Comparison of healing intra-articular fracture of distal femur using a Kirschner wire and autologous fibrin glue in an animal model
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Considering different surgical techniques for the fixation of osteochondral intra-articular fracture, the present study aimed to compare the efficacy of autologous fibrin glue and Kirschner wire (KW) on an osteochondral fracture in the left lateral condyle of Dutch rabbits with a control group. After 6 weeks, macroscopic and microscopic evaluation showed that autologous fibrin glue is easier and faster with a higher number of bone trabecula (\(P < 0.05\)), whereas the healing rate and cellularity of the healing site were not different between the two groups (KW and glue). The use of autologous fibrin glue can be an alternative to KW fixation in the fixation of osteochondral fractures.

Introduction

Bone is an ever-changing tissue and consists of biologic active cells, integrated in a rigid framework. When a fracture occurs, the cellular contents of the bone, including osteoclasts, osteoblasts, and mesenchymal cells, are activated. The healing process of the bone includes various interconnected mechanisms that can be influenced by biomechanical, biochemical, cellular, and hormonal elements [1]. However, the healing of osteochondral lesions has several problems as the entry of synovial fluid into the fractured area and movement at the fracture surface may prevent callus formation. In addition, the existence of no vessel in the articular cartilage limits the healing potential [2].

Thus, fixation of articular fractures is a challenging issue as the standard methods, including Kirschner wire (KW) or screws in the joint, can interfere with the joint’s motion and cause joint stiffness. Because of the proximity of the growth cartilages to joints in young children, fixation devices in articular fractures can cause additional growth problems. Use of fibrin-based tissue adhesives has been introduced as a potentially useful method for fixation of these challenging fractures [3,4].

Fibrin glue or sealant, composed of clot-forming proteins, including fibrinogen, factor XIII and fibronectin, and thrombin [3] with added platelet rich plasma (PRP), enhances the healing properties by platelet-mediated release of cytokines and growth factors [5,6], which enhances neovascularization [7,8] and can theoretically seal the area and block the entry of the synovial fluid into the fracture site [9]. Autologous fibrin glue causes no adverse allergic reaction or transmission of viral or prion agents, is more cost-effective than the traditional forms, and improves cell migratory patterns, sustainability, and morphology by their immunologic content [10]. Some studies have suggested that using fibrin glue instead of KW can eliminate the mechanical block of joint movement and growth plate damages caused by fixation devices in children [11]. However, possible drawbacks to the use of autologous fibrin glue include the amount of blood needed for the generation of the glue, nonstandard quality of the glue because of the difference in individuals’ blood protein profile and the risk of contamination.

Because of the significance of healing osteochondral fractures, this study aimed to compare the efficacy of PRP augmented autologous fibrin glue with KW fixation in healing osteochondral fractures of the distal femur of rabbits.

Materials and methods

Animals

Thirty adult male Dutch white rabbits, each weighing 2–2.2 kg, were selected for this experiment, which were kept under standard laboratory conditions (temperature 20–22°C, with relative humidity, 14 h light and 10 h darkness) in 60 × 60 × 60 cm cages under specified pathogen-free conditions with free access to water and food at laboratory animal unit. Mature rabbits, with closed growth plates, were divided randomly into three equal groups of 10 rabbits; one group received KW fixation and the second group received fibrin glue, which were compared with each other and with a control group.
The protocol of the present experiment was approved by Ethics Committee of Shiraz University of Medical Sciences.

**Fibrin glue preparation**

A peripheral blood sample was obtained before the surgery, which was centrifuged for 10 min at 2800 rpm in 21°C for separation of PRP. Platelet counts were then measured for each rabbit, yielding a standard mean PRP platelet number. The fibrinogen solution was subsequently prepared using plasma according to the autologous-donor production method [12]. Autologous thrombin was prepared using the remaining plasma. Briefly, calcium gluconate was added to PRP, mixed, and allowed to stay. The fibrin glue was formed by mixing the cryoprecipitated fibrinogen, thrombin, and calcium gluconate during the surgery; 100 µl of this mixture was used for the fixation of each osteochondral fracture.

**Surgery**

Anesthesia was induced by an intramuscular injection of 10 ml/kg ketamin (Ketalar; Parke Davis, Mumbai, Maharashtra, India) and 8 mg/kg xylazin (Rompun; Bayer AC, Leverkusen, Germany), and a preoperative dose of intramuscular cefazolin sodium (50 mg/kg) was injected for prophylaxis of infection. All surgical procedures were performed under aseptic conditions by a single surgeon. Under general anesthesia, after preparing and draping, the joint was exposed through direct incision on the lateral condyle of the left femur and an osteochondral cylindrical fragment, 10 mm in diameter and 6 mm in depth, was harvested from the lateral condyle by a standard size trocar. Then, the fragment was relocated to the fracture site that was fixed by a KW size 1 in KW group (Fig. 1), whereas in the control group, the osteochondral fragment was relocated to the site of fracture only, without any extra fixation method. In the glue group, the osteochondral fragment was relocated and fixed by autologous fibrin glue. Finally, the capsule and periosteum were repaired and the skin was sutured by nylon 4–0 and dressing was performed. Rabbits received preoperative antibiotics and postoperative analgesic and antibiotic for 72 h; they were kept and taken care of under standard conditions for 6 weeks.

After this period, the rabbits were killed using an overdose of diethyl ether. The lateral condyle of the left femurs was excised and sectioned in the coronal and sagittal planes and the fragment and its surrounding bones were observed for fracture healing. Then, the specimens were fixed in 10% neutral buffer, decalcified in 15% buffered formic acid, and embedded in paraffin for histopathological examination.

**Macroscopic evaluations**

The healing of the fragment was described by direct observation as union of the bone trabecula in the two coronal and sagittal planes of sections; when the osteochondral fragment was firmly connected to the crater, it was considered united and when easily detached, like a loose body, it was considered nonunited.

**Microscopic evaluations**

Two 5-µm thick sections were prepared from each specimen and stained with hematoxylin and eosin. The sections were scored in a blinded manner by a pathologist according to the Parisi et al. [13] and Emery et al. [14] scoring system. The presence of hyaline cartilage, fibrocartilage, woven bone, and trabecular bone at the callus was investigated. The number of trabecular bones in ×100 magnification of the microscope was counted in three different fields and scored as follows: grade 0 (absent), grade 1 (<30 trabecula), grade 2 (30–40 trabecula), and grade 3 (>40 trabecula). The presence of granulation tissue and chronic inflammatory cell infiltration was also scored as grade 0, 1, 2, and 3 for absent, mild, moderate, and severe states, respectively.

**Statistical analysis**

Analysis was carried out using SPSS software, version 18.0 for windows (SPSS Inc., Chicago, Illinois, USA). χ²-Test or Fisher exact test was used for nonparametric values including microscopic variables and macroscopic evaluation results. P values of less than 0.05 were considered statistically significant.

**Results**

Of the 30 rabbits, three died without any specific cause (one in the glue group and two in the KW group) and were excluded from the study. Finally, there were 27 rabbits: eight in the KW group, nine in the glue group, and 10 in the control group. Overall, the osteochondral fracture healing rate was 100% in the KW group, 77.8% in the glue group, and 40% in control group. The difference
in the healing rates between the KW group and the control group was statistically significant ($P=0.013$). No statistically significant difference was found between the healing rate of the control and the glue group, and also between the KW and the glue group. Although no statistically significant difference was found between the healing rate of the glue group and the KW group, the healing fracture rate in the glue group (77.8%) is clinically important compared with the control group (40%). The mean duration of surgery in the glue group was shorter than the pin group (6 vs. 8 min).

In terms of the trabecular bone density, in the glue group, 85.7% of samples had grade 3 and the remaining had grade 2. In the pin group, 25 and 75% of the samples showed healing grade 3 and grade 2, respectively. In the control group, all the samples had grade 1 (Table 1 and Fig. 2). There was a statistically significant difference between the mean bone trabecula number in the healed area between the KW and the control groups ($P<0.05$). In terms of other histological findings, the healed articular surface showed the presence of mixed hyaline cartilage and fibrocartilage, with no significant difference between the severity of inflammatory cell infiltration and granulation tissue formation in the healing site of different groups.

**Discussion**

In this study, the results of autologous fibrin glue usage for fixation of osteochondral fracture in animal model were compared with KW, whereas the glue group was not significantly different from the control group and the KW group in terms of osteochondral fracture healing, the mean time of surgery was shorter in the fibrin glue than the KW group, and the cellularity of healed fracture showed improvement in the fibrin glue group.

Similar to the present study, some researchers have compared several fixation methods. In one study, tensile strength of fibrin sealant and KW for fixation of osteochondral fractures of the knee were compared in dogs and the results of the study reported that fibrin sealants provide less initial tensile strength, but greater mean maximum tensile strength, compared with KW, and concluded that fibrin sealant can be used as an alternative method of fixation for small fragments [15]. Although it was shown previously that fibrin sealants can enhance revascularization and chondrocyte migration, the osteogenic potential of fibrin glue is not fully described [16,17]. Plaga et al. [18] compared three fixation methods for unstable osteochondral knee fractures [K-wires, fibrin glue (commercial) and polydioxanone pins] without immobilization and reported 29% healing in the no-fixation group, 50% in the fibrin glue, 86% in the polydioxanone pins, and 100% in the KW groups. The results of the present study also reported a similar healing rate in KW group, but a higher healing rate in the fibrin glue group. This finding could be because of the addition of PRP to the fibrin glue in our method and enhanced healing properties of the glue material as Nair et al. [19] have reported that activation of platelet aggregation by thrombin and calcium can form a three-dimensional fibrin scaffold, rich in growth factors and proteins, which might be promoting osteogenic differentiation.

Several studies have evaluated the efficacy of fibrin glue as a modern alternative because of the disadvantages of

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<th>Type of fixation (group)</th>
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<th>2</th>
<th>3</th>
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<tr>
<td>Fibrin glue (%)</td>
<td>0.0</td>
<td>14.3</td>
<td>85.7</td>
<td>100</td>
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<tr>
<td>KW (%)</td>
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<td>25</td>
<td>100</td>
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<tr>
<td>Control (%)</td>
<td>100</td>
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The mean number of trabecular bones in the glue group is more than that in the KW group ($\times100$ hematoyxlin and eosiin).

*aTrabecula density in the healing area in $\times100$ microscopical field: (a): low density (>30 trabecula), (b): intermediate density (30–40 trabecula), and (c): high density (>40 trabecula).
standard methods [3,4]. In one study, the role of fibrin adhesive sealant has been investigated in the repair of osteochondral fracture of the radial head and the femoral condyle in dogs that reported a 100% union rate and stimulated faster fracture repair by the fibrin sealants [20], whereas the present study reported a 77.8% gross union rate. The difference in the results of studies might be because of differences in animal healing properties, different glue ingredients, and different fracture details. In addition, in the present study, we created a relatively stable and smaller osteochondral fracture and did not immobilize the operated knees, which could have led to a difference in the healing rate.

We found a higher rate of fracture healing in the fibrin glue group (77.8%) compared with the control group (40%). Although the difference was not statistically significant, this finding may be clinically useful for considering fibrin glue fixation for small osteochondral fractures in difficult cases. Compared with other studies, the strength of this study is the use of PRP augmented autologous fibrin glue and histologic documentation of the quality of the fracture healing. However, the present study had some limitations, including the limited number of samples, which could affect the results, in addition to interobserver bias caused by minor differences in the induced fracture. Moreover, we did not apply immobilization that could affect the results. Therefore, it is suggested that future studies compare fracture healing rates using different fibrin sealants, either commercially available or autologous forms, and determine the effect of joint immobilization on healing the osteochondral fracture fixed by fibrin-glue.

**Conclusion**

Autologous fibrin-glue is a viable and effective material for fixation of osteochondral lesions in a rabbit model with a higher mean number of bone trabecular production in the bone healing area. Future studies can highlight the clinical advantages of fibrin glue method for the treatment of osteochondral fractures.

**Acknowledgements**

The authors thank Dr Mahjub Vahedi and Omid Kohi Hosseinabadi at the Laboratory Animal Center, Shiraz University of Medical Sciences, for their sincere assistance. They also thank Dr Nasrin Shokpouri at the Center for Development of Clinical Research of Nemazee Hospital for editorial assistance.

This work was supported financially by the Transplant Research Center, Shiraz University of Medical Sciences, Shiraz, Iran, under the thesis proposal submitted at no. 120.

**Conflicts of interest**

There are no conflicts of interest.

**References**