ALLOTRANSPLANTATION OF
FROZEN IRRADIATED MENISCI
IN RABBITS

by
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fulfillment of the requirements for the degree of Masters of Science in
Experimental Surgery.

ABSTRACT

The meniscus is essential for normal knee function. Previously considered unimportant, removal was performed when injured. This frequently led to degenerative arthritic changes. Today surgeons attempt to repair or minimally resect torn menisci, however many patients are encountered with irreparable tears or previously removed menisci. In these patients meniscal transplantation may prevent degenerative arthritis from developing.

Problems facing transplantation are ability to store tissues and prevent disease transmission. To overcome this, freezing and irradiation effects were examined on 60 rabbits undergoing medial meniscal transplantation. Fresh, frozen, and frozen-irradiated allografts were followed up to 17 months. 10 other rabbits underwent meniscectomy alone.

Zero-time studies revealed that freezing with or without irradiation rendered cells non-viable with no changes in mechanical properties. At long term follow-up, all grafts healed, and showed repopulation with metabolically active cells determined radioautographically. Mechanical properties were unchanged, except for a decreased time constant in the frozen group. Degenerative changes were not significantly different among groups.
RÉSUMÉ

Le ménisque est essentiel au bon fonctionnement du genou. Autrefois considéré peu important, on procédait à son exérèse lorsqu’il était endommagé. Cette pratique mena à des changements de type arthritique dégénéréscent. De nos jours, les chirurgiens essaient de réparer le ménisque ou tout au plus, de procéder à une résection très limitée. Cependant, plusieurs patients ont des déchirures irréparables du ménisque, ou ont déjà subi une méniscectomie. Chez ces patients, la transplantation du ménisque peut prévenir le développement d’une arthrite dégénéréscente.

Par contre la transplantation est sujette à deux problèmes majeurs: la conservation des tissus, et la prévention de maladies transmissibles. Afin de surmonter ceux-ci, les effets de la congélation et de l’irradiation furent observés sur 60 lapins qui ont subi une transplantation du ménisque médial. Tous les greffons frais, congelés, et congelés-irradiés furent suivis sur une période allant jusqu’à de 17 mois. 10 autres lapins subirent une méniscectomie.

Les études à temps zéro ont démontré que la congélation avec ou sans irradiation a rendu les cellules non viables, mais n’a pas changé les propriétés mécaniques. A long terme, tous les greffons ont cicatrisé et ont démontré une repopulation de cellules métaboliquement actives déterminées autoradiographiquement. Aucune des propriétés mécaniques n’a été changée à l’exception d’une réduction de la constante temps pour les greffons congelés. Les changements dégénéréscents ne furent pas statistiquement différents parmi les groupes.
PREFACE

The experimental work in this thesis was done while the candidate was under the supervision of Dr. David J. Zukor in the Department of Surgery, Division of Orthopaedics, McGill University, from July 1988 to June 1989.

All work contained in this thesis except where expressly stated is the work of the candidate. The rabbit model for allograft transplantation was first designed and a pilot study performed by Dr. David Zukor in 1986 (presented 1988).

The present study was designed by Dr. David Zukor. The first several surgical transplant procedures were performed by him with the assistance of Dr. Michel Daigle (research fellow at the time) several months prior to the candidate taking over the project.

The undertaking of the fabrication of the mechanical testing devices by the Department of Biomedical Engineering at Ecole Polytechnique was initiated solely by the consultation and request of Dr. Zukor. From this request for devices to mechanically test our specimens Mr. Pierre Duval fabricated these mountings with the collaboration of Dr. Zukor. The fabrication of such devices, including the testing of our zero-time meniscal specimens,
constituted the basis for Mr. Duval's Masters of Science degree thesis.

All mechanical testing, including pilot study, zero-time studies, and allograft studies were performed with the collaboration and guidance of Dr. Zukor and the candidate.

Histologic slide preparation and review and grading of histologic specimens was performed by Dr. Indrojit Roy at St. Mary's Hospital, Montreal. Radioautography slide preparation, development, and photography were performed by the candidate with the initial assistance of Ms. Nora Shepard, Shriners Hospital for Crippled Children, Montreal, who showed the candidate the technique. Review and interpretation of radioautographic material was performed by the candidate.

Electron microscopic studies on bovine meniscal tissue were performed by Dr. Isaac Farine (deceased) in Tel-Aviv, Israel. Dr. Farine graciously gave us these electron micrographs as an adjunct to our work. To the best of our knowledge this work is not published elsewhere.

This thesis was prepared by the candidate. Portions of the work contained in this thesis have been presented or published elsewhere. References for these are as follows:

Duval P. Characterisation des propriétés biomechaniques des menisques du genou à l'état normal et irradié Mémoire de maîtrise, Université de Montréal, Nov., 1989.

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To the Department of Orthopaedics, and the Research Institute at the Royal Victoria Hospital for jointly supporting my work with their research stipend. Both of which were always willing to lend a hand or give advice.

To Pierre Duval, Hocine Yahia, and Gilbert Drouin at the Institut de Genie Biomedical, Ecole Polytechnique for their work on the engineering and testing of our allografts. To Drs. Robin Poole and John Mort who were members of my advisory committee and constructive in their help.

To Dr. Indrojit Roy for his patience and skill in the arduous task of reviewing the histologic specimens.

To the memory of Dr. Isaac Farine, Tel-Aviv Israel, whose untimely passing during our research is a great loss to all of us. His efforts are not forgotten.
To Nora Shepard for her kind teaching in the technique of radioautography, and allowing me to use her facilities and equipment. To Wendy Spanier, for much needed help, advice and support from her vast knowledge and abilities with computers.

To Guy De Bouvries for assistance whenever I needed a helping hand.
DEDICATION

to Jodi, my beautiful and devoted wife;

for helping me by your nurturing, support, and endless understanding
all the way through my residency.
Without you I never would have made it.

to my lovely daughters Roni Brooke and Jaclyn Nicole:

who were both born during this time,
you represent freshness and the best things in life.
You are the reasons we do this.

and

to my late father Robert L. Rubins;

who gave me the inspiration to be everything I am today.
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ABBREVIATIONS

A° .................................. Angstrom unit ($10^{-10}$)
ANOVA .......................... Analysis of variance.
°C ................................. Degrees Celsius
DMEM ............................. Dulbecco’s Modified Eagles Medium
DMSO .............................. Dimethyl sulfoxide
Eh ................................. Corrected modulus of elasticity
EM ................................. Electron microscopy
er ................................. endoplasmic reticulum
ε_{ve} ............................. Viscoelastic deformation
F/U ................................. follow-up
GAG(S) ........................... Glycosaminoglycan(s)
GIBCO ............................ Grand Island Biomedical Corporation
Gly ................................. glycogen
Go ................................. golgi apparatus
HBSS .............................. Hanks Balanced Salt Solution
kg ................................. kilogram
Fi ................................. Frozen Irradiated (Allograft group)
Fr ................................. Frozen (Allograft group)
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<td>Fs</td>
<td>Fresh (Allograft group)</td>
</tr>
<tr>
<td>mcl</td>
<td>Medial collateral ligament</td>
</tr>
<tr>
<td>mg(s)</td>
<td>Milligram(s)</td>
</tr>
<tr>
<td>mi</td>
<td>Mitochondria</td>
</tr>
<tr>
<td>ml(s)</td>
<td>Milliliter(s)</td>
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<tr>
<td>mm(s)</td>
<td>Millimeter(s)</td>
</tr>
<tr>
<td>mos</td>
<td>Months</td>
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<tr>
<td>MPa</td>
<td>Millipascals</td>
</tr>
<tr>
<td>Mrad</td>
<td>Megarad (10^6 rads)</td>
</tr>
<tr>
<td>Ms</td>
<td>Meniscectomy (group)</td>
</tr>
<tr>
<td>Na₂³⁵SO₄</td>
<td>Sodium sulphate (radiolabelled sulphur)</td>
</tr>
<tr>
<td>n</td>
<td>Number</td>
</tr>
<tr>
<td>N</td>
<td>Newton (unit of force)</td>
</tr>
<tr>
<td>PG</td>
<td>Proteoglycan</td>
</tr>
<tr>
<td>PMMA</td>
<td>Polymethylmethacrylate (cement)</td>
</tr>
<tr>
<td>RPMI-1640</td>
<td>Rockwell Park Memorial Institute Media # 1640</td>
</tr>
<tr>
<td>s.d.</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>τ</td>
<td>Time constant</td>
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<tr>
<td>uCi</td>
<td>MicroCuries</td>
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<tr>
<td>Va</td>
<td>Vacuoles</td>
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ORIGINAL CONTRIBUTIONS TO KNOWLEDGE

I. In rabbits both viable and non-viable meniscal allografts heal fully to the host synovium.

II. In rabbits, meniscal allografts rendered non-viable by freezing with or without irradiation undergo graft repopulation with viable metabolically active cells.

III. At zero-time (immediately post freezing, or freezing and irradiating) there are no significant differences in the mechanical properties (time constant, modulus of elasticity, viscoelastic behaviour) of rabbit meniscal allografts as determined by indentation and traction (tensile) testing. (This was in collaboration with the Ecole Polytechnique, Institut du Genie Biomedical, Universite de Montreal).

IV. Post-transplantation, healed fresh, frozen, and frozen-irradiated allografts show no significant differences in mechanical properties except for a significant decrease in the time constant for the frozen allografts.

V. Medial meniscal allografts in the rabbit model need not be viable at the time of transplantation to heal, survive and function.
INTRODUCTION

Menisci are recognized to be important functional components of the knee joint. They are involved in many aspects of joint mechanics. Functions include: load transmission across the joint, joint stability, guiding the cam shaped femoral condyles through their polycentric range of motion, shock absorption, and lubrication and nutrition of articular cartilage by spreading a thin film of synovial fluid over the condylar surfaces.

The load bearing function is now accepted to be one of the most important roles of the menisci. Medial meniscectomy has been performed in the past for meniscal injuries sustained during sports or other trauma. Medial meniscectomy alters the stress distribution across the joint such that the load is doubled in the femur and increased seven-fold on the tibial plateau (Radin et al, 1984). These stress alterations which occur after meniscectomy result in damage to the articular cartilage leading to degenerative arthritis (Cox 1975; Fairbank, 1948; Walker, 1975) and to instability of the knee (Slocum and Larson, 1968). This phenomenon is known as the "Post-meniscectomy Syndrome".

The important role of the meniscus has been well established now. Clinically surgeons are making every attempt to repair torn menisci, or only partially
resect the damaged meniscus in lieu of total meniscectomy and its inherent deleterious effects on the knee joint. Despite these efforts, a significant number of relatively young patients without menisci (either due to previous meniscectomy or acutely torn and irreparable menisci) are encountered by orthopaedic surgeons.

It is in this group of patients that a meniscal replacement, either prosthetic or a transplant, would be most beneficial. Prosthetic replacement of the meniscus has had very limited success to date (Kenny, 1983; Toyonaga, 1983). In contrast, transplantation of the meniscus has been performed successfully both in humans and animals (Jackson 1990; Milton 1990; Weisemeier 1988; Zukor and Farine 1988; Zukor and Cameron).

This problem has been the basis for much interest in clinical and experimental work on the healing, repair, and possible replacement of the meniscus both in animals and in humans.

Meniscal transplantation research has been largely focused on the preservation of cells during storage periods to maintain viability in the grafts. Such stored grafts have a decreasing viable cell population with increased length of storage (Arnoczky and McDevitt, 1988). These studies have not focused on methods of secondary sterilization to prevent the possibility of
disease transmission.

This study examines the feasibility of deep freezing meniscal allografts for long term storage. The effect of irradiation, which is used to sterilize these tissues, was examined for any deleterious effects on the functional or mechanical properties of these menisci.

Unprotected freezing, and irradiation, are known to destroy cell viability. However it has been shown that certain skeletal tissues need not have viable cells at the time of transplantation. Healing and functional metabolic activity can be demonstrated by cells which have repopulated from the host (Arnoczky, 1986; Brown, 1982; Bright, 1981; Curtis, 1985; Shino, 1984). Should this occur with menisci, then perhaps great efforts at preservation of meniscal allografts are unnecessary.

The effects of irradiation on the mechanical properties of the meniscus have never been examined prior to the undertaking of this study. To this end we had solicited the collaboration of the Department of Biomedical Engineering at the Ecole Polytechnique in Montreal. Through a collaborative effort, specialized mechanical testing devices were designed and fabricated to specifically test the mechanical properties of the rabbit medial meniscus allograft.
These designs were unique and an original innovation in the testing of cartilaginous type tissue. These housings tested the fully intact specimen (whole meniscus) reproducing the physiologic situation, as opposed to the common method of testing only small strips or fragments.

The ultimate goal is to develop a method of storing and sterilizing meniscal allografts to allow the possibility of meniscal transplantation in an elective fashion from banked menisci. Ideally, the process would prevent disease transmission from allografts, without functionally damaging the tissue.

This study addresses these issues by determining if deep freezing (-70°C) and gamma irradiation (2.5 Mrads) can be employed to effectively store and sterilize meniscal tissue, without significant deleterious effects on the mechanical properties of the meniscus. The meniscal transplants could then function effectively to prevent the degenerative changes seen in the "Post-Meniscectomy Syndrome".
REVIEW OF THE LITERATURE

ANATOMY

Figure 1 displays the basic anatomy of the human knee joint. The left figure displays an anterior view of the knee with the patellar ligament complex in place. The illustration on the right shows this complex reflected inferiorly to expose the interior of the joint. The articulating surfaces of the femur and patella are seen. Synovium and joint capsule are present. Removing this synovium and with the knee in flexion (figure 2), the important supporting structures are demonstrated.

The femur and the tibia are linked by four ligaments: two inside the joint, the anterior and posterior cruciate ligaments, and two outside the joint, the medial (tibial) and lateral (fibular) collateral ligaments. The joint capsule (articular capsule) encloses all of this and adds to the stability of the joint. The curved femoral condyles which arise from the end of the femur, articulate with the concave surfaces of the tibial plateaus. These articular surfaces are covered by a thin layer of hyaline (articular) cartilage. It is through these surfaces that load transmission occurs.
Figure 1. The human knee joint. Anterior views display ligamentous structures (left) and interior of joint (right). (From Clemente 1981).
Figure 2. Human knee joint in flexion (left) displays cruciate ligaments and menisci. Top view (right) shows menisci on tibial plateaus. (From Clemente 1981).

Figure 3. Top view of menisci displaying their ligamentous attachments. (From Seedhom 1979).
The femoral condyles have a complex geometry, as they are convex in front to back (sagittal) and side to side (coronal) planes. Their radii in the sagittal plane are smaller at the back than the front, thus creating a cam effect as the joint is moved through its range of motion (the collateral ligaments and capsule become more taut in full extension). The condyles are also inclined toward each other, being more widespread at the back than in the front. This factor causes the condyles to displace the menisci more laterally as the knee is flexed. The groove between the condyles is the patellofemoral groove, which is where the patella articulates. Its posterior or articulating surface is also lined with hyaline cartilage. The patello-femoral ligamentous complex also stabilizes and supports the knee.

Looking at the interior of the knee joint from above, the menisci are seen (figure 2, right). The menisci are fibrocartilaginous crescent (semi-lunar) shaped wedged structures that are interposed between the articular surfaces of the femoral condyles and the tibial plateaus. They are firmly attached to the tibia both anteriorly and posteriorly via ligamentous structures which are attached to their anterior and posterior horns. Along the periphery they are adherent to the synovium. The medial meniscus becomes broader in its posterior aspect and is nearly semi-lunar in shape. In distinction, the lateral meniscus being almost circular, covers nearly the entire articular surface of the lateral tibial plateau. At the front of the joint the two menisci are linked
together by the transverse genicular ligament.

The medial meniscus is more constrained in its mobility on the tibial surface than the lateral meniscus. The horn attachments are further apart than the lateral meniscus, the medial coronary ligaments shorter, and the meniscus is firmly tethered to the medial collateral ligament (figure 3). The lateral meniscus has no such similar attachment to the lateral collateral ligament. The result of these attachments is that the medial meniscus is more limited in mobility. The lateral meniscus is much freer over the condyle during the flexion-extension cycle. The excursion of the lateral meniscus on the tibia is approximately 10 mm. compared with a 2 mm. excursion for the medial meniscus in humans (Brantigan and Voschell, 1941). These factors are important in the mechanics of load transmission as will be seen later in the analysis of load bearing across the knee.

**STRUCTURE**

The cells of the meniscus synthesize three important elements which comprise the basic structure of the meniscal fibrocartilage: collagen fibres, elastic fibres, and proteoglycans. The interaction and organization of these elements determines the nature and mechanical properties of the tissue.
Collagen

Collagen fibres of the meniscus are almost 98% type I collagen (Eyre & Muir 1975; Eyre & Wu, 1983), similarly seen in tendons. Type III and occasionally type V collagen is seen on the surface (Eyre & Wu, 1983). These fibres are made up of individual fibrils of varying cross sectional diameter (Ghadially et al. 1983). Using polarised light microscopy, Bullough (1970) described three orientations of the collagen fibres: circumferential, radial, and oblique (figure 4). Subsequent researchers support these findings (Cameron, 1972; Ghadially, 1978).

Figure 4. Collagen fibre structure of meniscus. The majority of the peripheral fibres are running circumferentially (From Bullough 1970).
The principle orientation of the fibres are circumferential, or in the longitudinal axis of the meniscus. This orientation withstands tension generated in the meniscus (the tension occurs in a circular fashion which generates a "hoop" stress). Bullough (1970) studied the tensile strength of different sections of the meniscus. He found that greater tensile strength correlated with increased longitudinal orientation of collagen fibres (parallel) to the tensile axis.

The radially oriented fibres act as "tie" fibres to resist longitudinal splitting of the collagen bundles of the meniscus (Bullough et al, 1970; Cameron, 1973). Wagner (1976) described their action as tension rods; therefore they contribute to resistance of compressive forces (Clark & Ogden, 1983). Aspden et al (1985) used polarised light microscopy, x-ray diffraction and patterns of artificial split lines to determine collagen fibril orientation in menisci from humans, dogs, and pig knee joints. He claimed that these results are more applicable to the mechanical function as they yield fibril orientations in macroscopic specimens. Electron microscopy only provides details of very small specific areas.

This orientation of fibres is suited for transmitting compressive loads in the knee joint. Equilibrium would be obtained if compressive forces were balanced by the circumferential stress (hoop stress). Aspden (1985)
postulated that radial fibres in this outer region bind the circumferential fibres so that they resist splitting apart by compression.

**Elastic Fibres**

Elastic fibres are known to be composed of an amorphous central core (electron-lucent), and a peripheral zone of short electron dense filaments of 10nm. Fibres with a central electron lucent core suggestive of mature elastic fibres were rare in rabbit meniscal tissue (Ghadially et al, 1978) and human meniscal tissue (Ghadially et al, 1983). Collections of 10 nm electron-dense filaments which are probably immature elastic fibrils were observed by them, and by our group (see figure 52). Elastin is present in 0.6% of dry weight of the human meniscus (Ingman, 1974).

Höpker (1986) postulated that elastin was important in the recovery of the original shape of the meniscus, as it is continually deformed with flexion and extension of the knee, especially in association with weight bearing (figure 5).
Figure 5. Lateral view of the human knee joint during flexion a and extension b. Alterations in the shape of the menisci during flexion are seen in top view c (in black). (From Hopker 1986).
Proteoglycans

Proteoglycans are important molecules in the extracellular matrix. They are protein cores to which are linked anionic glycosaminoglycan chains (GAG) (Muir, 1969). These GAGs are long chain polysaccharides containing acid groups, such as carboxyl and sulphate. Proteoglycans are produced by the chondrocytes in articular cartilage and fibrochondrocytes in menisci.

The ability of articular cartilage to withstand compressive forces is conferred mainly by its proteoglycan content (Kempson et al., 1976). The elastic properties of articular cartilage are determined by the large size and the high anionic charge of the proteoglycan (Hascall, 1977). McNicol and Roughley (1980) demonstrated that human menisci contain proteoglycan molecules of similar size and glycosaminoglycan content to those of articular cartilage, however in lower quantities.

Meniscal proteoglycans are of two types: (I) large molecules containing chondroitin sulphate and keratan sulphate which can aggregate into groups of 20-250 molecules by interacting with hyaluronic acid (Hardingham, 1981); (II) smaller size molecules which contain dermatan sulphate and lack the ability of interacting with hyaluronic acid. The body of the meniscus in humans and dogs contains mainly the first type (chondroitin and keratan
sulphate), whereas the fibrous attachment has more of the second type (dermatan sulphate) (Adams and Ho, 1987).

Differences in proteoglycans of meniscal and hyaline cartilage of the same species have been previously demonstrated (McNicol and Roughley, 1980; Roughley et al., 1981; Adams & Muir, 1982). Webber et al (1984) were the first to characterize newly synthesized proteoglycan in rabbit meniscal tissue. They too demonstrated structural and physical differences between the meniscal PG and its hyaline cartilage counterpart. The PGs were smaller, and existed as monomers even under associative conditions (addition of hyaluronic acid).

Experimentation by altering PG aggregates using hyaluronidase showed no alterations in the stress/strain relationship of meniscal tissue. This suggested that GAGs in the form of PGs do not contribute to the tensile strength of meniscal fibrocartilage (Ghosh and Taylor, 1987). Their mechanical role is that of providing resistance to compression as has been demonstrated for articular cartilage (Kempson et al., 1973; 1976). The actual PG content of the meniscus is very low, less than 1% on a dry weight basis. Histologic (Gosh et al., 1983) and biochemical (Adams & Muir, 1981) studies reveal that the macromolecules are not uniformly distributed in the extracellular matrix. The peripheral aspects where collagen is most thick and highly aligned are most
devoid of PGs. In contrast PG localization is most abundant along the inner edge with pockets in the central meniscal region. This arrangement of PGs supports a mechanical role of the menisci, whereby compressional load is translated into a circular stress or "hoop" stress.

**VASCULARIZATION**

Early studies on the vascularity of the menisci did not describe in great detail the fine vascular anatomy of the meniscus and perimeniscal tissues (Danzig, 1981; Scapinelli, 1968; Crock, 1967; Davies, 1948). Embryologic studies demonstrated that early in its development the meniscus has blood vessels throughout its substance (Clark & Ogden, 1983; Kaplan, 1955). Gradually during post-natal life the inner portion becomes avascular. It has been theorized that weight bearing and knee motion is responsible for this progressive loss of vascularity (DeHaven, 1981).

Arnoczky (1982) brilliantly described the microvascular anatomy of the meniscus using India ink perfusion and a modified Spalteholz tissue clearing technique (figures 6-7). He demonstrated the blood supply arising from branches of the medial, lateral, and middle geniculate arteries. These arteries give rise to a perimeniscal capillary plexus which originates in the capsular and synovial tissues of the joint. This supplies the peripheral 10-
Figure 6. Frontal section of medial compartment of knee Branching vessels from perimeniscal capillary plexus (PCP) can be seen penetrating peripheral border of medial meniscus. (F) femur, (T) tibia. (From Arnoczky, 1982).

Figure 7. Superior view of medial meniscus following vascular perfusion with India ink and tissue clearing technique. Note vascularity at periphery of meniscus as well as at anterior and posterior horns. (From Arnoczky 1982).
25% of the menisci, with the inner 75% being avascular. In addition, he described a peripheral vascular synovial fringe which is intimately associated with the superior and inferior edge of the menisci, but does not give off penetrating branches to the meniscal substance. The anterior and posterior horn attachments of the menisci are covered with well vascularized synovial tissue and thus have a good blood supply.

**HISTOLOGY OF THE MENISCUS**

**General Observations**

From the work of Ghadially et al (1978, 1983) using electron microscopy to examine the ultrastructure of rabbit semilunar cartilages, and normal and torn menisci in humans; several facts and observations were made:

1. There are no constant regional ultrastructural differences between sections of the anterior, middle, and posterior regions of the menisci in humans.
2. Morphological differences were found between surface layers (both superior and inferior) and the deeper or middle parts in all regions of the meniscus.
3. Anoxic or early autolytic changes, such as dilatation of the rough endoplasmic reticulum and mitochondrial swelling, are invariably present in autopsy specimens and to a lesser degree in surgical specimens.
There are several cell types described in the meniscus: (1) Chondrocytes; (2) Fibroblasts; (3) cells of intermediate form which are difficult to classify either as chondrocytes or fibroblasts ("transitional" cells); (4) Myofibroblasts (only in injured portions of three torn menisci as described by Ghadially et al 1983); (5) Mast cells (only in the outer parts of the menisci); and (6) degenerate and necrotic cells.

**Chondrocytes**

In the superficial zone of the meniscus the chondrocytes resemble those of Zone I of articular cartilage. They are ovoid or fusiform in profile with a few short cell processes, occasionally a circular profile is seen. Chondrocytes of the middle or deep zone resemble those of Zone II and III of articular cartilage as they have a rounded or polygonal profile when sectioned. Occasional elongated profiles similar to those in the superficial zone are seen. Organelles including rough endoplasmic reticulum, golgi apparatus and mitochondria are seen. A nuclear fibrous lamina is sometimes seen.

The cells are set in a fine pericellular matrix (composed of fine collagen fibres. This gives the appearance of a "halo" around the cell (figures 49-50).
Fibroblasts and Intermediate cells

Fibroblasts are commonly seen in the fibrofatty region where the menisci blend with the joint capsule, but within the depth of the meniscus actual fibroblasts are rare. They are spindle-shaped cells with abundant cytoplasm enclosing much endoplasmic reticulum (see figs. 47-48). Cell processes do not emanate from the surface as in chondrocytes, but elongated processes do arise at the poles of the cell. No fine textured pericellular matrix exists as for chondrocytes.

Intermediate cells are difficult to classify as either of the above types. They are plump polygonal cells with a fair amount of rough endoplasmic reticulum, little or no territorial matrix, but show occasional short cell processes from the cell surface. Such cells are found in both the superficial and deeper zones.

General Matrix

The extracellular matrix is composed of collagen fibrils primarily. These are intertwined within a sparse interfibrillar matrix with protein-polysaccharide material (proteoglycans) and their associated filaments.
Territorial Matrix ("halo")

This is a pericellular region around chondrocytes which is fine textured and sparse. It contains proteoglycan material with associated short filaments, and sparse collagen fibrils. It gives chondrocytes the appearance of having a halo around the cell (see figures 49-50).

Surface of The Normal Meniscus

An electron dense surface coat is seen on the surface of the normal meniscus cartilage (figure 47). This same coat was seen on the surface of normal articular cartilage (Ghadially 1982). He suggested that this coating is made up of extruded material and debris from the cartilage (i.e. degraded metabolites) matrix, as it has no other route of discharge.

Nature of Cells

The bulk of cells in the human meniscus (Ghadially, 1983), and almost all the cells in the rabbit meniscus (Ghadially, 1978) resemble chondrocytes more than fibroblasts. Reasons stated by Ghadially are: "(1) Cells in menisci more resemble chondrocytes rather than fibroblasts because many of them are rounded and have short processes coming off their surface; (2) the oval and
elongated cells seen mainly near the cell surface (which are probably regarded as fibroblasts by light microscopists) more resemble Zone 1 articular cartilage than fibroblasts; (3) characteristic proteoglycan particles as seen in hyaline cartilage and epiphyseal cartilage, are found in both the territorial and general matrix; and (4) although the territorial matrix may at times be sparse, it can be demonstrated in most instances. This is in contrast to what is seen in fibrous tissue, where a specialized territorial matrix does not occur around fibroblasts and proteoglycan particles are of rare occurrence and often undetectable (Ghadially, 1982).

FUNCTIONS OF THE MENISCUS

Sutton in 1897 described the menisci as the functionless remains of leg muscle origins which formerly arose intra-articularly. There have been many views on the exact functions, or lack thereof, pertaining to the menisci in the early literature. Part of this stems from the fact that with removal of these structures, the patient or even an athlete, was able to return to strenuous activities. In the last few decades it has become unequivocally clear that the meniscus has many important functions, the most important of which is load bearing and protection of articular surfaces from contact stresses.
The meniscus has been said to be instrumental in the following functions:

1. Transmission of load across the knee joint
2. Shock absorption of dynamic loads
3. Minimizing surface contact stresses (of articular cartilage)
4. Joint stabilization
5. Lubrication and nutrition of articular surfaces

**Transmission of load**

There are multiple biomechanical studies in the literature using cadaveric specimens (Ahmed & Burke, 1983; Krause et al., 1976; Seedhom & Hargreaves, 1979; Shrive et al., 1978; Walker & Erkman, 1975) which demonstrate that the intact menisci transmit from 50-99% of the total load acting across the joint in static and dynamic phases of the joint motion.

The material properties of the meniscus itself are very important in determining the actual amount of the load that they will transmit as Hefzy (1983) described using a mathematical model of the meniscus.

This relationship of mechanical properties of the meniscus to the degree of force transmission is one of prime importance, and is the basis for the
mechanical studies undertaken in this thesis. To fully understand how forces are transmitted, both in the normal and abnormal state, several studies are presented.

The mechanism of load transmission in the knee joint was first described by Fairbank (1948). Seedhom (1979) articulated this mechanism clearly. If one looks at a cross-section of the knee joint through the lateral meniscus, it is seen to be tightly interposed between the adjacent articulating surfaces of the femoral condyle and tibial plateau (figure 8). As load is applied through the femur, the meniscus because of its wedge-shaped form and slippery lubricated surface, is displaced radially to the periphery. The excursion is limited by the strong anchoring ligaments of the anterior and posterior horns, the fibrous capsule, and the medial collateral ligament. This force is then translated into a circumferential tensile stress or "hoop stress" (Aspden, 1985). Recall that the main orientation of the collagen fibres are circumferential (Bullough, 1970) which provide the maximum tensile strength along the axis of force. The remaining load is transmitted by areas of direct contact between articular cartilage of the condyles.

If however, a tibial attachment were severed, then the acting forces would drive the meniscus radially with minimal resistance; thus the load would be borne by the articulating surfaces solely and none by the meniscus.
Seedhom and Hargreaves (1979) experiment measured the load transmitted by normal menisci, meniscectomized knees, and simulated "bucket handle" torn menisci. The fraction of the load carried by the intact meniscus was 70-99%. The stiffness of both the meniscus and articular cartilage are non-linear, therefore the fraction of the load carried by the meniscus varies with the amount of load applied. They found that under no-load conditions the whole meniscus made contact with the femoral condyle.
If there were various types of tears created in the meniscus, none of which would completely interrupt the circumferential nature of the meniscus, then a significant amount of load is still transmitted. If the knee was completely meniscectomized, (or the meniscus made completely redundant by severing both horn attachments) then the area of contact between the femur and tibia was reduced and the stress increased greatly. If a bucket handle type tear was removed from the meniscus (partial meniscectomy), it was found that the stresses were increased more so than with intact menisci, but much less than the meniscectomized knee.

Radin (1984) used a photoelastic technique to compare the effects of meniscectomy, longitudinal tears of the meniscus, and retention of a meniscal rim on the degree of stress and its distribution in the medial compartment of the knee. His results showed menisci act to distribute stress within the knee joint, and in the absence of menisci this stress is concentrated and increased. If a peripheral rim of tissue were retained then the outer and middle areas were protected from abnormal stresses, however this tended to increase the stress acting near the intercondylar notch. An undisplaced longitudinal tear in the substance of the meniscus showed no obvious change in the magnitude or distribution of stress in their model, thus concurring with the current view that undisplaced tears should not be resected.
In a mathematical model of a toroid which represents the shape of the meniscus Aspden (1985) defined horizontal boundaries of positive and negative shear stress. These areas coincide with the most common location of observed longitudinal and horizontal tears of the meniscus in-vivo. This model also demonstrated that the most vulnerable area to injury is the posterior segment.

Walker and Erkman (1975) using a spatial location and casting model found the load bearing area to be approximately 6 cm² on each condyle. This finding is of the same order as that described by Kettlekamp (1972). Without menisci however this area is reduced to just 2 cm² per condyle exemplifying the increased amount of stress per area that must be borne.

Ahmed and Burke (1983) designed a complex apparatus which simulates the external static forces and torques acting on the knee in-vivo. They demonstrated that a significant amount of the load is transmitted by intact menisci. Using a thin film transducer, they displayed the contact areas and thus areas of stress distribution in cadaver knees. In knees that were totally meniscectomized the area of contact was reduced by 50-70% and the resultant stress per unit area was drastically increased (figure 9).
Figure 9. The effect of load on pressure distribution (left). Effect of meniscectomy on pressure distribution is shown at right (From Ahmed and Burke 1983).
In summary, post-meniscectomy, the contact area is reduced which leads to greatly increased stress concentration on the articular surfaces. This leads to fibrillation and wear of articular cartilage (i.e. arthritic changes).

First described by Fairbank (1948), osteophytic ridging, and flattening of the femoral condyles with narrowing of the joint space results from loss of the meniscus. Much support confirming that these changes are indeed related to the loss of the meniscus has been offered. Kettlekamp (1972) notes that the degenerative changes that occur first arise on the areas of direct contact of the tibial plateau and femoral condyle. Only later do they arise on the areas that were originally covered by the meniscus. Partial meniscectomy leads to less severe degenerative changes, the degree varying proportionately with the amount resected (DiStefano, 1980; Cox, 1975).

**Joint Stabilization**

Menisci have been ascribed a joint stabilization function (Brantigan, 1941). They are thought to facilitate complex movements into three planes of motion—hinge, glide and rotation (Canham, 1986). An interesting statement was made by Last (1972) "the chief role of the menisci is in rotation; the only mammal unable to rotate a knee (the fruit bat) is the only mammal lacking menisci". Radin (1972) suggested the menisci act as washers, easing rotation
of the femur on the tibia. Despite this, knees from which menisci have been removed are still able to rotate.

Joint congruency is also increased by the presence of menisci (Simon, 1983). This helps not only to stabilize the knee, but also acts in load transfer.

**Lubrication and Nutrition**

MacConaill (1932) first proposed a role of lubrication to the menisci. This was based on the theory of hydrodynamic lubrication. Menisci contribute to cartilage nutrition and lubrication by spreading a thin film of synovial fluid over the condylar articular cartilage of the femur.

The primary route of cartilage nutrition is via the synovial fluid. Torzilli (1983) describes two mechanisms for the passage of nutrients:

1. Passive diffusion of elements in solution. The diffusion rate is dependant on the concentration gradient. Large molecules are excluded on the basis of size.

2. Transport in solution by convection of liquid due to repetitive loading and unloading of the cartilage (and meniscus). This creates a pumping action which exchanges fluid from the cartilage aqueous phase with the synovial space fluid phase.
The second route of supply of nutrients is by way of blood supply which as shown above, is limited. Recently Bird and Sweet (1987) demonstrated a system of canals in meniscal cartilage of calves and young infants. These canals open onto the surface of the meniscus, and when joints were filled with india ink, the ink was noted to be in canal-like structures deep within the substance of the meniscus. These canals may play a role in the transport of synovial fluid within the meniscus, or may carry nutrients from the synovial fluid or the blood vessels to the avascular sections of the meniscus.

MENISCAL HEALING AND REPAIR

The first recorded attempt at meniscal repair was performed by Annandale in 1883 when he sutured a torn anterior horn of a medial meniscus to its former peripheral attachment. The patient returned back to work 10 weeks later.

In 1936 King published his classic article on the healing of semilunar cartilage by performing some simple experiments on the menisci of dogs. He demonstrated that surgically induced longitudinal lacerations in the substance of the meniscus did not heal. However, if the cut was through to the peripheral synovium, or a longitudinal cut in the substance was extended to
communicate with it, then it would heal.

Similar studies have been repeated more recently in which larger defects have been created in the meniscus and the healing process examined. Heatley (1980) excised peripheral segments of menisci in rabbits. These segments then healed with a highly cellular but fibrous tissue. Cells proliferated from the synovium and migrated to and along the cut edge of the meniscus. The cells then created a circumferential rim from which healing took place toward the center, filling in the defect. This repair process was expedited if the synovium was sutured to the cut edge of the meniscus. The gap is lessened and the sutures were thought to act as "bridges" for synovial cells according to Heatley. Occasional transformation of the avascular fibrous tissue to small regions of fibrocartilage was seen.

Arnoczky (1983) confirmed Kings' initial findings and took them one step further. Longitudinal lesions in the menisci of dogs failed to heal. Arnoczky created a vascular access channel, a tract that extended from the synovium to the lesion and therefore bisected it. These lesions were found to heal completely in 10 weeks. The vascular response originated in the perimeniscal capillary plexus.

The synovium is considered essential in this reparative process and is likely
the source of the new cells. Experimental work has shown that the meniscal regeneration that is occasionally observed in rabbits is prevented if a synovectomy is simultaneously performed with the meniscectomy (Kim, 1979).

Veth et al (1980) found in rabbits that surgically induced wedge shaped defects communicating with the synovium healed, the tissue containing fibrocartilage but being avascular. Longitudinal cuts in the substance did show some healing, but with avascular fibrous scar. This type of healing was not facilitated even if sutures were placed. They confirm the fact that invasion of cells is necessary for repair.

The rabbit shows extreme potential for repair of even longitudinal cuts in the avascular regions of the meniscus. This potential for healing in the substance does not occur in the avascular portion in human menisci.

Veth et al (1983) also used synovial flaps and carbon fibre implants to reconstruct the same surgically induced lesions as their prior experiment. The synovial flap induced healing with mainly fibrous but some fibrocartilage type tissue. With the carbon fibre implant massive fibrosis took place and morphologically the meniscus was distorted. An interesting finding though was the fact that the cells seem to be oriented parallel to the carbon fibres.
(circumferentially).

Based on the fact that the meniscus is capable of healing defects in its substance in the presence of vascular tissue, Arnoczky (1986) used an exogenous fibrin clot to heal discrete stable defects. The fibrin clot provides a matrix for repair, and the stimulus for healing. The repair was populated by fibrocytic cells, but was eventually remodelled to fibrocartilage. This suggests that the clot acted as a chemotactic stimulus, however the origin of the cells is unclear.

Attempt at meniscal replacement has met with little success. Replacement of the meniscus with a silastic prosthesis in the rabbit (Kenny et al, 1983) revealed no significant difference over the meniscectomized knee with respect to degenerative changes noted.

Similarly, meniscal substitution with a teflon-net (Toyonaga, 1983) which allows for ingrowth of fibrocartilage cells, has been performed. Degenerative changes of the tibia were still noted in the teflon-net group, but overall osteoarthritic changes were less than the meniscectomy group. Evidence of rejection of the teflon fibres was manifested by lymphocytic and giant cell infiltrates around the fibres.
More recently experimental work in the transplantation of whole menisci has shown promising results. Cryopreserved meniscal allografts transplanted in dogs knees (Arnoczky 1984) were observed to heal completely to the peripheral synovium and to the horn attachments by 4 weeks. Gross morphology was normal except the surface was more dull than the pearly white appearance of the normal meniscus. There were areas of cell death in the center of the allograft. By five months the gross morphology was normal. Histologically the grafts were hypercellular near the articular surfaces. There were no degenerative changes on the surfaces of the meniscus. While grossly normal, there were mild degenerative changes of the articular cartilage beneath the allograft. Initially isotope studies indicating proteoglycan production (determined by analysis for labelled sulphated glycosaminoglycans) showed diminished uptake versus controls. By twelve weeks post-transplantation the uptake was similar to controls.

The mechanical properties of these cryopreserved allografts were examined and found to be similar to control menisci (Schmidt, Arnoczky et al, 1986). The material properties of the meniscus appear to be unaffected by the cryopreservation process.

Lyophilized, irradiated menisci and deep frozen menisci were transplanted in sheep (Weisemeier et al, 1988). Results were assessed macro and
microangiographically, electron microscopically and biomechanically. Slightly superior results were seen in the lyophilized group. In both groups, and at all time intervals, no mechanical differences between treated and fresh menisci were seen.

Keating (1988) has reported on a series of 36 meniscal transplants in goats comparing fresh, freeze dried, and cryopreserved menisci. Animals were sacrificed 3 and 7 months post-operatively and menisci assessed via light and electron microscopy. It was seen that none of the fresh allografts healed and that they destroyed all knee joints into which they were transplanted. The cryopreserved menisci healed, and at 7 months the articular surfaces of the joint were grossly normal. In the freeze dried menisci, only 2 animals showed complete incorporation of the meniscus with no effusion and destruction of the joint.

Jackson (1990) performed meniscal transplantation in 30 goats. Autografts, fresh allografts, and cryopreserved allografts were implanted. In contrast to Keatings' findings, all groups healed without any rejection or destruction of joints. Grossly and microscopically all implanted menisci differed little from control menisci. Viability was best in the two allograft groups with the fresh faring better than the cryopreserved. There were however, biochemical changes in uronic acid content suggestive of alterations at the cellular level.
In most of the series described a strong limitation has been the relatively small number of experimental animals and short length of follow-up. Zukor et al (1988) tried to develop a model in the rabbit, which because of its smaller size and decreased cost would allow larger series with lengthened follow-up.

Zukors' model involved transplanting the medial meniscus in rabbits. They performed 51 allografts comparing fresh, frozen (-70°C), and dimethyl sulfoxide (DMSO) cryopreserved frozen (-70°C) allografts. Animals were sacrificed 1 to 9 months post-transplantation. Macroscopic and histological evaluation revealed encouraging results in all three groups with the fresh transplants faring slightly better than the others. 90% of the menisci were seen to heal completely. 83% maintained normal length though only 20% maintained normal width. No rejection or destruction of knee joints occurred, even in the fresh group. None of the rabbits developed significant degenerative articular cartilage changes. This was thought to be extremely important as the rabbit knee is known to be extremely sensitive and an excellent model for osteoarthritis. Minimal surgical intervention such as arthrotomy alone, and most certainly meniscectomy leads to degenerative arthritis in the rabbit (Floman 1980; Shapiro 1979; Moskowitz 1973).
In humans the data regarding isolated meniscal transplantation is very limited. A single case report of a fresh meniscal allograft with a one year follow-up has been recently published. In the German literature, a series of 14 patients with freeze-dried meniscal allografts with a mean follow-up of 6 months has been published (Wirth 1986). Although initial results were encouraging, recently this group abandoned freeze drying as a "storage" technique because of significant late failures.

To date the largest series of patients with meniscal allografts appears to be that of Dr. Allan Gross (Toronto). Zukor et al (1988) presented the results of a prospective review of 33 meniscal allografts performed in conjunction with fresh osteochondral allografts most often of the proximal tibia. Clinical success has been achieved in 75% of the patients who had follow-up greater than one year. More specific evaluation of the menisci was possible via a "second look" arthroscopy in 8 patients (10 menisci). Four other transplanted menisci have been assessed at the time of open arthrotomy for various other surgical procedures. Generally, the menisci have been noted to be structurally sound and functional as late as 8.5 years following transplantation.

Transplantation of the meniscus appears to be the most attractive option for meniscal replacement in cases where repair is not possible or the meniscus has
been previously resected. Proper size matching is important for the meniscal transplant to be maximally functional in performing the above mentioned functions. Rudan et al (1988) have categorized menisci as to size. Two-hundred and four menisci in the University of Toronto Bone Bank have been carefully examined, measured and classed. Simultaneous measurements of the proximal tibia were performed. In a computer analysis they found that human menisci could be categorized into sizes and that these sizes correlated with proximal tibial geometry.

In summary, from experimental studies on the biology, vascularity, and reparability of the meniscus, transplantation is a feasible method of meniscal replacement. This is borne out by the experimental studies and to a lesser extent by the small human experience.

To implement such a program in humans, many important issues must be addressed. While fresh tissue transplants probably represent the "gold standard", they are associated with certain theoretical and real problems. These include the immunology, the risk of disease transmission, logistical problems in procuring menisci from healthy donors and implanting them into recipients within a short time frame, and finally the difficulty of matching donor and recipient for proper size.
Thus, being able to establish a bank of menisci with a sizing classification such that donor and recipient could be technically well matched is extremely relevant to this problem. To perform such a procedure in an elective fashion there must be a way of storing these tissues until time of use without damage to them. Lastly the tissue must be "sterilizable" to prevent any possibility of disease transmission, while ensuring the tissue is not denatured (functionally) in the process.

**Effects of Irradiation**

Gamma irradiation in a dose of 2.5 megarads is widely employed by musculo-skeletal tissue banks, on the recommendation of the American Association of Tissue Banks (Friedlander 1979). This dose has been shown to kill most micro-organisms without significantly altering the mechanical properties of the tissues (bone, fascia).

Homograft aortic valves sterilized with 2.5 Mrads of gamma irradiation yielded no positive bacteriologic cultures, and at this dose was found to not alter the structure or physical characteristics of these valves (Gibbons 1974).

Previous work has shown that irradiation of cartilage destroys the
chondrocytes and that repopulation from the host occurs (Donald 1986).

In addition to the effects on the viability of the cell, irradiation may affect the tissue matrix present which would result in a change in its mechanical behaviour. Several authors have examined the effects of gamma irradiation on polymers (Charlesby 1960; English 1986; Phillips 1988) and certain living tissue (Charlesby 1960). Charlesby stated:

"long chain polymers can serve as simple models for biological materials and indeed many of the irradiation effects observed in radiobiology are closely parallel to those found in simple long chain polymers."

Irradiation causes two phenomena to occur in polymeric chains. Firstly, high energy radiation imparted to these chains causes chain scission (figure 10) (English 1986). The greater the chain length, the more susceptible the tissue by sheer probability of having all chains affected. The principal effect of irradiation is to excite electrons which are in chemical bonds, breaking them either in the main strand or lateral branches. The bonds may then reform immediately in which case the structure is not affected, or new combinations of bonds occur forming new cross-links. Cross-links may actually increase strength, but may cause tissue to become brittle. Both processes occur simultaneously in a complex fashion, the net result may be a weaker tissue, or a stronger but more brittle tissue (Phillips 1988).
Irradiation of polymers produces two main effects: chain scission, which reduces strength; and crosslinking, which increases strength but frequently leads to embrittlement. The two effects are usually combined in a complex interrelationship.

**Figure 10.** A. Effects of irradiation on polymers (from English 1986). B. Effects of irradiation on polymers (from Phillips 1988).
English (1986) reports the effect of elevation of temperature and irradiation may be synergistic, i.e. the net effect of a combination of the two processes is greater than a simple additive effect. This may be explained by the fact that with increased temperature, chains are more mobile and flexible which allows for greater cross-linking to occur.

In the meniscus, fibres and chains are bathed in aqueous solution which allow lateral chains to dissociate and recombine, and main strands to move. If the tissue is frozen, then the mobility of the main strands is lessened and the degradation of tissue by new cross-links should be less than at normal or elevated temperatures (personal communication with Dr. Potier researcher in the Department of Genetics, Ste. Justine Hospital, Montreal).

Preservation Techniques

Deep-freezing or freeze-drying techniques have been widely utilized for the storage of connective tissue allografts (Arnoczky, 1986; Bright, 1981; Brown, 1982; Shino, 1984; Tomford, 1982;1983;1984). This technique however, results in the destruction of the cellular components of the tissue, while decreasing its antigenicity (Arnoczky, 1986; Brown, 1982; Curtis, 1985; Shino, 1984).
Studies on bone (Brown, 1982), fascia (Bright, 1981; Curtis, 1985), and tendon (Arnoczky, 1986; Shino, 1984) both experimentally and clinically demonstrate that the tissues need not have viable cells at the time of initial transplant to ultimately function normally in recipient sites. Black (1981) and Tomford (1982) have suggested that articular cartilage allografts require intact cells to preserve their mechanical function. From this reasoning, authors have tried to maximally preserve cell viability in the storage techniques (Arnoczky, 1988; Schachar, 1982; Tomford, 1982-4).

In DMSO cryopreserved menisci transplanted by Arnoczky (1988), the viable cell population was significantly decreased. The number of cells and metabolic activity was seen to decrease with increased length of storage time. Zukor (1988) found no significant difference in terms of healing or degenerative changes among the DMSO cryopreserved or frozen menisci. This suggests that perhaps the meniscus being fibrocartilage may behave more like tendon or fascia and not require viable cells at the time of transplant to function normally. This tissue may become repopulated with cells from the host (Bright, 1983; Campbell, 1963; Nikolaou, 1986).

This study was undertaken in an attempt to address the issues of the feasibility of meniscal transplantation. More specifically, to determine if meniscal allografts can be stored for elective use, and sterilized to prevent
the possibility of disease transmission.

SPECIFIC AIMS

This study was a progression of previous work in the field of meniscal transplantation. It was an attempt to overcome some of the shortcomings of previous work (i.e. small series, short follow-up) as well to utilize a new technique (developed in collaboration with the Ecole Polytechnic) for measuring mechanical properties of the intact meniscus after treatment, in a more physiologic manner.

Objectives of the study were:

1. The assessment of healing, viability, and survival of transplanted fresh and "stored" menisci in rabbits at long term follow-up.

2. Specific assessment of the effects of gamma irradiation (2.5 Mrads) on the biological and mechanical properties of rabbit menisci.

3. The effects of meniscectomy versus transplantation with respect to degenerative changes of the articular cartilage of the knee joint in rabbits.
The working hypotheses were:

1. There is no difference in the healing of the meniscal allografts amongst the different groups. These menisci will continue to protect the articular cartilage from stress concentration as will be assessed by degenerative changes.

2. The mechanical properties of the menisci are not significantly affected by freezing or gamma irradiation.

3. The transplanted animals will fare better than the meniscectomy group in terms of degenerative changes.
MATERIALS AND METHODS

ANIMALS

Male White New Zealand rabbits weighing approximately 3.5 kg. each were utilized in this study (Reimans Fur Ranches, Ste. Agatha, Ontario). All meniscal allografts involved only the medial meniscus. 70 rabbits were divided into 4 treatment groups as follows:

Group I
20 rabbits received fresh meniscal allografts. Pairs of rabbits were operated on simultaneously and menisci were exchanged between rabbits.

Group II
20 rabbits received frozen (-70°C) banked meniscal allografts. These menisci were stored for periods of 1 to 4 weeks.

Group III
20 rabbits received frozen (-70°C) and irradiated (2.5 Mrads) banked meniscal allografts.
Banked menisci were kept frozen during the irradiation process with dry ice to avoid thermal effects and received 2.5 megarads of Gamma radiation. Dry ice always remained present in the insulated packaging at the end of the procedure, assuring there was no significant rewarming of specimens.

**Group IV**

10 rabbits underwent unilateral medial meniscectomies *without* reconstruction. This group serves as a control group. These animals were operated on first to create a bank of menisci. When these menisci were later transplanted the recipient animals' menisci were excised for repletion of the meniscal bank inventory.

**OPERATIVE TECHNIQUE**

All surgery was performed at the Royal Victoria Hospital animal facility. All procedures were performed under general anaesthesia consisting of intramuscular injections of Atropine (0.05mg/kg, Squibb), Atrevet (acepromazine maleate 0.5mg/kg), and Ketamine (25mg/kg, Parke-Davis).

Knees were shaved and prepped with providine solution. Animals were placed in pairs in the supine position, stabilized on the operating table and draped in a sterile fashion.
A medial parapatellar incision was employed. The medial collateral ligament was identified and carefully protected. At no time was this ligament detached or divided (thus not interfering with knee joint stability). Parallel transverse arthrotomies were performed just superior and inferior to the anterior portion of the medial meniscus (figure 11).

**Figure 11.** Surgical technique. Medial parapatellar incision exposing medial collateral ligament (mcl) and joint capsule. Arthrotomies have exposed the medial meniscus (m).

The anterior horn was visualized and divided from its bony attachment (figure 12). Dissection was then continued posteriorly allowing identification of the posterior horn of the meniscus which was then divided. The meniscus was removed intact (figure 13). A plane between the postero-medial capsule and adjacent musculature was then developed.
Figure 12. Medial meniscus has been detached at anterior horn and passed under medial collateral ligament. Femur (left) and tibia (right) are seen.

Figure 13. Medial meniscus after excision (right) and a lateral meniscus for comparison (left). Anterior horn is toward bottom of illustration.
At this point the allograft tissue was prepared for transplantation (figure 14). In the case of frozen, or frozen-irradiated tissue, the specimens were thawed rapidly prior to the procedure via immersion in room temperature Ringers lactate solution (Abbott Pharmaceuticals).

Figure 14. Medial meniscal allograft thawed and ready for implantation.

The meniscal allograft was implanted and carefully sutured into place utilizing slowly absorbable suture material (6-0 P.D.S. Polydioxinone. Ethicon. Figure 15).
Three suture points were generally utilized, one anteriorly, one posteriorly, and one at the mid portion. This was supplemented with additional sutures if required. The principles of meniscal repair were utilized, that is, sutures were buried within the substance of the meniscus and all knots were located outside of the joint. The wound was then closed in layers using absorbable suture material. No wound dressing was applied.

Prophylactic intramuscular penicillin V (phenoxymethyl penicillin) 0.5cc was injected once pre-operatively, and daily (0.25cc) for 5 days post-operatively.
POST-OPERATIVE COURSE

Post-operatively the animals were allowed to recover from anaesthesia and then are returned to their cages. No immobilization of the limbs was utilized. Wounds were examined daily in this period for any evidence of infection or dehiscence. Any abnormalities were recorded.

The animals were allowed full normal cage activities for a period of six to seventeen months at which time they were sacrificed.

At the time of analysis animals were sacrificed with 1.5 ml intravenous T-61 euthanasia solution.

GRAFT PROCUREMENT

Post-sacrifice the animals were placed in the supine position. An extensive longitudinal incision was made through the skin extending approximately 3 cm above and below the knee joint. The flap was then bluntly dissected posteriorly exposing the entire knee joint (joint capsule fully intact) with adjacent femur and tibia.
The contralateral unoperated knee was similarly dissected for comparison, and procurement of control tissue (for histologic, radioautographic and mechanical studies). The joint capsule was then opened carefully and all the stabilizing ligaments divided to fully expose the interior of the knee joint. At this point the transplanted or operated knee was photographed and evaluated macroscopically on a 12 point scale assessing the following four parameters:

I. Meniscus size- Length and width in comparison to normal contralateral controls

II. Healing- the degree to which the transplanted tissue became attached peripherally to the host synovium along the rim of the meniscus.

III. Brightness/color- the normal meniscus is glossy, smooth, and pearly white. Points were lost for opacity, hyperemia and roughness.

IV. Degenerative changes (Articular cartilage)- the meniscal allograft was excised via sharp dissection along the plane between the meniscus and the peripherally attached synovium allowing full exposure of the articular surface. The articular surface of the medial femoral condyle and corresponding tibial plateau were examined for the presence of osteophyte
formation, pitting, and erosion of cartilage.

PROCESSING OF ALLOGRAFTS

Of the specimens procured, one half were processed for histological and autoradiographic studies. The remaining allograft tissues were subjected to mechanical testing.

HISTOLOGY AND AUTORADIOGRAPHY

Immediately following procurement, photography, and macroscopic scoring the menisci were individually placed into incubation tubes containing 5 ml of Dulbecco’s modified Eagle’s medium (GIBCO) and Na$_2$SO$_4$ (10uCi/ml) (ICN biomedical, Montreal, Can.). Specimens were then incubated in a Dubnoff metabolic incubator for 7 hours at 37°C. Following incubation specimens were washed with Dulbecco’s medium three times. Meniscal tissue was then fixed in 10% buffered formalin (Fischer Scientific) and embedded in paraffin. Five micron thick sections were cut and mounted on glass slides. Alternate sections were processed for histologic staining and autoradiography.
Autoradiography

Mounted slides were coated with Kodak NTB\textsubscript{2} photographic emulsion, air dried, and placed in light and moisture proof boxes at 4°C for 21 days. After this exposure period, the slides were placed in Kodak D170 developer for 5 min, washed with distilled water, and then placed in a fixer for 10 minutes (24\% sodium thiosulphate) at 18°C. Slides were then washed in tap water for several minutes, rinsed in distilled water, air dried, and finally stained with Safranin-O (to stain proteoglycan and nuclear material).

The sections were then examined under light microscopy. A gross qualitative assessment of metabolic activity was made by comparing experimental sections with fresh unoperated control specimens.

Plain slides were dipped in emulsion for each batch processed to assess background (background control). In addition, 3 frozen, 3 frozen-irradiated, and 2 fresh menisci were processed at zero-time to determine zero-time cell population.
Histology

Histologic sections were mounted in a similar fashion to autoradiographic specimens, and stained with Hematoxylin and Eosin. These histologic sections were then examined in an objective, blinded fashion by a pathologist with a special interest in musculoskeletal pathology (Dr. Indrojit Roy, St. Mary’s Hospital, Montreal). Assessment was based on the following five parameters:

1. **Cell Necrosis** - based on loss of nuclear stain, empty lacunae, fragmented nuclei and pyknosis.

   **Scoring:**
   
   - 0 = None
   - 1+ = <20% of cells
   - 2+ = 20-50%
   - 3+ = >50%

2. **Synovial Changes**

   **Scoring:**
   
   - 0 = Normal
   - 1+ = Mild hyperplasia
   - 2+ = Moderate hyperplasia
   - 3+ = Marked hyperplasia
3. **Papillary Proliferation** - fibrous type on inner aspect of meniscus

**Scoring:**
- 0 = None
- 1+ = Mild
- 2+ = Moderate
- 3+ = Marked

4. **Inflammation** - presence of lymphoid cells

**Scoring:**
- 0 = None
- 1+ = Mild
- 2+ = Moderate
- 3+ = Marked

5. **Vascularity** - presence of vessels in and around the meniscus

**Scoring:**
- P = Present
- A = Absent

In certain specimens, routine histology was utilized to examine the articular cartilage of the tibial plateau and femoral condyles adjacent to the meniscal allograft to verify the presence or absence of degenerative changes.
MECHANICAL TESTING

Mechanical testing of meniscal tissue was performed to assess for changes in viscoelastic properties secondary to irradiation and or freezing.

Prior to the undertaking of this study there were no reports in the literature on the effects of irradiation on the mechanical properties of meniscal tissue. In collaboration with the Biomechanical Research Group from the Institute of Biomedical Engineering at the Ecole Polytechnique (at the Universite de Montreal, Montreal, Quebec), specialized jigs and mechanical testing devices were developed to specifically measure mechanical properties of the rabbit meniscus (Duval 1989). The rabbit meniscus by virtue of its small size, dictates the inherent technical difficulties involved.

INDENTATION TESTING

This experimental housing was designed to test the viscoelastic properties of the meniscus. The central zone of the meniscus is important in supporting compressive forces. Thus the viscoelastic properties of this region determine the capacity to absorb the dynamic forces that are applied. Indentation testing is designed to measure these properties.
The apparatus provides a fixed surface and reproducible method of applying a load to a specific region of the meniscus. The mounting applies a load oriented normal (90°) to the meniscal surface via a plunger which is supported by ball bearings and deformation curves are recorded. From these curves, parameters calculated are:

(i) Instantaneous elastic response
(ii) Total displacement
(iii) Time Constant
(iv) Viscoelasticity

INDENTATION MACHINE

The indentation housing is composed of 6 major components (figure 16-17):

1. Plunger
2. Adjustable support
3. Displacement transducer
4. Load application module
5. Inclinable indentation base
6. Signal analyzer (analog to digital conversion)
Figure 16. Indentation testing machine. Meniscus is housed in physiologic solution. Plunger is in contact with surface. As step-load is applied, transducer records displacement.

Figure 17. Close-up of housing displaying meniscus in place in preparation for testing.
SPECIMEN PREPARATION

Post sacrifice menisci were procured as previously described. Additionally they were debrided of synovial tissue and immersed in HBSS (GIBCO). Both right and left medial menisci were procured, the contralateral serving as a control.

ZERO-TIME SPECIMENS

Menisci were then refrigerated to -70°C in separate specimen containers. For zero-time studies, menisci were kept frozen during the irradiation process with dry ice to avoid thermal effects. Menisci were irradiated with 2.5 Mrad of Gamma radiation. Dry ice always remained in the insulated packaging at the end of the procedure, assuring there was no significant rewarming of specimens.

Specimens were rewarmed rapidly by immersion in HBSS at room temperature at the time of testing.
MOUNTING OF SPECIMENS

The medial meniscus was then fixed to the inclinable base with cyanoacrylate glue (Crazy glue™) on its inferior aspect (tibial side) and then bathed continuously in HBSS. The plunger was then oriented perpendicular (90°) to the surface in a precisely determined site on the inner posterior third 1 mm from the free edge as described by Duval (1989) (figures 16-17).

After a stable state was obtained a step load of 1.5 MPa was applied axially in a delicate but rapid fashion (0.24 sec.). The plungers displacement with respect to time was then recorded.

PARAMETERS MEASURED

The curve recorded displays both viscoelastic and elastic regions. From these, indices of global rigidity, viscoelasticity, and time constants (the time required for the plunger to attain a specific percentage of the total viscoelastic deformation) were calculated.
TRACTION TESTING

Specialized mounting devices were developed to permit tensile testing of peripheral fibres in the meniscus, while retaining the physiologic structure and geometry. This was an innovative and uniquely designed method for this purpose.

The unit is composed of 5 elements (figures 18-19):

1. Custom formed condylar surfaces
2. U-support
3. Meniscal anchoring blocks
4. Ommitronix traction machine
5. Signal analyzer (analog to digital conversion)

The analog signal from the transducer is converted to digital by an IBM-PC type computer with a LABMASTER acquisition card.
Figure 18. Traction testing mounting device. 1. Vitallium condylar casts. 2. U-support. 3. Positioning rod. 4-5. Adjustment ring and nut. 7. Meniscal anchoring block. 8. Set screws. (Adapted from Duval, 1989).
Figure 19. Ommitronix tensile testing machine (top). Signal analyzing system (bottom).
Facsimiles of femoral and tibial condylar surfaces were formed in vitallium from molds of these structures. High temperature polyethylene plastic was added to the contact surface to diminish friction (figure 20).

![Figure 20](image)

Figure 20. Close up views of vitallium cast facsimiles of the articular surfaces of the femoral and tibial condyles. Surfaces are polyethylene coated to decrease friction. (Adapted from Duval 1989).

Ideally, the meniscal tissue was procured by including tibial bone blocks to which both the anterior and posterior anchoring ligaments are attached. These blocks were then embedded in PMMA (polymethylmethacrylate) cement which was housed in a inoxydizable steel cylinder. The condylar
components were then articulated with the meniscal graft and held it as traction was applied by the machine in a linear fashion. This recreates the in-vivo situation where peripheral displacement generates a circumferential stress or "hoop stress". The force was then increased incrementally up to failure point (rupture of meniscus). The computer recorded force and displacement. From this, rigidity can be calculated.

At zero time both indentation and traction tests were performed, as the meniscal specimens were procured with their bony attachments. Post transplantation, because of the lack of firm bony attachment of the anterior and posterior horns to the tibia, only indentation testing was performed. This represents a technical disadvantage of the rabbit model-which because of its small size precludes procurement and reattachment of meniscal-bone block preparations (i.e. "implants").
Unpaired Students t-test was utilized for comparison of two means.

One-way ANOVA was used to make comparisons of one variable amongst several groups (Armitage 1987). A $p$ value < 0.05 was taken to indicate statistical significance.

In graphic representation of data, error bars for arithmetic means are always shown as ± one standard deviation.
RESULTS

A total of 71 animals were operated on during the study period. There were no wound infections in any of the operated animals. There were 12 deaths (17%) during the period, however only 3 occurred within the first month post-operative for a survival and follow-up rate of 83% (Table 1).

There were 5 deaths in each of the fresh (5/22) and frozen (5/21) allograft groups, 2 in the frozen irradiated group (2/19), and none in the meniscectomy group (0/9).

Table 1. Characteristics of Groups

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Deaths</th>
<th>Survival%</th>
<th>Mean F/U (mos.)</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fs</td>
<td>22</td>
<td>5</td>
<td>77</td>
<td>14.38</td>
<td>11.5-17</td>
</tr>
<tr>
<td>Fr</td>
<td>21</td>
<td>5</td>
<td>76</td>
<td>11.5</td>
<td>9.75-13</td>
</tr>
<tr>
<td>Fi</td>
<td>19</td>
<td>2</td>
<td>89</td>
<td>9.63</td>
<td>6.25-12.75</td>
</tr>
<tr>
<td>Ms</td>
<td>9</td>
<td>0</td>
<td>100</td>
<td>12.25</td>
<td>9.5-13.75</td>
</tr>
<tr>
<td>Total</td>
<td>71</td>
<td>12</td>
<td>83</td>
<td>11.94</td>
<td>6.25-17</td>
</tr>
</tbody>
</table>
MACROSCOPIC FINDINGS

Macroscopic analysis performed immediately post-sacrifice assessed healing of the meniscus to the periphery, size (length and width) of the meniscus compared to contralateral controls, color of the meniscus, and degenerative changes of the distal femoral condyle and proximal tibial plateau. Best score for each category is 3 points, thus allowing a maximum possible score of 12 points for a normal meniscus.

Figures 21-25 demonstrate specimens from all groups at specific times of follow-up.

Figure 21. Unoperated control menisci demonstrating normal size, color, and peripheral attachment to synovium. Medial meniscus is seen on right side of figure.
Figure 22. Fresh (Fs) transplant at 15 months (on right). Note normal size and appearance. Healing to both horn remnants has occurred. Total score was 10.25.

Figure 23. Frozen transplant (Fr) at 10.25 months (on right). Good size, color, and healing are shown. Total score was 9.25.
Figure 24. Frozen Irradiated (Fi) transplant at 12 months (on left). Healing and size are perfect with slight discoloration and degenerative changes occurring. Total score was 10.5.
Figure 25. Frozen-irradiated allograft at 6.25 months (on left) with femoral condyles shown. Size and healing were perfect, slight hyperemia of anterior horn and tibial degenerative changes were noted. Total score was 9.75.
MENISCECTOMY GROUP

The meniscectomy group displayed an interesting phenomenon. In 3 out of 9 meniscectomized knee joints there was a varying degree of meniscal regeneration (figures 26-28). These three regenerated menisci displayed an excellent gross morphology (shape and size very similar to native tissue), losing marks for color and degenerative change only. Their follow-up time ranged between 9.5 - 12.0 months post-meniscectomy. Histologically these menisci were distinct from native menisci. They were comprised of more spindly fibrocyte type cells and were less "chondroid" in nature. In the remaining Ms knees there were varying minor degrees of tissue regeneration, at best thin rims of fibrous-like tissue.

The degenerative changes seen in the meniscectomy group were not significantly different whether a medial meniscus regenerated or not.
Figure 26. Meniscectomy at 12 months. Large osteophytic rim is seen. There is a slight attempt at regeneration of meniscus near horns.
Figure 27. Meniscal Regeneration at 12 months. Meniscus (top) shape is not normal. Degenerative changes of tibia are seen. Total score was 8.75.
Figure 28. Meniscal regeneration at 12.25 months (right). Thin rim of normal looking tissue with marked degenerative changes was seen. Total score was 8.5.
MACROSCOPIC RESULTS

The total group scores ± standard deviation for the 4 macroscopic parameters are illustrated in table II and figure 29.

Table II. Total Macroscopic Score

<table>
<thead>
<tr>
<th>Group</th>
<th>Score ± s.d.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh (Fs)</td>
<td>9.56 ± 0.46</td>
</tr>
<tr>
<td>Frozen (Fr)</td>
<td>10.39 ± 0.29</td>
</tr>
<tr>
<td>Frozen Irrad (Fi)</td>
<td>9.18 ± 0.51</td>
</tr>
</tbody>
</table>

Figure 29. Macroscopic score represents the sum of all 4 macroscopic parameters. Maximum possible score is 12 points. Scores are represented as ± standard deviation.
Results indicate that all groups were quite similar overall and there was no statistically significant difference.

Evaluating each of the macroscopic parameters individually, all had no significant difference amongst groups except for degenerative changes. Figures 30-33 display these findings.

**Figure 30.** Scores represent the means ± s.d. of the macroscopic parameter healing. Maximum score is 3 points.
**Figure 31.** Scores represent the means ± s.d. of the macroscopic parameter size. Maximum possible score is 3 points.

**Figure 32.** Scores represent means ± s.d. of the macroscopic parameter color. Maximum score is 3 points.
For the degenerative changes, all 4 treatment groups are examined (including Ms group), as an objective was to determine if the meniscal transplantation protected from the degenerative changes seen after meniscectomy alone. Results indicate that there is a statistically significant difference among these groups, with the Fr group faring better than both Fs and Fi but not significantly better than the Ms group.
HISTOLOGIC FINDINGS

The menisci were stained with Hematoxylin and Eosin as well as Toluidine Blue and assessed for cell necrosis, synovial changes, papillary projection and inflammation. The presence of vascularity was also examined for, but virtually every meniscal transplant showed evidence of vascularization. This ranged from capillaries to larger size vessels.

The scoring system in this category is such that full marks would be a score of zero, and points were added to a maximum of 3 per category for any of the abnormal changes found.

Table III represents the number of specimens examined histologically and radioautographically amongst the different transplant groups, as well as total score ± standard deviation for all histologic parameters.

<table>
<thead>
<tr>
<th>Table III. Total Histologic Score</th>
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<tbody>
<tr>
<td>Group</td>
</tr>
<tr>
<td>Fresh (Fs)</td>
</tr>
<tr>
<td>Frozen (Fr)</td>
</tr>
<tr>
<td>Frozen Irrad (Fi)</td>
</tr>
</tbody>
</table>
Figurc 34. Scores represent the sum of all 4 parameters evaluated. Best possible score is 0. Points are added for abnormalities noted. Scores are represented as means ± s.d.

All groups were similar with respect to total histologic scoring as shown in figure 34.

There were no significant differences among transplant groups for cell necrosis, synovial changes (hypertrophy), papillary projection and inflammation (see figures 35-37).
**Figure 35.** Scores represent means ± s.d. for the histologic parameter cell necrosis. Best possible score is 0. Points are added for amount of cell necrosis seen. No significant difference is noted.

**Figure 36.** Scores represent means ± s.d. for the histologic parameter synovial changes. Best possible score is 0. Points are added for abnormal changes noted.
Inflammation

Inflammation i.e., the presence of lymphoid cells which is an indicator of transplant rejection, was virtually not seen in any of the transplant groups. There was only one meniscus per group that displayed any evidence of lymphoid type cells in the periphery and they were very few at most. Occasionally giant cells or macrophages were seen around foreign bodies—presumably either suture material or talc from gloves.
RADIOAUTOGRAPHY

The autoradiographic demonstration of incorporation of radioactive \(^{35}\)SO\(_4\) isotope indicates not only individual cell viability, but also that cells are metabolically functional and actively producing proteoglycan. This also determines that these cells are chondrocytic in nature.

Zero-Time Fresh (Fs)

At time zero fresh control tissue was seen to be quite cellular exhibiting strong uptake of \(^{35}\)SO\(_4\) isotope. This is evidenced by the marked density of granules overlying the cells. Figure 38 represents a Fs control medial meniscus fibrocartilage at zero time. Figures A and B represent the same field at different depths of focus to demonstrate the cells and their overlying granules. The slight cleft formation that is seen among the collagen fibres is an artifact as a result of fixation.
Figure 38. Autoradiograph of Fs (fresh) meniscus fibrocartilage at Zero-Time Cellular plane (A) and overlying granule layer (B) represent same field with different planes of focus. Strong uptake of $^3$H is seen x250.
Zero-Time Frozen (Fr) Tissue

Frozen tissue at zero time displays minor histologic changes. Cell nuclei are at times pyknotic, with occasional empty lacunae denoting cell loss. On the whole, histologically, the cellularity and individual cells appear quite normal (figure 39A). However, looking at the autoradiograph (figure 39B) there is absolutely no cell viability or metabolic activity exhibited in these normal appearing cells as assessed by $^3$S incorporation. Thus the freezing process has destroyed cell viability. Granules seen represent background exposure of the photographic emulsion as determined by blank controls. Figure 39 A and B display the same field in different focal planes to demonstrate granule (photographic emulsion) layer.
Figure 39  Autoradiograph of Frozen (Fr) medial meniscus fibrocartilage at zero-time. Figs. A and B display same field in different planes of focus to demonstrate granule layer. Note complete absence of metabolic activity. x250
Zero-Time Frozen Irradiated (Fi) Tissue

At zero-time frozen irradiated (Fi) menisci appear histologically similar to the frozen (F) group. They display some loss of cells as noted by the presence of empty lacunae, and pyknosis of nuclei, but grossly have an almost normal appearance. Figure 40 shows a representative example of Fi group meniscal fibrocartilage at time-zero.

Despite normal looking histology these cells have absolutely **no viability or metabolic activity** as manifested by lack of incorporation of $^{35}$SO$_4$. Figures A and B are the same field in different planes of focus to demonstrate the cellular and photographic emulsion layers. Granules seen in figure 40B represent background only as determined by blank controls.
Figure 40. Histology and autoradiograph of Frozen Irradiated meniscus at zero-time. Figs. A and B display same field with different focal planes to display granules. No metabolic activity is present. x250
TRANSPLANT GROUPS

Fresh Allografts

Healed fresh allograft menisci displayed normal histologic appearance of cells and normal metabolic activity at all times of procurement. In relation to zero-time fresh menisci, cellularity and metabolic activity qualitatively appears as great.

A fresh allograft procured at 11.5 months post-transplant is shown in figure 41. The graft is very cellular, with individual cells showing extremely dense granules overlying the individual fibrochondrocytes.

Figure 41. Autoradiograph of fresh (Fs) meniscal allograft at 11.5 months post-transplantation. Cellularity is normal with heavy granule activity indicating metabolic activity. x250
An allograft at 15 months is shown below (figure 42). The cellularity is somewhat less dense than the previous example, due to different sectioning of the meniscus. The cells are metabolically very active as manifested by the density of granules above them.

Figure 42. Autoradiograph of Fresh (Fs) meniscal allograft at 15 months post-transplant. Normal cellularity with metabolically active cells is seen x250

Frozen Allografts

In contradistinction to zero-time frozen (Fr) allografts which displayed no viability or metabolic activity, all (Fr) allografts procured post-
transplantation (9.75-13 months) revealed viable, metabolically active cells which incorporate $^{35}$SO$_4$ (figures 43-44). The allografts contained no viable cells after the freezing process, therefore these newly synthesizing cells must have repopulated the graft from host tissue.

The cellularity of the allograft tissues varied from normal to slightly less cellular than fresh (Fs) or that of control tissue. Grossly the individual cells appear to be slightly less metabolically active as manifested by the less dense granule layer overlying the cells.

![Figure 43](image)

**Figure 43.** Autoradiograph of a Frozen (Fr) allograft at 10.5 months post-transplant. In contrast to zero-time, tissue is now viable with metabolically active cells incorporating radiolabelled sulfate. x2.50
Frozen Irradiated Allografts

At zero-time the frozen-irradiated allografts were rendered non-viable by the process of freezing and sterilization. No metabolic activity was displayed. In all the allografts procured post-transplant (6.5-12.75 months) new activity was demonstrated by active $^{35}$SO$_4$ uptake into the cell (figures 45-46). Thus cells have repopulated the graft, most certainly arising from the host tissue.
Figure 45. Autoradiograph of Frozen Irradiated (Fi) allograft at 8.5 months post-transplantation. Graft is repopulated with host tissue cells and is metabolically active. x250

Figure 46. Autoradiograph of Frozen Irradiated (Fi) allograft at 9 months post transplantation. Note presence of metabolically active cells. Repopulation of allograft has occurred from host tissue. x250
ULTRASTRUCTURE OF MENISCAL TISSUE

As the histology immediately post-treatment (freezing or irradiation) is generally not significantly different amongst groups, we thought that there may be changes at a more molecular or cellular organelle level. To explore this idea the ultrastructure of the cell was examined.

In collaboration with Dr. I. Farine (Tel Aviv, Israel. Deceased) examined the effects of freezing and irradiation on the cellular ultrastructure of bovine meniscal tissue using transmission electron microscopy. (For a review of previously described ultrastructure see review of literature pg. 18).

The following illustrations display the ultrastructure of normal fresh control bovine meniscal tissue, as well as treated tissue. All illustrations are original works that were prepared by Dr. Farine.

Fresh (Fs) Meniscal Tissue

Figure 47 displays the surface (s) of the meniscus showing the electron dense (d) layer, and fibroblast cells (f). There is no pericellular matrix present, i.e. no "halo" as seen around chondrocytes. Collagen fibres can be seen running in different orientations.

At higher magnification, the spindle-shaped fibroblast displays rough endoplasmic reticulum, glycogen (Glyc), and lipid material (LI) (figure 48).
Figure 47. Electron micrograph of Fresh (Fs) meniscal tissue. Surface (s) of meniscus showing dense (d) material, and fibroblast cells (f) are shown. Collagen fibres are seen in different orientations. x 10,000

Figure 48. Electron micrograph of Fresh (Fs) meniscal tissue. Fibroblast (f) is seen with lipid droplet (li), glycogen (Glyc), and rough endoplasmic reticulum. S = surface. x 20,000
A typical chondrocyte with its round nucleus and pericellular matrix or "halo" is depicted in figure 49. Note the processes or villi (v) that emanate from the surface of the cell; these are specific features that define this cell as a chondrocyte.

![Figure 49](image)

**Figure 49.** Electron micrograph of Fresh (F) meniscal tissue chondrocyte. Note characteristic pericellular "halo" (matrix) and cellular processes (v) coming off surface of cell. x 6,000

This same chondrocyte shown at higher power displays all the organelles quite clearly (figure 50). Mitochondria (mi) are easily seen as well as the Golgi apparatus (Go), and rough endoplasmic reticulum (er). Glycogen is seen near the nucleus. The "halo" is better visualized as a loose matrix consisting of fine collagen fibres.
Figure 50. EM of Fresh (Fs) meniscal tissue chondrocyte displaying mitochondria (mi), the Golgi apparatus (Go), rough endoplasmic reticulum (er), glycogen stores (Gly), and the halo (H) consisting of fine collagen fibrils. x 6000

Figure 51. Electron micrograph of fresh (Fs) meniscal tissue displaying collagen fibres in longitudinal section. Regular periods of 600 Å are seen. x 40,000
Collagen fibres in fresh tissue have a regular, uniform appearance when seen longitudinally. The width of the period in this specimen is 600 Å (figure 51). When cut in cross section, collagen fibrils are seen to vary in size between 300 - 1500 Å in diameter as demonstrated in figure 52.

![Figure 52](image)

**Figure 52** Electron micrograph of fresh (Fs) meniscal tissue displaying normal collagen cut in cross-section. Individual collagen fibrils vary in diameter from 300-1500 Å. Elastic fibrils are seen in upper field. x75,000

This finding has been reported in human menisci (Ghadially et al, 1983) and in rabbit menisci (Ghadially et al, 1978). Silva (1969, 1970) noted a similar situation in discs and menisci, that is, different size collagen fibrils were mingled together to form fibres and lamellae. He pointed out that such an arrangement renders these structures efficient broad-banded shock
absorbers, absorbing energy over a wide range of vibration frequencies.

**Frozen Meniscal Tissue**

Meniscal tissue frozen at -70°C for 2 weeks is shown in both superficial (figure 53) and deep layers (figure 54). The surface of the meniscus shows an absence of the electron dense layer, and the cells appear degenerated, swollen and denser than normal. Collagen fibres appear unchanged.

![Figure 53 EM of frozen (-70°C) meniscal tissue. Surface shows absence of dense material. Fibroblast cells (f) are denser, show swelling and evidence of degeneration. Fine collagen fibres are seen. x 4,000.](image-url)
The deeper section shows pyknosis of the nucleus and vacuolization of the cell secondary to freezing.

**Figure 54.** Electron micrograph of frozen (Fr) (-70°C) meniscal tissue deep layer. Nucleus is pyknotic with vacuolization of cytoplasm and degeneration of cell. Collagen fibres are in normal array. x 10,000.

The collagen fibres do not manifest any significant deleterious effects of freezing. They remain in regular uniform periods of 550 Å (figure 55). In cross-section they are similarly unchanged, displaying heterogeneity of fibril diameter (figure 56).
Figure 55. Electron micrograph of frozen (at -70°C) meniscal tissue. Collagen fibrils maintain their orderly array and uniformity of periods (550 Å). x 60,000

Figure 56. Electron micrograph of frozen (at -70°C) meniscal tissue. Collagen fibrils are cut in cross section, displaying normal features and heterogeneity of fibril diameter (750 - 1800 Å). x 62,000
**Irradiated Meniscal Tissue**

Fresh tissue (without freezing) was subjected to 2.5 mrad of gamma irradiation to determine the effects of irradiation alone on the cell ultrastructure. The chondrocyte shown (figure 57) retains its general appearance, but has many signs of degeneration: *Cytoplasm* is large and contains many vacuoles (Va). The mitochondria (m) are swollen, while the endoplasmic reticulum is mostly preserved with ribosomes on its surface. Vesicles (Ve) are seen released into the matrix. *Nucleus* is pyknotic (dense, irregular in shape). *Pericellular matrix* - the "halo" is sparse, with few collagen fibres.

*Figure 57. EM of irradiated (2.5 mrad) fresh meniscal tissue. Cell retains form however, cytoplasm contains large vacuoles (Va), swollen mitochondria and has released vesicles (Ve). Endoplasmic reticulum (e.r.) is preserved.*
Collagen fibrils appear unchanged by radiation alone as figures 58-59 depict.

**Figure 58.** Electron micrograph of Gamma irradiated (2.5 mrads) fresh meniscal tissue. Collagen fibrils are in regular uniform periods of 600 Å. x 40,000

**Figure 59.** Electron micrograph of Gamma irradiated (2.5 mrads) fresh meniscal tissue. Collagen fibres seen in cross-section appear normal and display diameters from 330 - 1600 Å as does non radiated tissue. x 60,000
Frozen and Irradiated Meniscal tissue

Meniscal tissue was frozen at -70°C and irradiated with 2.5 mrad of Gamma irradiation. While the matrix of the tissue appears normal, the cells show degenerative changes similar to either freezing or irradiation alone (figure 60). At the surface of the meniscus there is a loss of the electron-dense material, and fine collagen fibres are seen only. The cells are swollen with dense nuclei.

**Figure 60.** Electron micrograph of frozen (-70°C) and irradiated (2.5 mrad) meniscal tissue. There is absence of the electron dense surface layer. The Fibroblast (f) is swollen, and its nucleus is pyknotic. x 13,000
In deeper zones the pericellular matrix becomes scant, and the nucleus is irregular and dense (figure 61).

**Figure 61.** Electron micrograph of frozen (-70°C) and irradiated (2.5 mrad) meniscus. The pericellular matrix (h) is sparse and the nucleus is irregular and dense (pyknotic). The collagen fibres are intact. x 20,000.

Collagen fibres remain intact and uniform after freezing and irradiating. The fibrils maintain a regular periodicity when seen in longitudinal section. In cross section they exhibit a similar heterogeneity of fibre diameters as the other groups. Overall they display no differences when compared with fresh, frozen, or irradiated tissue (figures 62-63).
Figure 62. Electron micrograph of frozen (-70°C) and irradiated (2.5 mrad) meniscal tissue. Collagen fibrils seen in longitudinal section display uniformity with regular periods of 550 Å. x 40,000

Figure 63. Electron Micrograph of frozen (-70°C) and irradiated (2.5 mrad) meniscal tissue. Collagen fibrils seen in cross-section displaying normal array. Diameters range from 370-1600 Å. x 60,000
MECHANICAL TESTING RESULTS

The parameters measured are defined as follows.

**Time constant (τ):** The time required for a material to achieve a specified percentage of its total deformation. e.g. $\tau_{63\%}$ is the time required to achieve 63% of total deformation.

**Viscoelastic deformation ($\epsilon_{VR}$):** the ratio of the viscoelastic displacement to the initial thickness of the material (beneath the plunger).

**Corrected modulus of elasticity ($E_m$):** The relationship between the force exerted per area on a surface, to the instantaneous elastic deformation. This has been corrected for the influence of the steel base which supports the meniscus during the testing.

ZERO TIME

Indentation testing of zero time specimens compared 8 fresh, 6 frozen, and 6 frozen irradiated menisci. No significant differences were observed for the time constants, viscoelastic behaviour, and total deformation among the three groups. A tendency to a slight lowering of the time constant was seen in
the irradiated group, but this was not significant.

**Table IV. Zero Time Specimens**

<table>
<thead>
<tr>
<th>Groups</th>
<th>$\tau_{20%}$ (sec)</th>
<th>$\tau_{37%}$ (sec)</th>
<th>$\tau_{50%}$ (sec)</th>
<th>$\tau_{61%}$ (sec)</th>
<th>$F_{ve}$ %</th>
<th>$d_{tot}$ (mm x 10$^3$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh</td>
<td>10 ± 4</td>
<td>26 ± 8</td>
<td>44 ± 11</td>
<td>75 ± 23</td>
<td>72 ± 7</td>
<td>30 ± 7</td>
</tr>
<tr>
<td>Frozen</td>
<td>9 ± 4</td>
<td>24 ± 9</td>
<td>40 ± 18</td>
<td>72 ± 45</td>
<td>67 ± 5</td>
<td>24 ± 8</td>
</tr>
<tr>
<td>Frozen Irrad.</td>
<td>6 ± 2</td>
<td>15 ± 6</td>
<td>27 ± 10</td>
<td>57 ± 62</td>
<td>65 ± 5</td>
<td>23 ± 7</td>
</tr>
<tr>
<td>Difference</td>
<td>N.S.</td>
<td>N.S.</td>
<td>N.S.</td>
<td>N.S.</td>
<td>N.S.</td>
<td>N.S.</td>
</tr>
</tbody>
</table>

Traction testing was performed on the zero time specimens only (n = 5 per group). There were no significant differences in the rigidity of the different groups.

**Table V. Rigidity of Menisci**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Rigidity (k) (N/mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh</td>
<td>97 ± 13</td>
</tr>
<tr>
<td>Frozen(Fr)</td>
<td>93 ± 11</td>
</tr>
<tr>
<td>Frozen Irrad(Fi)</td>
<td>101 ± 14</td>
</tr>
<tr>
<td>Difference</td>
<td>N.S.</td>
</tr>
</tbody>
</table>
TRANSPORT GROUPS

Indentation testing compared the transplant specimen with the contralateral control meniscus. The number of specimens per group was limited by technical reasons (in some instances the geometry was altered by small changes in shape rendering it impossible to duplicate the orientation of the testing device to allow fair comparison).

The Fresh group displayed no statistically significant differences in modulus of elasticity, viscoelastic deformation, and time constant. A tendency to lowering of the time constant is seen in the fresh group (i.e., the time to achieve a given % of total deformation is lessened, and therefore the tissue is more "deformable").

Table VI. Indentation Parameters (Fs) Group

<table>
<thead>
<tr>
<th></th>
<th>( n = 5 )</th>
<th>( E_h ) (Mpa)</th>
<th>( \tau ) (secs)</th>
<th>( \epsilon_{ve} ) %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh(Fs)</td>
<td></td>
<td>3.84 ± 1.88</td>
<td>24 ± 19</td>
<td>35 ± 12</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>3.83 ± 1.36</td>
<td>47 ± 24</td>
<td>29 ± 5</td>
</tr>
<tr>
<td>Difference</td>
<td></td>
<td>N.S.</td>
<td>N.S.</td>
<td>N.S.</td>
</tr>
</tbody>
</table>
The Frozen group displayed a significant difference in the lowering of the time constant. The modulus of elasticity, and the viscoelastic deformation were the same (i.e., the specimens are more "deformable" than controls).

Table VII. Indentation Parameters (Fr) Group

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Frozen (Fr)</th>
<th>Control</th>
<th>Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>$E_H$ (MPa)</td>
<td>2.93 ± 0.78</td>
<td>4.03 ± 0.45</td>
<td>N.S.</td>
</tr>
<tr>
<td>$\tau^*$ (secs)</td>
<td>41 ± 26</td>
<td>67 ± 22</td>
<td>Signif$^*$</td>
</tr>
<tr>
<td>$\varepsilon_{VP}$ (%)</td>
<td>36 ± 10</td>
<td>28 ± 8</td>
<td>N.S.</td>
</tr>
</tbody>
</table>

* $n=6$
$^p < 0.05$

The Frozen irradiated menisci displayed no significant differences for all three parameters calculated, although there was a tendency to a non-significant increase in the time constant (i.e., they are less "deformable" than controls).
Table VIII. Indentation Parameters (Fi) Group

<table>
<thead>
<tr>
<th></th>
<th>$E_h$ (MPa)</th>
<th>$\tau$ (secs)</th>
<th>$\epsilon_{VF}$ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frozen Irrad. (Fi)</td>
<td>3.98 ± 1.02</td>
<td>59 ± 37</td>
<td>17 ± 8</td>
</tr>
<tr>
<td>Control</td>
<td>3.95 ± 0.72</td>
<td>41 ± 16</td>
<td>24 ± 11</td>
</tr>
<tr>
<td>Difference</td>
<td>N.S.</td>
<td>N.S.</td>
<td>N.S.</td>
</tr>
</tbody>
</table>
SUMMARY OF RESULTS

Histologic and Radioautographic Findings

From the histologic, both light and electron microscopic, and radioautographic examinations performed several findings are important.

1. Although cells and tissues appear histologically normal at time zero after either freezing, or freezing and irradiation, these cells are not metabolically active and not viable.

2. Ultrastructural examination under EM reveals that cells and their organelles are damaged leading to cessation of metabolic activity and cell death.

3. The tissue matrix, i.e. the collagen structures are not significantly affected by either freezing, irradiation or a combination of both.

4. At long-term follow-up, cells in all transplant groups are viable and metabolically active, synthesizing proteoglycan. The frozen and frozen-irradiated menisci had non-viable cells at time zero. Therefore repopulation of these grafts from host tissue must be occurring.
5. Gamma irradiation in the dose of 2.5 Mrads did not prevent revascularization of the meniscus and subsequent repopulation, viability, and function.

6. No significant immunologic reactions to the transplanted tissues were seen.

7. Arthritic type degenerative changes were the least in the frozen (Fr) transplant group. Among the other treatment groups including the meniscectomy (Ms) group there were no significant differences.

**Mechanical Testing Results**

1. At zero time there were no significant differences in the mechanical properties of each group secondary to freezing and freezing with irradiation. A non-significant lowering of the time constant was seen.

2. Post-transplantation there were no significant differences in modulus of elasticity, viscoelastic deformation, and time constant for all transplanted allografts except for the frozen (Fr) allografts which displayed a significant lowering of the time constant (they were more "deformable" than controls).
3. The Fs group menisci displayed a non-significant decrease in the time constant, and increase in visco-elasticity.

4. The Fi group menisci displayed a non-significant increase in the time constant and a decrease in visco-elasticity.

5. Overall modulus of elasticity appears to be unchanged among all allograft groups.
DISCUSSION

Once thought to be a vestigial structure, it is now unrefuted that the meniscus is important in knee joint mechanics. The most important of its functions may be its role in load bearing. When injured, few options were available to the surgeon in the past and meniscectomy would be performed. Meniscectomy was not as benign a procedure as originally thought, for it was the basis for the degenerative arthritic changes which developed over time (Cox 1975; DiStefano 1980; Fairbank 1948; Kettlekamp 1972). The mechanism behind these changes is the greatly increased stress concentration on the articular cartilages of the femur and tibia which arise post-meniscectomy (Ahmed 1983; Radin 1984; Seedhom 1979; Walker 1975). This results in the "Post-Meniscectomy Syndrome".

As a result of this knowledge a more conservative approach to meniscal injuries came about. Surgical treatment attempted to repair the tears, or at most only partially excise the meniscus, as even a narrow but intact rim can still function to bear a significant portion of the load (Seedhom 1979).

Despite this, surgeons are still often faced with young patients with severely damaged and irreparable menisci, or those who have undergone previous
menisectomy. A form of replacement of the meniscus was the next logical step.

As synthetic replacement of the meniscus has on the whole been unsuccessful (Kenny 1983; Toyonaga 1983), interest in the possibilities of meniscal transplantation have been stimulated. This is in part incited by the successful utilization of connective tissue allografts, such as bone (Zukor 1989; Brown 1982,) tendon (Arnoczky 1986; Shino 1984; 1988), and fascia (Bright 1981) both clinically and experimentally.

The ability to store and bank connective tissue allografts is advantageous. The widespread use of connective tissue allografts in musculo-skeletal surgery has led to much examination of storage and preservation techniques for these tissues. Deep-freezing or freeze-drying (lyophilization) are mainly employed (Arnoczky 1986; Barad 1982; Bright 1983; Brown 1982; Curtis 1985; Shino 1984; Tomford 1982-84).

The cellular components of these tissues are destroyed by the majority of these preservation techniques. However, it is clear that in tissues such as tendon and bone viable cells at the time of transplantation are not necessary for graft success.
Reports have stated that articular cartilage requires viable cells to maintain their mechanical function (Black 1979; Tomford 1983), and this has been the basis for focusing on preservation techniques which maintain cell viability in cartilaginous tissue including the meniscus. Cryopreservation with cytoprotective agents (e.g. dimethyl sulfoxide) and controlled rate freezing techniques have shown that some viability can be maintained, but the population of cells decreases with time.

The fibrocartilaginous meniscus is quite different than articular cartilage. To this end it may in fact behave like other more fibrous connective tissue allografts (tendon, fascia) in terms of not requiring viable cells at time of transplantation to survive and function mechanically. The graft may undergo repopulation of cells from host tissue as seen in these other tissues.

We wanted to examine meniscal transplantation using frozen allografts which would allow long term storage. An important issue is secondary sterilization of these tissues to decrease the possibility of disease transmission. We chose gamma irradiation which has been shown to destroy most infectious agents in the doses currently used for bone and other allografts as recommended by the American Association of Tissue banks.

Additionally, freezing and irradiation are known to reduce tissue antigenicity
thereby advantageously minimizing the possibility of transplant rejection.

The rabbit model for allotransplantation of the medial meniscus was developed for several reasons. The rabbit knee has been considered to be analogous to the human knee by several authors (Shaw and Martin, 1962) both functionally and anatomically. A subtle difference in functional use is that this animal spends most of its time with its hind legs in flexion. The rabbit knee has been utilized in studies on knee joint mechanics for its similarities to the human knee. It is also a good model for degenerative arthritis, as even a simple arthrotomy may lead to arthritic changes (Floman 1980; Shapiro 1979; Moskowitz 1973).

The sensitivity of the knee to the development of arthritic changes lends itself well to the evaluation of various allograft techniques, since the essence of transplantation is to prevent the "Post-Menisectomy Syndrome".

In terms of size the rabbit is small, and therefore is relatively easy to house and maintain in large numbers. This factor allows for study of a greater series of specimens.

However, the small size makes the transplantation procedure technically more difficult, and this may contribute to some of the degenerative changes
noted which were seen to be not significantly less than the meniscectomized knee. Transplantation includes first a meniscectomy, then an implantation of the allograft. Clearly this is more traumatic than simple meniscectomy alone. The joint is exposed for a longer duration, and articular cartilage has been shown to be extremely sensitive to drying in open air, even 30 minutes of exposure without irrigation can cause necrosis of the articular cartilage (Mitchell and Shepard, 1989).

Due to its small size actual fixation of the anterior and posterior horns of the meniscus to the ligamentous remnants in the recipient was unable to be performed. The relevance of this is understood by recalling the mechanism of load transmission as discussed previously. Force is transmitted axially and is translated into a circumferential stress (parallel with the longitudinal fibres) or a hoop stress as the meniscus is forced out radially. However this requires a constrained meniscus that is firmly secured at the horns to the bony surfaces. At least initially there is no such constraint in the allografts, allowing a "redundant" meniscus analogous to the example shown in figure 5 where subsequently no load is transmitted by the meniscus. At sacrifice post-transplantation however, there were many examples where the meniscal allograft was completely healed to the ligamentous attachments of the recipient knee, and thus could functionally load bear (see fig. 15). Those that did not display this healing are in part explained by size mismatch, where too
small a graft was implanted, thus the horns are never quite in contact with the ligament insertion sites. The time required for this healing to the ligaments is unknown, but is likely to require several months. The significance of this is that the knee prior to full incorporation of the allograft behaves like a knee with a redundant or severely torn meniscus that does not bear load. This results in increased stress concentration. This is probably the major factor explaining why the allografts knees are not significantly superior over the meniscectomy group in terms of degenerative changes.

We did not immobilize the limbs post-operatively as one would after surgery in humans, as the rabbit will chew through almost any material. Therefore adequate time for healing before load bearing does not occur. This fact may further contribute to the degenerative changes noted.

A factor which is important in the proper function of the meniscus and subsequent prevention of arthritic changes is size matching of meniscus to curvature and dimensions of the condyles. Subtle differences did occur among the rabbits, more marked in some cases. The only control we had over this was to order and utilize animals of the same approximate weight (all approx. 3.5 kg.). Animals while being housed either pre or post-operatively grew at different rates. Though often not evident grossly, this must have resulted in a degree of size mismatch.
The small size factor limited our ability to perform traction testing to zero-time fully intact specimens only, as they were procured with the ligamentous attachments and accompanying bone blocks. A larger model such as the dog or goat would allow for such a procurement where the allograft bone blocks could be rigidly fixed with screw fixation.

**Macroscopic Results**

All transplants performed showed excellent healing of the graft to host synovial sites. This indicates that allogeneic meniscal tissue is well incorporated into the recipient.

We did not observe any evidence of graft rejection or destruction (of grafts and joints) in all groups including the fresh transplant groups which theoretically are more "antigenic" than either frozen or frozen irradiated menisci. Cartilage is known to be "immunologically privileged".

Inbred rabbits were not utilized in this study. However in obtaining rabbits from the same breeder they are likely to have some degree of inbreeding and thus genetic similarity. This would act to diminish transplant rejection.
Degenerative Changes (Articular Cartilage)

The pilot study previously performed by Zukor (1988) displayed very little degenerative change in the transplant groups. The fact that degenerative changes were seen in all allograft groups post-transplantation in this study is probably related to a more thorough examination of the specimens (in the pilot study only photographs were examined). An improvement in technique in this study was to fully remove the meniscus and examine the underlying cartilage. This better displays subtle changes in the articular cartilage both in exposed and covered regions, as well as manifesting smaller osteophytic changes which arise peripherally. In addition, the follow-up period was much longer (6.5-17 months versus 1-9 months in pilot study) allowing for the development of these changes to occur.

Degenerative changes were found to be statistically significantly different when all allograft groups including the meniscectomy group are compared. Statistical evaluation demonstrated the frozen group to be superior to other allograft groups, though not significantly better than the meniscectomy group. Histologic evaluation does not explain this, as all allograft groups were not statistically significantly different for all parameters evaluated. Perhaps the mechanical testing results shed light on this.
If one looks at the mechanical testing results, the only statistically significant difference noted was a lowering of the time constant for the frozen allografts. This lowering translates into a more deformable tissue. This increased deformability of the tissue may dampen compressive loads more than the fresh allograft group. Radiation changes at the molecular level of proteoglycan or collagen chains may be a cause of a more "brittle" allograft and thus result in more degenerative changes in the irradiated group.

**Meniscectomy Group**

A phenomenon fairly unique to the rabbit is that of meniscal regeneration. This is not known to occur in humans routinely. In 1944 Smillie did describe several patients who after total meniscectomy did regenerate a form of a meniscus. They were thinner, flat shaped and composed of fibrous tissue only. Isolated instances of regeneration report frail structures with haphazard collagen orientation, not circumferential (Sisk 1987).

In this study 3 out of 9 knees developed almost normal looking regenerated menisci. In other instances only thin rims of tissue were noted. Importantly this attempt always occurred from the periphery inwards, and was always rim-like in nature, suggesting it came from the synovium. This has been proven by previous work where synovectomy with meniscectomy prevented this
regeneration from occurring (Kim 1979). The development of this meniscus may minimize some of the degenerative changes that would otherwise occur, as previous mechanical load bearing studies have demonstrated that in the presence of an intact rim (i.e. a bucket handle tear) significant fractions of the load are still transmitted by the meniscus (Seedhom 1979; Radin 1984).

Histologic and Radioautographic Findings

Treatment of menisci with freezing or freezing and irradiation produces only minor differences in the microscopic appearance of the tissues at zero-time. Some cells show pyknosis, and occasional empty lacunae are seen which denote cell death. Notwithstanding this, it is difficult to appreciate the actual changes that have occurred without viability studies, as the histology alone may be deceptive and lead to false assumptions of viability.

We chose to use radiolabelled $^{35}$SO$_4$ for radioautography as the sulfate moiety is taken up by chondrocytes and incorporated in glycosaminoglycan (GAG) synthesis. These GAGs are utilized in proteoglycan production. Only chondrocytes are capable of proteoglycan production. Metabolic activity if seen indicates that the cells are chondrocytes of the meniscus. Thus uptake of $^{35}$SO$_4$ indicates not only cell viability, but that the cells are metabolically active and capable of proteoglycan production.
Interpretation was made qualitatively only, as true inferences about the number of metabolically active cells, or the degree of metabolic activity cannot be made. The sections of the menisci examined were not rigidly controlled for as this was technically difficult. Therefore actual cell variation may be a reflection of normal differences in cellularity in different regions of the meniscus.

Many radioautographic assays were done in this study, and there were many parameters difficult to control (isotope half-life, photographic emulsion background can be controlled for with isotope decay charts and control background slides for different batches of emulsion). To make comments on actual "metabolic activity" of each cell, one would need to assay for GAG synthesis via GAG extraction and scintillation counting. However this has been shown not to correlate with actual viable cell number, as percentage level of biosynthetic activity was not nearly the same as the percentage of incorporating cells (Arnoczky 1986).

The radioautography performed shows unequivocally that at zero-time all treated allograft specimens are totally devoid of any viable and metabolically active cells. Therefore these allografts were transplanted with non-viable cells.
While zero-time studies displayed no viability whatsoever, both frozen and frozen-irradiated healed allografts displayed metabolically active cells post-transplantation. The fresh healed allograft manifests strong metabolic activity as well.

This new metabolic activity can only be explained by ingress of new cells from the host (Arnoczky 1986; Bright 1983; Campbell 1963; Nicolaou 1988). The most likely source in this model is the host synovium, as we have seen that the synovium is responsible for meniscal regeneration in the rabbit (Kim 1979).

In observing the frozen and frozen-irradiated groups, grossly they appear to be less cellular early on in the follow-up. Then later on the transplants "globally" seemed to show an increase in the number of cells seen in a given field (this in mind that true comparisons are not controlled for). The histology post-transplant examined in a blinded fashion, displayed no significant differences for cell necrosis, synovial hypertrophy, and inflammation.

An important but expected finding was the lack of transplant rejection observed. Lymphoid cells are indicative of transplant rejection. Indeed, lymphoid cells were virtually non-existent in the allograft specimens upon
Histologic evaluation. Cartilage has long been known to be an "immunologically privileged" tissue and the implantation of fresh cartilage allografts has not been associated with immunologic reaction (Aston 1986; Campbell 1963; Tomford 1982). Cartilage is known to be only weakly antigenic, the most active immunologic component being the chondrocyte. These cells are effectively sequestered in the proteoglycan matrix and are therefore insulated from immune cells and immunoglobulins, which are too large to diffuse through. Additionally, cartilage (and the meniscus) is relatively non-vascular, therefore effector cells cannot permeate the tissue well when intact. In theory only gradual release of antigenic material occurs in non-vascularized allografts, which may allow tolerance to develop in the host toward the graft.

EM Studies

The electron microscopic work on bovine meniscal tissue by Dr. Farine examines in more detail the possible effects of freezing and irradiation on the ultrastructure of the tissue.

These studies very clearly show the marked changes that occur in the cells due to freezing alone, irradiation alone, or both in combination. Cells show pyknosis of nuclei, swelling and vacuolization of the cytoplasm, and
mitochondrial swelling. Despite these changes at the cellular level, there were no detectable changes in the collagen fibre array (alignment), or the size of the fibrils in all treated tissue when compared to control.

Therefore freezing and irradiation cause cellular changes leading to cell death without affecting the tissue matrix. This further strengthens the results seen in the mechanical testing.

**Mechanical Testing**

No studies in the literature have previously described the effects of irradiation on meniscal fibrocartilage prior to the conception of this study. In addition most previous methods of biomechanical testing of menisci examined sections from the meniscus only. Utilized here is a new method (developed in collaboration with the Dept. of Biomedical Engineering at Ecole Polytechnique, Montreal. Duval 1989) which closely simulates the normal physiology of the intact meniscus.

Zero-time studies displayed no significant differences in time constants, viscoelastic behaviour, and total deformation for 22 specimens examined. A non-significant lowering of the time constant was seen for the frozen irradiated group. Traction testing revealed no significant differences in
rigidity for the 15 specimens evaluated.

Biomechanical studies performed on the healed allograft specimens post-transplantation manifested no significant differences for modulus of elasticity, and viscoelastic deformation. The frozen allograft group displayed a significant lowering of the time constant, which translates to a more "deformable graft" than its control. Though not significant, the frozen irradiated menisci displayed a tendency to an increase in the time constant, or, that these menisci are less "deformable" than controls. This may be explainable in part by the known effects of irradiation on polymer chains, i.e. the increased cross-linking that occurs after side chain breakage. This may lead to a "stiffer" but more brittle tissue (English 1986).

The number of grafts studied were limited by technical reasons. The testing devices required very exact and reproducible specimen shapes to properly compare the grafts. The shape of some of the grafts were somewhat altered making this difficult. Increased numbers of specimens would improve the validity of the statistics.

While our mechanical testing was somewhat limited by technical reasons specific to the rabbit model, the methodology developed would appear to be
valid and valuable for future studies including those with a larger animal model.

Summary

The fate of non-viable frozen and frozen-irradiated meniscal allografts is such that they repopulate with host cells, heal and continue to function. We can postulate that from these findings, meniscal allografts need not be viable at the time of transplantation to heal, survive and function. They act as a scaffold into which new cells can migrate. Perhaps the host synovium serves as the source of cells. Presumably these are pluripotent mesenchymal cells which can then differentiate and become chondrocytes.

Efforts at cryopreservation of this tissue are probably not necessary, as viable cells are not critical to the success of allotransplanted menisci.

The inferences from this study suggest that meniscal transplantation in humans can be refined. The ability to store the tissues by simple freezing allows one to perform the procedure in an elective fashion. Sterilization with gamma irradiation minimizes the concern of disease transmission while not significantly affecting the biomechanical properties of the tissue. Classification of a bank of meniscal sizes would ensure proper size matching.
Practically, meniscal transplantation has already been performed in limited numbers in humans. It is likely that with further refinements and experience this procedure will represent the alternative to meniscectomy, and prevent the Post-Meniscectomy syndrome.

Directions for future work are in progress to label host cells and show tissue of origin and route of migration of repopulating cells.

CONCLUSIONS

I. There is no difference in the healing of fresh, frozen, or frozen irradiated meniscal allografts in rabbits.

II. The ultimate healing, incorporation and function of meniscal transplants in rabbits does not require the presence of viable cells at the time of implantation.

III. Repopulation of these grafts occurs with host cells which are
metabolically active, synthesizing proteoglycan.

IV. Freezing with or without irradiation does not appear to have a significant effect on the biomechanical properties of these allografts at zero-time.

V. Freezing with or without irradiation does not appear to have a significant effect on the biomechanical properties of these allografts at long term follow-up, except for a decrease in the time constant for frozen allografts.

VI. In comparing the effects of transplantation versus meniscectomy (in terms of preventing joint deterioration, significant differences could not be shown as the rabbit model limits the ability to firmly fixate the meniscus at time-zero.

VII. The rabbit model for medial meniscal transplantation is useful, but has certain technical limitations.
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