Injury, Int. J. Care Injured 46 (2015) 649–654

Contents lists available at ScienceDirect

Injury

journal homepage: www.elsevier.com/locate/injury

Novel rat model of nonunion fracture with vascular deficit

M. Roberto-Rodrigues [a], R.M.P. Fernandes [a], R. Senos [a,b,*], A.C.D. Scoralick [a], A.L. Bastos [a], T.M.P. Santos [c], L.P. Viana [a], I. Lima [c], M.A. Guzman-Silva [d], J.R. Kfoury-Júnior [b]

[a] Department of Morphology, Universidade Federal Fluminense, Niterói, Brazil
[b] Department of Surgery of Veterinary Faculty, Universidade de São Paulo, São Paulo, Brazil
[c] Department of Nuclear Engineering, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil
[d] Department of Pathology, Universidade Federal Fluminense, Niterói, Brazil

ARTICLE INFO

Article history:
Accepted 19 January 2015

Keywords:
Atrophic
Bone healing
Delayed union
Experiment design
Femur
Histology
Intramedullary pin
Microcomputed tomography
Non-union
Pseudoarthrosis

ABSTRACT

Nonunion fractures occur frequently in humans, with profound implications (medical and non-medical). Although there are numerous animal models to study pathogenesis and treatment of nonunion fractures, there is apparently the lack of a definitive model for atrophic nonunion fracture. Therefore, the objective was to develop a low-cost rat model of nonunion fracture with a vascular deficit that enabled standardized quantitative analysis of bone growth and regeneration. The model was developed with two surgeries, performed apart. The first involved osteotomy of the femur diaphysis, removal of periosteum and endosteum, isolation of the fracture site using a latex artefact (Penrose drain tube), and reduction of the fracture using an intramedullary pin, whereas the second surgery was to remove the latex artefact. Based on radiographic imaging, micro-CT and histological analyses done 125 days after the fracture was induced, there was clear evidence of atrophic nonunion fracture, without pin migration or specimen loss. Perceived advantages of this model included low cost, ease of reproducibility, lack of specimen loss, and, finally, the potential to assess bone growth and regeneration under poor vascular conditions.

Introduction

Failure of a fracture site to consolidate, defined as a nonunion fracture, occurs in approximately 5–10% of human fractures in North America and the United Kingdom, with an estimated one hundred thousand cases per year in the United States [1–4]. In addition to medical aspects, this condition often has enormous functional, social and financial implications [2,4–6]. For example, the National Health Service of the United Kingdom estimated that the costs for treatment of nonunion fractures ranged from £7000 to £79,000 per person affected, excluding financial implications of morbidity and loss of income [3].

Soft tissue lacerations surrounding a fracture site, including the periosteum and vascular deficits, can produce an atrophic nonunion [1,2,7], which are often the most difficult type of nonunion fracture to treat [8].

Numerous animal models of nonunion fracture have been developed, to understand the pathogenesis of this condition and improve treatment [9–15]. Nevertheless, most of those models create purely mechanical disturbances, but not the metabolic pertubations that result in atrophic nonunion fracture [9]. Furthermore, at least some atrophic nonunion fracture models are not well suited for testing treatments [9,16].

Previous study has proven that the removal of the bone marrow [17] is certainly an incisive technique with good results on promoting the nonunion; however, spontaneous cases of atrophic nonunion are not usually attributed to bone marrow deficiency.

Although cauterization of 2 mm of periosteum on the fracture board more closely approximates clinical cases, this results in cortical bone with and without periosteum in the same diaphysis. Consequently, this complicates studies of bone growth and regeneration that include standard quantitative analysis [18], as periosteum promotes bone regeneration [1].

The objective of this study was to develop a novel rat model of nonunion fracture with vascular deficit to support studies on atrophic nonunion healing.

Methods

Ethical aspects

Experimental procedures were consistent with ethical principles for animal research and were reviewed and approved under

* Corresponding author at: Department of Morphology – UFF, Rua Professor Hernani Melo 101, Niterói CEP 24210-130, Brazil. Tel.: +55 21999660220.
E-mail address: rafaelsenos@yahoo.com.br (R. Senos).

http://dx.doi.org/10.1016/j.injury.2015.01.033
0020–1383/

protocol number 170/2012 by the Bioethics Commission of the Universidade Federal Fluminense.

Animals and conditions

Six adult Wistar rats (*Rattus norvegicus*), 3 months of age and 350–400 g, were acquired from the Centre for Laboratory Animals of the Universidade Federal Fluminense. Throughout the study, rats were individually housed in polypropylene cages (30 cm × 20 cm × 14 cm), with 12/12 night/day cycles, 21 °C and 50% relative humidity. All rats had *ad libitum* access to a complete rat feed (Nuvilab®) and filtered water.

Surgical technique

The nonunion fracture model was established in the left femur, using two surgical procedures.

Operative technique #1

The anesthesic protocol was adapted from previous publishing [19]. Acepromazine (0.75 mg/kg, IM) was given as a pre-anaesthetic,

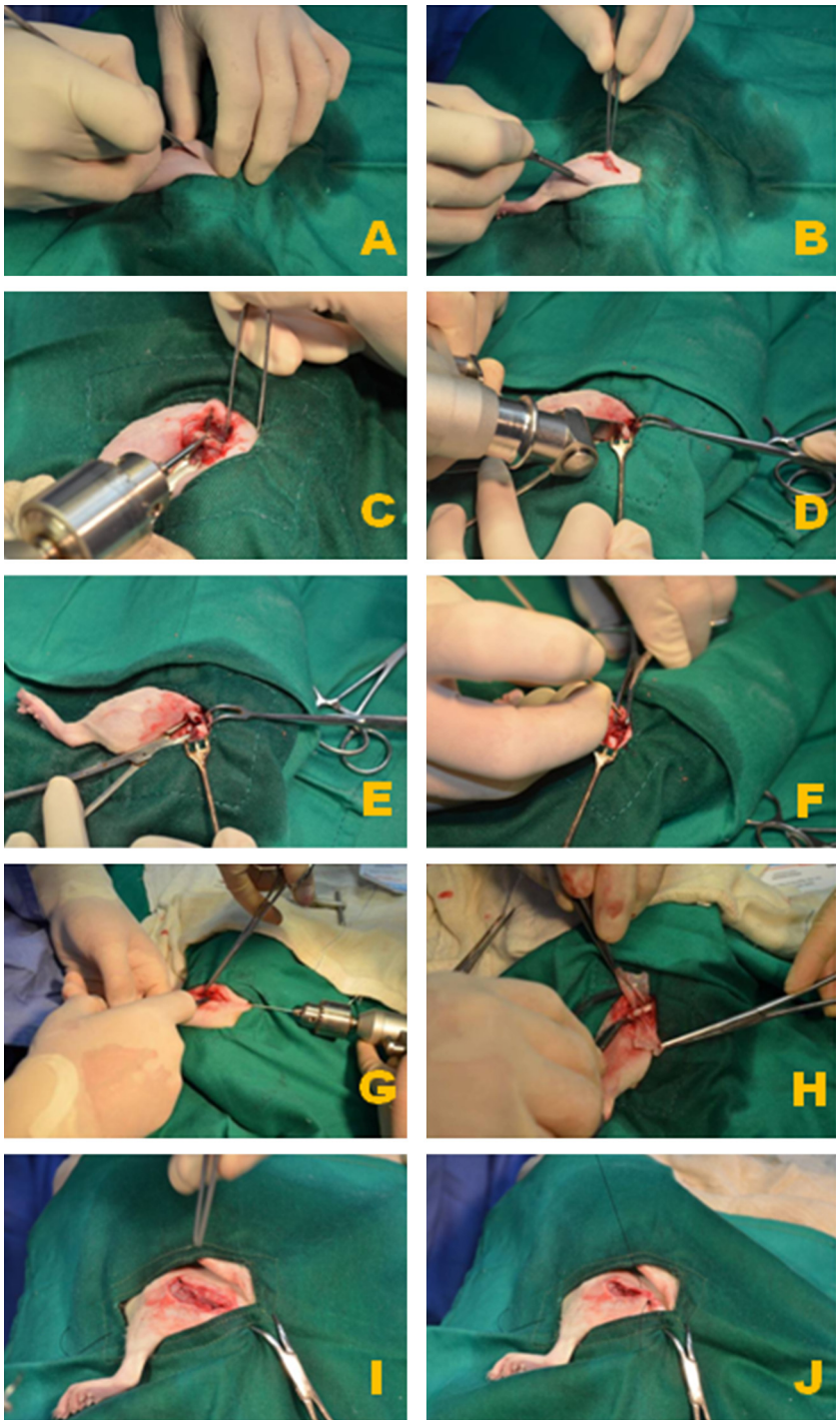

Fig. 1. Operative technique #1. (A and B) Surgical approach to the femur diaphysis; (C) periosteum removal; (D and E) controlled transverse fracture on femur diaphysis; (F) endosteum removal; (G) fracture reduction with intramedullary pin; (H) installation of the latex artefact; and (I and J) sutures.