# A multiscale study on strain-rate dependent separated responses of collagen structures and interfascicular matrix in frozen - thawed tendons.

Movement is a natural and physiological need of every human being. It is also the foundation of a healthy life, as regular physical activity significantly reduces the risk of premature death caused by obesity, diabetes, cancer, as well as cardiovascular and neurodegenerative diseases. On the other hand, excessive exercise intensity, human-to-human contact in some sports disciplines, or simple accidents may cause injuries to the human musculoskeletal system. The most common injuries are those of soft tissue structures such as tendons or ligaments. One of the most widely described injuries in the medical literature is tearing of the anterior cruciate ligament (ACL) which requires surgical intervention and the application of an allogenic tissue graft to repair. An example of soft tissue which is commonly used to prepare such as grafts, is the Achilles tendon.

Soft tissues, intended for biostatic allogeneic grafts, are obtained from deceased donors and then processed in tissue banks, where they are sterilized with ionizing radiation and further preserved by deep-freezing (-80°C). As shown in previous studies tissue storage at -80° C causes necrosis of the living cells and loss of tissue immunogenicity. Therefore, the obtained tissue is biologically inactive, which minimizes the risk of transplant rejection and eliminates the need for biological matching between the donor and the recipient. It also eliminates the need to use immunosuppressive drugs after reconstructive surgery. The most common procedure for the processing, storage, and distribution of tendon tissue for the purpose of transplantation involves two freezing/thawing (F/T) cycles from the moment of its collection to its implantation into the recipient's body. Subsequent tissue thawing is usually related to tissue sterilization or necessary serological and microbiological tests. Unfortunately, under unfavorable circumstances, it may be necessary for the tissue to be frozen and thawed more than twice. Such a situation may occur in cases of time-consuming multiorgan donors, in cases of inconclusive results and repeated serological and microbial tests, or when planned surgeries have to be cancelled and the sterile packaging of thawed tissue had not yet been tampered with. In such situation, there arise a reasonable doubt whether, or to what extent, a repeated freezing of the tissue will affect its biomechanical properties. A variety of medical cases, for which multiply F/T have to be applied, arises a strong need for throughout studies, which may help to fully understand the influence on multiply F/T on mechanical, biochemical, and structural properties of soft tissues at each level of their hierarchy.

The main objective of this study is to investigate a quasi-static and strain-rate dependent response of the interfascicular matrix (IFM) separated from the responses of collagen structures in multiply frozen - thawed (F/T) tendons. The study is designed as a continuation and extension of the research previously carried out by the Applicant, which clearly proved the negative impact of repeated freezing and thawing on the mechanical stability of the tendon considered as a whole. The next step of that research is proposed in this study and aims to answer the following research questions: What is the individual mechanical and biochemical response of various components of the tendon tissue subjected to multiply F/T cycles? Is it possible to separate the response derived from the collagen phase of the IFM response to understand the mechanisms responsible for tissue damage during multiple freezing? To what extent are the elastic properties of the tendon related to the mechanical response of its collagen substructures, and to what extent with the viscous properties seen in the IFM? The main research hypothesis assumes that progressive deterioration of the viscous properties of the tissue (considered as a whole) with subsequent F/T cycles is largely the result of changes in mechanical strength and biochemical properties of the IFM, especially in terms of non-collagenous content of the matrix.

According to the hypothesis of the Applicant, the following F/T cycles may interfere with the levels of three essential IFM's proteins called decorin, aggrecan and versican, which are strictly related to the mechanical response (decorin) of the matrix and are deeply related to the its water content of IFM (aggrecan, versican). In addition, since tissue is a viscoelastic material and thus exhibits strain-rate-dependent behavior, the Applicant wants to answer the supplementary research question: If, how, and to what extent, the multiple F/T influence the strain-rate sensitivity of the tendon tissue and its individual components? The research hypothesis for the supplementary objective of this study assumes that the influence of F/T cycles on strain-rate sensitivity may be more intense in the case of viscoelastic IFM than for elastic collagen substructures.

Separation of the response derived from the collagen phase of the IFM response may be a key to understanding the mechanisms of damage at the lowest levels of hierarchy of the tissue subjected to multiple F/T. This, in turn, may help to develop protocols of tissue processing in such a way to influence and control selected mechanical properties of tissue allografts and thus prepare patient-specific tissue grafts. The idea of personalized tissue allografts would provide a higher quality of distributed tissue transplants and thus a better quality of life for post-operative patients.