Ultrastructure of periprosthetic Dacron knee ligament tissue

Two cases of ruptured anterior cruciate ligament reconstruction

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Light- and electron-microscopic investigations were performed on two failed Dacron ligaments that had been removed from 2 patients shortly after failure of the implant 2–3 years after reconstruction of the anterior cruciate ligament. Two different cell populations and matrices were correlated with closeness to the Dacron threads. Fibroblasts surrounded by connective tissue with collagen fibrils were located far from the Dacron threads. Roundish cells, appearing to be myofibroblasts surrounded by a more lax connective tissue and elastic fibers, were found close to the Dacron threads. The presence of myofibroblasts and the matrix differentiation could be attributed to the different mechanical forces acting on the Dacron and on the connective tissue because of their different coefficients of elasticity. The sparse occurrence of inflammatory cells in the synovial membrane and in the connective tissue surrounding the Dacron supports the biologic inertness of this artificial material. However, the repair tissue was not structured to resist tension stresses.

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We report light- and electron-microscopic examinations of the cellular elements and the matrix that have invaded synthetic Dacron ligaments in 2 patients operated on because of a ruptured anterior cruciate ligament.

Material and methods

Two males, aged 23 and 26 years, were operated on for anterior cruciate ligament insufficiency with open reconstruction using high-strength Dacron (Meadox Medicals, Inc.). A double drill-hole (tibial and femoral) technique was used without augmentation and fixation to the bone with double staples. The isometric femoral area was always checked, and a notchplasty was performed to prevent graft abrasion and impingement.

At 26 and 31 months after the operations, the patients had a sudden recurrence of the instability, with one or more episodes of giving-way during sports activities. Arthroscopy, 3 and 5 days later, revealed a complete rupture of the ligament in both patients; the prosthesis appeared to be coated with a synovial-like fold (Figure 1).

The ligaments were removed and fixed in 4 percent glutaraldehyde in cacodylate 0.1M buffer at pH 7.2, for 6 hours. The ligaments were fragmented and fixed in an OsO_4 1 percent solution in 0.1M cacodylate buffer at pH 7.2, dehydrated in ascending concentrations of cold ethanol, passed through cold propylene oxide, and embedded in epoxy resin.

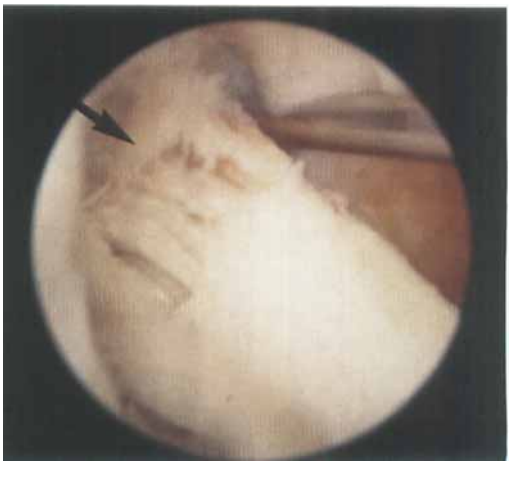

Figure 1. The arrow indicates the complete rupture at the proximal edge. The ligament is coated with a synovial tissue.

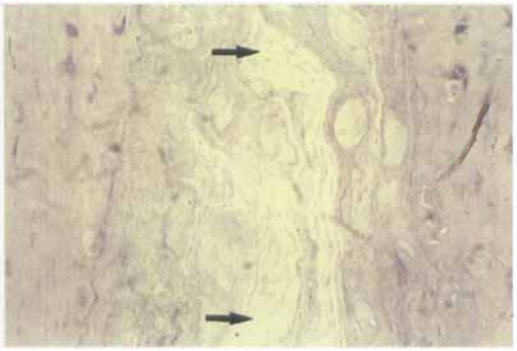

Figure 2. Near the Dacron filaments (arrows), roundish cells are visible; and far from the Dacron fibrils, elongated fibroblast-like cells are present. LM, ×10.

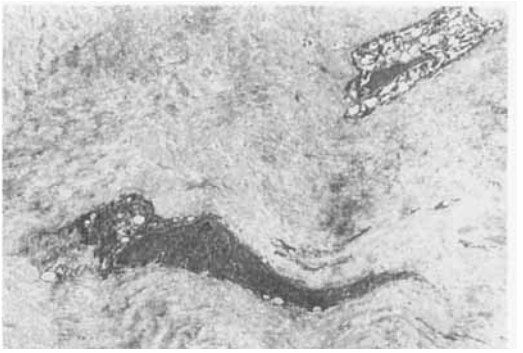

Figure 3. Elongated fibroblast-like cells populating the newly formed tissue far from the Dacron threads. EM, ×2,800.

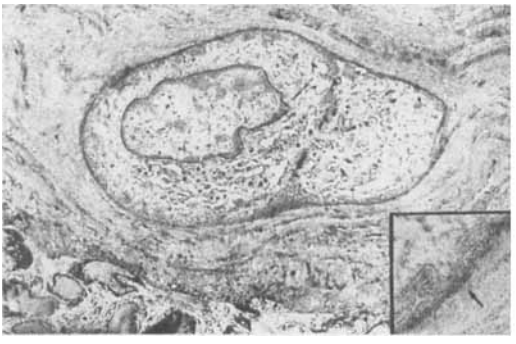

Figure 4. Roundish cells with abundant secretory organelles are evident near the Dacron threads. The cells' nuclei are strongly euchromatic. Around the cell membrane, the matrix is characterized by numerous randomly disposed microfilaments. EM, ×2,800.

Insert: The cell membrane of roundish cells is characterized by a condensed border that is reminiscent of an adhesive structure (arrow), ×28,000.

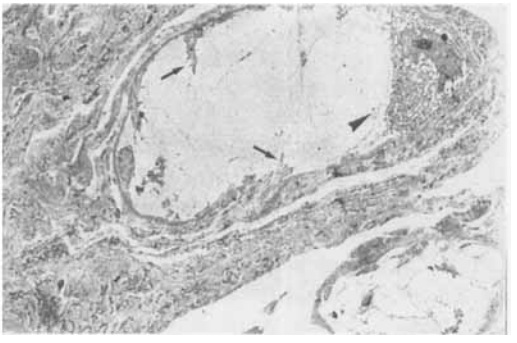

Figure 5. Dacron threads can also be seen in direct contact with roundish cells (pointer). Cell flaps invading the Dacron cracks (arrows). EM, ×1,200.

Semithin (0.5 μ) sections were stained with 1 percent toluidine blue 0.5 percent borate, pH 5.4, for light microscopic studies. Thin sections were stained with uranyl acetate-sodium bismuth (Riva 1974) and examined with a Jeol 100S electron microscope.

Results

Light microscopy

The Dacron threads were easily recognized as transparent areas that appeared to be roundish in transverse sections and randomly distributed in longitudinal sections. The diameter of the Dacron threads was about 20–25 μ. The synovial membrane showed no pathologic changes and only a few inflammatory cells. Near the Dacron threads, roundish cells were always evident, and far from the threads elongated fibroblast-like cells were seen. However, the sections contained mostly connective matrix (Figure 2). The matrix was particularly toluidine blue positive near the Dacron threads.

Electron microscopy

Also at the electron-microscopic level, the different cell populations could be distinguished around the Dacron. The first one, represented by elongated fibroblast-like cells, presented a heterochromatic nucleolus and a cytoplasm that was characterized by some secretory organelles with rough endoplasmic reticulum, Golgi apparatus, and roundish secretion vesicles (Figure 3). These cells were enveloped in a well-organized connective matrix. The matrix was composed of collagen fibrils organized into bundles

that were oriented parallel to the cell surface. Collagen fibrils presented a regular 680 Å longitudinal periodicity, and measured about 300 Å in diameter.

The second cell population was represented by large roundish cells showing an euchromatic nucleolus and a well-organized secretory apparatus in the cytoplasm (Figure 4). Inside the cytoplasmic membrane, a thin, electron-opaque, amorphous border (reminiscent of an adhesive structure), about 300 Å thick, "highlighted" the membrane (Figure 4). Around the cell membrane, a 2,000–3,000 Å thick space—characterized by thin, aperiodic, randomly oriented filaments—was evident. Around this pericellular zone, periodic collagen fibrils, collected in bundles, are present (Figure 4). Between the collagen bundles, amorphous electron-transparent areas can be seen. These amorphous areas, with sparsely and randomly distributed, thin aperiodic filaments, could be interpreted as an incompletely structured elastin. The roundish cells often adhered, by large extensions, to the Dacron threads; and thin cell flaps were seen inside the cracks of the Dacron filaments (Figure 5). Dacron threads were also in contact with the connective matrix—organized into periodic collagen fibrils. This contact was mediated by a strongly electron-opaque amorphous material 200–400 Å thick.

Discussion

The failure of the Dacron prostheses was probably due to the mechanical abrasion of the ligament against the intercondylar roof (Gerdes et al. 1982, Claes et al. 1985), although a notchplasty had been performed. Our observations in the human material were comparable with those obtained in light microscopic studies in sheep (Jenkins 1978), horses (Goodship et al. 1980), and rats (Ralis and Foster 1981), with fibroblasts and collagen bundles abundantly oriented along the longitudinal axis of the ligament, as in a normal cruciate ligament. However, the 300 Å diameter of the collagen fibrils was quite different from those described in humans by Dale et al. (1972), Danylchuk et al. (1978), and Amiel et al. (1984), who found 1,500–2,500 Å diameter fibrils. The amorphous areas with thin, aperiodic filaments that we interpreted as incompletely structured elastin do not seem to occur in human ligaments (Danylchuk et al. 1978, Amiel et al. 1984).

The second cell type can probably be interpreted as myofibroblast-like cells: this opinion can be supported by the adhesive structures seen on the cell membrane and by the amorphous or microfibrillar pericellular space. This material is very similar to the basal lamina that normally envelops myofibroblasts (Inoue 1989). Roundish cells in periprosthetic tissue were also reported by Goodship et al. (1980) and Dahlstedt et al. (1990) in carbon fibers and in Goretex ligaments, and were interpreted as dividing or inflammatory cells.

These cells seem similar to those populating vascular grafts, where a myofibroblast population differentiated from perivascular mesenchymal cells around or inside the arterial Dacron grafts (Tazzi et al. 1981).

This interpretation is supported by Amis et al. (1982): they observed that around Dacron implants no inflammatory reaction is present if compared with carbon implants, and also Goodship et al. (1985) described only rare inflammatory cells around and inside the Dacron prosthesis.

One of the possible factors stimulating the differentiation of fibroblasts into myofibroblasts may be represented by micromovements between the Dacron threads and the peri-Dacron tissue due to their different coefficient of elasticity (Goodship et al. 1985, Park et al. 1985). This could explain the different types of tissue organization near and far from the Dacron threads.

It is possible to conclude that the Dacron prosthesis is well tolerated, but that the periprosthetic tissue assumes the characteristics of a coating tissue without any mechanical properties related to tension stresses (Amis et al. 1982). The Dacron prosthesis may act as a substitution that, bypassing tension stresses, inhibits the formation of a stress-resistent connective tissue.

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