Exp Biomed Res 2020; 3(1): 18-29 DOI: 10.30714/j-ebr.2020157450

EXPERIMENTAL BIOMEDICAL RESEARCH
http://www.experimentalbiomedicalresearch.com

Original Article

Influence of articular arthroscopy-like washout on fracture healing of intra-articular fractures; animal experiment

Osman Orman[1] · Kahraman Ozturk[1] · Mehmet Baydar[1] · Ethem Guneren[2] · Elif Taslidere[3] · Mujgan Orman[4] · Omer Ozel[5]

[1]*Department of Hand Surgery, Baltalimani Metin Sabanci Bone and Joint Diseases Education and Research Hospital, University of Health Sciences, Istanbul, Turkey.*
[2]*Department of Plastic and Reconstrictive Surgery, Bezmialem Vakif University, Faculty of Medicine, Istanbul, Turkey*
[3]*Department of Histology and Embryology, Inonu University, Faculty of Medicine, Malatya, Turkey*
[4]*Department of Radiology, Medicana International Hospital, Istanbul, Turkey*
[5]*Department of Orthopedics and Traumatology, Başkent University, Faculty of Medicine, Istanbul, Turkey*

ABSTRACT

Aim: To examine whether the application of intra-articular lavage during arthroscopic joint fracture surgery can disturb fracture union and cartilage healing.

Metods: Twenty New Zealand rabbits were then randomly divided into 3 groups; these groups consisted of 2 surgical groups including eight rabbits and a control group consisting of 4 rabbits. After both rear limbs exposed with a medial parapatellar incision, medial femoral condyle was fractured. Four groups were created by doing anatomic reduction or non-anatomic reduction and making irrigation or no irrigation. (Group 1: Fixed by creating a gap and no Irrigation; Group 2: Fixed by creating a gap and irrigation; Group 3: Fixed with complete reduction and no irrigation; Group 4: Fixed with complete reduction and irrigation) X-rays of both knees of all rabbits were taken at the end of the second week and at the end of the eighth week. The operated knees were collected for histopathological analysis.

Results: Radiological data show a significant difference in the level of ossification between the groups in the 2nd week; however, this difference was lost in the 8th week. Histopathologically, at the end of week 8, it was observed that the subchondral bone tissue was incompletely renewed in all the groups. The cartilage tissue of the joint surface was not fully formed and renewed and that it did not completely coalesce with the old cartilage tissue in all of the groups. Compared with the other groups, the group that fracture was anatomically reducted with no irrigation (Group 1), the cartilaginous tissue layer formed was thicker while the surface of the tissue was flatter.

Conclusion: There were no adverse effects of intra-articular lavage on fracture union and cartilage healing in an in vivo environment. Nonetheless, the findings of this study should be confirmed with a larger sample size.

Keywords: Intra-articular fracture, arthroscopy, irrigation, fracture union, cartilage healing.

✉ Dr. Osman Orman,
Department of Hand Surgery, Baltalimani Metin Sabanci Bone and Joint Diseases Education and Research Hospital, Istanbul, Turkey
E-mail: osmanorman@baltalimani.gov.tr
osmanorman@gmail.com
Received: 2019-09-22
Accepted: 2019-12-06 / Publication Date: 2020-01-01

Introduction

In recent years the number of arthroscopic joint surgeries has increased dramatically, becoming an important part of orthopedic surgeries. The use of arthroscopy in fracture treatment is considered to be more advantageous compared to open surgery for determining the fracture type and associated soft tissue injury as it is less harmful to surrounding tissues and provides better reduction [1]. Fractures in the tibia plateau [2-5] and eminence [6-8]; ankle [9-11]; femoral head [12,13]; shoulder glenoid [14]; tuberculum majus [15]; distal clavicle [16]; elbow radial head [17], coronoid [18], capitellum [19]; wrist distal radius [20-22] and scaphoid [23-25] were reported to be successfully treated by arthroscopy assisted surgical techniques.

During arthroscopy, the joint surfaces and fracture zone are irrigated with excess of 0.9 % isotonic NaCl or Ringer's Lactate solutions for a few hours. It has been reported that irrigation with pressure lavage can break the healed fractures in the metaphyseal area but there is no evidence that application of saline solution into the joint without pressure for a long time and in high volume is harmful for the unification of fractures [26]. In addition, application of NaCl and Ringer Lactate to the solid cartilage during arthroscopy does not have any harmful effects but their effect on the broken cartilage is still unknown [27,28]. In this study we have examined whether the application of intra-articular lavage during arthroscopic joint fracture surgery can disturb fracture union and cartilage healing.

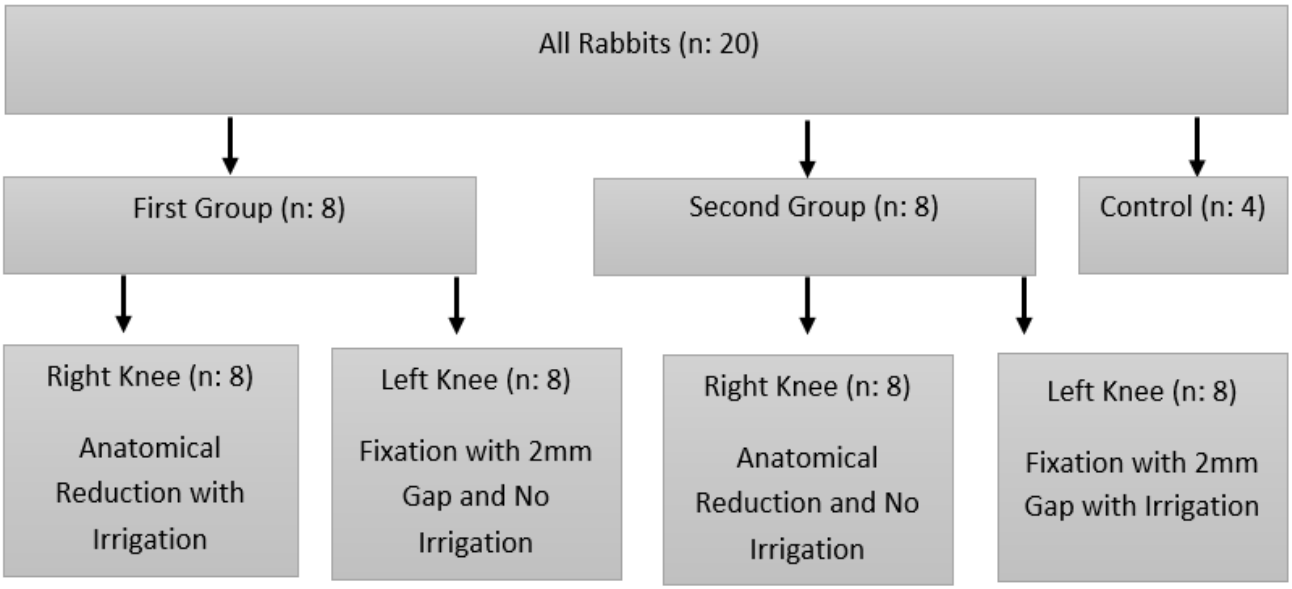
Materials and Methods

The study was approved by the Bezmialem Vakif University Animal Experiments Local Ethics Committee (Decision no: 2015/220). Twenty New Zealand rabbits were used which weighed between 2400 g - 2800 g and were 9 - 15 months of age. Prior to the surgery, the rabbits were acclimatized for three days and fed on a regular diet. The rabbits were then randomly divided into 3 groups; these groups consisted of 2 surgical groups including eight rabbits and a control group consisting of 4 rabbits (Graphic 1). Both knees of all rabbits were used in the study in order to reduce the number of subjects.

Surgical protocol

Thirty minutes before the operation, the antibiotic Cefazolin Na (50 mg IM) was applied prophylactically. Meloxicam (10 mg / kg) was administered subcutaneously on the day before the surgery and for 3 days postoperatively. The operations were conducted under general anesthesia using Ketamine (35-40 mg/kg) IM following sedation with Xylazine (3-5 mg/kg). After both rear limbs were shaved, they were fixed with a clip, covered with sterilized dressing and the knee joint was exposed with a medial parapatellar incision. The medial femur condyle was fractured at 45 degrees oblique and osteotomy was initiated from the middle of the joint. For the first group of rabbits, after the fractures created in the right knee underwent anatomical reduction without leaving a gap, they were fixed with one 2.7 mm cortical screw and 2 cannulas, which transversed the joint, were inserted. The wound was tightly stitched and made waterproof. The fracture of the left knee was fixed with a 2.7 mm screw, leaving a

Graphic 1. Grouping of subjects.



2 mm gap between the fracture lines and they were closed without any cannula placement. For the second group of rabbits, after the fractures created in the right knees underwent complete anatomical reduction, they were fixed with a 2.7 mm cortical screw and closed without placing cannulas. The fractures of the left knee were fixed with a 2.7 mm screw with a 2 mm gap and 2 cannulas, which transversed the joints, were placed. The wounds were tightly closed without leaving a gap and made waterproof.

Thirty minutes after finishing the surgery and before ending the anesthetic procedure, the cannulated knees were irrigated by infusing 1 liter of normal saline solution for 30 minutes and the cannulas were pulled out (Figure 1, 2). The rabbits were followed up on a regular diet for 8 weeks. During the follow up, rabbits that exhibited the presence of distal localization of the fracture, loss of fixation, development of infection, significant reduction lost and a weight loss of more than 20% [29,30] were excluded from the study.

X-rays of both knees of all rabbits were taken at the end of the second week and at the end of the eighth week followed by euthanasia with high dose Xylazain and Ketamine. The operated knees were collected for histological (Giemsa and fluorescent) analysis.

Radiological Analysis

Four dials around the bone containing the mineralized external callus were evaluated in anteroposterior and lateral direct radiographs taken at the end of week 2 and week 8 (Table 1) [29] .

Histopathological analysis

The collected tissues were fixed in 10% neutral buffered formalin and decalcified in a 10% EDTA (pH 7.4) decalcification solution. After decalcification, the tissues were rinsed with distilled water and an alcohol series (70%, 90%, 96% and 100%) followed by incubation with xylene and embedding in paraffin. 5mm thick sections prepared in a microtome were placed on positively charged slides. The sections were stained with hematoxylin & eosin and histopathologically analyzed using a light microscope (Nikon Eclipse i5, Tokyo, Japan). Histological findings were scored as previously

described by Wakitani et al [31]. The sections were rated for: 1) Cell morphology (maximum 4 points), 2) Matrix Staining Intensity (maximum 3 points), 3) Surface Regularity (maximum 3 points), 4) Thickness of Cartilage (maximum 2 points) and 5) Integration of donor with host adjacent cartilage (maximum 2 points). The maximum score was calculated as 14 points.

Statistical analysis

The non-parametric Kruskal Wallis and post-hoc Dunn Multiple tests were used for statistical comparison of the four surgical groups with each other. If any significant differences were seen in Kruskal Wallis Test, then post-hoc Dunn test was applied at the second stage in order to confirm the difference.

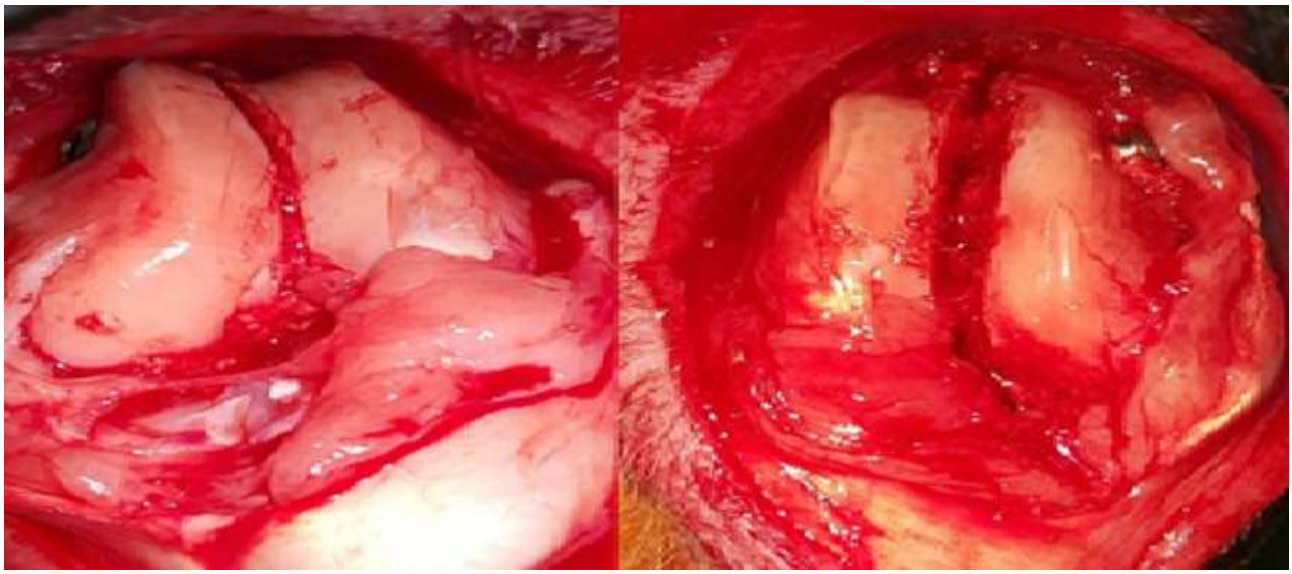


Figure 1. Intra-operative images, a) Right knee fixed without leaving a gap. b) Left knee fixed with 2mm gap.

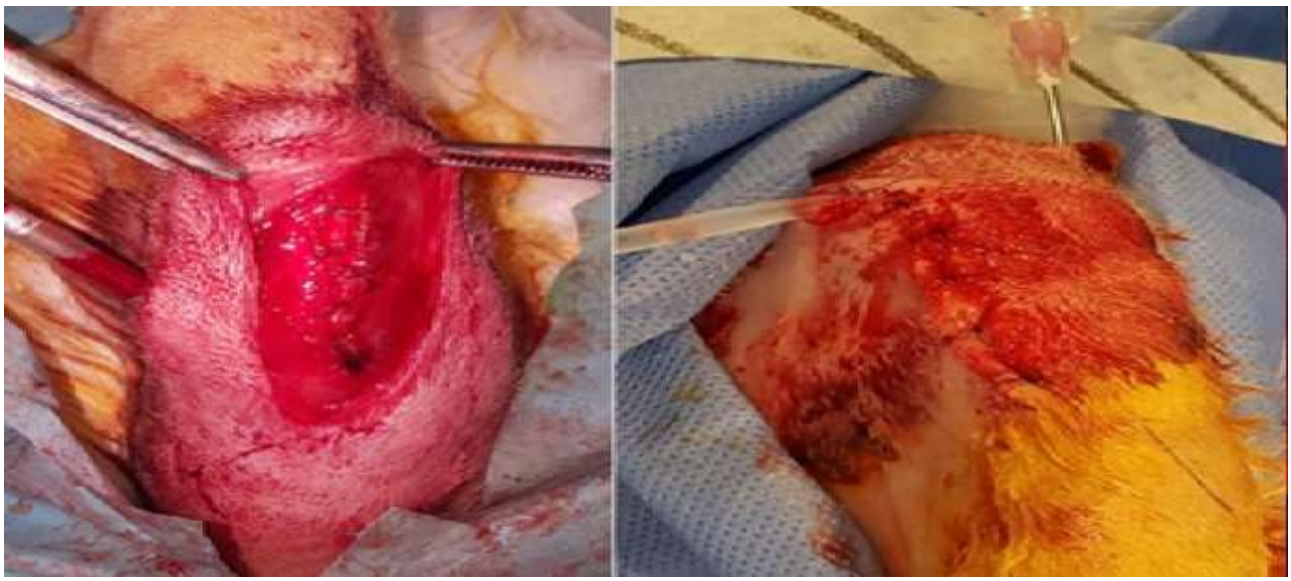


Figure 2. a) The joint capsule tightly closed with a cannula placement. b) In-knee irrigating; with 18 gauge injector, the liquid is drained from the plastic cannula while 0.9% saline solution is injected into the joint.

Table 1. Radiological evaluation scale.

0	No bridged mineralized callus appearing on any scale
1	A brilliant mineralized callus seen on a scale
2	Bridged mineralized callus seen on a scale
3	Triple bridged mineralized callus seen on a scale
4	Four bridged mineralized callus seen on a scale

Results

The study was initiated with 20 rabbits with the aim to assign eight subjects to each group. Bilateral femur diaphysis fracture developed in 1 subject, unilateral femur diaphysis fractures developed in 3 subjects; infection occurred on one side of the femur in 2 subjects; while one rabbit died during the study. The 7 subjects mentioned above were replaced with new subjects. The study was completed at the end of week 8 when all of the rabbits were sacrificed and histological specimens were obtained from them. After histological examination, those subjects with pseudoarthrosis and loss of implant position were excluded as well. Subject populations that were evaluated for each group are shown in Table 2.

The level of ossification was evaluated from the radiographic data obtained in the second and the eighth weeks and graded according to the method outlined in Table 2; the mean values of ossification for the different groups are given in Table 3. A significant difference (Kruskal-Wallis test) was found in the level of ossification between the groups in the 2nd week; however, this difference was lost in the 8th week. Additionally, the ossification data from the groups at Week 2 were subjected to a post-hoc Dunn's test. We observed significant differences between the first (G-NI) and fourth (C-I) groups; the fourth group exhibited less ossification in early time point. No significant differences were found in other comparisons (Table 4).

At the end of week 8, it was observed that the subchondral bone tissue was incompletely renewed in all the groups. A comparison of the extent of subchondral bone tissue renewal indicated an increase in Groups C-NI and C-I when compared to Groups G-NI and G-I.

Unlike the incomplete formation of new bone tissue in the subchondral area, an increase in connective tissue and vascularization were observed in all of the groups. When the

Table 2. Group names, abbreviations and number of samples.

Group	Process	Abbreviation	Number
1	Fixed by creating a gap and no irrigation	G-NI	7
2	Fixed by creating a gap and irrigation	G-I	6
3	Fixed with complete reduction and no Irrigation	C-NI	5
4	Fixed with complete reduction and Irrigation	C-I	7
5	Control		8

Table 3. Radiographic median values of the groups.

Groups		Median	Min	Max
Group 1 G-NI	2nd Week	1.00	0.00	1.00
	8th Week	3.00	2.00	4.00
Group 2 G-I	2nd Week	1.00	0.00	1.00
	8th Week	3.00	2.00	4.00
Group 3 C-NI	2nd Week	1.00	0.00	1.00
	8th Week	3.00	1.00	4.00
Grup 4 C-I	2nd Week	2.00	1.00	2.00
	8th Week	3.00	2.00	4.00

cartilage tissue of the joint surface was examined, it was observed that the cartilage tissue was not fully formed and renewed and that it did not completely coalesce with the old cartilage tissue in all of the groups. In Group C-NI, the new tissue, which was formed on the joint surface, was generally fibro-cartilage in nature and in some areas hyaline cartilage tissue was observed. In other groups, hyaline cartilage tissue formation was not observed but there was the formation of extensive connective tissue. Compared with the other groups, the cartilaginous tissue layer formed in Group C-NI was thicker while the surface of the tissue was

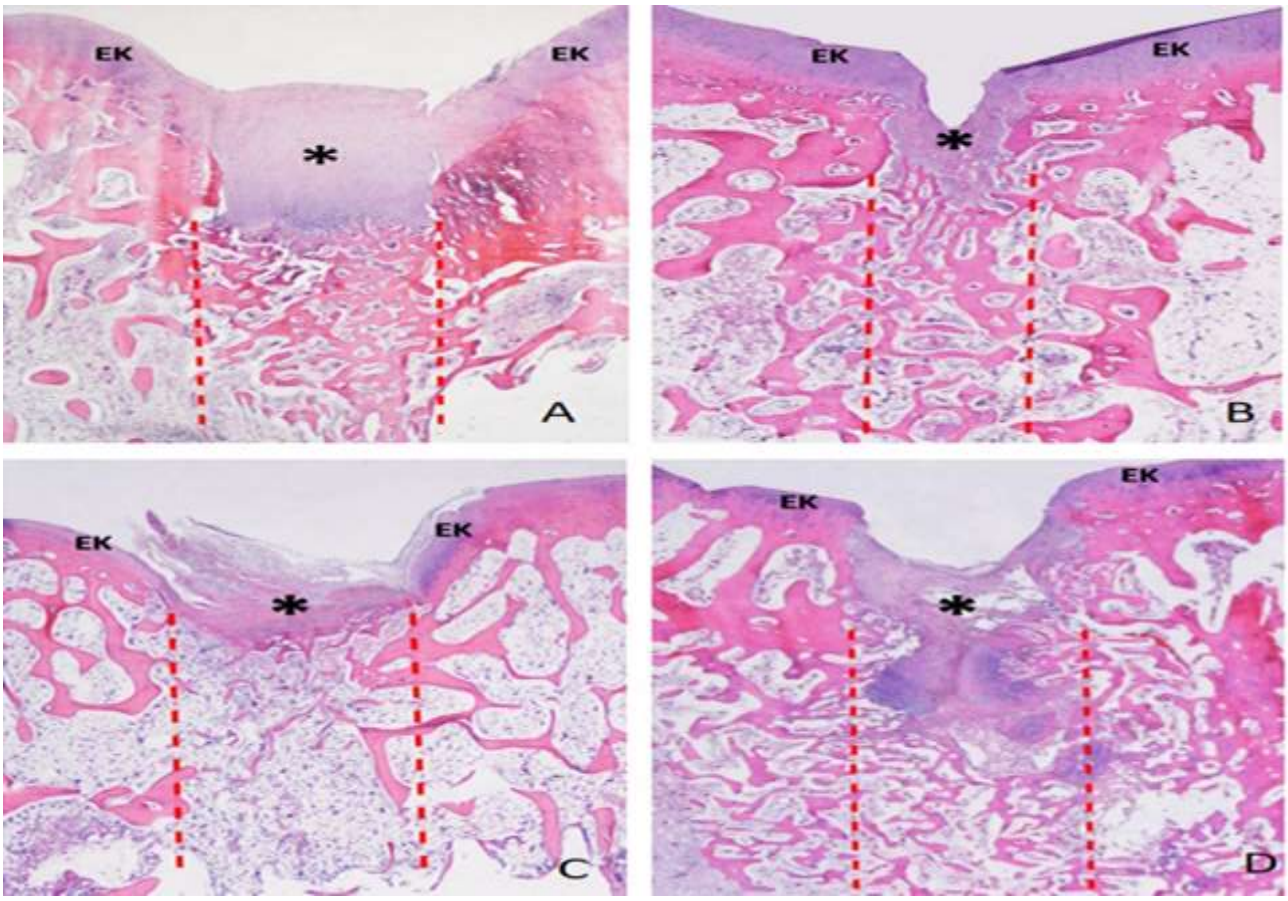


Figure 3. Histologic analysis of sections taken from the knee joints by staining with hematoxylin & eosin. In groups C-I **(B),** G-NI **(C)** and Group G-I **(D),** the area between the old cartilage tissue (EK) on the joint surface has a fibrous connective tissue feature (*) with a new developing density and a subchondral ossification area (red striated area) which is below this part. In group C-NI (A), fibrocartilage tissue (*) containing both connective tissue and newly formed hyaline cartilaginous tissue (*) and newly developed subchondral ossification area (red striated area) has been observed. (C-NI: Time reduction, no irrigation, C-I: Complete reduction, irrigation available, G-NI: Intermittent fix, no irrigation, G-I Reduction with gap with irrigation. Graphic 1. Grouping of subjects.

flatter. Additionally, the newly formed tissue was better fused with cartilaginous tissues adjacent to it. Statistical evaluation of the data and the Wakitani scoring indicated there was incomplete improvement in all groups; nonetheless, the Group C-NI demonstrated the best histological parameters for healing (Tables 5-8, Figure 3).

Discussion

Intra-articular fractures are compulsive fractures because they require both anatomic fracture reduction and rigid fixation; moreover it is often difficult to reach the fracture area. Even minor problems may affect the clinical results negatively. A better understanding and experience of arthroscopic methods may lead to

Table 4. Statistical comparison of the week 2 and week 8 data in different the groups.

	Kruskal Wallis Chi-square Test		Post-hoc Dunn Test					
	K-W Value	*P* Value	Group 1 - 2	Group 1 - 3	Group 1 - 4	Group 2 - 3	Group 2 - 4	Group 3 - 4
2 nd Week	10.336	0.016	1.000	1.000	0.024	1.000	0.272	0.065
8th Week	1.155	0.764	Not suitable for further evaluation.					

p<0.05, K-W: Kruskal Wallis

Table 5. Median values of ossification percentages at week 8.

Groups	Median	Minimum	Maximum
Group 1 (G-NI)	12.70	9.22	19.92
Group 2 (G-I)	11.81	2.03	33.68
Group 3 (C-NI)	24.44	21.46	29.33
Group 4 (C-I)	20.43	16.69	32.29

Table 6. Comparisons of ossification percentages at week 8 in different the groups

	Kruskal Wallis Chi-Square Test		Post-hoc Dunn Test					
	K-W Value	*P* Value	Group 1 - 2	Group 1 - 3	Group 1 - 4	Group 2 - 3	Group 2 - 4	Group 3 - 4
Percent of Ossification	10.053	0.018	1.000	0.065	1.000	0.106	0.353	0.231

K-W: Kruskal Wallis Value.

Table 7. Mean values according to Wakitani 31, 32 histological grading scale at eighth week.

		Cell Morphology	Matrix Staining	Surface consistency	Cartilage Thickness	Combining of the donor tissue with recipient neighboring cartilage.	Total
Group 1 (G-NI)	Median	4.00	3.00	3.00	2.00	2.00	2.00
	Maximum	4.00	3.00	3.00	2.00	2.00	14.00
	Minimum	2.00	2.00	1.00	1.00	1.00	7.00
Group 2 (G-I)	Median	3.50	3.00	3.00	2.00	2.00	13.50
	Maximum	4.00	3.00	3.00	2.00	2.00	14.00
	Minimum	2.00	2.00	1.00	2.00	1.00	8.00
Group 3 (C-NI)	Median	2.00	1.00	2.00	2.00	1.00	9.00
	Maximum	2.00	2.00	3.00	2.00	2.00	10.00
	Minimum	1.00	1.00	1.00	0.00	0.00	3.00
Group 4 (C-I)	Median	3.00	2.00	3.00	2.00	2.00	12.00
	Maximum	4.00	3.00	3.00	2.00	2.00	14.00
	Minimum	2.00	2.00	1.00	1.00	1.00	8.00

Table 8. Statistical comparison of cell morphology and matrix staining in the groups.

	Kruskal Wallis Chi-Square Test		**Post-hoc Dunn Test**					
	K-W Value	*P*	Group 1- 2	Group 1-3	Group 1-4	Group 2-3	Group 2-4	Group 3-4
Cell Morphology	10.710	**0.013**	1.000	**0.016**	1.000	**0.038**	1.000	0.349
Matrix Staining	12.873	**0.005**	1.000	**0.016**	1.000	**0.007**	0.632	0.432
Surface Consistency	4.832	0.185	Not suitable for further evaluation.					
Cartilage Thickness	3.470	0.325	Not suitable for further evaluation.					
Combining of the donor tissue with recipient neighboring cartilage.	7.435	0.059	Not suitable for further evaluation.					
Total	9.371	**0.025**	1.000	0.054	1.000	**0.035**	1.000	0.552

more extensive use of arthroscopy in intra-articular fracture surgery. Arthroscopically assisted fixation of intra-articular fractures has the following advantages over traditional fixation methods: a) much better visualization and full reduction despite minimal invasiveness, b) increased clinical improvement due to diagnosis and c) repair of other injuries accompanying the fracture [1]. Nevertheless, there are inherent limitations of arthroscopy-assisted intra-articular fracture treatment the most important of which are the long learning curve and the material for fixation. Our study was designed to examine another possible limitation, the effect of lavage during arthroscopy on the healing of fractures and cartilage tissue.

There are four separate mechanisms responsible for the healing of fractures: Enchondral ossification in which the fracture hematoma plays a role, intramembranous ossification in which periostea is responsible, appositional ossification and ossification through the direct Haversian system. These mechanisms contribute to fracture healing at various rates and may be affected by variables such as fracture shape, location, stability, and fixation type. Usually, in fractures that undergo anatomical reduction and rigid fixation, the healing is primarily through a more direct Haversian system (primary ossification) rather than hematomas. On the other hand, fractures that undergo healing without anatomic reduction and rigid fixation heal with hematoma by (secondary ossification) enchondral ossification [33]. Joint cartilage defects, on the other hand, heal with fibrous tissue formation. We tried to prevent secondary ossification at the osteotomy sites by removing the hematoma from the environment via arthroscopy like closed irrigation. In preliminary studies, some cases were created to remove the fracture hematoma. Park et al reported that after osteotomy in the rabbit tibia diaphysis, union was delayed or never developed with open irrigation in the first and the second day [34]. Dirschle et al observed a delay in union by 20 – 30% at early time points when pressureless irrigation with syringes and high pressure irrigation system were used after femur medial condyle osteotomy [35]. Cartilage tissue healing constitutes the subject of many animal experiments; Mitchell et al. [36] reported that medial femoral condylar fractures of the rabbit femur healed with hyaline cartilage when compression was done; however, without compression they healed with fibrous cartilage.

Although the effect of lavage solutions on natural cartilage tissue has been investigated previously, to our knowledge, no study in the published literature has examined the effects of arthroscopic lavage on fractured cartilage and bone tissue [27,28]. Rabbits were considered as appropriate subjects for this study since the Haversian system of rabbits are similar to human bones and because the cost and care of rabbits are more reasonable compared to larger animals. Our experimental setup was based on the femur medial epicondyle osteotomy and fixation method described by Mitchell et al. [36] Unlike other published studies; lavage after osteotomy was carried out by providing a closed lavage environment in order to simulate the arthroscopic environment [26,34,35]. In the rabbit knees that were fixated with gap and underwent irrigation (group 2), we expected to observe a decrease in fracture union tissue quantity and deterioration of the quality of cartilage tissue that were likely related to the removal of the hematoma; however, in the histological examinations, there was no significant difference between the groups in the percentage of ossification. Also when we

examined the cartilaginous tissue, we observed that the Wakitani score was lower in the second group compared to the third group where complete reduction was carried out without any irrigation. We also observed that cell morphology and matrix staining scores were lower in the second group. However, we also observed similar data in the first group of animals where a gap was created and the wound was not irrigated. For this reason, we think that the healing disorder of the cartilaginous tissue was caused by the created gap, and not by washing. We did not observe any harmful effect of washing on fracture and cartilage healing in our study.

The major weaknesses of our study are the low number of subjects and the use of both knees of the subjects. In addition to this, radiologic studies were performed with direct X-ray because of the lack of access to micro-computerized tomography, which would show the rabbit bone structure better.

Conclusion

There were no adverse effects of intra-articular lavage on fracture union and cartilage healing in an *in vivo* environment. Nonetheless, the findings of this study should be confirmed with a larger sample size.

Funding: *There is no financial support and sponsorship*

Conflict of Interest: *The authors declare that they have no conflict of interest.*

ORCID iD of the author(s)

Osman Orman /0000-0002-9177-2457
Kahraman Ozturk /0000-0002-7644-659X
Mehmet Baydar /0000-0002-1020-1207
Ethem Guneren /0000-0002-5981-7010
Elif Taslidere /0000-0003-1723-2556
Mujgan Orman /0000-0002-9629-0211
Omer Ozel /0000-0002-6859-017x

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