HISTOLOGICAL STUDY OF THE DOCKING SITE AFTER BONE TRANSPORT. TEMPORAL EVOLUTION IN A SHEEP MODEL.

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Abstract

Introduction:

Bone transport appears to be a solution for segmental bone defects; specifically, the “docking site” is where the transported segment meets the target segment at the end of the process. A lack of its consolidation is one of the major causes of failure for this technique. Many studies have been performed in order to enhance the consolidation of the docking site, but histological changes occurring in it remain unknown. The aim of this study was to determine microscopic changes present in this area, from distraction to remodeling, in order to clarify the best options to facilitate the success of this technique.

Materials and Methods:

Ten adult sheep were submitted to bone transport using an Ilizarov external fixator. Histomorphometry and immunohistochemical studies were performed in the docking site to determine the main types of ossification, the evolutions of tissues and blood vessels and the distributions of collagen I and II.

Results:

Ossification was mainly intramembranous with some areas of endochondral ossification. Fibrous tissue was predominant until 98 days after surgery. The area occupied by blood vessels increased until 50 days after surgery, when it decreased slowly until the end of the study.

Conclusions:

As far as the authors know, this is the first histological study performed in the docking site reporting the complete evolution of tissues until the end of remodeling, showing results contrary to those published by others authors. This could help to clarify information about its union and may be useful for future investigations about techniques for improving the consolidation of the docking site in humans.

Keywords

Docking site, Bone transport, Ilizarov, Ossification, Intramembranous

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1. Introduction

Treatment of segmental bone defects as a consequence of multiple processes (tumors, fractures, etc.) continues to be a challenge for orthopedic surgeons. Several techniques (vascularized allografts, Papineau, etc.) have been performed in order to solve this problem. Bone transport appears to be a solution [1-6]. It consists of the transport of a bone segment (transportable segment) through the intercalary defect, filling the gap with autologous bone similar to the previous one. The site where the transported segment meets the target segment at the end of bone transport is called the “docking site”, and the newly formed bone is called the “regenerate”.

Problems related to consolidation of the docking site are the main cause of failure with this technique [7-9]. Therefore, many surgical techniques have been developed to facilitate this consolidation [7, 10-12], including closed and open procedures, that do not show a significant difference in results when they are compared with each other [9].

While the regenerate has been widely studied [1, 2, 6, 10, 13-21] both biomechanically and histologically and its ossification is known to be mainly intramembranous [1-3, 10, 20, 22], there are only few studies about the docking site [8-11, 23].

To our knowledge, there are only two studies concerning the histological characterization of the docking site [10, 23] , and only one [10] reports data about types of ossification. Garcia et al. [10] presented a model of bone transport in lambs performed over a plate, concluding that the docking site shows endochondral ossification. Nagashima et al. [23] histologically described a canine model. However, there are no histological studies of the docking site in long bones under physiological conditions.

It is also known that a good blood supply is mandatory for bone transport success. Many published studies refer to vascularization in limb lengthening [24-28] and make a general description of the vascular distribution, showing an overall increase of vascularization compared to the contralateral limb. However, there are very few studies about vascularization after bone transport [29]. In addition, there are no studies reporting the vascular evolution at the docking site beyond the histomorphometric studies reported by Garcia [10].

The knowledge of the histological and vascular processes occurring in the docking site during and after bone transport could help to clarify the most appropriate method for enhancing its consolidation procedure.

The purpose of the present study is to analyze the histological changes that are present in the docking site and its vascularization from the surgery to the end of remodeling, implementing the same principles as in humans. The knowledge about type or types of ossification occurring in the docking site will help to understand the processes going on during the last phase of bone transport in order to avoid the problems related to its consolidation.

2. Material and Methods

2.1. Animals

Ten female skeletally mature female Merino sheep with a mean weight of 53.9 ± 8.9 kg and aged 3-5 years old were included. Each animal was sacrificed at a unique time (17, 22, 29, 35, 37, 50, 79, 98, 161 and 525 days after surgery). All of them had a metatarsal length of at least 14 cm and a minimal diameter of 10 mm in the thinner area. Animals without those requirements, with wounds or scars from previous injuries, with bone deformities affecting the gait, with local or systemic infections that could complicate the postoperative period and with 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 in accordance with our institution's Animal Laboratory Committee Guidelines and was under the supervision of a veterinarian team.

2.2. Surgery

Under general anesthesia, an optimized Ilizarov external fixator [15, 17-19] was attached to the non-osteotomized diaphysis of the right hind metatarsus of the sheep. The fixator was positioned using six stainless steel 4 mm Schanz screws that were drilled proximally and distally to the subsequent osteotomies lines and two 3 mm diameter Kirschner wires for the transportable bony segment (Fig. 1). All of them were drilled in a standardized position using a Driver Device for Wires and Pins [18]. The device design was based on a comprehensive anatomical study performed in laboratory specimens by positioning the screws and K-wires in an angular arrangement to achieve a high torsional and bending rigidity and avoiding neurovascular or tendinous injuries.

All Schanz screws were predrilled with a 2.5 mm drill bit. K-wires were also predrilled with a 2 mm drill bit and were inserted in the transportable bony segment in a mediolateral direction. Once the wires were placed, the driver device was removed, and the external fixator was assembled on the screws.

Three osteotomies were performed using an oscillating saw and a guide in order to create a defect of 15 mm and a bone transportable segment of 25 mm in length. Finally, the transportable bony segment was led proximally to close the saw gap, resulting in a defect of approximately 15 mm. A periosteum located in the area of the osteotomy was previously retracted and protected.

The distraction of the callus was 1 mm per day in one step after 7 days of latency and continued over 15 days. All animals were sacrificed with the distractor placed in the hind metatarsus, except for the 525 days sheep. A second surgery was undergone in this case at 192 days, removing the distractor and allowing it to live freely until the day of the sacrifice. After euthanasia, the metatarsal bone was disjointed and frozen until samples were processed for histological study.

2.3. Macroscopic study

A macroscopic study was performed in order to determine the size of docking site and its correct alignment. The size of the docking site was calculated as the width of the new ossification zone from the mobile proximal end to the fixed distal end. The translation of the segment was measured in millimeters when it was presented.

2.4. Histological study

For histological purposes, morphometric and immunohistochemical studies were performed.

Metatarsal bones were decalcified and sectioned longitudinally with a razor blade in order to obtain two blocks from half of the limb: the regenerate and the docking site. The regenerate block contained a portion of the proximal segment (base segment), the entire regenerate and a portion of the distal segment (transported segment) and was used in a previous study [15]. This study focuses on the docking site block, which contained a portion of the transportable segment (proximally) and a portion of the target segment (distally). The blocks were embedded in descaling solution and paraffin, to obtain 2x2 cm pieces. Each piece was then cut into 4 µm thick sections. Blocks containing the regenerate were employed in another parallel work.

All measurements for morphometric and immunohistochemical studies were performed measuring area percentages with microscope images using the ImageJ 1.47V program (Wayne Rasband, National Institutes of Health, USA; Java 1.6.0_20 32 bit) [21, 30, 31].

2.4.1. Morphometric study

The sections were mounted on microscope slides and stained with Hematoxylin and Eosin and Masson Trichrome with aniline blue (Bio-Optica, Milan, Italy) following the manufacturer’s instructions.

Microscopic docking site morphology was examined to evaluate the percentages of fibrous tissue and trabecular bone, as well as types of ossification (intramembranous vs endochondral), over time, based on microscopic observation at 10X, 20X, 40X, 100X and 200 X augmentations.

- Tissue measurements

Microphotographs of an entire longitudinal section of the docking site were used to specifically quantify area percentages of fibrous tissue and trabecular bone, as well as necrotic tissue when it is present.

- Ossification types

To quantify the percentages of areas of each type of ossification (intramembranous vs endochondral), every longitudinal section of the docking site was studied. Three microphotographs randomly taken of the docking site were used for the counts, obtaining a detailed description of the types of ossification and their localization.

2.4.2. Immunohistochemical study

Immunohistochemical studies were performed in order to determine the expressions of collagen types I and II and the development of the vascularization in the docking site. Measurements were performed in the entire area of the docking site. It was carried out using an indirect immunoperoxidase system. Tissue slices were deparaffinized in xylene baths and rehydrated in alcohol. Inhibition of endogenous peroxidase was performed by incubation in 3% hydrogen peroxide in methanol for 30 min followed by extensive washing in phosphate-buffered saline (PBS). In order to block non-specific reactions, the incubation in 10% goat serum in PBS (30 min at room temperature) was also performed prior to incubation in primary antibodies. Specific primary antibodies, anti-collagen type I (intramembranous ossification), type II (endochondral ossification) and anti VIII factor (it marks the walls of blood vessels and defines the evolution of vascularization) were used. After incubation with a primary antibody (18 hours at 4 ° C) the tissue was washed in PBS and the secondary biotinylated antibody was applied (30 minutes at room temperature). Immunoreactive areas against collagen types I and II were described, and areas anti VIII factor were measured using three photomicrographs per animal at 20X magnification (0.34 mm^2/photomicrograph). Immunohistochemical studies could not be performed in the 161 and 525 days sheep because of the low immunogenicity of the tissue against the antibodies in the mature bone.

3. Results

All animals completed the study without any complications regarding the distractor or the surgical wound. Only one sheep had a swollen metatarsus during the latency period and was successfully treated with oral anti-inflammatory. Weight bearing on the operated limb was allowed from the beginning of the study, and the animals limped only one or two days after the surgery.

3.1. Macroscopic description

The average length of the docking sites was 3.3 mm (2-5 mm). All presented good alignment, except for the 37 days sheep, which showed a lateral translation of 2 mm (Figs. 2A-2B). In the 29 days sheep, the regenerate presented a slight lateral translation during transport with mostly cortical tissue and little medullar cavity.

3.2. Histological study

3.2.1. Morphometric study

- Tissue measurements

The percentages of trabecular bone and fibrous tissue presented in the docking site are shown in Figure 3. From the beginning of the study to 98 days after surgery, the average percentage of fibrous tissue was 92.19%. It was at 161 days after surgery (140 days since the end of distraction) when an increase in the percentage of bone trabeculae was observed (20.96%). Fibrous tissue decreased over time, and 525 days after surgery, the docking site was completely ossified. No trabecular bone is detected in the 525 days sheep (it has remodeled to cortical bone) and thus is not represented in Figure 3.

- Ossification types

Microscopic study of the docking site at 20X, 40X, 100X and 200X in the 98 days sheep showed a total absence of ossification areas. Only dense connective tissue was observed. The remaining animals showed areas of intramembranous ossification (Fig. 4), except for the 22 and 50 days sheep, where some areas of endochondral ossification were found (60% and 10% of the ossification areas, respectively). However, those endochondral areas were situated outside of the osteotomies, next to cortical areas; therefore, they might not be considered part of the docking site. Intramembranous areas were more numerous in all animals (except for the 22 days sheep) but were smaller than the endochondral areas. Additionally, ossification started subperiosteally in all animals. The first endosteal areas were identified at 35 days post-surgery, and all of them were intramembranous until the end of the study.

3.2.2. Immunohistochemical study

The immunohistochemical study showed expression of collagen type I in all animals except in the 98 days sheep. All ossification areas appeared to be subperiosteal. Only the 22, 35 and 50 days sheep showed expressions of collagen type II; these foci appeared in the same situation that previous morphometrical studies have described (Fig. 5). Therefore, they might be excluded from the docking.

Temporal evolution of vascularization is shown in Figure 6. A significant increase of the vascular area was identified in the animal sacrificed immediately after distraction. This vascular increment remained until 37 days after distraction, and 50 days after surgery, it showed a slow decrease. When the percentage of area occupied by blood vessels was studied by region (proximal, central, distal), it was higher in the peripheral areas than in the central area of the docking (Fig. 6).

4. Discussion

Although consolidation of the docking site is a frequent source of problems, histological changes occurring in it remain unknown. In this paper, the morphological and ossification features of the docking site in an experimental model of bone transport performed on sheep have been assessed. As far as the authors know, this is the first study showing the histological changes and vascularization of the docking site from the day of surgery until the end of remodeling, using an Ilizarov external fixator and following the same principles as those applied in humans.

Because of the impossibility of conducting the study in real patients and to ensure reliable results closer to humans, animals with similar morphological characteristics and a similar load bearing distribution were selected. The small sample size might affect comparison outcomes, although the difficulty of feedlot and animal care and costs must be considered. To minimize interindividual differences, animals of the same sex and similar weights and ages were used. Although the bone defect was not as complex as the bone injuries observed in clinical practice, the study was carried out under ideal physiological conditions in order to obtain histological results as close as possible to those in human patients.

Regarding the distribution of the different tissues in the docking, the area occupied by fibrous tissue was more than 90% until 98 days post-surgery, when an increment in bone trabeculae was detected. This was in concordance with Nagashima et al. [23]. In that work, lamellar bone was not reported until 12 weeks after distraction, representing 45% of the tissues presented in the docking site, and consolidation of the docking site was obtained in only half of the animals. Although the area occupied by the bone trabeculae in the present study was not large (maximum detected 20,96% of the total present newly formed tissues), it could be the result of the difference in follow up time between the two older animals (161 and 525 days).

The presence of this large amount of fibrous tissue was in concordance with previous works about the consolidation of the docking site [7, 9, 12, 23, 32, 33], where the presence of a fibrous connective cap was identified [7, 9-11, 23]. Initially, it was theorized that ossification of the docking site during bone transport followed the same principles as regular fracture healing [7]. However, the repair cascade that follows the fracture does not occur at the docking site because both bone ends are approached gradually, and the time for consolidation has been lengthened over the conventional healing process [5, 33-38]. This connective cap has also been the basis of the development of several studies comparing different techniques to enhance the consolidation of this area[7-9], without showing significant differences in consolidation rates or time of fixation between closed and open techniques [8, 9]. However, there is an inclination to perform a secondary debridement surgery on the docking site because these are usually complex patients and surgical techniques on this area are an attempt to accelerate consolidation. This paper confirms that consolidation of the docking site occurs despite the connective cap and agrees with authors who defend closed techniques in order to enhance the consolidation of the docking site [8, 9, 33].

Intramembranous ossification was predominant spatially and temporally in all animals in the docking site, except for the 22 days sheep. These findings are in contrast with those described by Garcia et al. [10], who reported predominant endochondral ossification. However, the distraction process was performed over a plate, and weight bearing was avoided during the first weeks; this is rarely used in daily clinical practice and may explain the different results. Moreover, it is known that the type of ossification present in fracture healing also depends on the stiffness of the osteosynthesis technique employed, and the biological response of the tissues to weight bearing in the operated limb appears to be a key factor for promoting consolidation in distraction regimens [1, 17-19, 39, 40].

Immunohistochemistry studies showed an increment in the percentages of area occupied by blood vessels in the first period, followed by a slow decrease from 50 days after surgery. Although the small size of the docking site makes the possibility of analyzing it by area difficult, its global temporary vascular pattern was found to be similar to the one described by several authors for the regenerate [13, 15, 24, 41, 42]. There are many studies about the changes in vascularization in limbs submitted to distraction [24-28], but works dedicated to the vascularization of the operated limb after a bone transport are scarce

[29], and none have focused on the docking. To our knowledge, this is the first study reporting the evolution of the vascularization of the docking site using immunohistochemical techniques.

It is known that the increase in the vascularization in limbs submitted to distraction osteogenesis [26, 28, 41, 43, 44] reaches levels up to nine times higher than the contralateral side and that compression over fibrous connective tissue occupying the docking site at the end of distraction induces ischemia. We hypothesize that the ischemia of the fibrous cap stimulates the vascularization, whose increase is also induced by the distraction stimulating the vascular invasion of the docking site. The time necessary for the vascular invasion of the connective cap could explain the delay in the consolidation of the docking site. If the high stability of the fixator that is used is added, it could explain the development of intramembranous ossification in this area.

This study of histological changes occurring in the docking site will help to explain the consolidation process and to research the effects of different strategies to achieve its union. It has been concluded that ossification of the docking site is predominantly intramembranous when a stiff Ilizarov fixator is used (620-695 N/mm) to perform bone transport. It could be hypothesized that its ossification is influenced by the stiffness of the fixator employed for the transport, similar to the regenerate. The vascularization also has an important role in ossification, and therefore, the delay in its onset lengthens the time for consolidation of the docking site. Experimental and clinical studies focusing on both factors are required in order to improve the outcomes that those techniques lack.

BIBLIOGRAPHY

[1] Claes L, Veeser A, Göckelmann M, Horvath D, Dürselen L, Ignatius A. A novel method for lateral callus distraction and its importance for the mechano-biology of bone formation. Bone. 2010;47:712-7.

[2] Fink B, Pollnau C, Vogel M, Skripitz R, Enderle A. Histomorphometry of Distraction Osteogenesis During Experimental Tiabial Lengthening. Journal of Orthopaedic Trauma. 2003;17(2):113-8.

[3] Ilizarov G. The Tension-Stress Effect on the Genesis an Growth of Tissues: Part II: The Influence of the Rate and Frequency of Distraction. Clinical Orthopaedics and Related Research. 1989(239):263-85.

[4] Ilizarov G. The Tension-Stress Effect on the Genesis and Growth of Tissues: Part I: The Influence of Stability of Fixation and Soft-Tissue Preservation. Clinical Orthopaedics and Related Research. 1989(238):249-81.

[5] Paley D, Catgni M, Argnani F, Villa A, Bendetti GB, Cattaneo R. Ilizarov treatment of tibial nonunions with bone loss. Clinical Orthopaedics and Related Research. 1989(241):146-64.

[6] Wanders N, Richards M, Steen H, Kuhn J, Goldstein S, Goulet J. Evaluation of the Mechanical Environment During Distraction Osteogenesis. Clinical Orthopaedics and Related Research. 1998(349):225-34.

[7] Giotakis N, Narayan B, Nayagam S. Distraction osteogenesis and nonunion of the docking site: Is there an ideal treatment option? Injury. 2007;38(S1):S100-S7.

[8] Lovisetti G, Sala F. Clinical strategies at the docking site of distraction osteogenesis: Are open procedures superior to the simple compression of Ilizarov? Injury. 2013;44(S1):S58-S62.

[9] Lovisetti G, Sala F, Miller AN, Thabet AM, Zottola V, Capitani D. Clinical reliability of closed techniques and comparison with open strategies to achieve union at the docking site. International Orthopaedics. 2012;36:817-25.

[10] García F, Picado C, Garcia S. Histology of the regenerate and docking site in bone transport. Arch Orthop Trauma Surg. 2009(129):549-58.

[11] Robinson P, Papanna M, Younis F, Khan S. Arthroscopic debridement of docking site in Ilizarov bone transport. Ann R Coll Surg Engl. 2010;92:437-43.

[12] Sala F, Marinoni E, Miller AN, Pesenti G, Castelli F, Alati S, et al. Evaluation of an Endoscopic Procedure for the Treatment of Docking Site Nonunion. Journal of Orthopaedic Trauma. 2013;27(10):569-75.

[13] Forriol F, Denaro L, Longo U, Taira H, Maffulli N, Denaro V. Bone Lengthening osteogénesis, a combination of intramembranous and endochondral ossification: an experimental study in sheep. Strat Traum Limb Recon. 2010(5):71-8.

[14] Krawczyk A, Kuryszko J, Wall A, Dragan S, Kulej M. Experimental studies on the effect of osteotomy technique on the bone regeneration in distraction osteogénesis. Bone. 2007(40):11.

[15] López-Pliego EM, Giráldez-Sánchez MA, Mora-Macias J, Reina Romo E, Domínguez J. Histological evolution of the regenerate during bone transport: an experimental study in sheep. Injury. 2016;47(S3):S7-S14.

[16] Mizuta H, Nakamura E, Mizumoto Y, Kudo S, Takagi K. Effect of distraction frequency on bone formation during bone lengthening. A study in chickens. Acta orthopaedica Scandinava. 2003;74(6):709-13.

[17] Mora-Macías J, Reina Romo E, Dominguez J. Distraction osteogenesis device to estimate the stiffness of the callus in vivo. Medical Engineering & Physics 2015.

[18] Mora-Macías J, Reina Romo E, López-Pliego M, Giráldez-Sánchez MA, Domínguez J. In vivo mechanical characterization of the distraction callus during bone consolidation. Annals of Biomedical Engineering. 2015.

[19] Mora-Macías J, Reina Romo E, Morgaz J, Domínguez J. In vivo gait analysis during bone transport. Annals of Biomedical Engineering. 2015.

[20] Sencimen M, Aydintug Y, Ortakoglu K, Karslioglu Y, Gunhan O, Gunaydin Y. Histomorphometrical analysis of new bone obtained by distraction osteogenesis and osteogenesis by periosteal distraction in rabbits. Int J Oral Maxillofac Surg. 2007;36:235-42.

[21] Singare S, Li D, Liu Y, Wang J. The effect of latency on bone lengthening force and bone mineralization: an investigation using strain gauge mounted on internal distractor device. Biomedical Engineering Online [Internet]. 2006 9 March 2006; 5:[8 p.]. Available from: www.biomedical-engineering-online.com/content/5/1/18.

[22] Kallio T, Vauhkonen M, Peltonene J, Karaharju E. Early bone matrix formation during distraction. A biochemical study in sheep. Acta Orthopedica Scandinava. 1994;65(4):467-71.

[23] Nagashima L, Rondon M, Zakhary I, Nagy W, Zapata U, Dechow P, et al. Bone regeneration and docking site healing after bone transport distraction osteogenesis in the canine mandible. Journal of Oral Maxillofacial Surgery. 2012;70(2):429-39.

[24] Aronson J. Temporal and spatial increases in blood flow during distraction osteogénesis. Clinical Orthopaedics and Related Research. 1994(301):124-31.

[25] Gil-Albarova J, Melgosa M, Gil-Albaroba O, Cañadell J. Soft tissue behavior during limb lengthening: an experimental study in lambs. Journal of Pediatric Orthopaedics Part B. 1997;6(4):266-73.

[26] Shevtsov V, Asonova S, Naumov A, Gordievskikh N, Kuznetsova L, Erofeev S. Comparative morphofunctional investigation of vascular pool of the muscles of the elongated extremity using different distractional regimens. La Chirugia degli organi di movimento. 2000;85(1):11-22.

[27] Shevtsov V, Asonova S, Yerofeyev S. Morphological characteristics of angiogenesis in the myosfacial tissues of a limb elongated by the Ilizarov method. Bulletin Hospital for Joint Diseases. 1995;54(2):76-84.

[28] Shevtsov V, Gordievskikh N, Bunov V, Petrovskaya N. Changes in blood flow during tibial thickening by the Ilizarov Method. Bull Exp Biol Med. 2002 134(6):525-7.

[29] De Coster T, Simpson A, Wood M, Li G, Kenwright J. Biologic model of bone transport distraction osteogenesis and vascular response. Journal of Orthopaedic Research. 1999;17(2):238-45.

[30] Sabri C, Richelme F, Pierres A, Benoliel A, Bongrand P. Interest of image processing in cell biology and inmunology. Journal of Immunological Methods. 1997;208:1-27.

[31] Schneider C, Rasband W, Eliceiri K. NIH Image to Imaje J: 25 years of image analysis. Focus on Bioimage Informatics. 2012;9(7):671-5.

[32] Paley D. Current Techniques of Limb Lengthening. Journal of Pediatric Orthopaedics. 1988;8(1):73-92.

[33] Paley D, Maar D. Ilizarov bone transport treatment for tibial defects. Journal of Orthopaedic Trauma. 2000;14(2):76-85.

[34] Cierny G, Zorn K. Segmental tibial defects: comparing conventional and Ilizarov methodologies. Clinical Orthopaedics and Related Research. 1994;301:118-23.

[35] De Coster T, Geblert R, Mikola E, Pirela-Cruz M. Management of Posttraumatic Segmental Bone Defects. Journal of the American Academy of Orthopaedic Surgeons. 2004;12:28-38.

[36] Green S. Skeletal defects: a comparison of bone grafting and bone transport for segmental skeletal defects. Clinical Orthopaedics and Related Research. 1994;301:111-7.

[37] Mekhail A, Abraham E, Gruber B, Gonzalez M. Bone Transport in the Management of Posttraumatic Bone Defects in the Lower Extremity. Journal of Trauma. 2004;56:368-78.

[38] Naggar L, Chevalley F, Blanc C, Livio J-J. Treatment of large bone defects with the Ilizarov technique. The Journal of Trauma. 1993;34:390-3.

[39] Cai G, Saleh M, Coulton L, Yang L. Distraction- resisting force during tibial diaphyseal lengthening and consolidation- a study on a rabbit model. Clinical Biomechanics. 2004(19):733-4.

[40] Claes L, Augat P, Schorlemmer S, Konrads C, Ignatius A, Ehrnthaller C. Temporary distraction and compression of diaphyseal osteotomy accelerates bone healing. Journal of Orthopaedic Research. 2008(26):772-7.

[41] Choi I, Ahn J, Chung C, Cho T. Vascular proliferation and blood supply during distraction osteogénesis: a scanning electron microscopic observation. Jouranl of Orthopaedic Research. 2000;18(5):698-705.

[42] Yasui N, Kojimoto H, Sasaki K, Kitada A, Shimizu H, Shimomura Y. Factors affecting Callus Distraction in Limb Lengthening. Clinical Orthopaedics and Related Research. 1993(293):55-60.

[43] Eckardt H, Lind M, Christensen K, Hansen E, Hvid I. Mid tibia distraction osteogénesis redistributes bone blood flow. Acta Orthopaedica 2005;76(4):459-64.

[44] Rowe N, Mehrara B, Luchs J, Dudziak M, Steinbrech D, Illei P, et al. Angiogenesis during mandibular distraction osteogénesis. Annals of Plastic Surgery. 1999;42(5):470-5.

Figure and Legends

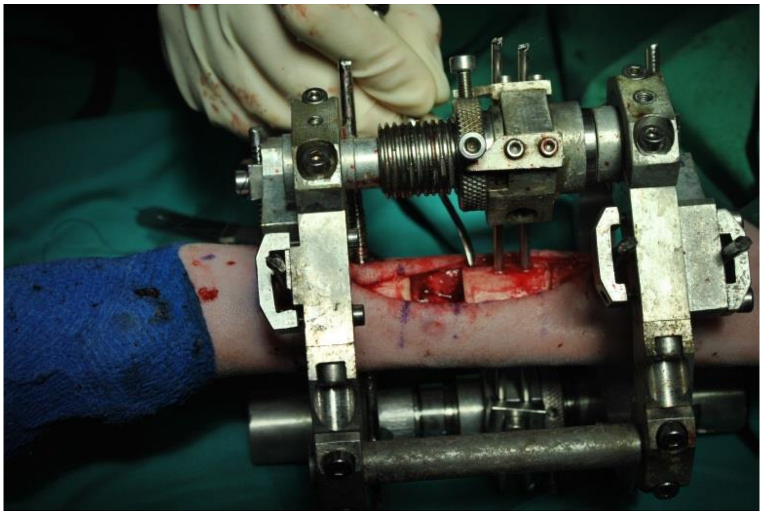
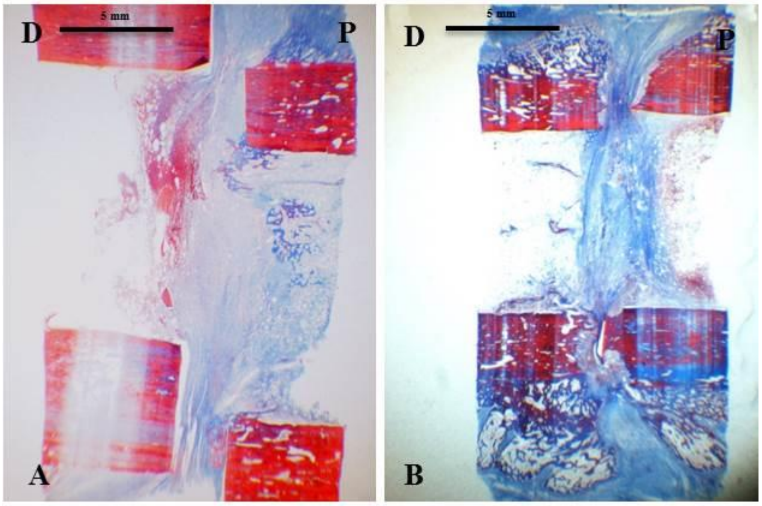


Fig. 1. Final appearance of the metatarsus once the fixator is placed on it.



Figs. 2A-2B. Microphotographs of an entire longitudinal section of the docking site. Masson Tricrome X10 magnification. (A) Lateral translation of 37 days sheep docking site. (B) Perfect alignment of 50 days sheep docking site. (P: proximal; D: distal).

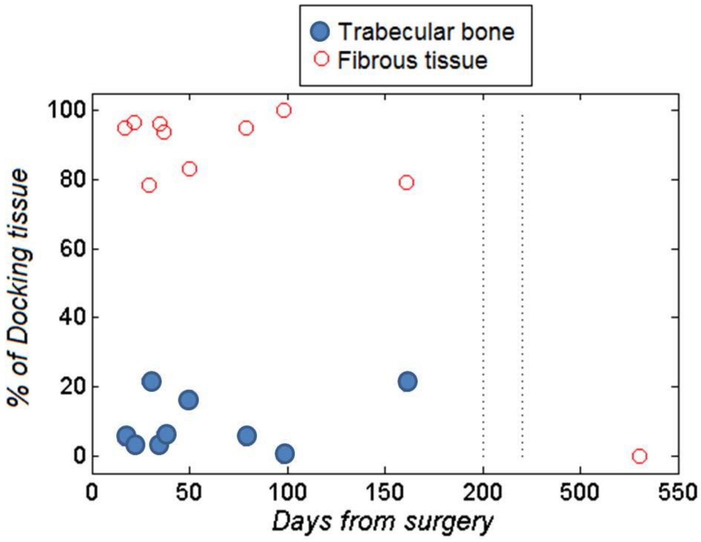


Fig. 3. Evolution of fibrous tissue and trabecular bone in docking site.

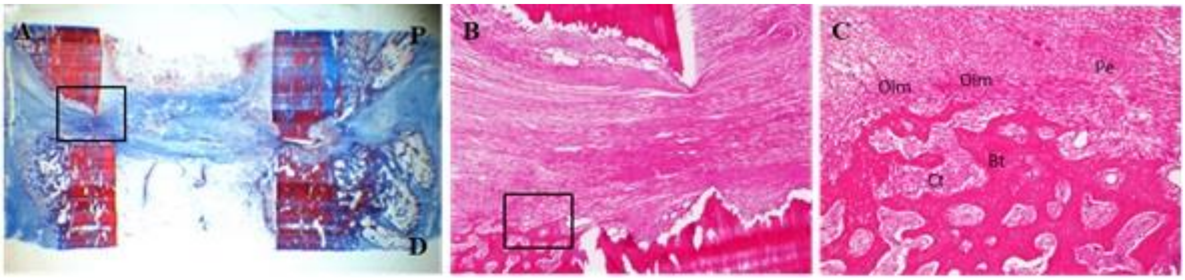


Fig. 4. Focus of Intramembranous ossification in the 50 days sheep. Study area is included in the square: (A) Masson Tricrome X6 magnification. (B) Hematoxilin-Eosin X20 magnification. (C) Hematoxilin-Eosin X40 magnification. (P: proximal; D: distal; Oim: intramembranous ossification; Pe: periosteum; Ct: conective tissue; Bt: bone trabeculae)

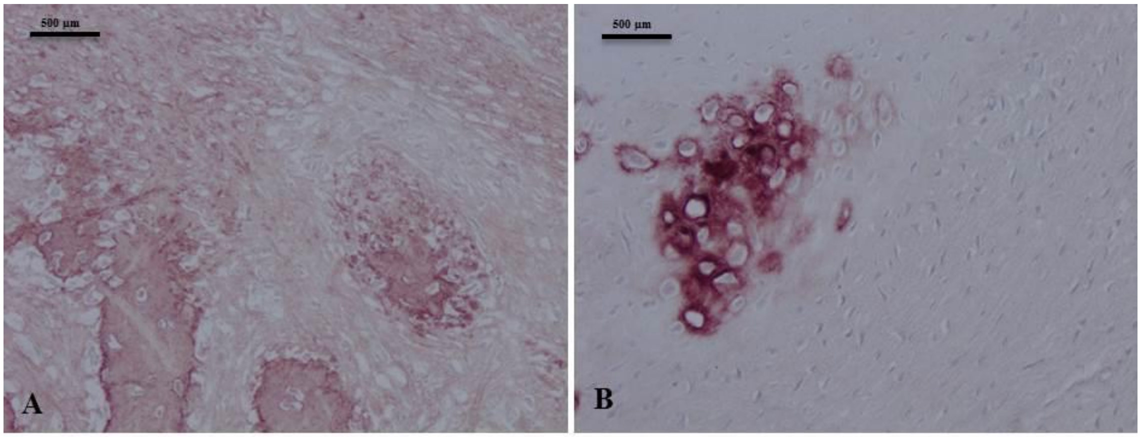


Fig. 5. 20X Magnification images of inmunohistochemical study of docking site in 50 days sheep. (A) Expression of type I collagen. (B) Expression of type II collagen.

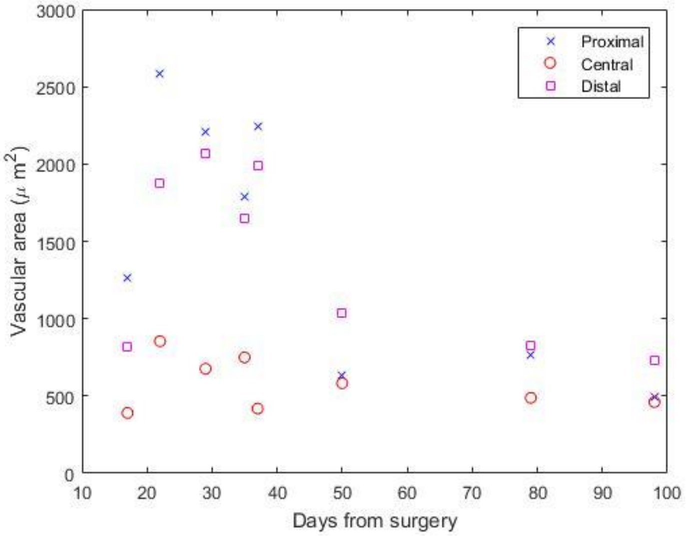


Fig. 6. Progression of the vascular area over time.