IRRADIATED MENISCAL ALLOTRANSPLANTS OF RABBITS : STUDY OF THE MECHANICAL PROPERTIES AT SIX MONTHS POSTOPERATION

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In this study, the long-term effects of gamma irradiation were investigated on the indentation properties of rabbits' meniscal allotransplants. Three groups, i.e. fresh, frozen and frozen irradiated allotransplants, were studied at 6 months postoperatively. Contralateral menisci served as controls in each group. Treatment effects were assessed using three mechanical parameters, i.e. compliance to long-term creep (J_R), compliance to rapid load (J_μ) and viscoelastic characteristic time (τ_i). Comparison of the three groups showed that compliance to long-term creep of the frozen irradiated menisci was significantly reduced. This suggests that at 6 months postoperatively, the frozen irradiated allotransplants were less viscous compared to fresh or frozen transplants.

Keywords : allotransplant ; gamma irradiation ; meniscus ; mechanical properties ; biomechanics.
Mots-clés : allogreffe ; irradiation gamma ; ménisque ; propriétés mécaniques, biomécanique.

INTRODUCTION

Following meniscectomy, deleterious alterations in the stress distribution of the knee occur, frequently leading to degenerative arthritis or the so-called "post-meniscectomy syndrome" (5, 8, 10, 13). Theoretically, replacement of the meniscus, either prosthetically or by transplantation, might alter this course and prevent the degenerative changes. Experimentally, prosthetic replacement of the meniscus has met with little success (9, 10, 17, 20). Conversely, transplantation of the meniscus in animals (3, 11), and to a lesser extent in humans (22), has been successful.

However, current fears about viral contamination in allotransplantation have necessitated sterilization using methods such as gamma irradiation. The International Atomic Energy Agency adopted 25 KGy as the standard irradiation dose for medical products (19). Data from our laboratory suggest that such low levels of irradiation produce no significant reductions in the immediate mechanical properties of frozen rabbit meniscal grafts (4). However, it is not known if the irradiated graft retains its inherent mechanical properties following transplantation. We investigated the gamma irradiation effect on the indentation properties of meniscal allotransplants after 6 months postoperatively.

MATERIALS AND METHODS

Thirty male mature New Zealand white rabbits, weighing 3.5 ± 0.1 kg., received allotransplants of the medial meniscus comprising three groups of 10 each : (i) Group I : fresh transplants ; (ii) Group II : frozen (- 70° C) transplants and (iii) Group III : frozen (- 70° C) and irradiated (25 KGy) menisci.

Each of the rabbits received a medial meniscus allograft in one of its knees. The meniscus of contralateral knee was not operated and served as a control.

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Surgical technique

Procedures were performed under general anesthesia utilizing intramuscular injection of atropine, ketamine and atrevet. A medial parapatellar incision was employed. Parallel transverse arthrotomies were performed just superior and inferior to the anterior portions of the medial meniscus. The anterior horn was visualized and divided from its bony attachment. Dissection was then continued posteriorly until the posterior horn of the meniscus was identified. It too was divided and the meniscus excised. The interval between the posteromedial capsule and the adjacent musculature was then developed. The meniscal allograft was then implanted utilizing three suture points, one posteriorly, one anteriorly and one at the mid portion. The wound was then closed in layers using absorbable material.

In the case of fresh transplants (Group I), pairs of rabbits were operated upon simultaneously and menisci exchanged between the two. In Groups II and III, banked menisci were used. These allografts were stored frozen (– 70°C) for at least one week prior to transplantation.

Following surgery, rabbits were allowed full weight bearing. At the end of the 6 month period, rabbits were killed by intravenous anesthetic overdose. The medial meniscus allografts and the controls (contralateral knee) were removed for biomechanical evaluation.

Table I. — Indentation properties of fresh meniscal allograft (Group I) [a]

Variable	Control	Fresh
J_μ ($\times 10^{-6}$ m²/N)	0.79 ± 0.27	0.78 ± 0.38
J_R ($\times 10^{-6}$ m²/N)	2.80 ± 0.98	3.10 ± 0.42
τ_i (sec)	33 ± 7.0	28 ± 3.0

[a] Data are presented as means ± SD.

Table II. — Indentation properties of frozen meniscal allograft (Group II) [a]

Variable	Control	Frozen
J_μ ($\times 10^{-6}$ m²/N)	0.74 ± 0.08	1.02 ± 0.27 [b]
J_R ($\times 10^{-6}$ m²/N)	2.50 ± 0.17	4.10 ± 0.18 [c]
τ_i (sec)	67 ± 10	41 ± 7.0 [d]

[a] Data are presented as means ± SD.
[b,c,d] $p < 0.05$ compared with control (based on paired Student's t-test).

Table III. — Indentation properties of frozen irradiated meniscal allograft (group III) [a]

Variable	Control	Frozen irradiated
J_μ ($\times 10^{-6}$ m²/N)	0.76 ± 0.13	0.78 ± 0.19 [b]
J_R ($\times 10^{-6}$ m²/N)	1.97 ± 0.17	2.14 ± 0.10 [c]
τ_i (sec)	68 ± 9.0	51 ± 10 [d]

[a] Data are presented as means ± SD.
[b,c,d] $p < 0.05$ compared with control (based on paired Student's t-test).

Biomechanical evaluation

Biomechanical testing was performed by an indentation creep test. The technique and instrumentation of indentometry have been described elsewhere (4). Principally, it is an indentor of 0.75 mm radius with a cylindrical tip (fig. 1). A 1.5 MPa test stress was applied over 0.24 sec. (rapid compliance) and then maintained for 3×10^3 sec. until creep reached an apparent maximum (compliance to long-term creep).

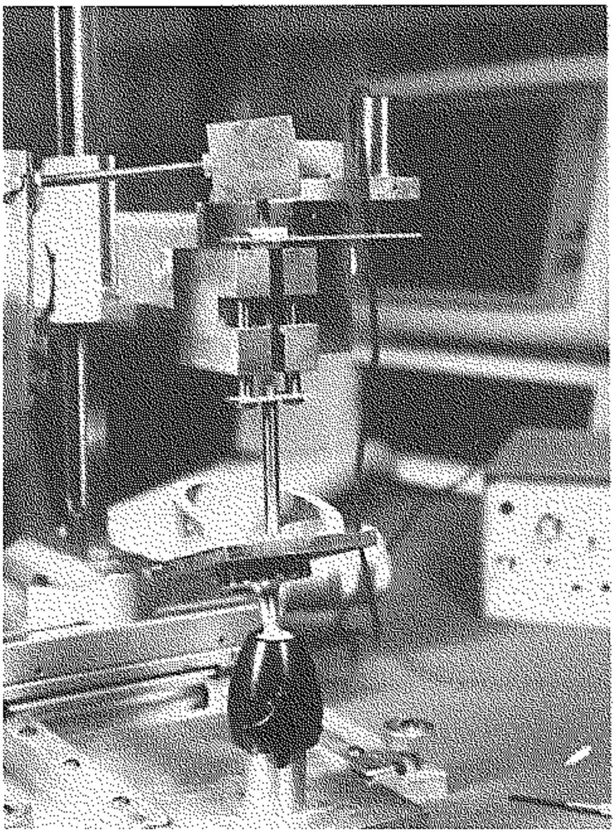

Fig. 1. — Photograph of the apparatus used for the indentation tests.

The relative radial location of each indentation test was at about 40% of the total radial width of the meniscus, measured from the external edge (fig. 2).

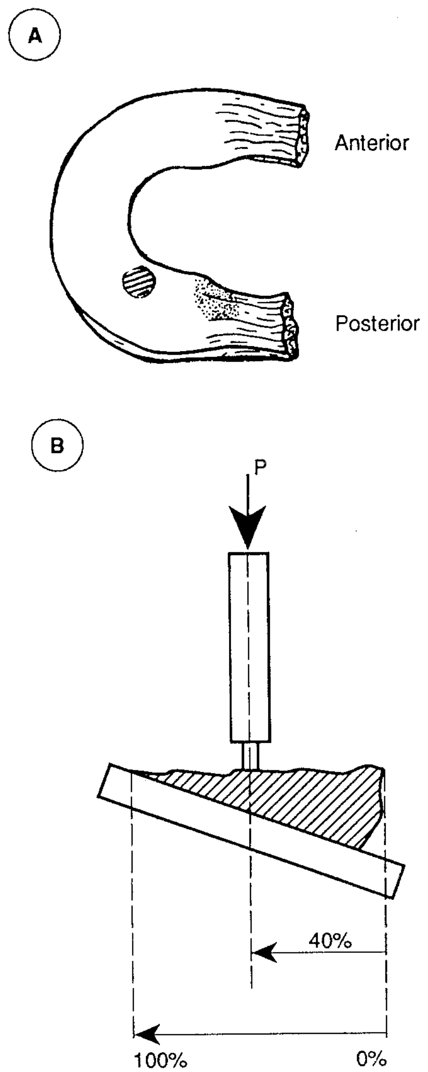

Fig. 2. — Indentation site. (a) schematic diagram of indentation location relative to meniscal geometry. (b) cross-sectional view showing the relative radial position of each indentation test.

Deformation of the meniscus was measured with an axially mounted linear variable differential transducer (LVDT). The deformation was recorded as a function of time (fig. 3). Three biomechanical parameters were measured : J_μ, the compliance of the tissue response to rapid load application (instantaneous deformation) ; J_R, the compliance calculated from the measurement of long-term total creep (equilibrium deformation) ; and τ_i, the characteristic time at which the deformation reaches 63% of the total creep.

Meniscus thickness values at the site of indentation were measured immediately after completion of indentation tests. This measurement was achieved by using a needle-shaped indentor and an LVDT to sense the moment when the indentor tip penetrates into the meniscus and when it contacts the base plate of the holder. The meniscus thickness was used in calculating the compliance values according to the formula (7) :

$$J = 4\,a\,K\,w\,/\,P\,(1 - \nu) \quad (1)$$

In this formula, creep compliance J is defined in terms of a, radius of area of indentation ; K = K (a/h, ν), a geometric scaling function, where h is the meniscus thickness and ν the Poisson's ratio ; w, deformation, and P, applied force.

Statistical analysis

Mean values and standard deviations were computed. Paired data were compared using a paired Student's t-test. Analysis of variance (ANOVA) was used to determine if the means were significantly different. Individual differences between groups were further evaluated using multiple t-tests, utilizing the Student-Newman-Keuls procedure. A confidence level of 95% was chosen to define significance.

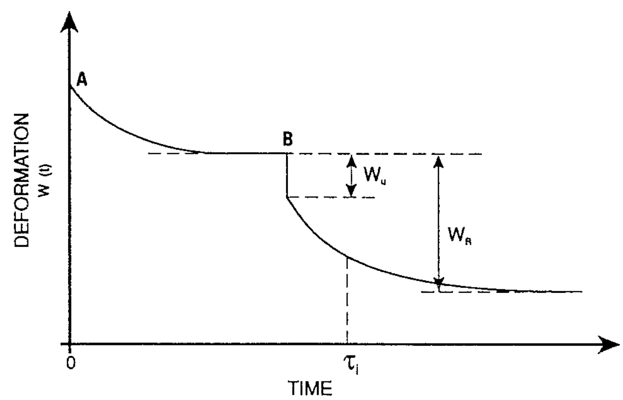

Fig. 3. — Creep indentation curve showing W_μ (instantaneous deformation) and W_R (equilibrium deformation) from which the compliances (J_μ and J_R) were calculated by using formula (1). τ is the characteristic time measured from B (point of application of one stress test).

RESULTS

By 6 months, all of the fresh transplants had almost completely healed to the peripheral attachments. In Group II, one of the frozen allograft menisci was degenerated. In Group III, two of the frozen irradiated meniscal allografts had been almost totally removed from the joint by granulation tissue.

The results of creep indentation tests after 6 months postoperatively are summarized in Tables I, II and III. There were no statistical differences in any of the three biomechanical parameters (J_{μ}, J_R and τ_i) between the fresh meniscal allotransplants and the contralateral controls. In Group II and Group III, there were, however, significant differences ($p < 0.05$) between frozen and frozen irradiated allotransplants and their fresh contralateral controls.

The compliance to rapid load (J_{μ}) in Group II was 37% more than controls and compliance to long-term creep (J_R) was 64% more than controls. In addition, the viscoelastic characteristic time (τ_i) was 38% less than controls.

In Group III, there were less increases in the compliances (J_{μ} and J_R) and less decrease in the characteristic time (τ_i) between frozen irradiated allotransplants and their contralateral controls.

Based on a two-way ANOVA, there were biomechanical differences between fresh, frozen and frozen irradiated allotransplants. Student-Newman-Keuls multiple comparison test was then used to compare the average values of the biomechanical parameters in the three groups of allotransplants. A significant decrease of the compliance to long-term creep (J_R) was found in frozen irradiated allografts ($p < 0.05$). However, there were no significant differences in both J_{μ} and τ_i between the three groups.

DISCUSSION

There have been many reports on the effects of freezing (2, 12, 21) and irradiation (6) on the mechanical properties of soft tissues such as meniscus and ligament. However, information on the influence of irradiation after implantation has not been documented.

Comparison between each group and its contralateral control showed no differences for fresh allotransplant for any of the three parameters measured. There were, however, differences between frozen and frozen irradiated and their contralateral controls. The compliance to long-term creep (J_R) and the compliance to rapid load (J_{μ}) were greater than those for contralateral controls. The viscoelastic characteristic time (τ_i) was lower than that for the control. With the assumption that meniscus has a biphasic response to creep testing like a cartilage (14). It could be argued that compliance to long-term creep (J_R) is related to the solid matrix (collagen and glycosaminoglycans), while J_{μ} and τ_i are affected primarily by the water content and permeability of the tissue.

Our findings indicate that frozen irradiated allotransplants undergo more changes than frozen allotransplants. This may be explained by the remodeling phenomenon occurring postoperatively. A change in the collagen architecture of the meniscus has been demonstrated after 6 months for both fresh and deep frozen grafts (2). On the other hand, repopulation with viable host cells occurred in frozen irradiated menisci allografts (1). Therefore, our findings suggest that the remodeling rate is faster in irradiated than in nonirradiated allotransplants.

The results of this study indicate that gamma irradiation produces significant reduction in the compliance to long-term creep (J_R). The significance of this finding is that the viscous part of the transplant (glycosaminoglycans content and their interactions with the collagen fiber network) is altered by irradiation.

One of the primary objectives of this study was to consider the long-term effects of irradiation on the rabbit meniscal allotransplants and their significance for clinical practice. Although it is difficult to extrapolate the results from rabbits to humans, it would seem reasonable to suggest that at 6 months after implantation, the frozen irradiated menisci were significantly less viscoelastic when compared to fresh or frozen transplants.

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SAMENVATTING

L. YAHIA, D. ZUKOR. Irradiatie van meniscus allotransplantaten bij het konijn : studie van de mechanische karakteristieken op de 6de postoperatieve maand.

In deze studie onderzoeken de auteurs de invloed op lange termijn van gamma-irradiatie van meniscus allotransplantaten op hun indentatie eigenschappen. Drie groepen — vers, bevroren en geïrradieerde bevroren allotransplantaten — werden onderzocht, 6 maanden postoperatief. De controlaterale menisci werden als controle-materiaal gebruikt bij elke groep. Het effekt van de behandeling werd geëvalueerd met behulp van 3 mechanische parameters, met name compliance voor lange termijn assimilatie (JR), compliance voor korte termijn (J) and viscoëlasticiteit (T1).
De vergelijking van de 2 groepen tonen aan dat de irradiatie van de allotransplantaten de lange termijn

compliance opmerkelijk vermindert. Dit laat vermoeden dat op de 6de postoperatieve maand de geïrradieerde bevroren allotransplantaten minder viscoëlastisch waren dan de verse of bevroren transplantaten.

RÉSUMÉ

L. YAHIA, D. ZUKOR. Allogreffes de ménisques stérilisés par rayon gamma chez le lapin.

Les auteurs ont étudié les effets à long terme de la stérilisation par irradiation gamma sur les propriétés d'indentation des allogreffes de ménisque de lapins. Trois groupes d'allogreffes ont été évalués après six mois d'implantation : frais, congelés et irradiés congelés. Les ménisques controlatéraux ont servi de contrôle. Nous avons utilisé trois paramètres mécaniques pour analyser l'influence du traitement : les compliances à court terme (J_{μ}) et à long terme (J_R), et le temps caractéristique de fluage (τ_i).

Nos résultats démontrent que l'irradiation des allogreffes congelées réduit la compliance à long terme d'une façon significative. Par conséquent, les performances viscoélastiques des allogreffes traitées par irradiation diminuent par rapport à celles des allogreffes fraîches ou congelées.