Craniofacial Distraction Osteogenesis

Effects of rhythm of distraction on bone regeneration

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Effects of rhythm of distraction on bone regeneration

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Craniofaciale distractie osteogenese

Effecten van ritme van distractie op botregeneratie

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~ This work may contain distracting elements ~

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Chapter

1

General Introduction

General Introduction

Distraction osteogenesis

Distraction osteogenesis (DO) is defined as the formation of new bone tissue between bone segments that are divided by an osteotomy and then gradually separated by exerting an external force to the mobile bone segment(s). The resulting callus tissue in the distraction gap will eventually mineralize, creating a new bridge of bone tissue between the osteotomy edges of the original bone segments. Elongated bones are the result (Fig. 1).

Short history

Although craniofacial DO has been used clinically since the early nineties, the first report on bone lengthening dates from more than a century ago. In 1905, Codivilla was the first to describe the process of limb lengthening.[1] Half a century later, the technique was rediscovered by Ilizarov, who made significant contributions to the refinement of orthopedic DO through his extensive clinical and experimental work.[2-5] In 1973, Snyder *et al* were the first to successfully apply the bone lengthening technique to the mandible of a dog.[6] Still, it was not until 1992 that mandibular DO was reported in human patients.[7] Nowadays, DO is widely accepted as a treatment for acquired and congenital deformities in the field of oral and maxillofacial surgery.

Indications

The indications for the application of craniofacial DO may include resection defects, post-trauma defects, alveolar bone heightening for dental implant placement, Pierre-Robin sequence, hemifacial microsomia, and syndromes associated with congenital anomalies such as obstructive sleep apnea syndrome, velocardiofacial syndrome, cerebral palsy, Treacher-Collins syndrome, Crouzon syndrome, Apert syndrome, Pfeiffer syndrome and Nager syndrome.[8]

Compared with conventional osteotomies, larger advancements of craniofacial bones are possible with distraction osteogenesis. In addition, it may replace bone grafts, so that no donor-site surgery is needed for the harvesting of bone (predominantly from the iliac crest), and there is no danger of donor bone rejection.

Furthermore, the gradual lengthening of the bone and the surrounding soft tissue results in better adaptation, decreasing the risk of relapse.

Distraction protocol

A distraction protocol consists of a number of sequential stages (Fig. 2). First, an osteotomy is required to divide the bone into two segments. This results in loss of continuity and mechanical integrity, triggering the repair process.

Second, a latency period is applied. During the latency period, which is the time between osteotomy and onset of distraction, a reparative callus is allowed to form. Mechanical integrity and blood supply are restored, and osteoprogenitor cells proliferate.

Third, the distraction phase commences. This phase is characterized by the application of external traction forces to the osteotomized bone segments using a distraction device (also called a 'distractor'). The resulting distraction of the callus creates a dynamic microenvironment. The tension created in the tissues has growth-stimulating effects, including the prolongation of angiogenesis with increased tissue oxygenation, and the increase of fibroblast proliferation with intensification of biosynthetic activity. The distraction phase itself consists of two important parameters: the rate of distraction and the rhythm of distraction. The former indicates the daily amount of excursion of the distracted bone segment, typically measured in millimeters per day. The latter indicates the number of distractor activations used to achieve the daily rate of distraction, which is usually one to four times a day.

Fourth, after cessation of distraction the consolidation phase starts and continues until the distraction device is removed. It represents the time required for complete mineralization of the regenerate (*i.e.*, the newly formed bone).

Last, the remodeling period following the consolidation phase represents the period from the application of full functional loading to the complete remodeling of the regenerate. The initially formed bony scaffold is reinforced by parallel-fibered lamellar bone, cortical bone and marrow cavity are restored, and Haversian remodeling normalizes the bone structure.

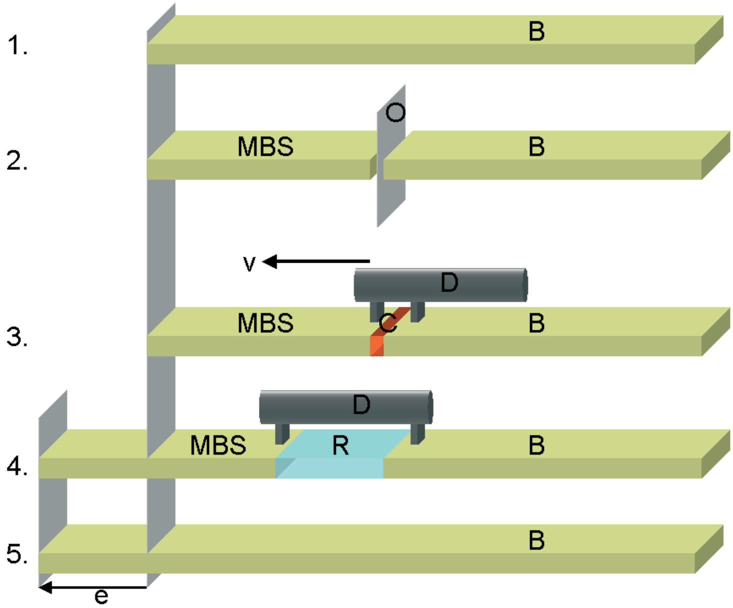


Figure 1
Schematic view of the process of distraction osteogenesis: 1. Bone (B), which needs to be elongated; 2. An osteotomy (O) is performed, creating a mobile bone segment (MBS) separated from the rest of the bone (B); 3. A distractor (D) is placed on the bone segments. Callus tissue (C) has formed between the bone segments. Activation of the distractor moves the mobile bone segment (MBS) parallel to the vector of distraction (v); 4. The resulting distraction gap is filled by newly formed tissue (regenerate; R), which will eventually calcify; 5. After complete mineralization of the regenerate the distraction gap is completely bridged by new bone. The total amount of elongation (e) depends on the length of the distraction period and the rate of distraction.

Biological foundation

The biological processes behind distraction osteogenesis have been extensively studied, and are summarized in various review papers.[9-11] In summary, after osteotomy, the loss of mechanical integrity in a skeletal segment triggers an evolutionary process of bone repair known as fracture healing. During the latency period, the sequence of events that take place are similar to that seen during fracture healing. However, during distraction the normal process of fracture healing is interrupted by the application of gradual external distraction forces to the callus. The tension in the callus tissue creates a metabolically active microenvironment in which new tissues are formed in a direction parallel to the external force (*i.e.*, the vector of distraction).

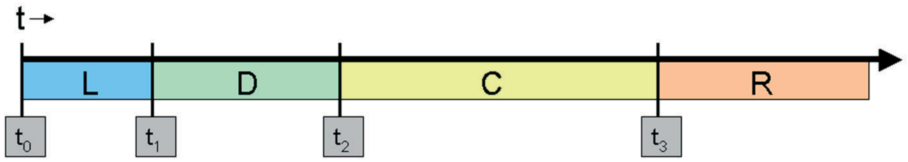


Figure 2

Sequential stages of a typical distraction protocol. Stage t_0 = osteotomy is performed, distractor is applied to the bone segments; Stage L = latency period, during which reparative callus is formed; Stage t_1 = end of latency period, start of distraction period; Stage D = distraction period, during which the mobile bone segment is distracted according to an appropriate rate and rhythm of distraction; Stage t_2 = end of distraction period, start of consolidation period; Stage C = consolidation period, during which the distractor stays fixed to the bone and is not being activated anymore; Stage t_3 = end of consolidation period, removal of distractor; Stage R = remodeling period, during which the bones are subjected to ongoing remodeling.

Various cytokines play an essential role in distraction osteogenesis. For instance, transforming growth factor (TGF) stimulates the processes of bone production and inhibits the processes of bone resorption. Bone morphogenetic protein (BMP), insulin-like growth factor (IGF), and fibroblast growth factor (FGF) stimulate the proliferation and differentiation of osteogenic cells. Vascular endothelial growth factor (VEGF) regulates the budding and growth of new blood vessels from existing vascular structures, which is essential as successful osteogenesis is largely dependent of adequate revascularization. Cells secrete extracellular matrix (ECM), which can be subdivided into collagenous ECM proteins (such as collagen type-I, type-II *etc.*) and non-collagenous ECM proteins (such as osteonectin, osteopontin, osteocalcin). These ECM proteins facilitate early bone formation and regulate ossification and bone remodeling.

Ossification may be endochondral, intramembranous, or transchondroid.[12] Normally, craniofacial DO regenerates will follow the route of intramembranous ossification. This requires stable fixation of the distraction device and a rate of distraction not exceeding 2 mm/d. Otherwise, cartilage will form first, which may later be replaced by bone tissue via endochondral ossification. An intermediate tissue type has also been reported, with chondrocyte-like cells and osteocyte-like cell coexisting in chondroid bone without a clearly distinguishable boundary.[12]

Distraction devices

Stable fixation of the distraction device is required to prevent pseudoarthrosis. The devices may be placed internally or externally. When selecting a distraction device, one has to take into account its advantages and disadvantages. External distractors are available in longer lengths than internal distractors, may provide excellent control of bone segment movement, and are easy to place, maintain, and remove. On the other hand, there is the risk of skin scarring and poor acceptance by the patient. Internal distractors may avoid skin scarring, while there is less negative psychosocial impact because the distractor is hidden. However, internal distractors may be more difficult to place. Some intraoral devices are not well tolerated by the patients. In addition, another surgical procedure is needed for removal, while it may also lack multidirectional adjustment capabilities when the vector of distraction has to be adjusted during the distraction phase.

Distraction devices that can perform automated non-stop (*i.e.*, continuous) distraction are desirable. Such devices only have to be activated at the start of the distraction phase, after which the device automatically performs distraction at a preset rate. At the end of the distraction phase the distractor will be terminated. The advantage of such a continuous distractor over a discontinuous distractor (which needs daily manual activation by patient, caretaker or physician) is that the outcome of distraction is independent of patient compliance; erroneous handling by the patient caused by physical or mental problems (or otherwise) is eliminated by the automated nature of continuous distraction. The patient is relieved from the stress of having to activate the distractor each time, which may be as often as four times a day.

Continuous distraction versus discontinuous distraction

Continuous distraction may also stimulate osteogenesis. In a dog model, Ilizarov used an automated distraction device to lengthen the tibia 0.017 mm every 24 minutes (*i.e.*, a (semi-)continuous distraction rhythm of 60 steps per day). He found that, at a given rate, the outcome of orthopedic DO improved when the rhythm of distraction was increased.[4] This suggests that continuous distraction would also improve the outcome of craniofacial distraction osteogenesis.

Despite the great potential of continuous distraction osteogenesis for craniofacial reconstructive surgery, relatively few studies have been performed to investigate the possibilities and effects of continuous distraction on craniofacial bone structures. The distraction devices were either spring-mediated[13-16] or utilized micromotor[17-20] or hydraulic mechanisms[21-26]. These studies reported the feasibility of continuous distraction, although some issues had to be resolved. For spring-mediated distraction, the vector of distraction may not be linear; the spring force is not constant and decreases towards the end when actually greater forces are needed to overcome increasing tissue resistance; a second surgery may be required for activation of the device; and length of distraction may be unpredictable.[13] For micromotor distraction gear break, fracture of the mandible, and postoperative infections were reported.[17] Infections were also reported in some studies[25,26] on hydraulically powered continuous distractors, although most studies were problem free[21,22,27]. In comparison with conventional distractors, continuous distraction devices usually consist of more hardware. To our knowledge, only few studies have actually compared continuous with discontinuous distraction.[22,26,27] However, the studies were performed in small numbers of pigs, which makes it difficult to interpret the statistical relevance of these data. Does continuous distraction actually result in better osteogenesis than conventional discontinuous distraction?

Aim of this study

The aim of this thesis was to quantitatively compare various rhythms of distraction using statistically relevant numbers of experimental animals, in order to identify the effect of the rhythm of distraction on bone regeneration. We were particularly interested in the comparison between continuous and discontinuous distraction.

We hypothesize that continuous distraction accelerates bone regeneration compared to discontinuous distraction.

Thesis outline

The results of our animal experimental studies on the effects of various distraction rhythms on bone regeneration are presented in the following thesis chapters.

Chapter 2 presents a review paper, which summarizes and reviews the currently available animal experimental data regarding the various distraction parameters. Can recommendations for the optimal distraction protocol be formulated, based on these data? Relatively little quantitative data are available on the effects of rhythm of distraction, especially regarding the comparison between discontinuous distraction and continuous distraction protocols. Our animal experimental studies, described in the following chapters, will study the effects of various distraction rhythms on regenerate quality.

In Chapter 3, our experiences with rabbits as the animal model of choice for craniofacial distraction osteogenesis research are discussed. Factors such as housing and handling as well as tolerance to surgical and experimental protocols are reviewed and compared with other animal models. Is the rabbit an appropriate animal model for research on craniofacial DO?

In Chapter 4, various discontinuous rhythms of distraction are compared with each other. Does triple daily activation of the distractor significantly increase regenerate quality compared with single daily activation (given the same rate of distraction)? Regenerates are quantitatively assessed by microcomputed tomography (micro-CT) in order to see whether there are significant differences in bone formation between these discontinuous distraction protocols. In addition, evaluation of the regenerate by radiographs is compared with evaluation by ultrasonographs. Do bone-fill scores based on ultrasonographs provide a more reliable predictive value for the amount of new bone formation than plain radiographs?

Chapter 5 introduces a custom-built continuous distraction device, which is used in an experiment on the effect of continuous distraction on bone regeneration. In scientific literature, relatively few reports can be found regarding the quantitative comparison of discontinuous and continuous distraction protocols. Therefore, data from the previous discontinuous distraction study were added to the continuous distraction data pool, in order to compare continuous with discontinuous distraction. Does continuous distraction significantly accelerate osteogenesis?

Chapter 6 discusses the quality of bone regenerates resulting from discontinuous and continuous distraction on a histological level. Bone tissue and various other tissues, as well as blood vessels, various cell types and mineral apposition rate were quantified. Again, comparison between discontinuous and continuous distraction protocols was made. Are there differences in histological characteristics and bone apposition dynamics between discontinuous and continuous distraction protocols?

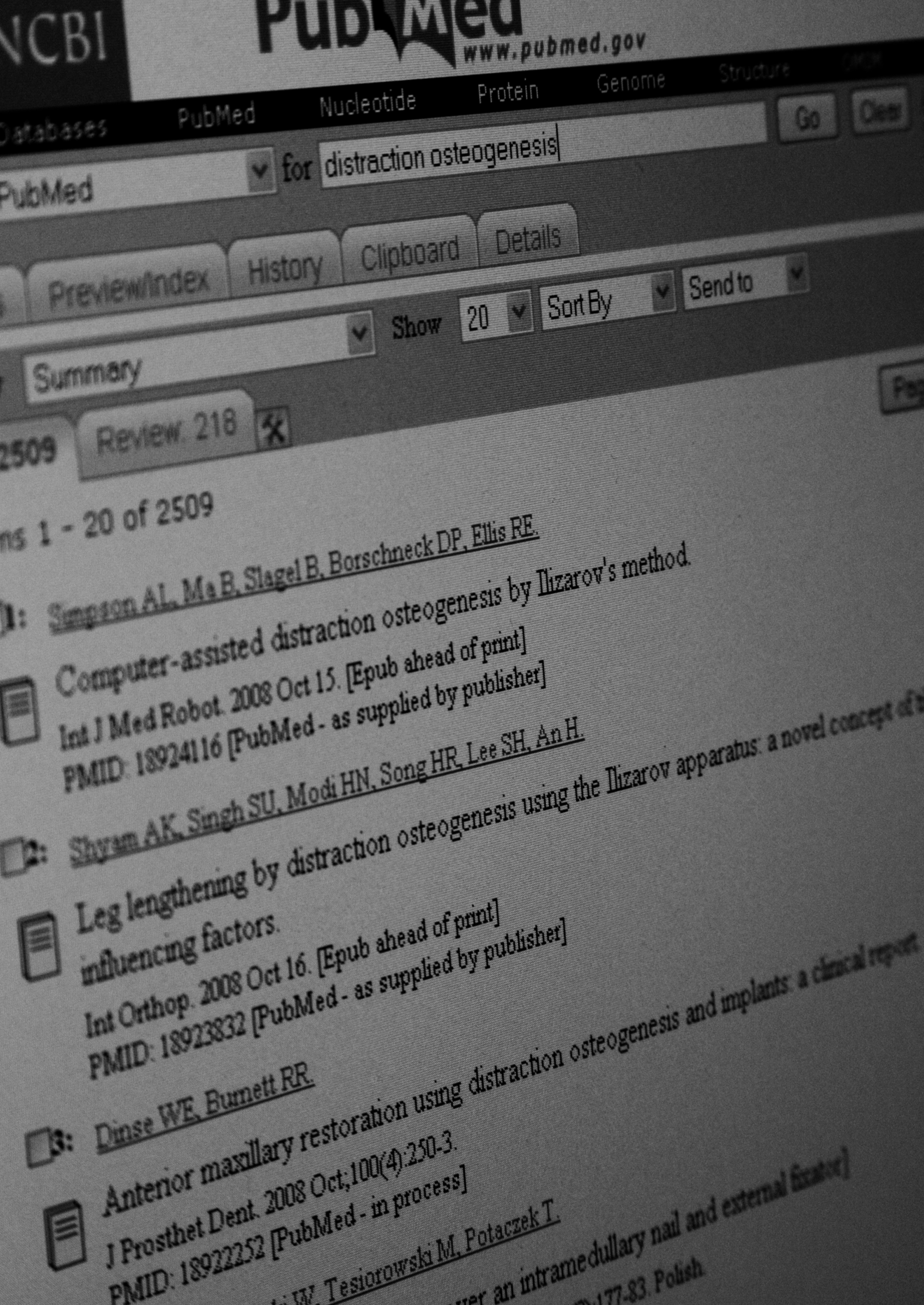
Chapter 7 presents an overview of the work in this thesis and discusses the key findings, their implications, and future perspectives.

References

1. Codivilla A. On the means of lengthening, in the lower limbs, the muscles and tissues which are shortened through deformity. Am J Orthop Surg 1905;(2):353-357.
2. Ilizarov GA. A method of uniting bones in fractures and an apparatus to implement this method. USSR Authorship Certificate 98471 1952;
3. Ilizarov GA. The principles of the Ilizarov method. Bull Hosp Joint Dis Orthop Inst 1988;48(1):1-11.
4. Ilizarov GA. The tension-stress effect on the genesis and growth of tissues: Part II. The influence of the rate and frequency of distraction. Clin Orthop Relat Res 1989;(239):263-285.
5. Ilizarov GA. The tension-stress effect on the genesis and growth of tissues. Part I. The influence of stability of fixation and soft-tissue preservation. Clin Orthop Relat Res 1989;(238):249-281.
6. Snyder CC, Levine GA, Swanson HM, and Browne EZ, Jr. Mandibular lengthening by gradual distraction. Preliminary report. Plast Reconstr Surg 1973;51(5):506-508.
7. McCarthy JG, Schreiber J, Karp N, Thorne CH, and Grayson BH. Lengthening the human mandible by gradual distraction. Plast Reconstr Surg 1992;89(1):1-8; discussion 9-10.

8. Chopra S, and Enepekides DJ. The role of distraction osteogenesis in mandibular reconstruction. Curr Opin Otolaryngol Head Neck Surg 2007;15(4):197-201.
9. Bouletreau PJ, Warren SM, and Longaker MT. The molecular biology of distraction osteogenesis. J Craniomaxillofac Surg 2002;30(1):1-11.
10. Al-Aql ZS, Alagl AS, Graves DT, Gerstenfeld LC, and Einhorn TA. Molecular Mechanisms Controlling Bone Formation during Fracture Healing and Distraction Osteogenesis. J Dent Res 2008;87(2):107-118.
11. Meyer U, Kruse-Losler B, and Wiesmann HP. Principles of bone formation driven by biophysical forces in craniofacial surgery. Br J Oral Maxillofac Surg 2005;44(4):289-295.
12. Yasui N, Sato M, Ochi T, Kimura T, Kawahata H, Kitamura Y, and Nomura S. Three modes of ossification during distraction osteogenesis in the rat. J Bone Joint Surg Br 1997;79(5):824-830.
13. Mofid MM, Inoue N, Tufaro AP, Vander Kolk CA, and Manson PN. Spring-mediated mandibular distraction osteogenesis. J Craniofac Surg 2003;14(5):756-762.
14. Idelsohn S, Pena J, Lacroix D, Planell JA, Gil FJ, and Arcas A. Continuous mandibular distraction osteogenesis using superelastic shape memory alloy (SMA). J Mater Sci Mater Med 2004;15(4):541-546.
15. Zhou HZ, Hu M, Hu KJ, Yao J, and Liu YP. Transport Distraction Osteogenesis Using Nitinol Spring: An Exploration in Canine Mandible. J Craniofac Surg 2006;17(5):943-949.
16. Zhou HZ, Hu M, Yao J, and Ma L. Rapid lengthening of rabbit mandibular ramus by using nitinol spring: a preliminary study. J Craniofac Surg 2004;15(5):725-729.
17. Schmelzeisen R, Neumann G, and von der Fecht R. Distraction osteogenesis in the mandible with a motor-driven plate: a preliminary animal study. Br J Oral Maxillofac Surg 1996;34(5):375-378.
18. Ploder O, Mayr W, Schnetz G, Unger E, Ewers R, and Plenk H, Jr. Mandibular lengthening with an implanted motor-driven device: preliminary study in sheep. Br J Oral Maxillofac Surg 1999;37(4):273-276.

19. Ploder O, Kanz F, Randl U, Mayr W, Voracek M, and Plenk H, Jr. Three-dimensional histomorphometric analysis of distraction osteogenesis using an implanted device for mandibular lengthening in sheep. Plast Reconstr Surg 2002;110(1):130-137; discussion 138.
20. Zheng LW, Cheung LK, Ma L, and Wong MCM. High-rhythm automatic driver for bone traction: an experimental study in rabbits. Int J Oral Maxillofac Surg 2008;37(8):736-740.
21. Kessler P, Wiltfang J, and Neukam FW. A new distraction device to compare continuous and discontinuous bone distraction in mini-pigs: a preliminary report. J Craniomaxillofac Surg 2000;28(1):5-11.
22. Kessler PA, Merten HA, Neukam FW, and Wiltfang J. The effects of magnitude and frequency of distraction forces on tissue regeneration in distraction osteogenesis of the mandible. Plast Reconstr Surg 2002;109(1):171-180.
23. Ayoub AF, and Richardson W. A new device for microincremental automatic distraction osteogenesis. Br J Oral Maxillofac Surg 2001;39(5):353-355.
24. Ayoub AF, Richardson W, and Barbenel JC. Mandibular elongation by automatic distraction osteogenesis: The first application in humans. Br J Oral Maxillofac Surg 2005;43(4):324-328.
25. Ayoub AF, Richardson W, Koppel D, Thompson H, Lucas M, Schwarz T, Smith L, and Boyd J. Segmental mandibular reconstruction by microincremental automatic distraction osteogenesis: an animal study. Br J Oral Maxillofac Surg 2001;39(5):356-364.
26. Wiltfang J, Kessler P, Merten HA, and Neukam FW. Continuous and intermittent bone distraction using a microhydraulic cylinder: an experimental study in minipigs. Br J Oral Maxillofac Surg 2001;39(1):2-7.
27. Kessler P, Neukam FW, and Wiltfang J. Effects of distraction forces and frequency of distraction on bony regeneration. Br J Oral Maxillofac Surg 2005;43(5):392-398.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distraction osteogenesis
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Clipboard
Details
Show
20
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Send to
Summary
2509
Review: 218
1 - 20 of 2509
1: Simpson AL, Ma B, Slagel B, Borschneck DP, Ellis RE.
Computer-assisted distraction osteogenesis by Ilizarov's method.
Int J Med Robot. 2008 Oct 15. [Epub ahead of print]
PMID: 18924116 [PubMed - as supplied by publisher]
2: Shyam AK, Singh SU, Modi HN, Song HR, Lee SH, An H.
Leg lengthening by distraction osteogenesis using the Ilizarov apparatus: a novel concept of
influencing factors.
Int Orthop. 2008 Oct 16. [Epub ahead of print]
PMID: 18923832 [PubMed - as supplied by publisher]
3: Dinse WE, Burnett RR.
Anterior maxillary restoration using distraction osteogenesis and implants: a clinical report.
J Prosthet Dent. 2008 Oct;100(4):250-3.
PMID: 18922252 [PubMed - in process]
Tesiorowski M, Potaczek T.
intramedullary nail and external fixator]
177-83. Polish.

Chapter

2

Recommendations for optimal distraction protocols for various animal models on the basis of a systematic review

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Abstract

Background: The principles of orthopedic distraction osteogenesis (DO) have been successfully applied to the craniofacial skeleton. However, the amount of latency time, the rate and rhythm of distraction, and the length of the consolidation period that are optimal for long bone distraction, may be suboptimal for craniofacial DO.

Objectives: The purpose of the present study is to provide recommendations for optimal distraction parameters in animal experimental research on craniofacial DO.

Material and methods: These recommendations are based on data from papers, added to the PubMed database between 1 January 1973 and 1 January 2007, that had investigated the outcome of DO resulting from variations in a single distraction parameter while standardizing the other distraction parameters.

Results: Although experimental animal group sizes were rather small especially in those studies that used large animals, and both skeletally mature and immature animals had been used, the (in most cases quantitative) data provided useful information on the optimal parameters in craniofacial DO. In general, a latency period may not be necessary at all. Distraction should be performed at a rate of 1 mm/d (this may be twice as slow when small animals such as rats are used) preferably using a continuous rhythm, followed by a six to eight-week consolidation period.

Conclusion: These recommendations can be used as basic guidelines for further animal experimental studies on craniofacial DO.

Introduction

Snyder *et al*[1] successfully applied the principles of long bone distraction osteogenesis (DO) to the craniofacial skeleton of a dog in the early seventies. Afterwards, many more animal experimental and clinical papers on craniofacial DO were published. Swennen *et al*[2,3] produced two excellent reviews on craniofacial DO covering the period between 1966 and 2000, giving a useful overview of the available clinical and animal experimental literature in that specific period of time.

In most cases, distraction parameters such as the amount of latency time, the rate of distraction, the rhythm of distraction, and the length of the consolidation period were directly adopted from orthopaedic DO and applied to the craniofacial skeleton. However, these parameters may be suboptimal for craniofacial structures. The purpose of the present study is to review the animal experimental papers, added to the PubMed database between 1 January 1973 and 1 January 2007, that addressed the outcome of DO resulting from variations in a single distraction parameter while standardizing the other distraction parameters. On the basis of these papers, we attempt to provide recommendations for optimal distraction parameters in animal experimental research on craniofacial DO. In our opinion, by giving an overview on the current knowledge in this field of research and by extracting recommendations from the available literature this paper can be useful for any researcher interested in animal experimental work on craniofacial DO.

Materials and methods

In the present study, we were interested in animal experimental research on distraction osteogenesis in craniofacial bones. A PubMed search (National Library of Medicine, NCBI) was performed for the period between 1 January 1973 and 1 January 2007, using combinations of the search terms ‘distraction osteogenesis’, ‘craniofacial’, ‘mandible’, ‘maxilla’, ‘midface’, and ‘cranial’. Only studies written in English were included.

Of the English-written papers that had been added to the PubMed database between 1 January 1973 and 1 January 2007, abstracts and full papers were screened to include only the publications that determined the outcome of craniofacial DO resulting from variations in a single distraction parameter while standardizing the other distraction parameters. Papers that did not meet these criteria were eliminated. Also, studies that had used adjuvants (such as the administration of exogenous growth hormones) to artificially influence the DO parameters were excluded from our analysis, because we were only interested in the basic response of the distracted tissues to variations in distraction parameters.

Of each of the selected papers, we recorded author names, study purpose, animal model, animal growth status, number of animals, experimental region, osteotomy type, latency time, rate of distraction, rhythm of distraction, and consolidation time. Also, we determined whether the conclusions were based on qualitative or quantitative data. On the basis of these data we attempted to provide recommendations for the optimal distraction protocol for a given animal model, which may prove useful for researchers with an interest in animal experimental research on craniofacial DO.

Results

A total number of 292 publications matched our search performance. After screening of the abstracts and full papers, we found that 54 papers had investigated the outcome of craniofacial DO resulting from comparisons of either different latency times, rates of distraction, rhythms of distraction, or consolidation times. These 54 papers were analyzed in detail. Seven different animal models had been used, with a total number of 1857 animals.

Dog model

A total number of 161 dogs had been used in 14 studies (14/54; 26 %) (Table 1).[4-17] The animals were skeletally mature (indicated by the descriptions ‘adult’, ‘skeletally mature’: range 10-20 kg) in six cases[8-11,14,16], while in two cases[5,13] the animals were skeletally immature (‘sub-adult’: 6-9 months of age, 2600-3400 g). In the remaining six studies[4,6,7,12,15,17] only age or weight was given, without explicitly mentioning the skeletal growth status. Eleven cases involved the osteotomy of the mandible prior to distraction. Three cases mentioned corticotomy of the mandible.

Table 1. Optimal distraction parameters for the dog model.

Parameter	Parameter values tested	Parameter value giving best outcome	Supported by (semi)quantitative analysis?	Number of animals per (sub)group
Latency (days)	-	-	-	-
Rate (mm/d)	0.5, 1	0.5[19], 1[19]	-	1[19]
Rhythm (1/24 h)	1, 2	1[19], 2[19]	-	1[19]
Consolidation (days)	0, 1, 7, 14, 28, 30, 42, 56, 60, 70, 84, 90	30[17, 43], 42[10, 11, 19, 42, 48], 56[5, 11, 12, 35, 39, 48], 60[17, 43], 90[18]	yes[5, 10-12, 17, 18, 39, 42, 43, 48]	1[10, 12, 19], 2[5, 10, 12, 35, 39, 42], 4[10-12, 17, 18, 43, 48], 5[11]

Superscript numbers refer to the reference numbers in the bibliography.

Goat model

Goats had been used in four studies (4/54; 7 %) (Table 2).[18-21] All 36 animals were skeletally mature ('young adult', 'adult': range 18-24 months, 18-25 kg). Corticotomy of the mandible prior to distraction was performed in all cases.

Table 2. Optimal distraction parameters for the goat model.

Parameter	Parameter values tested	Parameter value giving best outcome	Supported by (semi)quantitative analysis?	Number of animals per (sub)group
Latency (days)	-	-	-	-
Rate (mm/d)	1, 2	1[20, 66]	yes[20, 66]	3[20], 4[66]
Rhythm (1/24 h)	-	-	-	-
Consolidation (days)	7, 14, 28, 56	28[54], 56[30]	yes[30, 54]	3[30, 54]

Superscript numbers refer to the reference numbers in the bibliography.

Pig model

The pig model had been used frequently (12/54; 22 %), with a total number of 221 animals (Table 3).[22-33] Animals were skeletally mature ('adult': no details on age or weight were given) in one case[27] and skeletally immature (described as 'skeletally immature': age 4-6 weeks, weight 20-25 kg, or as having 'mixed dentition': range 4-8 months, weight 25-30 kg) in 11 cases[22-26,28-33]. In all cases, the mandible was chosen as the experimental region. Osteotomy of the mandible was performed in six cases, while corticotomy was mentioned in the other six papers.

Table 3. Optimal distraction parameters for the pig model.

Parameter	Parameter values tested	Parameter value giving best outcome	Supported by (semi)quantitative analysis?	Number of animals per (sub)group
Latency (days)	0, 4	0[6, 16, 58]	yes[6, 16, 58]	2[6, 16, 58], 3[58]
Rate (mm/d)	1, 2, 4	1[6, 16, 23, 40, 56-58, 64, 65]	yes[6, 16, 23, 40, 56-58, 64, 65]	1[56, 57], 2[6, 16, 23, 40, 57, 58, 64, 65]
Rhythm (1/24 h)	1, continuous	continuous[25, 63]	yes[25, 63]	1[25, 63], 2[63]
Consolidation (days)	0, 8, 14, 16, 24-28, 56, 84, 90	24[23, 64, 65], 28[25], 24-28[16], 90[56, 57]	yes[16, 23, 25, 56, 57, 64, 65]	1[16, 25, 56, 57], 2[16, 23, 56, 57, 64, 65]

Superscript numbers refer to the reference numbers in the bibliography.

Rabbit model

A total number of 143 rabbits had been used in six studies (6/54; 11 %) (Table 4).[34-39] The animals were skeletally mature ('young adult', 'adult', 'skeletally mature': range 2.8-4 kg) in five cases[34-36,38,39]. In the remaining case[37] skeletal growth status was not mentioned explicitly but weight (2.75 kg) was given. In all cases the region of experiment was the mandible on which either corticotomy (four cases) or osteotomy (two cases) was performed.

Table 4. Optimal distraction parameters for the rabbit model.

Parameter	Parameter values tested	Parameter value giving best outcome	Supported by (semi)quantitative analysis?	Number of animals per (sub)group
Latency (days)	0, 2, 4, 5, 7, 10	5[1], 7[47]	yes[47]	4[1], 12[47]
Rate (mm/d)	0.9, 1, 2, 2.7, 3	0.9[8], 1[2, 51]	yes[8]	3[8], 6[2], 6-7[51]
Rhythm (1/24 h)	1, 2	1[2], 2[2]	-	6[2]
Consolidation (days)	0, 1, 7, 14, 28, 42, 56, 70	14[8], 56[27]	yes[8, 27]	3[8], 5[27]

Superscript numbers refer to the reference numbers in the bibliography.

Rat model

Eleven studies (11/54; 20 %) used the rat model (Table 5).[40-50] A total number of 1089 rats were used in these studies. Skeletally mature ('adult': 3 months of age, 250-350 g) rats were used in seven studies[40-42,45,47,50, 46], while skeletally immature ('early growth', 'growing': 4-6 weeks of age, weight 100-178.1 g) rats were used in two studies[47,49]. This included one study that used both growing and non-growing animals[47]. Skeletal growth status was not mentioned explicitly in three other papers[43,44,48], but age or weight was mentioned (3 months, 350-380 g). In all cases of distraction the mandible was osteotomized.

Rhesus monkey model

A total number of 39 rhesus monkeys were used in three studies (3/54; 6 %) (Table 6).[51-53] The monkeys were skeletally immature ('young': 4 years of age, weighing 5-7 kg) in one study[51]. In the remaining two studies[52,53], skeletally mature ('mature', 'adult': range 6-9 years, 6-8 kg) animals were used. Distraction followed osteotomy of the mandible (one case) or the midface (two cases).

Sheep model

In four studies (4/54; 7 %) the sheep model was used (Table 7).[54-57] In total, 58 sheep were involved. Skeletally mature ('adult': 3 years of age) sheep were used in one study[54]. Skeletally immature ('growing') animals were used in another study[55]. The remaining two studies[56,57] did not explicitly mention skeletal growth status, but gave only age and weight (12 months old, weighing 45 kg). Distraction was performed on the mandibles in all cases, following osteotomy (three cases) or corticotomy (one case).

Table 5. Optimal distraction parameters for the rat model.

Parameter	Parameter values tested	Parameter value giving best outcome	Supported by (semi)quantitative analysis?	Number of animals per (sub)group
Latency (days)	0, 3, 5, 7	5[38]	yes[38]	6[38]
Rate (mm/d)	0.2, 0.25, 0.4, 0.5, 0.6, 1	0.2[26, 31, 32, 37, 45, 62], 0.4[26, 31, 32, 45, 62], 0.5[38], 0.6[26, 45, 62]	yes[26, 31, 32, 37, 38, 45, 62]	6[38], 7-8[37], 8[45], 8-9[26, 31, 32, 62]
Rhythm (1/24 h)	1, 2, 4	2[38]	yes[38]	6[38]
Consolidation (days)	0, 2, 3, 6, 10, 14, 16, 17, 24, 28, 30, 31, 38, 42, 56	16[26], 17[37], 24[32, 45, 62], 28[34, 61], 30[26], 31[37], 38[32], 42[41, 44]	yes[26, 32, 34, 37, 41, 44, 45, 61, 62]	5[41], 6[34], 7-8[37], 8[44, 45], 8-9[26, 32, 62], 13[61]

Superscript numbers refer to the reference numbers in the bibliography.

Table 6. Optimal distraction parameters for the rhesus monkey model.

Parameter	Parameter values tested	Parameter value giving best outcome	Supported by (semi)quantitative analysis?	Number of animals per (sub)group
Latency (days)	-	-	-	-
Rate (mm/d)	-	-	-	-
Rhythm (1/24 h)	-	-	-	-
Consolidation (days)	0, 14, 28, 30, 42, 60, 84, 90, 180	84[60], 90[7, 9]	yes[7, 9, 60]	1[7, 9], 2[7, 9, 60], 3[7, 9], 4[9, 60]

Superscript numbers refer to the reference numbers in the bibliography. Studies were performed on the mandible[60] and the midface[7, 9].

Table 7. Optimal distraction parameters for the sheep model.

Parameter	Parameter values tested	Parameter value giving best outcome	Supported by (semi)quantitative analysis?	Number of animals per (sub)group
Latency (days)	0, 4, 7	0[55]	yes[55]	6[55]
Rate (mm/d)	1, 2, 3, 4	1[13, 59]	yes[13, 59]	2[59], 6[13]
Rhythm (1/24 h)	-	-	-	-
Consolidation (days)	30, 60, 90, 180	90[15]	yes[15]	2[15]

Superscript numbers refer to the reference numbers in the bibliography.

Discussion

The intramembranous bones of the skull have a different vascular supply compared to long bones. Therefore, DO parameters suitable for orthopedic DO might be suboptimal for craniofacial DO. The purpose of the present study is to provide recommendations for optimal distraction parameters in animal experimental research on craniofacial DO. These recommendations are based on data from papers that had investigated the outcome of DO resulting from variations in a single distraction parameter while standardizing the other distraction parameters. On the basis of the animal experimental literature, added to the PubMed database between 1 January 1973 and 1 January 2007, we found the following parameter values to result in the best outcome for craniofacial DO:

(1) Dog model: a distraction rate of 0.5-1 mm/d, achieved by one or two daily activations, followed by 30-90 days of consolidation. No data on latency were found.
(2) Goat model: a distraction rate of 1 mm/d, followed by 28-56 days of consolidation. No data on latency and rhythm of distraction were found.
(3) Pig model: a 0-day latency period, a distraction rate of 1 mm/d, achieved by a continuous rhythm of distraction, followed by a 24-90 day consolidation period.
(4) Rabbit model: a 5-7 day latency period, a distraction rate of 0.9-1 mm/d, a rhythm of distraction of 1 or 2 activations a day, followed by a 14-56 day consolidation period.
(5) Rat model: a 5-day latency period, a distraction rate of 0.2-0.6 mm/d, achieved by two daily activations, followed by 16-42 day consolidation period.
(6) Rhesus monkey: a consolidation period of 84-90 days. No data was found on latency, rate of distraction and rhythm of distraction.
(7) Sheep model: a 0-day latency period, a distraction rate of 1 mm/d, followed by a 90-day consolidation period. No data was found for rhythm of distraction.

The choice of an appropriate animal model depends on the level of hypothesis testing, as mentioned by Siegel & Mooney[58]. Seven different animal models had been used in the studies that we reviewed, ranging from small animals such as rats to larger animals such as pigs. Their primitive bone and soft tissue responses are considered similar to human bone and soft tissue responses.[58] Primates, which are phylogenetically closest related to humans, had also been subjected to craniofacial DO studies, but a phylogenetically close relationship is not always required for optimal extrapolation of animal experimental research to the human situation. Much more important are the similarity of anatomy, physiology, and mechanical properties of bone in a given animal model. Rhesus monkeys are appropriate animal models for maxillary DO because of their suitable maxillary anatomy and similarity to clinical applications in humans.[52] However, a pig mandible can be as appropriate as a primate mandible. Rabbits represent the best compromise between size of the mandible and cost.[59] Moreover, rabbits show patterns of bone accretion and peak bone mass profiles similar to those of humans, as well as true skeletal maturity.[60] Larger animals have the advantage of larger craniofacial structures, which allow the use of the same distraction devices used for human patients or large experimental distraction devices. In addition, anatomy and physiology are highly similar to the human situation in certain cases, while some of these models can be relatively inexpensive and easy to handle.[30,61] Advantages of a dog model include a sufficiently large maxillary sinus, a splanchnocranium comparable to the human, and a typical hypoplastic maxilla comparable to human class III conditions with reversed overjet and in cleft lip and palate patients.[61] Sheep are mainly selected as animal model for their size, which also allows successive biopsies.[62,63] Nevertheless, small animals such as mice and rats prove very useful for studies on molecular biology[41], for large numbers of animals for statistical power are possible.

Skeletal growth status is an important factor to take into consideration when studying the regeneration rate of distracted bone. Swennen *et al*[3] suggested to distinguish between skeletally mature and skeletally immature animals. Growing (skeletally immature) animals may have higher bone regeneration and remodeling potential than non-growing (skeletally mature) animals have. However, aging may not always have an effect on DO in certain cases, as has been shown in a rat tibial DO model.[64] In the present paper, all studies on craniofacial DO have been screened for specification of the skeletal growth status. Twelve of the 54 papers gave no explicit statement concerning skeletal (im)maturity. Instead, only age or body weight was given. Although standardized weight charts exist, these parameters can be prone to erroneous judgments in relation to skeletal growth status. The best way to confirm skeletal (im)maturity is by radiographic assessment of the growth plates.

Animal experimental research often involves skeletally mature animals to elucidate the fundamental biological processes behind DO. Interestingly, in clinical use, DO is often applied in a pediatric population. It is possible that optimal distraction protocols for skeletally mature subjects are suboptimal for skeletally immature subjects. Therefore, skeletally immature animal models should be used more often, to able to compare the bone regeneration process during DO in growing versus non-growing subjects. For example, a higher rate of distraction could be applied in growing subjects because of the higher bone regeneration potential. This may result in shorter treatment times.

Usually, a latency period is part of a distraction protocol. During this period between the osteotomy and the start of distraction a reparative callus is formed. Blood supply is restored and cellular proliferation takes place. In long bone distraction, latency periods may take up to a week or more. However, pig and sheep studies have shown that a latency period may not be necessary at all in craniofacial DO. In fact, 0-day latency protocols resulted in mandibles of equal quality when compared to 4-day latency protocols.[22,23,55] Also, masseter muscles adapted well to mandibular elongation in a 0-day latency protocol.[24] When surrounding soft tissues are able to adapt well to distraction, relapse of the distracted bone is less likely to occur, improving the long-term success rate of DO.

Interestingly, a 0-day latency period had also been tested in a rat model, but the outcome was inferior to that of a 5-day latency period, which appeared to be the optimal latency period for the rat model.[40] Similarly, 5-7 days of latency were considered optimal in rabbit models, suggesting that latency is necessary in order to allow healing of the soft tissues and that bone mineralizes at a higher rate when a latency period is used.[34,37] Why the latency period can be eliminated in one animal model, whereas it seems to be necessary in another, is not yet fully understood. The bones of the skull have a richer blood supply than long bones have, so it is likely that latency periods can be shorter for craniofacial DO than for orthopedic DO. Whether it can be completely eliminated without significantly compromising bone quality and quantity is a question that may need more investigation. Elimination of the latency period would reduce the total treatment time, though.

More consensus can be found concerning the optimal rate of distraction. In a canine tibial DO model Gavriil Ilizarov, who performed ground-breaking work in DO research, had already reported that a rate of 1 mm/d led to more favorable results than either a slower rate (resulting in premature consolidation) or faster rate (resulting in retarded osteogenesis and detrimental changes in surrounding soft tissues).[65] In the animal experimental papers that we studied the optimal rate of distraction also appears to be 1 mm/d for all animal models, with exception of the rat model that requires a slower rate of distraction (0.2-0.6 mm/d). This does not seem strange, considering the far smaller size of rats compared to the other animal models. Despite its small size, the rat appears to be very useful because large numbers of experimental animals are possible. In addition, they are relatively cheap and easy to house in experimental animal facilities. Large numbers of experimental animals also allow for detailed statistical analyses, which is more difficult to apply on the small group sizes when larger animal models are used. For example, no qualitative and quantitative differences in bone formation were found in a canine mandibular distraction study using rates of 0.5 and 1 mm/d, but the authors acknowledged that this may had been the result of the limited number of animals in each experimental group.[14]

Ilizarov's report on canine tibial DO also mentioned that at a given rate of distraction a greater frequency of distraction provided a better outcome.[65] In craniofacial DO research this was supported by some reports, but not confirmed by others. For example, no difference between one or two daily activations (at a rate of 1 mm/d) was found in the rabbit model, suggesting that a single activation a day would be more convenient to the patient and clinician.[38] However, small subgroup sizes were used in that study, and only qualitative data were presented. Similarly, a dog model showed no difference between a protocol with either one or two daily activations.[14] These data were not supported by statistical analysis, though. Any possible significant difference or trend towards significant difference may have been left unnoticed. The limited number of animals in each group and the relatively small variation in the distraction protocol were mentioned as reasons why no relation between rhythm of distraction and the quality and quantity of newly formed bone in a canine mandibular DO model was found.[14] However, the direct influence of the rhythm of distraction on bone regeneration has been illustrated by comparisons between discontinuous and continuous rhythms of distraction.[26,27] Although these pig studies used small group sizes, the results clearly showed that bone regeneration was accelerated by continuous distraction compared to bone regeneration by discontinuous distraction (one activation a day).[26,27] Also, considerably lower distraction forces were required for continuous distraction.[26,27] Increasing the rhythm of distraction creates a more constant tension by which tissues become more metabolically activated. This principle is called the Law of Tension-Stress.[66] Thus, a high rhythm of distraction could reduce the consolidation time needed to sufficiently mineralize the regenerated bone before distractor removal.

The most difficult parameter to interpret from the data is the optimal amount of consolidation time prior to removal of the distractor and full loading of the regenerate. Many studies have determined the quality and quantity of newly formed bone after various consolidation periods and were therefore very useful. All data indicated that bone quantity and quality increased after longer consolidation times. On the basis of the analyzed studies, consolidation periods ranging from two weeks to three months have shown the best results. In none of the cases the regenerated bone had obtained the quality or quantity that normal bone has. Remodeling towards normal bone characteristics may take up to a year or more. Concerning shortening DO treatment by reducing consolidation time, the difficulty is to keep the consolidation period just long enough for the regenerate to sufficiently mineralize in order for it to tolerate full mechanical loading. Only three papers explicitly provide suggestions for the optimal consolidation time before removal of the distractor.[8,16,18] In a goat model for mandibular DO, after eight weeks of consolidation the distractor can be removed and the mandible should be exposed to adaptive functional exercise.[18] A dog model for mandibular DO showed that at six weeks of consolidation the decision can be made to remove the distraction device.[8,16]

Accurate and reliable techniques to determine adequacy of bone fill when making the decision to remove a distractor have to be further investigated.[30] Suggestions have been made to combine data obtained from plain radiography, ultrasonography, ultrasonometry, and biomechanical testing to make a rational decision to remove distraction devices in a timely manner.[30] Also, dual emission x-ray absorptiometry (DXA) could be applied as a predictor to determine the most appropriate time for distractor removal.[56]

In conclusion, on the basis of the animal experimental papers that we analyzed, we attempted to formulate recommendations for craniofacial DO protocols for animal experimental research. Although experimental animal group sizes had been rather small especially in those studies that used large animals, and both skeletally mature and immature animals had been used, the (in most cases quantitative) data provided useful information on the optimal parameters in craniofacial DO for a given animal model. In general, rat and rabbit studies showed that a latency period of five to seven days is sufficient to allow initial tissue healing before starting active distraction, although pig and sheep studies showed that a latency period is not necessary at all. More research is needed to determine whether such a 0-day latency protocol can also be successfully applied in other animal models. Quality and quantity of newly formed bone increased when distraction is performed continuously at a rate of 1 mm/d, although for smaller animals the rate may be half as high. A consolidation period of six to eight weeks should be enough for the regenerate to sufficiently mineralize before removal of the distractor, but techniques which allow reliable assessment of the stiffness of the regenerate should be further investigated in order to truly optimize the timing of distractor removal. These recommendations can be used as basic guidelines for further animal experimental studies on craniofacial DO.

References

1. Snyder CC, Levine GA, Swanson HM, and Browne EZ, Jr. Mandibular lengthening by gradual distraction. Preliminary report. Plast Reconstr Surg 1973;51(5):506-508.
2. Swennen G, Schliephake H, Dempf R, Schierle H, and Malevez C. Craniofacial distraction osteogenesis: a review of the literature: Part 1: clinical studies. Int J Oral Maxillofac Surg 2001;30(2):89-103.
3. Swennen G, Dempf R, and Schliephake H. Cranio-facial distraction osteogenesis: a review of the literature. Part II: Experimental studies. Int J Oral Maxillofac Surg 2002;31(2):123-135.
4. Cano J, Campo J, Gonzalo JC, and Bascones A. Consolidation period in alveolar distraction: a pilot histomorphometric study in the mandible of the beagle dog. Int J Oral Maxillofac Implants 2006;21(3):380-391.
5. Makarov MR, Harper RP, Cope JB, and Samchukov ML. Evaluation of inferior alveolar nerve function during distraction osteogenesis in the dog. J Oral Maxillofac Surg 1998;56(12):1417-1423; discussion 1424-1415.
6. Fisher E, Staffenberg DA, McCarthy JG, Miller DC, and Zeng J. Histopathologic and biochemical changes in the muscles affected by distraction osteogenesis of the mandible. Plast Reconstr Surg 1997;99(2):366-371.

7. Miao J, Li G, Zhao P, Chen G, Teng L, and Ling Y. 2/3 osteotomy for lengthening the mandible in dogs by gradual distraction. J Craniomaxillofac Surg 1997;25(6):301-304.
8. Smith SW, Sachdeva RC, and Cope JB. Evaluation of the consolidation period during osteodistraction using computed tomography. Am J Orthod Dentofac Orthop 1999;116(3):254-263.
9. Cope JB, and Samchukov ML. Regenerate bone formation and remodeling during mandibular osteodistraction. Angle Orthod 2000;70(2):99-111.
10. Cope JB, and Samchukov ML. Mineralization dynamics of regenerate bone during mandibular osteodistraction. Int J Oral Maxillofac Surg 2001;30(3):234-242.
11. Cope JB, Samchukov ML, and Muirhead DE. Distraction osteogenesis and histogenesis in beagle dogs: the effect of gradual mandibular osteodistraction on bone and gingiva. J Periodontol 2002;73(3):271-282.
12. Gosain AK, Kalantarian B, Song LS, Larson JD, Jenkins CA, and Wilson CR. Comparison of canine mandibular bone regeneration by distraction osteogenesis versus acute resection and rigid external fixation. Plast Reconstr Surg 2004;114(6):1490-1499; discussion 1500-1491.
13. Gomez DF, Sant'Anna EF, Leven RM, Ostric SA, Figueroa AA, Royston TJ, Sumner DR, and Polley JW. Microstructural and strength evaluation of regenerate tissue during the consolidation period after vertical mandibular ramus distraction. J Craniofac Surg 2005;16(5):805-811.
14. Hasse AR, Porksen M, and Zimmermann CE. Bilateral mandibular distraction in adult dogs with an epiperiosteal distractor. Br J Oral Maxillofac Surg 2005;43(2):105-112.
15. Park B-W, Kim J-R, Lee J-H, and Byun J-H. Expression of nerve growth factor and vascular endothelial growth factor in the inferior alveolar nerve after distraction osteogenesis. Int J Oral Maxillofac Surg 2006;35(7):624-630.
16. Ryoyama D, Sawaki Y, and Ueda M. Experimental study of mechanical analysis in mandibular lengthening. Application of strain gauge measurement. Int J Oral Maxillofac Surg 2004;33(3):294-300.
17. Sant'Anna EF, Gomez DF, Sumner DR, Williams JM, Figueroa AA, Ostric SA, Theodoru S, and Polley JW. Micro-computed tomography evaluation of the

glenoid fossa and mandibular condyle bone after bilateral vertical ramus mandibular distraction in a canine model. J Craniofac Surg 2006;17(3):611-619.

18. Li J, Hu J, Wang D, Tang Z, and Gao Z. Biomechanical properties of regenerated bone by mandibular distraction osteogenesis. Chin J Traumatol 2002;5(2):67-70.
19. Hu J, Tang Z, Wang D, and Buckley MJ. Changes in the inferior alveolar nerve after mandibular lengthening with different rates of distraction. J Oral Maxillofac Surg 2001;59(9):1041-1045; discussion 1046.
20. Tang ZL, Hu J, Li JH, and Zou SJ. Effect of nerve growth factor and Schwann cells on axon regeneration of distracted inferior alveolar nerve following mandibular lengthening. Chin J Traumatol 2004;7(2):81-86.
21. Zou S, Hu J, Wang D, Li J, and Tang Z. Changes in the temporomandibular joint after mandibular lengthening with different rates of distraction. Int J Adult Orthodon Orthognath Surg 2001;16(3):221-225.
22. Glowacki J, Shusterman EM, Troulis M, Holmes R, Perrott D, and Kaban LB. Distraction osteogenesis of the porcine mandible: histomorphometric evaluation of bone. Plast Reconstr Surg 2004;113(2):566-573.
23. Troulis MJ, Glowacki J, Perrott DH, and Kaban LB. Effects of latency and rate on bone formation in a porcine mandibular distraction model. J Oral Maxillofac Surg 2000;58(5):507-513; discussion 514.
24. Castano FJ, Troulis MJ, Glowacki J, Kaban LB, and Yates KE. Proliferation of masseter myocytes after distraction osteogenesis of the porcine mandible. J Oral Maxillofac Surg 2001;59(3):302-307.
25. Kessler P, Neukam FW, and Wiltfang J. Effects of distraction forces and frequency of distraction on bony regeneration. Br J Oral Maxillofac Surg 2005;43(5):392-398.
26. Kessler PA, Merten HA, Neukam FW, and Wiltfang J. The effects of magnitude and frequency of distraction forces on tissue regeneration in distraction osteogenesis of the mandible. Plast Reconstr Surg 2002;109(1):171-180.

27. Wiltfang J, Kessler P, Merten HA, and Neukam FW. Continuous and intermittent bone distraction using a microhydraulic cylinder: an experimental study in minipigs. Br J Oral Maxillofac Surg 2001;39(1):2-7.
28. Thurmuller P, Troulis MJ, Rosenberg A, and Kaban LB. Changes in the condyle and disc in response to distraction osteogenesis of the minipig mandible. J Oral Maxillofac Surg 2002;60(11):1327-1333.
29. Perrott DH, Rahn B, Wahl D, Linke B, Thurmuller P, Troulis M, Glowacki J, and Kaban LB. Development of a mechanical testing system for a mandibular distraction wound. Int J Oral Maxillofac Surg 2003;32(5):523-527.
30. Kaban LB, Thurmuller P, Troulis MJ, Glowacki J, Wahl D, Linke B, Rahn B, and Perrott DH. Correlation of biomechanical stiffness with plain radiographic and ultrasound data in an experimental mandibular distraction wound. Int J Oral Maxillofac Surg 2003;32(3):296-304.
31. Zimmermann CE, Harris G, Thurmuller P, Troulis MJ, Perrott DH, Rahn B, and Kaban LB. Assessment of bone formation in a porcine mandibular distraction wound by computed tomography. Int J Oral Maxillofac Surg 2004;33(6):569-574.
32. Zimmermann CE, Thurmuller P, Troulis MJ, Perrott DH, Rahn B, and Kaban LB. Histology of the porcine mandibular distraction wound. Int J Oral Maxillofac Surg 2005;34(4):411-419.
33. Thurmuller P, Troulis MJ, Rosenberg A, Chuang S-K, and Kaban LB. Microscopic Changes in the Condyle and Disc in Response to Distraction Osteogenesis of the Minipig Mandible. J Oral Maxillofac Surg 2006;64(2):249-258.
34. Aida T, Yoshioka I, Tominaga K, and Fukuda J. Effects of latency period in a rabbit mandibular distraction osteogenesis. Int J Oral Maxillofac Surg 2003;32(1):54-62; discussion 63.
35. Komuro Y, Takato T, Harii K, and Yonemara Y. The histologic analysis of distraction osteogenesis of the mandible in rabbits. Plast Reconstr Surg 1994;94(1):152-159.
36. Stewart KJ, Lvoff GO, White SA, Bonar SF, Walsh WR, Smart RC, and Poole MD. Mandibular distraction osteogenesis: a comparison of distraction rates in the rabbit model. J Craniomaxillofac Surg 1998;26(1):43-49.

37. Singare S, Li D, Liu Y, Wu Z, and Wang J. The effect of latency on bone lengthening force and bone mineralization: an investigation using strain gauge mounted on internal distractor device. Biomed Eng Online 2006;518.
38. Al Ruhaimi KA. Comparison of different distraction rates in the mandible: an experimental investigation. Int J Oral Maxillofac Surg 2001;30(3):220-227.
39. Cheung LK, Zheng LW, and Ma L. Effect of distraction rates on expression of bone morphogenetic proteins in rabbit mandibular distraction osteogenesis. J Craniomaxillofac Surg 2006;34(5):263-269.
40. Paccione MF, Mehrara BJ, Warren SM, Greenwald JA, Spector JA, Luchs JS, and Longaker MT. Rat mandibular distraction osteogenesis: latency, rate, and rhythm determine the adaptive response. J Craniofac Surg 2001;12(2):175-182.
41. Rowe NM, Mehrara BJ, Dudziak ME, Steinbreck DS, Mackool RJ, Gittes GK, McCarthy JG, and Longaker MT. Rat mandibular distraction osteogenesis: Part I. Histologic and radiographic analysis. Plast Reconstr Surg 1998;102(6):2022-2032.
42. Mehrara BJ, Rowe NM, Steinbrech DS, Dudziak ME, Saadeh PB, McCarthy JG, Gittes GK, and Longaker MT. Rat mandibular distraction osteogenesis: II. Molecular analysis of transforming growth factor beta-1 and osteocalcin gene expression. Plast Reconstr Surg 1999;103(2):536-547.
43. Liu ZJ, King GJ, and Herring SW. Alterations of morphology and microdensity in the condyle after mandibular osteodistraction in the rat. J Oral Maxillofac Surg 2003;61(8):918-927.
44. King GJ, Liu ZJ, Wang LL, Chiu IY, Whelan MF, and Huang GJ. Effect of distraction rate and consolidation period on bone density following mandibular osteodistraction in rats. Arch Oral Biol 2003;48(4):299-308.
45. Williams BE, King GJ, Liu ZJ, and Rafferty KL. Sequential histomorphometric analysis of regenerate osteogenesis following mandibular distraction in the rat. Arch Oral Biol 2005;50(5):497-506.
46. Liu ZJ, Anderson MW, Gu GM, and King GJ. Apoptosis in the regenerate produced by mandibular osteodistraction in the mature rat. Orthod Craniofac Res 2005;8(1):41-51.

47. Shin JY, Liu ZJ, and King GJ. Trabecular organization in mandibular osteodistraction in growing and maturing rats. J Oral Maxillofac Surg 2005;63(1):77-86.
48. Okafuji N, Liu Z-J, and King GJ. Assessment of cell proliferation during mandibular distraction osteogenesis in the maturing rat. Am J Orthod Dentofac Orthop 2006;130(5):612-621.
49. Shibazaki R, Maki K, Tachikawa T, Shibasaki Y, Hinton RJ, Carlson DS, and Opperman LA. Changes in parathyroid hormone-related protein and 3-dimensional trabecular bone structure of the mandibular condyle following mandibular distraction osteogenesis in growing rats. J Oral Maxillofac Surg 2005;63(4):505-512.
50. Warren SM, Mehrara BJ, Steinbrech DS, Paccione MF, Greenwald JA, Spector JA, and Longaker MT. Rat mandibular distraction osteogenesis: part III. Gradual distraction versus acute lengthening. Plast Reconstr Surg 2001;107(2):441-453.
51. Wang XX, Wang X, and Li ZL. Effects of mandibular distraction osteogenesis on the inferior alveolar nerve: an experimental study in monkeys. Plast Reconstr Surg 2002;109(7):2373-2383.
52. Cheung LK, Zhang Q, Zhang ZG, and Wong MC. Reconstruction of maxillectomy defect by transport distraction osteogenesis. Int J Oral Maxillofac Surg 2003;32(5):515-522.
53. Cheung LK, and Zhang Q. Radiologic characterization of new bone generated from distraction after maxillary bone transport. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2003;96(2):234-242.
54. Gaggl A, Schultes G, Regauer S, and Karcher H. Healing process after alveolar ridge distraction in sheep. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2000;90(4):420-429.
55. Tavakoli K, Walsh WR, Bonar F, Smart R, Wulf S, and Poole MD. The role of latency in mandibular osteodistraction. J Craniomaxillofac Surg 1998;26(4):209-219.
56. Farhadieh RD, Gianoutsos MP, Dickinson R, and Walsh WR. Effect of distraction rate on biomechanical, mineralization, and histologic properties of an ovine mandible model. Plast Reconstr Surg 2000;105(3):889-895.

57. van der Meulen JH, Borschel GH, Lynch JB, Nicklin S, Ho KC, Gianoutsos MP, Walsh WR, and Kuzon WM, Jr. The effect of rate of distraction osteogenesis on structure and function of anterior digastric muscle fibers. Plast Reconstr Surg 2005;115(3):831-837.
58. Siegel MI, and Mooney MP. Appropriate animal models for craniofacial biology. Cleft Palate J 1990;27(1):18-25.
59. Al-Sebaei MO, Gagari E, and Papageorge M. Mandibular distraction osteogenesis: a rabbit model using a novel experimental design. J Oral Maxillofac Surg 2005;63(5):664-672.
60. Norris SA, Pettifor JM, Gray DA, and Buffenstein R. Calcium metabolism and bone mass in female rabbits during skeletal maturation: effects of dietary calcium intake. Bone 2001;29(1):62-69.
61. Stalmans K, Van Erum R, Verdonck A, Nadjmi N, Schepers E, Schoenaers J, and Carels C. Cephalometric evaluation of maxillary advancement with an internal distractor in an adult boxer dog. Orthod Craniofac Res 2003;6(2):104-111.
62. Lewinson D, Maor G, Rozen N, Rabinovich I, Stahl S, and Rachmiel A. Expression of vascular antigens by bone cells during bone regeneration in a membranous bone distraction system. Histochem Cell Biol 2001;116(5):381-388.
63. Lewinson D, Rachmiel A, Rihani-Bisharat S, Kraiem Z, Schenzer P, Korem S, and Rabinovich Y. Stimulation of Fos- and Jun-related genes during distraction osteogenesis. J Histochem Cytochem 2003;51(9):1161-1168.
64. Aronson J, Gao GG, Shen XC, McLaren SG, Skinner RA, Badger TM, and Lumpkin CK, Jr. The effect of aging on distraction osteogenesis in the rat. J Orthop Res 2001;19(3):421-427.
65. Ilizarov GA. The tension-stress effect on the genesis and growth of tissues: Part II. The influence of the rate and frequency of distraction. Clin Orthop Relat Res 1989;(239):263-285.
66. Ilizarov GA. The tension-stress effect on the genesis and growth of tissues. Part I. The influence of stability of fixation and soft-tissue preservation. Clin Orthop Relat Res 1989;(238):249-281.

Chapter

3

Rabbits as a model for research into craniofacial distraction osteogenesis

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Abstract

Background: Various factors play a role in determining the appropriate animal model for craniofacial research.

Objectives: In this paper, the rabbit is evaluated as a suitable animal model for biological research on craniofacial distraction osteogenesis. Our experiences with housing and handling, surgical and experimental protocols as well as comparison with other animal models as known in the literature are discussed. Furthermore, in this paper, we introduce and describe (the use of) a continuous hydraulic distractor on the nasal bones of the rabbit.

Material and methods: Fifty-two skeletally mature New Zealand White rabbits were used. Forty-two of the fifty-two operations were uneventful. Complications occurred in ten of the fifty-two cases, of which two were actually animal-related, while the other eight were distractor-related. During the experiments animals maintained a good health. The distraction procedures were well tolerated by the rabbits.

Results: The rabbit model is excellent for use in biological research on craniofacial DO. Specifically, the nasal bones are easily surgically accessible, size and shape of the nasal bones allow various commercially available as well as custom-made distractors to be easily attached to the bones, animal care and housing are relatively simple and inexpensive, and tissue harvesting for further analyses is no problem due to the manageable size and shape of the rabbit skull compared with smaller and larger laboratory animals.

Conclusion: Therefore, we recommend the rabbit model for biological research on craniofacial distraction osteogenesis.

Introduction

In 1905, Codivilla introduced a method for elongating lower limbs that were shortened due to deformity.[1] Five decades later, this technique, now known as distraction osteogenesis (DO), was rediscovered by Ilizarov, whose extensive fundamental and clinical research led to significant improvements in orthopedic bone lengthening. His principles of bone lengthening are still used in today's orthopedic surgery.[2-4]

Not only orthopedic surgeons recognized the potential of DO for the correction of bone deformities; in 1972, Snyder *et al* were the first to successfully apply the principles of DO in the mandible of a dog.[5] Two decades later, McCarthy *et al* were the first to report on the lengthening of the human mandible by means of DO.[6] Nowadays, DO is widely accepted as a method for correcting bone deformities in oral and maxillofacial surgery.

Although fundamental research in craniofacial DO has been conducted since the 1970s, experimental work remains necessary to improve the outcome of craniofacial DO treatment. Animal experimental research on the biomolecular processes underlying DO, refinement of the distraction protocol, improvement of distraction devices and diagnostic methods for evaluating the newly formed bone is required for better understanding and optimizing craniofacial DO.

The choice for an animal model depends on various factors.[7] While animal models may resemble the mechanical and physiological human situation, they provide only an approximation, with each animal model having unique advantages and disadvantages.[8] Reviews of the animal experimental literature show that a large diversity of animal models has been used for fundamental research on craniofacial DO.[9,10] These animal models included rats, rabbits, cats, dogs, goats, sheep, pigs, and monkeys.

This paper focuses on the rabbit as appropriate animal model for biological research on craniofacial distraction osteogenesis, in particular distraction of the nasal bones. We hope to aid researchers who are interested in conducting animal experimental research on this topic and are looking for a suitable animal model. From our point of view, the rabbit model balances cost and utility very well. Our experiences with housing and handling, surgical and experimental protocols as well as comparison with other animal models as known in the literature are discussed.

The focus of this paper is to give attention to the animal model itself; the data of the experimental work will be presented in a separate paper.

Furthermore, we introduce a hydraulic continuous distraction device, which was tested on the nasal bones of the rabbit. Continuous distraction has been studied in previous studies. The continuous rhythm of distraction would result in better and accelerated bone formation. Relatively very little studies have compared the conventional discontinuous distraction with continuous distraction. Kessler *et al*[11] and Wiltfang *et al*[12] investigated this topic in pigs, however the numbers of pigs were low. Pigs are relatively large experimental animals, making them expensive. In our studies, we have not only compared discontinuous distraction with continuous distraction rhythms, but we also did this in large numbers of animals per group, enabling us to do statistical analyses on the data.

Lastly, because of the small but still manageable size, and the convenient size and shape of its nasal bones, the rabbit is suggested as most appropriate animal model for biological research on craniofacial distraction osteogenesis and for testing new types of distraction devices.

Materials and methods

Animal model and housing

Fifty-two skeletally mature New Zealand White rabbits (*Oryctolagus cuniculus*) (Harlan Netherlands BV, Horst, the Netherlands) with a mean weight (SD) of 2766 g (307 g) at the start of the experiments were used. Upon arrival at the Erasmus MC animal house facility, the rabbits were allowed to acclimatize to diet (Teklad 2030/2930, Harlan Netherlands BV, Horst, the Netherlands) and housing for at least a week. Their general appearance, food uptake and activity were monitored throughout the experiments. Dependent on the requirements of the experiment, the rabbits were housed in large plastic boxes (which are open at the top) either individually (approximately 1 m^3 of space per rabbit) or in groups of three (approximately 2 m^3 of space per three rabbits). Food and water were given *ad libitum*. Box floors were covered with bedding and cage enrichment. All animals were housed in a temperature-controlled room with a 12-hour light/12-hour dark schedule.

Preanaesthetic and anesthetic agents and procedures

Preoperatively, the animals were anaesthetized with an intramuscular (IM) injection (0.5 ml/kg) of medetomidine hydrochloride (Domitor 1 mg/ml, Pfizer Animal Health BV, Capelle aan den IJssel, the Netherlands). The nasal region was shaved and cleaned with 70 % ethanol. Subsequently, general anesthesia was maintained using a mixture of oxygen and halogenated ether (1-3 % Isoflurane; Pharmachemie BV, Haarlem, the Netherlands), which was administered through a facemask.

Surgical procedure

During surgery, animals were placed on heating pads (Barkey Autocontrol, Adquipment Medical, Hellevoetsluis, the Netherlands) at approximately 38 ºC to prevent hypothermia. They were operated on in prone position under sterile conditions. After a longitudinal incision of approximately two centimeters down the median line, the skin and periosteum were reflected to expose the nasal bones (Fig. 1).

Three types of distraction devices have been used in our craniofacial DO studies (Fig. 2). The Type-1 distractor (TRACK 1.0, KLS Martin, Tuttlingen, Germany) is a manually-activated distractor, which was used in 33 of the 52 cases. The Type-2 distractor is an adapted Leone spring-activated distractor (Orthocom BV, Odijk, the Netherlands), which was used in three of the 52 cases. The Type-3 distractor is a custom-made hydraulic distractor, which was used in 15 of the 52 cases. In one case, no distractor was applied due to death of the rabbit before commencement of the surgery.

The distractor was positioned on the exposed nasal bones. When the correct orientation of the vector of distraction was confirmed, holes for the distractor fixation screws were drilled under copious irrigation with saline solution. These drill holes later also served as markers to determine the area of distraction. After temporary removal of the distractor, a complete osteotomy perpendicular to the internasal suture was performed with a reciprocating saw, approximately one centimeter rostrally to the frontonasal suture. After the distractor had been reapplied, it was slightly activated to ensure that the osteotomy was complete and the bone segment was mobile. Subsequently, the distractor was returned to its initial closed position. The skin and periosteum were repositioned and sutured. Directly after surgery, the animals were given a subdermal injection of 0.05 ml/kg buprenorphine hydrochloride (Temgesic 0.3 mg/ml, Schering-Plough BV, Utrecht, the Netherlands) for pain management. No additional pain management was needed afterwards.

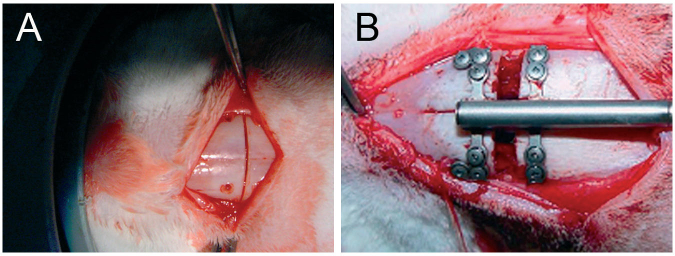

Figure 1

(A) After exposure of the nasal bones the holes for the distractor were drilled and a complete osteotomy was performed, creating a mobile bone segment. (B) After placement of the distractor, mobility of the bone segment was tested to confirm completeness of the osteotomy.

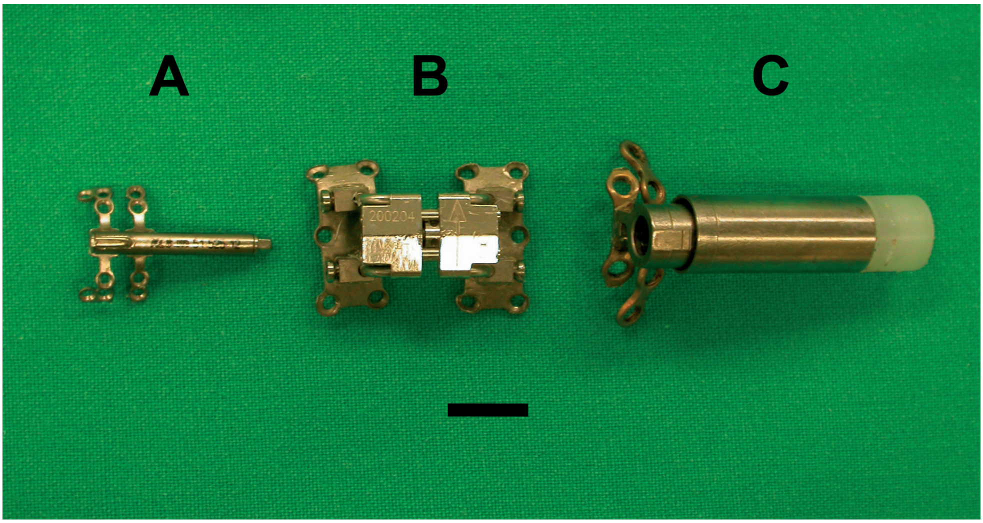

Figure 2

Three types of distraction devices were used. (A) Type-1: a manually activated distractor. (B) Type-2: a spring-activated distractor. (C) Type-3: a custom-made hydraulic distractor. The length of the scale bar represents 1 cm.

Distraction protocol and post-experimental procedures

All rabbits were subjected to three days of latency, eleven days of distraction and twenty-one days of consolidation. At the end of the consolidation phase, the rabbits were sedated (0.5 ml/kg IM, 1 mg/ml Domitor, Pfizer Animal Health BV, Capelle aan den IJssel, the Netherlands) and euthanized by intravenous injection (0.5 ml/kg) of sodium pentobarbitone (Euthesate 200 mg/ml, CEVA Sante Animale BV, Naaldwijk, the Netherlands). By using a band saw the regenerated bone tissue was easily harvested for further preparation and analyses. Bone quantity and quality were determined *post-mortem* by plain radiography, ultrasonography, microcomputed tomography and histology. The results of these analyses are discussed in more detail in another paper[13], as the aim of the present paper is to describe our rabbit model.

The experimental protocol was approved by the Animal Experiments Committee under the national Experiments on Animals Act and adhered to the rules laid down in this national law that serves the implementation of "Guidelines on the protection of experimental animals" by the Council of Europe (1986), Directive 86/609/EC.

Results

Forty-two of the fifty-two (80.8 %) rabbits underwent the surgical and experimental protocols without complications. All types of distractors fitted well on the nasal bones of the rabbits; screws and fixations plates were stable in all cases. Animals maintained a good health during the experiments. The distraction procedures were well tolerated by the rabbits. During activation of the distractors the rabbits were calm and easy to handle, and no sedation or pain medication was needed before or after device activation. There were no wound infections that compromised the animals' health. The rabbits displayed normal activity and food uptake. Generally, there was no considerable loss of body weight at the end of experiment compared with the body weights at the start of the experiments (Fig. 3). Neither individual housing nor group housing gave any problems.

Complications occurred in ten (19.2 %) of the fifty-two cases of which only two were actually animal-related (3.8 %), while the other eight were distractor-related (15.4 %). The animal-related complications included the following events: (a) One animal (1.9 %) died after administration of the preanaestheticum (rabbit EUN2). The actual surgery had not yet commenced at that moment. (b) One rabbit (EVL5; 1.9 %) had an epileptic seizure during surgery. After the seizure had been brought under control, the surgery was successfully continued. The distractor-related complications included the following events: (c) In one case, the skin of the rabbit (EXU8; 1.9 %) had moved completely over the distractor. Therefore the distractor (Type 1) could not be activated. An extra surgery was needed to expose the distractor again, in order to activate it. (d) The distractor (Type 1) was obstructed in two cases (3.8 %) at two and three days before the end of the distraction period (rabbits ESQ5 and ETF9, respectively). The obstruction was such that further activation of the distractor was impossible. (e) In five cases (9.6 %), device failure was seen using two Type-2 distractors (rabbits FOD9 and FNY9) and three Type-3 distractors (rabbits FZN2, GMB2, and GNW9). No distraction was achieved in these cases; examination of the distraction area and the distractors revealed that the obstructions were all due to technical failure of the distractor, rather than premature fusion of the distracted bone segments. Although the nasal bones are relatively thin (approximately 2-3 mm of cortical bone), distractor stability was good in all cases; therefore the success rate was 100 %.

Discussion

Many animal models have been used in craniofacial DO research, including rats, rabbits, cats, dogs, goats, sheep, pigs, and monkeys. Limiting the diversity of animal models used in craniofacial DO research would facilitate the comparisons between reports that have used the same animal model. Data pools will become more robust, as more data for one specific animal model on a specific research subject will become available. For example, complete information on (optimal) distraction parameters can be found for rats, rabbits and pigs, while for other animal models this information is still incomplete.[9] It may be wise to focus on rats, rabbits, or pigs when conducting craniofacial DO research. Based on our experiences, we would like to promote the rabbit model for biological research on craniofacial DO. In many cases, the rabbit has multiple advantages over other animal models.

The selection of an animal model not only needs to take into account ethical implications, availability of the animals, costs, and maintenance and handling issues,[14] but also whether the focus is mechanical or biological.[15] Larger, but more expensive animals may be more suitable when biomechanics is of interest, because they more closely approximate the size and bone anatomy of the human. However, they offer little advantage for investigating biological questions. In addition, tissue harvesting and processing may also be more difficult due to the larger size. Smaller animals may be more useful in studying biological questions, and by virtue of their limited costs, easy handling, and faster healing time will provide a larger data pool from which to base results.[15]

While advantageous for housing, the small size of generic animals such as mice and rats, may give difficulties with finding or custom-making a suitable distraction device. Also, long-term studies which require several biopsies, or large blood samples, are impossible in such a small animal.[16] Dogs and pigs may be larger, but the dog's status as pet can contribute to emotional difficulties, while pigs are expensive, and can be loud and aggressive, making housing and handling more difficult.[16]

Rabbits are excellent alternatives, because they are the smallest laboratory animals still large enough to allow for the use of commercially available distractors, which eliminates the need for spending time and budget on designing custom-built distraction devices. Furthermore, housing and handling are relatively easy compared with the larger laboratory animals. The rabbit model represents the best compromise between cost and size.[17] Moreover, rabbits show patterns of bone accretion and peak bone mass profiles similar to those of humans, as well as true skeletal maturity.[18] Costs for animal caretaking are relatively low compared with pigs; besides the difference in purchase price (dependent on *e.g.* age, weight, sex) rates for housing rabbits are approximately five times lower than for pigs. This may be an important factor when long-term studies are involved.

When basic biological processes of craniofacial distraction osteogenesis need to be studied, the rabbit nasal bones have shown to be suitable experimental sites.[19-21] The nasal bones are easily surgically accessible, while shape and size allow for the use of commercially available distractors as well as custom-made experimental distractors. Also, in contrast to the mandible there are no large muscle groups attached to the nasal bones. Therefore the vector of distraction is not influenced and relapse is not likely to occur. Tissue harvesting and processing can be done without difficulties due to the manageable size.

Obviously, it is unlikely that a single animal model can act as the ideal animal model for research in craniofacial distraction osteogenesis, with each animal model having its distinctive advantages and disadvantages. In this respect, rabbits are considered to be one of the most difficult and unpredictable species of laboratory animals to anaesthetize, because of the sensitivity of the respiratory centre to the paralyzing action of anesthetics, the extremely narrow range between anesthetic and lethal doses and the large inter-rabbit variability to the depressant action of conventional anaesthetics.[22]

Despite these potentially complicating factors, we did not encounter considerable problems with anesthesia by means of a facemask. The animal's state of anesthesia was constantly monitored by checking the rate and depth of respiration as well as the paw reflex. Endotracheal intubation could have been used instead of a facemask. However, we chose not to intubate the rabbits, because this is more complex than applying a facemask, which would result in longer operating time. Also, there is a higher risk of damaging the respiratory tract when intubating. Inhalation anesthesia via a facemask is less complicated and less invasive.

Mortality and morbidity rates were low. One rabbit died, before the start of the actual surgery. Another rabbit had an epileptic seizure during surgery. These were the only animal-related complications (3.8 % of the total number of rabbits). During distraction, the rabbits were very cooperative and distractors were easily accessible. In contrast to mandibular or maxillary distraction, distraction of the nasal bones does not alter the morphology of the jawbones. Therefore, the rabbits were able to eat and drink as they were used to, and maintained or gained weight, which normally indicates a good general health. In addition, when using a relatively small distraction device like the Type-1 distractor, female rabbits can safely be housed in a group.

The experimental results of the use of the continuous distractor are presented in a separate paper. Continuous distractors are of special clinical interest, for the continuous rhythm of distraction enhances and accelerates bone formation in the distraction area.[3,12] This means increased regenerate quality in a shorter time span compared with the conventional distractors, which can only deliver discontinuous distraction rhythms (typically 1-4 activations a day). Consolidation time will be reduced, significantly shortening the total treatment time for the patient. This decreases the physical and psychosocial burden for the patient, increasing the patient's quality of life during the treatment time. Continuous distraction also has the advantage of automatic activation of the distractor, so the patient or caretaker is relieved from the task of manually activating the distractor every day. This also means that incorrect use is prevented. Continuous distraction provides many advantages over discontinuous distraction, however, more research is needed to develop clinically usable continuous distractors, which are small and light enough to be carried around for a long period of time. A sophisticated feedback mechanism that allows the distractor to cover a constant pre-set distance under any circumstances (varying soft tissue resistance for example) is also required.

In conclusion, by virtue of its good balance between cost and utility, we recommend the rabbit model as it has shown to be excellent for use in biological research on craniofacial DO. Specifically, the nasal bones are easily surgically accessible, size and shape of the nasal bones allow various commercially available as well as custom-made distractors to be easily attached to the bones, animal care and housing are relatively simple and inexpensive, and tissue harvesting for further analyses is no problem due to the manageable size and shape of the rabbit skull.

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References

1. Codivilla A. On the means of lengthening, in the lower limbs, the muscles and tissues which are shortened through deformity. Am J Orthop Surg 1905;(2):353-357.
2. Ilizarov GA. The principles of the Ilizarov method. Bull Hosp Joint Dis Orthop Inst 1988;48(1):1-11.
3. Ilizarov GA. The tension-stress effect on the genesis and growth of tissues: Part II. The influence of the rate and frequency of distraction. Clin Orthop Relat Res 1989;(239):263-285.
4. Ilizarov GA. The tension-stress effect on the genesis and growth of tissues. Part I. The influence of stability of fixation and soft-tissue preservation. Clin Orthop Relat Res 1989;(238):249-281.
5. Snyder CC, Levine GA, Swanson HM, and Browne EZ, Jr. Mandibular lengthening by gradual distraction. Preliminary report. Plast Reconstr Surg 1973;51(5):506-508.
6. McCarthy JG, Schreiber J, Karp N, Thorne CH, and Grayson BH. Lengthening the human mandible by gradual distraction. Plast Reconstr Surg 1992;89(1):1-8; discussion 9-10.
7. Siegel MI, and Mooney MP. Appropriate animal models for craniofacial biology. Cleft Palate J 1990;27(1):18-25.
8. Pearce AI, Richards RG, Milz S, Schneider E, and Pearce SG. Animal models for implant biomaterial research in bone: a review. Eur Cell Mater 2007;13:1-10.
9. Djasim UM, Wolvius EB, van Neck JW, Weinans H, and van der Wal KGH. Recommendations for optimal distraction protocols for various animal models on the basis of a systematic review of the literature. Int J Oral Maxillofac Surg 2007;36(10):877-883.
10. Swennen G, Dempf R, and Schliephake H. Cranio-facial distraction osteogenesis: a review of the literature. Part II: Experimental studies. Int J Oral Maxillofac Surg 2002;31(2):123-135.
11. Kessler PA, Merten HA, Neukam FW, and Wiltfang J. The effects of magnitude and frequency of distraction forces on tissue regeneration in

distraction osteogenesis of the mandible. Plast Reconstr Surg 2002;109(1):171-180.

12. Wiltfang J, Kessler P, Merten HA, and Neukam FW. Continuous and intermittent bone distraction using a microhydraulic cylinder: an experimental study in minipigs. Br J Oral Maxillofac Surg 2001;39(1):2-7.
13. Djasim UM, Wolvius EB, Van Neck JW, Van Wamel A, Weinans H, and Van Der Wal KGH. Single versus triple daily activation of the distractor: No significant effects of frequency of distraction on bone regenerate quantity and architecture. J Craniomaxillofac Surg 2008;36(3):143-151.
14. Egermann M, Goldhahn J, and Schneider E. Animal models for fracture treatment in osteoporosis. Osteoporos Int 2005;16 Suppl 2:S129-138.
15. Liebschner MA. Biomechanical considerations of animal models used in tissue engineering of bone. Biomaterials 2004;25(9):1697-1714.
16. Newman E, Turner AS, and Wark JD. The potential of sheep for the study of osteopenia: current status and comparison with other animal models. Bone 1995;16(4 Suppl):277S-284S.
17. Al-Sebaei MO, Gagari E, and Papageorge M. Mandibular distraction osteogenesis: a rabbit model using a novel experimental design. J Oral Maxillofac Surg 2005;63(5):664-672.
18. Norris SA, Pettifor JM, Gray DA, and Buffenstein R. Calcium metabolism and bone mass in female rabbits during skeletal maturation: effects of dietary calcium intake. Bone 2001;29(1):62-69.
19. Movassaghi K, Altobelli DE, and Zhou H. Frontonasal suture expansion in the rabbit using titanium screws. J Oral Maxillofac Surg 1995;53(9):1033-1042.
20. Parr JA, Garetto LP, Wohlford ME, Arbuckle GR, and Roberts WE. Implant-borne suture expansion in rabbits: a histomorphometric study of the supporting bone. J Biomed Mater Res 1999;45(1):1-10.
21. Li M, Park SG, Kang DI, Eppley BL, Sadove AM, and Coleman JJ, 3rd. Introduction of a novel internal spring-driven craniofacial bone distraction device. J Craniofac Surg 2004;15(2):324-328.
22. Bivin WS, and Timmons EH Basic Biomethodology. In*:* S. H. Weisbroth, R. E. Flatt, and A. L. Kraus (eds.), The Biology of the Laboratory Rabbit, pp. 73-90. New York: Academic Press, Inc.; 1974.

Chapter

4

Single versus triple daily activation of the distractor: No significant effects of frequency of distraction on bone regenerate quantity and architecture

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Abstract

Background: The frequency of distraction may influence the outcome of distraction osteogenesis (DO), but little studies have focused on the effect of this distraction parameter on bone regenerate quality in craniofacial DO.

Objectives: To study the effect of two different frequencies of distraction on the quantity and architecture of bone regenerate using micro-computed tomography, and to determine whether radiographic and ultrasonographic bone-fill scores provide reliable predictive value for the amount of new bone in the distraction area.

Material and methods: Twenty-six skeletally mature rabbits underwent three full days of latency, after which midface distraction was started. Low-frequency group (n=12): a distraction rate of 0.9 mm/d achieved by one daily activation for eleven days to create a 10 mm distraction gap. High-frequency group (n=12): *idem*, but three daily activations were used instead of one. Control group (n=2) underwent no distraction. After twenty-one days of consolidation, bone-fill in the distraction area was assessed by means of ultrasonography and radiography. Micro-computed tomography was used to quantify new bone formation and bone architecture.

Results: Relative bone volume (BV/TV) showed a tendency towards a difference ($P = 0.09$) between the low and high-frequency groups. No significant differences were found for bone architecture. No significant correlation between BV/TV values and bone-fill scores was found.

Conclusions: An increase in rhythm from one to three activations daily does not create significantly more bone. Bone-fill score values provided no reliable predictive value for the amount of new bone formation.

Introduction

Distraction osteogenesis (DO) is the process of *de novo* bone formation by means of gradual traction of the soft callus between separated bone segments. When subjected to distraction, bone tissue becomes highly metabolically activated as long as tension is maintained, resulting in osteogenesis[1]. In addition, histogenesis (*i.e.* the adaptation of surrounding soft tissues to the distraction forces) is stimulated[1], minimizing the risk of relapse. Over recent years, craniofacial DO has gained wide acceptance as the treatment of choice for correcting both acquired and congenital craniofacial deformities. However, DO treatment may take several months, which can be an uncomfortably long period for the patient.

Optimizing distraction parameters such as the length of the latency period, the rate of distraction, the frequency of distraction, and the amount of consolidation time may significantly shorten the total treatment time for craniofacial DO. Although the frequency of distraction may influence the outcome of DO, relatively few animal experimental studies have focused on the effect of this parameter on bone regenerate quality while standardizing the other parameters. More fundamental research is therefore needed on the effect of the frequency of distraction on bone regenerate quality in craniofacial DO.

The present study aimed to assess the effect of two different frequencies of distraction on the quantity and bone architecture of the bone regenerate in a rabbit midfacial distraction model. Micro-computed tomography was used to collect quantitative data. We also determined whether radiographic and ultrasonographic bone-fill scores provided reliable predictive value for the amount of new bone formation in the distraction area.

Material and methods

Animals and surgical procedure

Rabbits were chosen as the animal model, because of the relatively low costs and simple handling compared with the larger animal models as well as the favorable size compared with the smaller animal models. Also, bone characteristics of the rabbit are known from the literature[2,3]. We used twenty-six skeletally mature female New Zealand White rabbits (*Oryctolagus cuniculus*) (Harlan Netherlands BV, Horst, the Netherlands), with a mean body weight (± SD) of 2754 g (± 268 g) on the day of operation. The experimental protocol was approved by the Animal Experiments Committee under the national Experiments on Animals Act and adhered to the rules laid down in this national law that serves the implementation of "Guidelines on the protection of experimental animals" by the Council of Europe (1986), Directive 86/609/EC.

After arrival at the Erasmus MC animal house facility, the animals were allowed to acclimatize to housing and diet for at least a week. Throughout the experiments they were monitored for general appearance, activity, and food uptake. They were fed and maintained in large boxes in groups of three. Box floors were covered with bedding and some cage enrichment. Preoperative and postoperative care was overseen by the animal facility's veterinarians and staff. The animals were weighed just before operation and just before sacrifice.

Preoperatively, the animals were anaesthetized with an intramuscular injection of medetomidine hydrochloride (Domitor, Pfizer Animal Health BV, Capelle aan den IJssel, the Netherlands). The frontonasal region was shaved and cleaned with 70 % ethanol. Subsequently, general anesthesia was maintained using 1-3 % Isoflurane (Pharmachemie BV, Haarlem, the Netherlands) in a 2:1 oxygen/nitrous oxide mixture administered through a facemask.

During surgery, animals were placed on heating pads at approximately 38 ºC to prevent hypothermia. They were operated on in a prone position under sterile conditions. After a longitudinal median incision of about 1.5 cm, the nasal bone was exposed by reflecting the overlying skin and periosteum. The nasal bone was chosen because it is large enough to fit distractors that are commercially available for clinical use, it is easily accessible, and there are no associated muscle groups which could interfere with the desired vector of distraction. The distractor (TRACK 1.0, Gebrüder Martin GmbH & Co. KG, Tuttlingen, Germany) was positioned on the exposed nasal bone. When the correct orientation of the vector of distraction was confirmed, holes were drilled for the distractor fixation screws under copious saline irrigation. A complete osteotomy perpendicular to the internasal suture was performed with a reciprocating saw, approximately 1 cm rostrally from the frontonasal suture. After the distractor had been stably fixed to the nasal bone using eight 4-mm screws (four each on the rostral and caudal side of the osteotomy), the distractor was slightly activated to ensure that the osteotomy was complete (Fig. 1). Subsequently, the distractor was turned to its initial closed position. The skin and periosteum were repositioned and sutured with Vicryl 5-0 (Ethicon Inc., Somerville, USA), allowing only the activation rod of the distractor to protrude through the skin.

Post-operatively, the animals were given buprenorphine hydrochloride (Temgesic, Schering-Plough BV, Utrecht, the Netherlands) for pain management. Food and water were given *ad libitum*. All animals were housed in a temperature-controlled room with a 12-hour light/12-hour dark schedule.

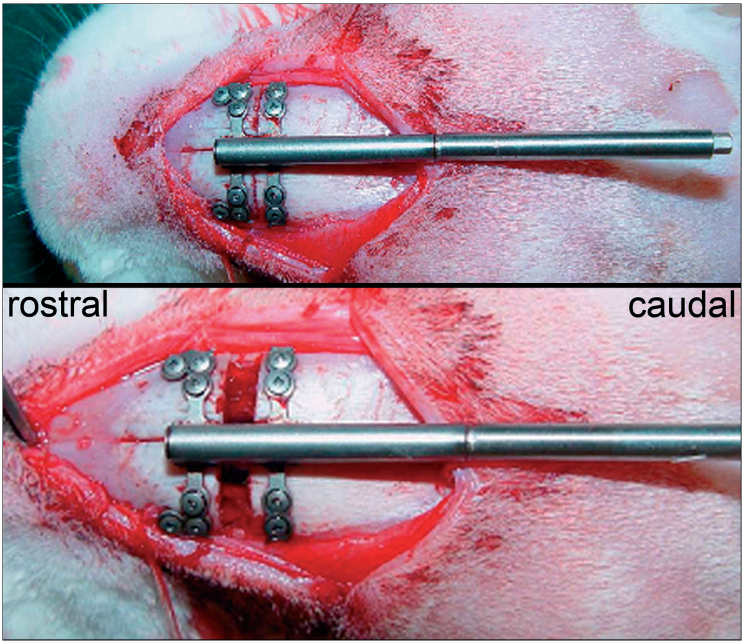

Figure 1
Dorsal view of the osteotomy site and the distractor *in situ* before and after activation.

Distraction protocol

The animals were randomly assigned to two experimental groups and one control group. All rabbits were allowed three full days of latency, after which distraction was started at a rate of 0.9 mm/day. The low-frequency group (n=12) underwent distraction at a rate of 0.9 mm/d achieved by a single daily activation (0.9 mm early in the morning) for 11 days to create a 10 mm distraction area. The high-frequency group (n=12) underwent distraction at a rate of 0.9 mm/d achieved by three daily activations (0.3 mm early in the morning, at noon, and late in the afternoon) for 11 days to create a 10 mm distraction area. The control-group animals (n=2) were subjected to the same surgical procedures; a distractor was applied as well, but these rabbits did not undergo active distraction. Unaffected parts of the nasal bones of these animals served as control samples. Following the distraction period, all rabbits had a 21-day consolidation period to allow mineralization of the regenerate. All animals were sacrificed after completion of the consolidation period by intravenous injection of sodium pentobarbitone (Euthesate, CEVA Sante Animale BV, Naaldwijk, the Netherlands).

Radiographic evaluation

Immediately after sacrifice, lateral radiographic images were taken of the distraction area with the distractor still attached. The images were taken with a General Electric Mobile 225 X-ray device (General Electric, Milwaukee, Wisconsin, USA), set at 40 kV, 0.80 s, and 100 mA.

Bone-fill scores were determined from the radiographs using a semiquantitative 4-point scale[4-6], with a score of “0” indicating “no bone fill”; a score of “1” indicating “bone fill, but less than 50 %”; a score of “2” indicating “more than 50 % bone fill, but less than 100 %”; and a score of “3” indicating “100 % bone fill”.

Ultrasonographic evaluation

Additionally, *post mortem* ultrasound images of the distraction area were acquired from twelve animals using a ProSound SSD-4000 ultrasound console (Aloka Holding Europe AG, Zug, Switzerland) to compare with the radiographic images. A 13 MHz linear array transducer was used to make B-mode images of the nasal bone.

Bone formation was assessed by determining the ultrasound reflection strength of the bone using bone-fill scores. A semiquantitative 4-point scale[4-6] was applied to the ultrasound images, with a score of “0” indicating “complete through-transmission of ultrasound waves, clear gap margins, and no echogenic material”; a score of “1” indicating “partial through-transmission of the ultrasound waves, identifiable gap margins, and less than 50 % echogenic material"; a score of "2" indicating "partial through-transmission of the ultrasound waves, partially obscured gap margins, and more than 50 % echogenic material"; and a score of "3" indicating "no through-transmission of the ultrasound waves, invisible gap margins, and 100 % echogenic material".

Micro-computed tomographic (micro-CT) analysis

After radiographic and ultrasonographic images had been taken, the distractor was removed and the distance between the anterior and posterior screw plates was measured. By subtracting 5 mm from this value (which is the distance between both screw plates in the closed condition), the corrected total distraction area was calculated. The distraction area, including a portion of the adjacent original bones, was resected using a band saw. The tissue blocks were stored in Burkhardt's fixative. *Post mortem* micro-computed tomographic scans of the tissue blocks were made with a SkyScan 1076 *in vivo* micro-CT scanner (SkyScan, Aartselaar, Belgium) and manufacturer's scanning software. Examination consisted of a scout view, selection of the distraction area, offline reconstruction and evaluation. Serial transverse scan images were made at a resolution of 18 µm. Reconstruction of the scans was made using Nrecon 1.3 software (SkyScan, Aartselaar, Belgium).

Reconstruction data were processed using CT Analyser 1.3.2.2 and CTvol 1.6 software (SkyScan, Aartselaar, Belgium) for 2D and 3D analysis. Interpolation between 2D-free-hand selections of the nasal bone area was used to create a 3D volume of interest (VOI) (Fig. 2). Selection was such that only the regenerate tissue of the distracted nasal bone between the caudal and rostral screw holes was included.

Within the VOI, the relative bone volume (BV/TV) was determined to quantify new bone formation. Relative bone volume (BV/TV) is the volume of mineralized bone (BV) in relation to the total tissue volume (TV) in the VOI, and can be used to allow comparisons of samples of variable sizes. To determine differences in bone formation within a regenerate, distraction areas were divided into three equal parts; a caudal, central, and rostral region. Each region itself was again equally subdivided (Fig. 3). To assess the architecture of the regenerate, we also determined bone-surface to bone-volume ratio (BS/BV), trabecular number (Tb.N), trabecular thickness (Tb.Th), and trabecular separation (Tb.Sp).

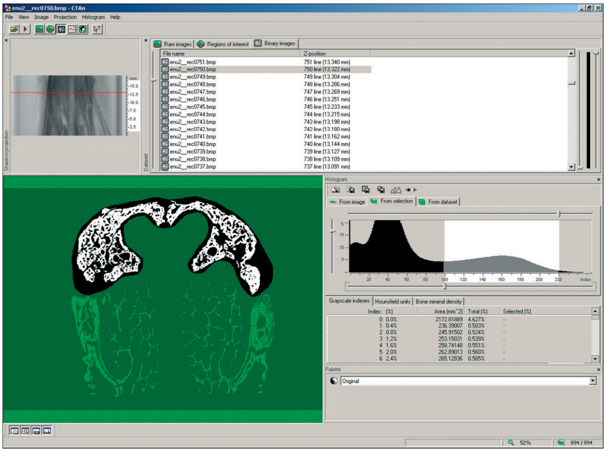

Figure 2

Transverse 2D image of the distraction area. A 3D volume of interest was created by interpolation of multiple 2D regions of interest. A 2D region was outlined by free-hand selection and included the area in which nasal bone regenerate was seen or expected.

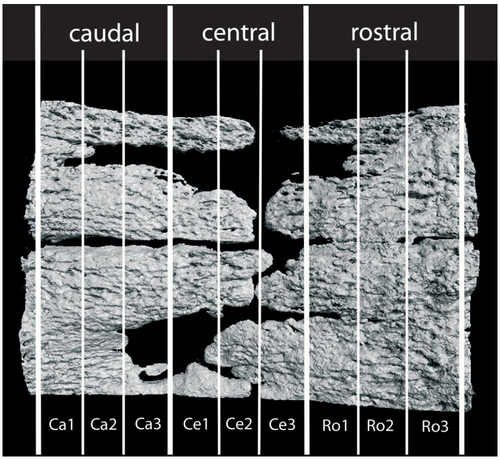

Figure 3

Graphic representation (dorsal view) of the division of the distraction area into a ‘Caudal’, ‘Central’, and ‘Rostral’ region. Each part itself is similarly subdivided into three parts. For each part, the relative bone volumes were assessed.

Statistics

Statistical analyses were conducted using SPSS 12.0 for Windows (SPSS Inc., Chicago, USA). For comparison of the parametric values between the low and high-frequency groups, the Mann-Whitney U test was used. Spearman's rank correlation coefficients were established to determine correlations between micro-CT parameters. The level of significance was fixed at $P < 0.05$.

Results

General

All animals tolerated the distraction procedures well. No infections or screw loosening occurred. None of the animals lost weight during the experiment, which illustrates normal feeding and drinking activity throughout the distraction protocol, suggesting that the animals did not suffer too much from the distraction procedures. In all groups there was a significant increase in body weight ($P < 0.01$) throughout the distraction protocol. Mean weight (± SD) of the low-frequency group was 2767 g (± 281 g) at the start of the experiment and 3292 g (± 219 g) at the end. The high-frequency group weighed 2700 g (± 250 g) at the start of the experiment and 3325 g (± 238 g) at the end. Between both groups, there was no significant difference in mean body weight increase.

Jamming of the distractor occurred in two animals after eight and nine days of distraction. Despite all efforts to keep the operation site as clean as possible during surgery, small blood clots or wound debris must have lodged inside the distractor body, which was confirmed by post-experimental examination; removal of the small blood clots or debris solved the jamming.

In another animal, activation of the distractor on the planned first day of distraction was made impossible, due to the skin that had completely covered the activation rod of the distractor despite our precautions. The activation rod had to be surgically exposed again, but the distractor could not be activated until the fifth postoperative day rather than the fourth. As a result, this rabbit had a slightly longer latency time, but it was still within the generally accepted latency time for rabbits (which can be up to seven days). Because rabbits have relatively loose skin, the activation rod should protrude through the skin sufficiently to prevent the skin from completely covering the distractor.

Fourteen of the twenty-four animals showed a distraction distance of approximately 10 mm (*i.e.*, ranging from 9 to 11 mm), while seven animals showed a smaller distraction distance (range 4 – 8 mm; three rabbits in the low-frequency group, four rabbits in the high-frequency group). Larger distraction distances (12 or 13 mm) were seen in three animals (two rabbits in the low-frequency group, one rabbit in the high-frequency group). Mean distraction distance was virtually the same for both groups.

Bone-fill score

Mean (± SD) radiographic bone-fill score was 1.9 (± 1.0) for the low-frequency group and 2.1 (± 1.1) for the high-frequency group. For both groups, radiographic evidence of bone formation was most common in the caudal region of the distraction areas, while bone formation was less common in the central region. There was no significant difference in mean radiographic bone-fill scores between the low and high-frequency groups.

Mean (± SD) ultrasonographic bone fill score was 1.8 (± 0.4) for the low-frequency group and 2.0 (no SD) for the high-frequency group. In most cases, through-transmission of ultrasound beams could be observed in the central region of the distraction area. Through-transmission appears when there is no bone to reflect the ultrasound beam. There was no significant difference between mean ultrasonographic bone-fill scores of both groups.

The individual bone-fill scores were plotted against their corresponding BV/TV values (Fig. 4). The scattergram did not reveal a significant relationship between BV/TV values and bone-fill scores in the experimental groups.

Micro-CT parameters

The highest BV/TV value (26.8 %) was found in the high-frequency group. Surprisingly, the high-frequency group also hosted the animal with the lowest BV/TV value (9.7 %). After twenty-one days of consolidation, BV/TV values of the experimental groups have not reached the BV/TV values seen in the control group.

Most bone formation at that time point had taken place in the caudal and rostral parts of the distraction areas (Fig. 5).

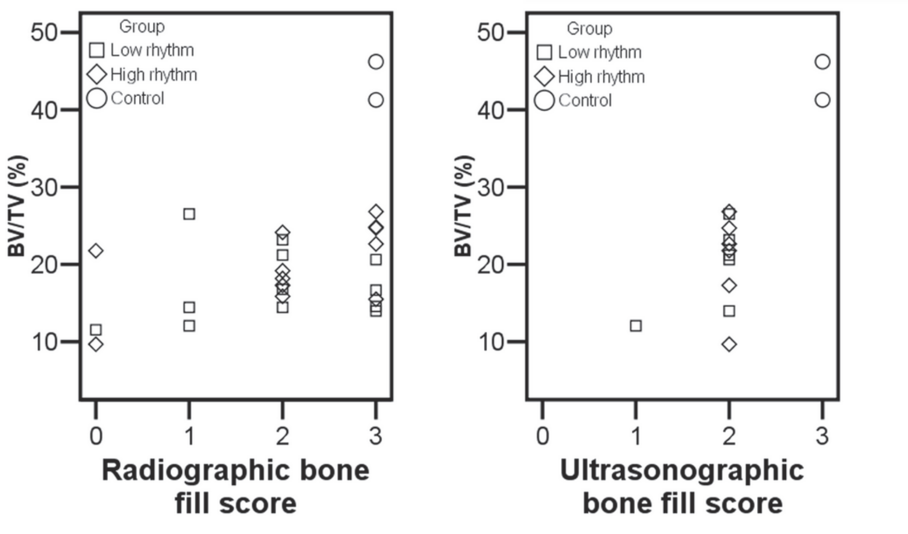

Figure 4

Scattergrams of radiographic bone-fill scores (n=26) and ultrasonographic bone-fill scores (n=14) plotted against their corresponding BV/TV values. BV/TV = mineralized bone volume/total tissue volume. No significant correlation between either radiographic bone-fill scores or ultrasonographic bone-fill scores and relative bone volumes was observed in the experimental groups.

Statistical analysis of micro-CT data did not reveal significant differences between the experimental groups concerning BV/TV (*P* = 0.09), BS/BV (*P* = 0.20), Tb.N (*P* = 0.39), Tb.Th (*P* = 0.25), or Tb.Sp (*P* = 0.33) (Fig. 6).

Data from the present study showed a significant positive correlation between BV/TV and BS/BV values in the low-frequency group ($r = 0.664$; $P < 0.05$) A positive correlation close to significance was also found between BV/TV and Tb.N in the low-frequency group ($r = 0.566$; $P = 0.055$). Furthermore, Tb.Th was negatively correlated with BV/TV for the low-frequency group ($r = -0.566$; $P = 0.055$). The low-frequency group also showed significant correlations between BS/BV and Tb.Th ($r = -0.951$; $P < 0.01$), and Tb.Sp and Tb.N ($r = -0.958$; $P < 0.01$). Tb.Sp and Tb.N showed significant correlation in the high-frequency group ($r = -0.951$; $P < 0.01$). No correlations were found between either radiographic bone-fill scores and BV/TV, or ultrasonographic bone-fill scores and BV/TV.

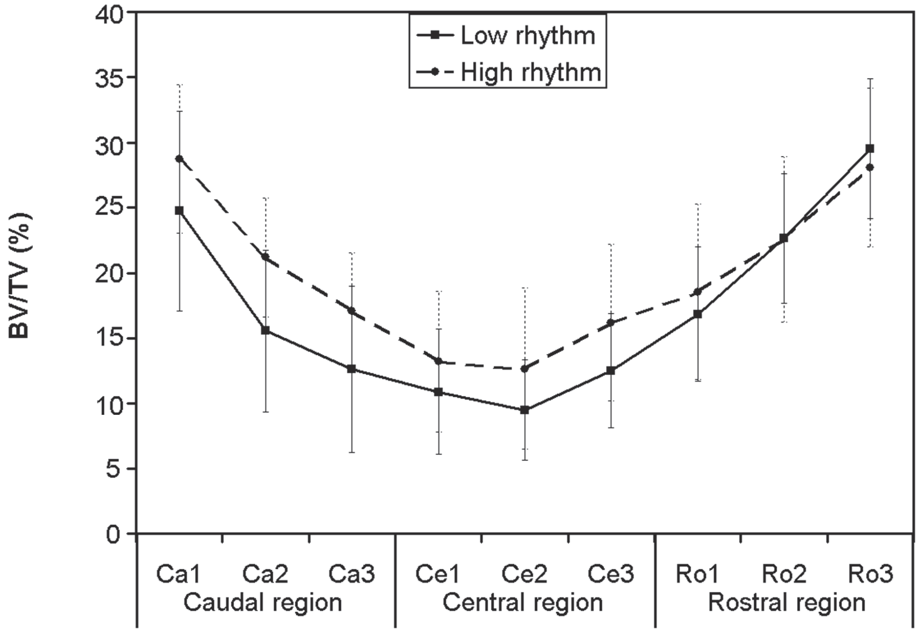

Figure 5

Mean BV/TV values in each subdivision of the distraction areas for the low-frequency group (solid line) and the high-frequency group (dashed line). Whiskers represent the standard deviations. After twenty-one days of consolidation, bone formation was most common in the caudal and rostral region of the distraction area. The central region of the distraction area showed the lowest relative bone volume, suggesting that this region is the last region to mineralize.

Discussion

Distraction osteogenesis has been widely used for correcting various craniofacial deformities for more than a decade[7]. However, a major disadvantage is the length of the treatment time, which can take months. To overcome this problem, fundamental research is required in order to optimize craniofacial distraction protocols. For instance, biomolecular studies give more insight into the expression patterns of various growth factors involved in osteogenesis[8-10]. It has been found that the rate of

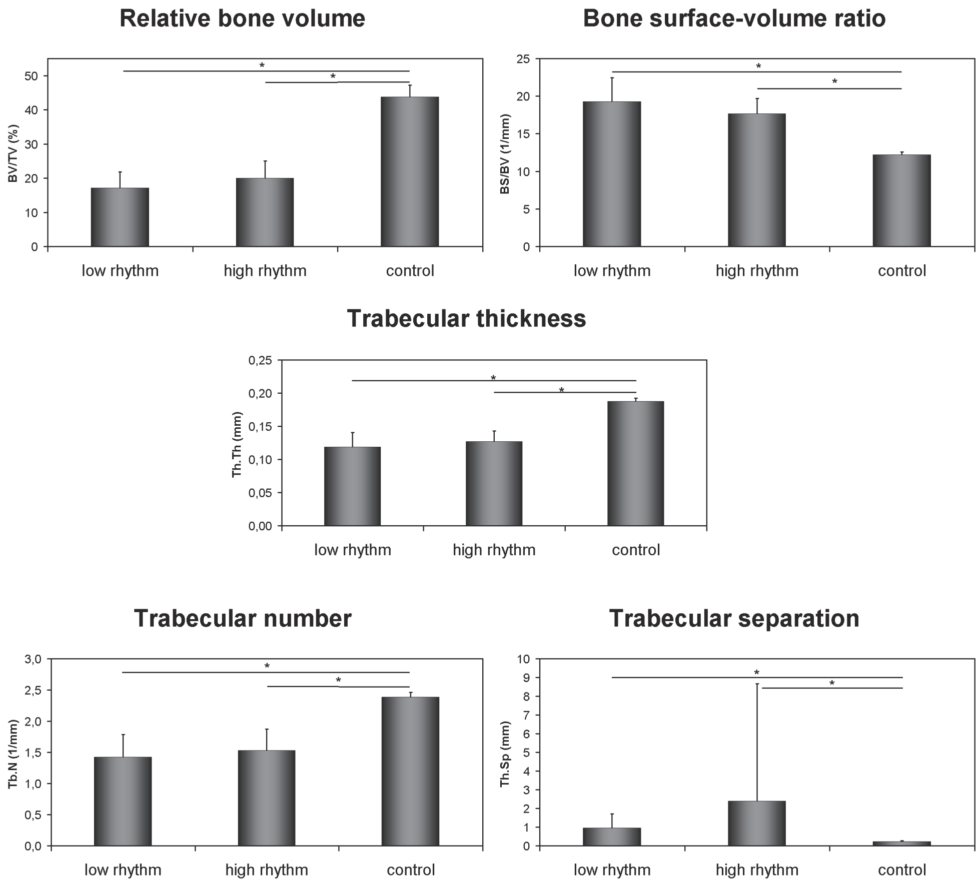

Figure 6

Comparison of micro-CT parameters. Bars represent means, whiskers show standard deviations. Significant differences between groups are indicated by asterisks (*$P < 0.05$).

distraction influences the expression of endogenous BMP-2[9]. By administrating recombinant human BMP-2, bone consolidation can be enhanced[11]. Furthermore, transplantation of autologous bone marrow mesenchymal stem cells has been shown to accelerate callus and bone formation, facilitating consolidation of the newly formed bone[12]. However, relatively few studies have focused on the effect of frequency on the distraction outcome.

The effect of distraction frequency on the outcome of craniofacial DO while standardizing the other parameters has been investigated in various animal models, such as dogs[13], pigs[14,15], rabbits[16], and rats[17]. In rats, it was found that the optimal protocol should include two daily activations of the distractor[17]. However, in a dog model and rabbit model there appeared to be no difference between one or two daily activations[13,16]. Extrapolated to the clinical situation, this would suggest that one daily activation is more convenient for the patient and the clinician than two[16]. However, the number of animals in the experimental groups in this study was small and data were not supported by statistical analysis; possibly a significant difference or trend may thus have remained unnoticed. In the dog study[13], relatively small variations in the distraction protocol were stated as possible reasons for finding no relationship between frequency of distraction and the quality and quantity of newly formed bone in a canine mandibular DO model.

Despite the use of relatively large numbers of rabbits, the present study shows that activating the distractor three times a day does not result in more bone formation when compared with a single daily activation. However, our quantitative micro-CT data show a tendency towards a difference in relative bone volume (P = 0.09) between the low-frequency group and the high-frequency group. In addition, compared with the low-frequency group the high-frequency group shows more resemblance to the control group concerning the bone architecture. Our data suggest that increasing the frequency of distraction while standardizing the other distraction parameters would result in regenerates with higher bone volume and improved bone architecture.

This is in agreement with studies that reported the use of continuous distraction in a pig model. Continuous distraction produced bone of higher quality, while mineralization was accelerated and lower distraction forces were needed[14,15]. To date, automatic distractors are not yet commercially available for clinical use, although an automatic distractor has successfully been tested in a patient[18]. Such automatic distractors may still be too inconvenient for large-scale clinical use, especially when compared with smaller spring-mediated distractors[19-22]. However, Mofid *et al* have suggested that spring-mediated distraction could be accompanied by non-linearity of the vector of distraction, decreasing force, and unpredictable distraction lengths.[20] Distractors which operate hydraulically[15,23,24] or by micromotor[25] may be better options. Such distractors should be able to lengthen bone in a controlled fashion, using non-stop distraction. Thus, for large-scale clinical use, more research is needed to produce automatic distractors that are significantly smaller and lighter. Patients would certainly benefit from automatic distractors, for these devices can perform continuous distraction, improving the distraction outcome without the need for the patient to manually activate the distractor.

Consistent with other reports[26,27], our micro-CT data demonstrate that bone formation was most common in the caudal and rostral region of the distraction area, indicating that it had started at the host-bone edges and progressed towards the centre of the distraction area. Normally, the volume of new bone should reflect the mechanical strength of the regenerate. Non-invasive quantitative computed tomography is considered to accurately predict bone stiffness[28]. For animal experimental studies, quantitative computed tomography proved to be very useful in quantifying various bone parameters[11,29-36]. However, inconvenience, cost and especially radiation exposure make it difficult to justify serial computed tomography scans for evaluation of the human distraction area[5,6]. For clinical use, alternative methods of regenerate assessment should be considered.

Clinically, bone regeneration in a craniofacial distraction area is usually evaluated by serial radiographs. On the basis of the radiographic data, the clinician decides when the distractor can be removed. However, several factors may lead to delay in removal of the distraction device and thus extend the total duration of treatment[5]. As an alternative to radiographic evaluation, ultrasound was proposed[5,6,37,38]. Ultrasonographic assessment of the DO wound may provide earlier evidence of bone formation than plain radiography, while the ease, minimal expense, lack of metal artefact, lack of radiation exposure, and lack of the need to sedate uncooperative young patients may all make ultrasound a more favorable tool for assessing bone healing[5]. However, because of the physical limitations of ultrasound in penetrating bone, it may not be a reliable method in later stages[37]. Ultrasonographic assessment of the distraction area should then be combined or replaced with radiographic assessment.

Interestingly, our data showed that neither radiographic bone-fill scores nor ultrasonographic bone-fill scores significantly correlated with relative bone volume data obtained from micro-CT measurements. We expected to find increasingly higher bone-fill scores for rabbits with increasingly higher relative bone volumes. Thus, in our study, the bone-fill scores provided no reliable predictive value for the amount of new bone formation. Possibly, the 4-point scale bone-fill score was not detailed enough to distinguish score “1” from “2” in some cases, resulting in some rabbits being scored “1”, while on the basis of their relative bone volume one would expect a score “2”, or *vice versa*. Although the semiquantitative 4-point bone-fill score has been used successfully in previous studies[4-6], we would recommend refinement of the bone-fill scores, for example by extending the number of points on the scale. Another possibility is to use quantitative morphometry instead of the rather subjective semiquantitative 4-point bone-fill scores to measure the area of new bone formation.

Regarding the ultrasonographic evaluation, a limitation may also have been that we assessed the regenerates at end-point, *i.e.* after 21 days of consolidation, by which time the regenerates had already partly mineralized. Such parts of mineralized bone might have obscured the non-mineralized areas of the regenerate, due to the bone penetration characteristics of ultrasound.

Another possible limitation in our ultrasonographic evaluation was the fact that we scored only twelve rabbits (six in each experimental group) using the ultrasonographic bone-fill scores, while radiographic evaluation was done for all rabbits (twelve in each experimental group). Unfortunately, the first six rabbits in each group did not undergo ultrasound evaluation, because during the experiments with these rabbits the facilities for performing ultrasound scanning had not yet become available. We can only speculate that correlations would have been clearer if all rabbits had undergone ultrasonographic evaluation.

Conclusion

Relative to a single daily activation, three activations a day do not significantly improve new bone formation, although there was a tendency towards a higher relative bone volume, and bone architecture showed a tendency towards a greater resemblance to the control group after three daily activations. Radiographic and ultrasonographic bone-fill scores provided no reliable predictive value for the amount of new bone formation.

Acknowledgements

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References

1. Ilizarov GA. The tension-stress effect on the genesis and growth of tissues. Part I. The influence of stability of fixation and soft-tissue preservation. Clin Orthop Relat Res 1989;(238):249-281.
2. Norris SA, Pettifor JM, Gray DA, and Buffenstein R. Calcium metabolism and bone mass in female rabbits during skeletal maturation: effects of dietary calcium intake. Bone 2001;29(1):62-69.
3. Parr JA, Garetto LP, Wohlford ME, Arbuckle GR, and Roberts WE. Implant-borne suture expansion in rabbits: a histomorphometric study of the supporting bone. J Biomed Mater Res 1999;45(1):1-10.
4. Troulis MJ, Coppe C, O'Neill MJ, and Kaban LB. Ultrasound: assessment of the distraction osteogenesis wound in patients undergoing mandibular lengthening. J Oral Maxillofac Surg 2003;61(10):1144-1149.
5. Thurmuller P, Troulis M, O'Neill MJ, and Kaban LB. Use of ultrasound to assess healing of a mandibular distraction wound. J Oral Maxillofac Surg 2002;60(9):1038-1044.
6. Kaban LB, Thurmuller P, Troulis MJ, Glowacki J, Wahl D, Linke B, Rahn B, and Perrott DH. Correlation of biomechanical stiffness with plain radiographic and ultrasound data in an experimental mandibular distraction wound. International J Oral Maxillofac Surg 2003;32(3):296-304.
7. McCarthy JG, Katzen JT, Hopper R, and Grayson BH. The first decade of mandibular distraction: lessons we have learned. Plast Reconstr Surg 2002;110(7):1704-1713.
8. Zhu WQ, Wang X, Wang XX, and Wang ZY. Temporal and spatial expression of osteoprotegerin and receptor activator of nuclear factor -kappaB ligand

during mandibular distraction in rats. J Craniomaxillofac Surg 2007;35(2):103-111.

9. Cheung LK, Zheng LW, and Ma L. Effect of distraction rates on expression of bone morphogenetic proteins in rabbit mandibular distraction osteogenesis. J Craniomaxillofac Surg 2006;34(5):263-269.
10. Knabe C, Nicklin S, Yu Y, Walsh WR, Radlanski RJ, Marks C, and Hoffmeister B. Growth factor expression following clinical mandibular distraction osteogenesis in humans and its comparison with existing animal studies. J Craniomaxillofac Surg 2005;33(6):361-369.
11. Yonezawa H, Harada K, Ikebe T, Shinohara M, and Enomoto S. Effect of recombinant human bone morphogenetic protein-2 (rhBMP-2) on bone consolidation on distraction osteogenesis: a preliminary study in rabbit mandibles. J Craniomaxillofac Surg 2006;34(5):270-276.
12. Qi M, Hu J, Zou S, Zhou H, and Han L. Mandibular distraction osteogenesis enhanced by bone marrow mesenchymal stem cells in rats. J Craniomaxillofac Surg 2006;34(5):283-289.
13. Hasse AR, Porksen M, and Zimmermann CE. Bilateral mandibular distraction in adult dogs with an epiperiosteal distractor. Br J Oral Maxillofac Surg 2005;43(2):105-112.
14. Kessler PA, Merten HA, Neukam FW, and Wiltfang J. The effects of magnitude and frequency of distraction forces on tissue regeneration in distraction osteogenesis of the mandible. Plast Reconstr Surg 2002;109(1):171-180.
15. Wiltfang J, Kessler P, Merten HA, and Neukam FW. Continuous and intermittent bone distraction using a microhydraulic cylinder: an experimental study in minipigs. Br J Oral Maxillofac Surg 2001;39(1):2-7.
16. Al Ruhaimi KA. Comparison of different distraction rates in the mandible: an experimental investigation. Int J Oral Maxillofac Surg 2001;30(3):220-227.
17. Paccione MF, Mehrara BJ, Warren SM, Greenwald JA, Spector JA, Luchs JS, and Longaker MT. Rat mandibular distraction osteogenesis: latency, rate, and rhythm determine the adaptive response. J Craniofac Surg 2001;12(2):175-182.

18. Ayoub AF, Richardson W, and Barbenel JC. Mandibular elongation by automatic distraction osteogenesis: The first application in humans. Br J Oral Maxillofac Surg 2005;43(4):324-328.
19. Idelsohn S, Pena J, Lacroix D, Planell JA, Gil FJ, and Arcas A. Continuous mandibular distraction osteogenesis using superelastic shape memory alloy (SMA). J Mater Sci Mater Med 2004;15(4):541-546.
20. Mofid MM, Inoue N, Tufaro AP, Vander Kolk CA, and Manson PN. Spring-mediated mandibular distraction osteogenesis. J Craniofac Surg 2003;14(5):756-762.
21. Zhou HZ, Hu M, Yao J, and Ma L. Rapid lengthening of rabbit mandibular ramus by using nitinol spring: a preliminary study. J Craniofac Surg 2004;15(5):725-729.
22. Zhou HZ, Hu M, Hu KJ, Yao J, and Liu YP. Transport Distraction Osteogenesis Using Nitinol Spring: An Exploration in Canine Mandible. J Craniofac Surg 2006;17(5):943-949.
23. Ayoub AF, and Richardson W. A new device for microincremental automatic distraction osteogenesis. Br J Oral Maxillofac Surg 2001;39(5):353-355.
24. Kessler P, Wiltfang J, and Neukam FW. A new distraction device to compare continuous and discontinuous bone distraction in mini-pigs: a preliminary report. J Craniomaxillofac Surg 2000;28(1):5-11.
25. Ploder O, Mayr W, Schnetz G, Unger E, Ewers R, and Plenk H, Jr. Mandibular lengthening with an implanted motor-driven device: preliminary study in sheep. Br J Oral Maxillofac Surg 1999;37(4):273-276.
26. Cope JB, and Samchukov ML. Regenerate bone formation and remodeling during mandibular osteodistraction. Angle Orthod 2000;70(2):99-111.
27. Cope JB, and Samchukov ML. Mineralization dynamics of regenerate bone during mandibular osteodistraction. Int J Oral Maxillofac Surg 2001;30(3):234-242.
28. Harp JH, Aronson J, and Hollis M. Noninvasive determination of bone stiffness for distraction osteogenesis by quantitative computed tomography scans. Clin Orthop Relat Res 1994;(301):42-48.
29. Gomez DF, Sant'Anna EF, Leven RM, Ostric SA, Figueroa AA, Royston TJ, Sumner DR, and Polley JW. Microstructural and strength evaluation of

regenerate tissue during the consolidation period after vertical mandibular ramus distraction. J Craniofac Surg 2005;16(5):805-811.

30. Cheung LK, and Zhang Q. Radiologic characterization of new bone generated from distraction after maxillary bone transport. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2003;96(2):234-242.
31. Cheung LK, and Zheng LW. Effect of recombinant human bone morphogenetic protein-2 on mandibular distraction at different rates in an experimental model. J Craniofac Surg 2006;17(1):100-108; discussion 109-110.
32. Shibazaki R, Maki K, Tachikawa T, Shibasaki Y, Hinton RJ, Carlson DS, and Opperman LA. Changes in parathyroid hormone-related protein and 3-dimensional trabecular bone structure of the mandibular condyle following mandibular distraction osteogenesis in growing rats. J Oral Maxillofac Surg 2005;63(4):505-512.
33. Sant'Anna EF, Gomez DF, Sumner DR, Williams JM, Figueroa AA, Ostric SA, Theodoru S, and Polley JW. Micro-computed tomography evaluation of the glenoid fossa and mandibular condyle bone after bilateral vertical ramus mandibular distraction in a canine model. J Craniofac Surg 2006;17(3):611-619.
34. Gosain AK, Kalantarian B, Song LS, Larson JD, Jenkins CA, and Wilson CR. Comparison of canine mandibular bone regeneration by distraction osteogenesis versus acute resection and rigid external fixation. Plast Reconstr Surg 2004;114(6):1490-1499; discussion 1500-1491.
35. Smith SW, Sachdeva RC, and Cope JB. Evaluation of the consolidation period during osteodistraction using computed tomography. Am J Orthod Dentofac Orthop 1999;116(3):254-263.
36. Zimmermann CE, Harris G, Thurmuller P, Troulis MJ, Perrott DH, Rahn B, and Kaban LB. Assessment of bone formation in a porcine mandibular distraction wound by computed tomography. Int J Oral Maxillofac Surg 2004;33(6):569-574.
37. Bail HJ, Kolbeck S, Krummrey G, Weiler A, Windhagen HJ, Hennies K, Raun K, and Raschke MJ. Ultrasound can predict regenerate stiffness in distraction osteogenesis. Clin Orthop Relat Res 2002;(404):362-367.

38. Hughes CW, Williams RW, Bradley M, and Irvine GH. Ultrasound monitoring of distraction osteogenesis. Br J Oral Maxillofac Surg 2003;41(4):256-258.

Chapter

5

Continuous versus discontinuous distraction: Evaluation of bone regenerate following various rhythms of distraction

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Abstract

Background: Activation of the distractor is usually done manually using a discontinuous rhythm of distraction. However, it is likely that continuous distraction improves the outcome.

Objectives: The aim of this study is to investigate continuous distraction osteogenesis (DO) of the nasal bones in a rabbit model, and to compare data from this continuous DO study with data from a previously conducted discontinuous DO study. In addition, radiographic and ultrasonographic bone-fill scores were determined to investigate whether these scores provided reliable predictive value for the amount of new bone formation in the distraction area.

Material and methods: Skeletally mature female New Zealand White rabbits were subjected to distraction of the nasal bones. A custom-made continuous distractor was used to perform automatic non-stop distraction. Bone data were obtained from radiography, ultrasonography and micro-computed tomography. Data from this experiment were compared with data from a previous study on discontinuous distraction rhythms.

Results: Ultrasonographic bone-fill scores correlated significantly to actual bone volume in contrast to radiographic bone-fill scores. Bone volume was significantly higher in the continuous DO group compared with the discontinuous DO groups.

Conclusions: Continuous distraction resulted in accelerated osteogenesis compared with discontinuous distraction. Furthermore, bone-fill scores based on ultrasonography showed a significant correlation with actual bone volumes.

Introduction

Distraction osteogenesis (DO) has been used regularly in oral and maxillofacial surgery since it was first reported by McCarthy *et al* in 1992.[1] The technique is based on the regenerative properties of bone. To elongate hypoplastic bone, the bone needs to be osteotomized, after which the bone segments are gradually pulled apart. The callus in the resulting gap between the bone segments is eventually replaced with calcifying tissue.

The success of the treatment depends on several factors. These include distraction parameters such as the latency time, the rate of distraction, the rhythm of distraction, and the consolidation time. Optimizing these parameters would result in improved and accelerated osteogenesis, and consequently in shorter treatment time. This would considerably increase the quality of life for the patient, knowing that treatment time can usually take several months, during which the patient can suffer physical and psychosocial problems.

Many studies have been conducted in order to find the optimal distraction parameters by varying one parameter while standardizing the others.[2] However, relatively little can be found regarding the optimal rhythm of distraction for craniofacial DO. The rhythm of distraction, defined as the number of daily activations of the distractor applied to achieve the desired daily excursion of the distractor (distraction rate; mm/d), varies between one and four activations (mostly at a rate of 1 mm/d; this implies an excursion varying between 1 – 0.25 mm per activation respectively).

In orthopedic DO research, Ilizarov found that increasing the rhythm of distraction improved osteogenesis.[3] His automated distraction device used a rhythm of 60 activations a day, resulting in accelerated bone formation in dog tibia compared with lower distraction rhythms. In craniofacial DO, it is likely that the outcome is also improved when high rhythms of distraction are used. However, activation of the distraction device is usually done manually by the patient, caretaker or clinician. Having to activate the distractor multiple times a day may be highly inconvenient, and increasing the number of manipulations of the distractor could also increase the risk of incorrect use of the distractor.

Continuous distraction, *i.e.* non-stop automated distraction, would resolve these issues. It enables the distractor to function without frequent interference by patient, caretaker or clinician because it only has to be activated at the start of the distraction phase and terminated at the end of the distraction phase. Automatic continuous distraction also allows a higher distraction rhythm, resulting in theoretically infinitely small excursions per activation. The small distraction steps would result in less damage to the distracted tissues, improving regeneration and adaptation of the tissues. Several studies have investigated continuous distraction, with devices that were spring-mediated[4-7], or were driven hydraulically[8-10] or by micro-motor[11-13]. However, to our knowledge only few studies have compared a conventional (*i.e.* discontinuous) distraction protocol with a continuous distraction protocol.[10,14] Findings from these porcine mandibular DO studies suggest that continuous DO is preferred over discontinuous DO. Although clinically relevant, the findings were based on small numbers of animals, possibly due to the use of the relatively expensive porcine animal model. Larger numbers of animals are desired in order to draw statistically relevant conclusions.

The aim of this study was to investigate bone regeneration following continuous distraction of the nasal bones in a rabbit model, using statistically relevant numbers of animals. Microcomputed tomography was used to collect quantitative data regarding bone parameters. Furthermore, data from this continuous DO study were compared with data obtained from a previously conducted discontinuous DO study.[15] In addition, radiographic and ultrasonographic bone-fill scores were determined to investigate whether these scores provided reliable predictive value for the amount of new bone formation in the distraction area.

Materials and methods

Animals and surgical procedure

Twelve skeletally mature female New Zealand White rabbits (*Oryctolagus cuniculus*) (Harlan Netherlands BV, Horst, the Netherlands) were used, with a mean body weight (± SD) of 2656 g (± 242 g) on the day of surgery. The experimental protocol was approved by the Animal Experiments Committee under the national Experiments on Animals Act and adhered to the rules laid down in this national law that serves the implementation of "Guidelines on the protection of experimental animals" by the Council of Europe (1986), Directive 86/609/EC.

After arrival at the Erasmus MC animal house facility, the animals were allowed to acclimatize to housing and diet for at least a week. Preoperatively and postoperatively, the rabbits were constantly monitored by the animal facility's veterinarians and staff for general appearance, activity, and food uptake. All animals were housed individually in large boxes in a temperature-controlled room with a 12-hour light/12-hour dark schedule. Food and water were given *ad libitum*. Box floors were covered with bedding and some cage enrichment. The animals were weighed just before surgery and just before sacrifice.

(Pre-)surgical procedures were as reported in previous work.[15] In summary, the frontonasal region was shaved and cleaned with 70 % ethanol after the animals were anesthetized with an intramuscular injection of medetomidine hydrochloride (Domitor, Pfizer Animal Health BV, Capelle aan den IJssel, the Netherlands). Subsequently, 1-3 % Isoflurane (Pharmachemie BV, Haarlem, the Netherlands) in a 2:1 oxygen/nitrous oxide mixture was administered through a facemask to maintain general anesthesia.

During surgery, animals were operated on in a prone position under sterile conditions while placed on heating pads at approximately 38 ºC to prevent hypothermia. The nasal bones were exposed by creating a longitudinal median incision (approximately 1.5 cm in length) and subsequently reflecting the overlying skin and periosteum. The continuous distractor was positioned on the exposed nasal bones according to the desired vector of distraction. Subsequently, holes were drilled for the distractor fixation screws under copious saline irrigation and a complete osteotomy perpendicular to the internasal suture was performed with a reciprocating saw, approximately 1 cm rostrally from the frontonasal suture. After stable fixation to the nasal bones was confirmed, the distractor was slightly activated to ensure that the osteotomy was complete. Subsequently, the distractor was turned to its initial closed position, followed by repositioning and closure of the periosteum and skin. Post-operatively, the animals were given buprenorphine hydrochloride (Temgesic, Schering-Plough BV, Utrecht, the Netherlands) for pain management.

Continuous distraction device

To achieve a continuous rhythm of distraction, a hydraulic distractor was custom-made to fit the nasal bone of the rabbit. The distractor consisted of two grade-5 titanium cylinders, two titanium screw plates (LOCK Plate 2.0, Synthes GmbH, Solothurn, Switzerland) and a connection for a catheter (Fig. 1a). The distractor was placed on the nasal bones using 6-mm screws (LOCK Screw Stardrive, Synthes GmbH, Solothurn, Switzerland) (Fig. 1b) and was connected by a Tygon-catheter (UNO Roestvaststaal BV, Zevenaar, the Netherlands) to a syringe (3ml Syringe Luer-Lock Tip, BD, Breda, the Netherlands), which was placed in a microprocessor-controlled syringe pump (Model 220, UNO Roestvaststaal BV, Zevenaar, the Netherlands).

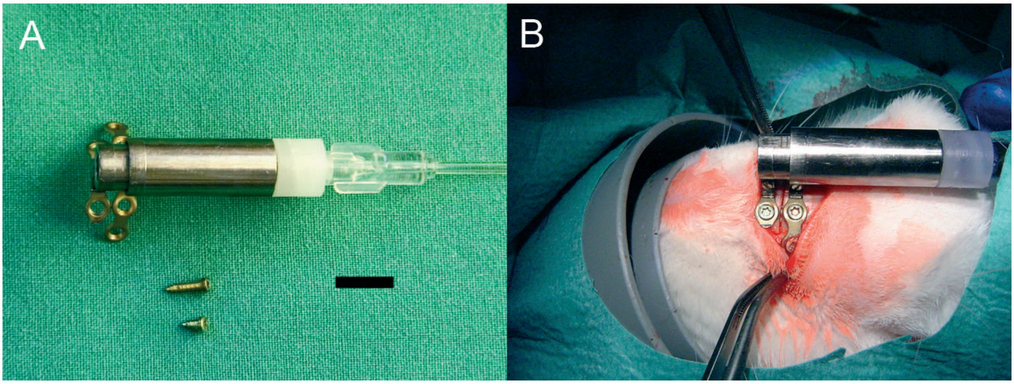

Figure 1

The continuous distraction device (A). The scale bar represents 1 cm. Placement of the continuous distractor on the rabbit nasal bones (B). Visual monitoring of the excursion was facilitated by marker lines separated 1 mm from each other.

The syringe, catheter and distractor together formed a closed system filled with hematoxylin, a purple fluid. Hematoxylin was used instead of colourless water or saline to be able to more easily find leakage or air bubbles if these would occur. The pump rate of the syringe pump can be varied; for this experiment, it was set to approximate a rate of distraction of 0.9 mm/day. The catheter was fixed to a swivel (Model ASP4, UNO Roestvaststaal BV, Zevenaar, the Netherlands) outside the rabbit box to reduce the stress on the catheter, and inside the rabbit box it was protected by a metal tether (Model BT03, UNO Roestvaststaal BV, Zevenaar, the Netherlands). To avoid stress on the catheter near the distractor end, the catheter is guided through a special rabbit jacket (UNO Roestvaststaal BV, Zevenaar, the Netherlands) to the distractor (Fig. 2).

Figure 2

Experimental continuous distraction set-up. Inside the rabbit box, the catheter is protected by a metal tether, which is attached to a special rabbit jacket.

Distraction protocol

All rabbits were allowed three full days of latency, after which distraction was started at a rate of 0.9 mm/day. One additional animal served as control; it was subjected to the same surgical procedures; a distractor was applied as well, but this rabbit did not undergo active distraction. Unaffected parts of the nasal bones of this animal served as control sample. Following the distraction period, all rabbits had a 21-day consolidation period to allow mineralization of the regenerate. All animals were sacrificed after completion of the consolidation period by intravenous injection of sodium pentobarbitone (Euthesate, CEVA Sante Animale BV, Naaldwijk, the Netherlands).

Radiographic evaluation

Immediately after sacrifice, a General Electric Mobile 225 X-ray device (General Electric, Milwaukee, Wisconsin, USA), set at 40 kV, 0.80 s, and 100 mA was used to take lateral radiographic images of the distraction area with the distractor still attached.

Bone-fill scores were determined using a semiquantitative 4-point scale [16-18] (Table 1).

Ultrasonographic evaluation

Additionally, a 13 MHz linear array transducer was used to make B-mode images of the distraction area using a ProSound SSD-4000 ultrasound console (Aloka Holding Europe AG, Zug, Switzerland) to compare with the radiographic images.

Bone formation was assessed using a semiquantitative 4-point scale [16-18] (Table 1).

Table 1. Semi-quantitative four-point scale used for the assessment of bone fill in the distraction area.

Bone-fill score	Radiographic evaluation	Ultrasonographic evaluation
0	No visible bone fill (0 %)	Complete through-transmission of US waves, clear gap margins, and no echogenic material
1	Visible bone fill, but less than 50 %	Partial through-transmission of the US waves, identifiable gap margins, and less than 50 % echogenic material
2	More than 50 % visible bone fill, but less than 100 %	Partial through-transmission of the US waves, partially obscured gap margins, and more than 50 % echogenic material
3	100 % visible bone fill	No through-transmission of the US waves, invisible gap margins, and 100 % echogenic material

Micro-computed tomographic (micro-CT) analysis

After radiographic and ultrasonographic images had been taken, the distractor was removed and total distraction distance was calculated. Tissue blocks containing the distraction area (*i.e.* the regenerated bone plus a portion of the adjacent original bones) were resected using a band saw and stored in Burkhardt's fixative.

Micro-computed tomographic scans of the tissue blocks were made with a SkyScan 1076 in vivo micro-CT scanner (SkyScan, Aartselaar, Belgium) and manufacturer's scanning software. Examination consisted of a scout view, selection of the distraction area, off-line reconstruction and evaluation. Serial transverse scan images were made at a resolution of 18 μm. Nrecon 1.3, CT Analyser 1.3.2.2 and CTvol 1.6 software (SkyScan, Aartselaar, Belgium) were used to reconstruct the data for 2D and 3D analysis. A 3D volume of interest (VOI) was created by applying interpolation between 2D-free-hand selections of the nasal bone area. Selection was such that only the regenerate tissue of the distracted nasal bone between the caudal and rostral screw holes was included.

Within the VOI, the relative bone volume (BV/TV) was determined to quantify new bone formation, as well as bone-surface to bone-volume ratio (BS/BV), trabecular number (Tb.N), trabecular thickness (Tb.Th), and trabecular separation (Tb.Sp).

Statistics

To compare continuous with discontinuous distraction, we have added the results of a previous study to the data pool.[15] These rabbits were subjected to the same surgical and experimental protocols, the only difference being the rhythm of distraction. In summary, in this study a (discontinuous) rhythm of one daily activation of the distractor was compared with a (discontinuous) rhythm of three daily activations.

In total, twelve rabbits underwent 'low-rhythm' discontinuous distraction (*i.e.*, single daily activation of the distractor at a rate of 0.9 mm/d), twelve rabbits underwent 'high-rhythm' discontinuous distraction (*i.e.*, triple daily activation at a rate of 0.9 mm/d), twelve rabbits underwent continuous distraction (*i.e.*, non-stop activation), and three rabbits were used as controls.

Statistical analyses were conducted using SPSS 12.0 for Windows (SPSS Inc., Chicago, USA). Equal variances were assumed and tested using Levene's homogeneity of variance test. For comparison of the parametric values between the two discontinuous DO groups and the continuous DO group, one-way ANOVA was used followed by LSD post-hoc testing. Spearman's rank correlation coefficients were established to determine correlations between micro-CT parameters as well as between bone-fill scores. Mean values are presented with their standard deviations (± SD). A value of $p<.05$ was considered significant.

Results

Continuous distraction experiments - General

The surgical procedures were well tolerated by the rabbits. During the experiments, there were no problems concerning the animals' health.

In two cases, the experiments had to be terminated preliminarily due to failure of two distractors. No distraction was achieved in these cases. The remaining ten rabbits completed the distraction protocol.

There was a significant increase in body weight (Wilcoxon signed ranks test, $p=.005$), suggesting that food and water uptake was not affected by the distractor or the distraction procedures.

Mean excursion of the distractor was 11.5 mm (± 2.1 mm).

Continuous distraction experiments – Bone-fill score

Mean bone-fill score by radiographic evaluation was 2.6 (± 0.7). Bone formation was most commonly present in the caudal and rostral parts of the regenerate, while evidence for bone formation could be observed less commonly in the central part.

Mean bone-fill score by ultrasonographic evaluation was 2.8 (± 0.4). Bone formation was observed most commonly in the caudal and rostral parts of the regenerate, while bone formation in the central part was observed more commonly compared with radiographic evaluation.

Continuous distraction experiments – Micro-CT parameters

Mean bone volume in the regenerate area was 26.2 % (± 4.4 %). Bone volumes ranged from 19.7 % to 33.8 %. Mean bone surface to volume ratio was 18.5/mm (± 2.1/mm), mean trabecular thickness was 0.12 mm (± 0.01 mm), mean trabecular separation was 0.45 mm (± 0.16 mm), and mean trabecular number was 2.1/mm (± 0.42/mm).

Continuous distraction *versus* discontinuous distraction

There were no significant differences between the two discontinuous DO groups regarding micro-CT bone parameters (Fig. 3). However, bone volume (BV/TV) in the regenerate area was significantly higher for the continuous distraction group than for

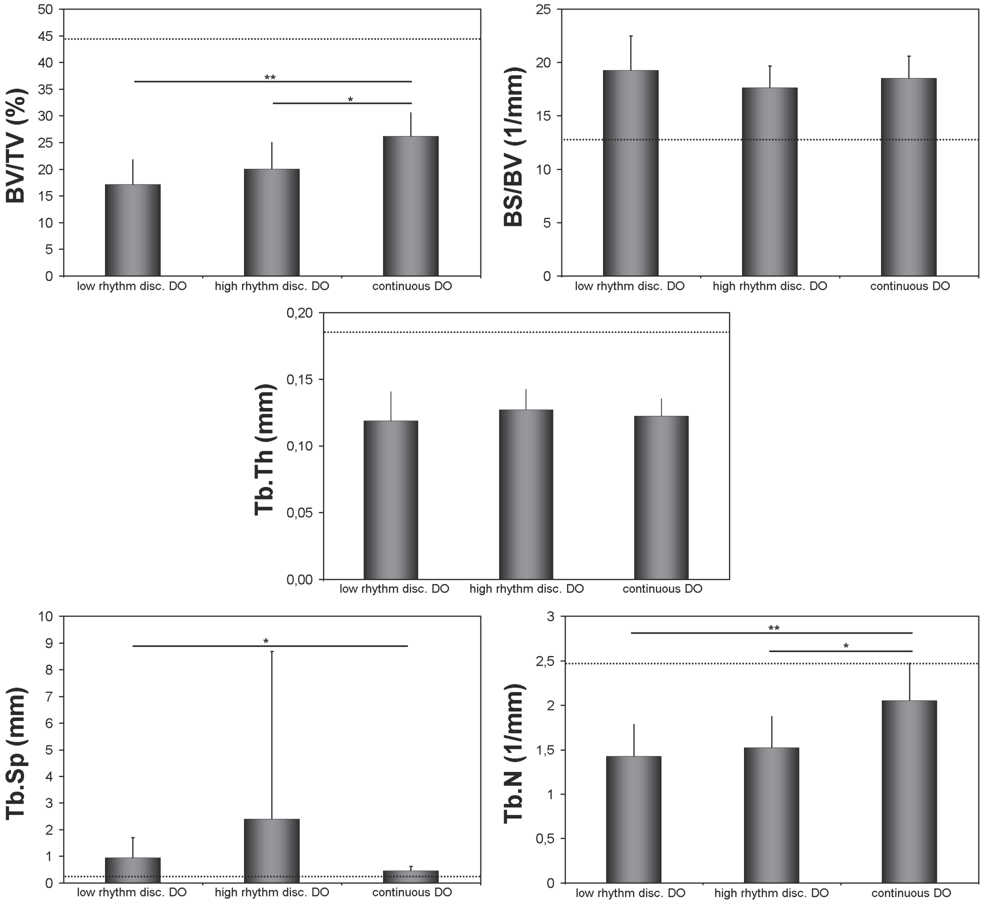

Figure 3

Continuous distraction resulted in significantly more bone volume, smaller trabecular separation, and higher trabecular number compared with discontinuous distraction. The dotted lines represent the mean control value. BV/TV = bone volume; BS/BV = bone surface/volume ratio; Tb.Th = trabecular thickness; Tb.Sp = trabecular separation; Tb.N = trabecular number. Double asterisks (**) indicate significance level $p<.01$, triple asterisks (***) indicate significance level $p<.001$.

the two discontinuous distraction groups (continuous versus discontinuous low-rhythm, *p*=.00007; continuous versus discontinuous high-rhythm, *p*=.004) (Fig. 3). In none of the cases, control values were reached (mean BV/TV control group; 44.4 % ± 2.7 %). In all groups bone volume was highest near the original host bone ends, and lowest in the centre.

Significant differences between the continuous DO group and the low-rhythm discontinuous DO group was found regarding trabecular number (*p*=.0003). Between continuous DO and high-rhythm discontinuous DO, significant difference was also found concerning trabecular number (*p*=.002).

A significantly positive correlation was found between BV/TV and Tb.N (continuous DO group, *ρ*=.758, *p*=.011). Significantly negative correlations were found between Tb.N and Tb.Sp (low-rhythm discontinuous DO group, *ρ*=-.958, *p*=.000001; high-rhythm discontinuous DO group, *ρ*=-.951, *p*=.000002), BV/TV and BS/BV (high-rhythm discontinuous DO group, *ρ*=-.063, *p*=.033), BV/TV and Tb.Sp (continuous DO group, *ρ*=-.758, *p*=.011), and BS/BV and Tb.Th (continuous DO group, *ρ*=-.939, *p*=.00005).

Radiographic *versus* ultrasonographic bone-fill score

Radiographic as well as ultrasonographic bone-fill scores from all rabbits were pooled and plotted in Fig. 4. Mean ultrasonographic bone-fill scores for the continuous DO group were higher than for both the low-rhythm discontinuous DO group (*p*=.002) and the high-rhythm group (*p*=.003). No significant difference between the groups was found regarding radiographic bone-fill scores.

A significant correlation was found between BV/TV and corresponding ultrasonographic bone-fill scores (*p*=.006, *ρ*=.571). No significant correlation was observed between BV/TV and corresponding radiographic bone-fill scores (*p*=.149; *ρ*=.253).

Discussion

In 1973, Snyder *et al* successfully applied Ilizarov's limb lengthening technique to the canine mandible for the first time.[19] Since then, many more experimental studies on craniofacial distraction osteogenesis (DO) have followed. In the early nineties,

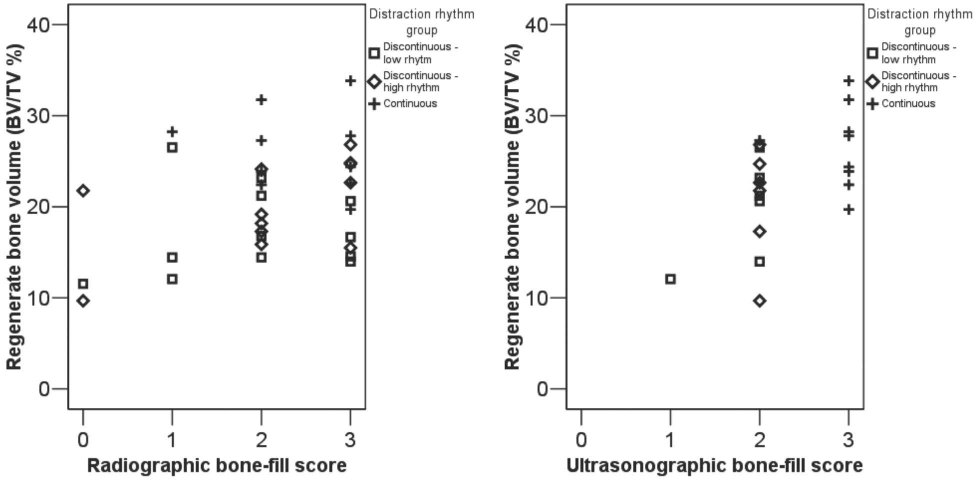

Figure 4

Pooled bone-fill score data. A significant correlation was found between BV/TV and ultrasonographic bone-fill scores was found.

experimental work by Karp *et al*[20,21] eventually led to the first report on the successful clinical application of mandibular DO in four young patients by McCarthy *et al*.[1] Craniofacial DO, being minimally invasive and associated with a low morbidity rate, proved to be a safe and effective clinical technique to correct bone defects in the skull.[22,23] In addition, animal experimental research elucidated the molecular mechanism of distraction osteogenesis, facilitating the use of targeted therapeutic manipulations to accelerate osteogenesis.[24,25] Despite the ever-growing amount of literature on craniofacial DO, relatively little is known on the effect of the rhythm of distraction on the outcome of craniofacial bone regeneration. The present study investigated whether a continuous rhythm of distraction would improve bone formation compared with a discontinuous rhythm of distraction, using a rabbit model.

In a canine tibia model Ilizarov[3] found that, at a given rate of distraction, increasing the rhythm of distraction resulted in a better outcome. Therefore, automated non-stop (*i.e.* continuous) distraction should show superior results over discontinuous distraction. Continuous distraction devices have been tested and were either spring-mediated[4-7], or based on hydraulic[8-10] or micromotor principles[11-13]. However, most of these studies solely investigated continuous distraction without making the comparison with discontinuous distraction rhythms.

The design of our continuous distraction device was based on a hydraulic pressure system. Spring-mediated distractors were considered, but spring-mediated devices may be prone to certain disadvantages, which include the non-linearity of the vector of distraction, decreasing spring force, a second operative procedure for activation of the shape memory alloy wire and unpredictable distraction lengths.[4] The use of a micromotor was technically more challenging, while technical problems such as gear break, bone fracture, or post-operative infections have been reported.[13] Based on current knowledge of the literature, hydraulic pressure-based devices have proved to be reliable.[8-10]

To our knowledge, the comparison of continuous and discontinuous distraction has been reported only by Kessler *et al*[14] and Wiltfang *et al*[10]. These studies were conducted in pigs. While pigs offer great advantage concerning the size and shape of craniofacial bone structures, they are expensive regarding purchase costs, housing and maintaining, while in addition handling can be difficult. As a result, the use of large numbers of animals for statistical power may not be possible when using such animal models.

For this reason, we chose to conduct our experiments on rabbits. Large numbers per group are possible, because rabbits are relatively inexpensive, while handling is simple. Specifically, we performed distraction on the nasal bones; these bones are easily surgically accessible, size and shape are suitable for the use of commercially available as well as experimental distractors, there is no interference with the vector of distraction by large muscle groups, and because the jaw bones are not affected food uptake during distraction is not influenced, which helps maintaining a good general health during the experiments.

The experimental set-up was well tolerated by the rabbits. The continuous distractor was well fixed on the nasal bones. The rabbit jacket and protective metal tether were sufficient to protect the catheter from being damaged. The purple-colored hematoxylin was useful in finding leakages if these had occurred during the experiments. The theoretical volume displacement was not always sufficient to produce the desired excursion of the distractor. By monitoring the excursion daily, the pump rate was adjusted if necessary, to match a distraction rate of approximately 1 mm/d. The excursion of the distractor could easily be monitored visually by using the scale on the distractor, which shows a marker line every millimeter.

The results of our continuous DO experiments were compared with data from a previous study[15], which showed that (at a constant distraction rate of 0.9 mm/d) a discontinuous distraction rhythm of three daily activations does not significantly improve osteogenesis compared with a discontinuous distraction rhythm of one daily activation. However, there was a tendency towards increased osteogenesis. Therefore, one may expect to find significant differences when continuous distraction is applied.

Our present data show that this is the case. After twenty-one days of consolidation, the continuous distraction protocol resulted in significantly more bone volume in the distraction area compared with the discontinuous protocols utilizing single or triple daily activation. This is in agreement with other studies that have compared continuous with discontinuous distraction.[8,10,14] An increase in bone formation was found in every subdivision of the regenerate area. Accelerated bone formation, particularly in the central part of the regenerate area (which normally is the last part to mineralize[26]), would result in a shorter consolidation period, and consequently a reduced total treatment time. The patient is allowed to remove the distractor at an earlier time point, relieving the patient of the physical and psychosocial burden that is associated with wearing and using a distractor. Thus, continuous distraction may considerably improve the patient's quality of life.

Besides the significant increase in bone volume, the micro-architecture of the regenerated bone showed significantly increased trabecular number in the continuous DO group compared with the discontinuous DO groups. No significant differences were found for trabecular thickness, trabecular separation and bone surface/volume ratio. Thus, at this stage of the ossification process, the increase in bone volume was predominantly due to an increase in numbers of trabeculae.

In the continuous DO group, significant positive correlation was found between bone volume and trabecular number, while significant negative correlation was found between trabecular separation and trabecular number. This implies that when the rhythm of distraction was increased, larger amounts of bone were formed; specifically, trabeculae increased in numbers, accompanied by a decrease in distances between the individual trabeculae, without the trabeculae becoming thicker. Comparable conclusions were reported in a canine mandibular DO study, which evaluated bone parameters after one and two months of consolidation.[27] Bone parameters tend to reach normal values after longer consolidation time. Our data show that this process is accelerated when using continuous distraction compared with discontinuous distraction.

Clinically, radiographs of the distraction area are used to evaluate the bone regeneration. However, some question the sensitivity of radiographs for bone formation in the distraction area.[17] Alternative methods for evaluating regenerated bone have been suggested in the literature. Among these is evaluation by means of ultrasonography.[17,18,28-30] Advantages of ultrasonographic evaluation over radiographic evaluation are that ultrasonographic assessment of the distraction area may provide earlier evidence of bone formation than plain radiography, while the ease, minimal expense, lack of metal artefact, and lack of radiation exposure may also make ultrasound a more favorable tool for assessing bone healing.[17] Complications can be detected early and precisely, while even differentiation between several kinds of complication is possible.[30,31]

Our data show a significant correlation between bone volumes (quantified by means of micro-CT) and corresponding ultrasonographic bone-fill scores. There was no significant correlation between bone volumes and corresponding radiographic bone-fill scores. The correlation coefficients imply that bone-fill evaluation by ultrasonography more closely relates to the actual bone volume. Ultrasonographic bone-fill scores are more appropriate to use as predictive values for the degree of bone fill in a distraction area than radiographic bone-fill scores are.

The (aforementioned) advantages of the use of ultrasonographs are considerable. However, because of the physical limitations of ultrasound in penetrating bone, it may not be a reliable method in later stages [28]. Ultrasonographic assessment of the distraction area should therefore always be combined with radiographic assessment. Nevertheless, the number of radiographs, and therefore the amount of radiation exposure, could be reduced by combining ultrasonography and radiography for bone-fill evaluation. More research is needed in order to optimize bone-fill evaluation by combined radiography and ultrasonography. This will help the clinician to optimize the timing of distractor removal and to prevent a patient from having to wear the distractor for an unnecessarily long period of time.

In conclusion, continuous distraction results in accelerated bone formation compared with single or triple daily activation. Furthermore, bone-fill scores based on ultrasonography show significant correlation with corresponding bone volumes obtained by means of micro-CT.

Acknowledgements

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References

1. McCarthy JG, Schreiber J, Karp N, Thorne CH, and Grayson BH. Lengthening the human mandible by gradual distraction. Plast Reconstr Surg 1992;89(1):1-8; discussion 9-10.
2. Djasim UM, Wolvius EB, van Neck JW, Weinans H, and van der Wal KGH. Recommendations for optimal distraction protocols for various animal models on the basis of a systematic review of the literature. Int J Oral Maxillofac Surg 2007;36(10):877-883.
3. Ilizarov GA. The tension-stress effect on the genesis and growth of tissues: Part II. The influence of the rate and frequency of distraction. Clin Orthop Relat Res 1989;(239):263-285.
4. Mofid MM, Inoue N, Tufaro AP, Vander Kolk CA, and Manson PN. Spring-mediated mandibular distraction osteogenesis. J Craniofac Surg 2003;14(5):756-762.
5. Idelsohn S, Pena J, Lacroix D, Planell JA, Gil FJ, and Arcas A. Continuous mandibular distraction osteogenesis using superelastic shape memory alloy (SMA). J Mater Sci Mater Med 2004;15(4):541-546.
6. Zhou HZ, Hu M, Yao J, and Ma L. Rapid lengthening of rabbit mandibular ramus by using nitinol spring: a preliminary study. J Craniofac Surg 2004;15(5):725-729.

7. Zhou HZ, Hu M, Hu KJ, Yao J, and Liu YP. Transport Distraction Osteogenesis Using Nitinol Spring: An Exploration in Canine Mandible. J Craniofac Surg 2006;17(5):943-949.
8. Kessler P, Wiltfang J, and Neukam FW. A new distraction device to compare continuous and discontinuous bone distraction in mini-pigs: a preliminary report. J Craniomaxillofac Surg 2000;28(1):5-11.
9. Ayoub AF, and Richardson W. A new device for microincremental automatic distraction osteogenesis. Br J Oral Maxillofac Surg 2001;39(5):353-355.
10. Wiltfang J, Kessler P, Merten HA, and Neukam FW. Continuous and intermittent bone distraction using a microhydraulic cylinder: an experimental study in minipigs. Br J Oral Maxillofac Surg 2001;39(1):2-7.
11. Ploder O, Mayr W, Schnetz G, Unger E, Ewers R, and Plenk H, Jr. Mandibular lengthening with an implanted motor-driven device: preliminary study in sheep. Br J Oral Maxillofac Surg 1999;37(4):273-276.
12. Zheng LW, Cheung LK, Ma L, and Wong MCM. High-rhythm automatic driver for bone traction: an experimental study in rabbits. Int J Oral Maxillofac Surg 2008;37(8):736-740.
13. Schmelzeisen R, Neumann G, and von der Fecht R. Distraction osteogenesis in the mandible with a motor-driven plate: a preliminary animal study. Br J Oral Maxillofac Surg 1996;34(5):375-378.
14. Kessler PA, Merten HA, Neukam FW, and Wiltfang J. The effects of magnitude and frequency of distraction forces on tissue regeneration in distraction osteogenesis of the mandible. Plast Reconstr Surg 2002;109(1):171-180.
15. Djasim UM, Wolvius EB, Van Neck JW, Van Wamel A, Weinans H, and Van Der Wal KGH. Single versus triple daily activation of the distractor: No significant effects of frequency of distraction on bone regenerate quantity and architecture. J Craniomaxillofac Surg 2008;36(3):143-151.
16. Troulis MJ, Coppe C, O'Neill MJ, and Kaban LB. Ultrasound: assessment of the distraction osteogenesis wound in patients undergoing mandibular lengthening. J Oral Maxillofac Surg 2003;61(10):1144-1149.

17. Thurmuller P, Troulis M, O'Neill MJ, and Kaban LB. Use of ultrasound to assess healing of a mandibular distraction wound. J Oral Maxillofac Surg 2002;60(9):1038-1044.
18. Kaban LB, Thurmuller P, Troulis MJ, Glowacki J, Wahl D, Linke B, Rahn B, and Perrott DH. Correlation of biomechanical stiffness with plain radiographic and ultrasound data in an experimental mandibular distraction wound. Int J Oral Maxillofac Surg 2003;32(3):296-304.
19. Snyder CC, Levine GA, Swanson HM, and Browne EZ, Jr. Mandibular lengthening by gradual distraction. Preliminary report. Plast Reconstr Surg 1973;51(5):506-508.
20. Karp NS, McCarthy JG, Schreiber JS, Sissons HA, and Thorne CH. Membranous bone lengthening: a serial histological study. Ann Plast Surg 1992;29(1):2-7.
21. Karp NS, Thorne CH, McCarthy JG, and Sissons HA. Bone lengthening in the craniofacial skeleton. Ann Plast Surg 1990;24(3):231-237.
22. McCarthy JG, Stelnicki EJ, and Grayson BH. Distraction osteogenesis of the mandible: a ten-year experience. Semin Orthod 1999;5(1):3-8.
23. McCarthy JG, Katzen JT, Hopper R, and Grayson BH. The first decade of mandibular distraction: lessons we have learned. Plast Reconstr Surg 2002;110(7):1704-1713.
24. Mehrara BJ, Rowe NM, Steinbrech DS, Dudziak ME, Saadeh PB, McCarthy JG, Gittes GK, and Longaker MT. Rat mandibular distraction osteogenesis: II. Molecular analysis of transforming growth factor beta-1 and osteocalcin gene expression. Plast Reconstr Surg 1999;103(2):536-547.
25. Rowe NM, Mehrara BJ, Dudziak ME, Steinbreck DS, Mackool RJ, Gittes GK, McCarthy JG, and Longaker MT. Rat mandibular distraction osteogenesis: Part I. Histologic and radiographic analysis. Plast Reconstr Surg 1998;102(6):2022-2032.
26. Cope JB, and Samchukov ML. Mineralization dynamics of regenerate bone during mandibular osteodistraction. Int J Oral Maxillofac Surg 2001;30(3):234-242.
27. Sant'Anna EF, Gomez DF, Sumner DR, Williams JM, Figueroa AA, Ostric SA, Theodoru S, and Polley JW. Micro-computed tomography evaluation of the

glenoid fossa and mandibular condyle bone after bilateral vertical ramus mandibular distraction in a canine model. J Craniofac Surg 2006;17(3):611-619.

28. Bail HJ, Kolbeck S, Krummrey G, Weiler A, Windhagen HJ, Hennies K, Raun K, and Raschke MJ. Ultrasound can predict regenerate stiffness in distraction osteogenesis. Clin Orthop Relat Res 2002;(404):362-367.
29. Hughes CW, Williams RW, Bradley M, and Irvine GH. Ultrasound monitoring of distraction osteogenesis. Br J Oral Maxillofac Surg 2003;41(4):256-258.
30. Juenger TH, Klingmueller V, and Howaldt HP. Application of ultrasound in callus distraction of the hypoplastic mandible: an additional method for the follow-up. J Craniomaxillofac Surg 1999;27(3):160-167.
31. Nocini PF, Albanese M, Wangerin K, Fior A, Trevisiol L, and Kretschmer W. Distraction osteogenesis of the mandible: evaluation of callus distraction by B-scan ultrasonography. J Craniomaxillofac Surg 2002;30(5):286-291.

Chapter

6

Histomorphometric comparison between continuous and discontinuous distraction osteogenesis

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Abstract

Background: Experimental research on improving the protocol for distraction osteogenesis (DO) has been performed extensively in the past, but little is known on the optimal rhythm of distraction. Findings in orthopedic literature showed that the outcome of DO is positively influenced by increasing the rhythm of distraction.

Objectives: The aim of this study is to quantitatively compare a continuous rhythm of distraction with discontinuous rhythms of distraction in a rabbit nasal bone DO model.

Material and methods: Tissue blocks of regenerated bone were harvested from thirty-eight young adult female New-Zealand White rabbits. After a latency period of three days, rabbits were subjected for eleven days to either single daily activation of the distractor at a rate of 0.9 mm/d, or triple daily activation at a rate of 0.9 mm/d, or continuous activation at a rate of 0.9 mm/d. After three weeks of consolidation, bone regenerates were analyzed by histomorphometry.

Results: The continuous DO group showed significantly ($p<.01$) more regenerate bone volume in the central part of the regenerate than the discontinuous DO groups. Higher osteoblastic activity was seen, as well as more blood vessels ($p<.05$). Bone volume and number of blood vessels correlated significantly in the central part of the regenerate ($p<.05$). Also, early mineral apposition rate was higher than late mineral apposition rate ($p<.05$).

Conclusion: Continuous DO significantly accelerates bone formation compared with discontinuous DO.

Introduction

Craniofacial distraction osteogenesis (DO) is the gradual lengthening of bone by applying controlled mechanical force in order to separate osteotomized bone segments. Originally developed in the field of orthopedic surgery, nowadays it has been widely used to treat congenital as well as acquired craniofacial bone defects.

Findings in orthopedic literature showed that the outcome of DO is positively influenced by increasing the rhythm of distraction[1]. This suggests that automated non-stop, *i.e.* continuous, distraction would also improve the outcome of craniofacial distraction osteogenesis. Continuous craniofacial distraction has been performed in a number of animal experimental studies, using various types of continuous distractors[2-10], but most of these studies have not quantitatively compared the results of a continuous DO protocol with a discontinuous DO protocol. Only few comparative quantitative analyses have been done in pig studies, but interpreting the statistical relevance of these data is difficult because of the small numbers of animals per group.

Therefore, the aim of our study was to quantitatively compare a continuous rhythm of distraction with discontinuous rhythms of distraction, using statistically relevant numbers of rabbits. Bone regenerates were analyzed histomorphometrically to identify the effects of rhythm of distraction on osteogenesis.

Material and methods

For this study, a total of thirty-eight young adult female New-Zealand White rabbits (Harlan Netherlands BV, Horst, the Netherlands) were used. Mean body weight (± SD) at the start of the experiments was 2742 g (± 282 g). The rabbits had been randomly assigned to one of four groups (Table 1). The surgical and experimental protocol have been described in previous studies[11,12]. In summary, an osteotomy was performed on the nasal bones. After the latency period, distraction was performed using low-rhythm discontinuous distraction, high-rhythm discontinuous distraction, and continuous distraction. After the consolidation period, regenerated nasal bone tissue was harvested for histology. The experimental protocol was approved by the

Animal Experiments Committee under the national Experiments on Animals Act and adhered to the rules laid down in this national law that serves the implementation of "Guidelines on the protection of experimental animals" by the Council of Europe (1986), Directive 86/609/EC.

Table 1. Distraction protocol

Group	N	Latency time (d)	Distraction time (d)	Distraction rate (mm/d)	Distraction rhythm (1/d)	Consolidation time (d)
Low-rhythm discontinuous DO	12	3	11	0.9	1	21
High-rhythm discontinuous DO	12	3	11	0.9	3	21
Continuous DO[a]	12	3	11	0.9	Non-stop	21
Control	2	-	-	-	-	-

[a] Two of the twelve rabbits were excluded from the analyses due to device failure.

Fluorescent labeling was used to measure mineral apposition rate (MAR). Calcein (15 mg/kg IM, Sigma-Aldrich Chemie BV, Zwijdrecht, the Netherlands) was administered on postoperative day 3, tetracycline (15 mg/kg IM, Sigma Aldrich Chemie BV, Zwijndrecht, the Netherlands) on postoperative day 15, and alizarin complexone (15 mg/kg IM, Sigma-Aldrich Chemie BV, Zwijndrecht, the Netherlands) on postoperative day 30.

At the 21st day of consolidation, endpoint was reached and rabbits were sacrificed. Tissue blocks of newly formed bone regenerate were harvested using a band saw (EXAKT 300 CP, EXAKT Apparatebau GmbH, Norderstedt, Germany). These tissue blocks also contained parts of the original bone adjacent to the initial osteotomy. The samples were stored in Burkhardt fixative and dehydrated in ascending ethanols using an automatic tissue processor (Leica TP 1020, Leica Microsystems BV, Rijswijk, the Netherlands) prior to impregnation in methylmethacrylate (MMA) monomer (Merck, Hohenbrunn, Germany). Tissue blocks were impregnated in 80 % (vol/vol) stabilized MMA, 1.0 % Lucidol (Sigma-Aldrich, Steinheim, Germany) as the initiator of polymerization, and dibutylphtalate (Merck, Hohenbrunn, Germany) for at least an hour in uncapped glass vials under vacuum, followed by the addition of N,N-dimethyltoluidine (a catalyst of polymerization) (Merck, Hohenbrunn, Germany) and embedding in capped glass vials at -17 °C for at least two days.

After polymerization, the plastic blocks were capped with resin (Technovit 3040, Heraeus Kulzer GmbH, Wehrheim, Germany), the glass vials were removed and the plastic blocks were placed in a sliding microtome (Reichert-Jung Polycut S, Leica Microsystems BV, Rijswijk, the Netherlands) equipped with a tungsten carbide knife (Reichert-Jung, Leica Microsystems BV, Rijswijk, the Netherlands) at a 40° angle. Sections of 7 μm thickness were cut, mounted on coated slides (Starfrost, Waldemar Knittel, Braunschweig, Germany) using a mixture of egg whites and glycerin, stretched with 50 % ethanol using a hot plate at 40 °C and stained with hematoxylin and eosin (H&E) and Goldner's trichrome.

At least one slide per animal was used for histomorphometry. Per slide, fifteen photos were taken using a digital camera (Leica DC300, Leica Microsystems BV, Rijswijk, the Netherlands) coupled to a light microscope (Leica IM500, Leica Microsystems BV, Rijswijk, the Netherlands). More specifically, three photos were taken in each of the five subdivisions of the distraction area; the caudal region, the caudocentral region, the central region, the rostrocentral region, and the rostral region (Fig. 1).

In each photo, the following primary parameters were quantified using image processing software, (ImageJ 1.37v, National Institute of Health, Bethesda, USA): total tissue volume (TV), mineralized bone volume (BV), fibrous tissue volume (Fb.V), osteoid volume (OV), bone surface (BS), number of blood vessels (Bv.N), volume of blood vessels (Bv.V), number of osteocytes (Ot.N), number of osteoblasts (Ob.N), and number of osteoclasts (Oc.N). From these primary parameters, secondary parameters were calculated: relative bone volume (BV/TV), relative fibrous tissue volume (Fb.V/TV), relative osteoid volume (OV/BV), relative number of blood vessels (Bv.N/TV), relative volume of blood vessels (Bv.V/TV), osteoblast-covered bone surface (Ob.S/BS), and osteoclast-covered bone surface (Oc.S/BS).

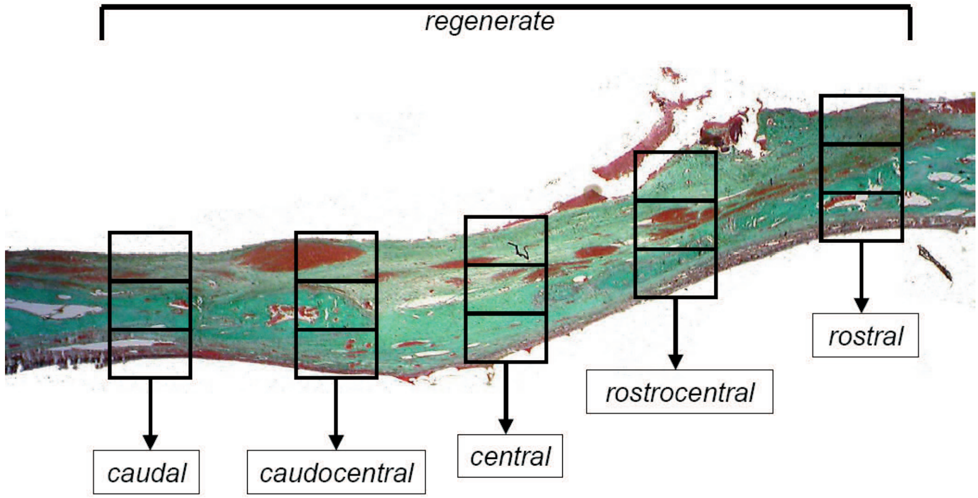

Figure 1
Overview of the regenerate. Three photos were taken in each of the five subdivisions of the regenerate. Goldner's trichrome stain, 10x2 magnification.

In addition, unstained sections were coverslipped using mounting medium (Fluoromount, BDH Laboratory Supplies, Poole, UK) for fluorescence microscopy. A Leica DFC 480 (Leica Microsystems BV, Rijswijk, the Netherlands) digital camera coupled to an Olympus AX70 (Olympus Nederland BV, Zoeterwoude, the Netherlands) microscope was used to take photographs of the sections under fluorescent light, using a FITC-filter for calcein, a DAPI-filter for tetracycline, and a TRITC-filter for alizarin complexone (Fig. 2). Mineral apposition rate (MAR) was determined by quantifying the average distance between the fluorescent labels using ImageJ software, and dividing the distance by the time between the respective fluorescent label administrations. MAR calculated from the distance between the calcein and tetracycline labels is indicated as 'early MAR', while 'late MAR' indicates the MAR calculated from the interlabel distance between tetracycline and alizarin complexone.

Data were stored in Excel 2000 (Microsoft Corporation, Redmond, USA) and statistically analyzed in SPSS 12.0 for Windows (SPSS Inc., Chicago, USA). The Mann-Whitney U test was used to identify significant differences. Spearman's rank correlation coefficients were established to determine correlations between bone parameters and vascular parameters. A p-value of $p<.05$ was considered significant.

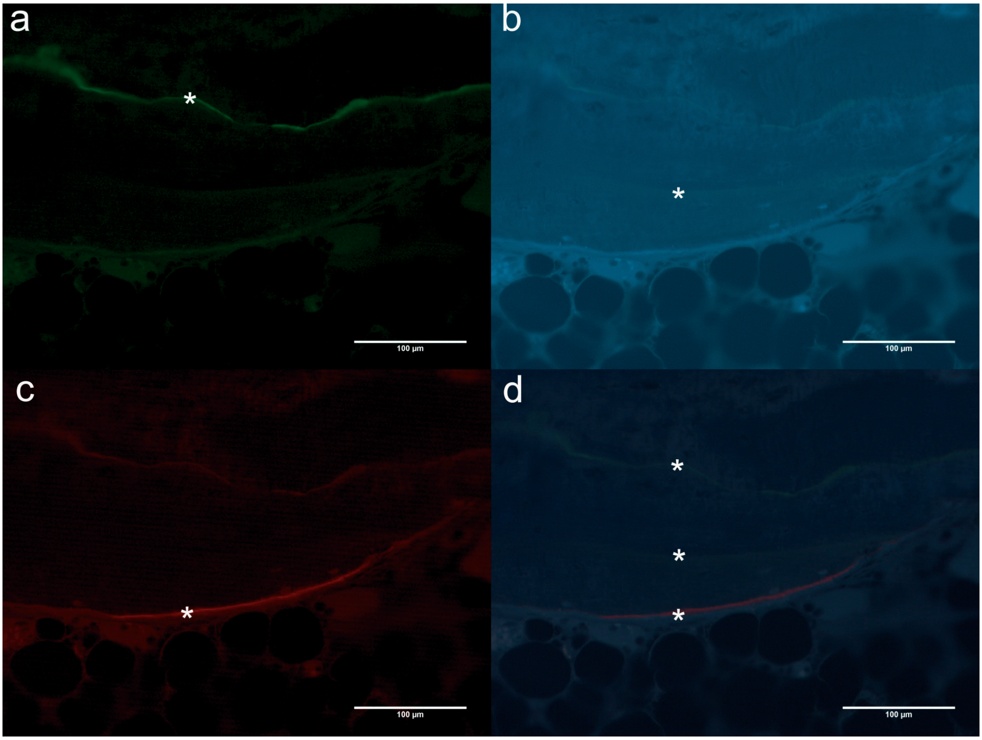

Figure 2

Fluorescent labels, indicated by asterisks (*); (a) calcein, (b) tetracycline, (c) alizarin complexone, (d) composite picture of all three labels. 10x20 magnification.

Results

Surgical and experimental procedures were well tolerated by the rabbits. In two cases, which both occurred in the continuous DO group, the experiments had to be terminated due to device failure and the two animals were excluded from further analyses.

General histology showed that bone trabeculae had been formed in the distraction area, surrounded by fibrovascular tissue (Fig. 3). As expected, bone trabeculae and fibrous tissue were aligned in the direction of the vector of distraction.

Osseous bridging was not yet completed after three weeks of consolidation. The central area of the regenerate showed the least amount of bone formation, indicating that bone formation had started from the host bone edges and progressed towards the center of the distraction area. At twenty-one days of consolidation, exclusively woven bone was seen in the regenerate. Large numbers of osteoblasts were found lining the newly formed bone tissue and depositing osteoid, while within the newly formed bone numerous osteocytes were observed. Occasionally, osteoclasts could be seen, but numbers were relatively low compared with osteoblast numbers. No chondroblasts were observed. No signs of endochondral ossification (*i.e.*, deposition of bone on a preexisting cartilage matrix) were seen, indicating that bone regeneration occurred by intramembranous ossification (*i.e.*, direct mineralization of matrix secreted by osteoblasts).

The results of the histomorphometric analyses are shown in Table 2. After twenty-one days of consolidation, bone volume was significantly higher in the central part of the distraction area of the continuous DO group compared with the central subdivision of the low-rhythm discontinuous DO group (p=.004) and the high-rhythm discontinuous DO group (p=.006), while it tended to be higher (p=.091) compared to the caudocentral subdivision of the high-rhythm discontinuous DO group. There were no significant differences concerning the other subdivision of the distraction area.

Conversely, the amount of fibrous tissue in the continuous DO group significantly decreased in the caudocentral subdivision (p=.049) and the central subdivision (p=.024) compared to the high-rhythm discontinuous DO group. It tended to be lower in the caudal (p=.056) and central (p=.065) subdivisions compared to the low-rhythm discontinuous DO group. Also, fibrous tissue volume tended to be lower in the low-rhythm discontinuous DO group compared with the high-rhythm discontinuous DO group in the rostrocentral subdivision (p=.085).

Osteoid volume did not show significant differences between the groups, although tendency towards decrease (p=.056) was observed in the rostral subdivision of the low-rhythm discontinuous DO group compared to the high-rhythm discontinuous DO group and in the caudal subdivision (p=.075) of the continuous DO group compared to the low-rhythm discontinuous DO group.

The continuous DO group showed significantly more osteoblastic surface than the low-rhythm discontinuous DO group in the caudal (p=.041) and the rostrocentral (p=.016) subdivision. Compared to the high-rhythm discontinuous DO group significant differences were observed in the rostrocentral subdivision (p=.040), while tendency towards increase was seen in the caudal subdivision (p=.067). There were no significant differences between the groups concerning osteoclastic surface.

The relative number of blood vessels was significantly higher in the rostral subdivision (p=.041) of the continuous DO group compared to the low-rhythm discontinuous DO group. A tendency towards increase was seen in the caudocentral subdivision (p=.074) of the high-rhythm group compared with the low-rhythm discontinuous DO group.

Bone volume and vascular parameters did not appear to correlate strongly for most parts of the regenerate. Combined group data for bone volume tended to correlate negatively with the number of blood vessels (p=.075) in the caudal area. Significant positive correlation (p=.046) was found in the central area, while it tended to correlate negatively (p=.083) in the rostrocentral area.

Fluorescent labeling (either calcein, tetracycline, or alizarin complexone) was always present in any subregion of the regenerate, with exception of the central subregion, which mostly lacked bone formation. Fluorescent labeling in the central subregion was only seen in one rabbit from the continuous DO group. Results for mineral apposition rate show that there were neither significant differences between the groups in early MAR nor in late MAR, with exception of a significantly higher late MAR (p=.033) in the caudal regenerate subdivision of the high-rhythm DO group compared with the continuous DO group. Within the groups, there were no significant differences between early and late MAR, with the exception of the continuous DO group, where early MAR was higher than late MAR in the caudal regenerate area (p=.027).

Discussion

The use of distraction osteogenesis was based on the Law of Tension-Stress, *i.e.* tissue regeneration principles established in studies on orthopedic lengthening of long bones[1,13]. Since its successful application in craniofacial DO, many experimental studies have contributed to the current knowledge on the underlying biological processes. Still, however, questions on this topic remain unanswered, requiring further research.

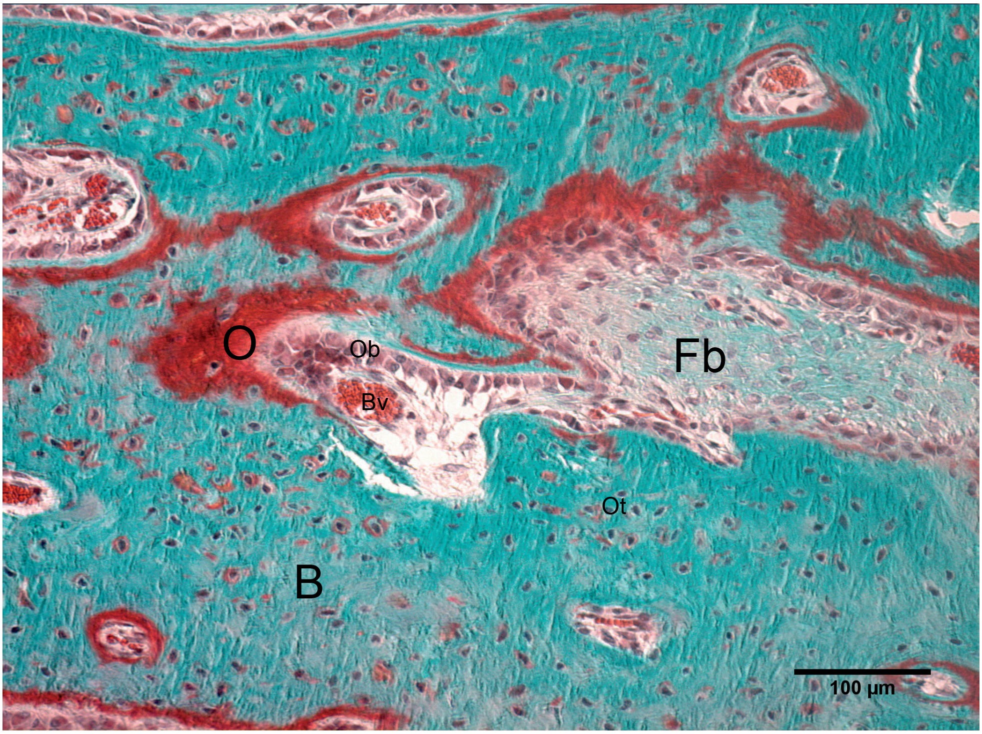

Figure 3

Example of a photo used for histomorphometry. B = mineralized bone; O = osteoid; Fb = fibrous tissue; Ob = osteoblasts; Ot = osteocytes. Goldner's trichrome stain, 10x10 magnification.

One of these questions concerns the influence of the rhythm of distraction on bone regeneration. In experimental craniofacial DO studies, only few studies have compared several rhythms of distraction in various animal models. However, these studies used discontinuous distraction protocols, involving distraction devices that required daily manual activation.

A relatively small number of experimental studies have investigated the use of automated continuous distraction devices. These distractors were either spring-mediated[2-5], powered hydraulically[6,8,14] or by micromotor[9,15,16]. Focusing solely on continuous distraction, none of these studies quantitatively compared the experimental continuous DO protocols with discontinuous DO protocols, except for some studies[6,8,17]. However, the small numbers of animals used in these porcine studies make interpreting its statistical value difficult.

To our knowledge, the present paper is the first to quantitatively compare continuous distraction with discontinuous distraction using statistically relevant numbers of animals. Our discontinuous distraction protocols involved single and triple daily activation of the distractor, while the continuous distraction protocol involved automated non-stop activation. All other parameters were standardized for all groups.

General histology showed bone formation progressing from the host bone edges towards the center, without signs of cartilage formation. The chosen rate of distraction (0.9 mm/d) was in accordance with the generally accepted optimal rate of distraction of 1 mm/d, and distractor stability was good throughout the experiments, eventually resulting in intramembranous ossification. These observations were in agreement with other reports[18-23].

Mineralization of regenerate bone usually starts at the host bone margins and progresses towards the centre in the final stages of ossification[24]. Interestingly, our study showed that bone volume was significantly higher in the central part of the distraction area in the continuous distraction group compared to the discontinuous distraction groups. Although the central part was still the last part to undergo mineralization in all cases, the higher bone volume in the continuous distraction group suggests accelerated osteogenesis. Inversely related, accelerated increase in bone volume resulted in accelerated decrease in fibrous tissue volume. Accelerated osteogenesis in the central part of the regenerate suggests earlier complete osseous bridging of the distraction gap, resulting in shorter consolidation time.

There was very little non-mineralized bone matrix compared to mineralized bone tissue in all groups. The ratio between osteoid volume and total tissue volume showed that osteoid volume was high in the low-rhythm discontinuous DO group and tended to be lower in the continuous DO group, however without showing significant differences. Possibly, higher amounts of osteoid had been present in the continuous group, but at endpoint (*i.e.*, after three weeks of consolidation) most of it was already turned over to mineralized bone tissue, while in the discontinuous distraction groups the mineralization process had not yet progressed to the same extent. This suggests accelerated bone mineralization in the continuous DO group, which is in agreement with data from a continuous DO study in pigs[8].

Osteoblast activity was determined by measuring the osteoblast-covered bone surface in relation to the total bone surface. Each group showed increasing

Table 2. Histomorphometric parameters

Parameter	Regenerate subdivision	Group			
		Low-rhythm discontinuous	High-rhythm discontinuous	Continuous	Control
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
BV/TV (%)	Caudal	46.0 ± 4.7	47.6 ± 7.6	49.5 ± 7.0	59.8 ± 11.3
	Caudocentral	26.7 ± 8.6	24.8 ± 11.1	34.4 ± 12.7	ND
	Central	9.8[a] ± 6.8	12.9[a] ± 9.6	28.8[a] ± 12.4	ND
	Rostrocentral	31.8 ± 12.9	23.6 ± 12.7	30.1 ± 11.8	ND
	Rostral	45.8 ± 16.1	45.2 ± 8.8	47.0 ± 4.3	60.5 ± 2.9
FV/TV (%)	Caudal	23.3 ± 5.1	29.2 ± 9.0	26.5 ± 4.0	17.0 ± 0.0
	Caudocentral	43.6 ± 13.4	51.5[b] ± 12.8	37.1[b] ± 13.9	ND
	Central	64.1 ± 14.7	67.2[b] ± 12.1	51.9[b] ± 18.8	ND
	Rostrocentral	40.9 ± 14.3	53.0 ± 15.7	44.7 ± 12.5	ND
	Rostral	26.3 ± 12.4	29.5 ± 5.2	27.4 ± 6.9	15.0 ± 9.9
OV/BV (%)	Caudal	5.2 ± 5.9	3.6 ± 4.3	2.2 ± 2.7	1.7 ± 0.4
	Caudocentral	5.9 ± 7.3	9.5 ± 16.1	4.0 ± 4.2	ND
	Central	35.4 ± 89.2	5.1 ± 8.2	2.9 ± 2.4	ND
	Rostrocentral	5.7 ± 8.0	2.1 ± 2.1	3.1 ± 3.1	ND
	Rostral	4.6 ± 5.8	2.3 ± 3.3	2.9 ± 3.1	2.8 ± 0.4
Bv.N/TV (1/mm^3)	Caudal	14.3 ± 8.9	15.7 ± 13.0	16.8 ± 12.0	13.5 ± 15.0
	Caudocentral	14.3 ± 7.3	20.7 ± 8.4	20.3 ± 8.4	ND
	Central	15.9 ± 7.8	15.2 ± 7.6	18.7 ± 16.5	ND
	Rostrocentral	18.0 ± 10.2	19.9 ± 10.0	15.4 ± 9.6	ND
	Rostral	8.9 ± 5.5	19.2 ± 14.7	17.0[c] ± 9.8	7.8 ± 0.6
Bv.V/TV (%)	Caudal	1.8 ± 1.1	1.8 ± 1.3	1.8 ± 0.9	4.8 ± 5.4
	Caudocentral	1.8 ± 1.2	3.4 ± 3.2	1.8 ± 1.9	ND
	Central	2.1 ± 1.5	3.2 ± 2.2	1.9 ± 1.6	ND
	Rostrocentral	1.6 ± 1.1	2.4 ± 1.7	1.6 ± 1.5	ND
	Rostral	1.1 ± 0.9	1.7 ± 1.3	1.6 ± 0.9	1.9 ± 1.5

Ob.S/BS (%)	Caudal	34.6[d] ± 17.4	38.0 ± 27.3	54.5[d] ± 19.5	40.0 ± 35.0
	Caudocentral	49.9 ± 23.5	63.8 ± 24.6	56.2 ± 27.3	ND
	Central	78.0 ± 24.4	67.2 ± 10.8	72.8 ± 20.1	ND
	Rostrocentral	51.7[d] ± 20.6	53.4[e] ± 23.4	77.1[d,e] ± 20.9	ND
	Rostral	39.3 ± 17.6	39.9 ± 22.7	49.4 ± 21.9	14.8 ± 20.9
Oc.S/BS (%)	Caudal	1.4 ± 1.5	0.7 ± 0.9	1.2 ± 1.0	4.9 ± 4.9
	Caudocentral	1.2 ± 1.3	0.9 ± 0.8	1.1 ± 1.8	ND
	Central	1.4 ± 3.0	1.1 ± 1.5	0.7 ± 1.5	ND
	Rostrocentral	1.7 ± 2.2	1.5 ± 1.8	1.3 ± 1.0	ND
	Rostral	1.2 ± 1.6	1.6 ± 2.3	2.4 ± 2.6	0.0 ± 0.0
MAR early (mcm/d)	Caudal	2.3 ± 1.4	3.1 ± 2.1	2.8[f] ± 1.2	2.5 ± 0.9
	Caudocentral	2.6 ± 1.6	6.0	4.5	ND
	Central	ND	ND	1.20	ND
	Rostrocentral	ND	3.3 ± 1.3	ND	ND
	Rostral	3.4 ± 0.5	2.9 ± 1.0	2.9 ± 1.6	2.9 ± 1.7
MAR late (mcm/d)	Caudal	2.0 ± 0.6	2.7[g] ± 1.2	1.6[f,g] ± 0.8	1.6 ± 0.7
	Caudocentral	1.6 ± 1.0	1.2 ± 0.2	2.1 ± 0.8	ND
	Central	ND	ND	4.53	ND
	Rostrocentral	1.0	1.7 ± 0.8	2.3 ± 1.1	ND
	Rostral	2.2 ± 1.3	1.7 ± 1.0	2.0 ± 1.2	1.7 ± 1.4

Summary of histomorphometric parameters. For the sham-operated control group, which underwent osteotomy but no distraction, the unaffected caudal and rostral areas in the histological slides are shown, thus excluding the central area where the osteotomy is located.

[a] higher BV/TV in continuous DO group compared with both discontinuous DO groups, p<.01

[b] lower FV/TV in continuous DO group compared with high-rhythm discontinuous DO group, p<.05

[c] larger bv.N/TV in continuous DO group compared with low-rhythm discontinuous DO group, p<.05

[d] larger Ob.S/BS in continuous DO group compared with low-rhythm discontinuous DO group, p<.05

[e] larger Ob.S/BS in continuous DO group compared with high-rhythm discontinuous DO group, p<.05

[f] higher late MAR compared with early MAR in continuous DO group, p<.05

[g] higher MAR in high-rhythm discontinuous DO group compared with continuous DO group, p<.05

ND = no data

osteoblast activity towards the centre of the distraction area. When compared between groups, significantly higher osteoblast activity was found in the continuous DO group in some parts of the regenerate. However, these differences were not found in the central parts, where they would have been expected regarding the higher bone volume found in the central parts. This suggests that increased osteogenesis is not necessarily the result of increased numbers of osteoblasts. The constant distraction forces during continuous DO may have stimulated higher activity of individual osteoblasts, resulting in more osteoid (and eventually more bone) than would have resulted from discontinuous DO. In discontinuous DO, osteoblast activity may only reach peak level directly after activation and decreases until the next activation cycle, when osteoblast activity increases and decreases again. During continuous DO, osteoblasts are subjected to constant distraction forces, stimulating osteogenesis continuously. Due to this higher activity, osteoblasts could produce relatively more bone tissue. This higher metabolic activity may be in part explained by the tension-stress effect, which is characterized by the stimulation of both proliferative and biosynthetic cellular functions[13]. Local deformations in the callus tissue caused by distraction may then serve as a key stimulus for osteogenesis via altering cell structure and extracellular matrix (ECM) densities to facilitate proliferation, differentiation and protein synthesis[25,26]. Our results show that at three weeks of consolidation there was no significant increase in numbers of osteoblasts, suggesting increased biosynthetic cellular activity of individual osteoblasts.

Fluorescent interlabel distances were measured to calculate MAR. Tetracycline tended to be weakly visible compared to the other fluorochromes, which appears to be a known problem[27]. Nevertheless, when present, tetracycline was sufficiently visible to perform measurements. Furthermore, in some cases labeling was absent mainly because of absent bone formation, which was mostly the case in the central areas of the regenerate. Labeling in the central subregion was observed only in the continuous DO group, indicating accelerated bone formation compared to the discontinuous DO groups. When possible, MAR was calculated for the period during the distraction period (early MAR) and for the period during the consolidation period (late MAR). Between groups, only late MAR appeared to be significantly higher in the caudal region of the high-rhythm discontinuous DO group compared with the continuous DO group. Within groups, only the continuous DO group showed a significantly higher early MAR compared to the late MAR, suggesting that more mineral apposition has occurred during the distraction phase compared to the consolidation phase. This may be the result of the mechanical stimulus that osteoblasts are subjected to during active distraction, in contrast to the static situation during the consolidation phase. These data suggest that mineral apposition is stimulated by continuous distraction, particularly during distraction and the early stage of consolidation.

Vascularization in the distraction area is considered to be essential for osteogenesis[28], and bone volume and number of blood vessels are known to correlate positively [29]. In the present study, bone volume showed significant positive correlation with the number of blood vessels in the central part, but not in the other parts of the regenerate. As the central part is the last part to mineralize, bone tissue in this part of the regenerate is of youngest age. Similar findings have been reported elsewhere[29], indicating that adequate blood supply is essential particularly in early bone formation. Vascularization depends on various angiogenic factors[20,30], of which levels increase during the early consolidation phase, and decrease during the late consolidation phase[22,31,32]. This suggests that vasculogenesis precedes osteogenesis. The endpoint in our experiments was at three weeks of consolidation. This may be a limitation to our study. Possibly, marked differences in vascular density between the groups had occurred during early consolidation, but these differences may have disappeared during late consolidation, and were therefore not measurable at endpoint. Nevertheless, it is likely that a similar pattern as seen in the central area at three weeks of consolidation had also occurred in the other regenerate areas in earlier stages of the consolidation period, with highest vascular density to be expected in the continuous DO group. This has eventually resulted in the observation that significantly more bone has formed in the continuous DO group.

Continuous distraction has been tested in a patient[33], but reports on the clinical use of continuous DO are scarce. To date, large-scale clinical use of continuous distraction devices is not yet possible. Such devices tend to be expensive in terms of costs for research and development as well as production and marketing, although the investments may prove worthwhile. Clinically, continuous distraction would offer considerable advantages. Because of the automated nature of continuous distraction, patients do not have to activate the distractor themselves, which prevents incorrect use. The continuous rhythm stimulates osteogenesis, resulting in accelerated bone formation. This may lead to considerably shorter consolidation periods. Lesser distraction forces are associated with continuous distraction[8,17] and thus may also reduce pain experienced by the patient, compared with the situation when the distractor is discontinuously activated once or twice a day. Further research needs to be done on designing patient-friendly continuous distractors.

Conclusion

In conclusion, continuous distraction results in significantly accelerated bone formation compared with discontinuous distraction.

Acknowledgements

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References

1. Ilizarov GA. The tension-stress effect on the genesis and growth of tissues: Part II. The influence of the rate and frequency of distraction. Clin Orthop Relat Res 1989;(239):263-285.
2. Mofid MM, Inoue N, Tufaro AP, Vander Kolk CA, and Manson PN. Spring-mediated mandibular distraction osteogenesis. J Craniofac Surg 2003;14(5):756-762.
3. Idelsohn S, Pena J, Lacroix D, Planell JA, Gil FJ, and Arcas A. Continuous mandibular distraction osteogenesis using superelastic shape memory alloy (SMA). J Mater Sci Mater Med 2004;15(4):541-546.
4. Zhou HZ, Hu M, Yao J, and Ma L. Rapid lengthening of rabbit mandibular ramus by using nitinol spring: a preliminary study. J Craniofac Surg 2004;15(5):725-729.
5. Zhou HZ, Hu M, Hu KJ, Yao J, and Liu YP. Transport Distraction Osteogenesis Using Nitinol Spring: An Exploration in Canine Mandible. J Craniofac Surg 2006;17(5):943-949.

6. Kessler P, Wiltfang J, and Neukam FW. A new distraction device to compare continuous and discontinuous bone distraction in mini-pigs: a preliminary report. J Craniomaxillofac Surg 2000;28(1):5-11.
7. Ayoub AF, and Richardson W. A new device for microincremental automatic distraction osteogenesis. Br J Oral Maxillofac Surg 2001;39(5):353-355.
8. Wiltfang J, Kessler P, Merten HA, and Neukam FW. Continuous and intermittent bone distraction using a microhydraulic cylinder: an experimental study in minipigs. Br J Oral Maxillofac Surg 2001;39(1):2-7.
9. Schmelzeisen R, Neumann G, and von der Fecht R. Distraction osteogenesis in the mandible with a motor-driven plate: a preliminary animal study. Br J Oral Maxillofac Surg 1996;34(5):375-378.
10. Ploder O, Mayr W, Schnetz G, Unger E, Ewers R, and Plenk H, Jr. Mandibular lengthening with an implanted motor-driven device: preliminary study in sheep. Br J Oral Maxillofac Surg 1999;37(4):273-276.
11. Djasim UM, Hekking-Weijma JM, Wolvius EB, van Neck JW, and van der Wal KGH. Rabbits as a model for research into craniofacial distraction osteogenesis. Br J Oral Maxillofac Surg 2008;*In Press.*
12. Djasim UM, Wolvius EB, Van Neck JW, Van Wamel A, Weinans H, and Van Der Wal KGH. Single versus triple daily activation of the distractor: No significant effects of frequency of distraction on bone regenerate quantity and architecture. J Craniomaxillofac Surg 2008;36(3):143-151.
13. Ilizarov GA. The tension-stress effect on the genesis and growth of tissues. Part I. The influence of stability of fixation and soft-tissue preservation. Clin Orthop Relat Res 1989;(238):249-281.
14. Ayoub AF, Richardson W, Koppel D, Thompson H, Lucas M, Schwarz T, Smith L, and Boyd J. Segmental mandibular reconstruction by microincremental automatic distraction osteogenesis: an animal study. Br J Oral Maxillofac Surg 2001;39(5):356-364.
15. Zheng LW, Cheung LK, Ma L, and Wong MCM. High-rhythm automatic driver for bone traction: an experimental study in rabbits. Int J Oral Maxillofac Surg 2008;37(8):736-740.
16. Ploder O, Kanz F, Randl U, Mayr W, Voracek M, and Plenk H, Jr. Three-dimensional histomorphometric analysis of distraction osteogenesis using an

implanted device for mandibular lengthening in sheep. Plast Reconstr Surg 2002;110(1):130-137; discussion 138.

17. Kessler PA, Merten HA, Neukam FW, and Wiltfang J. The effects of magnitude and frequency of distraction forces on tissue regeneration in distraction osteogenesis of the mandible. Plast Reconstr Surg 2002;109(1):171-180.
18. Farhadieh RD, Gianoutsos MP, Dickinson R, and Walsh WR. Effect of distraction rate on biomechanical, mineralization, and histologic properties of an ovine mandible model. Plast Reconstr Surg 2000;105(3):889-895.
19. Choi IH, Chung CY, Cho TJ, and Yoo WJ. Angiogenesis and mineralization during distraction osteogenesis. J Korean Med Sci 2002;17(4):435-447.
20. Bouletreau PJ, Warren SM, and Longaker MT. The molecular biology of distraction osteogenesis. J Craniomaxillofac Surg 2002;30(1):1-11.
21. Liu ZJ, Anderson MW, Gu GM, and King GJ. Apoptosis in the regenerate produced by mandibular osteodistraction in the mature rat. Orthod Craniofac Res 2005;8(1):41-51.
22. Hu J, Zou S, Li J, Chen Y, Wang D, and Gao Z. Temporospatial expression of vascular endothelial growth factor and basic fibroblast growth factor during mandibular distraction osteogenesis. J Craniomaxillofac Surg 2003;31(4):238-243.
23. Zimmermann CE, Thurmuller P, Troulis MJ, Perrott DH, Rahn B, and Kaban LB. Histology of the porcine mandibular distraction wound. Int J Oral Maxillofac Surg 2005;34(4):411-419.
24. Cope JB, and Samchukov ML. Mineralization dynamics of regenerate bone during mandibular osteodistraction. Int J Oral Maxillofac Surg 2001;30(3):234-242.
25. Morgan EF, Longaker MT, and Carter DR. Relationships between tissue dilatation and differentiation in distraction osteogenesis. Matrix Biol 2006;25(2):94-103.
26. Feng X, Tuo X, Chen F, Wu W, Ding Y, Duan Y, and Lin Z. Ultrastructural cell response to tension stress during mandibular distraction osteogenesis. Br J Oral Maxillofac Surg 2008;46(7):527-532.

27. Sun TC, Mori S, Roper J, Brown C, Hooser T, and Burr DB. Do different fluorochrome labels give equivalent histomorphometric information? Bone 1992;13(6):443-446.
28. Fang TD, Salim A, Xia W, Nacamuli RP, Guccione S, Song HM, Carano RA, Filvaroff EH, Bednarski MD, Giaccia AJ, and Longaker MT. Angiogenesis is required for successful bone induction during distraction osteogenesis. J Bone Miner Res 2005;20(7):1114-1124.
29. Amir LR, Becking AG, Jovanovic A, Perdijk FBT, Everts V, and Bronckers ALJJ. Formation of new bone during vertical distraction osteogenesis of the human mandible is related to the presence of blood vessels. Clin Oral Implants Res 2006;17(4):410-416.
30. Pacicca DM, Patel N, Lee C, Salisbury K, Lehmann W, Carvalho R, Gerstenfeld LC, and Einhorn TA. Expression of angiogenic factors during distraction osteogenesis. Bone 2003;33(6):889-898.
31. Byun J-H, Park B-W, Kim J-R, and Lee J-H. Expression of vascular endothelial growth factor and its receptors after mandibular distraction osteogenesis. Int J Oral Maxillofac Surg 2007;36(4):338-344.
32. Schumacher B, Albrechtsen J, Keller J, Flyvbjerg A, and Hvid I. Periosteal insulin-like growth factor I and bone formation. Changes during tibial lengthening in rabbits. Acta Orthop Scand 1996;67(3):237-241.
33. Ayoub AF, Richardson W, and Barbenel JC. Mandibular elongation by automatic distraction osteogenesis: The first application in humans. Br J Oral Maxillofac Surg 2005;43(4):324-328.

Chapter

7

General Discussion

General Discussion

Introduction

Since oral and maxillofacial surgeons have recognized the potential of distraction osteogenesis (DO), it has become a widely accepted method for correcting acquired and congenital deformities. Currently used parameter values were adapted directly from orthopedic DO, but may need to be optimized for craniofacial DO.

Our specific interest was in the role of the rhythm of distraction on osteogenesis. The primary aim of this thesis was to investigate the effects of rhythm of distraction on bone regeneration. We attempted to answer the following questions: What is the current knowledge on this topic in animal experimental literature, and can recommendations for the optimal distraction protocol be formulated, based on these data? Does triple daily activation of the distractor result in significantly more bone formation compared with single daily activation (given the same rate of distraction)? Does continuous distraction significantly accelerate osteogenesis compared to discontinuous distraction? Are there differences in histological characteristics and bone apposition dynamics between discontinuous and continuous distraction protocols? Do bone-fill scores based on ultrasonographs provide a more reliable predictive value for the amount of new bone formation than plain radiographs? Is the rabbit an appropriate animal model for research on craniofacial DO?

In the present chapter, the results of our studies will be discussed, as well as the limitations of our studies, the implications of the animal experimental data for clinical use, and future perspectives.

Review of the animal experimental literature

Can recommendations for the optimal distraction protocol be formulated, based on the available experimental literature? To answer this question, a systematic review of the animal experimental literature was performed (Chapter 2). The review showed that several animal experimental studies focused on finding optimal values for latency time, rate of distraction, rhythm of distraction, and consolidation time by investigating the effects of variations in a single distraction parameter (while keeping the other parameters constant). This systematic overview of the current scientific knowledge may help researchers in choosing the most appropriate distraction protocol for a specific animal model.

Usually a latency period of five to seven days is used prior to the start of distraction. However, because of the rich blood supply in craniofacial bones, a latency period may not be necessary at all, as demonstrated in pig and sheep models[1-4]. These data conflict with data found in rat and rabbit studies, where latency periods of 5-7 days are regarded necessary for optimal osteogenesis.[5-7] Why these data contradict is not yet fully understood. However, if latency time can be eliminated the total treatment time can be reduced.

Consensus was found on the optimal rate of distraction, which was approximately one millimeter per day for most animal models. Slower rates (<0.5 mm/d) would normally result in premature consolidation, while faster rates (>1.5 mm/d) would result in pseudoarthrosis or delayed ossification. Exception was found for rats, where a rate between 0.2 – 0.6 mm/d was considered optimal. A daily rate of 1 mm/d resulted in poor bone quality, presumably due to distraction-induced disruption of newly forming blood vessels.[6] As a rate of 1 mm/d equals a daily lengthening of 4 % of the mandibular length of adult (Sprague-Dawley) rats, the resulting disruption of newly forming blood vessels may cause a profound and persistent hypoxic zone of injury.[6] Comparably slow rates were not tested in the other animal models, and effects of such rates were thus not reported.

Data on the optimal consolidation time gave more insight into the optimal timing for distractor-removal. It was found that six weeks was the minimum time that the regenerate should be allowed before device removal, although too heavy loads should still be avoided for a period of time after distractor-removal.[8-10]

Regarding the rhythm of distraction, relatively little data was available on the effects of rhythm of distraction on regenerate quality compared with the other parameters. Ilizarov once found that (at a given rate of distraction) the outcome of long bone distraction improved when a higher rhythm of distraction was used.[11] Therefore, one might argue that this could apply for craniofacial bones as well. However, it is reported that a distraction rhythm of two daily activations does not differ from a distraction rhythm of one daily activation (given the same rate of distraction).[12,13] Single daily activation would then be more convenient to the patient or clinician, as bone formation does not improve by doubling the number of daily activations.

However, these studies were performed in small groups of animals. Therefore, the statistical relevance of these data is hard to interpret. The systematic review revealed that no study had ever quantitatively compared the effects of various rhythms of distraction on bone regeneration using statistically relevant numbers of experimental animals. Does osteogenesis actually improve with increasing rhythms of distraction? This issue was addressed in Chapters 4 to 6.

Discontinuous distraction

The difference between one or two daily activations might not be significant[12,13], but does this also apply for higher distraction rhythms? A distraction protocol with more daily activations should be considered, but the practical use of discontinuous distraction is limited by the number of daily manual activations that is considered reasonable. Can osteogenesis be accelerated by increasing the rhythm of distraction within these practical limits? To answer this question, we have compared two discontinuous distraction protocols: one using single daily activation of the distractor (*i.e.*, low-rhythm discontinuous distraction), the other using triple daily activation of the distractor (*i.e.*, high-rhythm discontinuous distraction) (Chapter 4). All other parameters were kept constant. A total of twenty-four rabbits were randomly assigned to one of the two experimental groups (*i.e.*, n=12 per group, which provided a statistically relevant group size). Bone formation was quantified by using micro-computed tomography (micro-CT). We found that increasing the distraction rhythm from single to triple daily activation did not significantly improve osteogenesis. This implies that there is no reason to increase the distraction rhythm from one to three daily activations.

However, the discontinuous distraction data did show a tendency towards more bone formation in the high-rhythm discontinuous distraction group compared to the low-rhythm discontinuous distraction group. It seems likely that increasing rhythms of distraction will show increasingly improved osteogenesis.

It has been reported that lower distraction forces are required when the rhythm of distraction increases.[14] Our (unpublished) torque data also showed that high-rhythm discontinuous distraction generally required less force than low-rhythm distraction. When increasing the frequency of activation, each activation represents a proportionally smaller advancement. Because of the smaller advancement per activation, less tissue resistance has to be overcome, and thus less distraction force is needed. Assuming that lower distraction forces results in less pain during the distraction procedure, increasing the distraction rhythm would be beneficial for the patient as it becomes easier to cope with the distraction procedures.

Ultrastructurally and histologically, bone formation followed the same pattern regardless of the rhythm that was used (Chapters 4 and 6). Osteogenesis started from the host bone edges, and progressed towards the center of the distraction area. The center of the distraction area was, therefore, the part that showed the latest bone formation. This is in accordance with the mineralization dynamics of bone regenerate in craniofacial distraction osteogenesis as reported by Cope *et al*.[15]

None of the rabbits showed complete osseous bridging of the distraction gap after three weeks of consolidation. As seen in the review of the literature (Chapter 2), it may take about six to eight weeks before consolidation is complete (*i.e.*, the distraction gap has been completely bridged by new bone tissue that is strong enough to bear loading). Had we chosen such a long consolidation period, no differences may have been observed at end-point, as all distraction gaps would have been completely bridged by new bone. To be able to differentiate between the experimental groups regarding bone formation rate and histology we have deliberately chosen a three-week consolidation time. This was the only end-point we have incorporated in our studies. More end-points may have provided more insight in the bone formation process at various stages of consolidation, but would also have required more rabbits (and thus more time and expenses) or smaller experimental groups (less rabbits per end-point, which would reduce the statistical power).

Continuous distraction versus discontinuous distraction

Although no significant differences were found in bone volume, there was a tendency towards higher bone volume when triple daily activation was applied (compared to single daily activation) (Chapter 4). Therefore, we hypothesized that significantly more bone formation would be found when continuous distraction is applied.

To investigate whether a continuous distraction protocol provides significant improvement of bone formation over the discontinuous protocols, distractors needed to be found that could perform automated continuous distraction. Spring-mediated devices were initially used because these were already commercially available and only had to be modified slightly to meet our needs (Chapter 3). These spring-mediated devices were able to deliver a force of 500 g, which was considered sufficient for our purposes. Unfortunately, during the *in-vivo* test phase it appeared that the available springs were not strong enough to achieve daily distraction at the desired rate of 1 mm/d.

Based on the torque data from the previous discontinuous DO experiments, distraction forces up to approximately 1800 g appeared to be required. Therefore, a customized continuous distractor was designed, which was powered by a syringe pump (Chapters 3 and 5). The syringe pump delivered a force of 18 kg, which was sufficient. The experimental set-up also included animal jackets, a syringe pump, syringes, tubes and tethers for tube protection (Fig. 1).

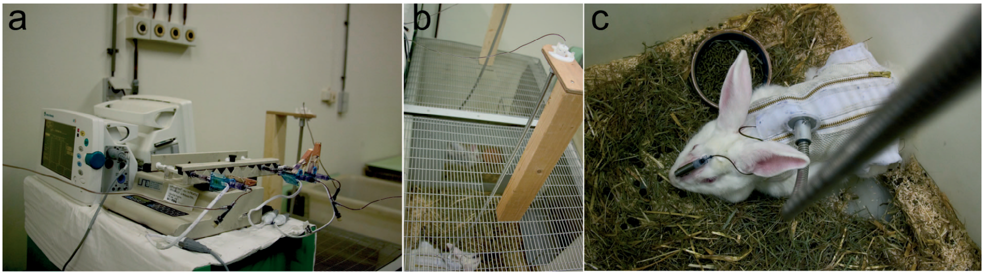

Figure 1

Experimental set-up: (a) The syringe pump can contain multiple syringes. Syringes and tubes are filled with a colored fluid, to be able to trace any possible leakages. (b) In the rabbit cage, the tube is protected by a metal tether, to prevent damage by the rabbit. The tether is long enough to allow the rabbit to reach any part of the cage. (c) The tether is connected to a jacket, and the tube is led through it to connect to the distractor.

During the experiments, the rabbits were allowed as much freedom of movement as possible. By trial and error, the procedure was modified where necessary until it was to our satisfaction. For instance, the initial flow rate (0.002 µl/h), which theoretically corresponded to the desired distraction rate of 1 mm/d, did not directly result in distraction. Apparently, pressure build-up at the start of distraction was delayed due to the slow flow rate. Therefore, flow rates needed to be adjusted regularly during distraction to maintain a distraction rate of 1 mm/d. Other complications during the test period included; pressure loss (and therefore no distraction), due to leakage or air bubbles in the system; jackets that did not fit the rabbits optimally, giving the rabbits a chance to escape from the jacket and damage the exposed tubes. All these issues were dealt with, or adequate measures were devised to cope with these issues would they occur again during the experiments.

Investigating the feasibility of automated continuous distraction is of great relevance, as continuous distraction would relieve the patient or caretaker from the task of activating the distractor. Up to date, manual activation is highly dependent on patient compliance; there is the risk of accidental erroneous handling of the device, such as turning it the wrong way; turning it too much or too little; not turning it at all because of fear or pain; or simply forgetting to turn it (this may particularly be the case when multiple daily activations are part of the protocol). Compliance to the distraction protocol is essential for the outcome of distraction.

Micro-CT analyses showed that continuous distraction resulted in accelerated bone formation compared with both low-rhythm and high-rhythm discontinuous distraction (Chapter 5). After three weeks of consolidation, bone volume in the continuous distraction group was significantly higher. Particularly, the higher bone volume was caused by an increase in trabecular number.

The histomorphometric analyses provided additional information about the organization of different tissues and cell types in the distraction area (Chapter 6). In some parts of the regenerate, higher osteoblast activity was seen as well as more blood vessels. The number of blood vessels appeared to correlate significantly positively with bone volume. Furthermore, mineral apposition rate was higher in the early stage of osteogenesis than in the late stage. Also, the continuous distraction protocol resulted in considerably more bone volume in the central part.

In a pig study, it was found that continuous distraction of the mandible resulted in completely osseous bridging after four weeks of consolidation, while after twelve weeks the regenerate had a ripe lamellar bony architecture, in contrast to discontinuous distraction.[14] In our studies none of the distraction gaps were completely bridged by mineralized bone after three weeks of consolidation, but the results imply that earlier complete osseous bridging can be expected when using a continuous distraction protocol compared to discontinuous distraction. This is clinically relevant, as accelerated osseous bridging, particularly in the central part of the regenerate, would reduce the consolidation period. Shortening the consolidation period, which usually may take up to several months, by accelerating osteogenesis would considerably benefit the patient.

Diagnostic methods

The methods for evaluating bone formation during distraction and consolidation need to be accurate, in order to be able to make a rational decision when to remove a distraction device. Removing the distractor too early, while the regenerated bone has not yet acquired the biomechanical properties to withstand full loading, may result in failure of the regenerate. In contrast, extending the neutral fixation period for too long may result in stress shielding, causing the bone to remodel accordingly to the absent mechanical stimuli (resulting in loss of bone volume), while the patient may also be subjected to physical or social limitations for a longer period of time. .

Several methods have been used in clinical and animal experimental studies to evaluate the regenerate. These include plain radiography, ultrasonography, ultrasonometry, microcomputed tomography (micro-CT), biomechanical testing, and dual emission X-ray absorptiometry (DXA).[16,17] In animal experimental research often plain radiography, ultrasonography, and micro-CT are preferred. In our studies, we have looked at the question whether ultrasonographic evaluation has a better predictive value for bone formation than plain radiographic evaluation (Chapters 4-6). A semi-quantitative 4-point score for bone formation was applied on both the radiographs and the ultrasonographs, after which correlation coefficients with the bone volumes as measured by quantitative micro-CT analysis were determined. While the 4-point semi-quantitative scores had been successfully used in other studies[17-19], they seem rather rough and inaccurate for such a delicate rhythm variable. We acknowledge that accuracy could have improved if a semi-quantitative score with more points had been used.

Results for the pooled data showed that ultrasonographic bone-fill scores correlated significantly stronger with the actual corresponding bone volumes (as measured by micro-CT) than plain radiographs did. These data imply that bone regenerate evaluation by ultrasonography more closely relates to the actual bone volume, and is therefore a better predictor for bone formation in the distraction area than plain radiography is.

The advantages of ultrasonographic evaluation over radiographic evaluation are that the higher sensitivity for changes in tissue characteristics may provide earlier evidence of bone formation, while the ease, minimal expense, and lack of radiation exposure may also make it a favorable tool.[18] Furthermore, complications can be detected early and precisely, and even differentiation between several kinds of complication is possible.[20,21] However, it should be stated that ultrasonographic evaluation can never completely replace radiographic evaluation. Because ultrasound is physically limited in penetrating mineralized bone tissue, it may not be a reliable tool in the later stages of consolidation. In the earlier stages of consolidation, ultrasonography has added value as it may reduce the total number of radiographs and therefore the total amount of radiation exposure. This may be relevant in clinical situations.

Rabbit model for craniofacial DO research

Many different animal models have been used for research on craniofacial distraction osteogenesis, ranging from rats to rhesus monkeys (Chapter 2). The rabbit model proved to be a suitable animal model for our research (Chapter 3). We acknowledge that it is unlikely that a single animal model will serve as the ideal model, but limiting the diversity of animal models used in craniofacial DO research would facilitate the comparisons between reports that have used the same animal model as more data for one specific appropriate animal model on a specific research subject will become available. For instance, the rat could serve as the animal model of choice when large numbers of animals are required because they are small, easy to handle, and relatively inexpensive. Disadvantages may be the size, which limits the amount of blood that can be collected, or limits the number of distractors that can be used.

Pigs on the other hand are more appropriate when physiology, size, and shape are relevant parameters; these resemble the human situation to a considerable degree. Any commercially available distractor for clinical use can also be used in the pig, saving costs for designing and producing custom-made distractors. Disadvantages are the high costs for purchase, housing and handling of the animals.

Positioned in between the former animal models, rabbits are large enough to fit a number of the currently available clinical distractors, but still small enough to be relatively inexpensive regarding costs for purchase and housing. This also facilitates the use of large numbers of rabbits for statistical power. Therefore, rabbits are regarded as the best compromise between cost and size.[22] In addition, bone accretion and peak bone mass are similar to those of humans, and rabbits also show true skeletal maturity.[23] Rabbits tolerate handling very well, which makes it less stressful for researchers and animal caretakers as well as the rabbits themselves.

The rabbits in our studies were subjected to nasal bone distraction. All rabbits were operated by the same researcher (U.M. Djasim). During our studies, one rabbit died after premedication was administrated prior to the actual surgery. Another rabbit suffered and survived an epileptic seizure during surgery. These were the only cases showing animal-related complications (4 % of the total number of rabbits that have been subjected to experiments). No other animal-related complications occurred; therefore mortality and morbidity rates were low.

The nasal bones were surgically easily accessible, while the shape and the size of the nasal bones also allowed for the use of currently commercially available distractors. No large muscle groups are attached to the nasal bones; therefore the vector of distraction was not influenced. In combination with adequate neutral fixation of the regenerate, the resulting stability prevented pseudoarthrosis. Nasal bone distraction did not affect mastication, enabling the rabbits to drink and eat normally. Nearly all rabbits gained weight, indicating good health and no stress. This assumes that the animals did not suffer from the distraction procedures. Also, the animal caretakers did not witness any severe pain or distress throughout the distraction experiments. In addition, the rabbits showed normal grooming behavior, which would less likely be seen in animals in severe pain or distress. There was no occurrence of meningitis or airway related complications. We did not investigate the relapse rate, but relapse was unlikely to have occurred during our experiments, due to the fact that the distractor provided a stable fixation throughout the complete consolidation phase. Also, tissue harvesting of the nasal bones as well as processing for micro-CT analyses and histology was uncomplicated. These factors make the rabbit a suitable animal model for research into craniofacial distraction osteogenesis.

Conclusion

Our studies provided quantitative data on the effect of distraction rhythms on regenerate quality and quantity, using statistically relevant numbers of experimental animals. The selected animal model was the rabbit, which was appropriate because of the good balance between size and cost. Nasal bone distraction was performed without complications. Data of discontinuous distraction experiments showed that bone regeneration did not markedly improve when triple daily activation of the distractor was used compared to single daily activation (given the same rate of distraction). However, a continuous distraction rhythm resulted in significantly more bone regeneration than both discontinuous distraction protocols. Based on the ultrastructural and histological data, we conclude that continuous distraction significantly accelerates osteogenesis in our model.

Future perspectives

It is known that tissue regeneration is compromised after radiotherapy, and the use of distraction osteogenesis in irradiated craniofacial bones has been studied before. These studies all used discontinuous distraction protocols. While in some studies no positive results were found[24-30], other studies did show the feasibility of distraction osteogenesis in irradiated bones[31-37]. This indicates that there is currently no consensus on the usefulness of distraction osteogenesis in irradiated bones. The number of studies dedicated to this topic is limited, due to the many problems that are associated with DO after radiotherapy. This may raise an interesting question for further research; does continuous distraction stimulate osteogenesis (or tissue regeneration in general) to such an extent, that it also compensates for the negative effects of radiotherapy on regenerative capabilities of tissues in patients who are treated for head and neck tumors?

In the case of oral cancer, treatment often consists of resection of the tumor, followed by radiotherapy. The amount of radiation that is received by the irradiated tissues is lethal to tumor cells, but also severely damages surrounding healthy tissues. Radiotherapy creates a hypoxic, hypocellular and hypovascular environment, which reduces the regenerative capabilities of the irradiated tissues. This may lead to bone defects, trismus, dysfunctional salivary glands, mucositis, and damage to the teeth and the mandibular condyle, for example.

Mutilation, as a result of tumor resection and radiation therapy, can lead to severe functional and esthetic problems. These patients are eligible for reconstructive surgery (such as distraction osteogenesis), however the outcome of the reconstructive treatment is negatively influenced by postoperative radiotherapy. Treatments that prevent and reduce radiation damage in the surrounding healthy tissues would considerably improve the patient's quality of life.

We are aiming to study treatments in animal models that optimize the quality of the irradiated tissues in order to improve the outcome of reconstructive surgery on irradiated tissues. Large bone defects due to tumor resection could then be treated using continuous distraction protocols. When distraction osteogenesis proves to be feasible there may be a lesser need for tissue transplantation, and after surgery the patient is allowed to leave the hospital sooner. No need for transplant surgery also means no donor site morbidity and associated pain and discomfort. Furthermore, the patient may be able to participate sooner in daily social and professional activities, while the shorter hospitalization time results in reduced health care costs for the patient.

To address this issue, we have designed a research project that will study the effects of various treatments on tissue quality after radiation therapy in an animal model, in order to use continuous distraction osteogenesis in irradiated bones. The aim of this study is twofold. The first part of the project focuses on optimizing the quality of the tissues after radiotherapy. Therapies that stimulate osteogenesis and histogenesis will be studied in a rat model. Rats are inexpensive regarding purchase costs and housing, and are easy to handle. We have extensive experience with anesthesiology and surgery in rats. The mandible of the rats will be exposed to a single radiation dose (the bioeffective equivalent of 45x2 Gy) which is considered sufficient to induce noticeable signs of radiogenic damage without creating an artificial bone defect.[38]

Subsequently, a number of treatments will be studied. (A) Rats will be subjected to twenty hyperbaric oxygen (HBO) therapy sessions (90 minutes each), during which the rats will breathe 100 % oxygen at a pressure of 2.4 ATA (*i.e.*, 2.4

times the atmosphere pressure at sea level).[39] This will result in increased partial pressure of oxygen in the irradiated tissues and increased oxygen transport capacity of the blood, which will stimulate tissue regeneration. (B) In addition, rats will be treated with RGTA (ReGeneraTing Agent). The synthetic heparan sulfate mimic RGTA-OTR4131 stimulates osteogenesis and vasculogenesis in bone defects.[40] RGTA-OTR4131 may play a key role in restoring the normal chemical signaling between the cells in a tissue leading to improved tissue regeneration. (C) Furthermore, bone morphogenetic protein-2 (BMP-2) is one of the most potent growth factors that play a role in inducing the differentiation of stem cells to osteoblasts, while vascular endothelial growth factor (VEGF) is essential for the formation of new blood vessels. Adequate blood supply is important for successful bone formation. Administration of exogenous BMP-2 and VEGF should therefore stimulate tissue regeneration. (D) Rats will also be subjected to a combination of these treatments. Bone and soft tissue will be analyzed by means of micro-CT, histology and immunohistochemistry. From the results, the optimal (combined) treatment for bone and soft tissue quality will follow.

The second part of the project focuses on the application of continuous distraction on irradiated bone. The results from the first part of the project are used to optimize the quality of bone and soft tissue prior to distraction. For this project, the rabbit may serve as a more appropriate animal model because currently available distractors fit easier to the rabbit craniofacial bones than to the rat craniofacial bones (which are considerably smaller). We have extensive experience with rabbit anesthesiology and surgery as well. A radiation protocol of 27 Gy divided in five fraction of 5.4 Gy will be used, which is equivalent to 50 Gy in 25 fractions.[31] 50 Gy is considered to be the threshold level where harmful effects of irradiation increase significantly.[41] Three months after the end of radiotherapy, a continuous distractor will be applied to the nasal bones and continuous distraction will be performed. At various time points, rabbits will be sacrificed in order to determine the quality of the bone regenerate and soft tissues. Eventually, the optimal timing for distractor removal will be determined.

These preclinical projects aim to optimize bone and soft tissue quality for reconstructive surgical treatment in general, and continuous distraction osteogenesis in particular. Benefits are that less tissue transplants are needed for reconstruction, and patients will have a shorter hospitalization and recovery time, meaning that they will be able to participate in social and professional activities earlier. In addition, expenses associated with the treatment are reduced due to the shorter hospitalization time. These factors will contribute considerably to the patient's quality of life.

References

1. Glowacki J, Shusterman EM, Troulis M, Holmes R, Perrott D, and Kaban LB. Distraction osteogenesis of the porcine mandible: histomorphometric evaluation of bone. Plast Reconstr Surg 2004;113(2):566-573.
2. Tavakoli K, Walsh WR, Bonar F, Smart R, Wulf S, and Poole MD. The role of latency in mandibular osteodistraction. J Craniomaxillofac Surg 1998;26(4):209-219.
3. Troulis MJ, Glowacki J, Perrott DH, and Kaban LB. Effects of latency and rate on bone formation in a porcine mandibular distraction model. J Oral Maxillofac Surg 2000;58(5):507-513; discussion 514.
4. Castano FJ, Troulis MJ, Glowacki J, Kaban LB, and Yates KE. Proliferation of masseter myocytes after distraction osteogenesis of the porcine mandible. J Oral Maxillofac Surg 2001;59(3):302-307.
5. Aida T, Yoshioka I, Tominaga K, and Fukuda J. Effects of latency period in a rabbit mandibular distraction osteogenesis. Int J Oral Maxillofac Surg 2003;32(1):54-62; discussion 63.
6. Paccione MF, Mehrara BJ, Warren SM, Greenwald JA, Spector JA, Luchs JS, and Longaker MT. Rat mandibular distraction osteogenesis: latency, rate, and rhythm determine the adaptive response. J Craniofac Surg 2001;12(2):175-182.
7. Singare S, Li D, Liu Y, Wu Z, and Wang J. The effect of latency on bone lengthening force and bone mineralization: an investigation using strain gauge mounted on internal distractor device. Biomed Eng OnLine 2006;5(1):18.
8. Li J, Hu J, Wang D, Tang Z, and Gao Z. Biomechanical properties of regenerated bone by mandibular distraction osteogenesis. Chin J Traumatol 2002;5(2):67-70.

9. Ryoyama D, Sawaki Y, and Ueda M. Experimental study of mechanical analysis in mandibular lengthening. Application of strain gauge measurement. Int J Oral Maxillofac Surg 2004;33(3):294-300.
10. Smith SW, Sachdeva RC, and Cope JB. Evaluation of the consolidation period during osteodistraction using computed tomography. Am J Orthod Dentofacial Orthop 1999;116(3):254-263.
11. Ilizarov GA. The tension-stress effect on the genesis and growth of tissues: Part II. The influence of the rate and frequency of distraction. Clin Orthop Relat Res 1989;(239):263-285.
12. Hasse AR, Porksen M, and Zimmermann CE. Bilateral mandibular distraction in adult dogs with an epiperiosteal distractor. Br J Oral Maxillofac Surg 2005;43(2):105-112.
13. Al Ruhaimi KA. Comparison of different distraction rates in the mandible: an experimental investigation. Int J Oral Maxillofac Surg 2001;30(3):220-227.
14. Kessler P, Neukam FW, and Wiltfang J. Effects of distraction forces and frequency of distraction on bony regeneration. Br J Oral Maxillofac Surg 2005;43(5):392-398.
15. Cope JB, and Samchukov ML. Mineralization dynamics of regenerate bone during mandibular osteodistraction. Int J Oral Maxillofac Surg 2001;30(3):234-242.
16. Farhadieh RD, Gianoutsos MP, Dickinson R, and Walsh WR. Effect of distraction rate on biomechanical, mineralization, and histologic properties of an ovine mandible model. Plast Reconstr Surg 2000;105(3):889-895.
17. Kaban LB, Thurmuller P, Troulis MJ, Glowacki J, Wahl D, Linke B, Rahn B, and Perrott DH. Correlation of biomechanical stiffness with plain radiographic and ultrasound data in an experimental mandibular distraction wound. Int J Oral Maxillofac Surg 2003;32(3):296-304.
18. Thurmuller P, Troulis M, O'Neill MJ, and Kaban LB. Use of ultrasound to assess healing of a mandibular distraction wound. J Oral Maxillofac Surg 2002;60(9):1038-1044.
19. Troulis MJ, Coppe C, O'Neill MJ, and Kaban LB. Ultrasound: assessment of the distraction osteogenesis wound in patients undergoing mandibular lengthening. J Oral Maxillofac Surg 2003;61(10):1144-1149.

20. Juenger TH, Klingmueller V, and Howaldt HP. Application of ultrasound in callus distraction of the hypoplastic mandible: an additional method for the follow-up. J Craniomaxillofac Surg 1999;27(3):160-167.
21. Nocini PF, Albanese M, Wangerin K, Fior A, Trevisiol L, and Kretschmer W. Distraction osteogenesis of the mandible: evaluation of callus distraction by B-scan ultrasonography. J Craniomaxillofac Surg 2002;30(5):286-291.
22. Al-Sebaei MO, Gagari E, and Papageorge M. Mandibular distraction osteogenesis: a rabbit model using a novel experimental design. J Oral Maxillofac Surg 2005;63(5):664-672.
23. Norris SA, Pettifor JM, Gray DA, and Buffenstein R. Calcium metabolism and bone mass in female rabbits during skeletal maturation: effects of dietary calcium intake. Bone 2001;29(1):62-69.
24. Clark CL, Strider J, Hall C, Ferguson HW, Armstrong KL, Runner RR, and Baur DA. Distraction Osteogenesis in Irradiated Rabbit Mandibles With Adjunctive Hyperbaric Oxygen Therapy. J Oral Maxillofac Surg 2006;64(4):589-593.
25. Tsuchiya H, Uehara K, Sakurakichi K, Watanabe K, Matsubara H, and Tomita K. Distraction osteogenesis after irradiation in a rabbit model. J Orthop Sci 2005;10(6):627-633.
26. Muhonen A, Saamanen AM, Peltomaki T, and Happonen RP. The effect of irradiation and hyperbaric oxygenation (HBO) on extracellular matrix of the condylar cartilage after mandibular distraction osteogenesis in the rabbit. Int J Oral Maxillofac Surg 2006;35(1):79-87.
27. Muhonen A, Haaparanta M, Gronroos T, Bergman J, Knuuti J, Hinkka S, and Happonen RP. Osteoblastic activity and neoangiogenesis in distracted bone of irradiated rabbit mandible with or without hyperbaric oxygen treatment. Int J Oral Maxillofac Surg 2004;33(2):173-178.
28. Muhonen A, Muhonen J, Minn H, Kulmala J, Klossner J, Teras M, Knuuti J, and Happonen RP. The effects of irradiation and hyperbaric oxygen on bone formation during rabbit mandibular distraction. Arch Oral Biol 2002;47(10):701-707.

29. Muhonen A, Peltomaki T, Hinkka S, and Happonen RP. Effect of mandibular distraction osteogenesis on temporomandibular joint after previous irradiation and hyperbaric oxygenation. Int J Oral Maxillofac Surg 2002;31(4):397-404.
30. Holmes SB, Lloyd T, Coghlan KM, and Newman L. Distraction osteogenesis of the mandible in the previously irradiated patient. J Oral Maxillofac Surg 2002;60(3):305-309.
31. Shao Z, Liu B, Liu Y, Liu W, Liu R, Peng Q, and Liu L. Distraction osteogenesis in the irradiated rabbit mandible. J Plast Reconstr Aesthet Surg 2006;59(2):181-187.
32. Gonzalez-Garcia R, Rodriguez-Campo FJ, Naval-Gias L, Sastre-Perez J, and Diaz-Gonzalez FJ. The effect of radiation in distraction osteogenesis for reconstruction of mandibular segmental defects. Br J Oral Maxillofac Surg 2007;45(4):314-316.
33. Girod A, Roger T, Breton P, and Bouletreau P. Experimental study of mineralization in mandibular bone distraction with irradiation during the consolidation phase. J Craniomaxillofac Surg 2005;33(6):386-394.
34. Muhonen A, Muhonen J, Lindholm TC, Minn H, Klossner J, Kulmala J, and Happonen RP. Osteodistraction of a previously irradiated mandible with or without adjunctive hyperbaric oxygenation: an experimental study in rabbits. Int J Oral Maxillofac Surg 2002;31(5):519-524.
35. Taub PJ, Bradley JP, and Kawamoto HK. Closure of an oronasal fistula in an irradiated palate by tissue and bone distraction osteogenesis. J Craniofac Surg 2001;12(5):495-499; discussion 500.
36. Gantous A, Phillips JH, Catton P, and Holmberg D. Distraction osteogenesis in the irradiated canine mandible. Plast Reconstr Surg 1994;93(1):164-168.
37. Price DL, Moore EJ, Friedman O, Garces YI, Kee AY, and Furutani KM. Effect of Radiation on Segmental Distraction Osteogenesis in Rabbits. Arch Facial Plast Surg 2008;10(3):159-163.
38. Niehoff P, Springer IN, Açil Y, Lange A, Marget M, Roldán JC, Köppe K, Warnke PH, Kimmig B, and Wiltfang J. HDR brachytherapy irradiation of the jaw - as a new experimental model of radiogenic bone damage. J Craniomaxillofac Surg 2008;36(4):203-209.

39. Williamson RA. An experimental study of the use of hyperbaric oxygen to reduce the side effects of radiation treatment for malignant disease. Int J Oral Maxillofac Surg 2007;36(6):533-540.

40. Lafont J, Blanquaert F, Colombier ML, Barritault D, Carueelle JP, and Saffar JL. Kinetic study of early regenerative effects of RGTA11, a heparan sulfate mimetic, in rat craniotomy defects. Calcif Tissue Int 2004;75(6):517-525.

41. Adamo AK, and Szal RL. Timing, results, and complications of mandibular reconstructive surgery: report of 32 cases. J Oral Surg 1979;37(10):755-763.

Chapter

8

Summary

Samenvatting

Summary

Distraction osteogenesis (DO) is defined as the formation of new bone tissue between bone segments that are divided by an osteotomy and then gradually separated by exerting an external force to the mobile bone segment(s). The resulting callus tissue in the distraction gap will eventually mineralize, creating a new bridge of bone tissue between the osteotomy edges of the original bone segments.

This bone lengthening technique was first reported in 1905 by Codivilla. Significant contributions to the refinement of DO were made by Ilizarov in the 1950s and onwards. In 1973, Snyder and co-workers succeeded in applying DO on the mandible of a dog. Two decades later, McCarthy and colleagues published the first report on the clinical use of craniofacial DO. Ever since, craniofacial DO has been a widely accepted tool for the treatment of acquired and congenital deformities of the skull bones.

A distraction protocol typically consists of the following stages; a latency period, a distraction period (during which the rate of distraction as well as the rhythm of distraction may influence the eventual outcome), the consolidation period, and the remodeling period. This thesis focused on the effect of the rhythm of distraction on bone regeneration.

In **Chapter 2**, a review paper was presented, which summarized and discussed the animal experimental papers on craniofacial DO published between 1973 and 2007. The included papers had all investigated the outcome of craniofacial DO resulting from variations in a single distraction parameter while standardizing the other distraction parameters. On the basis of these data, recommendations were given that can be used as basic guidelines for further animal experimental studies on craniofacial DO. In short, a latency period of 5-7 days showed the best results, although some studies had equally good results with no latency period at all. A rate of distraction of approximately 1 mm/d was recommended, but can be twice as slow in rats. A distraction rhythm of one or two daily activations of the distractor were considered to be sufficient, although pig studies suggested that a continuous rhythm of distraction would accelerate the bone formation process. After a consolidation period of 6-8 weeks, the distractor may safely be removed.

In most cases, animal experimental (sub)group sizes were rather small. This work in the present thesis was performed on statistically relevant numbers of animals per group. The animal model of choice was the rabbit. Our experiences with the rabbit as a model for research into craniofacial DO were reported in **Chapter 3**.

In total, 52 skeletally mature New-Zealand White rabbits were used. In ten cases (19 % of total), complications developed during surgery or experiments. Of these ten cases, only two (4 %) showed animal-related complications. The other eight cases (15 %) involved distractor-related complications. Rabbits have several advantages over other animal models regarding research into craniofacial DO. Rabbits are the smallest laboratory animals still large enough to allow for the use of commercially available distractors, which eliminates the need for spending time and budget on designing and producing custom-built distraction devices. Housing and handling are relatively easy. Patterns of bone accretion and peak bone mass profiles are similar to those of humans. Also, rabbits show true skeletal maturity. For our experimental purposes, the nasal bones were easily accessible, while shape and size allowed for the use of commercially available distractors as well as custom-made experimental distractors. The vector of distraction was not influenced due to the absence of large muscle groups attached to the nasal bones. Although it is unlikely that a single animal model can be ideal for research into craniofacial DO, the rabbit may be the best compromise between cost and size.

In **Chapter 4**, two distraction rhythms that are generally applied in conventional distraction protocols were compared with each other. While all other distraction parameters were standardized, one group of rabbits underwent distraction using single daily activation of the distractor, while another group underwent distraction using triple daily activation of the distractor. Bone architecture parameters did not change significantly. Therefore, an increase in distraction rhythm from single to triple daily activation of the distractor did not significantly enhance or accelerate osteogenesis.

The effects of a continuous rhythm of distraction on bone regeneration were investigated in **Chapter 5**. A custom-made continuous distraction device, based on hydraulic principles, was introduced. This device was able to perform automated non-stop distraction. The results of the continuous DO experiments were compared with the results from the previous discontinuous DO experiments. It was found that continuous distraction resulted in accelerated bone formation compared with discontinuous distraction. Furthermore, bone-fill scores based on ultrasonography showed a significant correlation with actual bone volumes, in contrast to bone-fill scores based on plain radiography.

Histomorphometric analysis revealed that continuous distraction resulted in higher osteoblastic activity and more blood vessels. Bone volume and number of blood vessels showed a significant correlation in the central part of the regenerate. There, a significantly higher bone volume was found compared with discontinuous distraction (**Chapter 6**). Normally, bone formation in this part of the regenerate would take longer time compared with the other parts of the regenerate. Therefore, accelerating bone formation, particularly in the central part, by using a continuous rhythm of distraction would likely result in shorter consolidation time, thereby reducing the total treatment time.

In conclusion, while increasing the rhythm of distraction from single to triple daily activation did not show significant improvements of the distraction outcome, a continuous rhythm of distraction showed accelerated bone formation compared with discontinuous rhythms of distraction.

Samenvatting

Distractie osteogenese (DO) is een behandeling waarbij nieuw botweefsel wordt gevormd tussen botsegmenten die van elkaar worden gescheiden door middel van een osteotomie en vervolgens geleidelijk uit elkaar worden gedreven. Het nieuw ontstane weefsel in de distractieruimte tussen de botsegmenten zal uiteindelijk mineraliseren, waardoor nieuw botweefsel de distractieruimte overbrugt.

Deze techniek voor het verlengen van botten (pijpbeenderen) werd voor het eerst beschreven door Codivilla in 1905. De techniek werd aanmerkelijk verfijnd door de inspanningen van Ilizarov in de jaren ’50 en later. In 1973 slaagden Snyder en collega’s er in om DO toe te passen in schedelbotten, in dit geval de onderkaak van een hond. Twee decennia later verscheen de eerste publicatie over succesvolle klinische toepassing van craniofaciale DO door McCarthy en collega’s. Sindsdien is craniofaciale DO een algemeen geaccepteerde behandeling voor het corrigeren van aangeboren en verworven botdefecten van schedelbotten.

Een distractieprotocol bestaat gewoonlijk uit een aantal stadia; een latentieperiode, een distractieperiode (waarbij de snelheid van distractie en het ritme van distractie een effect kunnen hebben op het eindresultaat), een consolidatieperiode en een remodelleringsperiode. Het onderzoek in dit proefschrift richtte zich op de effecten van het ritme van distractie op botregeneratie.

In **Hoofdstuk 2** werden dierexperimentele studies naar craniofaciale DO, gepubliceerd tussen 1973 en 2007, geanalyseerd en bediscussiëerd. De geïncludeerde studies onderzochten het eindresultaat van craniofaciale DO, waarbij gevariëerd werd in één enkele distractie parameter terwijl alle andere parameters werden gestandaardiseerd. Op basis van de gevonden data werd getracht aanbevelingen te formuleren die kunnen worden gebruikt als richtlijnen voor verder dierexperimenteel onderzoek naar craniofaciale DO. Samengevat; een latentietijd van 5-7 dagen leverde beste resultaten op, alhoewel enkele studies even goede resultaten behaalde zónder latentietijd. Een distractiesnelheid van circa 1 mm/d werd aangeraden, maar kan in het geval van ratten gehalveerd worden. Een distractieritme van één of twee dagelijkse activaties van de distractor is voldoende, hoewel resultaten uit enkele varkensstudies suggereren dat een continu distractieritme het botvormingsproces versnelt. Na een consolidatietijd van 6-8 weken kan een distractor veilig verwijderd worden.

In de meeste studies werd gebruik gemaakt van vrij kleine dierexperimentele (sub)groepen. Het werk in dit proefschrift is uitgevoerd met statistisch relevante aantallen proefdieren per groep. Het gekozen diermodel was het konijn. Onze ervaringen met het konijn als diermodel voor onderzoek naar craniofaciale DO werden beschreven in **Hoofdstuk 3**.

In totaal werden 52 konijnen van het ras Witte Nieuw-Zeelander gebruikt. In tien gevallen (19 % van het totaal) ontwikkelden zich complicaties tijdens de operatie of het experiment. Van deze tien gevallen lag de oorzaak van de complicaties bij slechts twee gevallen (4 %) bij het dier. In de acht andere gevallen (15 %) waren de complicaties distractor-gerelateerd. Konijnen hebben verscheidene voordelen ten opzichte van andere diermodellen met betrekking tot onderzoek naar craniofaciale DO. Konijnen zijn de kleinste laboratoriumdieren die nog groot genoeg zijn voor het gebruik van commerciëel verkrijgbare distractoren, zodat er geen noodzaak is om tijd en budget te besteden aan het ontwikkelen en produceren van op maat gemaakte distractoren. Konijnen zijn relatief gemakkelijk te huisvesten en te hanteren. Enkele botfysiologische aspecten zijn vergelijkbaar met die van de mens. Voor onze experimentele doeleinden werden de neusbotten gebruikt; deze waren chirurgisch gemakkelijk toegankelijk, terwijl de vorm en het formaat geschikt waren voor zowel commerciëel verkrijgbare distractoren als experimentele distractoren. De vector van distractie werd niet beïnvloed aangezien er geen grote spiergroepen aan de neusbotten hechten. Hoewel het onwaarschijnlijk is dat één enkel diermodel kan fungeren als het ideale model voor onderzoek naar craniofaciale DO, biedt het konijn het best mogelijke compromise tussen kosten en grootte.

In Hoofdstuk 4 werden twee distractieritmen, die vaak worden gebruikt in conventionele distractie protocollen, met elkaar vergeleken. Terwijl alle andere distractieparameters werden gestandaardiseerd, onderging een groep konijnen distractie aan de hand van één dagelijkse activatie van de distractor, terwijl een andere groep konijnen distractie onderging aan de hand van drie dagelijkse activaties van de distractor. Botarchitectuur-parameters veranderden niet significant. Derhalve resulteerde een toename van distractieritme van één enkele dagelijkse activatie naar drievoudige dagelijkse activatie van de distractor niet in significant verbeterde of versnelde osteogenese.

De effecten van een continu distractieritme op botregeneratie werden onderzocht in **Hoofdstuk 5**. Een op maat gemaakte continue distractor, gebaseerd op hydraulische principes, werd geïntroduceerd. Deze distractor was in staat om automatisch non-stop distractie uit te voeren. De resultaten van de continue distractie-experimenten werden vergeleken met de resultaten van de discontinue distractie-experimenten. Continue distractie bleek botformatie te versnellen in vergelijking met discontinue distractie. Tevens bleken botscores gebaseerd op ultrasonografische beelden een significante correlatie te vertonen met de daadwerkelijke botvolume-waarden, in tegenstelling tot botscores gebaseerd op röntgenfoto’s.

Histomorfometrische analyse toonde aan dat continue distractie resulteerde in hogere osteoblast-activiteit en meer bloedvaten. Botvolume bleek significant te correleren met het aantal bloedvaten in het centrale deel van het regeneraat. Daar was de hoeveelheid nieuw gevormd bot groter vergeleken met de discontinue distractie-groepen (**Hoofdstuk 6**). Normaliter duurt, vergeleken met de andere delen van het regeneraat, langer voordat in het centrale deel van het regeneraat bot wordt gevormd. Door de versnelde botvorming als gevolg van continue distractie, met name in het centrale deel van het regeneraat, kan de consolidatieperiode worden ingekort, zodat ook de totale behandeltijd kan worden gereduceerd.

Uit de gegevens kan worden geconcludeerd dat het verhogen van het distractieritme van één enkele dagelijkse activatie naar drievoudige dagelijkse activatie van de distractor het eindresultaat niet significant verbetert. Echter, een continu ritme van distractie resulteert in versnelde botvorming vergeleken met discontinue ritmen van distractie.

Chapter

9

Bibliography

Bibliography

Complete list of references, sorted in alphabetical order.

Adamo AK and Szal RL. Timing, results, and complications of mandibular reconstructive surgery: report of 32 cases. Journal of Oral Surgery 1979;37(10):755-763.

Aida T, Yoshioka I, Tominaga K and Fukuda J. Effects of latency period in a rabbit mandibular distraction osteogenesis. International Journal of Oral and Maxillofacial Surgery 2003;32(1):54-62; discussion 63.

Al Ruhaimi KA. Comparison of different distraction rates in the mandible: an experimental investigation. International Journal of Oral and Maxillofacial Surgery 2001;30(3):220-227.

Al-Aql ZS, Alagl AS, Graves DT, Gerstenfeld LC and Einhorn TA. Molecular Mechanisms Controlling Bone Formation during Fracture Healing and Distraction Osteogenesis. Journal of Dental Research 2008;87(2):107-118.

Al-Sebaei MO, Gagari E and Papageorge M. Mandibular distraction osteogenesis: a rabbit model using a novel experimental design. Journal of Oral and Maxillofacial Surgery 2005;63(5):664-672.

Amir LR, Becking AG, Jovanovic A, Perdijk FBT, Everts V and Bronckers ALJJ. Formation of new bone during vertical distraction osteogenesis of the human mandible is related to the presence of blood vessels. Clinical Oral Implants Research 2006;17(4):410-416.

Aronson J, Gao GG, Shen XC, McLaren SG, Skinner RA, Badger TM and Lumpkin CK, Jr. The effect of aging on distraction osteogenesis in the rat. Journal of Orthopaedic Research 2001;19(3):421-427.

Ayoub AF and Richardson W. A new device for microincremental automatic distraction osteogenesis. British Journal of Oral and Maxillofacial Surgery 2001;39(5):353-355.

Ayoub AF, Richardson W and Barbenel JC. Mandibular elongation by automatic distraction osteogenesis: The first application in humans. British Journal of Oral and Maxillofacial Surgery 2005;43(4):324-328.

Ayoub AF, Richardson W, Koppel D, Thompson H, Lucas M, Schwarz T, Smith L and Boyd J. Segmental mandibular reconstruction by microincremental automatic distraction osteogenesis: an animal study. British Journal of Oral and Maxillofacial Surgery 2001;39(5):356-364.

Bail HJ, Kolbeck S, Krummrey G, Weiler A, Windhagen HJ, Hennies K, Raun K and Raschke MJ. Ultrasound can predict regenerate stiffness in distraction osteogenesis. Clinical Orthopedics and Related Research 2002;404:362-367.

Bivin WS and Timmons EH: Basic Biomethodology; in S. H. Weisbroth, R. E. Flatt, A. L. Kraus, (eds), The Biology of the Laboratory Rabbit; Academic Press, Inc., 1974, pp 73-90.

Bouletreau PJ, Warren SM and Longaker MT. The molecular biology of distraction osteogenesis. Journal of Cranio-Maxillofacial Surgery 2002;30(1):1-11.

Byun J-H, Park B-W, Kim J-R and Lee J-H. Expression of vascular endothelial growth factor and its receptors after mandibular distraction osteogenesis. International Journal of Oral and Maxillofacial Surgery 2007;36(4):338-344.

Cano J, Campo J, Gonzalo JC and Bascones A. Consolidation period in alveolar distraction: a pilot histomorphometric study in the mandible of the beagle dog. International Journal of Oral and Maxillofacial Implants 2006;21(3):380-391.

Castano FJ, Troulis MJ, Glowacki J, Kaban LB and Yates KE. Proliferation of masseter myocytes after distraction osteogenesis of the porcine mandible. Journal of Oral and Maxillofacial Surgery 2001;59(3):302-307.

Cheung LK and Zhang Q. Radiologic characterization of new bone generated from distraction after maxillary bone transport. Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology and Endodontology 2003;96(2):234-242.

Cheung LK, Zhang Q, Zhang ZG and Wong MC. Reconstruction of maxillectomy defect by transport distraction osteogenesis. International Journal of Oral and Maxillofacial Surgery 2003;32(5):515-522.

Cheung LK and Zheng LW. Effect of recombinant human bone morphogenetic protein-2 on mandibular distraction at different rates in an experimental model. Journal of Craniofacial Surgery 2006;17(1):100-108; discussion 109-110.

Cheung LK, Zheng LW and Ma L. Effect of distraction rates on expression of bone morphogenetic proteins in rabbit mandibular distraction osteogenesis. Journal of Cranio-Maxillofacial Surgery 2006;34(5):263-269.

Choi IH, Chung CY, Cho TJ and Yoo WJ. Angiogenesis and mineralization during distraction osteogenesis. Journal of Korean Medical Science 2002;17(4):435-447.

Chopra S and Enepekides DJ. The role of distraction osteogenesis in mandibular reconstruction. Current Opinion in Otolaryngology and Head and Neck Surgery 2007;15(4):197-201.

Clark CL, Strider J, Hall C, Ferguson HW, Armstrong KL, Runner RR and Baur DA. Distraction Osteogenesis in Irradiated Rabbit Mandibles With Adjunctive Hyperbaric Oxygen Therapy. Journal of Oral and Maxillofacial Surgery 2006;64(4):589-593.

Codivilla A. On the means of lengthening, in the lower limbs, the muscles and tissues which are shortened through deformity. American Journal of Orthopedic Surgery 1905;2:353-357.

Cope JB and Samchukov ML. Regenerate bone formation and remodeling during mandibular osteodistraction. Angle Orthodontist 2000;70(2):99-111.

Cope JB and Samchukov ML. Mineralization dynamics of regenerate bone during mandibular osteodistraction. International Journal of Oral and Maxillofacial Surgery 2001;30(3):234-242.

Cope JB, Samchukov ML and Muirhead DE. Distraction osteogenesis and histogenesis in beagle dogs: the effect of gradual mandibular osteodistraction on bone and gingiva. Journal of Periodontology 2002;73(3):271-282.

Djasim UM, Hekking-Weijma JM, Wolvius EB, van Neck JW and van der Wal KGH. Rabbits as a model for research into craniofacial distraction osteogenesis. British Journal of Oral and Maxillofacial Surgery 2008a;*In Press.*

Djasim UM, Wolvius EB, Van Neck JW, Van Wamel A, Weinans H and Van Der Wal KGH. Single versus triple daily activation of the distractor: No significant effects of frequency of distraction on bone regenerate quantity and architecture. Journal of Cranio-Maxillofacial Surgery 2008b;36(3):143-151.

Djasim UM, Wolvius EB, van Neck JW, Weinans H and van der Wal KGH. Recommendations for optimal distraction protocols for various animal models on the basis of a systematic review of the literature. International Journal of Oral and Maxillofacial Surgery 2007;36(10):877-883.

Egermann M, Goldhahn J and Schneider E. Animal models for fracture treatment in osteoporosis. Osteoporosis International 2005;16 Suppl 2:S129-138.

Fang TD, Salim A, Xia W, Nacamuli RP, Guccione S, Song HM, Carano RA, Filvaroff EH, Bednarski MD, Giaccia AJ and Longaker MT. Angiogenesis is required for successful bone induction during distraction osteogenesis. Journal of Bone and Mineral Research 2005;20(7):1114-1124.

Farhadieh RD, Gianoutsos MP, Dickinson R and Walsh WR. Effect of distraction rate on biomechanical, mineralization, and histologic properties of an ovine mandible model. Plastic and Reconstructive Surgery 2000;105(3):889-895.

Feng X, Tuo X, Chen F, Wu W, Ding Y, Duan Y and Lin Z. Ultrastructural cell response to tension stress during mandibular distraction osteogenesis. British Journal of Oral and Maxillofacial Surgery 2008;46(7):527-532.

Fisher E, Staffenberg DA, McCarthy JG, Miller DC and Zeng J. Histopathologic and biochemical changes in the muscles affected by distraction osteogenesis of the mandible. Plastic and Reconstructive Surgery 1997;99(2):366-371.

Gaggl A, Schultes G, Regauer S and Karcher H. Healing process after alveolar ridge distraction in sheep. Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology and Endodontology 2000;90(4):420-429.

Gantous A, Phillips JH, Catton P and Holmberg D. Distraction osteogenesis in the irradiated canine mandible. Plastic and Reconstructive Surgery 1994;93(1):164-168.

Girod A, Roger T, Breton P and Bouletreau P. Experimental study of mineralization in mandibular bone distraction with irradiation during the consolidation phase. Journal of Cranio-Maxillofacial Surgery 2005;33(6):386-394.

Glowacki J, Shusterman EM, Troulis M, Holmes R, Perrott D and Kaban LB. Distraction osteogenesis of the porcine mandible: histomorphometric evaluation of bone. Plastic and Reconstructive Surgery 2004;113(2):566-573.

Gomez DF, Sant'Anna EF, Leven RM, Ostric SA, Figueroa AA, Royston TJ, Sumner DR and Polley JW. Microstructural and strength evaluation of regenerate tissue during the consolidation period after vertical mandibular ramus distraction. Journal of Craniofacial Surgery 2005;16(5):805-811.

Gonzalez-Garcia R, Rodriguez-Campo FJ, Naval-Gias L, Sastre-Perez J and Diaz-Gonzalez FJ. The effect of radiation in distraction osteogenesis for reconstruction of mandibular segmental defects. British Journal of Oral and Maxillofacial Surgery 2007;45(4):314-316.

Gosain AK, Kalantarian B, Song LS, Larson JD, Jenkins CA and Wilson CR. Comparison of canine mandibular bone regeneration by distraction osteogenesis versus acute resection and rigid external fixation. Plastic and Reconstructive Surgery 2004;114(6):1490-1499; discussion 1500-1491.

Harp JH, Aronson J and Hollis M. Noninvasive determination of bone stiffness for distraction osteogenesis by quantitative computed tomography scans. Clinical Orthopedics and Related Research 1994;(301):42-48.

Hasse AR, Porksen M and Zimmermann CE. Bilateral mandibular distraction in adult dogs with an epiperiosteal distractor. British Journal of Oral and Maxillofacial Surgery 2005;43(2):105-112.

Holmes SB, Lloyd T, Coghlan KM and Newman L. Distraction osteogenesis of the mandible in the previously irradiated patient. Journal of Oral and Maxillofacial Surgery 2002;60(3):305-309.

Hu J, Tang Z, Wang D and Buckley MJ. Changes in the inferior alveolar nerve after mandibular lengthening with different rates of distraction. Journal of Oral and Maxillofacial Surgery 2001;59(9):1041-1045; discussion 1046.

Hu J, Zou S, Li J, Chen Y, Wang D and Gao Z. Temporospatial expression of vascular endothelial growth factor and basic fibroblast growth factor during mandibular distraction osteogenesis. Journal of Cranio-Maxillofacial Surgery 2003;31(4):238-243.

Hughes CW, Williams RW, Bradley M and Irvine GH. Ultrasound monitoring of distraction osteogenesis. British Journal of Oral and Maxillofacial Surgery 2003;41(4):256-258.

Idelsohn S, Pena J, Lacroix D, Planell JA, Gil FJ and Arcas A. Continuous mandibular distraction osteogenesis using superelastic shape memory alloy (SMA). Journal of Materials Science: Materials in Medicine 2004;15(4):541-546.

Ilizarov GA. A method of uniting bones in fractures and an apparatus to implement this method. USSR Authorship Certificate 98471 1952.

Ilizarov GA. The principles of the Ilizarov method. Bulletin of the Hospital for Joint Diseases Orthopaedic Institute 1988;48(1):1-11.

Ilizarov GA. The tension-stress effect on the genesis and growth of tissues. Part I. The influence of stability of fixation and soft-tissue preservation. Clinical Orthopedics and Related Research 1989a;238:249-281.

Ilizarov GA. The tension-stress effect on the genesis and growth of tissues: Part II. The influence of the rate and frequency of distraction. Clinical Orthopedics and Related Research 1989b;239:263-285.

Juenger TH, Klingmueller V and Howaldt HP. Application of ultrasound in callus distraction of the hypoplastic mandible: an additional method for the follow-up. Journal of Cranio-Maxillofacial Surgery 1999;27(3):160-167.

Kaban LB, Thurmuller P, Troulis MJ, Glowacki J, Wahl D, Linke B, Rahn B and Perrott DH. Correlation of biomechanical stiffness with plain radiographic and ultrasound data in an experimental mandibular distraction wound. International Journal of Oral and Maxillofacial Surgery 2003;32(3):296-304.

Karp NS, McCarthy JG, Schreiber JS, Sissons HA and Thorne CH. Membranous bone lengthening: a serial histological study. Annals of Plastic Surgery 1992;29(1):2-7.

Karp NS, Thorne CH, McCarthy JG and Sissons HA. Bone lengthening in the craniofacial skeleton. Annals of Plastic Surgery 1990;24(3):231-237.

Kessler P, Neukam FW and Wiltfang J. Effects of distraction forces and frequency of distraction on bony regeneration. British Journal of Oral and Maxillofacial Surgery 2005;43(5):392-398.

Kessler P, Wiltfang J and Neukam FW. A new distraction device to compare continuous and discontinuous bone distraction in mini-pigs: a preliminary report. Journal of Cranio-Maxillofacial Surgery 2000;28(1):5-11.

Kessler PA, Merten HA, Neukam FW and Wiltfang J. The effects of magnitude and frequency of distraction forces on tissue regeneration in distraction osteogenesis of the mandible. Plastic and Reconstructive Surgery 2002;109(1):171-180.

King GJ, Liu ZJ, Wang LL, Chiu IY, Whelan MF and Huang GJ. Effect of distraction rate and consolidation period on bone density following mandibular osteodistraction in rats. Archives of Oral Biology 2003;48(4):299-308.

Knabe C, Nicklin S, Yu Y, Walsh WR, Radlanski RJ, Marks C and Hoffmeister B. Growth factor expression following clinical mandibular distraction osteogenesis in humans and its comparison with existing animal studies. Journal of Cranio-Maxillofacial Surgery 2005;33(6):361-369.

Komuro Y, Takato T, Harii K and Yonemara Y. The histologic analysis of distraction osteogenesis of the mandible in rabbits. Plastic and Reconstructive Surgery 1994;94(1):152-159.

Lafont J, Blanquaert F, Colombier ML, Barritault D, Carueelle JP and Saffar JL. Kinetic study of early regenerative effects of RGTA11, a heparan sulfate mimetic, in rat craniotomy defects. Calcified Tissue International 2004;75(6):517-525.

Lewinson D, Maor G, Rozen N, Rabinovich I, Stahl S and Rachmiel A. Expression of vascular antigens by bone cells during bone regeneration in a membranous bone distraction system. Histochemistry and Cell Biology 2001;116(5):381-388.

Lewinson D, Rachmiel A, Rihani-Bisharat S, Kraiem Z, Schenzer P, Korem S and Rabinovich Y. Stimulation of Fos- and Jun-related genes during distraction osteogenesis. Journal of Histochemistry and Cytochemistry 2003;51(9):1161-1168.

Li J, Hu J, Wang D, Tang Z and Gao Z. Biomechanical properties of regenerated bone by mandibular distraction osteogenesis. Chinese Journal of Traumatology 2002;5(2):67-70.

Li M, Park SG, Kang DI, Eppley BL, Sadove AM and Coleman JJ, 3rd. Introduction of a novel internal spring-driven craniofacial bone distraction device. Journal of Craniofacial Surgery 2004;15(2):324-328.

Liebschner MA. Biomechanical considerations of animal models used in tissue engineering of bone. Biomaterials 2004;25(9):1697-1714.

Liu ZJ, Anderson MW, Gu GM and King GJ. Apoptosis in the regenerate produced by mandibular osteodistraction in the mature rat. Orthodontics and Craniofacial Research 2005;8(1):41-51.

Liu ZJ, King GJ and Herring SW. Alterations of morphology and microdensity in the condyle after mandibular osteodistraction in the rat. Journal of Oral and Maxillofacial Surgery 2003;61(8):918-927.

Makarov MR, Harper RP, Cope JB and Samchukov ML. Evaluation of inferior alveolar nerve function during distraction osteogenesis in the dog. Journal of Oral and Maxillofacial Surgery 1998;56(12):1417-1423; discussion 1424-1415.

McCarthy JG, Katzen JT, Hopper R and Grayson BH. The first decade of mandibular distraction: lessons we have learned. Plastic and Reconstructive Surgery 2002;110(7):1704-1713.

McCarthy JG, Schreiber J, Karp N, Thorne CH and Grayson BH. Lengthening the human mandible by gradual distraction. Plastic and Reconstructive Surgery 1992;89(1):1-8; discussion 9-10.

McCarthy JG, Stelnicki EJ and Grayson BH. Distraction osteogenesis of the mandible: a ten-year experience. Seminars in Orthodontics 1999;5(1):3-8.

Mehrara BJ, Rowe NM, Steinbrech DS, Dudziak ME, Saadeh PB, McCarthy JG, Gittes GK and Longaker MT. Rat mandibular distraction osteogenesis: II. Molecular analysis of transforming growth factor beta-1 and osteocalcin gene expression. Plastic and Reconstructive Surgery 1999;103(2):536-547.

Meyer U, Kruse-Losler B and Wiesmann HP. Principles of bone formation driven by biophysical forces in craniofacial surgery. British Journal of Oral and Maxillofacial Surgery 2005;44(4):289-295.

Miao J, Li G, Zhao P, Chen G, Teng L and Ling Y. 2/3 osteotomy for lengthening the mandible in dogs by gradual distraction. Journal of Cranio-Maxillofacial Surgery 1997;25(6):301-304.

Mofid MM, Inoue N, Tufaro AP, Vander Kolk CA and Manson PN. Spring-mediated mandibular distraction osteogenesis. Journal of Craniofacial Surgery 2003;14(5):756-762.

Morgan EF, Longaker MT and Carter DR. Relationships between tissue dilatation and differentiation in distraction osteogenesis. Matrix Biology 2006;25(2):94-103.

Movassaghi K, Altobelli DE and Zhou H. Frontonasal suture expansion in the rabbit using titanium screws. Journal of Oral and Maxillofacial Surgery 1995;53(9):1033-1042.

Muhonen A, Haaparanta M, Gronroos T, Bergman J, Knuuti J, Hinkka S and Happonen RP. Osteoblastic activity and neoangiogenesis in distracted bone of irradiated rabbit mandible with or without hyperbaric oxygen treatment. International Journal of Oral and Maxillofacial Surgery 2004;33(2):173-178.

Muhonen A, Muhonen J, Lindholm TC, Minn H, Klossner J, Kulmala J and Happonen RP. Osteodistraction of a previously irradiated mandible with or without adjunctive hyperbaric oxygenation: an experimental study in rabbits. International Journal of Oral and Maxillofacial Surgery 2002a;31(5):519-524.

Muhonen A, Muhonen J, Minn H, Kulmala J, Klossner J, Teras M, Knuuti J and Happonen RP. The effects of irradiation and hyperbaric oxygen on bone formation during rabbit mandibular distraction. Archives of Oral Biology 2002b;47(10):701-707.

Muhonen A, Peltomaki T, Hinkka S and Happonen RP. Effect of mandibular distraction osteogenesis on temporomandibular joint after previous irradiation and hyperbaric oxygenation. International Journal of Oral and Maxillofacial Surgery 2002c;31(4):397-404.

Muhonen A, Saamanen AM, Peltomaki T and Happonen RP. The effect of irradiation and hyperbaric oxygenation (HBO) on extracellular matrix of the condylar cartilage after mandibular distraction osteogenesis in the rabbit. International Journal of Oral and Maxillofacial Surgery 2006;35(1):79-87.

Newman E, Turner AS and Wark JD. The potential of sheep for the study of osteopenia: current status and comparison with other animal models. Bone 1995;16(4 Suppl):277S-284S.

Niehoff P, Springer IN, Açil Y, Lange A, Marget M, Roldán JC, Köppe K, Warnke PH, Kimmig B and Wiltfang J. HDR brachytherapy irradiation of the jaw - as a new experimental model of radiogenic bone damage. Journal of Cranio-Maxillofacial Surgery 2008;36(4):203-209.

Nocini PF, Albanese M, Wangerin K, Fior A, Trevisiol L and Kretschmer W. Distraction osteogenesis of the mandible: evaluation of callus distraction by B-scan ultrasonography. Journal of Cranio-Maxillofacial Surgery 2002;30(5):286-291.

Norris SA, Pettifor JM, Gray DA and Buffenstein R. Calcium metabolism and bone mass in female rabbits during skeletal maturation: effects of dietary calcium intake. Bone 2001;29(1):62-69.

Okafuji N, Liu Z-J and King GJ. Assessment of cell proliferation during mandibular distraction osteogenesis in the maturing rat. American Journal of Orthodontics and Dentofacial Orthopedics 2006;130(5):612-621.

Paccione MF, Mehrara BJ, Warren SM, Greenwald JA, Spector JA, Luchs JS and Longaker MT. Rat mandibular distraction osteogenesis: latency, rate, and rhythm determine the adaptive response. Journal of Craniofacial Surgery 2001;12(2):175-182.

Pacicca DM, Patel N, Lee C, Salisbury K, Lehmann W, Carvalho R, Gerstenfeld LC and Einhorn TA. Expression of angiogenic factors during distraction osteogenesis. Bone 2003;33(6):889-898.

Park B-W, Kim J-R, Lee J-H and Byun J-H. Expression of nerve growth factor and vascular endothelial growth factor in the inferior alveolar nerve after distraction osteogenesis. International Journal of Oral and Maxillofacial Surgery 2006;35(7):624-630.

Parr JA, Garetto LP, Wohlford ME, Arbuckle GR and Roberts WE. Implant-borne suture expansion in rabbits: a histomorphometric study of the supporting bone. Journal of Biomedical Materials Research 1999;45(1):1-10.

Pearce AI, Richards RG, Milz S, Schneider E and Pearce SG. Animal models for implant biomaterial research in bone: a review. European Cells and Materials Journal 2007;13:1-10.

Perrott DH, Rahn B, Wahl D, Linke B, Thurmuller P, Troulis M, Glowacki J and Kaban LB. Development of a mechanical testing system for a mandibular distraction wound. International Journal of Oral and Maxillofacial Surgery 2003;32(5):523-527.

Ploder O, Kanz F, Randl U, Mayr W, Voracek M and Plenk H, Jr. Three-dimensional histomorphometric analysis of distraction osteogenesis using an implanted device for mandibular lengthening in sheep. Plastic and Reconstructive Surgery 2002;110(1):130-137; discussion 138.

Ploder O, Mayr W, Schnetz G, Unger E, Ewers R and Plenk H, Jr. Mandibular lengthening with an implanted motor-driven device: preliminary study in sheep. British Journal of Oral and Maxillofacial Surgery 1999;37(4):273-276.

Price DL, Moore EJ, Friedman O, Garces YI, Kee AY and Furutani KM. Effect of Radiation on Segmental Distraction Osteogenesis in Rabbits. Archives of Facial Plastic Surgery 2008;10(3):159-163.

Qi M, Hu J, Zou S, Zhou H and Han L. Mandibular distraction osteogenesis enhanced by bone marrow mesenchymal stem cells in rats. Journal of Cranio-Maxillofacial Surgery 2006;34(5):283-289.

Rowe NM, Mehrara BJ, Dudziak ME, Steinbreck DS, Mackool RJ, Gittes GK, McCarthy JG and Longaker MT. Rat mandibular distraction osteogenesis: Part I. Histologic and radiographic analysis. Plastic and Reconstructive Surgery 1998;102(6):2022-2032.

Ryoyama D, Sawaki Y and Ueda M. Experimental study of mechanical analysis in mandibular lengthening. Application of strain gauge measurement. International Journal of Oral and Maxillofacial Surgery 2004;33(3):294-300.

Sant'Anna EF, Gomez DF, Sumner DR, Williams JM, Figueroa AA, Ostric SA, Theodoru S and Polley JW. Micro-computed tomography evaluation of the glenoid fossa and mandibular condyle bone after bilateral vertical ramus mandibular distraction in a canine model. Journal of Craniofacial Surgery 2006;17(3):611-619.

Schmelzeisen R, Neumann G and von der Fecht R. Distraction osteogenesis in the mandible with a motor-driven plate: a preliminary animal study. British Journal of Oral and Maxillofacial Surgery 1996;34(5):375-378.

Schumacher B, Albrechtsen J, Keller J, Flyvbjerg A and Hvid I. Periosteal insulin-like growth factor I and bone formation. Changes during tibial lengthening in rabbits. Acta Orthopaedica Scandinavica 1996;67(3):237-241.

Shao Z, Liu B, Liu Y, Liu W, Liu R, Peng Q and Liu L. Distraction osteogenesis in the irradiated rabbit mandible. Journal of Plastic, Reconstructive & Aesthetic Surgery 2006;59(2):181-187.

Shibazaki R, Maki K, Tachikawa T, Shibasaki Y, Hinton RJ, Carlson DS and Opperman LA. Changes in parathyroid hormone-related protein and 3-dimensional trabecular bone structure of the mandibular condyle following mandibular distraction osteogenesis in growing rats. Journal of Oral and Maxillofacial Surgery 2005;63(4):505-512.

Shin JY, Liu ZJ and King GJ. Trabecular organization in mandibular osteodistraction in growing and maturing rats. Journal of Oral and Maxillofacial Surgery 2005;63(1):77-86.

Siegel MI and Mooney MP. Appropriate animal models for craniofacial biology. Cleft Palate Journal 1990;27(1):18-25.

Singare S, Li D, Liu Y, Wu Z and 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 2006;5(1):18.

Smith SW, Sachdeva RC and Cope JB. Evaluation of the consolidation period during osteodistraction using computed tomography. American Journal of Orthodontics and Dentofacial Orthopedics 1999;116(3):254-263.

Snyder CC, Levine GA, Swanson HM and Browne EZ, Jr. Mandibular lengthening by gradual distraction. Preliminary report. Plastic and Reconstructive Surgery 1973;51(5):506-508.

Stalmans K, Van Erum R, Verdonck A, Nadjmi N, Schepers E, Schoenaers J and Carels C. Cephalometric evaluation of maxillary advancement with an internal distractor in an adult boxer dog. Orthodontics and Craniofacial Research 2003;6(2):104-111.

Stewart KJ, Lvoff GO, White SA, Bonar SF, Walsh WR, Smart RC and Poole MD. Mandibular distraction osteogenesis: a comparison of distraction rates in the rabbit model. Journal of Cranio-Maxillofacial Surgery 1998;26(1):43-49.

Sun TC, Mori S, Roper J, Brown C, Hooser T and Burr DB. Do different fluorochrome labels give equivalent histomorphometric information? Bone 1992;13(6):443-446.

Swennen G, Dempf R and Schliephake H. Cranio-facial distraction osteogenesis: a review of the literature. Part II: Experimental studies. International Journal of Oral and Maxillofacial Surgery 2002;31(2):123-135.

Swennen G, Schliephake H, Dempf R, Schierle H and Malevez C. Craniofacial distraction osteogenesis: a review of the literature: Part 1: clinical studies. International Journal of Oral and Maxillofacial Surgery 2001;30(2):89-103.

Tang ZL, Hu J, Li JH and Zou SJ. Effect of nerve growth factor and Schwann cells on axon regeneration of distracted inferior alveolar nerve following mandibular lengthening. Chinese Journal of Traumatology 2004;7(2):81-86.

Taub PJ, Bradley JP and Kawamoto HK. Closure of an oronasal fistula in an irradiated palate by tissue and bone distraction osteogenesis. Journal of Craniofacial Surgery 2001;12(5):495-499; discussion 500.

Tavakoli K, Walsh WR, Bonar F, Smart R, Wulf S and Poole MD. The role of latency in mandibular osteodistraction. Journal of Cranio-Maxillofacial Surgery 1998;26(4):209-219.

Thurmuller P, Troulis M, O'Neill MJ and Kaban LB. Use of ultrasound to assess healing of a mandibular distraction wound. Journal of Oral and Maxillofacial Surgery 2002a;60(9):1038-1044.

Thurmuller P, Troulis MJ, Rosenberg A, Chuang S-K and Kaban LB. Microscopic Changes in the Condyle and Disc in Response to Distraction Osteogenesis of the Minipig Mandible. Journal of Oral and Maxillofacial Surgery 2006;64(2):249-258.

Thurmuller P, Troulis MJ, Rosenberg A and Kaban LB. Changes in the condyle and disc in response to distraction osteogenesis of the minipig mandible. Journal of Oral and Maxillofacial Surgery 2002b;60(11):1327-1333.

Troulis MJ, Coppe C, O'Neill MJ and Kaban LB. Ultrasound: assessment of the distraction osteogenesis wound in patients undergoing mandibular lengthening. Journal of Oral and Maxillofacial Surgery 2003;61(10):1144-1149.

Troulis MJ, Glowacki J, Perrott DH and Kaban LB. Effects of latency and rate on bone formation in a porcine mandibular distraction model. Journal of Oral and Maxillofacial Surgery 2000;58(5):507-513; discussion 514.

Tsuchiya H, Uehara K, Sakurakichi K, Watanabe K, Matsubara H and Tomita K. Distraction osteogenesis after irradiation in a rabbit model. Journal of Orthopaedic Science 2005;10(6):627-633.

van der Meulen JH, Borschel GH, Lynch JB, Nicklin S, Ho KC, Gianoutsos MP, Walsh WR and Kuzon WM, Jr. The effect of rate of distraction osteogenesis on structure and function of anterior digastric muscle fibers. Plastic and Reconstructive Surgery 2005;115(3):831-837.

Wang XX, Wang X and Li ZL. Effects of mandibular distraction osteogenesis on the inferior alveolar nerve: an experimental study in monkeys. Plastic and Reconstructive Surgery 2002;109(7):2373-2383.

Warren SM, Mehrara BJ, Steinbrech DS, Paccione MF, Greenwald JA, Spector JA and Longaker MT. Rat mandibular distraction osteogenesis: part III. Gradual distraction versus acute lengthening. Plastic and Reconstructive Surgery 2001;107(2):441-453.

Williams BE, King GJ, Liu ZJ and Rafferty KL. Sequential histomorphometric analysis of regenerate osteogenesis following mandibular distraction in the rat. Archives of Oral Biology 2005;50(5):497-506.

Williamson RA. An experimental study of the use of hyperbaric oxygen to reduce the side effects of radiation treatment for malignant disease. International Journal of Oral and Maxillofacial Surgery 2007;36(6):533-540.

Wiltfang J, Kessler P, Merten HA and Neukam FW. Continuous and intermittent bone distraction using a microhydraulic cylinder: an experimental study in minipigs. British Journal of Oral and Maxillofacial Surgery 2001;39(1):2-7.

Yasui N, Sato M, Ochi T, Kimura T, Kawahata H, Kitamura Y and Nomura S. Three modes of ossification during distraction osteogenesis in the rat. Journal of Bone and Joint Surgery British Volume 1997;79(5):824-830.

Yonezawa H, Harada K, Ikebe T, Shinohara M and Enomoto S. Effect of recombinant human bone morphogenetic protein-2 (rhBMP-2) on bone consolidation on distraction osteogenesis: a preliminary study in rabbit mandibles. Journal of Cranio-Maxillofacial Surgery 2006;34(5):270-276.

Zheng LW, Cheung LK, Ma L and Wong MCM. High-rhythm automatic driver for bone traction: an experimental study in rabbits. International Journal of Oral and Maxillofacial Surgery 2008;37(8):736-740.

Zhou HZ, Hu M, Hu KJ, Yao J and Liu YP. Transport Distraction Osteogenesis Using Nitinol Spring: An Exploration in Canine Mandible. Journal of Craniofacial Surgery 2006;17(5):943-949.

Zhou HZ, Hu M, Yao J and Ma L. Rapid lengthening of rabbit mandibular ramus by using nitinol spring: a preliminary study. Journal of Craniofacial Surgery 2004;15(5):725-729.

Zhu WQ, Wang X, Wang XX and Wang ZY. Temporal and spatial expression of osteoprotegerin and receptor activator of nuclear factor -kappaB ligand during mandibular distraction in rats. Journal of Craniomaxillofacial Surgery 2007;35(2):103-111.

Zimmermann CE, Harris G, Thurmuller P, Troulis MJ, Perrott DH, Rahn B and Kaban LB. Assessment of bone formation in a porcine mandibular distraction wound by computed tomography. International Journal of Oral and Maxillofacial Surgery 2004;33(6):569-574.

Zimmermann CE, Thurmuller P, Troulis MJ, Perrott DH, Rahn B and Kaban LB. Histology of the porcine mandibular distraction wound. International Journal of Oral and Maxillofacial Surgery 2005;34(4):411-419.

Zou S, Hu J, Wang D, Li J and Tang Z. Changes in the temporomandibular joint after mandibular lengthening with different rates of distraction. International Journal of Adult Orthodontics and Orthognathic Surgery 2001;16(3):221-225.

Chapter

10

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Acknowledgements / Dankwoord

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Urville

Chapter

11

Curriculum Vitae

Curriculum Vitae

Urville Djasim was born on April 9th, 1979 in Dijkveld, Suriname. At the age of 1, he and his parents moved to The Hague, the Netherlands. He graduated at the Interconfessioneel Makeblijde College (Gymnasium, pre-university education) in Rijswijk in 1997.

In the summer of 1997, he started studying Biopharmaceutical Sciences at Leiden University, but decided to switch to Biology in 1998, also at Leiden University. During his final years as a Biology student, he worked as a student assistant for the department of Evolutionary Morphology. For his graduation project, which resulted in a publication in an international journal of avian science, he studied the hearing system of Southeast Asian swiftlets.

In 2004, he successfully finished his Biology study and started his PhD research at the department of Oral and Maxillofacial Surgery of the Erasmus MC (the Erasmus University Rotterdam Medical Center) under supervision of prof.dr. K.G.H. van der Wal. After finishing his PhD research and thesis in 2008, he will continue his work for the department of Oral and Maxillofacial Surgery as a post-doctoral researcher.

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Chapter

12

PhD Portfolio

PhD Portfolio Summary

Summary of PhD training and teaching activities

Name PhD student:	Urville M. Djasim
Erasmus MC department:	Oral and Maxillofacial Surgery
Research school:	MUsculoskeletal Science Center (MUSC)
PhD period:	August 2004 – August 2008
Promotors:	Prof.dr. KGH van der Wal and Prof.dr. SER Hovius
Supervisors:	Dr. JW van Neck and Dr. EB Wolvius

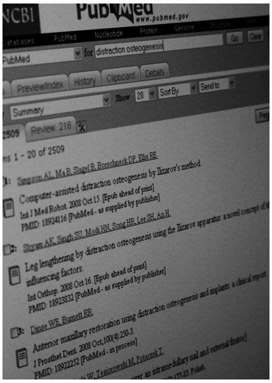
1. PhD training:	**Year**	**Workload**
1.1 General academic skills:		
English Biomedical Writing and Communication	Nov 2006 - Mar 2007	53 hours
1.2. Research skills:		
-		
1.3. In-depth courses (e.g. Research School, Medical Training):		
-		
1.4. Presentations:		
1.4.1. Oral presentations:		
De effecten van diverse parameters op craniofaciale distractie in dierexperimentele studies. Rotterdamse kijk op craniofaciale chirurgie. 20e Wetenschappelijke Vergadering van de Nederlandse Vereniging voor Schisis en Craniofaciale Afwijkingen (NVSCA). Rotterdam (the Netherlands)	Nov 2005	38 hours
Comparison of various rhythms in distraction osteogenesis: an experimental approach. Distraction osteogenesis: from basic research to clinical practice. Clinical applications in Orthopaedics, Plastic and Reconstructive Surgery, and Oral and Maxillofacial Surgery. Rotterdam (the Netherlands)	Nov 2006	38 hours
Het effect van verschillende ritmen van distractie op regeneraatkwaliteit: een microcomputertomografische analyse. 21e Wetenschappelijke Vergadering van de Nederlandse Vereniging voor Schisis en Craniofaciale Afwijkingen (NVSCA). Utrecht (the Netherlands)	Nov 2006	38 hours

Evaluatie van het konijn als diermodel voor onderzoek naar craniofaciale distractie osteogenese. Voorjaarsvergadering van de Nederlandse Vereniging voor Mondziekten, Kaak- en Aangezichtschirurgie (NVMKA). Zeist (the Netherlands)	Mar 2007	38 hours
Histomorfometrische analyse van discontinue versus continue distractie. 51e Najaarsvergadering van de Nederlandse Vereniging voor Mondziekten, Kaak- en Aangezichtschirurgie (NVMKA). Rotterdam (the Netherlands)	Nov 2007	38 hours
Craniofaciale distractie osteogenese: continu of discontinue bewegen? Symposium Surgically Assisted Rapid Maxillary Expansion. Rotterdam (the Netherlands)	Jun 2008	38 hours
Continuous versus discontinuous distraction osteogenesis. XIX Congress of the European Association for Cranio-Maxillo-Facial Surgery (EACMFS). Bologna (Italy)	Sep 2008	38 hours
Kwantitatieve vergelijking tussen continue en discontinue distractie osteogenese. 52e Najaarsvergadering van de Nederlandse Vereniging voor Mondziekten, Kaak- en Aangezichtschirurgie (NVMKA). Eindhoven (the Netherlands)	Nov 2008	38 hours
1.4.2. Poster presentations: Histomorphometric analysis of bone regeneration after distraction osteogenesis. 16th Conference of the Dutch Society for Biomaterials and Tissue Engineering (NBTE). Lunteren (the Netherlands)	Dec 2007	38 hours
1.5. International conferences: XIX Congress of the European Association for Cranio-Maxillo-Facial Surgery (EACMFS). Bologna (Italy)	Sep 2008	38 hours
1.6. Seminars and workshops: MUSC (MUsculoskeletal Science Center) retreat	Dec 2004	8 hours
Distraction Osteogenesis: from basic research to clinical practice. Clinical applications in Orthopaedics, Plastic and Reconstructive Surgery, and Oral and Maxillofacial Surgery	Nov 2006	8 hours
1.7. Didactic skills: -		
1.8. Other: **1.8.1. Publications:** Djasim UM, Wolvius EB, Van Neck JW, Weinans H, Van der Wal	2007	152 hours

KGH: Recommendations for optimal distraction protocols for various animal models on the basis of a systematic review of the literature. International Journal of Oral and Maxillofacial Surgery 36(10):877-883, 2007		
Djasim UM, Wolvius EB, Van Neck JW, Van Wamel A, Weinans H, Van der Wal KGH: Single versus triple daily activation of the distractor: No significant effects of frequency of distraction on bone regenerate quantity and architecture. Journal of Cranio-Maxillofacial Surgery 36(3):143-151, 2008	2008	152 hours
Djasim UM, Hekking-Weijma JM, Wolvius EB, Van Neck JW, Van der Wal KGH: Rabbits as a model for research into craniofacial distraction osteogenesis. British Journal of Oral and Maxillofacial Surgery (*in press*)	2008	152 hours
Djasim UM, Wolvius EB, Bos JATh, Van Neck JW, Van der Wal KGH: Continuous versus discontinuous distraction: Evaluation of bone regenerate following various rhythms of distraction. Journal of Oral and Maxillofacial Surgery (*accepted*)	2008	152 hours
Djasim UM, Mathot BJ, Wolvius EB, Van Neck JW, Van der Wal KGH: Histomorphometric comparison between continuous and discontinuous distraction osteogenesis. Journal of Cranio-Maxillofacial Surgery (*submitted*)	2008	152 hours
1.8.2. Grants: Stichting Anna Fonds	2006	78 hours
Fonds NutsOhra	2008	78 hours
1.8.3. Erasmus PhD Association Rotterdam: 1st Prize winner of the Erasmus PhD Association Rotterdam (EPAR) Photo Competition	2008	
2. Teaching activities: **2.1 Lecturing:** College, third-years' medical students	2006, 2007, 2008	3 hours
2.2. Supervising practicals and excursions: -		
2.3. Supervising Master's theses:		
Supervision over student research project "Application of distraction	Mar 2007 -	608 hours

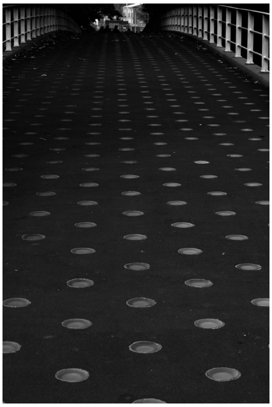
osteogenesis techniques on the nasal bone of the rabbit: continuous versus discontinuous distraction”	Oct 2007	
2.4 Other: -		

Chapter 1, page 6:
Sign near the Museum Boijmans Van Beuningen points in the direction of the Erasmus MC. A good place to start this thesis.



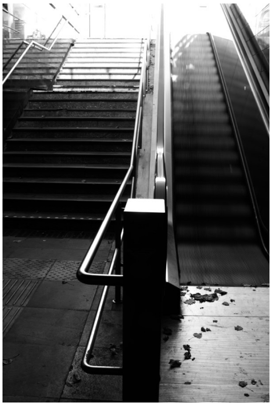
Chapter 2, page 18:
Screenshot of the PubMed website, a service of the U.S. National Library of Medicine and the National Institutes of Health. PubMed is an important database for scientific literature.

Chapter 3, page 40:
Two of the “Three Rabbits”; bronze statues of rabbits by the artist Tom Claassen, near the Kunsthal (Hall of Art) and the Natuurhistorisch Museum Rotterdam (Rotterdam Museum of Natural History).



Chapter 4, page 54:

Pedestrian bridge with built-in spotlights, near the Museumpark. The bridge symbolizes the newly formed bone tissue that has bridged the distraction gap after distraction osteogenesis, while the spotlights resemble osteocytes.



Chapter 5, page 76:

Exit of the subway station near the Erasmus MC. Discontinuous and continuous distraction rhythms are embodied by the stairs and the escalator, respectively.

Chapter 6, page 98:

With a bit of fantasy, the columns of this gallery at the Nederlands Architectuurinstituut (Netherlands Architecture Institute) resemble the numerous slides that were used for histomorphometrical analyses.

Chapter 7, page 118:

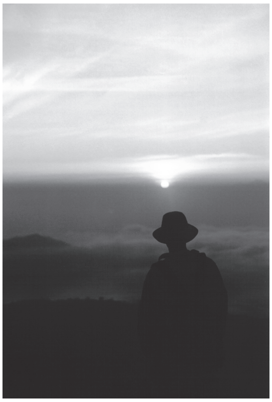
This convex mirror provides a wide-angle view over a large part of a construction site near the Erasmus MC. The Erasmus MC itself is visible in the background of the mirror image.

Chapter 8, page 138:

Part of the Erasmus Bridge, where all cables come together. The eye-catching cable-stayed Erasmus Bridge links the north and the south of Rotterdam.

Chapter 9, page 146:

Reading man, in a gallery in Bologna, Italy.



Chapter 10, page 164:

The sky is the limit. All the support I received during my PhD research lifted me up to great heights. Thanks again! (Self-portrait, on top of Gunung Batur, Bali, Indonesia.)

Chapter 11, page 170:

Athlete running the Rotterdam marathon.

Chapter 12, page 174:

Photo of the Erasmus Bridge. This photo won the First Prize in the 2008 Photo Competition of the Erasmus PhD Association Rotterdam (EPAR).

All photos were taken by Urville Djasim.