HUMAN ACL REMODELING AFTER STRESS DEPRIVATION—A HISTOLOGICAL EVALUATION

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INTRODUCTION:

Mechanical loading is critical for maintaining normal functions of musculoskeletal tissues, such as ligaments. Stress deprivation can cause abnormal metabolism in these tissues, resulting in loss of normal structure and function. This issue has been widely studied in vivo with animal immobilization models and in vitro cell cultures. However, to our knowledge, no human in vivo model has yet been established. The goal of this study was to introduce a human stress in vivo deprivation model of ruptured anterior cruciate ligament (ACL) model and to evaluate the model histologically.

METHODS:

The model design is based on the fact that in total tearing ACL, the ligament remnant will be stress deprived. When those samples are collected and ranked over time, they should demonstrate a remodeling process after stress deprivation. In this study, ACLs were harvested from 25 athletes (10 males at 17.4 ± 2.4 years old and 15 females at 23.5 ± 10.2 years old) with noncontact ACL tears, who were undergone surgical anterior cruciate ligament reconstructions. The time between injury and surgery varied from 1 day to 3 years. In order to avoid post-injury effects, all samples were taken from an area distal to rupture ends identified visually during surgeries and confirmed by histological inspection.

The samples were fixed in 10% buffered formalin and then embedded in paraffin. Five micron-thick sections were cut and followed by hematoxylin and eosin (H & E) staining.

Histological slides were examined under microscopy with normal and polarized lights. In order to avoid the interference from inflammatory infiltration in acute injury samples, only areas without obvious inflammatory infiltration were evaluated. For each sample, ten disparate areas under oil lens were evaluated to determine the total cell number. The extracellular matrix was also evaluated by the crimp pattern of collagen fibers and space between fibers [1].

RESULTS:

The fibroblast cell number showed no significant change until 8 weeks after stress deprivation, after which cell density increased until one year after stress deprivation (Fig.1). After 8 weeks, collagen fibers began to lose their normal crimp pattern and regular organization, combined with widening of the space between fibers with time (Fig.2).

DISCUSSION:

To our knowledge, this was the first human model to study ACL remodeling after stress deprivation. The information from this model will assist in understanding the effects of mechanical loading on human ligaments.

Our finding, that the degeneration of extracellular matrix occurred after stress deprivation, indicated that mechanical loading is critical for the maintenance of the normal structure of ACL. The cell numbers and the integrity of the extracellular matrix remained stable until after 8 weeks of stress deprivation. This suggested that the normal structure of ACL can remain intact for a period of time after stress deprivation. The cell number increased after 8 weeks and the degeneration of the extracellular matrix occurred after 8 weeks of stress deprivation inferred that a relationship might exist between these two phenomena.

Previous animal immobilization studies have shown that the widening of space between collagen bundles as well as fragmentation of collagen fibers appeared after 6 weeks of stress deprivation [1]. Those phenomena were also observed in our study, but after 8 weeks duration. Another rabbit ACL immobilization study showed that the fibroblasts changed their shape from ovoid to spindle-like with fusiform nuclei after 9 weeks [2]. However, such a change was not observed in our study.

Factors other than stress deprivation in the model should to be considered in this study. Previous literature has shown that inflammatory reaction, as well as repairing and remodeling after injury exists in the rupture end of torn ACL [3]. In order to avoid the post-injury effects, all ACL samples in this study were harvested far from the rupture ends during surgeries using visual observation. In our study, it was found that focal inflammatory infiltrates also existed in some ACL samples from non-injury sites, especially in those from acute injury patients. However, during histological evaluation, those areas with obvious inflammatory infiltrates were excluded.

In summary, a human ACL model was developed to directly assess damaged ligament remodeling after stress deprivation. The results suggested that no histological changes occurred until 8 weeks after stress deprivation.

REFERENCES:

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[2] Newton, P., et al., Matrix 10 (1990), 314-319.
[3] Murray, M. et al., J. Bone Jiont Surg Am 82 (2000), 1387-1397.

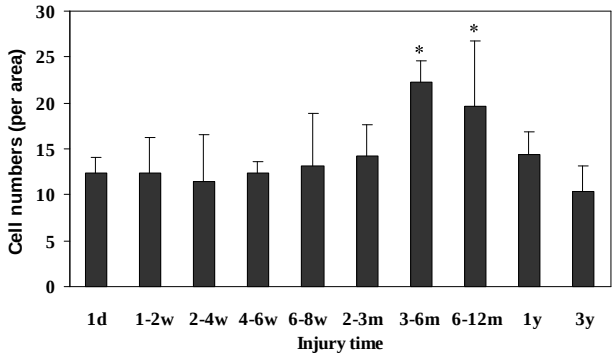

Fig.1. Cell density of ACL increased dramatically after 8 weeks of stress deprivation, and dropped after 1year deprivation (P<0.01).

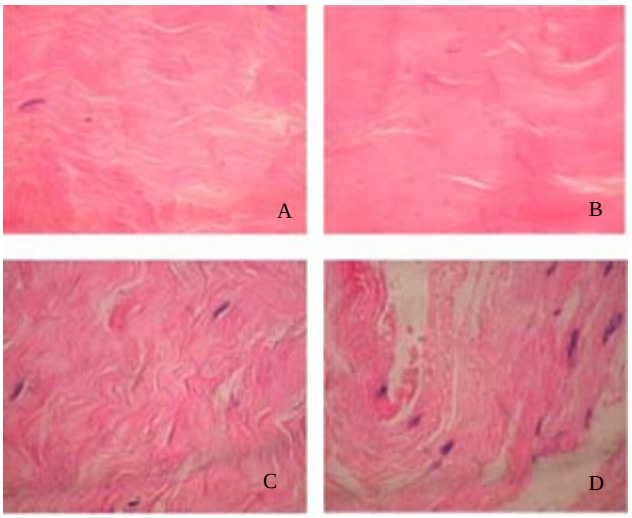

Fig.2. H & E staining of ACL after stress deprivation for different times. A) 1 day, B) 8 weeks, C) 9 weeks and D) 3 years.