies have demonstrated the efficacy of prophylactic regimens using either nonsteroidal anti-inflammatory drugs (NSAIDs) or low-dose external beam irradiation (XRT) to prevent HO formation following total hip arthroplasty and surgical treatment of acetabular fractures, the HO prophylactic potential of any of these treatment modalities in the setting of trauma or trauma related amputation has never been assessed. This study was aimed at investigating the effectiveness of the NSAID indomethacin and irradiation, in the prevention of HO formation following extremity blast amputation in a rat model.

Methods: 36 Sprague-Dawley rats were subjected to blast amputation of a hindlimb via a column of propelled water following detonation of a submerged explosive. There were 12 controls, 12 animals received an oral suspension of indomethacin at a dose of 3 mg/kg for 10 days starting on operative day, while another 12 received a single dose 8 Gy of irradiation to the amputated stump on the third postoperative day. All wounds were treated with bulb syringe irrigation, minimal debridement of skin edges, and primary closure of fascia and skin. Serial radiographs were done until euthanasia at 24 weeks, at which time HO severity was quantified as (0) absent, (1) mild, (2) moderate, or (3) severe, and HO type qualified as contiguous with the residual stump or as distinct bony islands, by independent graders.



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Results: One animal in the irradiation group died 2 weeks postoperatively and was not replaced. One animal in the control and indomethacin groups, and 2 animals in the irradiation group developed persistent granuloma-like lesions on their residual stumps. These animals all had radiographic evidence of HO. The mean HO severity was 1.5, 1.14, and 1.97 in the control, indomethacin, and irradiation groups, respectively. The qualitative means of HO type were 1.5, 1.47, and 2.12 in the control, indomethacin, and irradiation groups, respectively. Kruskal-Wallis one-way analysis of variance revealed no significant difference in HO severity or type between both treatment groups and control.

Conclusion: While indomethacin and XRT used prophylactically have shown efficacy in the prevention of HO in non-blast extremity injuries, these interventions when administered in a similar fashion seem not to effect any change in the development of HO in the setting of blast-injured extremities with resultant amputation. This revelation may be indicative of the provocation of inciting stimuli so overwhelming that conventional interventions are ineffective. The effect of NSAIDs administered pre-blast, earlier XRT post-blast, or both treatment modalities combined immediately after blast injury warrants further study.

The Surgeon's Catch-22: A Prospective Study on Inflammation, Wound Failure, and Heterotopic Ossification in Combat Wounds

*Donald N. Hope, MD; Jonathan A. Forsberg, MD; Benjamin K. Potter, MD;
Elizabeth M. Polfer, MD; Eric A. Elster, MD, FACS;*

*Walter Reed National Military Medical Center, Bethesda, Maryland, USA;
Naval Medical Research Center, Silver Spring, Maryland, USA*

Background/Purpose: After a decade of war, we have observed an increase in both combat-related injury survival, as well as a paradoxical increase in injury severity, mainly due to the effects of blasts. These severe injuries leave a devastating impact on each patient's immune system, resulting in massive upregulation of the systemic inflammatory response. In this setting, the timing of wound closure is made based mainly on subjective determinations and the surgeon's anecdotal experience. However, closing a wound either too early or too late can be problematic. By examining the inflammatory mediators, preliminary data suggest that it may be possible to correlate complications such wound failure and heterotopic ossification (HO) with distinct systemic and local inflammatory profiles. We asked whether systemic or local markers of inflammation could be used as an objective means to estimate the likelihood of wound failure and/or HO in patients sustaining combat-related injuries.

Methods: 200 combat-wounded active duty servicemembers who sustained high-energy extremity injuries were prospectively enrolled between 2007 and 2013. In addition to injury-specific and demographic data, we quantified 24 cytokines and chemokines in the serum and wound effluent during each debridement. Correlations were investigated between these markers and wound failure or HO.

Results: The relationships between inflammatory proteins and wound-specific outcomes varied throughout the debridement process. For patients who formed HO, serum interleukin (IL)-3 ($P = 0.002$), serum IL-12p70 ($P = 0.0013$), effluent IL-3 ($P = 0.02$), and effluent IL-13 ($P = 0.006$) were independently associated with HO formation. Both serum ProCT ($P = 0.03$) and effluent IL-6 ($P = 0.02$) correlated with wound failure.

Conclusion: We identified correlates of wound-specific complications such as wound failure and HO by characterizing the patient's systemic and local inflammatory response. Models designed to codify these interactions and estimate the likelihood of complications such as wound failure and HO must accommodate nonlinear relationships that vary over time.

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**BSFF SYMPOSIUM 4:
BIGGER DATA - BIGGER PROBLEMS?**

Moderators: *Mohit Bhandari, MD, PhD, FRCSC*
Gerard P. Slobogean, MD, MPH, FRCSC

3:45 pm	What is Big Data? <i>Gerard P. Slobogean, MD, MPH, FRCSC</i>
4:00 pm	NHS Database- What It Can and Cannot Do <i>Peter V. Giannoudis, MD</i>
4:10 pm	Scandinavian Data-Ongoing Challenges and Successes <i>Frede Frihagen, MD, PhD</i>
4:20 pm	Designing Studies That Utilize Large Databases: The Basics <i>Mary L. Forte, PhD</i>
4:30 pm	The Future of Large Scale Databases: Will They Replace the Clinical Trial? <i>Saam Morshed, MD, PhD</i>
4:40 pm	Discussion

NOTES

See pages 99 - 147 for financial disclosure information.

INORMUS Invited Paper - Trauma Worldwide Data Set*Mohit Bhandari, MD, PhD, FRCSC*

NOTES

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Changing the System: Improving Outcome From Major Trauma by Developing a National System of Regional Major Trauma Networks

Christopher G. Moran, MD, FRCS(Ed)¹; Maralyn Woodford²; Fiona Lecky, FRCS, MSc, PhD²; Antoinette Edwards, BA²; Timothy Coats, MBBS, FRCS, MD²; Keith Willett, MD, FRCS³;

¹NHS England, Nottingham University Hospital, Nottingham, United Kingdom;

²Trauma Audit and Research Network, University of Manchester, Manchester, United Kingdom;

³NHS England, Oxford, United Kingdom

Background/Purpose: International evidence suggests that trauma care improves with organized trauma systems. In England, trauma care for the entire population (58.5 million) was reorganized in 2012 with the development of Trauma Networks. All hospitals in the country were designated as either Major Trauma Center (MTC; Level-1: n = 26) or Trauma Units (TU; Level-2), or Local Emergency Hospitals (LEH; Level-3). Prehospital care was also reorganized so that patients identified with major trauma were taken directly to the MTC if within 45 minutes travel time from accident. Other patients are taken to the nearest TU for resuscitation and expert triage before secondary transfer to the MTC. All Level-3 centres are bypassed. This paper reviews the early results.

Methods: From April 2008 until April 2014, data on 118,801 patients with an ISS >8 was prospectively collected by the national Trauma Audit and Research Network (TARN). The probability of survival was calculated using a model including ISS, age, blood pressure on arrival at hospital, and Glasgow Coma Scale (GCS). The odds ratio of survival (+95% confidence interval) was then calculated for each year and normalized to the year 2008-2009.

Results: From 2008 until 2011 there was a no significant change in the odds of surviving major trauma in England. However, following introduction of the Major Trauma Networks there was a significant ($P < 0.008$) 19% increase in the odds of survival during the first year of the new system and a further 17% increase in the odds of survival during the second year as the system matures so that the odds of survival for the population is now 1.36 compared to 2008. Process measures within the trauma system have shown significant increases in reception by an attending-led trauma team, more rapid intubation and CT scan, and increased use of tranexamic acid and massive transfusion protocols (all $P < 0.001$).

Conclusion: We believe this is the first attempt at an organized change in the system for major trauma care on a national level and covering a population of over 50 million. We have observed a significant improvement in the care process together with a significant improvement in the odds of surviving. This demonstrates that improvements seen in smaller state or regional trauma systems can be translated into a national trauma system with similar improvements for the whole population.

Increased Systemic Complications in Open Femoral Shaft Fractures Are Associated with the Degree of Soft-Tissue Injury Rather Than New Injury Severity Score (NISS) Values: A Nationwide Database Analysis

Christian D. Weber, MD¹; Rolf Lefering, PhD²; Thomas Dienstknecht, MD¹;

Philipp Kobbe, MD, PhD¹; Richard M. Sellei, MD¹; Frank Hildebrand, MD, PhD¹;

Hans-Christoph Pape, MD, PhD, FACS¹; Trauma Registry of the German Trauma Society;

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²Institute for Research in Operative Medicine (IFOM), University of Witten/Herdecke, Cologne-Merheim Medical Center, Cologne, Germany

Background/Purpose: In blunt high-energy trauma, the degree of soft-tissue injuries associated with femoral shaft fractures may vary. The objective of this study was to assess the impact of open versus closed soft-tissue injuries associated with femoral shaft fractures on major systemic complications and mortality after trauma.

Methods: In this prospective cohort study from a population-based trauma database, patients with femoral shaft fracture (AO/OTA-32) were divided into the following groups: closed femoral shaft fracture (CFSF) and open femoral shaft fracture (OFSF). Open soft-tissue injuries were classified according to the Tscherne classification. Data of demographic, injury, therapy, and outcome characteristics (eg, multiple organ failure [MOF], sepsis, mortality, length of stay [LOS]) were collected and analyzed using SPSS.

Results: Data from 32,582 trauma victims were documented in a nationwide trauma registry between January 1, 2002 and December 31, 2013. Among 5761 trauma patients (NISS 30 ± 14 points), 4423 belonged to the CFSF group (77%) and 1338 belonged to the OFSF group (23%). Open fractures were separated into I° (334, 28.1%), II° (526, 44.3%), III° (309, 26%), and IV° (19, 1.6%). OFSF are associated with an increased risk for hemorrhagic shock (HS), higher resuscitation requirements, and increased in-hospital and intensive care LOS, but not with increased injury severity according to NISS, sepsis, or mortality. The prevalence of MOF, sepsis and mortality increased with the degree of open soft-tissue injury.

Conclusion: Open femur fractures were not associated with higher injury severity scores (NISS), but with an increased risk for HS, higher resuscitation requirements, MOF, and increased length of stay (LOS). The incidence of sepsis and mortality increased with the degree of open soft-tissue injury. The treatment of OFSF seems to be more complex and time-consuming, but the risk for major clinical complications (eg, sepsis, mortality) seems to be comparable for both groups.

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Anatomic Region and the Risk of Adverse Events in Orthopaedic Trauma: An Analysis of 19,000 Patients

Cesar S. Molina, MD; Rachel V. Thakore, BS; Eduardo J. Burgos, MD;
William T. Obremskey, MD, MPH, MMHC; Manish K. Sethi, MD;
Vanderbilt University, Nashville, Tennessee, USA

Purpose: Little data exist exploring adverse events in orthopaedic trauma surgery. As our health-care system creates potential reimbursement implications for perioperative complications through readmission penalties, etc, it is increasingly important to turn our attention to this issue. Through the American College of Surgeons National Surgical Quality Improvement Program (ACS-NSQIP) database, we sought to compare adverse events in orthopaedic trauma procedures by anatomic region (upper extremity [UE], hip and pelvis [HP], and lower extremity [LE]) and to evaluate the impact of anatomic region on the overall rate of complications.

Methods: The ACS-NSQIP prospective database was used to identify a total of 91 CPT codes representing 19,028 orthopaedic trauma patients from 2005-2011. These patients were then divided into three anatomic regions: UE ($n = 4925$), HP ($n = 5273$), and LE ($n = 8830$). Perioperative minor and major complications were recorded and include wound dehiscence, superficial surgical site infection, pneumonia, urinary tract infection, death, deep wound infection, myocardial infarction, deep venous thrombosis, pulmonary embolism, peripheral nerve injury, sepsis, and septic shock. A comparison in perioperative complications between the three groups was performed using c^2 analysis. We used a multivariate analysis that controls for age, medical comorbidities, American Society of Anesthesiologists (ASA) status, operative time, baseline functional status, and anatomic region to evaluate risk factors for complications.

Results: A total of 19,028 orthopaedic trauma cases were divided into three anatomic regions: 25.9% ($n = 4925$) UE, 27.7% ($n = 5273$) HP, and 46.4% ($n = 8830$) LE. Table 1 shows the difference in age, ASA scores, and complication rates between the three groups. Statistically significant differences were identified when comparing demographics between HP and UE patients; these include the number of patients in each group over 65 years of age (85% vs. 32.2%), ASA >2 (78.9% vs. 32.4%), and diabetes (17.9% vs. 11.1%) ($P = 0.01$). No other variables were significantly different among the groups. After controlling for several important individual patient factors, hip and pelvis patients are nearly four times more likely to develop any perioperative complication than upper extremity patients (odds ratio [OR]: 3.79, 95% confidence interval [CI]: 3.01-4.79, $P = 0.01$). Also, patients in the LE group are three times more likely to develop any complication versus UE patients (OR: 2.82, 95% CI: 2.30-3.46, $P = 0.01$). The table shows the differences in patient age and ASA status as well as presents the overall complication rates:

Table 1. Patient Demographics/Characteristics and Rates of Complications*

Anatomic Region	Mean Age*	Mean ASA Score*	Complication Rate*
Upper extremity (n = 4925)	55.4 ± 19.2	2.16 ± 0.77	3.0% (148)
Hip / pelvis (n = 5273)	79.3 ± 14.0	2.95 ± 0.67	19.0% (1002)
Lower extremity (n = 8830)	70.2 ± 19.3	2.77 ± 0.77	14.2% (1251)

* $P < 0.005$

Conclusion: There is an alarming difference in complication rates among anatomic regions in orthopaedic trauma patients. Even after controlling for several variables, patients undergoing procedures to the LE are almost three times more likely to develop a complication than patients in the UE group. Those undergoing procedures to the HP are almost four times more likely to develop a complication than patients in the UE group. While some of these results are explained by age and ASA status, further studies are required to explain the impact that anatomic region has on the overall complication rate.

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**BSFF SYMPOSIUM 5:
ADVANCES IN ARTICULAR CARTILAGE INJURY AND TREATMENT
- WHERE WE ARE AND WHERE WE'RE GOING**

Moderators: *Joseph Borrelli Jr, MD*
Susanna Chubinskaya, PhD

7:30 am	Cartilage's Response to Injury <i>Dominik Haudenschild, PhD</i>
7:40 am	Current/Futures Chondroprotective Products <i>Susanna Chubinskaya, PhD</i>
7:50 am	Chondroplasty/Microfracture/Cells for Treatment of Cartilage Injuries <i>Seth Gasser, MD</i>
8:00 am	OsteoChondral Allografts in 2014 <i>James Stannard, MD</i>
8:10 am	Joint Preservation: Treatment of Intra-articular Malunions <i>Christian Krettek, MD, FRACS</i>
8:20 am	Discussion

NOTES

ΔThe Dose-Response Effect of the Mast Cell Stabilizer, Ketotifen Fumarate, on Post-Traumatic Joint Contractures

*Prism Schneider, MD, PhD, FRCSC; Herman Johal, MD, MPH; Andrew R. Buckley;
Kevin A. Hildebrand, MD, FRCSC;
University of Calgary, Calgary, Alberta, Canada*

Purpose: Posttraumatic joint contracture (PTJC) is a debilitating complication following an acute fracture or intra-articular injury, which can lead to loss of motion at the affected joint. Prior research has shown that, in a rabbit model, treatment with ketotifen can significantly reduce the severity of joint contracture; however, the doses used previously were 0.5 mg / kg or 1.0 mg / kg twice daily, and there was no dose-response relationship shown over this narrow range. Prior to clinical testing in humans, knowledge of the dose-response relationship is required and an optimal dose range needs to be identified where contracture reduction is maximized while side effects are avoided. We hypothesize that there will be a linear dose-response effect of ketotifen on posttraumatic contractures and joint capsule properties, using an in vivo rabbit model.

Methods: After obtaining IRB approval, an in vivo model of PTJC of the knee was created, using a combination of intra-articular injury and internal immobilization in skeletally mature New Zealand White rabbits. Five groups of animals were studied ($n = 10$ per group): a nonoperative control group, a group with the operatively created PTJC and no pharmacological treatment (operative contracture group), and three groups with the operatively created PTJC treated with a mast cell stabilizer, ketotifen fumarate, at doses of 0.01 mg / kg, 0.1 mg / kg, and 5.0 mg / kg ketotifen twice daily (the 0.01-mg / kg, 0.1-mg / kg, and 5.0-mg / kg ketotifen groups). After 8 weeks of immobilization, flexion contractures were measured using a custom rabbit-knee-gripping device, attached to a hydraulic materials testing machine (MTS, Eden Prairie, MN) and the posterior aspect of the joint capsule was harvested for immunohistochemical quantification of myofibroblast and mast cell numbers.

Results: Flexion contractures developed in the operative contracture group and the severity of the contractures exhibited a dose-response reduction in all three of the groups treated with 0.01 mg / kg, 0.1 mg / kg, and 5.0 mg / kg of ketotifen, respectively; however this effect was greatest between the 0.01-mg / kg and 0.1-mg / kg doses. The joint capsule myofibroblast and mast cell numbers in the operative contracture group were significantly increased compared with the values in the control group ($P < 0.001$), and the myofibroblast and mast cell numbers in the 0.1-mg / kg and 5.0-mg / kg ketotifen groups were significantly reduced compared with the values in the operative contracture group ($P < 0.001$). There were 2 implant failures, 1 deep infection resulting in death, and 2 pre-experimental deaths during the acclimatization period.

Conclusion: The use of a mast cell stabilizer, ketotifen fumarate, reduces the biomechanical and cellular manifestations of joint capsule fibrosis in a rabbit model of posttraumatic joint contracture and a dose-response relationship was identified. Reduction in biomechanical and cellular contracture properties began at the 0.1-mg / kg dose, which is lower than previously studied doses. This study suggests that an inflammatory pathway, mediated by mast cell

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activation, is involved in the induction of joint capsule fibrosis after traumatic injury. The range of doses used in this study includes both human-equivalent dosing and the currently used therapeutic dose for the treatment of asthma in humans.

ΔPresence and Degree of Matrix Metalloproteinases and Aggrecan Breakdown Products in the Setting of Acute Intra-Articular Fracture

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University of Utah Department of Orthopaedics, Salt Lake City, Utah, USA

Background/Purpose: Matrix metalloproteinases (MMPs) are a group of enzymes that play a role in tissue remodeling and have the ability to degrade articular cartilage. Aggrecan is a proteoglycan found in the extracellular matrix of cartilage. Proteolytic cleavage of aggrecan leads to aggrecan fragments that may be associated with cartilage degradation. Prior studies have demonstrated an association between the degree of inflammatory osteoarthritis and the presence of MMPs and presence of aggrecan breakdown products, suggesting a causative role in those diseases. The presence or absence of these compounds in the acute setting of articular fracture has not been established. Previous study of inflammatory cytokines demonstrated a local response, but did not examine these compounds. This study is designed to evaluate the presence of MMPs and aggrecan breakdown products following intra-articular tibial plateau fracture.

Methods: After IRB approval, investigators prospectively aspirated synovial fluid from the injured and uninjured knees of 45 patients between the ages of 18 and 60 years with tibial plateau fractures. Patients with open fracture, history of autoimmune disease, preexisting arthritis, or presentation greater than 24 hours from injury were excluded. The 20 patients who required spanning external fixator followed by definitive fixation were aspirated at both surgeries. The concentrations of MMP-1, -2, -3, -7, -9, -10, -12, and -13 were quantified using multiplex assays. Additionally, aggrecanase-cleaved aggrecan fragments were quantified using a sandwich ELISA (enzyme-linked immunosorbent assay) with an α-ARGS monoclonal antibody. Repeated-measures analysis of variance was used to test for differences on the log-transformed variables.

Results: We enrolled 45 patients (14 females, 31 males), with an average age of 42 years (range, 20-60). There were 24 low-energy (OTA 41B or Schatzker 1-3, all OTA 41B) tibial plateau injuries and 21 high-energy (Schatzker 4-6) tibial plateau injuries. Of the high-energy fractures, 6 were OTA 41B3 and 15 were OTA 41C. There were significantly higher concentrations of MMP-1 ($P < 0.001$), MMP-3 ($P < 0.001$), MMP-9 ($P < 0.001$), MMP-10 ($P < 0.001$), MMP-13 ($P = 0.001$), and aggrecan breakdown ($P = 0.021$) in injured knees as compared to uninjured knees. Only MMP-9 ($P = 0.05$) was found to be significantly greater in high-energy as compared to low-energy injuries. Interestingly, aggrecan breakdown ($P = 0.007$) was significantly greater in low-energy as compared to high-energy injuries. MMP-1 ($P < 0.001$), MMP-3 ($P = 0.026$), MMP-12 ($P < 0.001$), and aggrecan breakdown ($P < 0.001$) were significantly greater at the time of the second procedure at an average of 9.5 days (range, 3-21 days) from initial surgery. Conversely, the concentration of MMP-9 was significantly less at the time of the second surgery ($P = 0.005$). Both MMP-10 and MMP-7 remained elevated at the second time point, but this trend was not statistically significant. While MMP-13 was not significantly elevated in the injured compared to uninjured knee at the time of initial injury, MMP-13 was significantly elevated at the second procedure ($P < 0.001$).

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Conclusion: There is a significant increase in aggrecan breakdown and presence of MMPs in the acutely injured knee compared to the control knee, demonstrating the response after joint trauma to be local. In contrast to other inflammatory mediators examined, only MMP-9 was acutely elevated in high-energy as compared to low-energy injuries, suggesting that this proteinase may be associated with greater acute joint destruction. Perhaps most importantly, most of the MMPs and amount of aggrecan breakdown continued to be elevated a mean of 10 days after injury, demonstrating that the cartilage is subjected to continued interaction with matrix degenerating proteases over a week after injury. Given these data, these factors merit further investigation for a potential role in the ultimate development of posttraumatic arthritis.

Pre-Injury Depletion of Macrophages Results in Increased Acute Joint Inflammation Following Articular Fracture

Karsyn N. Bailey; Bridgette D. Furman, BS; Kelly A. Kimmerling, MS; Chia-Lung Wu, PhD; Janet L. Huebner; Virginia B. Kraus, PhD; Farshid Guilak, PhD; Steven A. Olson, MD; Duke University Medical Center, Durham, North Carolina, USA

Background/Purpose: Posttraumatic arthritis (PTA) is an accelerated form of arthritis that occurs following joint injury. Following articular fracture, C57BL/6 mice showed significant signs of PTA, whereas MRL/MpJ mice, a superhealer mouse strain, exhibited less severe joint degeneration. In order to elucidate the link between macrophages and PTA, a transgenic mouse strain that allows for the specific depletion of macrophages was used in this study. The Macrophage Fas-Induced Apoptosis (MAFIA) mouse strain expresses the inducible Fas-suicide gene, which facilitates apoptosis of macrophages following the administration of AP20187. We hypothesized that locally depleting synovial macrophages in the MAFIA mice would reduce joint synovitis following articular fracture. The objective was to characterize the role of macrophages in acute joint inflammation and synovitis following articular fracture.

Methods: Male MAFIA mice (Jackson Laboratories) were obtained at 6 weeks of age and then aged to skeletal maturity at 16 weeks, at which point the left hindlimb was subjected to a moderate articular fracture as previously reported. Macrophages were locally depleted at 2 days prior to fracture, immediately following fracture, or 2 days post fracture. The AP20187 ($n = 6$ per time point), which induces macrophage apoptosis in the MAFIA mice, or the carrier solution ($n = 3$ per time point) were delivered via a single 6- μ L intra-articular injection. The mice were sacrificed 7 days post fracture, the limbs were harvested, and serum and synovial fluid were collected and stored at -80° for future analysis. All hindlimbs were formalin fixed and scanned with micro-CT (SkyScan 1176, Bruker BioSpin). The limbs were then processed and paraffin embedded using standard techniques. Histological sections of the joint were stained using hematoxylin and eosin, and synovitis was quantified using a modified synovitis grading scheme for mouse tissue. A multifactorial analysis of variance was used to assess synovitis by group and time, with limb as a repeated measure.

Results: Articular fractures were successfully created in all mice. For synovial inflammation, fracture in the experimental limb led to increased total synovitis scores compared to contralateral control limbs in all groups ($P < 0.05$). Additionally, with predepletion (Day -2), mice that received AP20187 had significantly higher joint synovitis in fractured limbs compared to carrier solution ($P = 0.05$). At the other two time points, where depletion occurred on the day of fracture (Day 0) or 2 days post fracture (Day 2), there was no significant difference in the level of synovitis between AP20187 and the carrier solution. Mice that received a local injection of AP20187 prior to fracture (Day -2) exhibited severe joint inflammation characterized by synovial infiltration with increased cellular density.

Conclusion: The observed changes at all points evaluated did not support our hypothesis; macrophage depletion did not reduce acute joint inflammation following articular fracture. Conversely, when depleted 2 days prior to fracture, joint inflammation increased following articular fracture. The massive influx of inflammatory cells was observed mostly in mac-

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rophage-depleted joints, suggesting that macrophages could be a modulator for recruiting inflammatory cells following injury. Although inflammation has degenerative effects on cartilage, our data suggest that macrophages are important for regulating synovial inflammation and bone maintenance after joint injury. This finding is significant for understanding the role of macrophages in inflammation and joint injury. Our data suggest that macrophages are important to maintain a controlled inflammatory response to joint injury.

Funding Sources: Arthritis Foundation Grant 5244, National Institutes of Health, Department of Defense.

**BSFF SYMPOSIUM 6:
OSTEOPOROSIS- ARE WE CLOSER TO GOLD STANDARDS?**

Moderator: *Theodore Miclau III, MD*

9:25 am	Fracture Models: Diaphyseal and Metaphyseal Healing <i>Volker Alt, MD</i>
9:35 am	Mechanical Testing: Selection of a Model <i>Loren Latta, PhD</i>
9:45 am	Medical Management <i>Joseph Lane, MD</i>
9:55 am	Atypical Femur Fractures – New Information <i>Kenneth A. Egol, MD</i>
10:05 am	Augmentation of Fixation: Selection of an Optimal Material <i>Meri Tibi Marmor, MD</i>
10:15 am	Discussion

NOTES

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Is There a Future for Femoroplasty in Hip Fracture Prevention?

Introducing Anisotropy Restoring Femoroplasty

*Edward K. Rodriguez, MD, PhD; Leandro Grimaldi, MD; Aidin Masoudi, MD;
Ara Nazarian, PhD;*

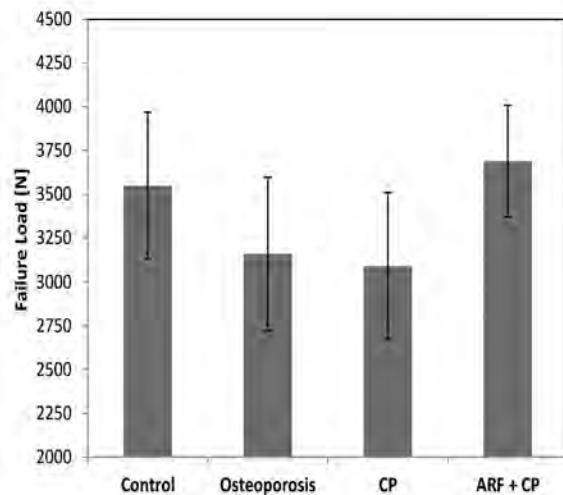
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Background/Purpose: There are presently no standard of care interventional procedures aimed at preventing hip fracture occurrence in geriatric patients. Prior work on femoroplasty has focused on attempts to increase fracture resistance of the osteoporotic proximal femur by insertion of polymethylmethacrylate (PMMA), or other polymeric isotropic fillers. Results thus far have been inconsistent and no method has been adopted clinically. We introduce Anisotropy Restoring Femoroplasty (ARF), a technique that aims to mimic proximal femur anisotropy as defined by trabecular architecture. ARF combines linear structural elements with calcium phosphate (CP) filler to result in increased overall fracture resistance and improved stiffness matching.

Methods: A proof of concept pilot study was performed in which a minimally invasive intraosseous ARF device prototype was designed and tested in a cadaveric porcine proximal femur model to test whether ARF is a viable option for hip fracture prevention. Four groups of 6 porcine proximal femurs each—(1) normal control, (2) detrabeculated and partially decalcified (simulated osteoporotic), (3) simulated osteoporotic with CP isotropic femoroplasty, and (4) simulated osteoporotic with ARF device plus CP—were instrumented and tested to failure to quantify maximum load tolerance and construct stiffness.

Results: Insertion of the ARF device plus CP restores load to failure and stiffness of the proximal simulated osteoporotic pig femur model to nearly normal values when tested to failure. The simulated osteoporotic group and the CP femoroplasty reinforced group (without ARF) exhibited similar failure load and stiffness, both significantly below the control group and the simulated osteoporotic group reinforced with the ARF device plus CP ($P < 0.05$ for both cases).

Conclusion: In this proof of concept pilot study, Anisotropy Restoring Femoroplasty results in increased fracture prevention potential when compared with traditional isotropic femoroplasty. A future minimally invasive procedure that effectively improves fracture resistance in osteoporotic proximal femur could result in a significant reduction of mortality, morbidity, and cost associated with geriatric hip fracture care.



See pages 99 - 147 for financial disclosure information.

Comparison of Femoral Head Rotation and Varus Collapse Between a Single and Integrated Dual Screw Intertrochanteric Hip Fracture Fixation Device Using a Chair Rise Biomechanical Model

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Purpose: This study was conducted to compare the efficacy of two intertrochanteric fracture fixation devices in preventing femoral head rotation and varus collapse using a cadaveric biomechanical model. We hypothesized that an integrated dual screw construct would confer greater stability than a single screw construct.

Methods: 11 matched pairs of cadaveric osteopenic female hemipelves (T-scores: -1.5 ± 0.5 ; age: 72.8 ± 5.8 years) were used. The hip joint and capsule were retained during soft-tissue dissection. An unstable intertrochanteric fracture without calcar support (OTA 31-A2) was created in each specimen using a customized jig prior to reduction and fixation with either a single screw (Gamma3, Stryker Orthopaedics, Mahwah, NJ) or integrated dual screw fixation device (InterTAN, Smith & Nephew, Memphis, TN) under fluoroscopic guidance (Fig. 1, inset). Specimens were secured in fiberglass resin and coupled to a custom biomechanical testing apparatus (Fig. 1) and subjected to 3 months of chair rise simulation using a combination of controlled pelvis rotation ($\pm 45^\circ$) and corresponding axial loading at 2:1 body weight (BW) ratio for 13.5 K cycles while allowing any passive internal-external femoral shaft rotation. Optoelectronic triads quantified varus collapse and rotation about the neck axis temporally throughout cycling. If specimens survived 3 months of simulated chair rise loading (13.5 K cycles at $2 \times$ BW), an additional 2 K cycles of loading was performed in $0.25 \times$ BW/250 cycle increments to a maximum of $4 \times$ BW or until failure.

Results: Femoral head rotation with the integrated dual-screw fixation construct was significantly less than the single screw construct after 3 months of simulated chair rise (3.2° vs. 24.5° , $P = 0.016$, see Fig. 2). Maximum femoral head rotation at the end of $4 \times$ BW loading or until failure was significantly less ($7\times$) for the integrated dual screw than the single screw construct (5.5° vs. 35.4° , $P = 0.006$). Varus collapse was significantly less with the integrated dual screw construct when compared to the single screw construct over the entire cyclic loading protocol (5.4° vs. 8.4° , $P = 0.021$, see Fig. 3).

Conclusion: An integrated dual screw construct confers significantly greater resistance to multiplanar femoral head rotation and varus collapse over 3 months of simulated chair rise. This laboratory study provides biomechanical evidence that an integrated dual screw fixation device may be favorable and provide more predictable fixation than single screw fixation for the treatment of unstable, extracapsular intertrochanteric fractures in the elderly patient population with compromised bone quality.

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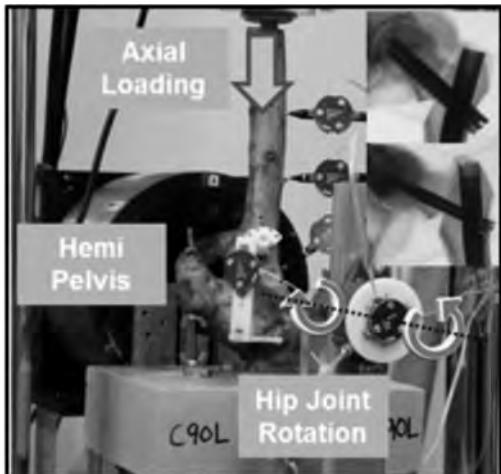


Fig. 1. Chair rise biomechanical test setup.

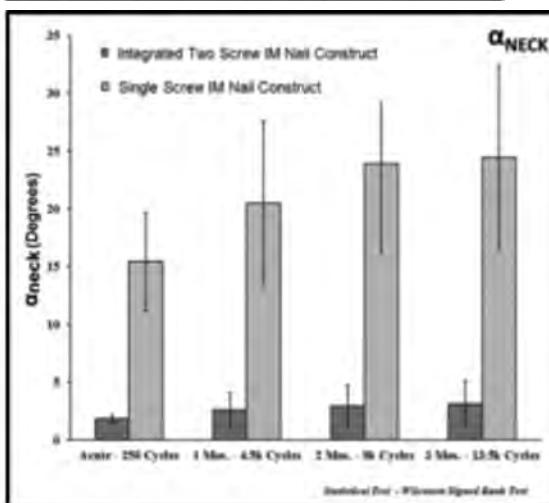


Fig. 2: Rotation about the neck axis (α_{neck}) over 13.5 K loading cycles.

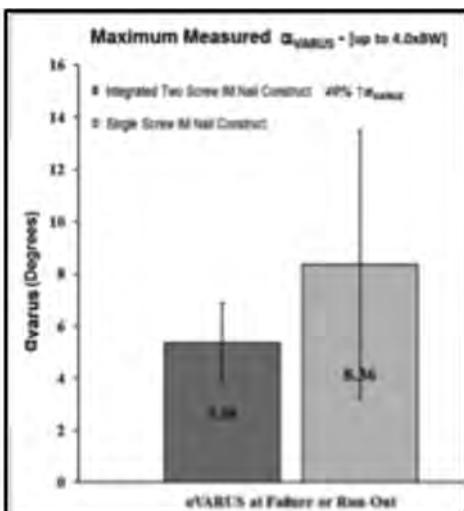


Fig. 3. Varus collapse (α_{varus}) measured over entire duration of cyclic loading.

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Traumatic Fracture Healing in Geriatric Mice Shows Decreased Callus Formation with Associated Deficiencies in Cell Cycle and Immune Cell Function

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Purpose: Geriatric fragility fractures often display reduced healing, which leads to substantial morbidity, mortality, and cost to the patient as well as cost to society. An improved understanding of the fundamental biological deficiencies of geriatric fracture healing will provide mechanistic insight into this disordered healing and provide rationally selected therapeutic targets. The purpose of this study was to develop a truly geriatric fracture model based on chronological age.

Methods: Traumatic, prestabilized, transverse, 3-point-bend tibia fractures were created in 5-month-old (m/o) mature young adults and 25-m/o geriatric C57BL/6 mice from the National Institute of Aging (NIA) colonies. Fracture calluses were harvested at 0, 5, 10, 15, 20, 25, 30, and 40 days post fracture (dpf) for analysis with micro-CT (vivaCT 40), histomorphometry (Safranin-O, Masson's Trichrome), immunohistochemistry (IHC; anti-Proliferating Cellular Nuclear Antigen [PCNA]), quantitative real-time polymerase chain reaction (qPCR), and microarray. Microarray data was uploaded into DAVID bioinformatics for gene set enrichment analysis (GSEA) and Cell Type Enrichment Analysis for Microarray Data (CTen) to evaluate cell populations.

Results: Geriatric mice produce a significantly reduced healing response. As early as 10 dpf, geriatric mice produced less cartilage and total callus. This blunted response in conjunction with delays in endochondral ossification led to diminished bone and callus formation throughout fracture healing (Figs. 1A and B). Despite the reduced total cartilage and bone produced by geriatric mice, the ratio of cartilage and bone relative to total callus produced was not significantly altered (Fig. 1C). Reflective of this, the relative expression of chondrogenic and osteogenic genes was similar. Staining for PCNA revealed decreased proliferation in mesenchymal stem cells in 25-m/o mice. Global gene expression analysis revealed differences in aged and young healing profiles most strikingly related to cell cycle and immune function. Cell cycle genes are highly upregulated in young mice at 0, 5, and 10 dpf, but upregulated in geriatric mice at 20 dpf.

Conclusion: Overall, the fracture-healing template appears to be intact in geriatric mice, much as it is in geriatric humans, but callus expansion is significantly hindered with additional temporal delay. Gene expression based analyses of cell populations demonstrate a reduced proliferative capacity of progenitor cells, which also highlighted differences in genes related to the cell cycle and immune response. Further exploration of the difference in progenitor and fracture callus cell populations and the healing environmental milieu is warranted to identify therapeutically targetable deficiencies in geriatric fracture healing.

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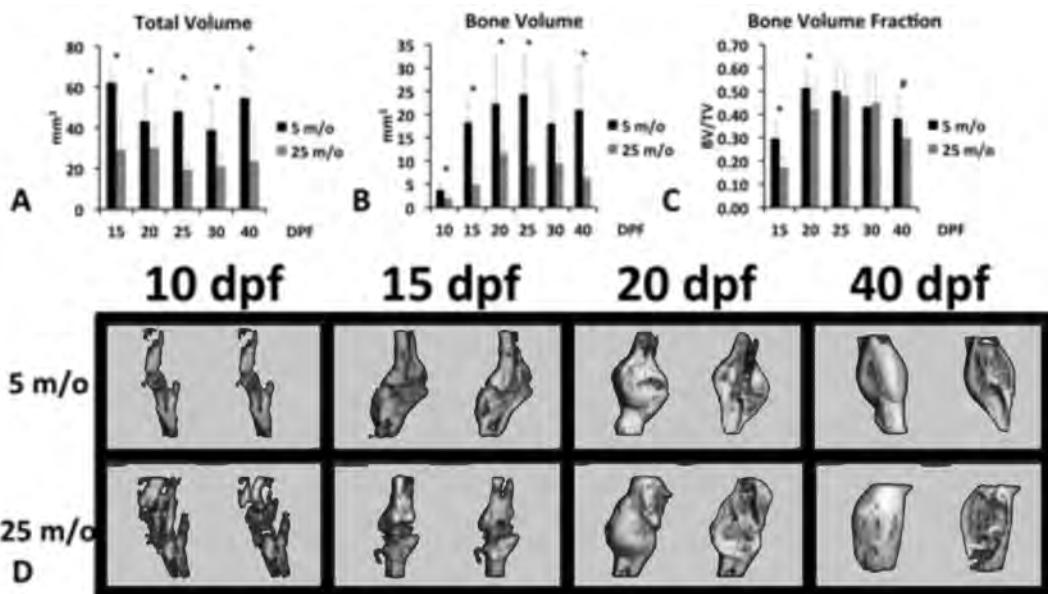


Fig 1. Micro-CT measurements of (A) total volume, (B) bone volume, and (C) bone volume fraction. (D) Representative whole callus and midcoronal plane images.