Original Article

Macroscopic and Histological Evaluations of Meniscal Allograft Transplantation Using Gamma Irradiated Meniscus: A Comparative *in Vivo* Animal Study

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Abstract

Background: Many studies suggest that the gamma irradiation decreases allograft strength in a dose-dependent manner. However, no study has demonstrated that this decrease in strength translates into higher failure rate in meniscal allograft transplantation (MAT). The aim of this study was to investigate the effects of gamma irradiation on macroscopic and histological alterations of transplanted meniscal tissue and joint cartilage after MAT.

Methods: Medial total meniscectomies were performed on the right knees of 60 New Zealand white rabbits. All meniscal allografts were divided into three groups (20 in each group) and then sterilized with 0 Mrad, 1.5 Mrad, or 2.5 Mrad of gamma irradiation. For each group, 5 menisci were randomly chosen for scanning electron microscopic (SEM) analysis and the remaining 15 were prepared for MAT surgeries. Forty-five right knees received MAT surgeries (0 Mrad group, 1.5 Mrad group, 2.5 Mrad group, 15 in each group), whereas the remaining 15 only received medial meniscectomy (Meni group). The left knees of the Meni group were chosen as the Sham group ($n = 15$). All the rabbits were sacrificed at week 24 postoperatively. Cartilage of the medial compartment of each group was evaluated macroscopically using the International Cartilage Repair Society (ICRS) score and then histologically using the Mankin score based on the Masson Trichrome staining.

Results: The SEM analysis confirmed that the meniscal collagen fibers would be significantly damaged as the dose of gamma irradiation increased. At week 24, the overall scores of macroscopic evaluations of the transplanted meniscal tissue showed no significant differences among the three groups receiving MAT surgeries, except for 2 in the 2.5 Mrad group presented partial radial tears at midbody. The ICRS scores and the Mankin scores showed the lowest in the Sham group and the highest in the Meni group ($P < 0.05$). For the three groups receiving MAT surgeries, the 2.5 Mrad group showed significant higher ICRS scores and Mankin scores than both the 0 Mrad group and the 1.5 Mrad group ($P < 0.05$). Whereas the 1.5 Mrad group presented similar results to the 0 Mrad group concerning both the ICRS scores and the Mankin scores.

Conclusions: The current *in vivo* animal study proved that although the meniscal collagen fibers were damaged after gamma irradiation, the failure rate of MAT surgeries might not significantly increase if the irradiation dose was <1.5 Mrad for New Zealand white rabbits.

Key words: Gamma Irradiation; Histological; Macroscopic; Meniscal Allograft Transplantation

Introduction

Menisci play important roles in weight bearing, shock absorption, and lubrication.[1] Meniscectomy has been shown to be associated with increased risk of early knee osteoarthritis.[2-4] Now-a-day, meniscal surgery aims to preserve meniscal tissue as much as possible.[5,6] However, in some cases damaged menisci cannot be repaired, and meniscectomy is inevitable. Therefore, an implant will be considered to replace the removed meniscus.

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Since the first successful meniscal allograft transplantation (MAT) reported in 1984, this operation has been used in patients worldwide.[7] Many studies have evaluated the clinical outcomes of MAT concerning pain and function so far, which generally agreed that it yielded good and excellent results.[8,9] However, concerns remain regarding the availability of graft and possible disease transmission.[10]

Tissue banks implement sterilization techniques to ensure graft sterility. Gamma irradiation, which has known bactericidal and virucidal properties, is currently the most popular option for sterilization of meniscal

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allograft.[11] However, studies have shown that gamma irradiation significantly alters the initial biomechanical properties of meniscal allograft in a dose-dependent manner: Doses as low as 2.0 Mrad have been proved to reduce the initial stiffness and strength of meniscal allograft.[12] It is unknown whether or not this alteration in biomechanical properties has influences on outcomes of MAT.

The purpose of this study was to investigate the effects of gamma irradiation on macroscopic and histological alterations of transplanted meniscal tissue and joint cartilage after MAT. Based on the results of previous studies, we hypothesized that the outcomes of MAT would become worse as the dose of gamma irradiation on meniscal allograft increased.

Methods

Study design

Sixty adult, female New Zealand white rabbits weighing 3.0–4.0 kg were used in this study. Our study received permission from the Institutional Animal Care and Use Committee of Beijing Jishuitan Hospital. The allograft menisci were harvested from the right knees of all rabbits after total medial meniscectomy and then divided into three groups (20 in each group). Each meniscus was treated with 0 Mrad, 1.5 Mrad, or 2.5 Mrad of gamma irradiation and frozen (−20°C) for 6–14 days. For each group, 5 menisci were randomly chosen for scanning electron microscopic (SEM) analysis. The remaining 15 were prepared for later MAT surgeries.

All rabbits first underwent total medial meniscectomy on the right knees. Then 45 of the right knees received MAT with the prepared meniscal allografts (0 Mrad group, 1.5 Mrad group, and 2.5 Mrad group, 15 in each group), whereas the remaining 15 right knees received no MAT surgeries (Meni group). In addition, the left knees of the Meni group were regarded as the Sham group (n = 15). All rabbits were sacrificed at week 24 after the operation for further analyses.

Surgical procedures

First, all rabbits were anesthetized with ketamine (35 mg/kg) and xylazine (5 mg/kg). No immunosuppressive agents were used in this study. Then the operative side was shaved scrubbed with povidone-iodine and aseptically draped, a 5–6 cm long medial parapatellar longitudinal incision was made. The medial meniscus was resected sharply along the periphery and detached from its anterior and posterior junction. Care was taken not to injure the medial collateral ligament, which was important for the postoperative stability of the knee joint. The treated meniscal allograft was thawed by immersion in sterile saline solution and then sutured to the adjacent synovium with nonresorbable No. 4–0 sutures (Ethicon, Somerville, NJ, USA). The anterior and posterior horns of the meniscal allograft were reattached to the appropriate ligamentous structures. The capsule, periarticular tissues, and skin were closed with No. 3–0 Vicryl sutures (Ethicon). For the Meni group, only total medial meniscectomy was performed. For the Sham group, the operation was performed on the medial compartment by the same approach required for the total meniscectomy but without removal of the meniscus.

Postoperative protocols

After wound closure, a bulky cotton dressing was applied to the operative side. The dressing was removed 48 h postoperatively. All the rabbits were given prophylactic antibiotics and allowed free cage activities without restriction of motions immediately after surgery. No intra-articular anesthesia or postoperative analgesics were given to the animals. Specific care measures were taken daily to keep the cages clean and to prevent exogenous infections. At 24 weeks postoperatively, each animal was sacrificed with an injection of 10 ml of thiopental through the ear vein. The operative knees of each rabbit were harvested from the hip joint for macroscopic evaluations and histological assessments.

Macroscopic evaluations of meniscus and joint cartilage

The joints were dissected with the femur separated from the tibia and the meniscus left attached to the tibia plateaus. Photographs were taken of the tibial plateau (Nikon 4600; Nikon Photo Products, Tokyo, Japan) with and without the menisci in place, and of the exposed femoral condyles.

The menisci were macroscopically evaluated with respect to the integration, implant position, horn position, shape, tears, surface, size, tissue, and synovial reaction.[7] Each parameter was scored from 1 to 3 based on the situation of the implants, with 1 denoted the best and 3 indicated the worst.

The joint cartilage of the femur and tibia were evaluated macroscopically according to the criteria of the International Cartilage Repair Society (ICRS) cartilage injury classification.[7] Cartilage of the medial femoral condyle (MFC) and the tibial plateau were analyzed. The medial tibial plateau (MTP) was divided into meniscus-covered (MC) and non-MC (NMC) regions.

Scanning electron microscopic analysis of meniscus

The menisci were thawed at room temperature. Each meniscus was cut into specimens of 12 mm × 12 mm (length × width) along the sagittal plane, which retained the upper and lower planes. Five specimens were randomly chosen for each meniscus. The specimens were repeatedly washed with physiological saline, dehydrated, sprayed with silver, and imaged at the upper plane under a SEM (SEM eXplore Locus SP, GE, USA). Tension, smoothness, orientation, and continuity of the meniscal fibers were evaluated. Each parameter was scored from 1 to 3 based on the situation of the meniscal fibers, with 1 denoted the best and 3 indicated the worst.

Histological assessments of joint cartilage

The osteochondral specimens were separated from the joints and fixed in 10% neutral buffered formalin (Sigma Diagnostics, St. Louis, MO, USA). Specimens were then decalcified in 10% ethylenediaminetetraacetic acid (Titriplex

III; Merck, Darmstadt, Germany) for 3 weeks. After that, the specimens were sectioned in the coronal plane at the midpoint of the tibial plateau and in the sagittal plane at the midpoint of the MFC. All the specimens were then dehydrated in alcohol and embedded in paraffin. Sections 4-μm thick were cut and then stained with Masson Trichrome.

Histological sections of the MTP were divided into MC and NMC regions. The MFC, MC, and NMC regions were graded with the Mankin grading system[7] for hyaline cartilage degeneration, respectively. This semiquantitative analysis was used to assess cartilage structure (0–6), cellular abnormalities (0–3), matrix staining (0–4), and tidemark integrity (0 or 1). A minimum score of 0 denotes no cartilage degeneration, with a higher score indicating more severe cartilage destruction and the maximum score being 14.

All the above experiments were scored by three attending pathologists who were blinded to the procedures and to the experimental groups. The mean score of all investigators was used for the final evaluation.

Statistical analysis

The Mann–Whitney rank test was used to compare macroscopic and histological scores between different groups and regions. All statistical analyses were performed with SPSS 18.0 (SPSS Inc., Chicago, IL, USA). Statistical significance was set at $P < 0.05$.

Results

At week 24, all the rabbits recovered well from the operation with no infection or complication. No evidence of gross intra-articular hemorrhage was noted in any joint.

Macroscopic evaluations of meniscus and joint cartilage

For the three groups received MAT surgeries, the gross appearance of the meniscal allografts was normal with a shiny white color and a smooth surface, except for 2 menisci in the 2.5 Mrad group showed partial radial tear at midbody. All of the allografts healed to their normal attachment sites, with no signs of disruption or gap formation. The scoring results for macroscopic evaluations of meniscus are shown in Table 1. Overall, no significant difference was found in terms of the total scores among the three groups. However, a significant difference was found for meniscal tears, with the 2.5 Mrad group presented the highest.

The ICRS scores are shown in Table 2. At week 24, the cartilage in the Sham group showed normal. However, for the Meni group, cartilage degeneration in the medial compartment, especially at the MFC and NMC regions of the MTP, were observed. The ICRS scores of the Meni group became higher for all the regions, with osteophytes detected in some specimens.

In addition, ICRS scores of the three groups received MAT surgeries ranged between those of the Sham group and the Meni group [Table 2], the 2.5 Mrad group revealed the highest,

Table 1: Macroscopic scoring results of meniscus tissue

	Mean (range)			*P*		
	0 Mrad group	1.5 Mrad group	2.5 Mrad group	0 vs. 1.5	0 vs. 2.5	1.5 vs. 2.5
Integration	2.4 (1–3)	2.5 (1–3)	2.6 (1–3)	0.855	0.786	0.679
Implant position	1.7 (1–3)	1.9 (1–3)	2.0 (1–3)	0.746	0.765	0.621
Horn position	2.5 (1–3)	2.6 (1–3)	2.7 (1–3)	0.643	0.856	0.719
Shape	2.5 (1–3)	2.6 (1–3)	2.7 (1–3)	0.786	0.679	0.467
Tears	1.9 (1–2)	1.9 (1–2)	2.9 (1–3)	0.978	0.015*	0.039*
Surface	1.7 (1–2)	1.8 (1–2)	1.9 (1–2)	0.489	0.896	0.768
Size	1.6 (1–2)	1.7 (1–2)	1.8 (1–2)	0.765	0.687	0.631
Tissue	1.4 (1–2)	1.5 (1–2)	1.6 (1–2)	0.896	0.695	0.725
Synovia	1.6 (1–2)	1.7 (1–2)	1.8 (1–2)	0.547	0.789	0.768
Total score	17.8 (10–25)	18.2 (11–25)	18.6 (12–26)	0.437	0.216	0.367

*Statistically significant ($P < 0.05$).

Table 2: ICRS scores and Mankin scores of the joint cartilage

Variable	Regions	Mean (range)					*P*		
		Sham group	0 Mrad group	1.5 Mrad group	2.5 Mrad group	Meni group	0 vs. 1.5	0 vs. 2.5	1.5 vs. 2.5
ICRS	MFC	1.2 (1–2)	2.6 (1–3)	2.7 (1–3)	3.0 (2–4)	3.7 (3–4)	0.657	0.013*	0.035*
	MC	1.4 (1–2)	2.4 (1–3)	2.5 (1–3)	3.1 (2–4)	3.6 (3–4)	0.489	0.021*	0.037*
	NMC	1.3 (1–2)	2.4 (1–3)	2.5 (1–3)	3.2 (2–4)	3.4 (3–4)	0.678	0.018*	0.028*
Mankin	MFC	3.4 (0–5)	6.1 (3–8)	6.5 (3–8)	8.1 (4–10)	10.5 (7–13)	0.743	0.023*	0.038*
	MC	4.6 (1–6)	5.7 (4–9)	6.0 (4–9)	8.7 (6–11)	9.9 (8–13)	0.867	0.036*	0.042*
	NMC	4.4 (1–6)	6.5 (5–10)	6.9 (5–10)	8.4 (6–12)	10.8 (7–14)	0.397	0.028*	0.037*

*Statistically significant ($P < 0.05$). ICRS: International Cartilage Repair Society; MFC: Medial femoral condyle; MC: Meniscal-covered region of medial tibial plateau; NMC: Nonmeniscal-covered region of medial tibial plateau.

whereas the 0 Mrad group showed the lowest. Specifically, no significant differences were shown between the 1.5 Mrad group and the 0 Mrad group in all the regions investigated. However, significant differences were detected between the 1.5 Mrad group and the 2.5 Mrad group in all the regions.

Scanning electron microscopic analysis of meniscus

The microscopic meniscal fibers of 0 Mrad group regularly arranged without significant disruption [Figure 1]. However, as the dose of gamma irradiation increased, fibers of the menisci showed an apparently irregular arrangement with some disrupted. The scoring results of SEM analysis of meniscus are shown in Table 3. Both the 1.5 Mrad group and the 2.5 Mrad group showed significantly higher total scores than the 0 Mrad group. In addition, a significant difference was also detected between the 1.5 Mrad group and the 2.5 Mrad group in terms of the total score.

Histological assessments of joint cartilage

The Mankin scores are shown in Table 2. Overall, the Sham group showed the lowest, whereas the Meni group showed the highest grade of degenerative changes based on Mankin scores in all the regions. In addition, Mankin scores of the three groups received MAT surgeries ranged between those of the Sham group and the Meni group, with the 2.5 Mrad group revealed the highest, whereas the 0 Mrad group showed the lowest. Specifically, no significant differences were shown between the 1.5 Mrad group and the 0 Mrad group in all the regions investigated. However, significant differences were detected between the 1.5 Mrad group and the 2.5 Mrad group in all the regions [Figure 2].

Discussion

The most important findings of this study could be summarized as follows: (1) The microscopic meniscal collagen fibers, detected under the SEM, were damaged as the dose of gamma irradiation increased. (2) However, no significant differences were found between the 1.5 Mrad group and the 0 Mrad group with regard to both the macroscopic and histological evaluation outcomes of the joint cartilage after MAT surgeries at 24 weeks postoperatively.

The use of musculoskeletal allograft in orthopedic procedures has increased considerably over the last decade.[13] Allograft tissue has become an increasingly popular graft choice for MAT surgeries and has been shown to offer good to excellent clinical outcomes.[14,15] However, one of the major concerns regarding allograft tissue is the potential risk of disease transmission. Since aseptically harvested and processed allograft tissue cannot be regarded as sterile, many tissue banks use sterilizing agents after the tissue has been harvested and processed.[16,17]

Gamma irradiation is perhaps the most popular sterilization method used by tissue banks.[18,19] Advantage includes its

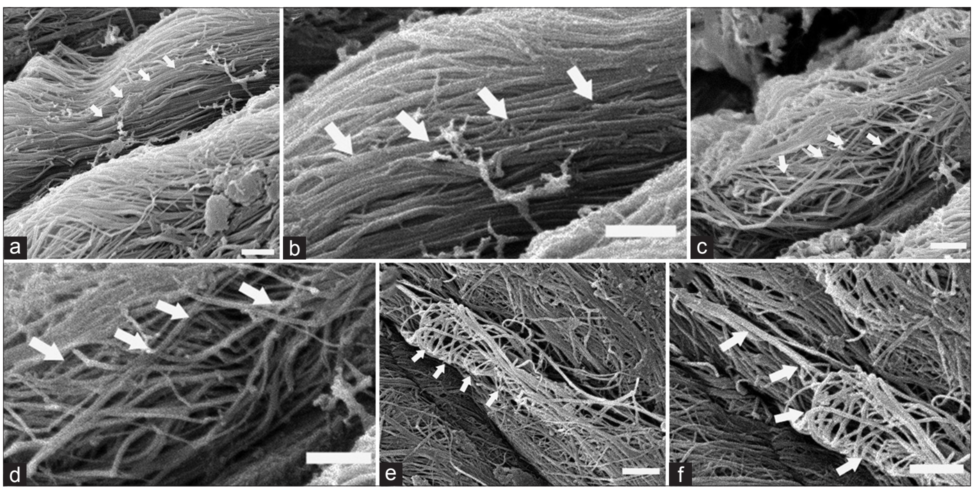

Figure 1: Observations of meniscal collagen fibers using scanning electron microscopy ([a, c, e] original magnification ×2500; [b, d, f] original magnification ×5000). Collagen fibers in 0 Mrad group (a and b) were smooth and closely arranged, with the orientation consistent (white arrows). However, some of the collagen fibers in 1.5 Mrad group (c and d) and 2.5 Mrad group (e and f) were rough and disconnected, with the orientation irregularly arranged (white arrows). The bars in a, c, e represent 5 μm; the bars in b, d, f represent 10 μm.

Table 3: Scanning electron microscopic analysis results of meniscus tissue

	Mean (range)			*P*		
	0 Mrad group	1.5 Mrad group	2.5 Mrad group	0 vs. 1.5	0 vs. 2.5	1.5 vs. 2.5
Tension	1.5 (1–2)	2.0 (1–3)	2.6 (2–3)	0.034*	0.017*	0.036*
Smoothness	1.6 (1–2)	2.0 (1–3)	2.5 (2–3)	0.031*	0.026*	0.019*
Orientation	1.8 (1–2)	2.2 (1–3)	2.7 (2–3)	0.038*	0.019*	0.014*
Continuity	1.4 (1–2)	2.2 (2–3)	2.8 (2–3)	0.048*	0.008*	0.028*
Total score	6.7 (4–8)	8.6 (5–10)	10.5 (8–12)	0.029*	0.003*	0.027*

*Statistically significant ($P < 0.05$).

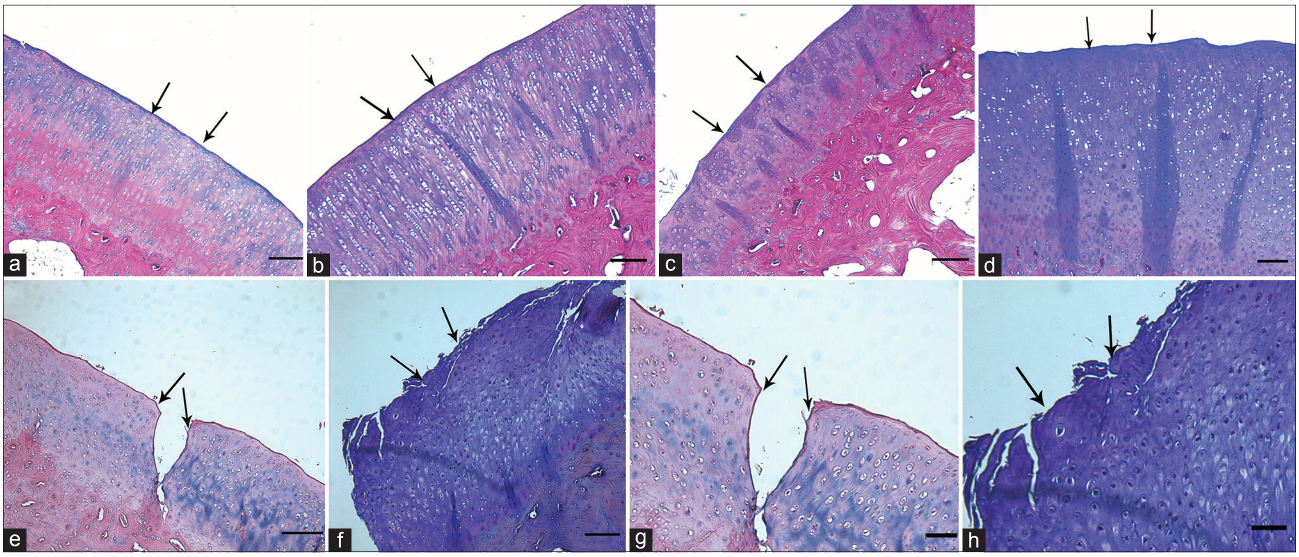

Figure 2: Histological appearance of articular cartilage surfaces of medial femoral condyle (MFC) and medial tibial plateau (MTP) of the three groups receiving meniscal allograft transplantation at week 24 ([a-f] original magnification ×200; [g and h] original magnification ×400). Smooth surface with no reduction of staining were displayed in both the 0 Mrad group (a and b) and the 1.5 Mrad group (c and d) for MFC and MTP regions (black arrows). However, (e-h) mild to moderate formation of gaps and significant reduction of staining were recognized in both the MFC and MTP regions in the 2.5 Mrad group (black arrows). The bars in a-f represent 20 μm; the bars in g and h represent 40 μm.

capability of tremendous tissue penetration that ensures the sterile effect throughout the entirety of musculoskeletal allograft. In addition, it is very effective against bacteria at relatively low dose (1.5–2.5 Mrad).[20] However, the dose of gamma irradiation that can be administered is limited by the potential adverse effects on meniscal musculoskeletal allograft. According to the literature, gamma irradiation has a dose-dependent manner on the biomechanical properties of allograft tissue.[21] Questions remain regarding the clinical implications of these findings.

The biomechanical behavior of the meniscus depends on its particular collagen organization.[22] An ideal sterilization method should not harm this fine structure array. In this study, the irradiated menisci allografts were analyzed under SEM. Results revealed that collagen fibers of the menisci might be damaged as gamma irradiation applied, confirming the potential risk of gamma irradiation on biomechanical properties of menisci. However, damages of the collagen fibers in 2.5 Mrad group was significantly more severe than those in the 1.5 Mrad group, indicating this negative effect also has a dose-dependent character as previously reported.

Whether the alterations in microscopic collagen fibers would result in significant higher failure rate in MAT surgeries has therefore become a major issue. To the best of our knowledge and belief, no study has clarified the relationship between the doses of gamma irradiation and the outcomes of MAT surgeries. According to the results, overall total scores of the macroscopic meniscal evaluation among the three groups receiving MAT surgeries showed no significant difference. Although the collagen fibers of menisci showed a higher degree of disarray with gamma irradiation, survival rate of menisci after MAT seemed not be influenced. On the other hand, 2 menisci of the 2.5 Mrad group showed partial radial tear at the midbody, which might also be explained by the alterations of meniscal collagen fibers detected under the SEM.

All the three groups received MAT surgeries slowed down the articular cartilage degeneration of the medial compartment. The ICRS scores and Mankin scores of the three MAT groups were significantly lower than those of the Meni group. It is encouraging that the MAT surgeries still showed good viability when using the gamma-irradiated menisci. Meanwhile, the 1.5 Mrad group showed similar chondroprotective effects to the 0 Mrad group. We found no significant difference in results of macroscopic and histological evaluation between these two groups in all the regions investigated. It seemed that the low dose of 1.5 Mrad might not damage the function of meniscal allografts. To date, an *in vitro* biomechanical study[18] has confirmed that 1.5 Mrad of irradiation does not significantly impair the biomechanical strength of tendon allografts compared with those without irradiation. In addition, a recent study[20] found that 1.5 Mrad of gamma irradiation could maintain both the proper biomechanical strength of bone tissues and achieve adequate sterilization. Costi *et al*.[19] also showed that 1.5 Mrad of gamma irradiation was unlikely to negatively affect the bone stability and therefore might guarantee successful clinical outcomes.

Despite the chondroprotective effect of the irradiated meniscal allograft, cartilage degeneration was not completely prevented. Significant differences in ICRS and Mankin scores were still found between the three MAT groups and the Sham group at week 24 postoperatively. This could be attributed to various reasons. One of the concerns was the material characteristics of the allograft. Rijk[22] concluded that alteration of the meniscus size would destroy the collagenous network, which altered not only the shape of the meniscus but its mechanical properties as well. In addition, the dose of gamma irradiation could also change the properties of the allograft. Studies have shown that more than 3.0 Mrad of irradiation would increase the brittleness of bone and significantly reduce the collagen content in bone tissue.[11,12] The use of relatively high-dose (>2.5 Mrad) gamma irradiated tendon allografts for anterior cruciate ligament reconstruction resulted in significantly decreased stability of the knee joint.[23] Curran *et al*.[20] proved that 2.0 Mrad of irradiation could reduce the biomechanical strength of allograft; this negative effect became more prominent as the dose of irradiation increased. In this study, the chondroprotective effect in the 2.5 Mrad group showed significant inferior outcomes compared to the other 2 MAT groups, which was similar to the previous findings in terms of the dose of irradiation.

The current study offers some advantages. First, this is the first *in vivo* animal study to investigate the doses of gamma irradiation on outcome MAT surgeries. Second, both macroscopic and histological evaluations were performed on menisci and joint cartilage using semiquantitative analyses, which were relatively more objective compared with previous findings. Third, all data were collected from qualified pathologists who were blinded to the experiments.

However, there were also some limitations in this study. First, all the research data were obtained from rabbits, which could not be directly transferred to humans. Second, to investigate the ideal dose of gamma irradiation on meniscal allograft, another group with dose of 1.0 Mrad would be beneficial. Despite of that, the 1.5 Mrad group showed similar outcomes compared with the 0 Mrad group in all the regions detected, indicating that the 1.5 Mrad would be an appropriate dose when sterilizing the menisci before MAT surgeries for New Zealand white rabbits. Third, another group with dose of >3.0 Mrad should be included as the maximum dose. Fourth, the *in vivo* histological results of meniscus should be given.

In conclusion, the current *in vivo* animal study proved that although the meniscal collagen fibers were damaged after gamma irradiation, the failure rate of MAT surgeries for New Zealand white rabbits might not significantly increase if the irradiation dose was <1.5 Mrad. Therefore, future MAT surgeries might recommend 1.5 Mrad as an ideal dose of gamma irradiation when sterilizing the meniscal allografts for New Zealand white rabbits.

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