

# Primary Fracture Healing in a Rat Model

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## INTRODUCTION

Current models of fracture repair in small animals mainly investigate secondary fracture healing via external callus formation. Primary fracture healing occurs when fracture fragments are apposed and rigidly immobilised. It accelerates functional recovery. The biological processes of these two fracture healing mechanisms differ. Primary fracture healing involves new bone formation without the intermediate phase of cartilage deposition<sup>1</sup>. Primary fracture healing can be considered to be an extension of the physiological repair and turnover of bone that is constantly occurring, whereby micro-fractures are located and removed by osteoclasts. New bone is then laid down by the ensuing osteoblasts following in the wake of the osteoclasts. Pharmacological agents such as bisphosphonates may have a profound effect on primary fracture healing but minimal or no effect on secondary fracture healing. A rodent model of primary fracture healing was developed.

## METHODS

**Animals:** All animal procedures were Home Office, UK approved and adhered to the Animals (Scientific Procedures) Act 1986. 10 skeletally mature ex-breeder male Sprague Dawley rats (Harlan, UK) with a mean( $\pm$ SD) weight of 512( $\pm$  24.3)g were used. Animals were humanely sacrificed at 6 weeks following rigid plate fixation of a transverse midshaft tibial osteotomy.

**Surgical Procedure:** Isoflurane inhalational anaesthesia (1-3%) was used. Pre-operative antibiotic prophylaxis (1 mL/kg Synulox), fluids (10ml/kg 0.9% saline) and analgesia (0.05mg/kg buprenorphine) administered subcutaneously. Positioned supine, the right hind limb is shaved, prepped with bethadine and sterile drape applied. The right hind leg is infiltrated with 0.4mls/kg of 1% Xylocaine prior to skin incision. Antero-medial approach to the tibia exploiting interval between *tibialis anterior* and *tibialis posterior*. A custom made 4-hole jig (Physics Workshop, Edinburgh University, UK) is applied to the flat medial surface of the tibia and four holes drilled using a shortened 1.0 mm drill bit powered with a hand-held Dremmel® Multitool under constant cool saline irrigation prior to creation of a transverse tibial osteotomy with a fine circular saw of 0.1mm thickness (RS Components, UK). The jig ensures compression at the osteotomy site during plate application. The periosteum is stripped circumferentially from the tibia at the level of the osteotomy. A pre-bent 4-hole stainless steel plate (Physics Workshop, Edinburgh University, UK) measuring 12mm x 3mm x 0.4mm is applied to the medial surface of the tibia with four M1.2 x 4mm stainless steel screws (PTS Ltd, East Grinstead, UK) to obtain rigid stability and compression across the osteotomy. Wound closure with 3-0 vicryl. Post-op Buprenorphine oral analgesia (0.3mg/kg B.D) was administered in jelly cubes for 24 hours.



**Figure 1: Tibial plating showing anatomical alignment and compression at the osteotomy site**

**Radiographs:** Antero-posterior radiographs of the right tibia obtained using a portable x-ray unit (Acu-Ray JR, Stern Manufacturing Toronto, Canada) with an output of 60 kV and exposure time of 0.1ms. Images were captured on digital x-ray plates (Fuji IP Cassette, Fuji Photo Film Co Ltd Japan). Radiographs were obtained at weekly intervals post-osteotomy and inspected qualitatively for callus formation and fracture union. Following sacrifice, right tibiae were dissected free of soft tissue and metalwork removed prior to capture of a standard lateral radiograph to eliminate the effect of fibula overlay. The visibility of the fracture line was observed and scored as totally visible, partly visible, or absent as previously reported<sup>2</sup>.

**Histology:** Tibiae from 5 animals were immersed in 10% formalin in phosphate buffered saline at pH 7.2-7.4 for 48 hours, then decalcified in EDTA at pH 7 and 37°C for 4 weeks with weekly changes of EDTA. 3 longitudinal 5µm coronal sections spaced 500µm apart were obtained

from each specimen. Sections were stained with Haematoxylin and Eosin (H&E) to assess the general morphology at the fracture site.

**Mechanical Testing:** In 5 animals tibiae were placed in 0.9% saline and frozen at -20°C prior to mechanical testing within 2 weeks. Bones were warmed to 37°C before stress test to failure was performed with a 4-point bend using a custom made rig (The University of Edinburgh Physics Workshop, Kings Buildings, Edinburgh, UK) incorporating a pivot in the upper loading position to ensure equal application of force at all 4 loading points in conjunction with a Zwick/Roell Z005 materials testing machine. The tibial midshaft cross section resembled a triangle. The cross-sectional moment of inertia was calculated using values derived from a combination of physical measurements with sliding calipers and scaled digital images of the fractured diaphysis. The 'Elastic Beam Bending Theory' was used to calculate the stress at failure.

## RESULTS

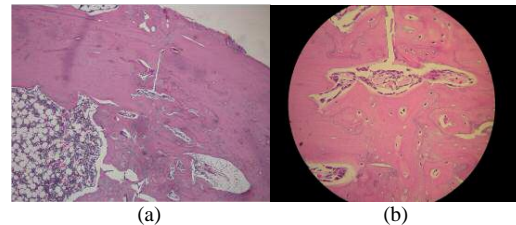
All rats were fully weight bearing within 12 hours. There were no post-operative wound complications. Serial radiographs revealed fracture union without callus formation. Fracture line visibility is shown in the table below. H&E stained slides showed minimal evidence of external callus formation. Osteonal units with leading osteoclasts were seen to traverse the fracture site indicating primary fracture healing. The mean( $\pm$ SD) stress at failure for the healing tibial osteotomies at 6 weeks was 37.4( $\pm$ 29.20)MPa.



**Figure 2: Plain radiographs (a) 2 wks, (b) 4 wks and (c) 6 weeks post-op show no external callus**

Totally Visible	Partially Visible	Absent
n=0	n=5	n=5

**Table 1: Visibility of fracture line on lateral x-ray following sacrifice and metal work removal**



**Figure 3: (a)H&E x5 show fragments aligned and apposed with no external callus, (b)H&E x40 'Cutting Cone' with leading osteoclast traversing fracture site**

## DISCUSSION

A simple reproducible model of primary fracture healing has been developed in a rat.

## REFERENCES

1. Perren SM. Physical and biological aspects of fracture healing with special reference to internal fixation. *Clin Orthop Relat Res* 1979-138:175-96.
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