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**14. ABSTRACT**
The **purpose** of this project is to characterize the differential effects of radiation on the two pathways of fracture healing in an established animal model of bilateral femur fracture repair. Since relocation to MUSC, the PI has been fully engaged in securing suitable facilities, collaborations, and related institutional regulatory approvals to implement the animal model at MUSC. This was accomplished in August, 2013. Surgical procedures consisting of bilateral femur fracture and repair have been completed on both cohorts of 18 animals in the year 1 SOW; all animals were imaged and have been sacrificed according to protocol schedule. Group I specimens are being analyzed with micro CT to assess callus volume and character. Group II specimens are being processed for RNA isolation and PCR array analysis. Year 2 SOW animal procedures in Group III will begin on schedule in the fifth quarter of the award.

**15. SUBJECT TERMS**
Fracture healing, bone healing, endochondral ossification, intramembranous ossification, irradiation, radiotherapy, pathologic fractures, bony metastasis, bone cancer, animal model, rat model

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**Introduction:**

We propose that there exists an optimal pathway of fracture healing that is relatively less impaired by the effects of radiation therapy, and that this pathway would logically be preferentially exploited in the treatment of pathologic fractures requiring radiation for local tumor control. Further, to the extent that the method of surgical fracture fixation dictates the pathway of fracture healing, we propose that there will likewise exist an optimal method of surgical fracture repair based on the induced biology of fracture healing that would logically be utilized in the setting of malignant skeletal disease where it is anticipated that radiation would be employed as postoperative adjunctive therapy. Accordingly, we will investigate the differential effects of radiation on the two pathways of bone healing and propose an optimal method of surgical fracture repair for managing malignant osteoporotic fractures that require external beam irradiation for local tumor control.

**Keywords:**
Fracture healing, bone healing, endochondral ossification, intramembranous ossification, irradiation, radiotherapy, pathologic fractures, bony metastasis, bone cancer, animal model, rat model

**Overall Project Summary:**

*Current objectives:* ***Bilateral femur fracture procedures have been completed on 36 study animals (18 in Group I and 18 in Group II) as outlined in Quarters 2 and 3 under Task 1.*** All animals have been survived out to predetermined time points, euthanized, and bilateral hindlimb tissue harvest has been accomplished producing ***a total of 72 specimens***. Thirty-six specimens from Group I are currently undergoing analysis by micro CT for histomorphometry as well as immunohistology to evaluate callus formation. The thirty-six specimens from Group II are being processed for RNA expression and PCR analysis to evaluate differential signaling of bone healing.

Figure 1. AP and lateral plain film radiographs of Sprague-Dawley rat post bilateral femur fracture procedure.

*Results:* We are currently analyzing harvested specimens from the first 36 study animals by micro CT imaging and RNA analysis to identify differences in fracture healing via the two pathways. *Specimens collected from Group I animals for RNA analysis have been held in order to facilitate batch processing. Analysis of these samples is currently underway.* Group II specimens are being processed for histomorphometry and immunohistology. ***Micro CT data acquired from Group II specimens during the last two months are included in supplemental appendices***.

*Progress and Accomplishments:* Facilities have been established for conduct of the bilateral femur model at MUSC and all necessary collaborations and institutional regulatory approvals have been obtained. We have completed bilateral surgical femur fracture procedures on all 36 animals indicated in Task 1 in the SOW for the first year of the study. Those harvested specimens are currently being processed per protocol as delineated under Tasks 2 and 3. The project is currently on schedule and Task 1 work specified for the 5$^{th}$ quarter will occur as planned. We do not anticipate any delays affecting the study in the near future.

**Key Research Accomplishments:** Animal experiments completed on schedule and processing underway with data becoming available on a rolling basis according to tissue processing needs and constraints.

**Conclusion:** Research work on schedule as proposed and planned. No results to report.

**Publications, Abstracts, and Presentations:** Nothing to report.

**Inventions, Patents and Licenses:** Nothing to report.

**Reportable Outcomes:** Nothing to report.

**Other Achievements:** The experience and training provided by this award during the past year directly contributed to the hiring of the past research resident to a position in the orthopaedic residency at the Medical University of South Carolina.

**References:** None.

## Appendices:

1. Micro CT Data – Group II animals.

| Sample Information | | | | Callus volume (CV) | | Bone Volume (BV) | | | CV/BV | | | EX/IN | Tissue mineral density (TMD) | | Tissue mineral density (TMD) | | Notes |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Group | | Housing time | Number | *External ($mm^3$)* | *Internal ($mm^3$)* | *Bone: ($mm^3$)* | *True: ($mm^3$)* | *External* | *Internal* | *Total* | | | *External (mg HA/ccm)* | *Internal (mg HA/ccm)* | *Bone: (mg HA/ccm)* | *True: (mg HA/ccm)* | |
| Control | Plate | 1 wk | 12 | 3.64 | 5.83 | 79.19 | 73.36 | 0.05 | 0.08 | 0.13 | 0.62 | | 762.78 | 1004.73 | 1201.68 | 1217.33 | 3D reconstruction done. |
| Control | Plate | 1 wk | 19 | 5.70 | 1.96 | 82.11 | 80.15 | 0.07 | 0.02 | 0.10 | 2.91 | | 839.72 | 880.56 | 1198.91 | 1206.69 | 3D reconstruction done. |
| Control | Plate | 1 wk | 20 | 3.72 | 2.32 | 71.55 | 69.23 | 0.05 | 0.03 | 0.09 | 1.60 | | 743.24 | 846.85 | 1206.04 | 1218.08 | 3D reconstruction done. |
| | | | **Ave** | **4.35** | **3.37** | **77.62** | **74.25** | **0.06** | **0.05** | **0.10** | **1.71** | | **781.91** | **910.71** | **1202.21** | **1214.03** | |
| | | | *St dev* | *1.17* | *2.14* | *5.45* | *5.51* | *0.01* | *0.03* | *0.02* | *1.15* | | *51.01* | *83.15* | *3.59* | *6.37* | |
| Control | Plate | 2 wk | 13 | 17.87 | 4.30 | 80.51 | 76.21 | 0.23 | 0.06 | 0.29 | 4.16 | | 855.74 | 853.43 | 1219.00 | 1239.63 | 3D reconstruction done. |
| Control | Plate | 2 wk | 17 | 10.33 | 3.04 | 80.29 | 77.25 | 0.13 | 0.04 | 0.17 | 3.40 | | 818.52 | 766.20 | 1217.70 | 1235.47 | 3D reconstruction done. |
| Control | Plate | 2 wk | 18 | 13.69 | 5.21 | 78.90 | 73.69 | 0.19 | 0.07 | 0.26 | 2.63 | | 786.48 | 908.99 | 1192.61 | 1212.66 | 3D reconstruction done. |
| | | | **Ave** | **13.96** | **4.18** | **79.90** | **75.72** | **0.18** | **0.06** | **0.24** | **3.39** | | **820.25** | **842.87** | **1209.77** | **1229.25** | |
| | | | *St dev* | *3.78* | *1.09* | *0.87* | *1.83* | *0.05* | *0.02* | *0.06* | *0.76* | | *34.66* | *71.98* | *14.88* | *14.52* | |
| Control | Plate | 4 wk | 6 | 13.02 | 3.61 | 70.05 | 66.44 | 0.20 | 0.05 | 0.25 | 3.61 | | 882.60 | 879.54 | 1069.55 | 1079.87 | 3D reconstruction done. |
| Control | Plate | 4 wk | 7 | 22.43 | 8.22 | 83.59 | 75.37 | 0.30 | 0.11 | 0.41 | 2.73 | | 894.54 | 857.78 | 1125.01 | 1154.15 | 3D reconstruction done. |
| | | 4 wk | 16 | 15.58 | 3.50 | 77.99 | 74.49 | 0.21 | 0.05 | 0.26 | 4.45 | | 881.86 | 888.06 | 1208.17 | 1223.21 | |
| | | | **Ave** | **17.01** | **5.11** | **77.21** | **72.10** | **0.23** | **0.07** | **0.30** | **3.60** | | **886.33** | **875.13** | **1134.24** | **1152.41** | |
| | | | *St dev* | *4.87* | *2.69* | *6.80* | *4.92* | *0.06* | *0.03* | *0.09* | *0.86* | | *7.12* | *15.61* | *69.77* | *71.68* | |
| Control | Nail | 1 wk | 12 | 4.70 | 2.66 | 89.42 | 86.76 | 0.05 | 0.03 | 0.08 | 1.77 | | 804.72 | 838.43 | 1234.37 | 1246.51 | 3D reconstruction done. |
| Control | Nail | 1 wk | 19 | 3.72 | 1.53 | 91.56 | 90.03 | 0.04 | 0.02 | 0.06 | 2.43 | | 732.50 | 863.15 | 1218.44 | 1224.48 | 3D reconstruction done. |
| Control | Nail | 1 wk | 20 | 1.99 | 2.62 | 88.37 | 85.75 | 0.02 | 0.03 | 0.05 | 0.76 | | 716.66 | 942.97 | 1219.00 | 1227.43 | 3D reconstruction done. |
| | | | **Ave** | **3.47** | **2.27** | **89.78** | **87.51** | **0.04** | **0.03** | **0.07** | **1.65** | | **751.29** | **881.52** | **1223.94** | **1232.81** | |
| | | | *St dev* | *1.37* | *0.64* | *1.63* | *2.24* | *0.02* | *0.01* | *0.02* | *0.84* | | *46.94* | *54.64* | *9.04* | *11.96* | |
| Control | Nail | 2 wk | 13 | 13.57 | 1.67 | 86.87 | 85.20 | 0.16 | 0.02 | 0.18 | 8.13 | | 795.46 | 885.93 | 1215.85 | 1222.32 | 3D reconstruction done. |
| Control | Nail | 2 wk | 17 | 6.24 | 1.12 | 87.19 | 86.07 | 0.07 | 0.01 | 0.09 | 5.57 | | 734.63 | 793.52 | 1230.20 | 1235.88 | 3D reconstruction done. |
| Control | Nail | 2 wk | 18 | 9.69 | 2.09 | 85.88 | 83.79 | 0.12 | 0.02 | 0.14 | 4.64 | | 793.43 | 875.93 | 1221.96 | 1230.59 | ***Nail broken inside.*** |
| | | | **Ave** | **9.83** | **1.63** | **86.65** | **85.02** | **0.12** | **0.02** | **0.13** | **6.11** | | **774.51** | **851.79** | **1222.67** | **1229.60** | |
| | | | *St dev* | *3.67* | *0.49* | *0.68* | *1.15* | *0.04* | *0.01* | *0.05* | *1.81* | | *34.55* | *50.71* | *7.20* | *6.84* | |
| Control | Nail | 4 wk | 6 | 37.36 | 1.75 | 72.14 | 70.39 | 0.53 | 0.02 | 0.56 | 21.35 | | 850.10 | 776.11 | 1154.18 | 1163.58 | 3D reconstruction done. |
| Control | Nail | 4 wk | 7 | 41.25 | 2.28 | 87.16 | 84.88 | 0.49 | 0.03 | 0.51 | 18.09 | | 820.46 | 943.43 | 1139.27 | 1144.53 | 3D reconstruction done. |
| Control | Nail | 4 wk | 16 | 34.73 | 1.90 | 82.96 | 81.06 | 0.43 | 0.02 | 0.45 | 18.28 | | 824.91 | 912.41 | 1190.02 | 1196.53 | 3D reconstruction done. |
| | | | **Ave** | **37.78** | **1.98** | **80.75** | **78.78** | **0.48** | **0.03** | **0.51** | **19.24** | | **831.82** | **877.32** | **1161.16** | **1168.21** | |
| | | | *St dev* | *3.28* | *0.27* | *7.75* | *7.51* | *0.05* | *0.00* | *0.05* | *1.83* | | *15.98* | *89.01* | *26.08* | *26.31* | |
| X-Ray | Plate | 1 wk | 1 | 7.81 | 2.77 | 80.65 | 77.88 | 0.10 | 0.04 | 0.14 | 2.82 | | 701.11 | 740.00 | 1070.75 | 1082.51 | 3D reconstruction done. |
| X-Ray | Plate | 1 wk | 2 | 2.81 | 3.18 | 71.35 | 68.17 | 0.04 | 0.05 | 0.09 | 0.88 | | 725.00 | 712.50 | 1078.99 | 1096.09 | 3D reconstruction done. |
| X-Ray | Plate | 1 wk | 8 | 5.78 | 2.43 | 81.59 | 79.16 | 0.07 | 0.03 | 0.10 | 2.38 | | 881.76 | 742.50 | 1201.96 | 1216.06 | 3D reconstruction done. |
| | | | **Ave** | **5.47** | **2.79** | **77.86** | **75.07** | **0.07** | **0.04** | **0.11** | **2.03** | | **769.29** | **731.67** | **1117.23** | **1131.55** | |
| | | | *St dev* | *2.51* | *0.38* | *5.66* | *6.01* | *0.03* | *0.01* | *0.02* | *1.01* | | *98.13* | *16.65* | *73.49* | *73.50* | |
| X-Ray | Plate | 2 wk | 3 | 8.34 | 15.40 | 99.33 | 83.93 | 0.10 | 0.18 | 0.28 | 0.54 | | 781.02 | 787.22 | 1065.48 | 1116.54 | 3D reconstruction done. |
| X-Ray | Plate | 2 wk | 9 | 1.10 | 3.27 | 73.46 | 70.19 | 0.02 | 0.05 | 0.06 | 0.34 | | 821.95 | 871.39 | 1268.72 | 1287.23 | 3D reconstruction done. |
| X-Ray | Plate | 2 wk | 15 | 8.99 | 2.91 | 84.41 | 81.50 | 0.11 | 0.04 | 0.15 | 3.09 | | 839.08 | 715.09 | 1182.80 | 1199.50 | 3D reconstruction done. |
| | | | **Ave** | **6.14** | **7.19** | **85.73** | **78.54** | **0.08** | **0.09** | **0.16** | **1.32** | | **814.02** | **791.23** | **1172.33** | **1201.09** | |
| | | | *St dev* | *4.38* | *7.11* | *12.99* | *7.33* | *0.05* | *0.08* | *0.11* | *1.53* | | *29.83* | *78.23* | *102.02* | *85.36* | |
| X-Ray | Plate | 4 wk | 10 | 5.15 | 15.20 | 98.70 | 83.50 | 0.06 | 0.18 | 0.24 | 0.34 | | 940.38 | 899.36 | 1123.07 | 1163.79 | 3D reconstruction done. |
| X-Ray | Plate | 4 wk | 11 | 12.30 | 1.58 | 78.79 | 77.21 | 0.16 | 0.02 | 0.18 | 7.78 | | 927.41 | 746.39 | 1189.93 | 1199.01 | 3D reconstruction done. |
| X-Ray | Plate | 4 wk | 14 | 14.07 | 10.90 | 79.25 | 68.35 | 0.21 | 0.16 | 0.37 | 1.29 | | 930.56 | 851.21 | 1162.42 | 1212.05 | |
| | | | **Ave** | **10.51** | **9.23** | **85.58** | **76.35** | **0.14** | **0.12** | **0.26** | **3.14** | | **932.78** | **832.32** | **1158.47** | **1191.62** | |
| | | | *St dev* | *4.72* | *6.96* | *11.36* | *7.61* | *0.07* | *0.09* | *0.09* | *4.05* | | *6.76* | *78.21* | *33.60* | *24.96* | |
| X-Ray | Nail | 1 wk | 1 | 4.79 | 3.55 | 84.17 | 80.62 | 0.06 | 0.04 | 0.10 | 1.35 | | 718.52 | 778.70 | 1198.72 | 1217.22 | 3D reconstruction done. |
| X-Ray | Nail | 1 wk | 2 | 4.44 | 0.80 | 81.86 | 81.06 | 0.05 | 0.01 | 0.06 | 5.55 | | 779.44 | 806.02 | 1144.09 | 1147.43 | 3D reconstruction done. |
| X-Ray | Nail | 1 wk | 8 | 6.49 | 2.00 | 96.42 | 94.42 | 0.07 | 0.02 | 0.09 | 3.25 | | 816.39 | 903.15 | 1234.00 | 1241.01 | 3D reconstruction done. |
| | | | **Ave** | **5.24** | **2.12** | **87.48** | **85.37** | **0.06** | **0.03** | **0.09** | **3.38** | | **771.45** | **829.29** | **1192.27** | **1201.88** | |
| | | | *St dev* | *1.10* | *1.38* | *7.83* | *7.84* | *0.01* | *0.02* | *0.02* | *2.10* | | *49.42* | *65.41* | *45.30* | *48.64* | |
| X-Ray | Nail | 2 wk | 3 | 8.66 | 3.37 | 89.23 | 85.86 | 0.10 | 0.04 | 0.14 | 2.57 | | 819.72 | 923.15 | 1156.50 | 1165.66 | ***Nail broken inside.*** |
| X-Ray | Nail | 2 wk | 9 | 4.27 | 0.76 | 72.29 | 71.53 | 0.06 | 0.01 | 0.07 | 5.62 | | 808.98 | 869.26 | 1271.22 | 1275.49 | 3D reconstruction done. |
| X-Ray | Nail | 2 wk | 15 | 15.72 | 2.68 | 82.74 | 80.06 | 0.20 | 0.03 | 0.23 | 5.87 | | 772.68 | 699.16 | 1217.15 | 1234.49 | 3D reconstruction done. |
| | | | **Ave** | **9.55** | **2.27** | **81.42** | **79.15** | **0.12** | **0.03** | **0.15** | **4.68** | | **800.46** | **830.52** | **1214.96** | **1225.21** | |
| | | | *St dev* | *5.78* | *1.35* | *8.55* | *7.21* | *0.07* | *0.02* | *0.08* | *1.84* | | *24.65* | *116.91* | *57.39* | *55.50* | |
| X-Ray | Nail | 4 wk | 10 | 34.07 | 5.87 | 68.22 | 62.35 | 0.55 | 0.09 | 0.64 | 5.80 | | 884.36 | 853.34 | 1132.05 | 1158.29 | 3D reconstruction done. |
| X-Ray | Nail | 4 wk | 11 | 31.36 | 1.98 | 104.60 | 102.62 | 0.31 | 0.02 | 0.32 | 15.84 | | 841.48 | 926.30 | 1204.09 | 1209.45 | 3D reconstruction done. |
| X-Ray | Nail | 4 wk | 14 | 22.12 | 0.61 | 83.60 | 82.99 | 0.27 | 0.01 | 0.27 | | | 851.39 | 800.19 | 1196.59 | 1199.50 | 3D reconstruction done. |

2. 3-D micro CT reconstructions comparing callus volume of femora healing via plate fixation versus IM nail fixation.

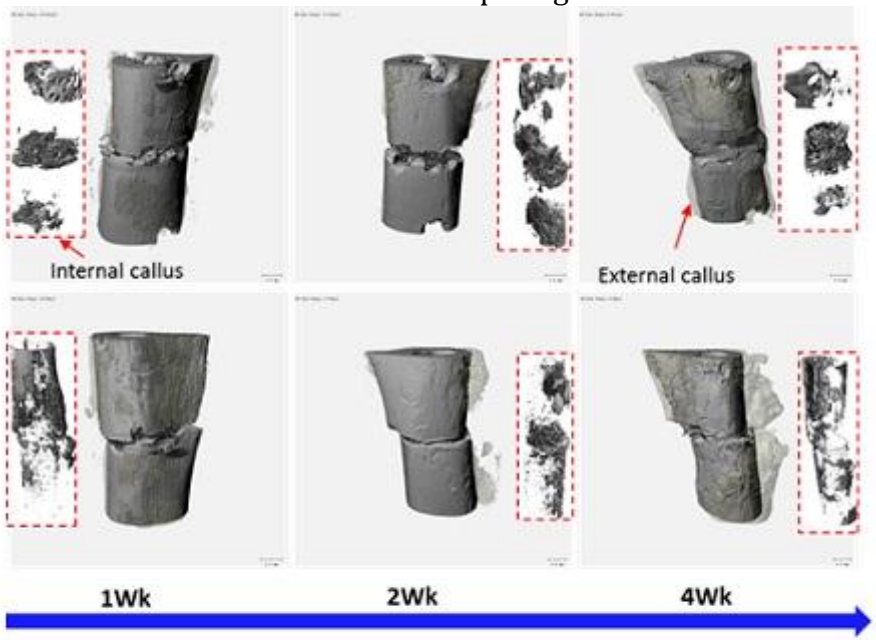


Figure 2. 3-D CT reconstruction of femora treated with IM nail or plate fixation at 1, 2 and 4 weeks. External callus is represented by the light grey portion surrounding the darker bone. Internal callus is represented in the outlined box.