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The effect of definitive continuous distraction employed with the Ilizarov type external fixation system on fracture healing: an experimental rabbit model

Ilizarov tipi eksternal fiksatörle belirli sürekli distraksiyonun kırık iyileşmesi üzerine etkisi: Tavşanda deneysel çalışma

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Amaç: Bu çalışmada Ilizarov tipi eksternal fiksatör sisteminde belirli sürekli distraksiyon (BSD) uygulayarak BSD'nin tavşan tibiasında kırık iyileşmesi üzerine etkisi araştırıldı.

Çalışma planı: Bir yaş üzerinde ve ağırlıkları 2800-4000 gr olan 15 adet Yeni Zelanda tipi tavşan, tibia ve fibulaya osteotomi uygulandıktan sonra üç gruba ayrıldı. Birinci grup normal Ilizarov tipi eksternal fiksatör uygulanarak izlendi. İkinci ve üçüncü grup tavşanlara, yaylarla modüle edilmiş Ilizarov tipi eksternal fiksatör sistemi ile sırasıyla ağırlığın %10'u ve %30'u kadar BSD uygulandı. Üç grup 12, 21, 28 ve 35. günlerde radyografilerle izlendi. Kırk beşinci günde tavşanların yaşamı sonlandırılarak kırıktaki kallus ve kaynama makroskobik ve mikroskobik olarak değerlendirildi. Radyografik değerlendirme Lane ve Sandhu'nun sistemine göre, histolojik değerlendirme ise Heiple ve ark.nın sistemine göre yapıldı. Sonuçlar Kruskal-Wallis testi ile değerlendirildi.

Sonuçlar: En yüksek radyografik ve histolojik puanlar sırasıyla 8.6 ve 11.6 ile ikinci grupta elde edildi ($p<0.05$). Üçüncü grubun radyografik ve histolojik puanları kontrol grubundan düşük bulundu.

Çıkarımlar: Ilizarov tipi eksternal fiksatör sistemi ile ağırlığın %10'u kadar BSD uygulaması kırık iyileşmesini olumlu etkilerken, daha fazla instabilitenin kırık iyileşmesini geciktirdiği sonucuna varıldı.

Anahtar sözcükler: Kemik uzatma/yöntem/enstrümantasyon; kemik kallusu/fizyoloji/radyografi; eksternal fiksatör; kırık fiksasyonu/enstrümantasyon; Ilizarov tekniği; osteogenesis, distraksiyon; tavşan; tibia/cerrahi.

Objectives: The objective of this study was to evaluate the effect of definitive continuous distraction (DCD) employed through an Ilizarov type external fixator on fracture healing in a rabbit tibia model.

Methods: Fifteen mature New Zealand white rabbits weighing 2.8 to 4.0 kg were randomly assigned to three groups following osteotomy of the tibia and fibula. The first group (controls) was treated only with the Ilizarov type external fixator while the second and third groups were subjected to DCD amounting to 10% and 30% of the body weight, respectively, being employed through modulated springs on the Ilizarov external fixation system. All the rabbits were evaluated by radiographies on the 12th, 21st, 28th, and 35th days following surgery and were sacrificed on the 45th day. Radiographic evaluations were made according to the Lane and Sandhu's scoring system, and histologic evaluations according to the scoring system proposed by Heiple et al. The results were analyzed using the Kruskal-Wallis test.

Results: The highest radiographic and histologic scores were obtained from the second group, being 8.6 and 11.6, respectively ($p<0.05$). Radiographic and histologic scores of the third group were lower than those of the control group.

Conclusion: Application of DCD of 10% of body weight through modulated springs on the Ilizarov external fixator improves fracture healing while a greater amount of instability results in delayed healing.

Key words: Bone lengthening/methods/instrumentation; bony callus/physiology/radiography; external fixators; fracture fixation/instrumentation; Ilizarov technique; osteogenesis, distraction; rabbits; tibia/surgery.

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Fracture healing has become one of the most interesting fields of traumatology during recent years. Increase in number of patients in parallel to the rise in the number of disasters and wars in the community, and individual injuries and accidents gave rise to requirements for several clinical and experimental studies, which aim to accelarate the healing time, and return patients much faster to their daily life and activities. Such studies contributed to comprehend many unknown details related to the cellular and immunological level of the healing process of fractures.

Terjesen and Johnson[1] have reported that in fractures where the fixation is not very rigid, micromobility increases the amount of external callus and it has a positive impact on the fracture union, however no detailed information was provided about the amount of instability. Ilizarov[2] studied the biological effects of distraction osteogenesis on the bone, and demonstrated the proliferation and biosynthetic effect of the gradual distraction. Furthermore, he reported that the longitudinal instability resulted in regeneration between the bone tips during the distraction osteogenesis.

In the present study, our objective was to examine the effect of controlled instability on the union and healing time of fractures applying a definitive continuous distraction (DCD) with an Ilizarov type external fixator.

Materials and method

The study was performed in the laboratory of experimental animals at the Medical Faculty of the Cumhuriyet University. Permission was obtained from the Ethical Board of the Medical Faculty of the Cumhuriyet University before the study. The external fixators used during the study were supplied by Tasarimmed.

In experimental studies, animals selected as subjects should have some specific characteristics. While generally large animals like rabbit and dog are selected for biomechanical studies on fractures, animals with a faster healing time like rats are preferred for cellular studies. [3-5] In the present study, we preferred rabbits as experimental animals.

Fifteen New Zealand rabbits over 1 years of age with a body weight of 2800 to 4000 grams were used in the study. The subjects were fed in the laboratory of experimental animals. They were divided into three groups of five without any gender differantiation. The study was completed with 13 rabbits as two rabbits in the first group died during the postoperative phase of the study.

Preoperative procedures

DCD was based on using an Ilizarov type of external fixators modified with a spring system. For this purpose, firstly the spring constant was calculated using the k=m.g/d formula.[6] In order to calculate the "k" value in the formula, a 1 kg weight (m) was hanged at the end of the spring; and it was observed that the spring was opened for 0.28 m (d). The "g" constant was taken as 9.8 in the formula, where it was calculated that k= 35 N/m (Figure 1).[6] And, then the rabbits were weighed. In order to determine the "x" in the formula of f=k.x which indicates the work a spring does, the "f" value was found. The weights of rabbits were converted to the weight in Newton by the f=kg.g formula. In order to do that, kilograms of the rabbits (kg) were multiplied by 9.8, the g constant.[6]

All subjects were randomly divided into three groups. In the first group, a normal fracture healing was evident following the administration of a normal Ilizarov type external fixator without any spring system (control group). The amount of DCD to be administered to the rabbits was scheduled amounting to 10% and 30% of the body weights of rabbits in the second and third groups, respectively. These

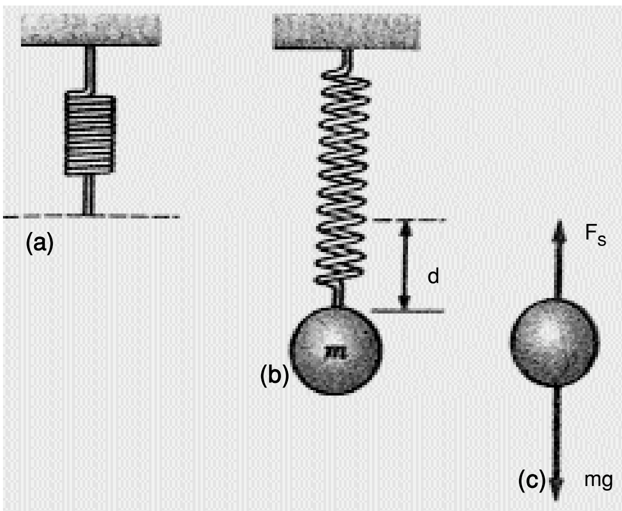

Figure 1. Calculation of the spring constant. Referred from Serway.[4]

Table 1. The body weights of rabbits in Newton and distraction amount administered to groups

Rabbit	Group	Kilograms (kg)	Weight in Newton	10% of body weight	30% of body weight	Amount of distraction (mm)
Rabbit 1	1	4.000	39.2	–	–	–
Rabbit 3	1	3.000	29.4	–	–	–
Rabbit 7	1	3.400	33.3	–	–	–
Rabbit 10	1	3.500	34.3	–	–	–
Rabbit 14	1	3.400	33.3	–	–	–
Rabbit 2	2	3.500	34.3	3.4	–	0.9
Rabbit 4	2	3.200	31.3	3.1	–	0.8
Rabbit 9	2	3.200	31.3	3.1	–	0.8
Rabbit 12	2	2.900	28.4	2.8	–	0.8
Rabbit 13	2	2.800	27.4	2.7	–	0.7
Rabbit 5	3	3.100	30.3	–	9.0	2.5
Rabbit 6	3	3.100	30.3	–	9.0	2.5
Rabbit 8	3	2.900	28.4	–	8.4	2.4
Rabbit 11	3	3.300	32.3	–	9.6	2.7
Rabbit 15	3	3.400	33.3	–	9.9	2.8

calculations were placed into the f=k.x formula to calculate the x value, i.e. to what extent the spring shall be tightened, for each rabbit. DCD was based on two components attached to the rods of the Ilizarov type external fixator. As two rods would have been used for fixation, it was administered by dividing the value into two in centimeter (Table 1).[6]

Based on this, calculations were made for the spring comprising the first component. The diameter of the rod was less than the diameter of the spring in order to enable better movement of the spring on the rod. The second component, which was attached to the system in order to transmit the definitive distraction onto the ring, was used as a springhead. The springhead was wide enough to move inside the nut fixing the ring to the rod and it was deeper than the length of the nut so that the DCD applied by the spring was transmitted to the ring with no compression when the nut was loosened for 1 mm. No instability problems developed as the nuts in the system

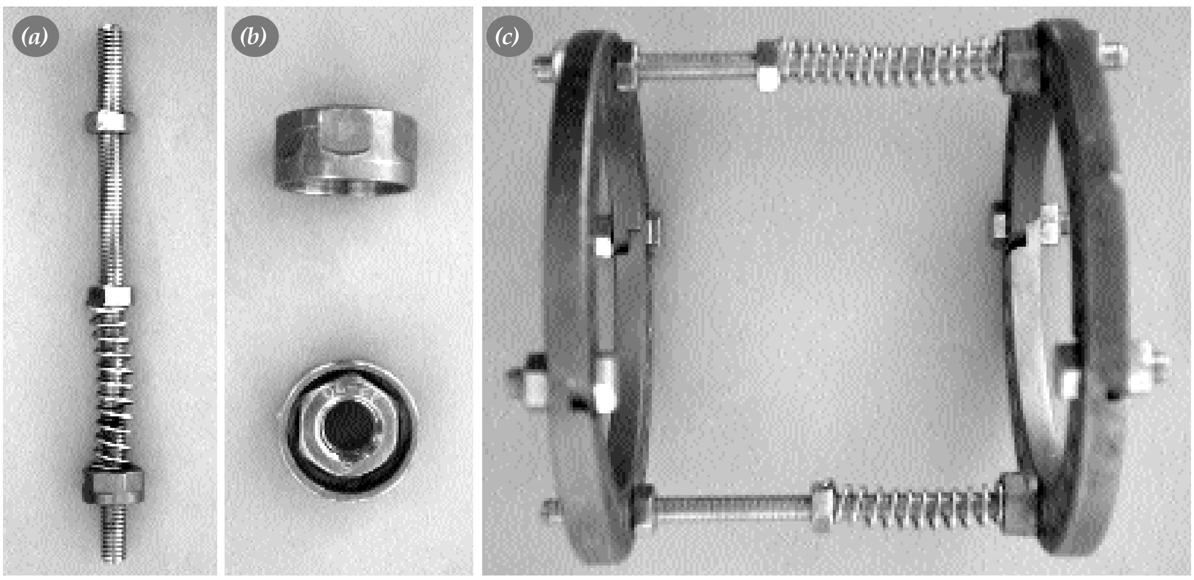

Figure 2. **(a)** Spring **(b)** Springhead **(c)** General appearance of the system

were loosened more than 1 mm. Compression which occurred in response to the distraction was also inhibited by means of the springhead attached. Consequently, the system was ready (Figure 2).

Surgical technique

TAll rabbits were administered intramuscular ketamine hydochloride (Ketalar®) 10mg/kg and xylazine hydochloride (Rompun®) 3 mg/kg for anaesthetic purposes. Following the anaesthesia, prospective sites for K-wires and osteotomy were shaved. The surgery area was dyed by povidon iodine (Biokadin®); and covered with sterile perforated drapes. During surgery, two rings, four 1.5 mm K-wires, eight sidebars for fixation to the ring, two rods interconnecting the rings, two springs and two springheads for DCD were used. After the wires being inserted, frame installed and stability established, skin and fascia were passed through anterolaterally from the medial intersection of cruris 1/3 proximal 1/3. Tibia and fibula underwent osteotomy by means of an osteotome we made of Steinman pin in our clinic. Skin and fascia were anatomically sutured using a 5/0 chrome catgut. Wound sites of the rabbits were dressed daily by betadine. All subjects received Ceftriaxon Disodium (Novosef®) 50 mg/kg/day for a period of three days in order to prevent infection.

The nut on the springhead was loosed for 1 mm at postoperative day 5. The spring was stretched to the extent, which was previously calculated, to initiate the DCD amounting to 10% of body weight in the second group and 30% of the body weight in the third group of rabbits.

A normal fixator was installed in the first group of rabbits, and a normal fracture healing was evident. This group was the control group. As the rabbit no. 7 died at postoperative day 14, and rabbit no. 14 at postoperative day 22 due to diarrhea, they were both excluded from the study. This group was completed with three rabbits as it was defined that data could be interpreted with three subjects in the Kruskal-Wallis test, which we used for statistical evaluations.

Radiographic analysis

Callus formation and union were monitored by direct radiographies taken at postoperative days 12, 21, 28, and 35 for each rabbit. The radiographies at day 35 were scored in accordance with the Lane and Sandhu scoring system [7] (Table 2).

Table 2. Radiographic scoring system[7]

Category	Points
Bone formation (the highest score is 4)	
No evidence of bone formation	0
Bone formation occupying 25% of the defect	1
Bone formation occupying 50% of the defect	2
Bone formation occupying 75% of the defect	3
Full gap bone formation	4
Union (the highest score is 4)	
Full fracture line	0
Partial fracture line	2
Absent fracture line	4
Remodelling (the highest score is 4)	
No remodeling	0
Remodelling of the intramedullary channel	2
Full remodelling of cortex	4
Sum of radiographic scores	12

Macroscopic analysis

All rabbits were sacrificied at postoperative day 45 by injecting 30 mg/kg Ketalar®. A second grade pin tract infection was found in two rabbits, one in the second and other in the third groups. Osteotomy line was removed for macroscopic analysis.

Histologic analysis

Histologic evaluation of fracture healing is composed of four phases in rabbits; first five days hematoma; days 5-14, chondrogenesis; days 14-21, endochondral ossification; and days 21 and over, remodeling.[8]

Bone regions including the defective sites were decalcified in a 20% reduced formic acid solution for a period of 48 hours following the 10% formaldehyde fixation for three days. After tensile testing, tissues were dehydrated by a series of increasing ethyl alcohol, and embedded into paraffin. The longitudinal sections of 3-5 _m obtained from these blocks by microtome were stained by hematoxylin-eosine and Mallory’s Azan, and then analyzed in the Carl Zeis Jenamed 2 photomicroscope.

The histologic analysis was performed using the histological scoring system developed by Heiple et al.[9] and Lane and Sandhu[7] (Table 3).[10] Scoring was carried out by an expert on histology, who was blind to the groups.

Table 3. Histologic scoring system[9]

Category	Point
Union (the highest score is 4)	
No sign of union	0
Fibrous union	1
Osteochondral union	2
Bone union	3
Complete reorganization	4
Spongiosa (the highest score is 4)	
No sign of cellular activity	0
Early bone formation	1
Active new bone formation	2
Reorganized spongiosa formation	3
Complete reorganized spongiosa	4
Cortex (the highest score is 4)	
Absence of cortex	0
Early detection	1
Initiation of formation	2
Reorganization in majority	3
Complete organization	4
Bone marrow (the highest score is 4)	
Not available	0
Detection of fibrinous material	1
Defect occupying more than half	2
Fully occupying the red bone marrow	3
Adult type fatty marrow	4
Sum of histologic scores	16

Statistical assessment

The mean values were calculated after completion of radiographic and histologic scorings for each rabbit. The significance of mean values between groups was evaluated using the Kruskal–Wallis test since it is the non-parametric correspondent of the variance analysis for independent groups.

Results

Results of macroscobic analysis

Group 1: A macroscopic analysis of bones at postoperative day 45 showed that full union was achieved in the fracture areas. No movement was observed in fractures.

Group 2: Callus was very good at postoperative day 45. No fracture line and no movement.

Group 3: The union was not efficient, there was minimal movement at the tip of fractures and callus tissue was not adequately formed.

Results of radiographic analysis

Group 1: At postoperative day 12, the cortexes were normal, no thickening was observed and formation of periosteal callus was evident.

At postoperative day 21, normal callus formation was evident; cortical expansion was shown; periosteal organization and osteal reaction were evident.

A postoperative day 28, callus was formed, periosteal reaction decreased, cortex started to become organized, irregularity disappeared and expansion reduced.

At postoperative day 35, organized bone formation and a fissure type of fracture line were evident.

Group 2: Callus formation was more evident at postoperative day 12 compared to the first group.

At postoperative day 21, callus development was increased at superior; periost reaction was evident; no cortical expansion.

At postoperative day 28, callus formation was expanded, periostal reaction was good and cortical expansion was present.

At postoperative day 35, organized bone formation and a fissure type of fracture line were evident.

Group 3: Cortex had an organized appearance at postoperative day 12; no callus formation.

At postoperative day 21, a linear and suspected mild callus formation was evident.

At postoperative day 28, only callus formation was evident without any expansion.

At postoperative day 35, callus organization was initiated, and expansion developed; a bilateral line was evident. However, some radiographs didn’t demonstrate disorganization and expansion in the cortex.

Results of histologic analysis

Group 1: No virtual union was present between the tips of fracture, and the consistency was provided by a relatively thin, weak and irregular trabecular angle. The irregular primitive bone trabeculae in this area mainly consisted of a basophilic matrix in the active area. No bone marrow was evident while there was a very weak fibrillation in the vicinity of fracture. In more medullar regions, bone marrow, which was partly supported by collagenous fibers and blood vessels, maintained its presence. In the internal medullary region (in spongiosa) development of trabecula was very weak, and it mainly consisted of isolated thin structures (Figure 3a).

On the other hand, in the histological preparation series, there were areas where the development of trabeculae in the spongiosa was partly more evident. The trabeculae in such areas had a structure of sometimes better organized and partly calcified. At the free end of trabeculae, well-organized, hyperactive osteoblasts with basophilic

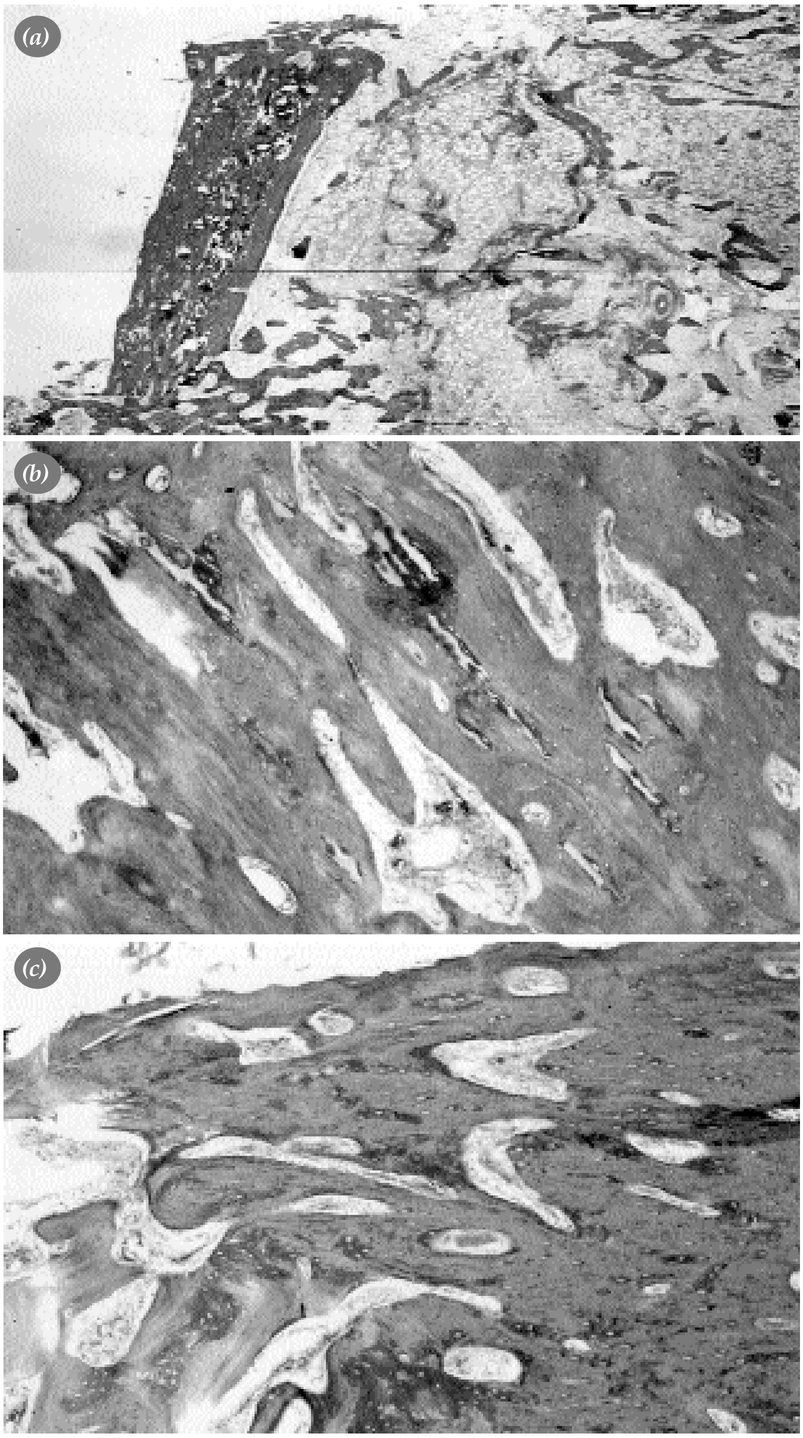

Figure 3. Histologic analysis of Group 1: (a) Development of a thin isolated trabecula in the inner medullary region is evident as well as weak trabeculae which have an irregular network structure between the fracture tips (x3.2, Mallory's azan). (b) Hypertrophic and basophilic osteoblasts are noticeable at the free tips of the trabeculae which demonstrate a more developed structure in some areas (x10, Mallory's azan). (c) The trabecullar osteon structure near the cortex area has a tendency to translate, and primitive trabecullar structure is evident in inner areas (left side) (x10, Mallory's azan).

staining characteristics were noticeable (Figure 3b).

While there was tendency to form a trabecullar osteon structure, especially in the vicinity of cortex, the basophilic areas in matrices were smaller. Among trabeculae, there were mesenchyme-like connective tissue cells and rarely thin collagenous fibrils as well as blood vessels (Figure 3c).

Group 2: The trabeculae in spongiosa had a more developed structure than in the first group. Although they had a nearly normal structure in terms of shape, and mostly had an acidophilic calcified matrix, they also included hypertrophic cartilage-like cells still surrounded by a basophilic matrix. Number of mesenchyme-like cells was reduced among trabeculae, and they were replaced by thin fibrils and fat cells (Figure 4a). In the sections near the cortical area, gaps between the trabeculae had been narrowed, and lamellar structuring was initiated. Some of lamellas had a circulatory order to shape up the osteon, yet they were still at the re-organization stage (Figure 4b).

Group 3: No trabeculae developed between fracture tips; however, the tips were covered by a fibrous connective tissue extending from periosteum to medulla and including intense collagenous fibers. Limited number of reorganized trabeculae was present in parallel to the cortex in the inner parts of the fractured compact bone. Those trabeculae had been mostly shaped by more cellular inner layer of the connective tissue extending inwards in the periosteum, and had a primary bone trabecullar structure. And, some connective tissue cells were differentiated from the osteoblasts lined up on the free surface of the fractured bone tips (Figure 5a).

No cortex development was observed in many areas. Instead, there was intense connective tissue. It was observed that periosteum contained intense connective tissue and rarely isolated bone trabeculae in such areas, which in fact mostly looked like a scar tissue (Figure 5b).

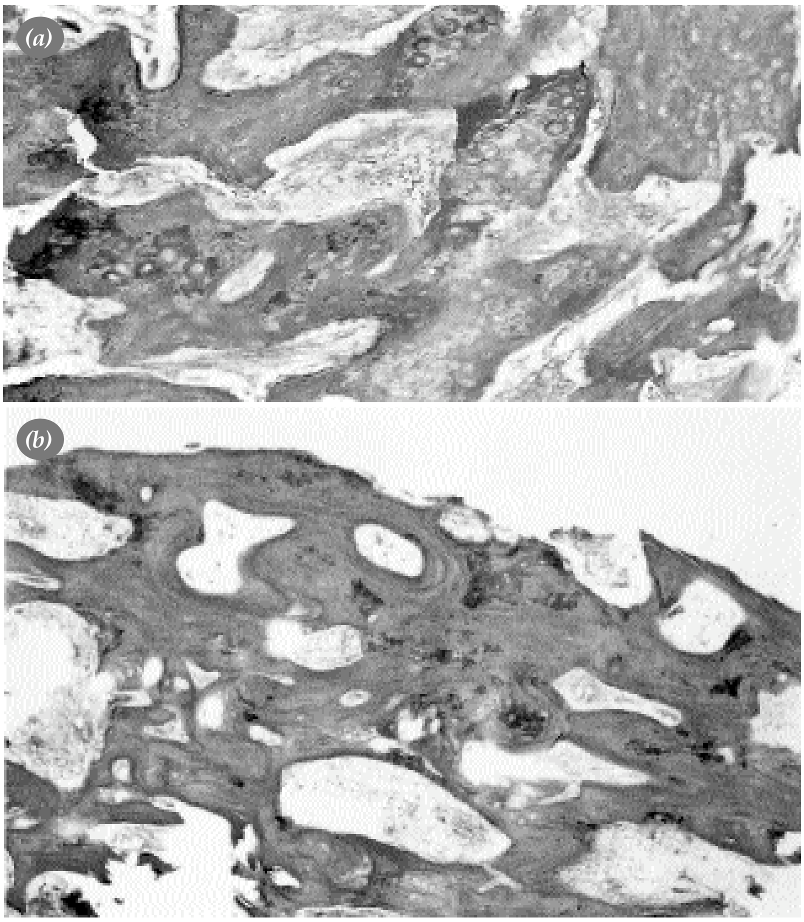

Figure 4. Histologic evaluations for Group 2: **(a)** Hypertrophic cells partly surrounded by basophilic matrix are evident in the well-developed trabaculae including acidophilic matrix in the spongiosa region. Fatty cells are noticeable among the trabeculae (x20, Mallory Azan). **(b)** A lamellar structuring and tendency to translate into osteon are evident in the cortical region (x10, Mallory Azan).

In some areas, mesenchymel cells were intense in the inner layer of the periosteum, and their graded differentiation was prominent. They differantiated from the more poligonal mesenchymel osteoblast-like cells, and constituted the majority of spongious trabeculae. Those primitive trabeculae intensely consisted of cells or extensive basophilic matrix; however, there was little or no acidiphilic matrix. Also existed are extensive cartilage areas among the primitive trabeculae (Figure 5c).

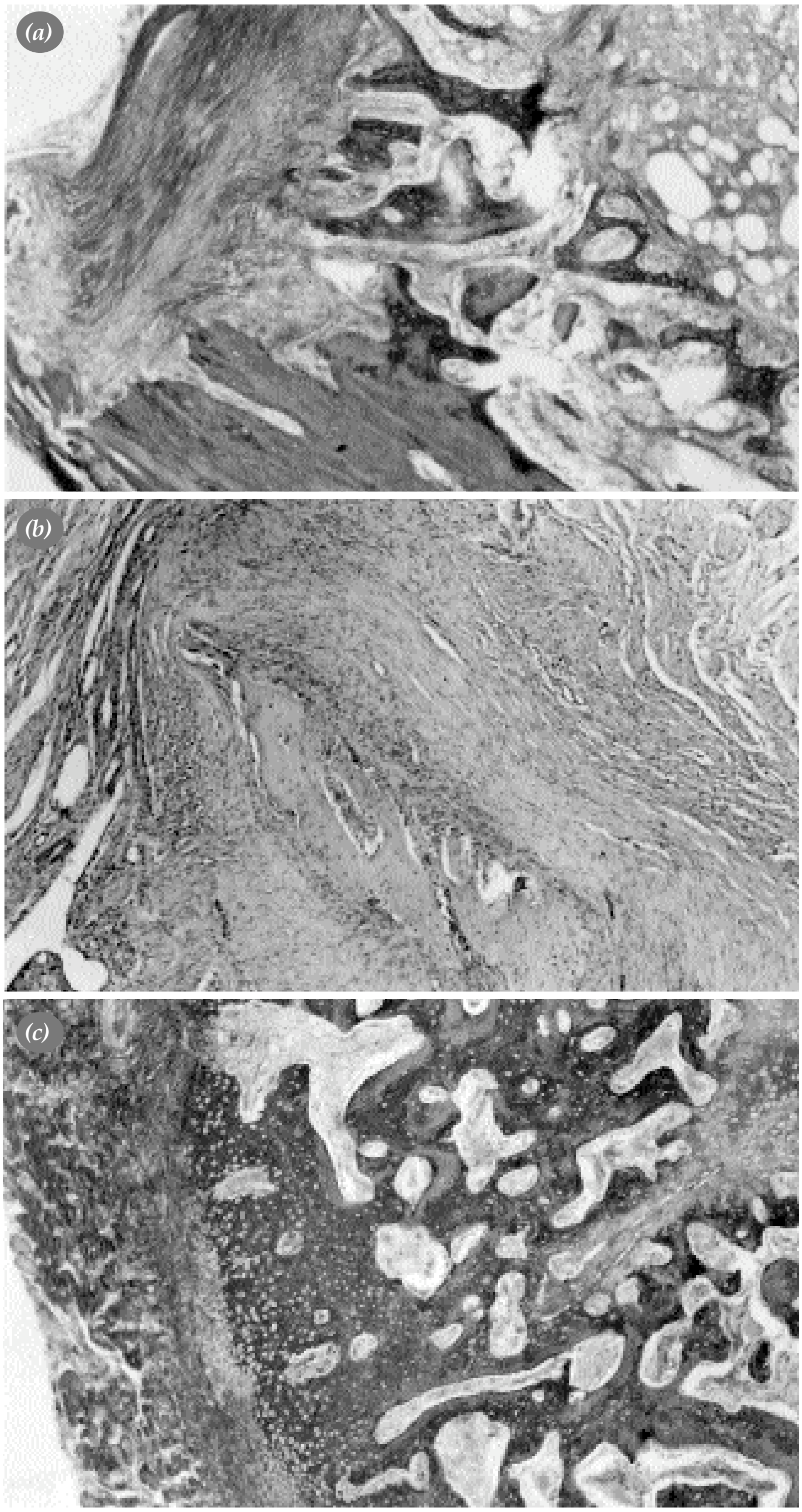

Figure 5. Histologic evaluations for Group 3: (a) Fractured bone tips are covered by intense connective tissue extending from the periosteum to the medulla, some of the cells in the inner layer differantiating and shaping the osteoblasts lined up at the ends of bones (x10, Mallory Azan). (b) In some regions, isolated trabaculae is surrounded by an intense connective tissue resembling a scar tissue (H-Ex10). (c) In addition to the graded cellular differantiation from the periosteum to the internal medullary region, primitive trabeculae with basophilic matrix and intense cartilage tissue are evident (G3x10, Mallory Azan).

Table 4. Mean values of the histologic and radiographic scores by groups

Group	Number of subjects	Histologic scoring		Radiographic scoring	
		Mean score	Standard deviation	Mean score	Standard deviation
Group 1	3	8.6667	0.5774	7.0000	0.0000
Group 2	5	11.6000	0.2449	8.6000	0.5477
Group 3	5	6.0000	0.7071	4.0000	0.7071

Results of statistical evaluations

The mean values and standard deviations of the histologic and radiographic scores were calculated for each of three groups. The mean values of histologic and radiographic scores for Group 1 were regarded as the normal union values for fractures. The mean values of radiographic and histologic scores for groups were significantly different ($p<0.01$; Table 4).

Discussion

Periosteum has an impact on the external callus formation, leading to proliferation of cells in the cambium layer with its characteristics of high osteoblastic and chondroblastic activity.[11] It is known that a wide incision and strip of the periosteum at the level of fracture delay the fracture healing.[12,13] In our study, we did our best to preserve the periosteal tissue in all groups in order not to disturb the impact of periosteum on fracture healing. In a study examining the effects of fixation rigidity on fracture healing, the tibial osteotomy line was fixed by an external fixator, adjusting it for low, moderate and high rigidity; and it was found that the amount of external callus was higher in the group with lower rigidity. However, no significant difference was found between the groups in terms of fracture healing at the end of week 6.[1] Wu et al.[14] have reported that fracture healing with an external fixator of rigid fixation was better and stability was an important factor. Terjesen and Johnson[1] indicated that in fractures with less rigid fixation micromobility increased the amount of external callus, having a positive impact on the fracture union. However, no information was provided about the extent of instability required.

In the present study, the nut activating the DCD mechanism was loosened for 1 mm in all rabbits in order to prevent any difference in the rigidity of the fixators. During follow-up, nuts of the fixators were regularly checked not to allow any instability.

De Bastiani et al.[15] also observed that the callus tissue was formed at postoperative week 3 in new fractures where they applied compression using a single-plane external fixator; and allowed weight bearing on the extremity, loosening the fixator. Thus, they have shown that fracture healing was better.

In the present study, a depth was provided a little over the nut in the springhead in order to prevent the effect of compression on fracture healing; therefore, the spring had a distraction to a specific extent, but no compression.

Chao et al.[16] suggested increased pin diameter, use of plenty of pins and reduction in the distance between the body of fixator and the fracture line in order to increase the rigidity of the external fixator; and highlighted the significance of periosteum and pin sites always being closer to the fracture. Based on these factors, the sites for K-wires were kept stable at each group. The closest point, i.e. approximately 5 mm distal of the tibial fibular compound was defined as the ostetomy line, and the osteotomy was performed through this point.

According to Paterson[17], Codvilla separated the bone lengthening into sections of osteotomy, stabilization, distraction and consolidation at the beginning of 1900's, and initiated the first studies on distraction osteogenesis. Ilizarov,[2] studied the biological effects of the distraction osteogenesis on the bone, and demonstrated the proliferation and biosynthetic effect of gradual distraction; also, he found that longitudinal instability resulted in regeneration between the bone ends during the distraction osteogenesis. Aronson and Harp[18] found that fracture gap and axial dynamization stimulated the fracture healing during distraction in an experimental study. Sen et al.[19] used a daily distraction speed of 1 mm in rabbits who underwent tibial lengthening by an Ilizarov type external fixator through callus distraction; and they stated that fixator must be stable and medulla should be preserved. Their results were consistent with Ilizarov. [20] The procedure applied in their study was indefinitive continuous distraction. A literature screening showed us that continuous distraction was applied four times a day as 0.25 mm, loosening the nut.[2]

Continuous distraction has been used in lengthening operations, particularly on callus. In the present study, the nut was loosened only once for 1 mm at day 5 and the fracture was monitored applying a distraction amounting up to 10% and 30% of the body weights of rabbits as we studied the effect of DCD on the fracture union.

Terjesen and Johnson [1] reported that micromobility in the fracture area increased the union of fracture and amount of callus. However, we didn't find any study related with the effect of controlled micromobility on the union of fracture during literature screening.

In the present study, it was found that administration of continuous distraction amounting to 10% of the body weight of rabbits by means of DCD system provided a better union than in the group who were applied normal Ilizarov type external fixator. We believe that this finding supports the view that micromobility increases union. As a result of macroscobic, radiographic and histologic analyses, we found that the difference between groups was significant ($p<0.01$).

Fracture healing is under the control of systemic and local factors. Several factors have positive impacts on fracture healing while some are negative.[4,5,21-23] It has been reported that administration of calcium sulphate in the osteodistraction area increased the bone formation and calcification.[24] We also believe that administration of DCD amounting to 10% of the body weight of rabbits had a positive impact on fracture healing.

In an experimental study with rabbits, Alho et al.[25] have reported that the union was prominent at day 28 and it was adequate at day 45. Aktuolu et al.[8] also demonstrated that complete union was achieved at approximately day 45 to 50 in rabbits. In the present study, the union time demonstrated by radiographs in the first and second groups were consistent with the results of above mentioned studies, which indicate that administration of 10% DCD has a positive impact on fracture healing, but it didn't affect the union time of fracture.

When the amount of distraction was increased up to 30% of the body weight of rabbits, we noticed that fibrous structures interfered with the fracture line and the fracture union was worse than in the other two groups depending on the instability, which was also evident in the histologic and radiographic scorings ($p<0.01$). Therefore, we concluded that amount of distraction should not be increased further as the administration of 30% DCD had a negative impact on fracture healing.

Several studies have shown that continuous distraction of fractures contributes to the primary fracture healing. Sen et al.[26] applied continuous compression using a Ilizarov type of external fixator in rabbits, and showed that compression is needed in order to achieve absolute stability and contact of fragments by osteosynthesis, and that rigid compression of the resulting callus activated the membraneous bone healing as well as primary bone healing. We think that the effect of definitive continuous compression on fracture union should also be studied using a similar system to DCD as in our study.

We believe that the DCD we applied in rabbits will have a pioneering drive for future experimental studies on the time and quality of fracture union, and then it will be developed to conduct clinical studies in later stages.

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