Tendons are the biological structures built with a connective tissue and its primary function is to transfer force generated by the muscles to the bones. The merit of the tendons is also causing the joints movement so the organisms with musculoskeletal system are able to move. The tendon is primarily composed with collagen, proteoglycan, glycoproteins, water and other cells. The major part in collagen content constitutes collagen type I (approx. 95%) which forms 60-85% of a dry tendon mass, and the remaining 5% consists collagen type III and V. Each tendon has a hierarchical structure - its composition starts from a single collagen molecules with the size nanometres (billionths of a meter), which are subsequently combining in microfibers called fibrils. Connected fibrils are giving the single collagen fibers and their size is “large” enough (1-20μm) that using a scanning electron microscope we are able to see the arrangement of each individual collagen fibre inside the specimen tendon. The collagen fibers are additionally surrounded by a cover of connective tissue, and then combined again to form the primary (first order) collagen bundles. Those bundles and again joined together forming the secondary structure (the second and the third order) giving in the end a "fully-fledged" tendon structure with a dimensions of several, and sometimes even a dozen centimetres. Healthy tendons are in the colour of shining white, and thanks to the contain of proteoglycans or mucopolysaccharides are able to attract water, maintain the require tendon shape and provide an appropriate tissue flexibility.

Tendon at rest has a slightly wavy form, but in the moment when the muscles, on which the tendon is attached, begin to work, it straightens and stretches. During human motion the tendon must be withstand on high loads resulting from the action of tensile, compressive or rotary forces. There is no doubt that athletes and physically active people are the social group with the highest exposure to physical stresses and therefore, the highest incidence of all kind tendon ailment. Unfortunately, insufficient preparation for workout (not enough warm-up), long-term repetitive or too rapid motions may cause a complete tendon rupture. In such cases, it may be necessary to perform a surgery procedure aims to reconstruct the damage tissue or replace it with a new one. The number of tendons transplantation and reconstruction procedures is increasing every year and techniques applied for those purposes are becoming much more sophisticated. Regardless the progress in the field of orthopaedic surgery, one fact remains unchanged - the tissue intended for transplantation has to be subjected to prior deep-freezing process. At this point, there may appear some reasonable doubts: will the freezing change the tissue properties? How fast and how long the tissue should be frozen? Will implanted thawed tendon will have the same strength as in the case of fresh tissue?

To find the answers to these questions, the scientists around the world are researching on the assessment of biochemical and biomechanical changes in tendon resulting from the repeated cycles of its freezing and thawing. The methodology of reported in the last 15 years studies is not consistent therefore none of the research group did not give a clear limit of a number of freezing/thawing cycles over which the tissue damage is so significant that tendon will be disqualified for further medical procedures. To explore the problem of the change of the frozen tendons properties, it is necessary to determine and deeply analyze the reasons for which this process causes an irreversible changes in the structure of the tendon tissue.

According to the authors of this project, the main cause of deterioration of the tendons properties during the freezing process is associated with a significant water content and the physico-chemical processes which the water is subjected to. So, if we dissected the tendon for transplantation and if it undergone a freezing process, the internal contain of the water (representing up to 60-80% of the wet tendon mass) will also start to freeze. Unfortunately, below a temperature limit of 3.98 ° C, a still liquid water begins to exhibit the phenomenon of anomalous thermal expansion. It means that the volume of water does not increase monotonically with increasing temperature in the entire area of a water liquid state but for a certain characteristic point (for 3.98 ° C) assumes the minimum value. At temperatures lower than this limit, the volume of water begins to rise rapidly with decreasing temperature and such a behaviour, among the behaviour all of chemical substances, is perceived as anomaly. An anomalous thermal expansion phenomenon is caused by a specific shape of water molecules and the existence of strong hydrogen bonds, which are breaking precisely in the area of anomalous. As the result of a bond breaking the disorder of liquid particles is rising, which consequently leads to an increase of the liquid volume. Moreover, when the ambient temperature drops to 0°C, the water (which is still increasing its volume!) begins to change the state of matter. This process, called water phase transition, causes the liquid water transformation into a solid state (ice) and as result the free water molecules are becoming bonded in a form of ordered structure called crystal lattice.

So now, if you think of water contained in the tendon in the way of a millions of paricles "trapped" between other tendon structures (fibrils, collagen fibres etc.) and subjected to cooling and freezing, the mystery of the "harmful water" should become clearer. After the crossing the temperature limit of the anomalous expansion, the volume of the water molecules begins rapidly increasing, blowing and pushing aside other internal tendon structures. This process is additionally intensified by a water phase transition which is following a few moments later. As a result of these two actions, the collagen fibres begin to modify their shapes, diameters and the mutual arrangement thereby they are changing the biochemical and biomechanical properties of the whole tendon.

The main goal of this project is to asses and evaluate the chemical and physical phenomena occurring in the tendon during freezing, causing microdamages inside its internal structure as a result of the anomalous temperature expansion of water. According to the proposed research hypothesis, the behavior of the tendon will be considered in two (described above) potentially problematic points – below the temperature 3.98°C (in the area of the water anomalous expansion) and at the edge of water-ice phase transition (at the 0 ° C). The authors decided to tackling this subject of research because of the strong belief that exactly these processes associated with crystallization of water in the tendons conjugated with the phenomenon of anomalous thermal and volumetric expansion of water are the main and the direct cause of the changes observed in the thawed tendon structure. The authors are deeply convinced that thorough investigation of the tendons freezing mechanism, including behaviour of inseparably water content, is crucial for appropriate assessment of the effects of the freezing process, and that this is the only way to point the clear line of usefulness of tendon grafts for further surgical treatment.

In order to thoroughly examined and analyse the phenomena occurring at each level at each level of the tissue hierarchy, the authors of this project will create a multiscale model of the tendon. For this purpose, by the use of molecular and chemomechanical techniques of modelling and simulation, we will develop models describing the various tendon structures represented in the diverse scales. The model developed for the atomic scale illustrating single collagen fibrils, and the microscale model representing a group of collagen bundles, will be combined in order to develop the mesoscale model of tendon able to describe its behaviour during freezing process from macroscale perspective. Developed model will also take into account the complex structure of an extracellular matrix which connects all other elements of tendon. . In order to include the processes occurring in tissue subjected to freezing process, it will also be necessary to develop models of water and ice for further description of the limit of a water phase transition.

Each developed model will be subjected to the computer simulation of freezing and the simulation method will be selected especially for each scale of representation - for the atomic scale it will be used a Molecular Dynamics method, for microscale a Coarse-Grained Molecular Dynamics method and for the macroscale model the Finite Element Method.

The created multiscale model of a frozen tendon will be subjected to the validation procedure to assess whether the developed computer model correctly reproduces the real tissue and if it will be able under simulation to predict the actual behaviour of real tendon. The validation will be based on the comparison between the results of the computer simulation with the results of the real laboratory experiments performed on samples of real frozen pig tendons. Validation procedure will include both: destructive and non-destructive tests, i.e.: visual assessment of a samples after SEM/TEM microscope, the observations of coloured longitudinal and cross-section of the samples under UV light, study the appearance of collagen fibres arrangement on the background of other connective tissue with Van Gieson staining and finally the mechanical tensile tests. As the final effect of the project we will obtain validated multiscale model of a tendon, able to simulate all the physical and chemical processes occurring in its hierarchical structure during freezing.

Obtained results will allow to gain a new, extensive knowledge about the underlying foundations of phenomena observed in the structure of the tendon subjected to freezing, thus the developed model will be a key factor in optimizing the speed of freezing for various collagen tissues. The choice of an appropriate conditions of freezing will significantly raise the realizable durability of the frozen tendon intended for transplantation, so after procedure the patient will have a higher comfort and safety of use the grafted tissue. Also, due to the fact, that presence of water in the soft tissues is a universal phenomenon the authors of the project are convinced that developed multiscale model on tendon, in particular, the methodology of its creation and simulation based on techniques of molecular and chemomechanical modelling, will be used for future research regarding the impact of freezing on various – healthy and diseased – tissues. Developed and validated models describing atomic, micro and macroscale will also provide a unique research ground for further studies related to the biochemistry and biomechanics of all soft tissues, therefore they will contribute to broadening of the current state of knowledge.