Nonuniformity of Collagen Ultrastructure Occurring in the ACL Graft
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Introduction: Anterior cruciate ligament (ACL) reconstruction is a widely used method to treat ACL injury. Clinically, rupture and laxity failure of grafts is still unsolved in this procedure. Stress reduction is known to adversely affect fibrous connective tissues. Stress shielding in normal patellar tendon results in a decrease in tensile strength and elastic modulus [1]. If the graft has a severe degenerative change after reconstruction, it will probably rupture even under normal loading conditions. The purpose of this study is to understand the ultrastructure changes of ACL graft by comparing it with that of the normal ACL. Our hypothesis is that a significant abnormality occurs in the collagen ultrastructure of ACL grafts which may weaken the structure.

Materials and Methods: ACL grafts from revision surgery were used in this study (n=6). All patients had instability and knee pain at the time of hospital admission, resulting from old injuries that were sustained more than 3 weeks previously. There was no severe impact directly to their operated knee joints after primary ACL reconstruction. The grafts included anterior tibialis allograft, hamstring autograft and patellar tendon autograft.

The torn grafts were incised during reconstruction and preserved for the atomic force microscopy (AFM) investigation. The feasibility of using the AFM for visualization of the ultrastructure of ACL has been demonstrated in our previous study [2]. After standard histology procedure, 10-μm sections underwent hematoxylin and eosin staining. Other 10-μm sections were immersed in xylen to dissolve the paraffin, then into absolute alcohol to remove the clearing agent. After rehydration, the sections were dried with air for AFM observation. Images were taken using the tapping mode of the AFM (Synergy ESPM, Novascan Technologies, Ames, IA). A tip with a cantilever length of 130 μm and a resonance frequency of 150 kHz was used (NSC35, Mikromasch USA, Portland, OR). The image was recorded with a 1 Hz scan rate and a 512×512 resolution. The gain for feedback loop was deliberately adjusted to get the best quality image. The dimension measure was calculated from images using Image Analysis software (Imaging Research, Inc., Ontario, Canada).

Results: The histological findings of the grafts on light microscopy examination showed densely populated spindle-shaped fibroblasts. Some areas revealed well-organized fiber bundles, but other areas showed a more or less wavy, irregular orientation of collagen fibers with spotty increases in cellularity and vascularity. Under AFM, images from different areas of the longitudinally cut graft showed different morphology. One 646 μm2 image showed a regular arrangement of collagen fibrils and their periods (Figure). In many areas, the fibrils were not well-arranged in a single direction, with some smaller fibrils oriented vertically to larger parallel fibrils (Figure). The average D-period was 65.8 nm from the longitudinally cut graft images, which is similar to that of normal ACL in the previous study. Morphometrical calculation revealed that the fibril diameters ranged from 30 nm to 330 nm. The diameters of the fibrils in the well-organized area peaked at between 180 nm and 260 nm, while those in disorganized areas peaked at between 45 nm and 90 nm.

Discussion: This study observed the micro-structure of ACL graft tissues collected from ACL revision surgery. Nonuniformity of collagen ultrastructure was clearly revealed by the AFM 3D images. Opposite from some areas with regular arrangement of fibrils, many other areas showed disorganization of collagen fibrils. From the biomechanical point of view, the disorganized fibrils may result in diminished mechanical strength of the grafts and increase the chance for rupture of the graft. The cause of the abnormality of the collagen ultrastructure in the graft is therefore our primary concern.

Normally, ACL graft tissues should undergo a sequence of biologic remodeling and incorporation with existing tissues. This remodeling process is accompanied by a loss of the graft tensile strength. The compromised ultimate strength of the graft at the completion of the remodeling process may be associated with diminished graft function [3]. These adaptive changes represent the response of the grafted tissue to its new intraarticular environment. This might result from stress-shielding by other healthy tissues.

Generally, failure of an ACL reconstruction may occur on the basis of either technical, mechanical, or biological factors. The samples used in this study excluded cases resulting from severe accidents. Therefore, we proposed that biological remodeling with the passage of time should be the possible reason for changes in ultrastructure. For future study, checking patient MRIs, x-rays and gait data might help us determine the stress-shielding mechanism causing the ultrastructure abnormality. Of course, other chemical or biological factors can not be excluded, and more multidisciplinary efforts are needed.

In summary, we demonstrated that nonuniformity and abnormality of collagen ultrastructure occurred in grafts of ACL reconstruction by using AFM technology. Three-dimensional images and analytic functions of AFM can refine the observations made by optical microscopy and provide detailed independent evidence of crossbridge structures of collagen fibers. By revealing the altered extracellular matrix structure and organization of fibrils, this study indicates that the stress-shielding may weaken the graft tissue structure and cause graft failure.

References: