Microanatomical study of replaced meniscus in the rabbit

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SUMMARY

We investigated clinical and morphological characteristics of replaced menisci after the transplantation of deep frozen meniscal allografts. We replaced medial menisci (in 18 New Zealand white rabbits) by meniscal grafts obtained from other rabbits. These grafts were kept in a deep freezer (3-5 weeks), thawed in sterile saline and transplanted. The menisci were removed and studied after 2 weeks (first group), 8 weeks (second group) and after 36 weeks (third group). Menisci from non-operated, contralateral knees served as controls. The tissue of the menisci investigated was processed using several histological and histochemical methods and analyzed by light microscope. Transplanted meniscal allografts retained a normal gross appearance, healed firmly through fibrovascular tissue to the recipient capsular tissue, and did not show macroscopic pathological changes. At the histological evaluation, in the first group the collagen fibers were irregularly oriented, with a low cellular population. In the second group, blood vessels were present, cellular repopulation and immature collagen fibers being observed. The third group had a more mature collagen tissue, with a significant cell repopulation. Deep-frozen meniscal allografts showed significant collagen remodeling with cellular and vascular ingrowth from the surrounding synovia. This suggests that used allografts function normally and protect underlying cartilage.

Key Words: Meniscus transplantation – Rabbits – Deep freeze preservation

INTRODUCTION

The significance of the menisci in knee joint function and maintenance is very well known. Removal of the meniscus induces changes in contact surfaces and changes in the distribution of forces in the joint, resulting in osteoarthrosis (Fukubayashi and Kurosawa, 1980). The late results following total meniscectomy are not satisfactory because only 10%-15% of patients are left without any discomfort or symptoms (Tapper and Hoover, 1969), and even 6% show some changes in the knee joint on radiological evaluation (Gear, 1967). These degenerative changes are particularly pronounced in concurrent and associated lesions of the meniscus and anterior cruciate ligament (ACL deficient knee) (Thompson and Fu, 1999). Therefore, wherever possible attempts should be made to keep the meniscus preserved.

The method of choice in meniscal lesions is meniscal repair -whenever possible- especially in lesions of the external meniscal part (Verdonk, 1997).

On the other hand, in total meniscectomy or in the case of extensive degenerative meniscal lesion where reconstruction is not possible, transplantation of the meniscus or partial meniscal replacement with collagen meniscus implants have been reported as an option (Verdonk, 1997; Fritz et al., 1996). These two procedures are significant since only 20% of meniscal lesions may be restored (sutured) (Verdonk, 1997). The use of artificial material in meniscus replacement is inappropriate and unnecessary because, given

its characteristics, applied frozen meniscal tissue is a fairly adequate and compatible replacement of injured or impaired menisci (Fabriciani et al., 1997). Transplanted meniscal allografts are able to "survive" in the articular space, to retain their structural and physiological characteristics, and do not undergo degenerative articular changes which would occur without meniscus (Fritz et al., 1996; Fabbriciani et al., 1997; Kohn et al., 1999).

In meniscal transplantation, the methods of processing and preservation of transplants are of the utmost importance. Three methods are currently used: cryopreservation, deep freezing and freeze-drying (Fabriciani et al., 1997).

The aim of our experimental study was to obtain data about the process of cellular repopulation and the repopulation and maturation of connective tissue (in transplanted menisci) as well as about the degree of maintenance of the knee joint cartilage underlying the transplanted meniscus. The evaluation included macroscopic, radiological and histological analysis of the transplants. With a view to obtaining a time-sequenced histological picture over a 9-month period, the rabbit knee was used as an experimental model.

Materials and Methods

In our study 18 adult male rabbits weighing 2-3 kg, were used as experimental animals (New Zealand breed from the Institute for Medical Research of the Military Medical Academy). In all experimental animals, medial menisci of the right knees were replaced by deep-frozen meniscal allografts provided by total meniscectomy from other matched rabbits under aseptic conditions.

Graft preservation method

Meniscal grafts, obtained under aseptic conditions, were preserved as follows: they were washed (immersed twice) in sterile phosphate-buffered saline (PBS), packed in sterile gauze, and stored in a deep-freezer at -170°C (storage technique). The appropriate size of the transplants was defined by clinical and radiological evaluation of the knee joint dimensions in the donor and recipient animals. At the time of allograft implantation, each meniscus was immersed in a sterile aqueous solution at 37°C and, after 2-3 minutes, was re-immersed in the same solution at room temperature. All procedures were performed under sterile conditions.

Surgical technique of meniscus transplantation

Experimental animals were anesthetized using a combination of Ketalar (0.2mg) and atropine sulfate (0.1mg/kg of body weight), with antibiotic treatment by Tolycar (0.5g). Under sterile conditions (the limb being shaved and cleaned with Povidone Iodide), the knee joint was entered through a medial parapatellar approach. The use of a tourniquet ensured a bloodless operative field. Following isolation and identification, a medial collateral ligament was detached from its femoral medial condylar insertion and displaced distally. Medial arthrotomy was performed to enable complete visualization of the medial knee compartment. The circumferential margin of the medial meniscus was detached sharply from insertion at the medial articular capsule and coronary ligament, as well as the anterior and posterior horns of the medial meniscus from its meniscal-ligamentous junctions. Previously obtained meniscal graft (preserved as described) was transplanted in the place of removed meniscus and sutured to the articular capsule and synovial membrane with 4-5 resorption sutures (PDS-3-0) placed horizontally from inwards toward the outside with the knots at the capsular external surface. The anterior and posterior meniscal horns were sutured at the insertions to ligament structures. Medial collateral ligament was reinserted at its insertion to the medial femoral condyle. Subcutaneous tissue and skin were sutured with 3-0 Vicryl.

The experimental animals were immobilized with fixed dressing that allowed them to rest on their legs only after they were free of pain. Antibiotics were administered for prophylactic purposes for 5-7 days following the surgery. The control group consisted of contralateral, normal knees. Clinical evaluation of the wound was performed in all cases.

Each operated knee was evaluated radiologically by the analysis of the anteroposterior and lateral views immediately before the experimental animal was sacrificed. The following parameters were analyzed: height of the articular space, presence of subchondral sclerosis and cysts, and the appearance of marginal osteophytes.

After 2, 8 and 36 weeks (groups I, II, III), the experimental animals (6 in each group) were sacrificed with an overdose of barbiturate and, after medial arthrotomy, macroscopic evaluation of the meniscus, cartilage and of synovial membrane was carried out. The color of the meniscus, the strength of the transplant union with the articular capsule, the presence of synovial hyperemia and of inflammatory signs as well as degenerative cartilaginous changes (tibial, femoral and patellar parts), and especially the cartilaginous portions underlying the transplanted meniscus, were analyzed.

Histopathological analysis of the transplants was performed thus: the entire meniscal trans-
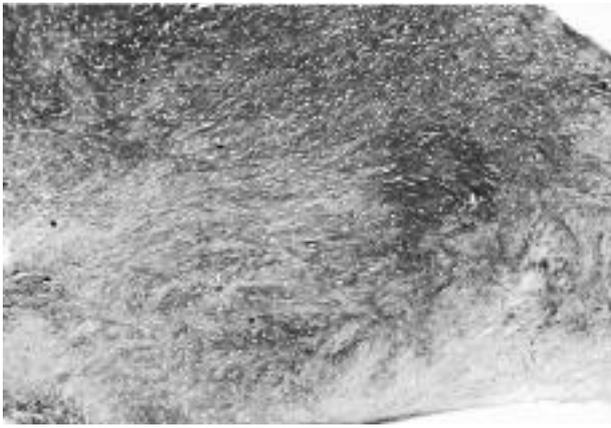
Fig. 1.- Meniscal allograft from the first group (sacrificed after 2 weeks), with a marked cell depopulation and unoriented collagen fibers (Masson Trichrome staining; x10).

Fig. 2.- Specimen of the allograft from the second group (sacrificed after 8 weeks), with pronounced cellular repopulation (Hematoxilin-Eosin - H&E; x10).

plants were excised, fixed in 10% buffered formaldehyde solution, dehydrated, cleaned and placed in a wax mold. 5µm thick sections were stained using the hematoxylin-eosin (HE), Periodic Acid Shift (PAS), Van Gieson, Paff-Halmi, Masson Trichrome and Von Kossa methods. Sections were analyzed by light microscope with and without polarization (Karl Zeiss) at different magnifications and characteristic details were photographed. Special attention focused on the histological features of the cartilage and of the bony parts of the tibial plateau after decalcination and the same histological methods were used to study medial menisci in the healthy controls.

RESULTS

After the transplantation, the experimental animals were *evaluated clinically*. Their body weight and temperature were measured and the wounds were inspected. No signs of wound infection, of any body weight loss or increased body temperature were found in any of the groups of experimental animals. Radiological evaluation of the antero-posterior and lateral views of operated knee joints revealed no degenerative changes in terms of subchondral sclerosis, cysts, osteophytes or narrowing of the intra-articular space within the groups studied.

In the first group of experimental animals (sacrificed after 2 weeks), *macroscopic evalua - tion* revealed a normal appearance of the transplanted meniscus, with a slightly modified color - much darker and less shiny. The attachment of the meniscus to the articular capsule was strong, the synovial membrane was hyperemic, and the cartilage of the femoral and tibial condyles was not changed.

In groups II and III (sacrificed after 8 and 36 weeks), respectively, the transplanted menisci had a normal appearance, color and strength, with no gross signs of infection.

Microanatomical analysis revealed a marked decrease in the cellar population, especially in the animals of group I. Degree-I degenerative changes in the cartilage were evident in two cases only after 4-5 months. There was no apparent subchondral bone; that is, a more extensive degree-IV destruction of cartilage.

In the animals of group I (sacrificed after 2 weeks), collagen fibers were not aligned in a parallel manner, and the lacunae were empty – a very small cellular population was observed (Fig. 1). Hypertrophic synovial membrane lacked signs of inflammation or immune response. Histological evaluation of the cartilage underlying the transplanted meniscus disclosed no pathologic changes.

In the animals of group II (sacrificed after 8 weeks), a cellular repopulation of fibrochondroblasts occurred, particularly at the meniscal circumference (Fig. 2), with a better orientation of collagen fibers in the form of immature, disorganized connective tissue (Fig. 3). Fibroblast proliferation was observed, and the meniscal transplants and joint capsules healed by fibrovascular scar tissue. The underlying bone was preserved and there were no signs of immune response or graft rejection.

In the group III animals (sacrificed after 36 weeks), cell repopulation was marked as was revascularization in the meniscal-capsular layer. Collagen fibers were young and immature (Fig. 4) and a small number of former collagen fibers persisted. Histological study revealed an almost normal appearance of the meniscus, with young connective tissue and preserved subchondral cartilage (Fig. 5), with no signs of degenerative changes.

Fig. 3.- Only a few old collagen fibers left (arrows) (second group sacrificed after 8 weeks; Masson Trichrome staining, x10).
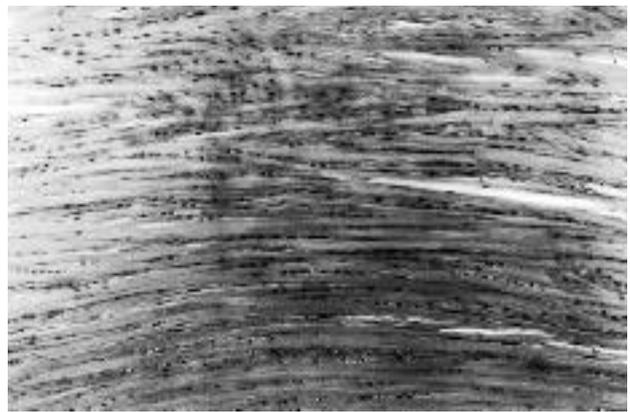
Fig. 4.- Young and immature collagen fibers in a specimen from the third group (sacrificed after 36 weeks; H&E; x25).
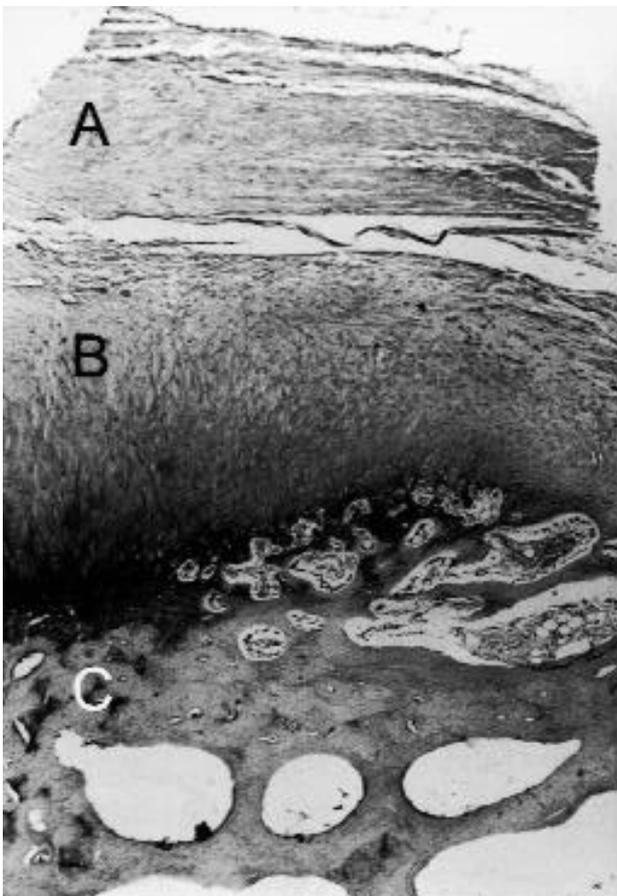
Fig. 5.- Under the meniscal transplant, preserved subchondral cartilage is visible. A- meniscus; B- cartilage; C- bone (third group- sacrificed after 36 weeks; H&E; x10).

DISCUSSION

The goal of meniscal transplantation is to ensure the survival of the implanted allograft in a joint space, achieve the morphology and function of a normal meniscus, and to prevent the cartilage destruction that would arise after total meniscectomy (Fabbriciani et al., 1997; Kohn et al., 1999; Arnoczky, Warren and McDevitt, 1989).

The absence of a significant immune response was striking in this study. Both meniscal tissue and cartilage are considered to be "immunologically privileged" due to avascularity, the relatively small number of cells, and their isolation from the host immune system (Fabbriciani et al., 1997; Ochi et al., 1995). Thus, we failed to find cellular or humoral responses, except for few local and minimal ones, as confirmed by histopathological analysis of the meniscal-synovial junction and synovial membrane. The synovial membrane was hyperemic but without any increased lymphocyte or plasma cell counts. These findings were the same for the deep-frozen and cryopreserved transplants (Langer et al., 1975).

Considering the time limitations involved in the possible transplantation of fresh meniscal allografts (4-6 hours following clinical death of the donor), technical problems related to the donor, the high risk of infectious disease transmission (Nemzek et al., 1994) -particularly HIV (Verdonk, 1997; Fritz et al., 1996)-, the problem of meniscal graft storage is of increasing interest. Currently, cryopreservation and deep-freezing are the most common techniques for allograft storage since the damage to extracellular matrix caused by these methods is minimal (Arnoczky et al., 1989; Gear, 1967).

The period of storage in deep-freeze has no effect on implant quality and utility. One of the advantages of deep-frozen allografts is their cost-effectiveness (inexpensive storage and no need for HLA typing), and additional sterilization by ethylene-dioxide and gamma rays decreases the infection rate to a minimum. This is essential since it has been shown that both bony ligamentous and meniscal allografts are able to transmit viruses (Buck et al., 1988; Buck et al., 1989). Deep freezing as a method has been shown to cause no harm or modification to the meniscal allograft microstructure.

However, all grafts, including allografts, serve as scaffolds for the ingrowth of cells and new

connective tissue from the adjacent synovial membrane and capsule. Even in fresh allografts the donor's living cells are vital for only 4 weeks and, finally, repopulation from the recipient occurs (Fabbriciani et al., 1997).

In our study, the significant degree of cell repopulation of the meniscus and transformation of fibroblasts into chondroblasts as well as the new connective tissue already developed after 8 weeks (Wada et al., 1998) were patent. From this standpoint, an allograft is an adequate scaffold, bearing in mind that its loading should be delayed until the connective tissue has matured. Time-conditioned cell repopulation of allografts and formation of new collagen fibers have been reported by several authors (Fabbriciani et al., 1997; Nagahara and Wada, 1994; Mikic, 1987; Mikic et al., 1993; Mikic et al., 1997; Bruns et al., 1998). These processes occur from the circumference towards the central meniscal part, during the period from the second week to the third month. After that time, the implant reaches attains the appearance of a normal meniscus, as seen in our study. Likewise, our experiments showed that new chondroblasts, chondrocytes differentiated from fibroblasts, could already be seen after a period of 12-16 weeks (Shibuya, 1999) and that the extracellular matrix was formed by these cells.

In our cases as well as in other studies (Fabbriciani et al., 1997; Bruns et al., 1998; Aagaard et al., 1999; Cummins et al., 1997), the cartilage was protected in the area overlain by the transplant. The uncovered part showed degenerative changes, as reported by Fabriciani et al. (1997).

Special attention should be paid both to an appropriate allograft volume and the meniscal allograft fixation technique. As well as other authors (Verdonk, 1997; Chen et al., 1996; Pollard et al., 1995; Shelton and Dukes, 1994; Lazovic et al., 1997a, 1997b; Alhalki et al., 1999; Messner, 1999; Goble et al., 1999), we have emphasized the significance of a proper surgical technique in terms of isometric graft positioning and rigid fixation of the implant. In larger experimental animals and humans, the application of meniscal graft carrying bony plaques of the anterior and posterior horns is possible, enabling rigid fixation of the graft (Vail et al., 1995).

In the light of the present findings, it may be concluded that meniscal allografts stored by deep freezing are the most suitable. Some limited and preliminary clinical trials (Verdonk, 1997; Goble et al., 1999) confirm the present findings to the effect that the transplant survives through the process of remodeling and that this successfully protects the underlying cartilage. However, further clinical investigations with prolonged follow-up periods are needed, as well as detailed evaluation of the mechanical characteristics of transplants.

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