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Histological evolution of the regenerate during bone transport: an experimental study in sheep

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KEYWORDS

Bone transport
Distraction osteogenesis
Regenerate
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ABSTRACT

Introduction: Bone transport (BT) for segmentary bone defects is a well-known technique as it enables correction with new bone formation, which is similar to the previous bone. Despite the high number of experimental studies of distraction osteogenesis in bone lengthening, the types of ossification and histological changes that occur in the regenerate of the bone transport process remain controversial.
Objective: The aim of this study is to provide the complete evolution of tissues and the types of ossification in the regenerate during the different phases of bone formation after BT until the end of the remodelling period.
Methods: A histological study was performed using ten adult sheep that were submitted to BT. The types of ossification as well as the evolution of different tissues in the regenerate were determined using histomorphometry and inmunohistochemical studies. The evolution of trabeculae thickness, osteoblast and osteoclast densities, relationship between collagen types and changes in vascularization were also studied.
Results: Ossification was primarily intramembranous, with some focus of endochondral ossification in isolated animals. The cell counts showed a progression of cellular activity from the periphery to the centre, presenting the same progression as the growth of bone trabeculae, whose trabeculae thickness was quadrupled at the end of remodelling. Inmunohistochemical studies confirmed the prevalence of type I collagen and the ratio of the Type I/Type II collagen ratio was found to be 2.48. The percentages of the vascularized areas were proximally higher than distally in all animals, but distal zone obtained higher rates than the central region.
Conclusions: Bone transport regenerate exhibits a centripetal ossification model and a mixed pattern with predominance of intramembranous over endochondral ossification. The data obtained resemble partially to those found in models of bone lengthening applied to large animals. This study provides a detailed structural characterization of the newly formed tissue, which may help to explain the development of the regenerate of bone transport in humans. It will also serve for future mechanobiological models that may aid research on the effect of loading or distractor stiffness in clinical results.

Introduction

The reconstruction of segmentary bone defects as a consequence of multiple processes (fractures and tumours) continues to be a difficult problem. Bone transport appears to be a solution for bone defects [1–3] by filling a gap with autologous bone with similar properties to the previous bone [4]. It involves the transport of a bone segment through an intercalary defect, which is filled with regenerated bone. The new bone is referred to as the "regenerate," and the site at which the transported segment meets the target segment at the end of bone transport is referred to as the "docking site."

Since the discovery of distraction osteogenesis, gradual distraction has been known to create a mechanobiological environment, which enables the stimulation of new bone formation. Although the development of various techniques has emphasized the need to control certain factors for success [3,5–7] (latency period, distraction rate, type of osteotom), the investigation of these points has been independently performed in different animal models with various distraction systems. This fact prevents a comparison and generates considerable controversy regarding the results including the histological findings [8–19]. Some authors only observed endochondral

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ossification, whilst pure intramembranous ossification was proposed by other authors [1,15,20,21]. A combination of endochondral ossification and intramembranous ossification has been noted in other investigations [13,14,22–26]. One study [27] reported the finding of transchondroid bone formation. Endochondral ossification was primarily reported in studies with small animal models [16,22,24–26], whilst intramembranous ossification was reported as the primary method in large animal models [10,11,13–15,28,29] (sheep and dogs). The difference in the mode of ossification can also be explained by the stiffness of the employed fixator. Monolateral mini-fixators, which are employed in most small animals, favour an increase in mobility in the regenerate, which causes the appearance of endochondral ossification foci. The differences between these distraction systems and the systems applied in humans and their anatomical characteristics (with different load distributions in humans) render small animal models inappropriate for human model studies [7,16,17,22,24,25,30].

Studies of bone transport have not addressed the complete histological evolution of the regenerate until the end of remodelling using the same principles that apply to humans (similar bone sizes, frames, distraction rate, and weight bearing). Considering that the average lengthening index is 1 month/cm [31], many investigators have not extended the follow-up time by more than three months [13,14,16,20,28,32]. However, the entire process in human tibia can continue from several months [31,32] to years (Paley [33] reported treatment times from six to 31 months depending on the patient). Other researchers extrapolate the results obtained in distraction osteogenesis in animal mandibles to long bones [21,32,34–36], in which the load conditions differ. In his study of limb lengthening in dogs, Fink [13] reported the morphometric evolution of the regenerate and bone trabeculae thickness. However, his histological studies were limited to the endosteal area of the regenerate, and the follow-up time did not exceed 50 days.

Therefore, the main purpose of this study is to provide the complete evolution of tissues in the regenerate after bone transport until the end of the remodelling period. The selected animal model is the sheep as it has healing conditions that are similar to the healing conditions of humans. An Ilizarov external fixator, which is similar to the external factor employed in humans, is applied, and load bearing is allowed since the day of the surgery on the operated limb. The histological changes, ossification types and the localization and progress during the entire process of BT are reported.

Material and methods

Animals

Ten skeletally mature female Merino sheep with a mean weight of 53.9 ± 8.9 kg and ages that range from 3–5 years old were included. The sheep were sacrificed at pre-established times (17, 22, 29, 35, 37, 50, 79, 98, 161 and 525 days after surgery). All sheep had a minimum metatarsal length of 14 cm and a minimal diameter of 10 mm in the thinner area. Animals without these requirements and with wounds or scars of previous injuries, bone deformities that affect their gait, local or systemic infections that may complicate the postoperative period and previous surgeries on the limbs were excluded.

The study was approved by the Ethics Committee for Animal Research of the University of Seville. Animal care was provided in accordance with our institution's Animal Laboratory Committee Guidelines and under the supervision of a veterinarian team.

Surgery

After general anaesthesia was administered, an optimized Ilizarov external fixator [37–39] was attached to the nonosteotomized diaphysis of the right hind metatarsus. The fixator was positioned using six stainless steel 4 mm Schanz screws, which were proximally and distally drilled to the subsequent osteotomies lines, and two 3 mm diameter Kirschner wires for the transportable bony segment. All parts were drilled in a standardized position using a driver device for wires and pins [38] (Figure 1a). The device design was based on a comprehensive anatomical study that was performed in laboratory specimens, in which the screws and K-wires were positioned in an angular arrangement to achieve a high torsional and bending stiffness whilst avoiding neurovascular or tendinous injuries.

Schanz screws were predrilled with a 2.5 mm drill bit. K-wires were inserted in the transportable bony segment in a mediolateral direction after predrilling with a 2 mm drill bit. When the wires were placed, the driver device was removed and the external fixator was assembled with the screws (Figure 1b).

After the fixator was applied, three osteotomies were performed using an oscillating saw and a guide to create a defect of 15 mm and a bone transportable segment with a length of 25 mm (Figure 1b). The periosteum located in the osteotomy area was previously retracted and protected. The transportable bony segment was proximally led to close the saw gap, which produced a defect of approximately 15 mm.

After seven days of latency, distraction of the callus began at 1 mm per day in one step and continued for 15 days.

When euthanasia was accomplished, the metatarsal bone was disjointed and frozen until the samples were processed for the histological study.

Histological study

For histological purposes, morphometric and inmunohistochemical studies were performed.

The metatarsal bones were decalcified and sectioned with a razor blade to obtain two blocks: the regenerate and the docking site. This study focused on the former. The regenerate block contained a portion of the proximal segment (base segment), the entire regenerate and a portion of the distal segment (transported segment). The blocks were

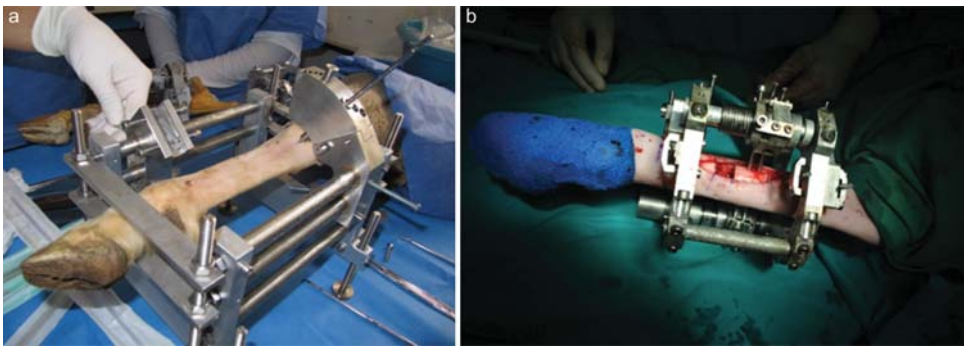

Fig. 1. (a) Driver device for wires and pins. Laboratory test. (b) Final appearance of the limb after assembling the distractor.