Biodegradation and Restitution of the Intercellular Matrix in Preserved Fragments of Dense Fibrous Connective Tissue After Their Grafting into the Recipient

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Results are presented from a study in which the structural arrangement of the intercellular matrix was examined in preserved connective tissue fragments (allografts) after their implantation into rabbits to repair posttraumatic space-occupying defects in the capsular-ligamentous complex of the knee joint. Stages of biodegradation and restitution undergone by the interstitial substance of connective tissues after the implantation of allografts are identified.

Key Words: *biodegradation; restitution; orderliness; connective tissue; allografts*

The degree of restitution and differentiation undergone by dense fibrous connective tissue (DFCT) after trauma depends on the extent to which the conditions prevailing during the posttraumatic period promote these processes [5]. The orientation and regularity of DFCT correlate with the anisotropy of its mechanical properties [1,4].

The functioning of connective tissue is intimately bound up with the activities of its cellular elements, such as the production of protein-carbohydrate complexes of the ground substance (proteoglycans and glycoproteins), the formation of fibrillary structures, and the regulation of metabolism, as well as with the structural stability of these elements. The three-dimensional structure of connective tissue is determined by fibroblasts in conjunction with fibrous components. Mechanical trauma disrupts the normal relationships existing in the connective tissue system. One method by which an environment favoring restitution of this system can be created is the repair of space-occupying defects with preserved DFCT fragments. After mechanical trauma of a joint resulting in abnormal configuration and interrelationships of its sliding surfaces, positive results cannot be expected unless the anatomic structure of the joint is restored to the maximum extent possible. Considerable promise is offered in this respect by the use of connective tissue allografts as bioplastic materials [2,3,6].

Although a large number of studies dealing with the posttraumatic restitution of connective tissue structures have been published, there are still no clear-cut criteria to rely upon in deciding which conditions are best for reparative regeneration of the connective tissue's cellular matrix and restoration of its integrity. Nor is it known for how long the injured joints should be immobilized and when a functional load can be safely imposed on them in cases where their capsular-ligamentous apparatus is reconstructed using auto- or alloplastic materials. In view of this, the chronic pathophysiological experiment with rabbits described here, in addition to being of interest to morphologists and pathophysiologists, may provide clinicians with a key to determining optimal times for the immobilization and loading of joints

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Fig. 1. Appearance of a connective tissue allograft on day 7 after plastic surgery to repair an experimentally produced defect in the tibial ligament. *a*) disorganized area of the collagenous-fibrous framework; *b*) tissue-specific structure of this framework characteristic of dense fibrous connective tissue. Polarization micrograph; ×64.

after plastic surgery without any risk of instability arising in the capsular-ligamentous complex.

MATERIALS AND METHODS

A total of 67 sexually mature Chinchilla rabbits were used. Under Hexenal anesthesia and sterile conditions, a full-thickness defect was produced in the collateral tibial ligament of the knee joint of each recipient rabbit from a medial parapatellar approach, and a DFCT fragment taken from a preliminarily killed rabbit and then kept for at least 10 days in 70% ethanol was sewn into the defect within 6 h of its production.

After surgery, the recipients were observed daily for their general condition, and the turgor of tissues surrounding the operated knee joint, the range of joint movement, local and general temperature responses, and the dynamic motor stereotype were determined at regular intervals. On postoperative days 7, 14, 21, 30, 60, 90, 180, and 360, rabbits were killed under Hexenal anesthesia and a segment of the limb with the operated knee joint was re-

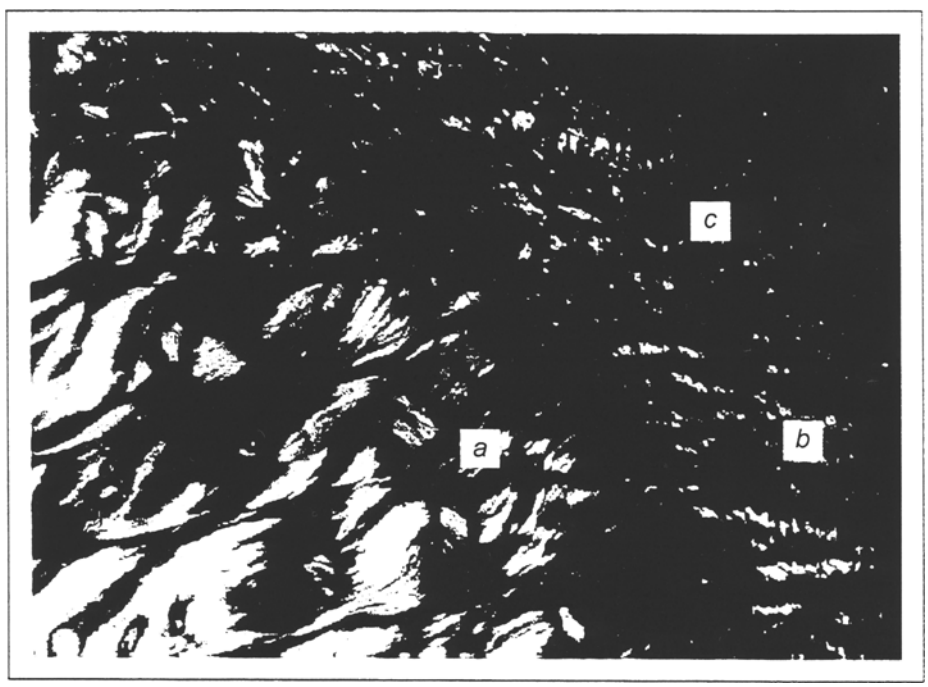

Fig. 2. Appearance of a connective tissue allograft on day 14 after plastic surgery. *a*) disintegrated fiber bundles in a marginal area of the allograft; *b*) homogenized collagenous-fibrous framework in the marginal area; *c*) fibrillary-microfibrillary framework of the regenerate forming in the area of contact between the implant and the recipient's tissue. Polarization micrograph; ×64.

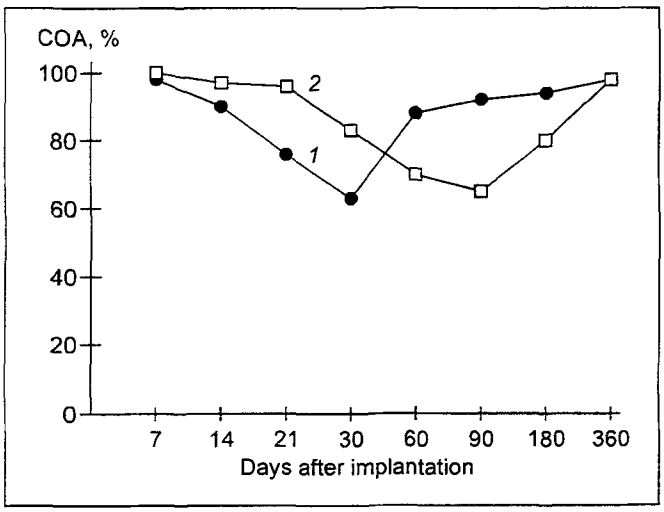

Fig. 3. Coefficients of optical anisotropy (COA) on different days after implantation of an allogeneic connective tissue fragment into the posttraumatic defect. *1* and 2) marginal and central areas of the allograft, respectively.

moved and fixed in 10% neutral formalin and Carnoy's fluid.

The arrangement and orientation of structures in the intercellular matrix were examined under an Amplivali Pol U polarizing microscope fitted provided with an SFN-10 microspectrophotometric attachment (LOMO, St. Petersburg) whose optical system contains a monochromator and microprobes.

At different times postimplantation, the coefficient of optical anisotropy (COA) was calculated by the formula: $COA=(I_{max}-I_{min})/I_{max}\times100\%$, where I_{max} and I_{min} are the maximal and minimal intensities of the polarized light that had passed through the test specimen.

The results were subjected to analysis of variance on a computer. The microscopic appearance of removed limb segments was described according to the following scheme: 1) type of collagenous framework (whether collagenous-fibrous, collagenous-elastic, fibrillary-microfibrillary, or other type); 2) signs of disorganization (disintegrated fiber bundles, fragmentation, homogenization); 3) orientation of the forming regenerate; and 4) orderliness of the intercellular matrix (as indicated by the COA value).

RESULTS

On day 7 after implantation, the DFCT fragments retained their general tissue-specific structure (Fig. 1), although the collagenous-fibrous framework appeared fragmented in the marginal areas of the fragment. First- and second-order fiber bundles with a high degree of double refraction and tortuous collagen fibers typical of DFCT were observed. Destruction of the interstitial substance led to a narrowing of the interbundle spaces. In the zone of contact between the donor and recipient tissues, the interstitial substance was so organized as to resemble structures with a fibrillary-microfibrillary collagenous framework and showed a relatively low capacity for double refraction.

On day 14, the intercellular matrix still retained its tissue-specific structure in the central areas of the implanted DFCT fragments, while fiber bundles of the collagenous framework showed signs of intensified disintegration in the marginal areas. The boundaries between second-order bundles were indistinct. More frequent alternation of collagen fiber bundles with areas of homogenized intercellular matrix were seen in the allografts (Fig. 2).

On day 21 postimplantation, second-order fiber bundles were observed to undergo intensive disintegration and some fragmentation in marginal areas of the allografts. In their interstitial substance, an increased front of fibrillary-microfibrillary structures with a high level of orientation was noted along with disorganization of the collagenous-fibrous framework.

By day 30, the fibrous framework of the allografts had become disorganized to a considerable extent. The interstitial substance in their marginal areas exhibited increased double refraction - an indication of intensified fibrillogenesis. Unidirectionally oriented collagen fiber bundles were in the process of formation.

On day 60, disorganization processes involved the implanted connective tissue fragments throughout their thickness. In the marginal zone, *de novo* formation of a collagenous-fibrous framework was seen. The central zone had a mosaic structure, disorganized areas of the implanted connective tissue fragment alternating with areas that retained their tissue-specific structure.

On day 90, continued disorganization of the intercellular matrix combined with remodulation of the developing regenerate at the site of donor tissue.

On day 180, continued morphogenesis of the newly formed collagenous-fibrous framework with the formation of first- and second-order fiber bundles was observed.

Finally, on day 360 postimplantation, increased remodulation of the regenerate was noted at the site of the implanted connective tissue fragment.

The shapes of polar figures and the temporal changes in polarization-optical characteristics after the implant operation pointed to a vigorous morphogenesis of interstitial substance proceeding at the site of biodegraded DFCT fragments. This substance closely resembled donor tissue in structure.

The COA in the marginal areas of allografts decreased between days 7 and 30 and then increased again to reach the initial value by day 360; in the central areas, the COA began to fall later, had the

lowest value on day 90, and then rose to the initial value (Fig. 3). The time course of this coefficient indicates that the recipient organism was striving to maintain its entropy at one level by guarding its life-support systems against an increase in entropy.

Polarization microscopy of marginal zones in the recipients' tissue beds after the implantation of DFCT fragments showed that the restorative process, having started in the marginal zone, then extended deep inside the implanted material.

The results we obtained led us to conclude that the changes undergone by the implanted DFCT fragment may be divided into three stages - a stage of disorganization (biodegradation), a stage of its replacement by the newly formed regenerate, and a stage of remodulation of the latter. The structural reorganization of DFCT fragments was accompanied by a decrease in their capacity for double refraction between days 14 and 30 after implantation and by an increase in this capacity from day 30 to day 360, i.e. the mechanical anisotropy of the allografts and, consequently, their mechanical strength were decreasing during the latter period. The mosaic pattern of biodegradation undergone by the intercellular matrix in the central areas of DFCT fragments and the start of its restitution compensated for the reduction in the strength of the fragments between days 30 and 90 postimplantation, despite the relatively low values of the integral COA in these areas. The lowered values of this coefficient obtained for the intercellular matrix of implanted DFCT fragments on days 14 and 30 suggest the need for limiting mechanical loads during that period or for developing devices capable of taking on, for a time, the functions of the capsular-ligamentous complex.

Under conditions of aseptic inflammation, biodegradation of the implanted DFCT, being a reaction of graft rejection that differs in principle from encapsulation, is a means of eliminating foreign material from the body.

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