Biomechanical Evaluation of Spontaneous Repair of Osteochondral Defects in the Rabbit Knee

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ABSTRACT

Background: Examination of cartilage repair in animal work is dependent upon the thickness and radius of the induced impalement. Full-thickness defects with a radius of 3 mm have been commonly used in animal studies to evaluate new procedures designed to improve the quality of articular cartilage repair. The aim of the present study was to define the biomechanical characteristics of the repair of 5×4 mm full-thickness osteochondral defects in adult male rabbits.

Methods: In a controlled clinical trial study 5 mm diameter and 4 mm deep osteochondral defects were drilled in the femoral patellar groove of twenty-one rabbits, and examined at 4, 8, and 16 weeks. The left knee was kept intact and was regarded as control. The knee joints were removed, and both legs were examined biomechanically by in situ indentation method at three time intervals (4, 8, 16 weeks). The instantaneous and equilibrium elastic modulus (after 900 second) were measured during the test.

Results: There were no differences in cartilage mechanical properties (instantaneous and equilibrium elastic-modulus) in different weeks (4, 8, 16 weeks) in the two groups (P=0.08). However, significant differences were seen between the experimental and control groups in 16 weeks in instantaneous elastic modulus (P=0.44). It suggests that new tissue in this group had more stiffness than control in 16 weeks.

Conclusion: Full-thickness osteochondral defect, measuring 5×4 mm in the patellar groove of the adolescent rabbit knee healed spontaneously.

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Introduction

Injury to articular cartilage in the major weight-bearing joints is an important problem in medicine [1]. From biomechanical point of view, the articular cartilage is a viscoelastic, non-homogeneous, un-isotropic tissue. Lack of vessels, nerves, lymphatic flow and metabolism are the major problems of the cartilage [2,3]. The main source of nutrition of the cartilage is from the synovial fluid and occasionally depends on the bone under the cartilage [4]. 61.50% of the knee arthroplasties are related to the cartilage defects. These defects are accompanied by diseases like osteoarthritis, rheumatoid arthritis and some congenital and metabolic diseases such as acromegaly, Paget, hemophilia. They can also occur after direct or indirect traumas and impact loading [4]. Usually after articular cartilage defects disabling symptoms such as pain, joint locking, and swelling are observed. It is believed that these defects can lead to progressive osteoarthritis [2]. Although articular cartilage has a highly organized structure necessary for its function, it lacks an intrinsic capability to repair defects more than 2-4 mm [5,6]. Spontaneous repair of the cartilage in superficial defects is impossible [4].
In the studies in which spontaneous repair of osteochondral defects were assessed in rabbits, defects of 3 mm in diameter and smaller are commonly being used. According to these studies, lesions in this size will repair spontaneously in 12 weeks, [3,7,8] but there are not enough data about larger lesions, so the aim of the study was to investigate long-term biomechanical changes (16 weeks) in spontaneous repair of the osteochondral defects (5×4 mm) in the patellar grooves of adolescent male rabbits.

Methods

This study was approved by the Ethical Committee on Animal Experiments of Shiraz University of Medical Sciences. Twenty one adult Dutch white male rabbits (2±0.40 kg, 4 months old) proliferated in Pasteur Institute of Iran were used for the study. It was a controlled clinical trial. The rabbits were kept about 2 weeks in animal lab to get used to its condition. Then, they were randomly divided into 3 groups (4, 8 and 16 weeks) by blocked randomization procedure. To get anesthetized, the rabbits were given an intramuscular injection of Ketamine (50 mg/kg) and xylazine (5 mg/kg). A longitudinal medial preparatellar incision was made in a sterile condition. The dissection was continued so that the distal end of the patellar groove (femoral trochlea) was exposed. A complete osteochondral defect (5 mm diameter and 4 mm in depth) was drilled in the weight-bearing central part of the patellar groove and then sutured with silk reversal cutting string.

The rabbits were kept in cages (1 m×0.75 m) freely, without any splint. Water and nutrition were available ad libitum. The left knee was kept intact as the control group. The rabbits of all 3 groups (4, 8, 16 weeks) were sacrificed with chloroform-soaked gauze. Then, the biomechanical study was performed.

The right femurs of both groups (control and experimental) were removed through the knee and were kept in plastic bags, frozen at -20°C [9]. From 1 hour before the indentation test, the specimens were put at room temperature, and kept moist with normal saline 0.90% throughout the test.

Indentation testing is a method used to analyze the mechanical properties of the articular cartilage in which the cartilage is put on the bone and investigated. (In situ study) [10,11].

Stress–relax method was used in this research [11]. A 1-mm diameter flat, nonporous indenter was vertically passed into the central patellar groove cartilage 0.20 mm at the rate of 0.50 mm/min in 30 ms and maintained constantly for 900s [12] (Zwick Z, Germany). Then, the instantaneous and equilibrium stress relaxation of the tissue was recorded. The load versus time curve was measured later at the 0.20 mm indentation and after the time. This test was performed in both intact and damaged legs.

Statistical Analysis

Kolmogorov-Smirnov test was performed to identify the normality of the variables in each group. AVONA was used for comparison of means and paired t-test was done for comparing two legs. For this analysis, statistical significance was considered at 0.05 levels.

Results

The results of the ANOVA test showed that there were no significant differences in the mean of the instantaneous and equilibrium elastic modulus among the 4th, 8th and 16th weeks in both groups (intact legs and injured ones) (P=8%) (Figures 1, 2).

On the other hand, the results of the paired t-test showed that there were no significant differences among the experimental and control groups at 4 and 8 weeks in the instantaneous and equilibrium indentation elastic modulus (P=0.07). In the 16th week only the instantaneous stiffness of the experimental group was significantly higher than that of the control group (Figure 3, 4).

Discussion

In this study, the biomechanical changes in spontaneous healing of the osteochondral defects of the patellar...
groove in rabbits (5×4 mm) was studied which showed that the defects of patellar groove in 5×4 mm will heal spontaneously. After the 4th week, there was no biomechanical difference among the experimental and control group. On the other hand, no difference was observed in the biomechanical properties of the cartilage among 4th, 8th and 16th weeks.

In a research on the repair of 3×3 mm osteochondral defects in rabbits, Qui and coworkers showed that there were no tissue or biomechanical differences among the 8th, 16th and 32nd weeks [13]. Wayne and coworkers showed in their research that in spontaneous repair of 3.50 mm depth osteochondral defects in the dog’s patella, there was no difference in the modulus among the 12th, 24th and 32th weeks [14]. The results of paired t-test indicated that there was a significant difference among the intact and injured legs, in the 16th week in instantaneous stiffness. This mean was more in the involved leg.

However, previous results do not confirm these issues. Douglas and colleagues studied the spontaneous repair of a 6×6 mm defect in the cartilage of the femoral medial condyle in 24 goats for 1 year. The contralateral legs in 18 goats were considered as the control group. The findings showed that the osteochondral defects in this size in goats did not repair spontaneously.

However, in studies of Hubergste and Breinan (in separate researches) defects in this size in the patellar grooves of dog and goat were completely repaired [15]. In another study [16], Shahgaldi worked on the repair of large osteochondral defects. 6×6 mm defects were performed in the left femoral lateral condyle of 16 goats and she studied their spontaneous repair for 6 and 12 months.

Convery in a research on the repair of large osteochondral defects in the femoral medial condyle of horses found that the osteochondral defect smaller than 3mm will completely heal after 12 weeks but the defects more than 9mm do not repair spontaneously [17]. The difference among the recent studies and previous ones is probably due to the difference in the region of the induced defects. For example in the previous studies, the defects were in the medial condyle of the femur and patella and in this study it was in the patellar groove. Another reason for the cartilage stiffness after 16 weeks is probably the replacement of the hyaline cartilage by fibrous tissue, which causes virtual cartilage stiffness. Future histological studies may confirm this claim.

Lack of a separate control group was a limitation of this research. Previous studies demonstrated that the altered loading of the contralateral leg during immobilization may cause biomechanical changes in the cartilage content in this leg (specially a decrease in proteoglycans) [19]. Because the matrix components of the cartilage are the most important reason of biomechanical efficiency of the cartilage [20], it is probable that remodeling of these contents is a reason of repairing. It is suggested that further studies be conducted with a separate control group for more precise data.

Conclusion

We conclude that the defect of 5×4 in patellar groove of rabbit will heal spontaneously. On the other hand after the 4th week, there was no biomechanical difference among the experimental and control group. Also no difference was observed in the biomechanical properties of the cartilage among 4th, 8th and 16th weeks. This result is an introductory study for human study, because injury to articular cartilage in the major weight bearing joints is a significant problem in medicine, especially in traumatic cases such as sport injury.

It should be noted as limitations that we did not survey and compare a large number of different sizes of lesions. Also we should consider the natural differences between rabbits and human beings. We aim to conduct this research in future on the animals which have more similarities with human beings, such as monkeys, chimpanzees, and similar animal models.

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References