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Bachelor's Thesis

"Design and Optimization of the Graft Pretension Protocol for the Reconstruction of the Anterior Cruciate Ligament (ACL)"

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Laura Quintana Quintana

Director

Oscar Martel Fuentes

Tutor

Arrate Muñoz Barrutia

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SUMMARY

Anterior cruciate ligament (ACL) rupture is one of the mayor concerns of today's professional high demand athletes due to its high probabilities of happening and slow recovery. In general, the grafts used in ACL reconstruction surgeries are tendons taken from the same patient in order to reduce the possibility of rejection. However, these soft tissue grafts share viscoelastic properties which produce an elongation of the graft after its implantation, which if too much can lead to an invalid surgery. Thus, in order to eliminate this undesired property, the tendon needs to be preconditioned after its removal from the patient and before the re-implantation. This procedure involves a graft board to be able to apply a specific load to the tendon, thus being able to elongate it before its final implantation in the affected person. However, this protocol has not been standardized to date, hence each surgeon proceeds in their own way.

The main goal of this project is to design and optimize a graft pretension protocol for the reconstruction of the anterior cruciate ligament. Digital Extensor Bovine tendons were used in order to test the quality and efficiency of the proposed protocols. Using a hydraulic machine, an ACL reconstruction surgery was simulated. A successful surgical procedure is determined by the graft elongation after its implantation in the knee, which if elongated too much invalids the reconstruction, not being able to achieve the natural ACL functions. Thus, out of all the parameters obtained, the most important one and so, taken into account for the discussion, is the elongation of the graft in the rehabilitation process. Using this parameter, protocols can be compared.

Two out of the three protocols proposed show to be statistical different from the control group. The third one produced a high elongation of the tendon grafts due to the small value load applied to them. As no significant difference was found between the two successful protocols, we can determine that the one implying less load would be the best one for its reproducibility in the operating room. In conclusion, a pretension protocol is beneficial to the grafts as it reduces post-surgery elongation and it has to consist of at least 89 N in order to be able to eliminate part of the graft's intrinsic viscoelastic properties.

Key Words

Anterior Cruciate Ligament (ACL), Pretension, Re-Tension, Initial tension.

DEDICATION

This work is dedicated to all those people, that in one way or another, have guided and supported me during these years of my university studies. Thus, in the following lines I would like to thank all of them.

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And finally I would also like to extend my gratitude to my friends, those university friends who have shared my multiple concerns and represented my daily support; those 'exchange students' friends who participated in making the third year of university the most wonderful adventure of my life; and finally, those life friends who, some in one ways and some in others, have been next to me at all times.

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1. INTRODUCTION

In this section the motivation that has driven us to carry out this project is going to be explained, as well as, the importance of such discoveries in the biomechanical field, our particular objectives and the impact that it is going to cause in our society.

1.1. Motivation

The knee is the central joint of the lower limbs of the human species. It is formed by the union of two important bones, the femur and the tibia, as well as a third small bone called patella. The remaining components are cartilage, ligaments, and fluids. Moreover, in its proximities, powerful muscles and tendons make possible the movement of the limb. Thus, the knee is designed to mainly perform the flexion and extension movements of the leg (Abulhasan, J., & Grey, M., 2017).

When some of these structures are damaged, issues arise. Knee problems are very common and can occur at any age, causing pain and difficulties when walking. As a result, they can interfere with many daily activities, ranging from practicing sports to seemingly trivial tasks like getting up from a chair and, thus, might have a significant impact on the affected person's life. One of the main causes of these issues are injuries to the ligaments, with a very common one occurring in the anterior cruciate ligament, also knowns as ACL.

The anterior cruciate ligament is recognized as one of the main stabilizers of the knee joint: It makes up for almost 85% of the knee's stability (Abulhasan, J., & Grey, M., 2017). Positioned in the knee joint, between the two biggest bones in the lower limbs of the body, its main function is to avoid a forward displacement of the tibia with respect to the femur. However, due to this important function and its location, it is usually overloaded and hence susceptible to injuries. These injuries often occur due to a sudden twisting motion, common in countless physical activities. As a result, ACL injuries have been a major focus of studies and research over the last decades, as the ones carried out by *Nurmi et al.* (Nurmi, J. T., Kannus, P., Sievänen, H., Järvelä, T., Järvinen, M., & Järvinen, T. L., 2004) and *Jaglowski et al* (Jaglowski, J. R., Williams, B. T., Turnbull, T. L., LaPrade, R. F., & Wijdicks, C. A., 2016). Scientists have been researching its importance and fundamental role for knee

stability, with its anatomy, physiology, biomechanics, assessment, risks, and rehabilitation being of particular interest.

Injuries are divided from moderate, such as small tears, to serious, when an entire torn of the ligament is performed. ACL controls the joint kinematics by guiding the instantaneous center of knee's orbit thus, it usually is reconstructed when torn. During that process, the injured ligament is detached and reconstructed with a tendon graft from the own patient's knee or from a lifeless donor. This is a low risk reconstruction surgery performed through small openings around the knee.

Although it is a really easy intervention, this procedure is not totally defined and there are some gaps that should be solved by the scientific community. The problem when proceeding to make an ACL reconstruction surgery using tendon autografts, lies within the tendon's intrinsic viscoelastic properties, making the soft tissue graft to elongate under certain loads and, thus, not returning to the original state. Because of that, before its insertion into the patient's knee, the tendon is preconditioned (i.e. submitted to some load), in an effort to remove these properties. However, non-consensus exists within the scientific community regarding this precondition protocol. This leads to the situation where each surgeon follows the protocol that seems most appropriate for him or her, without any regulation or standardized method.

1.2. Objectives

It has been hypothesized that, given the appropriate treatment, it is possible to totally remove the intrinsic viscoelastic properties of tendons for ACL reconstruction surgeries (Jisa, K. A., Williams, B. T., Jaglowski, J. R., Turnbull, T. L., LaPrade, R. F., & Wijdicks, C. A. , 2015). Thus, the main objective of this project is to design and optimize a graft pretension protocol for the reconstruction of the anterior cruciate ligament. Therefore, Digital Extensor Bovine tendons will be used in order to test which of the proposed protocols fulfil these requirements.

1.3.Socio-Economic Impact

On average, about 3 out of 10,000 inhabitants suffer from an ACL injury every year, highlighting the importance of standardized treatment. Specifically, in Spain, a study was conducted in the year 2001 counting 16,821 annual ACL Reconstruction surgeries (Vaquero, J., Haro, J. A. C., & Campos, F. F. , 2008). This results in 4 surgeries per 1,000 inhabitants per year if all tears had been operated. Moreover, between 5% and 25% of ACL reconstructions fail, being the biological failure (12.3%) the second cause of an invalid surgery, and traumatic failures (9.8%) and ruptures in the implant (2.1%) the following ones (Batista, J., Maestu, R., Sánchez, G. G., Logioco, L., Gutman, J., & Paunovich, J., 2010). These percentages reflect a great significance of the problem.

With the development of this project, we want to assure that further ACL reconstruction surgeries do not fail because of the aforementioned tendon graft elongation. Significant improvements of the process could result in higher quality performances of the implanted grafts, reducing the need for additional reconstructions, which, in turn, allows saving resources, both financial and biological.

In this section it has just been explained the importance of finding a protocol which better eliminates the intrinsic viscoelastic property of tendon grafts for a ACL reconstruction surgery, as well as the importance that this discovery would represent in the biomechanical field.

2. BACKGROUND

Knee stability is carried out by every single structure surrounding the joint. Ligaments and muscles enable the knee to support the countless movements and activities it is exposed to. Whenever the knee is injured, the understanding of these structures should be carried out in order to be able to understand the problem.

Therefore, in the following chapter the knee joint and all the components that constitute it are going to be explained. Specifically, the anterior cruciate ligament is going to be described in detail. Moreover, it is going to be explained as well its injury and its further treatment, including the different grafts types, fixation and surgery procedure.

2.1. Joint Description

Whenever two bones or a bone and a cartilage stablish a connection by articulating with each other, a joint is formed (Anatomy and Physiology 9.1 Classification of Joints, 2019). The type of connection determines the joint's stability and movement, which are related one to another. Short movement between bones is achieved by stable joints, whether high range of movements is performed by the lest stable ones. Thus, joints are classified by two aspects: their structure and their function.

On the one hand, the classification of joints established by their structure determines the type of connection made by adjacent surfaces articulating two bones. One option are fibrous joints, where the contiguous bones attach to each other by fibrous tissue, like ligaments. The second one are the cartilaginous joints; the bones are connected by hyaline cartilage or fibrocartilage. Lastly, for the synovial joint, the articulating surfaces of the bones are connected through a synovial fluid filled cavity. On the other hand, the classification of joints established by their movement is divided into three subgroups: immobile (synarthrosis), lightly movable (amphiarthrosis) or freely movable (diarthrosis) joints. (Anatomy and Physiology 9.1 Classification of Joints, 2019).

In the case of the knee, stability and control are maintained throughout a variety of situations that imply load over the joint. Structurally it is classified as a synovial joint and functionally as a freely-movable, arthrosis joint. Synovial joints are further classified based on the

different types of movement they enable. The knee, being a rather complex joint, is formed by two articulation joints: a condylar joint between the femur and tibia, and a saddle joint between the patella and femoral condyles. These articulations allow the knee to move in the three different planes: in the sagittal plane, flexion and extension are performed; in the transverse plane, internal and external rotation can be executed and finally; in the frontal plane, varus and valgus stresses are achieved. Overall, they offer a range of motion of six degrees of freedom, as we can appreciate in Figure 1 (Abulhasan, J., & Grey, M., 2017).
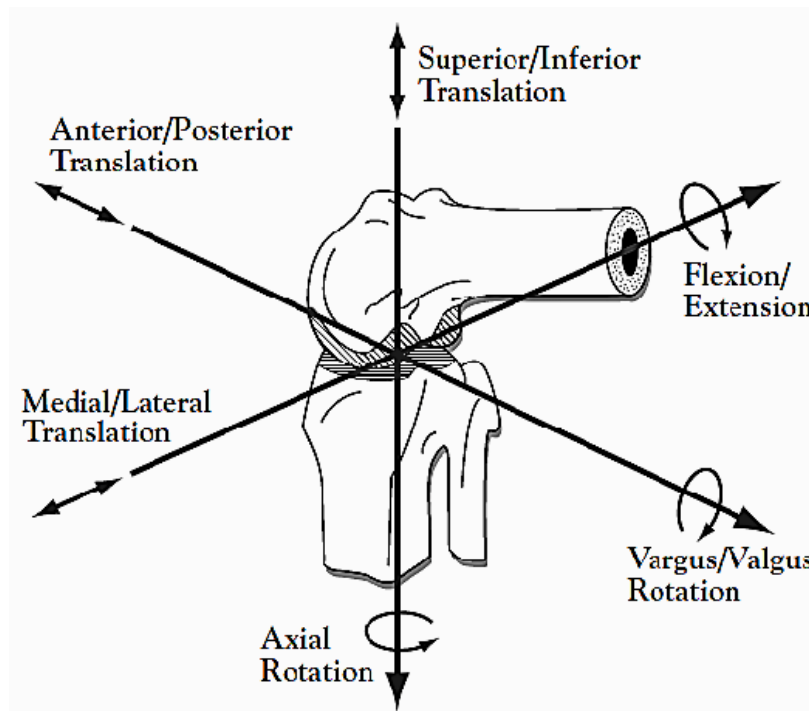
Figure 1. Scheme of the six degrees of motion of the knee (Komdeur, 2002)

Anatomically, the knee is made out of four main components: bones, cartilage, ligaments, and muscles.

2.1.1. Bones and Muscles

As mentioned before, the knee is assembled by two articulations. The one in the middle of the femur and tibia which carries most of the body mass, and the one that joins together patella and femur which enables a transfer without friction of the force caused by contraction of the quadriceps femoris muscle over the knee (The lower limb, 2019).

Additionally, muscles encircling the knee joint, as well as the ones in the hip, play an important role when talking about stability (Schmidler, 2018). The elementary objectives of these muscles is to perform the movements required for the six different degrees of freedom and collaborate with the neuromuscular system in order to control the motion of the knee (Abulhasan, J., & Grey, M., 2017). Thus, muscles around the knee can be further classified depending on the number of articulations that they interact with per movement performed. Most of them make use of just one articulation for every single movement, so they are considered monoarticular. However, biarticular muscles are also found when some of them interact in the hip joint at the same time as in the knee.

The function of the ventral part of the knee is to extend the knee and it is basically formed by the quadriceps muscles. The opposite movement, to flex the knee, is carry out by the dorsal part of the knee formed by the hamstring muscles and the medial musculature too. Additionally, the rotation of the knee is performed by the semitendinosus muscles. Lastly, in the group of biarticular muscles which act as knee flexors and hip extensors, we can find the ones in the lateral part of the knee, as well as the semimembranosus and the semitendinosus ones (Abulhasan, J., & Grey, M., 2017).

On one side, with respect to nerves, the knee joint is composed by several branches of nerves (obturator, femoral, tibial, and common fibular) which innervate the different components of the knee. On the other side, the blood supply provided by the arteries network is divided into the medial and the lateral menisci (cartilage) of the knee, being the first one higher. Thus, lateral menisci injuries require more time to be able to get over it (Abulhasan, J., & Grey, M., 2017).

2.1.2. Ligaments and Cartilage

Although ligaments and muscles around the knee operate together to surely move and stabilize the joint, it obtains the majority of its stability thanks to the ligaments while the muscles work as secondary components. This primary knee stabilizers are composed by fibrous tissue connecting bones between them (Brakeville, 2017). The knee is composed by four main ligaments, two collateral (medial and lateral) and two cruciate (anterior and

posterior) ones. Figure 2 shows the structure of the most important ligaments of the knee. Their description is given in the following paragraphs:

- The anterior cruciate ligament, ACL: Its main function is to avoid anterior tibial translation.
- The posterior cruciate ligament, PCL: It also avoids displacement of the tibia, but this time posteriorly to the femur (Anterior Cruciate Ligament (ACL), 2019).
- The medial collateral ligament, MCL: Its main function in extension and external rotation is to become tight, and oppositely to loose in flexion and internal rotation. This way it avoids exaggerated valgus stress and thus, provides stability.
- The lateral collateral ligament, LCL: It also avoids too much valgus stress and external rotation when knee flexions by running the lateral part of the knee from the femur to the fibula.

Additionally, there are smaller ligaments surrounding the joint as the capsular, anterolateral, arcuate and posterior oblique ligaments. All together, they maintain the stability of the knee (Types of Knee Ligaments, 2018).
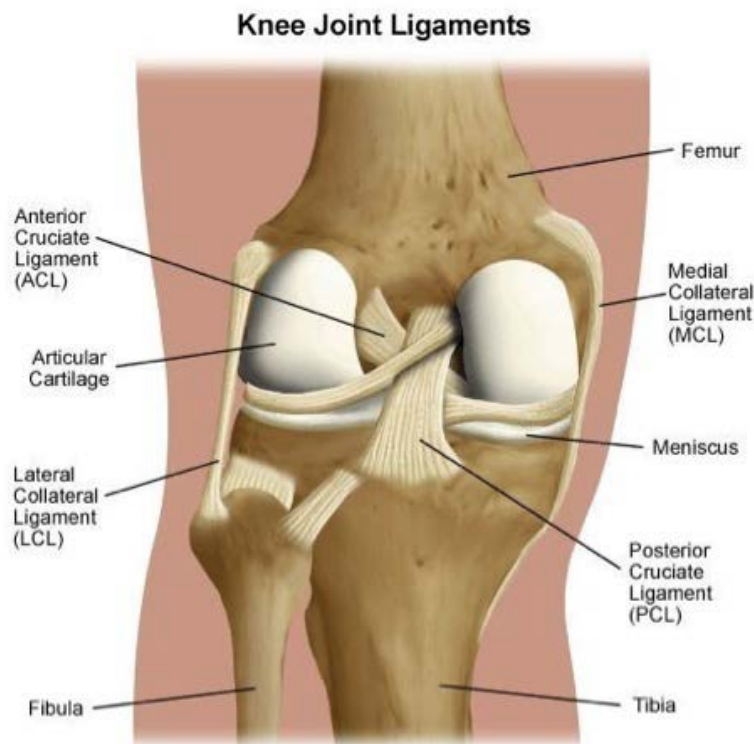
Figure 2. Left Knee anatomy – Ligaments (Types of Knee Ligaments, 2018)

Among all of them, the ACL is described as the main stabilizer of the knee, considered to proportionate around the 85% of the joint stabilization and thus, allowing for gentle flexion and rotation of the knee (Abulhasan, J., & Grey, M., 2017).

2.2. ACL Definition

Displacement of the tibia, anterior or rotationally to the femur, is resisted due to this important ligament in the knee, the ACL. As illustrated in Figure 3, it is mainly composed by two essential bundles, the smaller one named anteromedial (AM) and the larger one, posterolateral (PL) (Anterior Cruciate Ligament (ACL), 2019). They are called according to their insertion into the femoral origin. Thus, on one hand, the anteromedial bundle occupies the proximal and anterior position, relaxing when the knee is extended and tightening when flexed, thus it avoids anterior tibial translation. And on the other hand, the posterolateral bundle occupies the distal and posterior location, and behaves oppositely to the anteromedial one, contributing to knee stabilization when full extended (Petersen W, 2017).
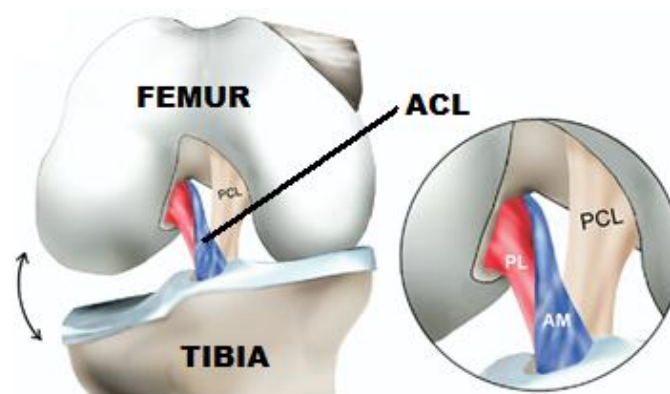
Figure 3. ACL composition. Anteromedial (AM) and Posteromedial (PM) bundles are marked. (Karthi, 2015)

Several nerve fibers arrive to the anterior cruciate ligament through the tibial nerve, specifically, through its dorsal articular branches. They infiltrate the capsule of the dorsal articulation, and spread over the ligament together with the synovial and periligamentous vessels (Anterior Cruciate Ligament (ACL), 2019). The 'ACL reflex' is an event which refresh muscle programs by the activation of these nerve fibers. People with an injured ACL suffer from weaker quadriceps femoris muscles contraction and exertion due to the lack of this event's feedback.

Moreover, regarding the blood supply of the ACL, it is maintained through the lateral and medial inferior geniculate artery which supplies with blood both, distal anterior and posterior cruciate ligaments. However, within the ligament, the quantity of blood vessels is not homogeneous, having one avascular region located within the fibrocartilage of the anterior part. This collaboration of low vascularity plus the existence of fibrocartilage makes the healing of a supposed ACL injury harder (Petersen W, 2017) .

2.3. ACL Injury

Athletes, especially those who practice skiing, football or basketball, are the type of people with more probabilities of suffering from an ACL injury. It is usually generated by a precipitated direction or speed modification having the foot rigidly leaned on the floor (cut-and-plant movement). Fast breaking moments, as well as falling from a jump, pivoting, twisting and direct colliding the knee are movements also associated with anterior cruciate ligament tears. However, contact collision ruptures are not as usual as the non-contact ones, representing the last ones the 70% compared to the other 30% of the first ones (Geeurickx, S., Campion, K., & Sareen, A., 2019).

Whenever a patient suffers from an ACL injury, knee instability is first experienced. Later, a cut down of the daily movements is produced, consequently implying a poor quality of life. Then, risk factors which may end in anterior cruciate ligament injuries are further divided into environmental factors and anatomical factors, explained in section 2.3.1.

2.3.1. Risk factors

Affected people may have suffered an ACL tear due to internal or external hazard factors, which are later described (Beynnon, B. D., & Shultz, S. J. , 2018).

External Factors

- Sport match competence: players are subjected to larger possibility of suffering an ACL tear while playing a game than training.

- Shoes and game field/court: although rising the rate of friction between the footwear and game field/court may correct sports' realization quality, it may have the potential to rise as well the possibilities of suffering from a ligament tear.
- Protective accessories: it is not clear whether using functional bracing conserves or not an ACL-deficient knee (Grade III sprain, later explained in section 2.3.2.).
- Weather: it possesses a direct consequence on the player suffering an ACL tear due to the possible mechanical reaction that may happen between the shoe and the game field/court due to adverse meteorological conditions.

<u>Internal Factors</u>

- Anatomy: some people may have more probabilities of suffering an injury due to a not normal posture or a lower limbs disposition which rise ACL strain values.
- Fatigue and loss of concentration may cause ACL injuries too.

In relation to biomechanics, when varus and internal rotation movements, the ligament is placed at higher risk. An adverse lower limb alignment followed by poor muscle protection and neuromuscular control would make the reaction of the ground to stress the knee joint during a cutting movement. This would lead the compressive load to laterally displace the femoral condyle and anteriorly shift the tibia as well as rotate it internally, hence producing an ACL injury as shown in Figure 4.
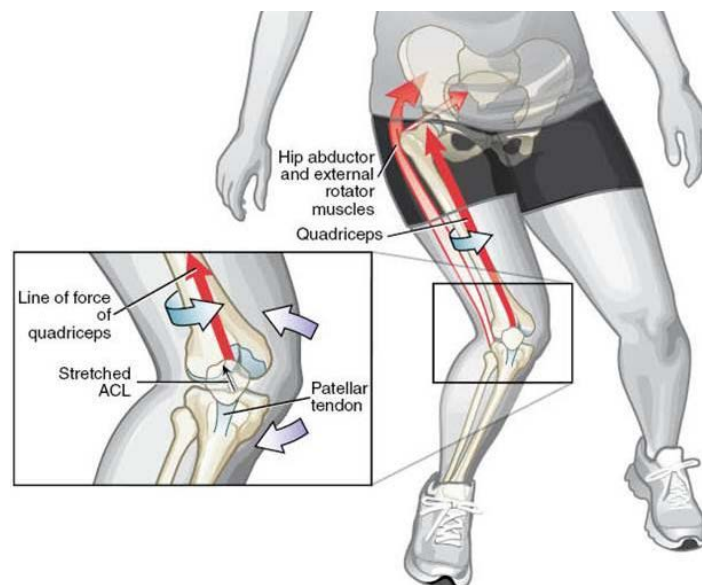
Figure 4. ACL Injury Movement (Musculoskeletal Key, 2016)

2.3.2. Grades of Injury

ACL injuries are classified in three groups depending on their level (Geeurickx, S., Campion, K., & Sareen, A., 2019).

- Grade I sprain: ACL fibers are not torn but just stretched thus, the stability of the knee is not achieved during daily exercise. It is also observable a small tenderness and swelling.

- Grade II sprain: Partial or (bleeding) incomplete torn of the ligament's fibers, thus making the affected person to feel pain and an unstable condition. Tenderness and swelling are also present as well as loss of function.

- Grade III sprain: Complete torn of the ligament's fibers is performed, hence the ACL is ruptured and it cannot dominate the movements of the joint. The patient's knee is usually tenderness and swelling, but the pain is not as big as is should be according to the injury level. Haemarthrosis appears in the first hours after the incident.
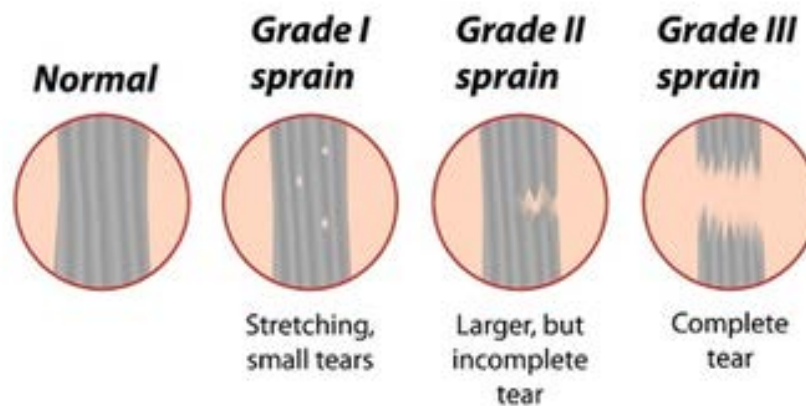
Figure 5. Grades of ACL injury (Tendon Injuries, 2019)

Moreover, children may suffer from a less probable fourth type of injury called anterior cruciate ligament avulsion. It happens whenever the ligament is totally torn from any of the bones where it is attached.

2.4. ACL Injury Treatment

As it is known already, the ACL plays an important role by stabilizing the knee joint and when injured, it becomes unstable and more damaged over time. However, surgeons may choose to reconstruct the ACL or not, depending on the factors enumerate next and described in more detail in Physiopedia (2019):

- Conservative treatment, meaning this not performing any surgery. It is chosen when the affected person is over the 35 years of age, has minimal anterior tibial displacement, has no anymore injuries in the zone, or does not perform a high exercise demand life. However, the long term quality of life is not the best one.
- Reconstruction treatment, meaning this performing a surgery. It is chosen when the affected person is below the 25 years of age, has an important anterior tibial displacement, has additional injuries in the zone, or requires a high performance life level.

The criteria above explained describes the majority of the cases of ACL injuries. Thus, treatment may be chosen individually for each patient by a specialized surgeon.

2.4.1. Grafts Types

Various tissues/grafts can be used to anatomically reconstruct the torn ACL, but there are two main types: autografts and allografts (Anterior Cruciate Ligament (ACL) Reconstruction, 2019).

<u>Autografts</u>

This kind of grafts are taken from the body of the affected person. Some examples are parts of the knee's extensor mechanism, patellar tendon, iliotibial tract, semitendinousus tendon, gracilis tendon, and menisci. The most common ones are described in the following paragraphs, as well as shown in Figure 6.

- Patellar Tendon: Around a third portion (9 or 10 mm) of the middle part of the patellar tendon is extracted as well as both pieces of the two bones where the tendon lies. Advantages of this graft type are its high performance replacing the native ACL as

well as their similar lengths. The bone blocks extracted alongside the tendon are used to serve as a join points where the ACL used to attach with the femur and tibia. This method allows a fast joint recuperation (6 weeks) due to the easier, earlier and stronger bone to bone, compared to soft tissue to bone, healing. But, side effects may appear as site morbidity or ventral joint pain. Moreover, Quad-Patellar autograft is also used sometimes.

- Hamstring Tendon: Two bundles are summed up into a single graft making a more powerful and stiffer conformation when compared with the other graft type just explained. Over time it has become more and more popular due to its technique improvements. Advantages are the lack of anterior joint, as well as post-surgery, pain and the small incisions needed to be performed in the patient's knee. However, especially in affected people with small muscles, the graft harvesting may be more difficult as well as its fixation into the knee joint needing the use of further material. Moreover, and as mentioned before, the healing of the soft tissue graft after its implantation will take longer (10/12 weeks) due to the soft tissue to bone fixation.
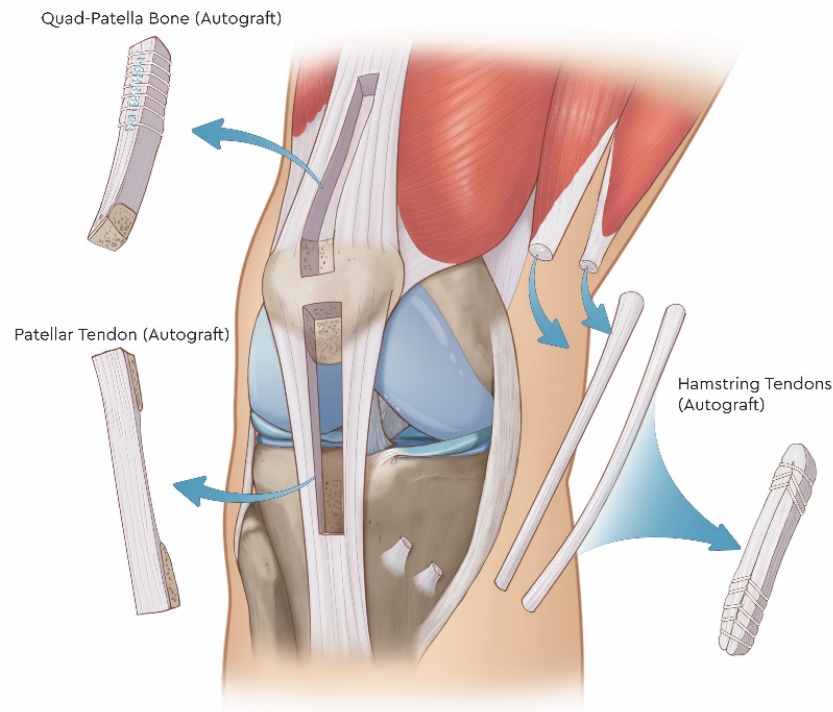
Figure 6. Possible Autografts obtainment (ACL Graft Options, 2019)

<u>Allografts</u>

This kind of grafts are taken from corpses or a donor tissue. Some biomechanical studies show that allografts are softer than patient's soft grafts. However, sometimes low allograft stiffness is enough for many affected people due to their little daily exercise.

Although, an allograft may not be the best choice for patients who will play highly demanding games, it does present a number of advantages. Namely, it presents a decreased duration of the surgery; It also eliminates the need to remove other tissues; The incisions performed are smaller and the post-operative pain is reduced when compared to reconstructions using autografts.

However, this kind of grafts also present some risks as disease transmission, decrease of tissue strength due to death of living cell during graft preparation, and longer soft tissue graft to bone healing period of time. Furthermore, allografts are not widely accessible as they are the most expensive ones.

Although autografts are the most frequently employed grafts (patellar bone & hamstring), no consensus has been made in the actual literature concerning which one provides the best properties. Thus, each doctor should choose a specific graft, depending on the best advantages and disadvantages for the patient.

There are also other kinds of grafts, like the xenografts taken from animals which are related with a great complexity coefficient, or the synthetics grafts, divided into biodegradable, permanent prostheses, and ligament augmentation devices. However, they are not used as frequently.

2.4.2. Fixation Types

An ideal fixation has to be strong enough to avoid failure, stiff enough to restore knee stability, and secure enough to avoid slippage from the fixation points. In the case of tendon graft fixation into the knee, the ideal one should also be biocompatible, safe, magnetic resonance image compatible, and allow for an easy revision. Taking into account these parameters there are two main fixation types: aperture fixation, which is at joint level, and suspensory fixation, which can be either cortical or cancellous (Martin, 2002).

On the one hand, an example of aperture fixation are the interference screws, shown in Figure 7. They are characterized by the amount of which the diameter of the screw exceeds the gap between the grafts and the tunnel. Interference screws should be at least 1mm wider than the tunnel diameter for soft tissue grafts. Nowadays, an atractive method consisting of bioabsorbable screws, which do not need to be removed, exists. However, disadvantages are that they might break during insertion, tissues reacting against them, and some fixation might be lost due to partial degradation. Until these issues are mostly resolved, they will remain in their status as under development.

Figure 7. Interference screws used for ACL fixation ((Chen, N. C., Brand Jr, J. C., & Brown Jr, C. H., 2007)

On the other hand, suspensory fixation-cortical methods are usually performed using endobuttons and thighropes. Endobuttons (Figure 8b) belong to the first generation of suspensory fixation and requires two parts: the insertion drilled to the diameter of the grafts and the connection of 4.5mm of diameter in the femoral tunnel. Tightropes (Figure 8c) belong to the second generation of suspensory fixation. When using this method, the loop length is reduced after flipping by tightening the rope and it also allows full length filling of graft part of the femoral tunnel.
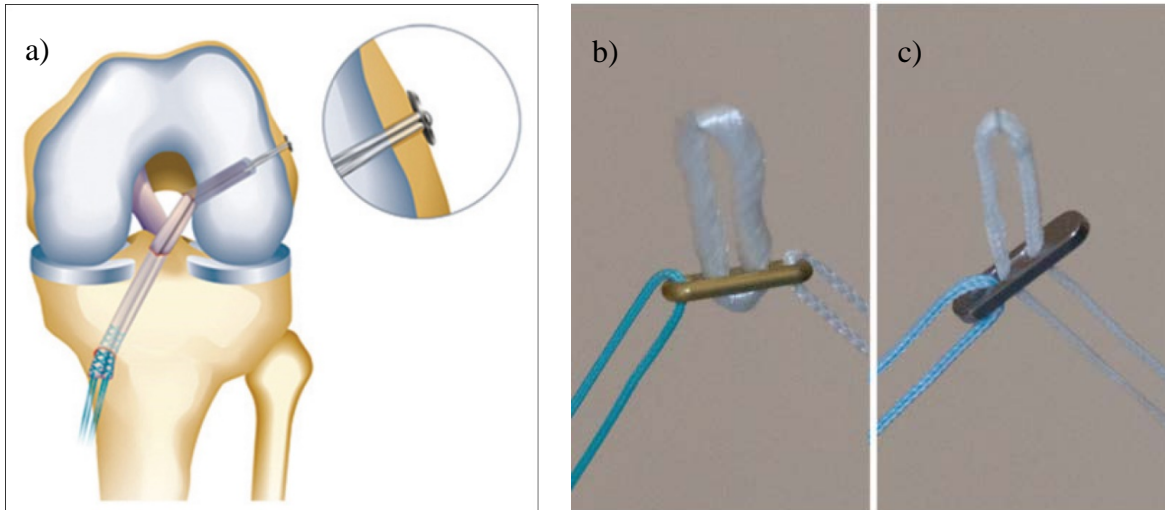
Figure 8. a) Suspensory fixation-cortical method representation (Mousavi, H., Maleki, A., & Nobakht, A., 2017). b) Endobutton representation and c) Tightrope representation (Petre, B. M., Smith, S. D., Jansson, K. S., de Meijer, P. P., Hackett, T. R., LaPrade, R. F., & Wijdicks, C. A., 2013)

Finally, it has to be taken into account that, in the early postoperational period, fixation is the weakest link where tibial fixation is at greater risk of failure. Thus, clinical results of various methods have to be taken into account to choose the most adequate for the patient.

2.4.3. ACL Reconstruction Procedure

A reconstructive surgery treatment will restore stability and will maintain the full active range of motion as well as resisting anteroposterior translation and rotational displacement. There are as many ACL reconstruction procedures as soft grafts types, fixation methods and surgery techniques that can be used. Due to the ACL's complexity, the placement of the ligament should not be modified during the surgery (Scheirs, D., 2019).

The intra-articular procedure should take place 10 weeks after the injury occurs. The main steps of the surgery, shown in Figure 9, are following explained:

1. Graft selection: As explained before, there are different types. The most commonly used autografts are patella grafts and hamstring tendon grafts (semitendinosus and gracilis).

2. Diagnostic arthroscopy: It is performed at the same time of any necessary meniscal repair. When the injury consists on a partial tear we have to be more careful as a displaced bucket could handle a complete tear. It is shown in Figure 9A.

3. Graft harvest: A clearly marked small incision from distal patella extending to the tibial tubercle is performed in order to obtain the soft tissue graft. To be able to perform this procedure an oscillating saw blade is usually employed, obtaining finally a triangular graft. It is shown in Figure 9B.

4. Graft arrangement: The graft obtained from the knee of the patient is filled inside a tube of 10mm length, later used in the femoral hole and another tube of 11mm length, this time for the tibial tunnel.

5. Intercondylar notch arrangement and notchplasty: Bone is hostile degraded by using an arthroscopic shaver. Later, if needed, more adjustments can be performed. It is shown in Figure 9C.

6. Installation of the tibial tunnel: The tibial tunnel should be made so the middle third part of the tendon graft resides inserted and does not make trouble in the interference with the roof of the intercondylar notch.

7. Installation of the femoral tunnel: After the installation of the tibial tunnel, the femoral tunnel is calculated in order to occupy another third of the soft tissue thus, creating a situation the most similar to a native ACL as possible.

8. Graft installation into the joint: After both tunnels have been performed, the graft is inserted with the help of an arthroscopic grasper. It is shown in Figure 9D.

9. Fixation of the graft: A nitinol pin is afterwards utilized to make firm the tendon graft within bone and tunnel. The amount of tension created over the graft in the installation process has direct causes over the rehabilitation process, and it is going to be one of the parameter further discussed. It is shown in Figure 9D.

10. Closure of the wound: Before the closure of the wound, the graft place is injected with 0.25% Marcaine. Then, with the knee in flexion, the wound is closed by using absorbable sutures. It is shown in Figure 9E.

In Figure 9, we can see a graphical summary of the ACL reconstruction procedure.
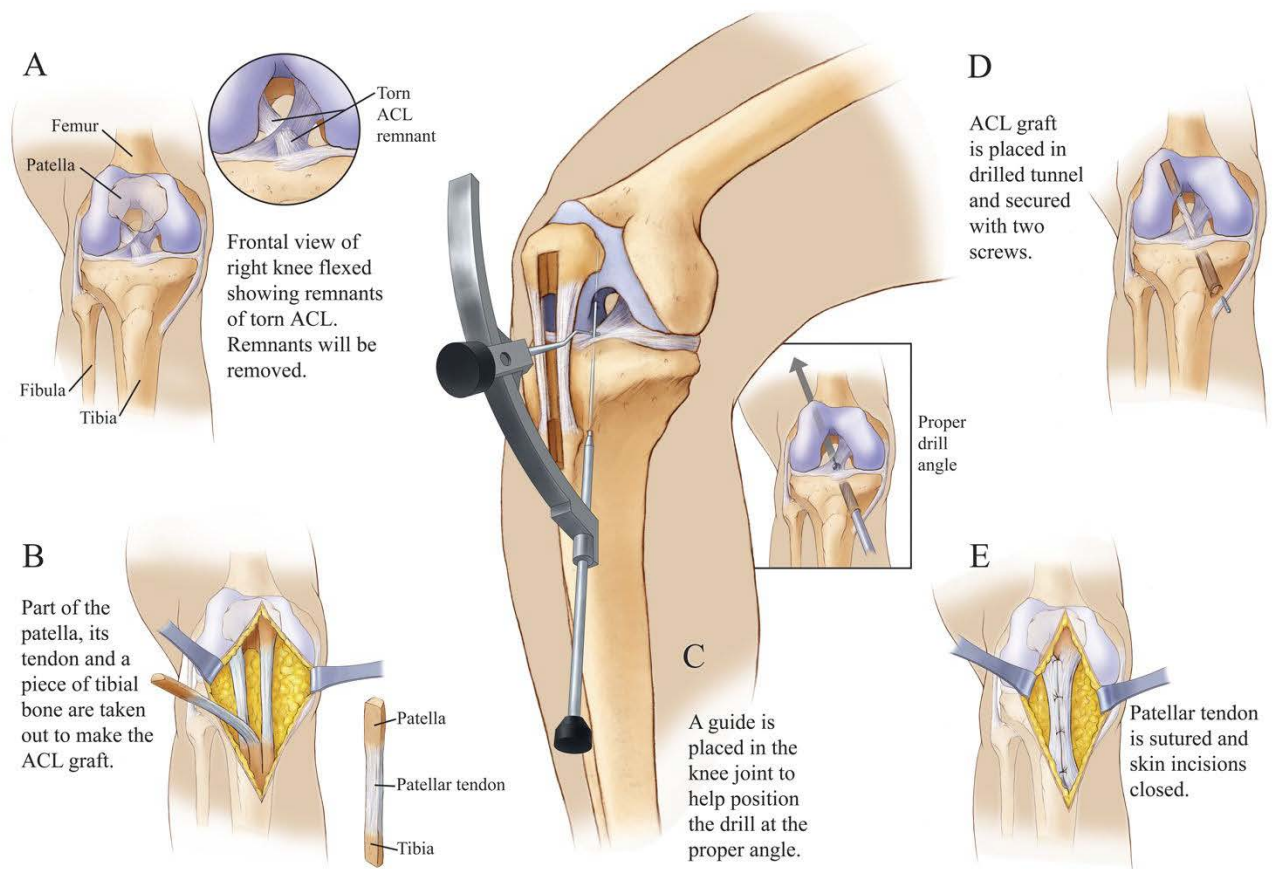
Figure 9. Anterior Cruciate Ligament (ACL) Reconstruction (Anterior Cruciate Ligament (ACL) Reconstruction, 2013)

In this section, it has been described the important role that the knee joint plays in our body, and so in our daily life. Specifically, the anterior cruciate ligament has been described in detail due to its importance when talking about knee stability. Thus, when damaged it supposes a big issue that should be solved, usually through an ACL reconstruction.

3. LITERATURE REVIEW

Before any experiment took place, a literature review was performed in order to discover the actual knowledge that the scientific community had already achieved. There were included several paper which main topics were to design a protocol for the ACL reconstruction surgery. Based on the current situation, a hypothesis was elaborated in order to proceed with the experiment.

3.1. Current situation

Tendon graft elongation, due to its viscoelastic graft properties, after its implantation into the knee is known as one of the main problem when talking about ACL reconstruction. Preconditioning protocols have been designed over time to reduce this effect which makes the tendon relax when submitted to stress. However, there is not consensus in the scientific community on which protocol is suited best. In this section, to gain a comprehensive overview about the current state of research, different articles and studies which have been conducted with respect to this question will be reviewed.

The first paper to be reviewed was written by by *Nurmi et al.* (2004). In their project, they made used of forty-two tendon grafts. Specifically, anterior tibialis tendons were subjected to two different protocols, cyclic and isometric preconditioning, plus the control, corresponding to no preconditioning at all to see whether preconditioning the graft is useful or not at all. The first two groups constituted the actual protocols proposed, where tendons were preconditioned. On the one hand, by designating a fluctuating load applied in several successive cycles (cyclic protocol) and, on the other hand, by designating a constant loading (isometric protocol). Grafts were performed these protocols before their fixation into the patient's knee.

Before any test was conducted, the authors decided to define some terms that are going to also be crucial during the development of our project. Firstly, we have *pretensioning,* defined as any load applied to the graft, commonly in a graft board, before its implantation in the patient's knee. Then, there is *preconditioning* (cyclic or isometric), which is the load applied to the soft tissue graft once it is fixed in one of the bone tunnels, usually the femoral one, but

not in the other one. The last specific term that they introduce is the *initial tensioning,* which defines the load applied to the tendon graft just before its final implantation into the tibial tunnel.

In the article, the pretensioning consisted of applying 15 minutes of 88N of tension to the grafts. Afterwards, the tendons were loaded into the mechanical testing machine and tested following the three protocols previously described. Lastly, to fix the grafts in the second tunnel, an initial load consisting on 80 N was performed, as *Yasuda et al* (1997) have proposed.

One of the purposes of the article was to make some research on the results of different preconditioning protocols to the tendon grafts stiffness after their implantation, by using interference screws, into the knee. After all, they determined that such protocols performed in the research did not enough removed the intrinsic viscoelastic property of the soft tissue grafts. However, it was defined that the initial tensioning significantly reduced the elongation after the reconstruction. Finally, they made an agreement deciding a high range of tension for safely fixing the grafts, although no consensus was made determining the best pretensioning, preconditioning and initial tensioning.

Another group of researchers that have also been investigating on this topic are *Jaglowski et al.* (2016). The goal of their project was to create a specific preconditioning protocol that will lead to a tendon graft the most similar to the former ACL as possible. With this objective in mind, they used bovine extensor tendons for each one of the different six proposed protocols. To obtain a n=6 they used thirty-six grafts. Half of the protocols were cyclic (10 cycles at 0.5 Hz between 10–80, 100–300, and 300–600 N), while the other half protocols were static (20 s at 80, 300, and 600 N) loading protocols. The way they defined the proposed protocols is similar to the test proposed in *Nurmi et al* (2004)., as cyclic and isometric preconditioning protocols are used. During the experiment, every single tendon was placed into a dynamic tensile machine for its vitro testing.

Over the course of the tests, graft stiffness values were calculated. The results of the protocols consisting on cycles from 300 to 600 N and the ones consisting on static loading 600 N obtained the highest stiffness values after the preconditioning was performed. A statistical equivalence was also found when comparing these methods to the stiffness of the former

ligament. Furthermore, elongations were also calculated. Furthermore, as a result, authors found that these preconditioning protocols produced an important reduction in the final elongation of the grafts compared to the other protocols which used to apply less load to the tendons.

Finally, as *Nurmi et al.* (2004) concluded too, actual preconditioning protocols applied over tendon grafts do not totally eliminate the viscoelastic property of tendons which implies a further elongation of the grafts after its implantation, thus not simulating the natural behavior of the ACL. But as seen in the results, higher load minimizes post-surgery elongation, thus showcasing the effectiveness of preconditioning the soft tissue grafts. However, optimal preconditioning protocols have not been defined yet.

In addition, *Lockwood et al.* (2017) try to define an optimal protocol for the preconditioning of the tendon grafts before its implementation in the patient's knee. By using semitendinosus–gracilis tendon grafts, which were bended to form a stronger conformation, they performed two precondition protocols implying a static high load (600 N). The first protocol consisting of a short period of time (20 s), and the second one of a larger period of time (15 min). Later, these tests would be compared to both, a protocol currently being carry out in surgeries (89 N for 15 min) and as it should always be, a protocol implying no preconditioning at all to work as control group. To conduct the tests, the tendons were securely placed into a servohydraulic dynamic tensile machine, where the in vitro experiments took place.

The "89 N for 15 min" and "600 N for 20 s" procedures produced comparable elongations during the simulated postoperative rehabilitation. The "600 N for 15 min" protocol derived in significantly less elongation at the cyclic loading than both the control group and the "89 N for 15 min" protocol. Hence, preconditioning load value and the period of time being applied, when increased, considerably resulted on less elongation of the graft after its implantation. It has also been observed an inverse relationship between elongation during preconditioning and its corresponding elongation after the surgery. In conclusion, it has been determined in this article that preconditioning of the tendon grafts is beneficial when performing a reconstruction surgery, both reducing post-surgery elongation and simulating native ACL stiffness.

Finally, the last group of authors that will be taken into consideration for the development of this project is going to be *Jisa et al.* (2015). The aim of their research was to look for a common denominator in actual literature, concerning the mechanical results and further surgical improvements, that best pretensions and preconditions tendon grafts for ACL reconstruction surgeries. This protocol, as explained above, has as a goal to find a protocol which achieve full viscoelastic property elimination from grafts, to avoid its relaxation and, therefore elongation, after an ACL reconstruction surgery. Just articles defining or contrasting different pretension and precondition protocols of tendons were eligible for admittance in the comparison. Five articles met the required criteria, being four of them in vitro biomechanical research and the other one, a histological study of affected person's tissue.

The usage of tendon grafts, specially hamstrings, for this type of surgery has raised in the last years and proved to be the chosen before other types of grafts in the majority of the worldwide performed reconstructions surgeries. For pretensioning techniques, although the majority of studies defined one of their pretension protocol to be in the window of 80-89 N, the totality of them can be placed at any point between 80 and 500 N. With respect to duration, protocols describing pretension were usually longer (from 30 s to 15 min) compared to standardized preconditioning protocols which were shorter (from 100 s to 5 min for static protocols and 100 s for cyclic protocols).

The most significant result of this article was the finding of similarities among different protocols defining pretension and precondition. However, the number of articles reviewed was not enough taking into account the variably of this kind of results. Thus, no consensus can be achieved, nor in magnitude or modality. Regardless, actual studies show the significant benefit effects of performing a protocol over the tendon grafts, reducing considerably their relaxation as well as better conserving them after the ACL reconstruction surgery.

3.2. Hypothesis

After the analysis of these articles, where different pretension, precondition and initial tension protocols were applied, we have come up with an own hypothesis.

First, it has to be distinguished between two possible tests which can be used to pretension the grafts. For the first test, the displacement is constant which leads to the loss of stress in the tendon to be the important parameter. The second test, on the other hand, is conducted in a way, which keeps the load constant, thus, the elongation becomes the relevant parameter. It has been seen that in an operating room the second type of test are not possible to be conducted, since the graft board used to pretension the grafts cannot be set to keep the force constant. Due to this fact, constant displacement tests have been chosen to pretension the soft tissue grafts. Furthermore, and with the same reasoning of being able to reproduce the method in a theater, we have decided to not make use of cyclic tests for the pretensioning, as they have shown low reproducibility.

After the pretensioning is applied to the tendons, several authors have agreed on simulating a transfer of the graft from the board to the knee by performing a discharge (Pilia, M., Murray, M., Guda, T., Heckman, M., & Appleford, M., 2015). However, an initial tension of 80 N as proposed by *Nurmi et al.* (2004) is an unrealistic force to be exercised during a surgery. Therefore, for this project, a more reasonable force of20 N (an equivalent of 2kg) will be used. This test is realized using a constant force, as it is the one being applied by the surgeon.

Finally, to be able to determine how good the proposed protocols perform, and as suggested by *Jaglowsi et al.* (Jaglowski, J. R., Williams, B. T., Turnbull, T. L., LaPrade, R. F., & Wijdicks, C. A., 2016) and Lockwood (Lockwood, W. C., Marchetti, D. C., Dahl, K. D., Mikula, J. D., Williams, B. T., Kheir, M. M., ... & LaPrade, R. F., 2017), a cyclic test is going to be conducted to all the tendon grafts after each protocol. This way, the process of rehabilitation of the patient's knee will be simulated. The lowest graft deformation in this last step would correspond to the best protocol.

In this section it has been described how several authors have achieved the problem proposed. Different points of view have been taken into account in order to develop a hypothesis that will reach the goal proposed, to design and optimize a graft pretension protocol for the anterior cruciate ligament reconstruction.

4. MATERIALS AND METHODS

In this section, the materials, processes, and protocols used and applied during the experiments are introduced.

4.1. Materials

Between the materials used in this experiment we can find the machine employed, the tendon grafts tested and the additional material needed in order to carry out the tests. All of them will be further described.

4.1.1. Machine

The testing of the different protocols took place in the Biomechanics Laboratory of the Mechanics Department at Las Palmas de Gran Canaria University. This laboratory was conditioned with a security chamber where the experiments took place. A Hydraulic Scale Fatigue Machine of 5 KN was used, with its characteristics being shown in Table 1. This tool is commonly known as Universal Dynamic Machine, as it allows to measure multiple material properties by submitting them to tensile and compression tests. It is actuated by a hydraulic system; thus proper refrigeration of the machine is required.

Table 1. Characteristic of the Hydraulic Scale Fatigue Machine of 5KN

Name	Hydraulic Scale Fatigue Machine of 5 KN
Model	EFH/5/FR
Brand	Microtest
Year of manufacturing	2003
Capacity	5KN
Serial Number	R5/034

For typical fatigue applications or dynamic behavior, the machine can be modified with multiple options like test accessories (jaws, tools, etc.) or transducers (force, deformation, etc.). The system of servo control and measurement SCM4000, developed for dynamic tests, offers a high degree of customization to the machines, with the possibility of carrying out tests with different functions (ramp, sine, triangular, etc.) as well as different modes of acquisition and treatment (Máquinas de Ensayo Servohidráulicas Serie EFH, 2019).

The EFH fatigue machine is composed of:

- A four column frame, usually with hydraulic system lifting of the upper bridge and locking or unlocking hydraulic-mechanical thereof on the chromed guide columns. The control of the positioning system of the upper bridge is electric.

- A servo cylinder with its equipment: double acting dynamic actuator / double shank, with built-in servo-valve block, position sensor, clamping flange, limit switches to name the most important ones.

- A hydraulic power station, whose capacity is determined by the dynamic performance of the machine: actuator speed, range of forces, etc.

- A set of commands and controls that include:

  o Electrical control and installation: drives, contactors, power stage of solenoid valves, alarms, signaling, signal connection, general feeds, etc.

  o Transducers for measurement and control: force, displacement, deformation, etc., as well as their corresponding electronic packaging.

  o Control, measurement and acquisition system: handled from a computer through software developed for this purpose (SCM4000) and adaptable to user needs.
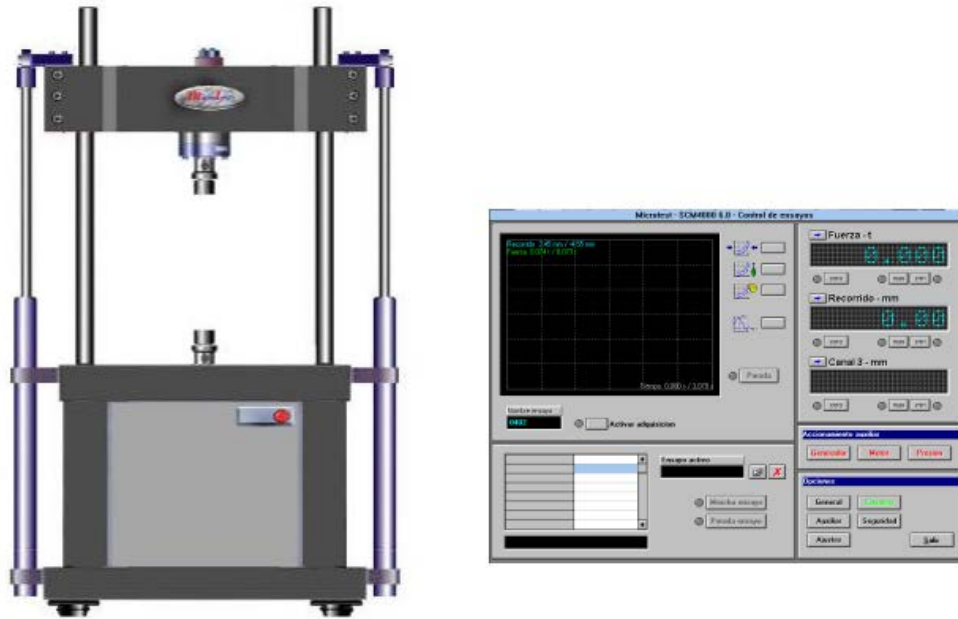
Figure 10. Example of EFH series Machine and Control System. mic (Máquinas de Ensayo Servohidráulicas Serie EFH, 2019)

Furthermore, a special grip system for the tested biologic material was incorporated into the machine, designed by the supervisor of this project, Oscar Martel. Toothed jaws, as seen in Figure 11, were designed to be placed in the machine to ensure the grip of the tendons that are going to be housed inside. Two toothed types jaws were tested, with the small-toothed ones proving to provide better grip. Hence, they were chosen to be used subsequently for the tests.

Figure 11. Specific jaws design for the Project. (Own Illustration)

4.1.2. Tendon Grafts

Tendon Grafts are required to design a protocol for the preconditioning of the soft tissue grafts for the ACL reconstruction in the human body. Since it is sought to conduct an experiment which represents an actual surgery the closest possible, the best option would consist of using tendons from a human cadaver. However, there were several reasons to prefer animal specimens instead of human ones. On the one hand, animal specimens offer the possibility of immediately freezing the tendons after their extraction. On the other hand, the age of the donor is less and more uniform than compared to possible human donors. The majority of human tissues obtained are from relatively old people, hence they behave different than young human tissue and, therefore, do not correctly simulate the behavior of these tendon grafts in a regular age patient. Lastly, it would be the difficulty to obtain human specimens. Due to all these aspects, many researchers, including us, have decided to use animal tendons, highlighting cattle and swine. In this very specific experiment, we have opted to use Common Digital Extensor Bovine tendons (Figure 12), because its similarities to the human patellar tendon usually used in ACL reconstructions.

Common digital
extensor tendon
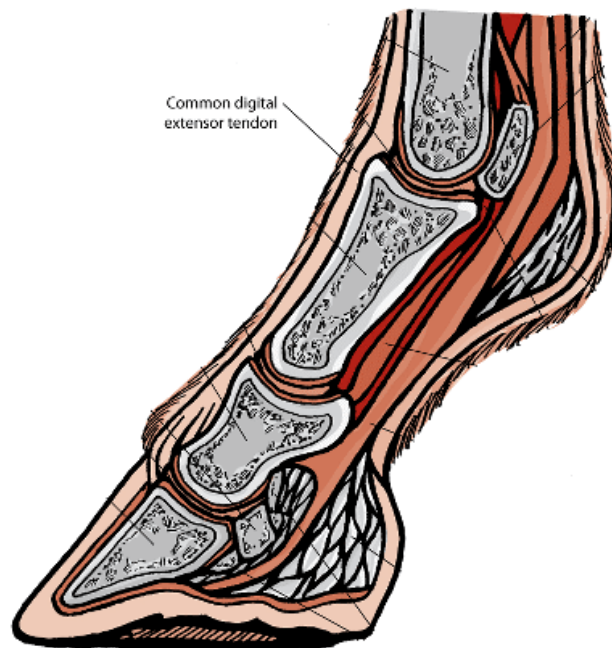
Figure 12. Anatomy of a bovine Leg. Common Digital Extensor Tendon at the upper left corner of the figure. (Reeves, 2014)

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The bovine lower limbs were obtained from the 'Matadero Insular de Gran Canaria' in compliance with all existing rules, later explained in section 7.1. These were taken right after the animals were slaughtered for other purposes. Once the legs were taken to the Laboratory of the University, the group of the three Extensor Digital Bovine tendons was extracted from every single limb and then, the tendons were separated into single. This procedure is shown in Figure 13.

Figure 13. Common Digital Extensor Tendon Extraction Process. (Own Illustration)

Afterwards, they were measured, both the width and the length. The width was measured by using a handmade calibrator and the length was measured by using a ruler (Figure 14). The exact length was going to be measure later, just before each test, so this first measurement was just to know what was going to be frozen.
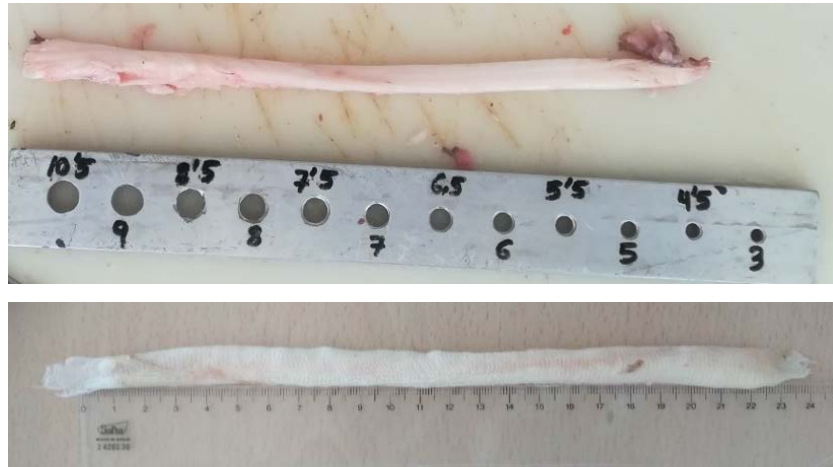
Figure 14. Tendon grafts measurement. (Own Illustration)

Moreover, they were marked, dated, and frozen at -20ºC. For the purpose of the experiment, 60 tendons of 6mm of width were used. This implied about 20 bovine limbs.

4.1.3. Additional Material

Although the Universal Dynamic Machine and the tendon grafts were the main components used for this project, there were also other materials needed. They are described below.

- Needle holder: also called needle driver. It is a surgical instrument commonly used by surgeons to hold a suturing needle to be able to close wounds (Chu, C. C., Von Fraunhofer, J. A., & Greisler, H. P., 1996). In this case, we used it to drive the graft through the jaws to be able to fix them in the machine.

Figure 15. Example of Needle Holder. (Own Illustration)

- Normal saline: It is a commonly used solution composed of 0.90% w/v of NaCl, 308 mOsm/L or 9.0 g per liter (Saline_(medicine), 2019). Its main applications are in the field of medicine. In this case, it was used to keep the grafts hydrated so they can keep their biological properties. Additionally, it shares characteristics with the synovial fluid inside the knee. Control of the tendon humidity was secured with high frequency, through sensing testing.

- Measurement elements: A regular ruler and a caliper were used to determine the length of the different tendon grafts. Firstly, the ruler was used, to measure the total length of the grafts in a roughly manner and then, once implemented in the machine, the free length of the tendons was measured with the use of an electronic caliper (Figure 16). The error proportionated by the ruler is 1 mm and the one by the electronic caliper is 0.005 mm.
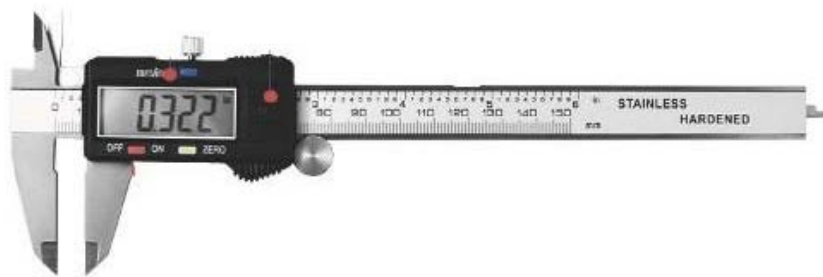
Figure 16. Example of Caliper. (Ryan, V., 2009)

Gloves, gauzes and other materials for sterilization were also utilized.

4.2. Methods

The methods consisted on the different tests followed in this experiment. The reproduction of the ACL reconstruction surgery into the hydraulic machine will be explained. Furthermore, the different protocols proposed will be described too.

4.2.1. Test types

The tests carried out in this project can be divided into two main groups. The first group is constituted by the pull-out tests, where the grafts are submitted to a constant load or

progressive elongation. The essay velocity in these tests is the one at which the element being tested is deformed. It has for unit mm/s or mm/min. The second group is the cyclic-load tests, where the grafts are loaded between two predetermined values. These ones are also called dynamic tests.

After defining the basics of any stress-elongation test, the specific steps of the project will now be determined. As mentioned before, it is sought to simulate a real ACL reconstruction protocol and, in order to achieve this goal, several steps that will be followed as if it was a real surgery were defined.

- Pretension: A pull-out test of progressive elongation will be applied to the tendons. As will be seen later, the pretension will consist of several re-tensions, to achieve that, after the determined load of each protocol is achieved, the position will be held for the corresponding time. After this time, a progressive elongation will be applied and held again. This happens as many times as each protocol requires. The step will simulate the pretension that can be performed clinically in a graft board (Figure 17), a table for the in vivo preparation of tendons.

Figure 17. Graft Board used for Tendon Grafts Pretension.Protocols. (Acufex Graftmaster III, 2019)

- Transfer to the patient: To simulate this process, a compression discharge test is performed until the force acting on the tendon is zero. The graft is left at rest for 5 minutes, a time that has been clinically estimated to be the duration of the tendon's extraction from the preparation table to its placement into the patient.

- Initial tension: An initial tension is applied to the soft tissue grafts. It corresponds to the one applied to the grafts just before being placed into the patient's knee.

- Post-surgery immobilization: Once the tendon is fixed in the knee of the patient, a process of postoperative immobility is simulated. It has been chosen to last for 30 minutes of rest in the position acquired after the initial tension applied.

- Rehabilitation: it is intended to simulate the process of rehabilitation of the knee after surgery. To this aim, trying to recreate the movement of the knee, a cyclic test will be performed. It will take into account the possible deformation that may occur in the graft after the surgery.

- Rupture: Finally, the tendon is ruptured by applying a tension force until the tendon slips or breaks. It was designed to determine the stiffness of the grafts after the test, being the slope of the linear part of the force-elongation graph. This part of the procedure is not significant for the results of the protocols. However, it is useful to know the stiffness of the tendon to compare it to the native ACL one.

In the following graph (Figure 18) it is shown the Stress – Elongation graph that defines all the parts of this experiment. Specifically, it is shown a protocol based on 89 N load and three re-tensions.
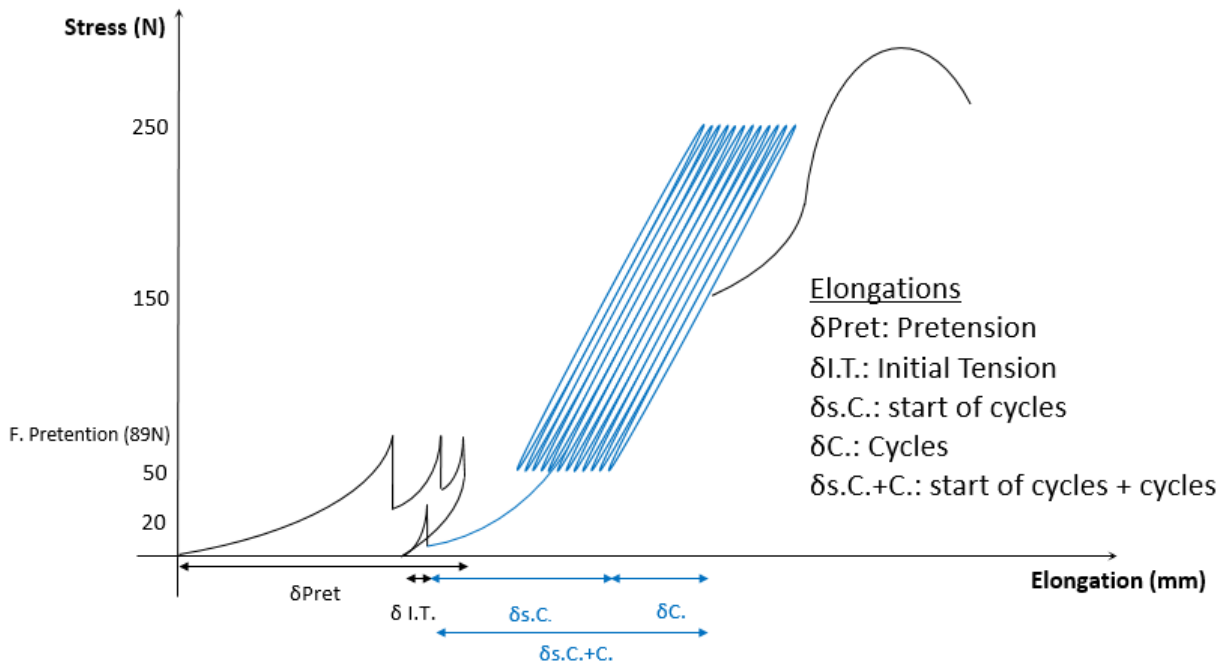
Figure 18. Stress - Elongation Graph of the Tendon Grafts. (Own Illustration)

The length, elongation, and stiffness in the different steps are going to be analyzed. However, the results that are going to be relevant for this project are the elongations produced once the graft is placed in the patient's knee, the ones marked in blue color in Figure 18, as we are trying to minimize the elongation produced after the surgery.

4.2.2. Protocols Proposed

The goal of this project is to get a protocol that effectively removes the non-linear viscoelastic property of tendons to use them as grafts replacements in ACL reconstruction surgeries. In other words, it consists of the minimum elongation of the graft after its implantation into the patient. To this aim, three different protocols where designed which will be compared to two other protocols used as a control group.

In earlier conducted trials, it was discovered that re-tension (i.e. stressing the tendon to a specific load several times) could be an effective way of preconditioning the graft. Thus, the first protocol proposed is the one suggested by most authors, 89 N for 15 minutes, but split into smaller fragments of time. Therefore, it was chosen to be 89 N, re-tensioned 7 times of 2 minutes each time. However, it was realized that a 7 re-tension protocol needed too much manual effort performed by an expert in the operating room. Thus, a protocol which does not need a lot of time nor load was desired, to easier be performed in a theater. So, a protocol was chosen consisting of 45N, re-tensioned 3 times, each lasting 3 minutes. And, finally, a higher load protocol was designed to enable to see whether different charges considerably change the results of the graft elongation in the rehabilitation process or not. Thus, 150 N with 3 re-tension periods of 3 minutes each was chosen as the third protocol.

To be able to compare these protocols with a standardized method, two control protocols were designed too. One, where only the cyclic procedure was applied, and another one, were also initial tensioning was performed. These two procedures were implemented to check which measures applied to the tendon were efficient. Table 2 summarizes the different protocols in terms of loads and times.

Table 2. Different Protocols description.

Protocol	Number of Samples Tested	Pretension	Initial tensioning	Start of cycles	Cycles
Control	10	-	-	150 N	50-250 N 1000 cycles
Control w I.T.	10	-	20 N – 1 min	150 N	50-250 N 1000 cycles
45N	10	45N re-tension 3 times 3 min each	20 N – 1 min	150 N	50-250 N 1000 cycles
89 N	10	89 N re-tension 7 times 2 min each	20 N – 1 min	150 N	50-250 N 1000 cycles
150 N	10	150 N re-tension 3 times 3 min each	20 N – 1 min	150 N	50-250 N 1000 cycles

4.3. Conditions of the Test

Firstly, the data acquisition will be described. The characteristics of each one of the tests will be explained, both their type and velocity. Thus, its reproducibility can be achieved. Secondly, it will be defined the process to record the data into the computer to further analyze it. Lastly, ways of failure of the experiment will be enumerated too.

4.3.1. Data Acquisition

The experiments had to be performed under certain conditions. First of all, to conduct the experiment, the Universal Dynamic Machine had to be set up. The toothed jaws were placed on the machine in the correct position, in order to avoid displacement of the jaws during the test. In the upper and lower part of the machine, springs are installed in which the jaws are placed. This way, one jaw will first be placed into its corresponding spring and later the second jaw will be placed while matching its teeth with the valleys of the first jaw. This way they will be matching each other's orientation (Figure 19). Once in their position, they will

we fixed pressing a lever and later protected by a methacrylate sheet. It is important for the sheet to be transparent so the tendon can be observed at every single moment. Furthermore, the tests will be carried out at room temperature of 20ºC, achieved by air conditioning.

Figure 19. Upper Jaw Placement. (Herrera, L., 2018)

While the machine was being settled up, the tendon was being defrosted. When it hit room temperature, it was bathed in natural saline. With the purpose of the tendon not drying, this solution is sprayed with a really high frequency during the test (around every 5 minutes). This procedure takes about 10 minutes and once the machine was set up and the tendon ready, the tests could be performed.

Just before the experiment started, its length was measured with the help of a ruler and then implemented into the machine with the help of the needle holders. By using the controls of the machine, the tendon was driven to slight tension. The part of tendon subjected to testing, the one in between the jaws, was further measured with the help of a caliper. The placement of the tendon can be seen in Figure 20.

Figure 20. Tendon placed into the Machine. (Herrera, L., 2018)

There are several procedures which were tested. To be able to have n=10 for the 5 protocol proposed, a number required for the results to have significance, we have to test 50 tendons.

As previously described in section 4.2.1., the first step of every test was the pretension. This step of the trial consisted of applying load to the tendon performing a control by position, which means maintaining this parameter as a constant. The tendon was subjected to the load required by each protocol at a constant speed of 10 mm/min and, when the corresponding force value was reached (e.g. 45N for protocol 45N), the tension was stopped and the tendon was hold in that position for the time indicated by each protocol. As we proposed re-tension procedures, once the time of the first step passed (e.g. 3 min for protocol 45N), it returned again to the indicated force at a speed of 1mm/min. This was repeated as many times as indicated for every protocol in the proposed protocols table (e.g. three times for protocol 45N).

Afterwards, the tendon was discharged to simulate the transfer to the patient. This was carried out by applying a compression test, using again a control by position at a constant speed of 3mm/min, until the tendon was no longer subjected to any force (0 N). Once this value was reached, this position was hold for five minutes.

The next step consisted of applying the initial tension to the graft, simulating its implementation into the patient, right before fixing it into the bones. A force of 20 N was selected, which will be applied with control by position at a constant speed of 2 mm/min. Since the control by position is not as precise, a loss of load would occur, thus the person execution the test would start and stop the tension test during one minute until the stabilization of those 20 N in the tendon was achieved.

Afterwards, the post-surgery immobilization was simulated was performed. The sample was kept in this position during 30 minutes, in which a loss of load would occur due to the viscoelastic natural properties of the tendon.

Finally, the process of rehabilitation of the knee in the patient after the reconstruction was simulated. A cyclic test was performed and the possible deformation that may occur in the graft after the operation was considered as a relevant result. This cyclic part consisted of two steps. As the cycles ranged from 50 N to 250 N, first the tendon must be loaded, performing a load control by maintaining the load at a speed of 5N/s, until reaching the middle load of 150 N. Later, a cyclic test consisting on 1000 cycles, 100 N of amplitude and 1 Hz of frequency was carried out. Thus, cyclical loads varied from 50 to 250 N. During this step, special attention was paid to hydrating the tendon with natural saline solution because it is more likely to dry.

Although it was not part of the protocol itself, the tendon was taken to rupture in order to measure its stiffness. To do this, a tensile force, using control by position at a constant speed of 10 mm/min, was applied until tendon breaks or slides.

Since biological material was handled, every time a test day ends, the toothed jaws and the parts that have been in contact with the tendons had to be removed and cleaned. The following Table 3 resumes the specific parameters used in each step of the test.

Table 3. Test steps parameters

Essay	Test types	Kind of control	Parameters	Time
Pretension	Tension	Position	10mm/min first and then 1mm/min	Defined by each protocol
Discharge	Compression	Position	3mm/min	5 min
Initial tensioning	Tension	Position	2mm/min	1 min
Start of Cycles	Tension	Load	5N/s	Until tendon reaches 150 N
Cycles	Senoidal	Load	100 N amplitude (50-250 N)	1000 cycles at 1Hz
Rupture	Tension	Position	10mm/min	Until tendon breaks

4.3.2. Data Collection

The collection of the data obtained through the tests was done using the same software with which the tests were controlled. To do this, the acquisition had to first be configured.
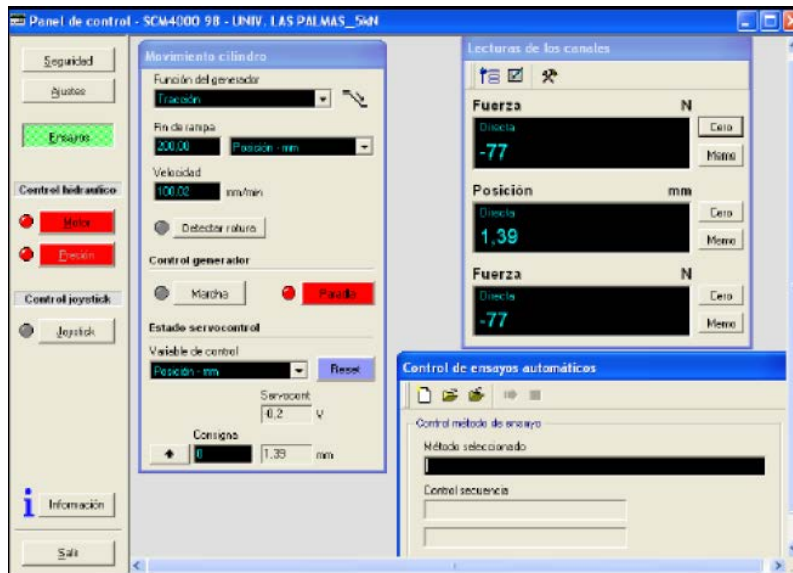
Figure 21. Data Collection Panel. (Herrera, L., 2018)

First of all, we had to set up a systematic name for each test carried out with the machine. This was done using the 'Control de ensayos automáticos' panel from Figure 21. Hence, we chose the following methodology: a T followed by the number of the tendon being tested, the date when the test was performed and the step of the experiment being collecting. For example, the file for the tendon 19 pretension made on the 9th of July of 2018 was named T19 09-07-18 Pretension. We had to be careful to store all the files in the same compartment so they were easily accessible later.

Afterwards, we have to ask to be able to visualize the graph on the screen, and so it will be stored in the file. If this step is skipped, not data is going to be collected. It has been chosen to visualize Stress (N) vs Elongation (mm). We also had to set up the control at which the step is going to be controlled, being this parameter different in the pull-out tests (Slope control) from the cyclic tests (Cycles control).

All this data was stored at a certain frequency set by the user. We chose different frequencies of data collection for each step, depending on whether we were interested in having more data points or not. We took into account that a higher frequency would mean more points taken per second, resulting in a bigger dataset. This was the reason why sometimes we did not chose the highest frequency, being quite challenging to work with those big files afterwards.

The following Table 4 summarizes the parameters used in every step of the experiment.

Table 4. Data Collection Parameters.

File Name	Control	Data collection frequency
T*(nº) date* Pretension	Slope Control	2 Hz
T*(nº) date* Initial-Tension	Slope Control	1 Hz
T*(nº) date* Start-of-Cycles	Slope Control	1 Hz
T*(nº) date* Cycles	Cycles Control, 1-1	100 Hz
T*(nº) date* Rupture	Slope Control	50 Hz

After everything was set up, the user should press the button 'Data recording' to start storing the data into the computer and the experiment could take place.

4.3.3. Test Failure

Although everything may have been set up correctly, the experiment sometimes fails and invalidates the current experiment, including all the steps done before. Only when the test fails at the rupture step, the whole experiment was taken into account, since that information was not totally required for the main project. Failure is the reason why 60 tendons were needed for 50 experiments.

There were two main causes of failure. The first one was when the graft slipped from the jaws and/or breaks. This usually happened during the cyclic part of the experiment, due to the high load at which the graft was submitted. To avoid this problem, it had to be assured before the experiment starts, that everything was tight. Therefore, the tendons should have a portion of themselves outside the jaws (facing the part not submitted to the experiment) so there was a smaller possibility of slipping. However, and due to the low precision of the machine used, even if everything was properly placed, a graft displacement from the jaws could occur.

The second cause of experiment abortion was when the data was not collected properly. It could, again, happen because of user or machine impairment. On the one hand, the user may have forgotten to set the data collection properly and/or press the 'Data recording' button. On the other hand, the machine may have also not be storing the data, due to its low precision. In both cases, the experiment was not valid anymore because of incomplete data sets for a single experiment.

5. RESULTS

In this section the processing of the data obtained before will be described and then, final results will be shown. Additionally, a statistical analysis will be performed in order to further discuss whether a method is better than the other ones.

5.1. Data Processing

After finishing the 50 proposed tests, we preceded to analyze de data obtained for each protocol. This consisted of extracting the files from the computer and work with them in Microsoft Excel Sheets. Test 19 will serve as an example to explain how the data analysis has been conducted. It corresponds to case 150 N with 3 re-tensions of 3 minutes each.

The files were taken from the computer and exported to excel. The first file analyzed is the pretension one, where pretension and the following discharge are included. The final value for the elongation of this part will be calculated subtracting the final elongation value of the process from the first one (δ Pret.). Because of the machine's impreciseness, a 5 N error has been taken into account for the value of 0 N.

The graph stress-elongation obtained is the following where the elongation is also defined:

$$\delta \text{ Pret. (mm)} = 3.71 - 0.00 = \textbf{3.71 mm}$$
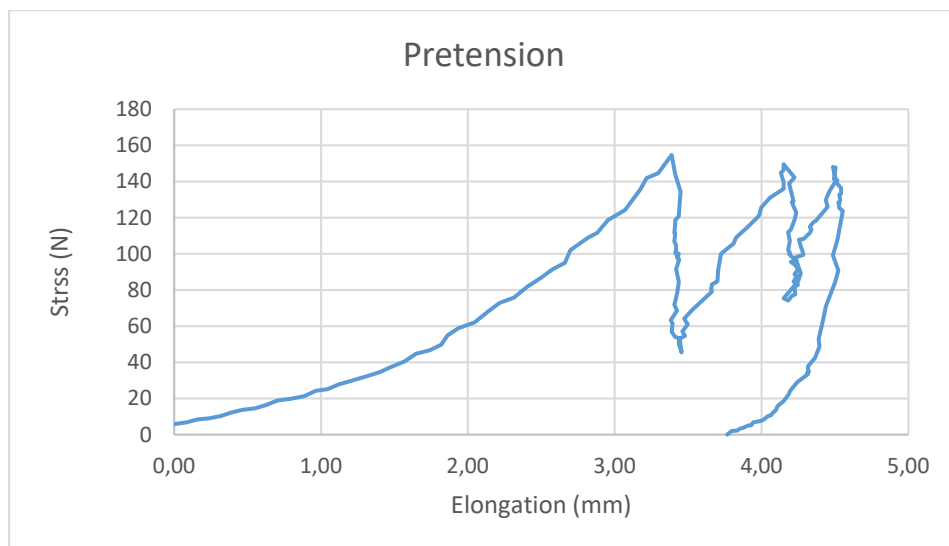
Figure 22. Pretension Graph (Own Illustration)

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The next file that we have defined in the console is the initial tensioning plus the 30 minutes of immobilization. The elongation of this step is calculated the same way as the previous step, the last value subtracted from the initial one (δ T.Ini.). Therefore, the graph looks as following:

$$\delta \text{ I.T. (mm)} = 3.88 - 3.69 = \textbf{0.19 mm}$$
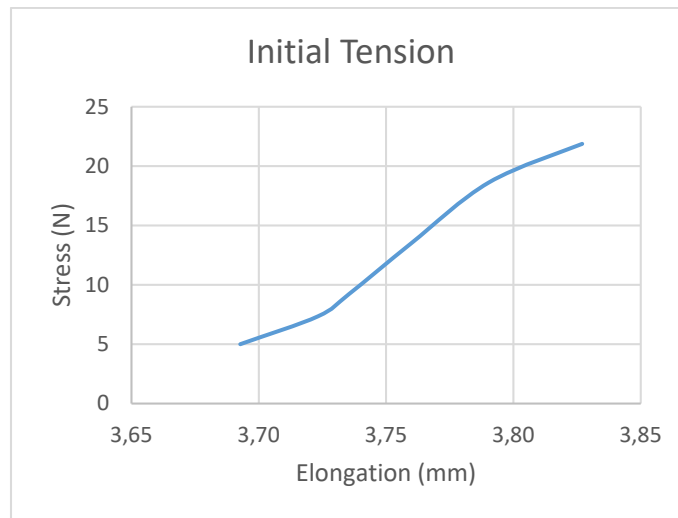
Figure 23. Initial Tension Graph (Own Illustration)

The third step correspond to the start of the cycles, loading the tendon until 150 N, which is the first step to take into consideration for the final results. The elongation is calculated one more time (δ s.C.).

$$\delta \text{ s.C (mm)} = 4.73 - 3.858 = \textbf{0.87 mm}$$
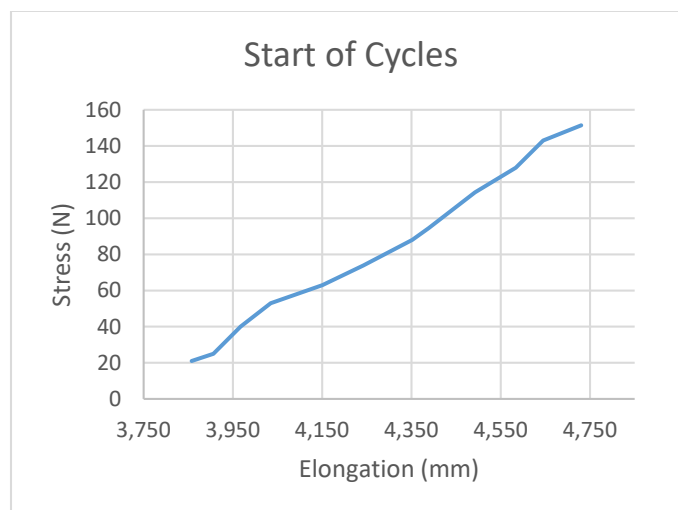
Figure 24. Start of Cycles Graph (Own Illustration)

Afterwards, the results of the cyclic treatment will be analyzed. Calculating the elongation in this step is a little bit trickier because it is not just taking the final value and subtract it from the first one. Cycle 1 and Cycle 1000 files are extracted from the console. Their corresponding absolute elongation are taken at the point of 150 N when going towards 250 N.

$$\delta \text{ C.1 abs (mm)} = 5.04 \text{ mm} \qquad \delta \text{ C.1000 abs (1000)} = 7.990 \text{ mm}$$

Then, the absolute value of the cycle 1 is subtracted from the previous steps, pretensioning, initial tensioning and start of cycles. Cycle 1000 absolute value is also subtracted from the previous steps, including this time Cycle 1 too.

$$\delta \text{ C.1 (mm)} = 5.04 - 3.71 - 0.19 - 0.87 = 0.27 \text{ mm}$$
$$\delta \text{ C.1000 (mm)} = 7.99 - 3.71 - 0.19 - 0.87 - 0.27 = 2.953 \text{ mm}$$

The total value of the elongation corresponding to cycles would be the elongation of Cycle 1000 plus the elongation of Cycle 1 (δ C.). The calculation and the corresponding graphs are showed as following,

$$\delta \text{ C (mm)} = 2.953 + 0.27 = \textbf{3.22 mm}$$

Furthermore, the stiffness of each cycle is also calculated by obtaining the equation of the slope of the graph.

$$\text{K1 (N/mm)} = 393.66 \text{ N/mm} \qquad \text{K1000 (N/mm)} = 456.68 \text{ N/mm}$$
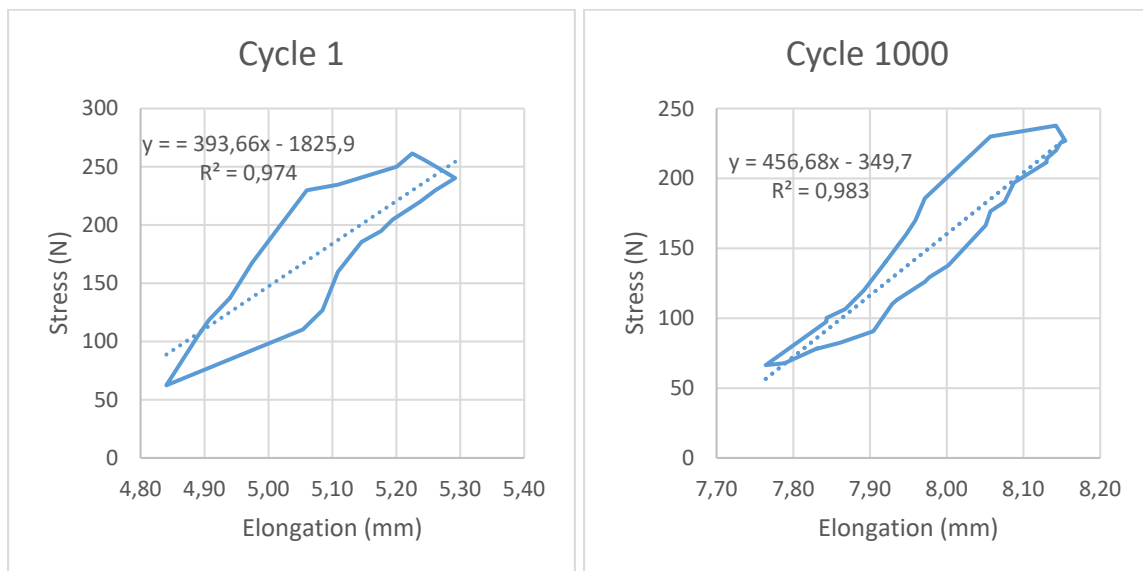
Figure 25. Cycles 1 and Cycle 1000 Graphs (Own Illustration)

The last step consists of analyzing the file containing the data of the final rupture of the tendon. The important parameter here is the stiffness. It is calculated by using the slope of the linear part of the stress-elongation graph. Therefore, a linear tendency line is drawn as well as its corresponding equation, always taking into account an R^2 of at least 0.95. The stiffness would be the slope of the equation.

$$K_{Rupture} \text{ (N/mm)} = 303.73 \text{ N/m}$$
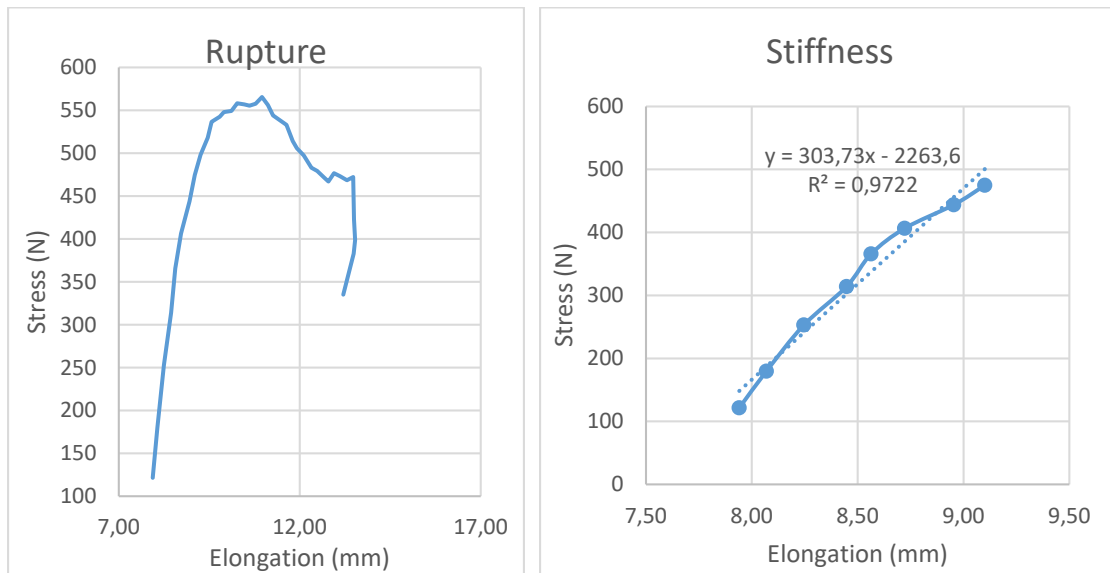
Figure 26. Final stiffness of the Tendon Graft taken from the Rupture of the Tendon (Own Illustration)

5.2. Data Obtained

In the following tables it is going to be shown the results obtained through all the 50 tendon grafts tests. They are classified in their acquisition order. First, the tendon sizes, then their elongations and finally their stiffness.

5.2.1. Tendon sizes

The free length of the tendons was measured once implemented inside the machine (F.L.). The length of the jaws was also taken into account to acquire the total length (F.L. + Jaws L.). Moreover, as we can appreciate from the table, the average diameter of the tendon grafts is 6mm as the width of a regular ACL (Asif, N., Ranjan, R., Ahmed, S., Sabir, A. B., Jilani,

L. Z., & Qureshi, O. A., 2016). All the results obtained from these measurements are shown in Table 5.

Table 5. Tendon length and width sizes

Essay	Protocol Type	F.L. (mm)	F.L. + Jaws L. (mm)	Diameter (mm)
T29	Contol	47.62	148	5.5
T30	Contol	40.52	141	6.5
T31	Contol	57.55	158	5.5
T32	Contol	61.45	161	6.5
T33	Contol	90.62	191	5.5
T34	Contol	58.22	158	6.5
T35	Contol	11.21	111	5.5
T36	Contol	68.97	169	6.5
T37	Contol	48.76	149	5.5
T39	Contol	44.94	145	6.5
Mean		**52.99**	**152.99**	**6.00**
Typical Deviation		**20.56**	**20.56**	**0.53**
T40	Control w I.T.	31.77	132	5.5
T41	Control w I.T.	20.19	120	6.5
T42	Control w I.T.	47.77	148	5.5
T43	Control w I.T.	60.22	160	6.5
T44	Control w I.T.	54.98	155	5.5
T45	Control w I.T.	76.37	176	6.5
T46	Control w I.T.	76.31	176	5.5
T47	Control w I.T.	108.55	209	6.5
T50	Control w I.T.	19.8	120	5.5
T51	Control w I.T.	38.14	138	6.5
Mean		**53.43**	**153.43**	**5.95**
Typical Deviation		**26.66**	**26.66**	**0.52**
T53	45N	47.89	148	6.5
T54	45N	62.19	162	5.5
T55	45N	33.23	133	6.5
T56	45N	92.01	192	5.5

T57	45N	51.53	152	6.5
T58	45N	39.84	140	5.5
T59	45N	92.49	192	5.5
T60	45N	48.21	148	5.5
T61	45N	56.28	156	5.5
T62	45N	53.72	154	5.5
Mean		**57.74**	**157.74**	**5.80**
Typical Deviation		**19.91**	**19.91**	**0.48**
T4	89 N	102.28	202	6
T5	89 N	37.45	137	6
T6	89 N	43.94	144	6
T7	89 N	60.26	160	6
T9	89 N	65.89	166	6
T11	89 N	70.02	170	6
T12	89 N	28.97	129	6
T13	89 N	113.78	214	6
T14	89 N	67.89	168	6
T16	89 N	92.27	192	6
Mean		**65.81**	**165.81**	**6.00**
Typical Deviation		**27.63**	**27.63**	**0.00**
T18	150 N	53.06	153	6
T19	150 N	20.58	121	6
T20	150 N	131.46	231	6
T22	150 N	39.32	139	6
T23	150 N	38.72	139	6
T24	150 N	70.23	170	5.5
T25	150 N	49.64	150	5.5
T26	150 N	66.13	166	5.5
T27	150 N	70.37	170	5.5
T28	150 N	50.92	151	5.5
Mean		**59.04**	**159.04**	**5.75**
Typical Deviation		**29.85**	**29.85**	**0.26**

5.2.2. Tendon Elongations

The elongations presented in Table 6 have already been defined in the graph of Figure 18 and also explained in section 5.1. However, there are also new elongation parameters. The displacement of the start of cycles plus the one of the cycles have been added to form the total elongation of the tendon after the surgical process (δ s.C+C.). Furthermore, the important displacements, i.e. the ones relevant to this study (δ s.C., δ C, δ s.C+C.), have been normalized to 75 mm, the average length of a human ACL (Colombet, P., & Graveleau, N. , 2015). The normalization has been calculated by dividing the elongation value by the total length of the tendon (F.L. + Jaws L.) and multiplying it by 75 mm. This way, the results which are shown in Table 6, can be translated to a normal ACL reconstruction surgery.

Table 6. Tendon graft elongations

Essay	Protocol Type	δ Pret. (mm)	δ I.T. (mm)	δ s.C. (mm)	δC. (mm)	δ s.C. (75 mm)	δC. (75 mm)	δ s.C+C. (75 mm)
T29	Control	-	-	3.69	4.12	1.873	2.095	3.968
T30	Control	-	-	3.58	8.64	1.909	4.613	6.522
T31	Control	-	-	4.40	4.60	2.092	2.187	4.280
T32	Control	-	-	4.36	8.95	2.027	4.156	6.183
T33	Control	-	-	4.14	5.95	1.630	2.339	3.970
T34	Control	-	-	3.79	8.40	1.797	3.982	5.778
T35	Control	-	-	3.65	3.97	2.462	2.677	5.139
T36	Control	-	-	9.08	5.40	4.031	2.396	6.427
T37	Control	-	-	3.65	5.43	1.840	2.738	4.578
T39	Control	-	-	3.85	4.02	1.990	2.078	4.067
Mean					5.95		2.93	5.09
Typical Deviation					1.99		0.95	1.05
T40	Control w I.T.	-	1.62	2.38	4.69	1.355	2.669	4.024
T41	Control w I.T.	-	1.39	2.81	5.72	1.755	3.567	5.323
T42	Control w I.T.	-	1.45	2.78	6.09	1.412	3.089	4.502

T43	Control w I.T.	-	2.06	2.77	4.69	1.294	2.193	3.487
T44	Control w I.T.	-	1.29	2.47	4.36	1.193	2.112	3.305
T45	Control w I.T.	-	1.46	2.95	5.31	1.256	2.256	3.513
T46	Control w I.T.	-	2.71	3.56	4.12	1.514	1.753	3.267
T47	Control w I.T.	-	1.70	3.39	5.28	1.218	1.900	3.118
T50	Control w I.T.	-	1.43	2.69	7.61	1.685	4.764	6.448
T51	Control w I.T.	-	1.55	2.48	6.22	1.346	3.378	4.723
Mean					**5.47**		**2.78**	**4.18**
Typical Deviation					**1.01**		**0.89**	**1.03**
T53	45N	-	0.32	1.67	3.40	0.848	1.723	2.571
T54	45N	2.09	0.65	1.76	6.44	0.813	2.979	3.792
T55	45N	1.61	0.19	1.31	7.06	0.735	3.977	4.712
T56	45N	1.87	0.39	1.99	6.03	0.777	2.355	3.133
T57	45N	2.73	0.65	2.06	7.95	1.021	3.933	4.954
T58	45N	2.20	0.64	1.97	6.33	1.057	3.394	4.452
T59	45N	2.64	0.53	2.28	8.40	0.887	3.274	4.161
T60	45N	2.03	0.72	2.03	10.12	1.025	5.123	6.148
T61	45N	2.63	0.54	2.12	6.87	1.019	3.295	4.314
T62	45N	2.32	0.40	2.04	7.37	0.994	3.597	4.591
Mean					**7.00**		**3.37**	**4.28**
Typical Deviation					**1.75**		**0.93**	**0.99**
T4	89 N	7.430	0.390	1.568	2.012	0.581	0.746	1.327
T5	89 N	2.410	0.220	0.879	4.151	0.480	2.265	2.745
T6	89 N	3.900	0.410	1.611	5.329	0.839	2.777	3.616
T7	89 N	6.950	0.400	1.336	2.934	0.625	1.373	1.998
T9	89 N	3.550	0.470	1.538	4.462	0.695	2.017	2.713
T11	89 N	3.750	0.360	1.209	4.901	0.533	2.162	2.695
T12	89 N	4.020	0.420	1.618	4.182	0.941	2.432	3.373

T13	89 N	3.24	0.48	1.44	1.59	0.505	0.558	1.063
T14	89 N	3.96	0.38	1.58	2.89	0.706	1.291	1.997
T16	89 N	5.11	0.47	1.79	5.17	0.697	2.017	2.715
Mean					**4.06**		**1.94**	**2.62**
Typical Deviation					**1.60**		**0.92**	**1.01**
T18	150 N	3.88	0.37	1.22	2.20	0.595	1.076	1.671
T19	150 N	3.71	0.19	0.87	3.22	0.542	2.002	2.544
T20	150 N	2.96	0.25	1.25	1.94	0.403	0.627	1.030
T22	150 N	3.45	0.21	1.15	2.88	0.617	1.552	2.169
T23	150 N	4.33	0.59	1.53	5.82	0.828	3.146	3.974
T24	150 N	3.39	0.45	1.28	1.70	0.565	0.748	1.313
T25	150 N	5.5	0.44	1.75	7.65	0.875	3.832	4.706
T26	150 N	4.79	0.33	1.42	3.36	0.639	1.519	2.158
T27	150 N	4.05	0.31	1.22	2.52	0.535	1.107	1.642
T28	150 N	4.33	0.35	1.12	2.40	0.555	1.194	1.749
Mean					**3.37**		**1.68**	**2.30**
Typical Deviation					**1.90**		**1.05**	**1.17**

5.2.3. Tendon Stiffness

The stiffness of the tendons was calculated as explained above (5.1.). In Table 7, we present the stiffness values for all the tendons which as we can appreciate round 200 – 300 N/mm. However, $K_{Rupture}$ for T4 to T16 is slightly lower than the others due to the higher period of such pretension protocol.

Table 7. Tendon graft Stiffness

Ensayo	Caso	K1 (N/mm)	K1000 (N/mm)	KRupture (N/mm)
T29	Control	257.1	257.7	219.4
T30	Control	292.4	302.1	233.4
T31	Control	238.5	259.2	223.3
T32	Control	236.6	236.2	455.1
T33	Control	200.1	199.4	174.1
T34	Control	228.98	216.07	198.15

T35	Control	362.6	386.5	316.0
T36	Control	215.8	204.3	174.1
T37	Control	254.9	249.5	216.7
T39	Control	257.8	278.8	266.4
Mean		**254.47**	**258.97**	**247.66**
Typical Deviation		**45.73**	**55.28**	**84.25**
T40	Control w I.T.	344.1	301.8	257.5
T41	Control w I.T.	264.3	253.9	223.4
T42	Control w I.T.	275.6	287.2	220.4
T43	Control w I.T.	264.6	253.2	223.6
T44	Control w I.T.	262.7	261.8	230.8
T45	Control w I.T.	210.8	190.3	175.4
T46	Control w I.T.	217.9	232.8	204.0
T47	Control w I.T.	190.9	197.9	185.3
T50	Control w I.T.	319.2	298.4	244.9
T51	Control w I.T.	299.4	273.7	254.0
Mean		**265.06**	**257.54**	**222.99**
Typical Deviation		**45.98**	**37.57**	**26.19**
T53	45N	307.5	323.2	277.7
T54	45N	252.6	200.0	174.4
T55	45N	346.8	333.5	245.6
T56	45N	210.1	192.7	175.4
T57	45N	292.0	308.2	263.8
T58	45N	301.0	310.8	240.9
T59	45N	212.5	192.1	153.0
T60	45N	273.2	247.8	214.1
T61	45N	249.9	259.0	232.9
T62	45N	247.3	239.0	227.0
Mean		**269.27**	**260.61**	**220.46**
Typical Deviation		**43.29**	**55.40**	**41.02**
T4	89 N	240.000	250.760	193.710

T5	89 N	350.350	197.650	98.608
T6	89 N	299.800	296.850	170.430
T7	89 N	279.070	280.540	158.410
T9	89 N	220.770	197.770	81.934
T11	89 N	245.870	149.260	58.350
T12	89 N	318.720	333.290	142.000
T13	89 N	195.9	193.7	141.5
T14	89 N	248.4	260.3	136.0
T16	89 N	204.0	178.1	98.3
Mean		**260.29**	**233.82**	**127.92**
Typical Deviation		**50.63**	**59.18**	**42.43**
T18	150 N	308.3	334.9	253.7
T19	150 N	393.7	456.7	303.7
T20	150 N	216.3	223.2	190.5
T22	150 N	317.6	339.4	257.8
T23	150 N	296.1	292.3	190.4
T24	150 N	279.7	306.6	253.3
T25	150 N	255.6	218.8	173.9
T26	150 N	255.9	271.7	233.4
T27	150 N	264.6	257.4	219.2
T28	150 N	292.4	286.3	243.7
Mean		**288.02**	**298.70**	**231.97**
Typical Deviation		**47.59**	**68.89**	**39.24**

5.3. Statistics

Every time data is acquired and worked with, a statistical analysis is required. In this specific case, we perform it in order to define whether the several methods tested are statistical different between them and so, which one is the best one. Therefore, we are going to perform a statistical analysis of the parameter we have defined to be the one that determines which method is superior to the other ones. It is the normalized elongation of the start of cycles plus the cycles (marked in blue in Figure 18) normalized to 75 mm. It describes the total

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elongation of the tendon graft after its implementation in the human knee, with the main goal of the experiment being to effectively reduce it.

This parameter has been extracted and plotted in Figure 27, based on the previously shown data tables.
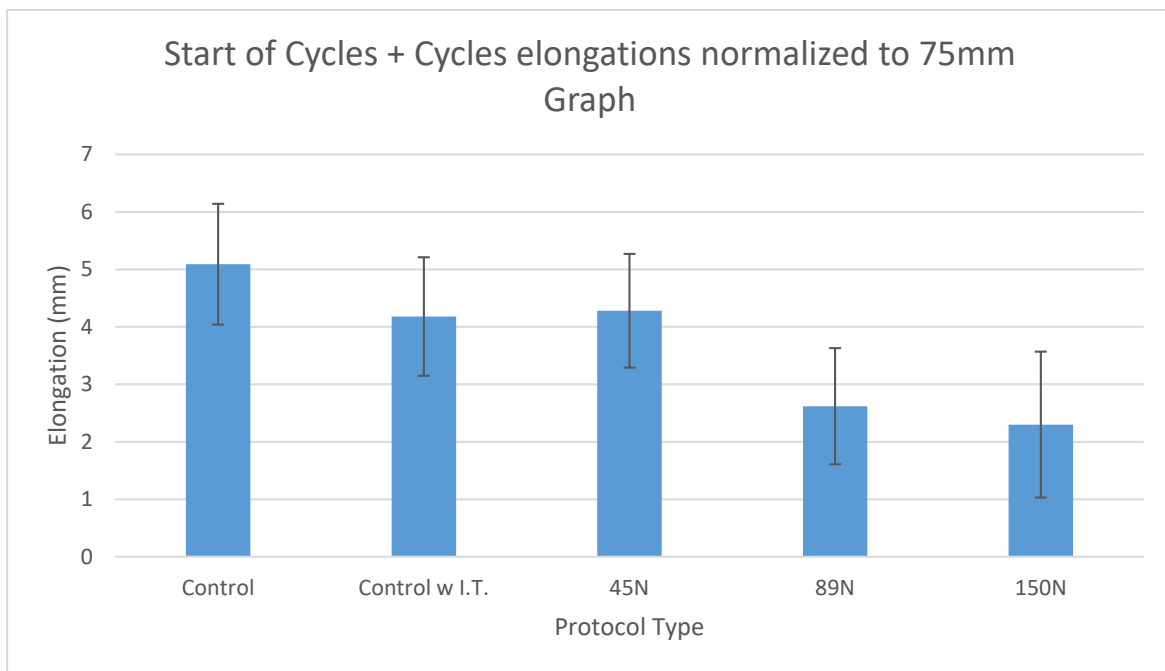
Figure 27. Start of Cycles + Cycles elongations normalized to 75mm Graph (Own Illustration)

From Figure 27, we can conclude that 150 N and 89 N are the best protocols while the control group performs the worst. Nevertheless, a statistical analysis has to be conducted to be able to claim that with certainty.

5.3.1. One-Way Anova

One-way Anova (analysis of variance) is a method usually employed to find statistically significant differences between the means of independent groups, being necessary at least three groups to be able to perform it. Based on the F distribution, it analyzes several means

and comes up with the result of which ones are determined as statically different (One way Anova, 2018). The means are equal if the null hypothesis is proved thus,

$$H_0: \mu_1 = \mu_2 = \mu_3 = \cdots = \mu_k$$

where μ = group mean, and k = number of groups.

However, whenever the one-way Anova has as a result a statistically significant value (p-value < 0.05), the alternative hypothesis, H_A, is taken into account (see Figure 28). In this second case, it is proved that there are at least there are two statistically significantly different group means (One way Anova, 2018).

a)

b)

p-value < 0.05

Null Hypothesis, H_O

Alternative Hypothesis, H_A
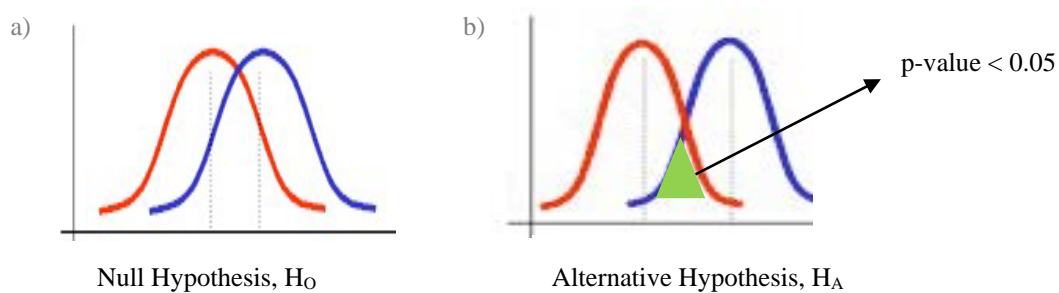
Figure 28. One-Way Anova Test. a) The means are not statistical different b) The means are statistical different achieving a   p-value of 0.05 (Own Illustration)

Hence, we can see in Figure 28a that if the group means have similar values, the variance between the group means is lower than the variance of the samples and, thus, the populations are consider equal. However, in Figure 28b, a higher variance ratio between the means, shown by the p-value<0.05, implies that samples were drawn from statistically significant different populations (Casella, 2008).

The one-way Anova test was then computed using an online software (Navendu, 2016). The results are shown in Table 8.

Table 8. One way Anova values obtained

Treatment	Control	Control w I.T.	45 N	89 N	150 N	Total
Observations N	10	10	10	10	10	50
sum $\sum x_i$	50.9120	41.7100	42.8280	24.2420	22.9560	182.6480
Mean $\overline{x}$	5.0912	4.1710	4.2828	2.4242	2.2956	3.6530
Sum of squares $\sum x_i^2$	269.1898	184.4969	192.1701	64.8531	65.1048	775.8148
Sample variance s^2	1.1096	1.1694	0.9718	0.6762	1.3786	2.2165
Sample std.dev. s	1.0534	1.0814	0.9858	0.8223	1.1741	1.4888
std. dev. of mean $SE_{\overline{x}}$	0.3331	0.3420	0.3117	0.2600	0.3713	0.2105

Source	Sum of squares SS	Degrees of freedom v	Mean square	F statistic	p-value
Treatment	60.8588	4	15.2147	14.3384	1.2698e-07
Error	47.7502	45	1.0611		
Total	108.6089	49			

After all, it can be observed that the p-value obtained from the F-statistic, is smaller than 0.05. It advices that group means are statistically significantly different.

In order to get to know which one of the group pairs have the different means, further analysis has to be done. Tukey HSD test would likely identify them.

5.3.2. Tukey HSD test

We have five different protocol results which are going to be applied the Tukey HSD test. It will be performed using the same online software as before (Navendu, 2016). Statistically significant differences are going to be tested for the ten pairs that can be obtained by all the possible combinations of the five treatments.

When performing the Tukey HSD Q statistic, it is first determined the critical value which this time is based on k=5 protocols and v=45 degrees of freedom. The error term is taken from the Studentized Range distribution which defines the probability distribution of studentized ranges for independent, identically distributed random variables (Stephanie, 2017). If we want to get a significance p-values of α=0.01 and 0.05, the Q critical values had to be obtained and further checked at several published tables of the inverse Studentized Range distribution, like the one at Duke University (qtable, Course sta110c, 1998).

$$Q_{\text{critical}}^{\alpha=0.01,k=5,v=45} = 4.8928 \text{ and } Q_{\text{critical}}^{\alpha=0.05,k=5,v=45} = 4.0186$$

Afterwards, it was settled up a Tukey statistic test for our group means in order to analyze them in comparison to the previously obtained Studentized Range distribution critical values. The online software stablished the Tukey confidence limits as documented in the NIST Engineering Statistics Handbook (Sematech, 2013) later making a simplified algebraic transformation. It calculated a specific value for each pair of group means being analyzed, which we define as the Tukey HSD Q-statistic:

$$Q_{i,j} = \frac{|\bar{x}_i - \bar{x}_j|}{s_{i,j}} \qquad \text{where,} \qquad s_{i,j} = \frac{\hat{\sigma}_e}{\sqrt{H_{i,j}}} \qquad i,j = 1,\dots,k; \ i \neq j$$

- $H_{i,j}$ is the harmonic mean of the number of observations in columns labeled i and j. When the sample sizes in the columns are equal, as it is in our case, their harmonic mean is the common sample size. This parameter varies across the compared pairs.

- $\hat{\sigma}_e = 1.0301$ is the square root of the Mean Square Error (1.0611) described in the previously explained one-way Anova test. This factor has the same value across all pairs.

In Table 9, results of the Tukey test of comparing whether $Q_{i,j} > Q_{critical}$ are shown following a color code. Green was set up for statically significant different group means, and red otherwise. Additionally, the obtained Q-statistic ($Q_{i,j}$) significance p-values are shown. Gleason is the author of the algorithm employed by the online software to obtain the critical values of the Studentized Range Distribution, as well as the $Q_{i,j}$ values (Gleason, J. R., 1999).

Table 9. Tukey HSD Test

Treatments pairs	Tukey HSD Q statistic	Tukey HSD p-value	Tukey HSD significance
Control vs Control w I.T.	2.8249	0.2838978	Insignificant
Control vs 45 N	2.4817	0.4136329	Insignificant
Control vs 89 N	8.1873	0.0010053	p-value < 0.01
Control vs 150 N	8.5821	0.0010053	p-value < 0.01
Control w I.T. vs 45 N	0.3432	0.8999947	Insignificant
Control w I.T. vs 89 N	5.3624	0.0038644	p-value < 0.01
Control w I.T. vs 150 N	5.7572	0.0016746	p-value < 0.01
45 N vs 89 N	5.7056	0.0018718	p-value < 0.01
45 N vs 150 N	6.1004	0.0010053	p-value < 0.01
89 N vs 150 N	0.3948	0.8999947	Insignificant

6. DISCUSSION AND CONCLUSIONS

In the following section it is going to be discussed the previously obtained results. Thus, would lead us to conclude which one of the protocols proposed in our hypothesis is the best one, and so would reduce the intrinsic viscoelastic property of tendon grafts. It is also going to be described the limitation found during the experiments as well as further approaches for this project.

6.1. Fulfilled Objectives

The main goal of this project was to determine a protocol that effectively removes the non-linear viscoelastic properties of tendons to be able to use them as soft tissue grafts for ACL reconstruction surgeries. For this, several preconditioning protocols have been designed, tested, and been further analyzed.

Different parameters have been taken into account during the tests to compare it with the one of a natural ACL. These are the initial length of the grafts, their elongation in every step of the experiment, and their stiffness. Further, we realized that the most important one, and the one we will take into account to decide whether one protocol is better than another one or not, is the normalized elongation at the start of cycles plus the one in the cycles. This representing, the elongation once the graft is inserted into the knee and is submitted to rehabilitation. These results have been normalized to the average size of human tendon grafts, 75 mm (Colombet, P., & Graveleau, N. , 2015), to be able to compare it with the results of a real surgery.

First of all, an ACL reconstruction is known as failed when the elongation of the tendon grafts are over 3 mm (Alford, J.W., & Bach, B.R. , 2005). If the tendon relaxes that much, it does not meet the function of the native ACL anymore, not being able to hold together tibia and femur. This failure may cause anterior movement of the tibia off the femur, as well as hyperextension of the knee. In our specific case, the bovine tendon used for the simulation were two times wider than human tendons, but when realizing a regular ACL surgery, they would use two human tendons for one graft, so both grafts are of similar width. However, when preparing a real ACL graft, they are usually bended in order to achieve a stronger

conformation. This is the reason why we should divide the elongation of our tendon grafts by two.

Taking into account previous considerations, we can determine that none of the results obtained in the five different methods failed; their normalized elongation at the start of cycle plus the cycles and divided by two resulting in values of 2.5±0.5, 2.1±0.5, 2.1±0.5, 1.3±0.5 and 1.2±0.6. Although all of them are under the 3 mm threshold (Alford, J.W., & Bach, B.R. , 2005), we can already see a tendency of which ones are going to be worse or better.

To be able to differentiate these methods, and statistic approach was used. First, we performed the one-way Anova method. This method is able to tell us if the several protocols differ from one another, taking into account a p-value lesser than 0.05. When performing the test, a value of 1.2698e-07 for the p-value was obtained and thus, lesser than the one previously determined (0.05).

Knowing that the protocols are different is not enough to be able to determine which protocol is the most suitable one to be implemented in operating rooms. Thus, further analysis was performed using the Tukey HSD Test. This test compares all the protocols two by two, obtaining in this way all the possible combination of pairs that exist between them (k=5 results in 10 different pairs). Similar to the Anova method, it is also based on a critical value that has to be reached to determine whether two methods as different or not.

First, we are going to analyze the pairs that did not achieve the p-value, that is, their Tukey HSD interference was insignificant and hence they are considered as equal protocols. On the one hand, these pairs are Control and Control w I.T.; Control and 45N; and last, Control w I.T and 45N. As expected in our hypothesis, the Control and the Control w I.T. are considered the same protocol and we can determine that applying an initial tension to the grafts is not enough to remove the undesired viscoelastic property. Pretensioning of the soft tissue grafts is totally required. However, it wasn't expected that the method 45N would be equal as the two Control protocols, implying that applying such small value of load to the tendons is not enough either. On the other hand, the last pair that is also consider as equal methods is 89 N and 150 N. From here, we can determine that load is not that important once it is over 89 N, as applying this load and 150 N produce the same effect on the tendon grafts.

Second, the pair of methods that did achieve the p-value and so are consider different treatments, serve as indicators to confirm that the protocols 89 N and 150 N are different to the Control, Control w I.T. and the 45N ones. With the help of this analytical tool, we are able to define the best two protocols, being 89 N and 150 N.

6.2. Conclusions

Although a single best protocol has not been achieved to totally remove the viscoelastic property of tendons used as grafts for ACL reconstruction, some discoveries have been made. Firstly, none of the protocols tried, not even the control group, are considered as failed surgery because its elongation after rehabilitation is shorter than 3 mm. Secondly, against some authors opinions (Nurmi, J. T., Kannus, P., Sievänen, H., Järvelä, T., Järvinen, M., & Järvinen, T. L., 2004), a pretension protocol is required to be applied to the tendons. This has been proved through the control group where pretensioning was not applied, thus leading to a much higher elongation in the cyclic tests and proving that the viscoelastic property of the tendon grafts was not removed enough. A 45 N pretension protocol was attempted, but the results obtained from it can be consider equal as the control group, so not contributing to any significant discovery. Finally, the last two out of the three protocols proposed were considered as successful, being these the 89 n and 150 N. And because this preconditioning of the soft tissue graft has to be done in a specially designed graft board while the surgery is taking place, the easiest successful protocol would be the best one, which was the 89 N.

In conclusion, it was discovered that in an ACL reconstruction it is beneficial for the soft tissue graft to be pretensioned before its implantation on the patient's knee by using a graft board. Such pretension protocol has to be of at least 89 N to be able to cause some impact reducing the graft viscoelastic property and so reducing the possibilities of graft post-surgery elongation.

6.3. Limitations

Although this project was performed successfully, it is true that several limitations have been found during its realization. The first one, and the most important one, is that reaching the end of the project we realized that tendons were slipping a little bit from the jaws. The partial solution we gave to this problem was to take as total length the one between the jaws plus the jaws length, as all of it was submitted to testing.

Furthermore, the low precision of the machine was an issue. We realized that the position sensor and the machine load accuracy were improvable for the small loads and positions being tested.

All these limitation, make us trust the comparisons between the protocols, as they were all performed in the same way, but not the absolute values obtained. Thus, the final results are not invalid. Further approaches to all these problems, have been though and explained in the next section, 6.4.

6.4. Future Work

After all this work have been done, the methodology of testing the tendons in a laboratory needs further improvement in an effort of trying to achieve similar conditions to an actual ACL reconstruction surgery. One possibility could be to test the tendon bended, as they are in a human graft. Researchers across the world are already working in that direction (Jaglowski, J. R., Williams, B. T., Turnbull, T. L., LaPrade, R. F., & Wijdicks, C. A., 2016), which promises progress in that field in the years to come. Therefore, new jaws have to be designed in order to achieve the new settlement. And it is necessary to ensure that soft tissue grafts do not slip from the new jaws, thus invalidating the experiment results. Moreover, to overcome the machine's inaccuracy, a new and more precise position sensor and load sensor have already been ordered.

With respect to new protocols that may be tested, we don't plan to change the initial tension load since it is done by a surgeon inside the patient's knee, thus values higher than 20 N not being realistic. Regarding the pretension protocol, we have proved that high load values are not suitable for this step, again because the difficulties of realizing it within the operating

room, while values lower than 89 N are not valid either because its inability to remove the undesired property. Therefore, we have decided the load of the protocols to be 89 N for all the protocols, as it is the best considered one in our study. However, it might be considered varying the time load is applied.

Furthermore, knowing that the protocol Control and Control w I.T. are considered equal, we decided to just use the Control w I.T. in order to compare it to the other protocols also tested in the new simulation. This way we can determine how much better is to perform a pretension over not doing it.

In this section it has just been discussed the best protocol, out of the ones previously proposed, being this the one of 89 N. However, the viscoelastic property wasn't reduced to the maximum so limitations and further approaches for the experiment were also described.

7. PLANIFICATION

In this last section, the planning of the project is going to be described. Firstly, the regulatory framework in which the project was embedded and secondly the budget that was needed to make it possible.

7.1. Regulatory Framework

There is a main reason why we decided not to work with human cadaveric tendons. The number of test that we could perform would be limited and the tendons would also be hard to obtain, thus not allowing us to perform this trial and error experiment.

The material used was of animal nature thus, all the legal regulations and technical standards had to be followed. Bovine Digital Extensor tendon was chosen because its similarities to human ACL soft tissue grafts. They were extracted from the lower part of bovine legs. The legs were obtained at the 'Matadero Insular de Gran Canaria' after completing an agreement between this intity and the University of Las Palmas de Gran Canaria.

The experiment we realized was conducted under the Legal Framework SANDACH (Subproductos De Origen Animal No Destinados a Consumo Humano) from April 2013. Regulation (CE) No. 1069/2009 categorizes animal by-products and derivative products in three categories depending on the risk they provide to the population, being the first category the one that encompasses the riskiest products and the third one the less risky products. The bovine tendons we used are included in the third category. Specifically, this third category includes products of animal origin or food products that contain the by-products not intended for human consumption because of commercial reasons, manufacturing or packaging defects, or other defects that do not pose a risk for human health.

As said in SANDACH, materials from the three categories may be used for research purposes under conditions that guarantee the control of risks, such as the prohibition of any subsequent use for other purposes and the obligation to remove by-products or derived products safely or return them to their place of origin.

Hence, this project and its materials followed all the regulations required.

7.2. Budget

As every research project, a budget is needed in order to be able to carry out the experiments proposed. Firstly, a material resources list was made and further investigation of their respective prices was achieved (Table 10).

Table 10. Breakdown of Material Resources costs.

Material Resources			
Concept	Cost (€)	Life cycle (years)	Amortization (€)
Hydraulic Fatigue Machine	30,000	25	500
Computer	3000	4	300
15 Bovine legs	*	*	*
Others**	50	-	50
		Total	850 €

*The bovine legs were obtained for free due to an agreement between the University of Las Palmas de Gran Canaria and the 'Matadero Insular de Gran Canaria'.

**This group contains the extra material explained in section 4.1.3.

Secondly, a human resources table was also performed (Table 11). Here, we took into account the price that junior and senior engineers would ask for in a company.

Table 11. Breakdown of Human Resources costs.

Human Resources			
Concept	Cost (€) /hour	Hours	Amount (€)
Undergraduate Researcher	12	400	4800
Supervisor Engineer	50	100	5000
		Total	9,800 €

Summing up the total quantities obtained from both tables, this project supposed a total budget of 10,650€

In this last section of the report, it was just explained the regulatory framework that regulates the material used during the whole project realization and its safely removal after the tests. Moreover, the budget employed in the material resources as well as in the human resources was also indicated and explained.

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