

Faculdade de Engenharia da Universidade do Porto



Functional Electrostimulation Applied in Assisted Rehabilitation after Anterior Cruciate Ligament Reconstruction

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Resumo

Este trabalho tem como objetivo desenvolver um protótipo de um sistema de eletroestimulação funcional combinado com terapia interferencial, para promover um melhor retorno à atividade de pacientes submetidos a reconstrução do ligamento cruzado anterior (LCA).

Após rotura do LCA e quando é necessária reconstrução deste ligamento, recorre-se a diferentes tipos de enxerto, tais como do tendão semitendinoso, patelar e quadrícipital. Naturalmente há a perda das características originais do ligamento ao substituí-lo por um enxerto, porém estes sofrem um processo de cura que envolvem a ligamentização e reinervação da estrutura utilizada e poderão ser recuperadas as características e funções do ligamento original. Na verdade, após lesão e reconstrução do ligamento acaba por se danificar e perder células sensoriais designadas de mecanoreceptores, o que consequentemente poderá afetar a capacidade de propriocepção e a estabilidade do joelho da pessoa lesionada.

Existem vários métodos de eletroestimulação que auxiliam na recuperação após reconstrução do LCA, porém podemos afirmar que não existe uma única abordagem terapêutica que seja totalmente eficaz. Uma das razões que poderá reduzir a eficácia destes métodos reside no facto de que a eletroestimulação não é realizada de forma verdadeiramente sincronizada com a atividade muscular do paciente, o que não facilita um apropriado estabelecimento de um *feedback* neurológico aquando da realização dos exercícios de reabilitação.

Com o sistema desenvolvido a estimulação da zona do joelho dos pacientes é feita apenas quando estes contraem os músculos do quadríceps, sendo que os pacientes iniciam cada sessão de tratamento com três séries de oito repetições do exercício “Quad Set”. Foi ainda elaborada uma interface gráfica de quadrados coloridos pela qual os pacientes guiam as suas ações. Posteriormente realizam o resto da sessão com os terapeutas, seguindo uma via mais convencional.

De modo a verificar os efeitos do sistema, foram usados um questionário inicial e um final de 10 perguntas que avaliavam particularmente dois aspetos - a capacidade de propriocepção e a estabilidade do joelho. De uma forma global, verificaram-se melhorias em 2 pacientes, 1 manteve-se sensivelmente igual e 1 piorou. No âmbito da propriocepção, 1 dos pacientes melhorou significativamente, num não se verificou diferenças entre os dois momentos e os outros 2 pioraram ligeiramente. No que diz respeito à estabilidade do joelho verificaram-se melhorias em 3 dos pacientes e 1 piorou ligeiramente. Pode-se afirmar que os resultados são inconclusivos sendo necessária a realização de mais estudos durante um período mais extenso e com uma amostra maior e mais homogênea.

Palavras chave: Eletroestimulação funcional, terapia interferencial, propriocepção, ligamento cruzado anterior.

Abstract

The aim of this study is to develop a prototype of a functional electrostimulation system combined with interferential therapy to promote a better return to activity in patients undergoing anterior cruciate ligament (ACL) reconstruction.

After rupture of the ACL and when reconstruction of this ligament is necessary, different types of graft are used, such as the semitendinosus, patellar and quadriceps tendons. Naturally, the original characteristics of the ligament are lost when it is replaced with a graft, but these undergo a healing process that involves ligamentisation and reinnervation of the structure used and the characteristics and functions of the original ligament can be recovered. In fact, after injury and reconstruction, the ligament ends up being damaged and losing sensory cells called mechanoreceptors, which can consequently affect the injured person's capacity for proprioception and knee stability.

There are various methods of electrostimulation that aid recovery after ACL reconstruction, but we can say that there is no single therapeutic approach that is totally efficient. One of the reasons that may reduce the effectiveness of these methods is that electrostimulation is not carried out in a way that is truly synchronised with the patient's muscular activity, which does not facilitate the proper establishment of neurological feedback when carrying out rehabilitation exercises.

With the system developed, stimulation of the patients' knee area is only carried out when they contract their quadriceps muscles, and the patients begin each treatment session with three sets of eight repetitions of the "Quad Set" exercise. A graphical interface of coloured squares was also designed to guide the patients' actions. They then carry out the rest of the session with the therapists, following a more conventional treatment.

In order to verify the effects of the system, a 10-question initial and final questionnaire was used to assess two aspects in particular - proprioception capacity and knee stability. Overall, there were improvements in 2 patients, 1 remained about the same and 1 worsened. In terms of proprioception, 1 of the patients improved significantly, in one there were no differences between the two moments and the other 2 worsened slightly. As far as knee stability is concerned, 3 of the patients improved and 1 worsened slightly. It can be said that the results are inconclusive and further studies need to be carried out over a longer period and with a larger, more homogeneous sample.

Keywords: Functional electrical stimulation, interferential therapy, proprioception, anterior cruciate ligament.

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ABBREVIATIONS

ACL	Anterior Cruciate Ligament
ADSC	Adipose-Derived Stem Cells
AMI	Arthrogenic Muscle Inhibition
CKC	Closed Kinetic Chain
EMG	Electromyography
FES	Functional Electrical Stimulation
IFT	Interferential Therapy
NMES	Neuro-Muscular Electrical Stimulation
PLC	Posterior Cruciate Ligament
PNF	Proprioceptive Neuromuscular Facilitation
RMS	Root Mean Square
ROM	Range of Motion
RTS	Return to Sports
SEP	Somatosensory Evoked Potentials
VEGF-A	Vascular Endothelial Growth Factor A

CHAPTER 1

1.1 INTRODUCTION

The injuries of the anterior cruciate ligament (ACL) are one of the most common lesions among athletes. They often occur when these perform movements involving sudden stops or changes in direction, jumping and landing.

Individuals undergoing anterior cruciate ligament (ACL) reconstruction are prone to experiencing sequelae, with the primary issue being arthrogenic muscle inhibition, characterized by reduced excitability of muscle motoneurons. In this scenario, after ACL reconstruction, it is common to observe Quadriceps Arthrogenic Muscle Inhibition (AMI). Following surgery, the loss of many mechanoreceptors occurs, thus affecting the feedback/feedforward mechanism. In other words, if the knee nervous communication pathways are compromised, the communication from the brain to the knee is also impacted. This can result in problems related not only to proprioception and knee positioning but also to potential compensatory adjustments, both muscular and visual, increasing time to recovery and the likelihood of injury recurrence. Furthermore, muscle strength can be adversely affected, with frequent observations of quadriceps strength deficits persisting for months and sometimes even years post-surgery.

Given these challenges, various methods and treatments have been developed, with neuromuscular electrical stimulation (NMES) standing out as a prominent approach. However, it cannot be asserted that a single treatment approach is entirely effective. One of the contributing factors to the reduced efficacy lies in the fact that stimulation is not applied in a truly synchronized manner, which does not facilitate the establishment of a proper neurological feedback during rehabilitation. In addition, interferential therapy has not been fully explored in post-operative ACL rehabilitation, and could possibly bring advantages in the regeneration and re-ervation of the ligament, as this is a structure that is located deep in the knee and some evidence suggests that interferential therapy allows the electric current to penetrate deeper into human tissues.

The aim of this dissertation is to develop a functional electrostimulation system combined with interferential therapy, using an electrostimulator (Sonopuls 992) and an auxiliary circuit developed by the researcher that collects the electromyographic signal from the quadriceps muscles and prevents/allows current to pass to electrodes positioned in the knee area, with current only passing when muscle contraction is detected.

The system developed by the researcher was used to apply an FES and interferential therapy protocol to 4 patients, using an isometric rehabilitation exercise (Quad Set) which simply consists of voluntary contraction of the quadriceps muscles. At the end of the application, the patients' progress was assessed using questionnaires that mainly evaluate proprioception and knee stability, which are directly related to the regeneration and re-innervation of the ligament.

This dissertation presents a theoretical foundation in the second chapter related to the anatomy of the knee joint and factors predisposing to ACL Injury, ACL reconstruction, rehabilitation after ACL injury, methods of assessing and monitoring recovery progress and tools and methods such as EMG signal collection and electrostimulation, and functional electrostimulation, in order to better understand the more practical side of this work - the application of FES.

The third chapter covers the process of developing the system, from the hardware to the software (muscle contraction detection algorithm), and the graphical interface through which patients guide their actions. Concepts such as gamification are mentioned with an example developed by the researcher, which could be tools to combine in future work. Also mentioned are the tools used to assess patient status (questionnaires), the associated results and conclusions, and some of the study's limitations.

Finally, the fourth chapter mentions the main conclusions of the work and some suggestions for future work.

CHAPTER 2 - STATE OF THE ART

Every year, around 1,4 million ACL injuries occur globally, mainly in sports involving quick changes in direction, twisting, and jumping. These injuries are impactful for athletes at all levels, causing prolonged recovery and sometimes preventing them from returning to their sport. Even after surgical reconstruction, individuals remain at risk of developing osteoarthritis at a younger age [1].

There are some human body systems that operate automatically and subconsciously maintain the body in its homeostatic state. One such system is the sensorimotor system, which controls joint stability during movement through a network of nerve signals.

After ACL injury, the two most common treatment and recovery methods are conservative treatment and ligament reconstruction.

This chapter presents some bibliography related to the anatomy of the knee and the factors predisposing to ACL injury, detailed information about some ACL reconstruction methods, the grafts used to substitute the ligament, common sequelae after injury and reconstruction of the ACL, some aspects of rehabilitation on this type of injury, electrostimulation as a tool in the process of recovery and its mechanisms and parameters, the process of regeneration and ligamentization of the graft and neuromuscular recovery.

2.1 ANATOMY OF THE KNEE JOINT AND FACTORS PREDISPOSING TO ACL INJURY

2.1.1 Knee Anatomy

The knee joint links the upper leg to the lower leg, and is the largest joint in your body. Knees play a crucial role in supporting the body weight and facilitating leg movement. They're involved in almost every leg-related activity, such as walking, running, and jumping. The key functions of your knees include:

- Enabling leg movement.

- Providing support when you stand and move.
- Contributing to stability and balance.

The knee is composed of various components, including bones, cartilage, ligaments, muscles, and nerves. Central in the knee's structure are the cruciate ligaments. They intersect each other, forming an X-shaped structure. These ligaments play a crucial role in regulating the forward and backward movements of the knee. Specifically, the anterior cruciate ligament (ACL) is positioned at the front of your knee, while the posterior cruciate ligament (PCL) is located behind the ACL, toward the rear of the knee. The ACL primarily regulates and stabilizes the forward movement of the knee. It helps to prevent the tibia from moving too far forward in relation to the femur. While it does have some influence on rotational stability, its main function is to prevent anterior translation of the tibia [2] .

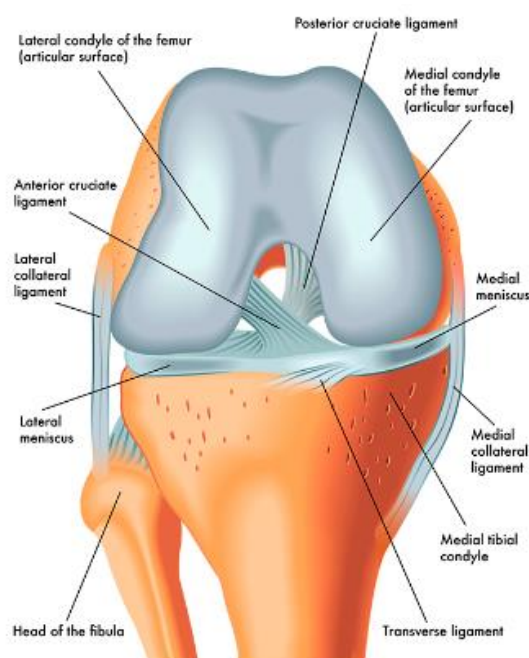


Figure 1: Knee Anatomy [3].

In the past, the ACL was thought to be a single and uniform structure. However, recent research suggests that it has two parts, called the anteromedial and posterolateral bundles. These parts are separated by a sheath of tissue and have different insertion locations and have unique functions that allow for the ACL's normal biomechanics. The anteromedial bundle is tight in flexion, whereas the posterolateral bundle is tight in extension [4].

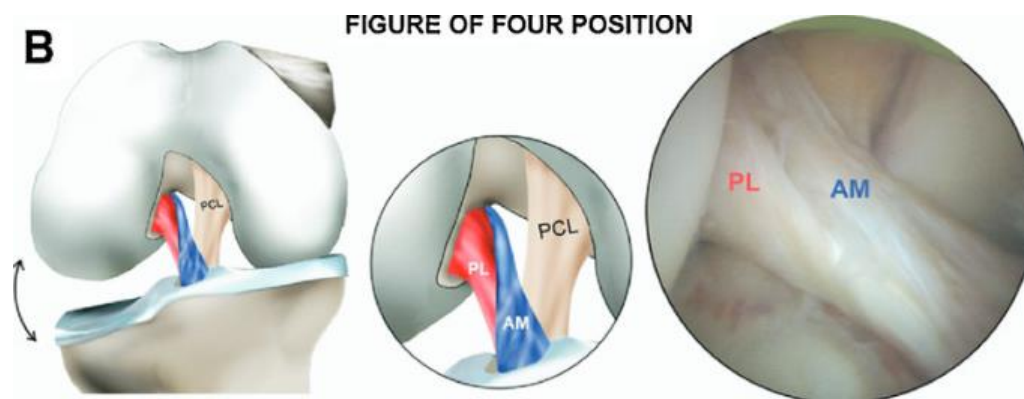


Figure 2: Anteromedial and posterolateral ACL's bundles[5].

Anatomical studies of the ACL and its bundles have found that the ACL's length can vary from 27 to 38 mm, depending on gender and the knee's position, and its width is typically 10 to 12 mm. The anteromedial bundle is about 6 to 7 mm wide, while the posterolateral bundle is about 5 to 6 mm wide [6].

In terms of histology, the anterior cruciate ligament (ACL) is composed of highly organized matrices of collagen type I (90%) and type III (10%), accounting for approximately 75% of its dry weight. Water constitutes 60% of its wet weight under physiological conditions [6].

When the knee is fully extended, the anterior cruciate ligament (ACL) plays a crucial role by absorbing 75% of the forward movement load, and this percentage increases to 85% when the knee is flexed between 30 and 90 degrees. The typical tensile strength of the ACL stands at approximately 2200 N, yet this strength is subject to change with age and repetitive mechanical stress [4].

The anterior cruciate ligament (ACL) is connected to nerve fibers from the posterior articular branches of the tibial nerve [7]. Most of these fibers are related to the blood vessels within the ligament and help control blood flow.

The sensorimotor role of the knee mechanoreceptors

Research has shown that the ACL, a key ligament in the knee, contains dense clusters of mechanoreceptors at its attachment points to bone, indicating its role in sensing joint position and movement. Interestingly, a connection between the brain and the ACL has been observed through electrical signals detected during ACL stimulation. When the ACL is injured, various sensorimotor problems can arise, including difficulties in sensing joint position (proprioception), weakness in muscles around the knee, and changes in muscle activation patterns [1]. The specific neurotransmitter receptors present in the ACL are.

- Ruffini receptors, which are sensitive to stretching, are mainly found on the surface of the ligament, particularly on the part connected to the thigh, where there's more movement [7]
- Vater-Pacini receptors, which are sensitive to quick movements, are located at both ends of the ACL, near the thigh and shin [8].

- Golgi-like tension receptors are situated close to where the ACL attaches and also beneath the lining of the joint [7].

These receptors (Ruffini, Pacini, and Golgi-like) are crucial for sensing the knee's position and movement, helping the brain understand the leg's posture. When nerve fibers in the proximal part of the ACL are activated, they affect the muscles around the knee, which is known as the ACL reflex. This reflex is vital for normal knee function and plays a role in updating how muscles work during movement [9].

2.1.2 ACL Injury Factors

When studying an injury, it is important to understand that they typically have a multifactorial nature, i. e., the same process can lead to different outcomes. According to the literature, there are mechanisms directly related to ACL injury such as controlling the valgus knee mechanism, external tibial rotation, anterior shear forces, and deceleration [10].

Therefore, athletes in sports such as football, basketball, volleyball, and skiing, where rapid changes of direction and ground impacts following jumps are prevalent, are more susceptible to this type of injury. However, as mentioned earlier, one process can lead to different outcomes, and we should not have a reductionist view because, in this case, there are no linear cause-and-effect relationships. We should broaden our perspective and consider the whole picture.

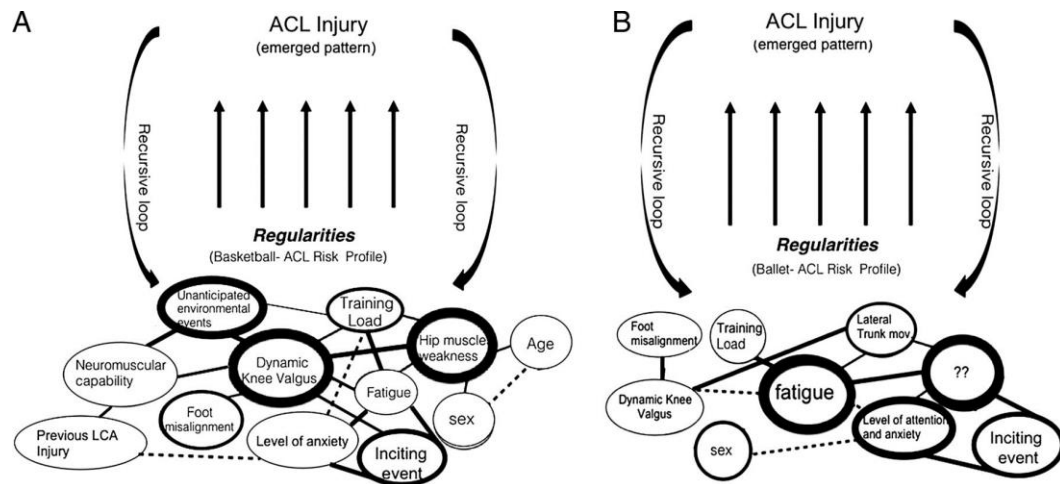


Figure 3: Factors that contribute to the ACL injury [11].

As can be seen in Fig. 3, we have two cases of ACL injury, with A being a basketball player and B being a ballet practitioner. In each of these cases, there are numerous factors that contribute to the injury. In the case of the basketball player, factors such as valgus knee movement, hip muscle weakness, and unpredictable environments are highly relevant. In the case of the ballet practitioner, factors such as fatigue, anxiety levels, and attention played a significant role in

triggering the injury. However, it is important to note that there are numerous factors in total that lead to injury in each of these individuals [11].

In accordance with the severity of the injury, it can be categorized into three grades [12].

- Grade 1: Characterized by a mild stretching of the ligament while maintaining the stability of the knee joint.
- Grade 2: Involves greater stretching of the ligament and may result in a partial tear.
- Grade 3: Complete rupture of the ligament, leading to instability of the knee joint.

2.2 ANTERIOR CRUCIATE LIGAMENT RECONSTRUCTION

2.2.1 Surgery

When an ACL injury occurs, there are two main treatment options: surgery or conservative (non-surgical) treatment. Surgery is advisable in cases where the individual with an ACL injury is young, participates in sports with frequent changes of direction and explosive movements, or if their daily life is compromised by knee instability.

Regarding conservative treatment some methods like targeted exercises to reduce swelling, control knee movement, strengthen muscles, and enhance stability, cold therapy and restrictive bracing are used [64]. However, the conservative path could lead to recurrent knee instability, meniscal injuries and early osteoarthritis in medium-long term [65].

The ACL does not have good natural healing capacity, which necessitates surgery, with ligament reconstruction being the standard procedure. In ACL reconstruction, a piece of tendon, called a graft, is used. This graft is secured inside the knee using tunnels created in the femur and tibia bones, replicating the position of the original ligament.

The surgery does not require open joint incisions. It is performed using arthroscopic techniques, which involve small incisions and a fiberoptic camera to visualize the area and specialized instruments to work inside the joint.

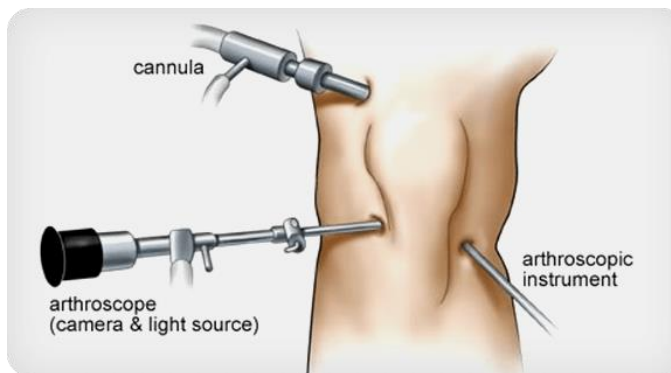


Figure 4: Arthroscopic technique for ACL reconstruction [13].

In 2018, Dr José Carlos Noronha and Dr João Pedro Oliveira described a modification of the original anterior cruciate ligament reconstruction technique [14]. It turns out to be a more economical way of performing the reconstruction. It is also an easy and reproducible all-inside ACL reconstruction technique in which the femoral socket is made through a standard anteromedial (AM) portal and the tibial socket is made through an anterolateral portal for inside-out tunnel drilling using a flexible reamer. As a result, it avoids the drill's retrograde aiming device and allows a final fixation of the graft at the desired degree of flexion.

This surgical technique has 6 steps:

- Step 1: Portal Configuration - Two arthroscopic portals are used for the surgery, 1 AM portal and 1 AL portal at the inferior pole of the patella.
- Step 2: Graft Harvest - Collection of the semitendinosus tendon (ST) graft with the knee flexed at 90 degrees and the ST is detached from its attachments, and prepared for use.
- Step 3: Graft Preparation - A fixation device is placed at both ends of the graft and the it is prepared and folded into a double-loop configuration.
- Step 4: Tibial Socket Creation - A drilling guide is used to create a hole in the tibia to secure the graft, a reamer is used to create a space for the graft and a wire is passed through the hole and exits through the AL portal.



Figure 5: Tibial socket arthroscopic view [14].

- Step 5: Femoral Socket Creation - The femoral attachment point of the ACL is identified and a drilling guide is used to create a socket in the femur at the marked point.
- Step 6: Graft Passage and Fixation - The graft is passed into both the femoral and tibial sockets, fixation devices are used to secure the graft to the bones and the graft is tensioned and checked for stability.

An ACL reconstruction (ACLR) should be considered only under the following conditions [15]:

- The patient experiences functional instability and complains of the knee giving way. In acute situations, it can be challenging to determine if there is functional instability. Hence, it is recommended to avoid ACLR in acute cases to reduce the risk of operating on asymptomatic patients.
- The knee exhibits minimal synovial (joint lining) inflammation, has full extension (0 degrees), demonstrates good patellofemoral mobility (equal on both sides), the patient can actively control the quadriceps muscle, and there is a proper walking pattern to prevent post-surgery arthrofibrosis (excessive scar tissue formation in the joint).
- The patient has a quadriceps strength deficit of no more than 20% compared to the healthy leg. A deficit of 20% or greater indicates a significant strength imbalance that may persist for up to two years after ACLR.

2.2.2 Grafts in ACL reconstruction

There are various options for tendon grafts that can be used to reconstruct the ACL, and in selecting a graft, it is important to consider that it should be stronger than the original ligament, allow secure fixation, be easy to remove if needed, and ideally not result in functional deficits upon removal.

Some of the options for grafts used in ACL reconstruction include the gracilis and semitendinosus tendons, patellar tendon grafts, and quadriceps tendon grafts [16].

The most common grafts used in ACL reconstruction are the hamstring tendon grafts (gracilis and semitendinosus tendons) and the patellar tendon grafts. A meta-analysis conducted by Samuelsen et al. (2017) concluded that when comparing these two types of grafts, hamstring tendon grafts have a slightly higher failure rate compared to patellar tendon grafts [17]. However, the failure rates were generally low in both cases, and the difference was small. In fact, there were no significant differences between the types of grafts in terms of knee stability, indicating that both options are viable.

Regarding the quadriceps tendon graft, it is increasingly being used in ACL reconstruction [18].



Figure 6: Skin incision in each tendon graft [19].

In fact, ligament reconstruction is a method that tends to have more favourable results in the problems described above, however, we must bear in mind that the effectiveness of ACL reconstruction ranged from 73% to 95%, while the rate of athletes returning to their pre-injury activity level ranged from 37% to 75% [20]. In particular, the success rate of returning to sport is very low.

One of the main causes of low results following ACL reconstruction is graft failure, which manifests as weaker mechanical characteristics compared to the original ACL. Studies have reported a graft failure rate ranging from 1.5% to 5.7% [21]. Also, the process of healing of the graft used to substitute the original ligament is very slow, leading to long lasting periods of rehabilitation and monitoring before being ready to start their normal physical life [22].

2.2.3 Arthrogenic Muscle Inhibition

According to a study by Diekfuss et al. (2018), individuals who do not experience an ACL injury have a stronger functional connection between the left primary sensory cortex and the right posterior cerebellar lobe responsible for balance and coordination. Thus, it can be stated that there is a predisposition to ACL injury in individuals with a reduced functional connection [23].

This can assist in rehabilitation planning and assign a more significant role to adaptive neuroplasticity to reduce coordination errors and the risk of injury. Therefore, it is essential to understand the compensations in sensory and visual-motor processing caused by ACL injuries. After an injury, afferent input is disrupted due to the loss of mechanoreceptors, resulting in altered somatosensory processing. Consequently, changes in proprioception occur, meaning that we no longer accurately perceive the position of the knee and how it is moving. This leads to a decrease in somatosensory contribution to motor control, resulting in impaired motor neuroplasticity due to altered efferent output and motor processing requiring more planning and visual feedback.

As a result, there is reduced stability in the absence of visual feedback, which affects movement control and becomes more reliant on visual feedback [24].

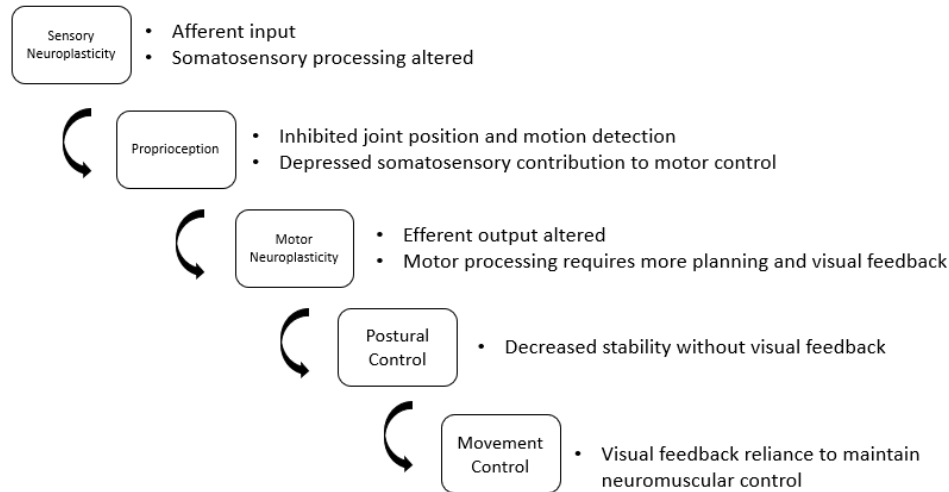


Figure 7: Successive responses after ACL rupture [24].

The loss of mechanoreceptors leads to arthrogenic muscle inhibition. In fact, after injury and surgery, there is a loss of some components such as intrafusal cells, the anterior cruciate ligament, and even some Golgi tendon organs. As can be seen in Fig. 8, there are several factors that contribute to arthrogenic muscle inhibition, including inflammation, swelling, ligament laxity, and receptor damage. These factors lead to other processes, such as the inhibition of group I nonreciprocal (Ib) interneurons, which consequently result in reduced excitability of quadriceps motoneurons, characterizing arthrogenic muscle inhibition [25].

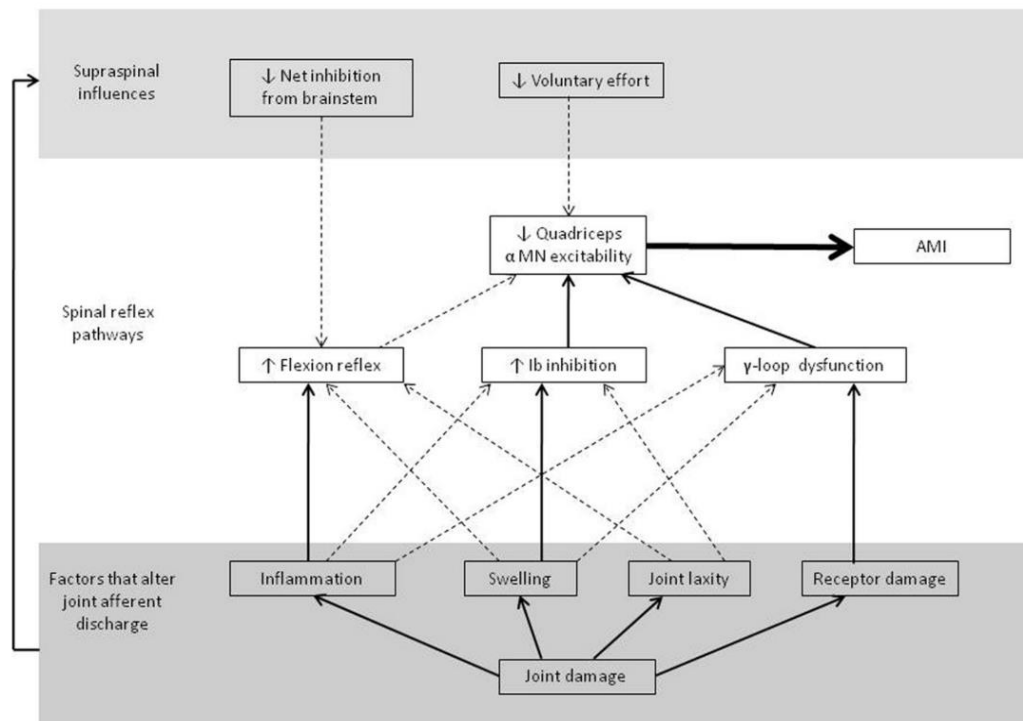


Figure 8: Factors and their relationships that may lead to arthrogenic muscle inhibition [25].

Certainly, due to immobilization, the strength levels of the lower limb are significantly impacted. In fact, deficits in quadriceps strength have been reported months to years after ACL reconstruction surgery [26].

2.3 REHABILITATION

2.3.1 Pre-surgery rehabilitation

When it comes to rehabilitation after anterior cruciate ligament (ACL) surgery, it is essential to understand not only the role of post-operative rehabilitation but also the role of pre-operative rehabilitation. According to the literature, the primary objectives of pre-operative rehabilitation are:

- Education and mental preparation;
- Achieving full knee extension;
- Pre-operative strength deficit quadriceps < 20%;
- A normal gait pattern;
- Minimal swelling.

To better understand the role of pre-rehabilitation, there are two important concepts related to a person's predisposition for a more favourable or less favourable recovery in the context of an ACL injury: "coper" and "non-coper." A person is characterized as a "coper" if they meet all the requirements represented in table 1 which correspond to well-being and daily activity perception questionnaires, bilateral differences within a maximum of 20%, and experiencing only one or no knee instability episodes.

Table 1: Coper classification tests [27].

Screening test	Coper classification threshold
Timed 6 meter hop	$\geq 80\%$
Knee Outcome Survey - Activities of Daily Living Score	$\geq 80\%$
Global Rating Score	$\geq 60\%$
Number of giveaway episodes	≤ 1

Copers achieve better long-term functional outcomes and return to activity, and sometimes they may not even require surgery. In this case, one of the objectives of pre-rehabilitation is to transform non-copers into copers. In fact, some studies confirm that this transition from non-coper to coper is possible, and according to the literature, the classification as a coper translates into satisfactory results for at least up to two years [27].

In this way, we can assert that, before ACL surgery, slowly advancing pre-surgery rehab should be seen as an important addition to standard care to enhance functional outcomes after the surgery [28].

2.3.2 Post-surgery rehabilitation

Utilizing a criteria-based, evidence-based framework for post-ACL surgery rehabilitation is crucial to systematically and effectively guide a patient through the recovery journey [29].

According to existing literature, rehabilitation following surgery can be categorized into three phases: the first phase, which is the initial post-operative stage; the second phase, termed the intermediate stage; and the third phase, focusing on sport-specific activities. Progression from one phase to the next, involving more demanding exercises, is determined by meeting specific clinical criteria [30].

In each phase of rehabilitation, there are specific objectives to achieve. Specifically, in the initial post-operative phase, the objectives include minimizing synovitis/effusion, achieving full knee extension (0°), regaining voluntary quadriceps control, and establishing an active dynamic gait pattern. In the second phase, the focus shifts to performing sport-specific tasks and physically demanding work without restrictions. In the third phase, the goal is to return to sports or physically demanding work [30].

During each of these phases, it is important to focus on training in terms of body functions and structures, such as mobility and strength, as well as activities and participation, including neuromuscular training, walking, and bicycling. Naturally, the training in these areas becomes progressively more challenging and complex as we advance through the phases. Detailed objectives, training recommendations, and phase progression criteria are specified in more detail in the attached document [30].

Strength training after an ACL surgery is a crucial aspect. Based on the training recommendations for each phase, some of the muscle strengthening exercises to be performed may include [30]:

- Phase 1: Straight leg raise, Leg-Press and Squat (ROM 0° - 60°), Step-up, Leg-Extension, Leg-Curl (for instance with elastic band) and concentric and eccentric strength training of the gluteal muscles, hamstrings and calf muscles such as ankle pump, heel slide, glute bridges and calf raises - note that throughout the weeks and depending on the response to the exercises, ROM can be gradually increased.
- Phase 2: Closed kinetic chain quadriceps exercises in full ROM range of motion, one-legged exercises (such as lunges or single-leg squats), Intensify strength training of the gluteal muscles, hamstrings and calf muscles, and decrease repetitions and increase resistance for all strength exercises.
- Phase 3: Intensify (sport) specific strength training.

When discussing rehabilitation protocols following ACL reconstruction, it's important to note that there are various protocols available, and they often differ in the number of phases they encompass. For example, Massachusetts General Hospital has developed a comprehensive rehabilitation protocol designed to provide guidance for clinicians in managing the post-operative course following ACL Reconstruction. This protocol is both time-based, taking into account the crucial aspect of tissue healing, and criterion-based, ensuring that specific milestones are met throughout the recovery process. This particular protocol comprises a total of six distinct phases:

- I. Immediate Post-Op (0-2 Weeks After Surgery)
- II. Intermediate Post-Op (3-5 Weeks After Surgery)
- III. Late Post-Op (6-8 Weeks After Surgery)
- IV. Transitional (9-12 Weeks After Surgery)
- V. Early Return to Sport (3-5 Months After Surgery)
- VI. Unrestricted Return to Sport (6+ Months After Surgery)

It's worth noting that these two protocols are just two of many rehab plans. These plans are customized to suit each patient's unique needs, considering the surgery type, patient goals, and what the surgeon prefers. These multi-phase plans play a crucial role in making sure that recovering from ACL surgery is both safe and successful.

When it comes to the recovery time after an ACL injury, it varies significantly, ranging from 6 to 12 months [27]. However, it's essential to assess the specific context. Typically, we need to adopt an approach that minimizes risks and maximizes benefits. Nevertheless, when dealing with high-performance athletes, there is a greater need to take more risks, which can sometimes lead to recurrences and a poorer recovery.

2.3.3 Anatomical and Physical Tests

2.3.3.1 KT-1000

The KT-1000 test is employed to provide an objective measurement of the degree of anterior movement of the knee joint between 20 and 30 degrees of knee flexion [31].



Figure 9: KT-1000 test [31].

After conducting extensive testing, the researchers were able to establish a distinct pattern: knees with an intact ACL or partial ACL tears exhibited less than 3 mm of increased anterior movement during the KT-1000 test. In contrast, all patients with a complete ACL tear displayed 3 mm or more of increased anterior movement when compared to their healthy, unaffected knee.

For this reason, the KT-1000 test is frequently used when there's uncertainty about whether a patient's ACL graft is torn or not. This test offers a more objective means of assessing a patient's potential knee instability, supplementing the information gathered from a physical examination to make a more accurate diagnosis.

2.3.3.2 Lachman Test

In the Lachman test, the patient should lie on their back. The knee being tested should be slightly bent, about 20 to 30 degrees. The examining doctor holds the patient's thigh with one hand and the lower leg with the other. The thumb of the hand holding the lower leg is placed on the front part of the patient's shin bone. To check the integrity of the anterior cruciate ligament (ACL), the examiner gently pulls the patient's lower leg forward while keeping the thigh steady.

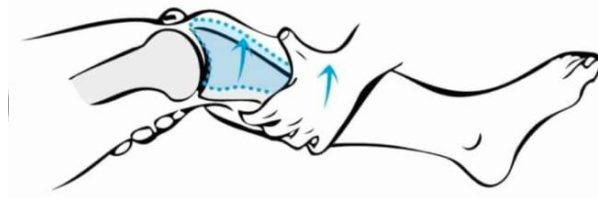


Figure 10: Lachman test [32].

When pulling the leg forward, a healthy ACL should prevent excessive forward movement of the shin relative to the thigh, resulting in a distinct and firm endpoint that the examiner can feel. The Lachman test is considered positive when there is too much forward movement of the shin, and no clear, firm endpoint is detected at the end of the motion [32].

2.3.3.3 Hop Test

Hop tests are the recommended choice for functional performance testing because they involve using the uninjured limb as a point of reference for comparing the injured limb. These tests also serve as a benchmark to assess when a person can safely leave rehabilitation and return to sports (RTS). Various hop tests, such as the single hop for distance, triple hop for distance, crossover hop for distance, and the 6-meter timed hop, have a strong body of research backing their reliability [33]. A limb symmetry index of >90% are often used as cut off scores for RTS [34].

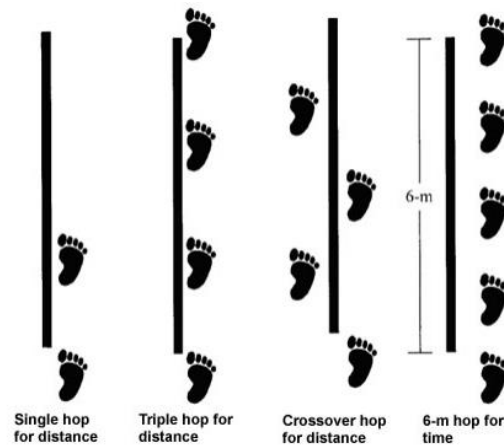


Figure 11: Hop tests [35].

2.3.4 Questionnaires

2.3.4.1 Return to Sport after Injury Questionnaire (ACL-RSI)

The ACL-RSI scale, was designed to assess psychological readiness for a return to sports after an Anterior Cruciate Ligament injury, helps identify specific psychological areas where patients might benefit from counselling alongside their physical rehabilitation. It consists of 12 questions that inquire about the psychological impact of resuming sports activities. These questions fall into three categories: risk assessment, self-confidence, and emotional well-being. Higher scores on this questionnaire indicate a greater level of psychological readiness and preparedness for returning to sports [36].

2.3.4.2 Subjective Knee Evaluation Form (IKDC)

The International Knee Documentation Committee (IKDC) Subjective Knee Form is a tool specifically created to evaluate various aspects of knee health. It consists of 18 carefully selected questions that aim to assess symptoms like pain, stiffness, swelling, joint locking, and instability in the knee. Additionally, some questions are designed to measure the functional abilities of the knee, including everyday activities like walking, running, jumping, and performing tasks such as climbing stairs, standing, kneeling, squatting, sitting, and getting up from a chair [37].

2.3.4.3 Quality of Life Questionnaire (ACL-QoL)

The ACL Quality of Life (ACL-QOL) is a tool that patients can use to express how an ACL injury has affected their overall well-being. This questionnaire comprises 32 questions covering five key areas: symptoms, participation in sports, work-related activities, social interactions, and emotional well-being. When answering these questions, patients provide a score that ranges from 0 to 160.

Higher scores reflect a better quality of life, with a score above 120 generally considered indicative of good quality of life, while a score below 80 typically suggests a lower quality of life [38].

2.3.4.4 Knee Outcome Survey Activities of Daily Living Scale (ADLS - KOOS)

The ADLS is a 14-item scale used to assess how knee symptoms impact a person's ability to carry out daily activities and specific functional tasks. Each item is rated on a scale from 0 to 5, where 5 means no difficulty, and 0 means unable to perform the task. The highest achievable total score is 70. To get the overall ADLS percentage rating, you sum up the scores, divide by 70, and then multiply by 100. A higher percentage indicates better functional ability. This scale is suitable for patients who either do not engage in sports or recreational activities or who are not yet at the level of participating in these activities [39].

2.4 GRAFT HEALING PROCESS AFTER ACLR AND RELATED ISSUES

2.4.1 4 Stages of tissue healing

When we talk about human tissue, such as muscle, tendons and ligaments healing, usually it follows 4 stages: bleeding, inflammation, proliferation and remodelling. During these stages, the damaged tissue is gradually replaced by a new one. In other words, the injured tissue is destroyed and is replaced by living tissue in the body. These phases don't happen one after the other neatly; they often overlap. So, the human body is constantly working to heal itself, sometimes in multiple ways at once.

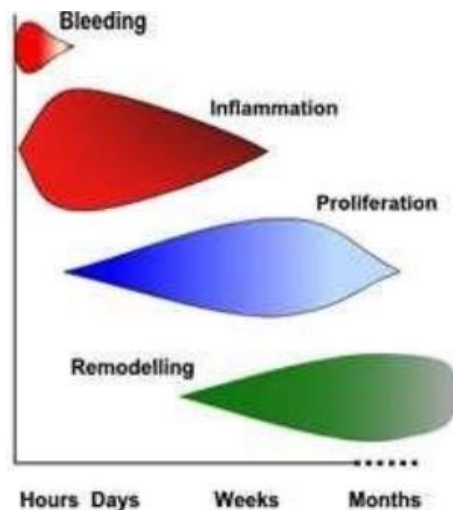


Figure 12: General stages of tissue healing [40].

2.4.1.1 Bleeding

The first stage of tissue healing is bleeding, that lasts about 24 hours, but the duration is related to the severity of the injury. Its function is protecting and reacting to the damaged. During this phase, various cells rush to the site to control bleeding. Thrombocytes and platelets work together to form clots, while leukocytes, a type of white blood cell, help fend off any potential infections. An effective management of this stage can reduce complications and promote better overall healing [40].

2.4.1.2 Inflammation

During this stage, which overlaps with the next stage (Proliferation), the focus is on protection and early repair. Typically, inflammation shows up with symptoms like swelling, bruising, warmth, pain, and limited function at the injury site. While inflammation used to be seen as something to minimize, recent research shows that is a crucial role in preparing the tissue regeneration.

Protection starts when injured cells release chemicals that initiate inflammation. The goal is to stimulate and regulate inflammation by widening blood vessels, which brings in lots of blood, oxygen, and nutrients to the injured area. This widening causes redness and swelling as blood rushes to the site. Pain pathways also activate during this time. Pain signals travel from the injury to the brain, prompting the brain to release chemicals or signal the injury to prevent further movement, enhancing protection.

Early repair marks the transition from inflammation to the proliferation phase. Inflammatory cells stick around so that when proliferation begins, there's a smooth transition and there is an overlap between the two stages [40].

2.4.1.3 Proliferation

Proliferation is the first phase of rebuilding itself. It begins around 48 hours after the injury and ends when most of the scar tissue has been formed.

In proliferation, specialized cells known as "blasts" arrive at the injury site to transform the fibrin-like matrix into a more organized structure. These blasts have the function to building a new tissue, which initially is disorganized and immature. For instance, if a bone is broken, new blood vessels may form to support the healing process. The blasts lay down this new tissue, whether it's bone, muscle, ligament, or another type, but it remains immature and unable to withstand normal forces.

Factors such as diet, exercise, and psychological variables, which could contribute to better outcomes in this stage, are being studied [40].

2.4.1.4 Remodeling

This stage is essential to transform the initially immature tissue, formed in proliferation stage into a more mature, organized structure. It's a gradual process that can take months to years,

depending on the type of tissue. During this phase, cells rearrange the disorganized tissue from the proliferation stage into a structured, aligned pattern, forming the final tissue type. This mature tissue is capable of withstanding the forces and stress exerted on it during movement [40].

2.4.2 Graft healing process after ACLR

After ACL reconstruction (ACLR), two crucial healing processes occur: tendon graft-to-bone tunnel healing and graft mid-substance remodelling, collectively known as graft healing. Initially, an inflammatory response is triggered, recruiting inflammatory cells to clear debris, that are waste material resulting from tissue damage, and release cytokines, which are small proteins secreted by various cells, including immune cells, that act as signalling molecules in the body that allows to regulate inflammation and act in immune responses, in other words they help orchestrate the healing process. These cytokines attract mesenchymal stromal cells (MSCs) to the bone tunnel and graft mid-substance. Subsequently, cell necrosis occurs within the implanted graft, followed by re-vascularization and repopulation of the tendon graft with MSCs. These cells differentiate and produce growth factors and extracellular matrix, facilitating the integration of the tendon graft into the bone tunnel. Meanwhile, with the assistance of inflammatory cells and MSCs, the graft mid-substance undergoes remodelling from a tendon to a ligament, a process known as ligamentization [41].

2.4.2.1 Graft-to-bone tunnel recovery

In the early stages of ACL reconstruction, attaching a flexible tendon graft to a rigid bone tunnel is a challenge. The key is to create a new attachment point, known as an "enthesis", which gradually transitions in structure, composition, and strength [42]. This ensures proper load distribution and prevents stress build-up at the junction. However, excessive inflammation can lead to the formation of a weaker scar tissue interface instead of a robust attachment site, resulting in subpar mechanical strength [43].

At the insertion where tendon meets bone, there are two types of connections: direct and indirect insertions. Direct insertion involves four gradual transition zones in order of gradual transition: tendon, uncalcified fibrocartilage, calcified fibrocartilage, and bone [42], while indirect insertion lacks fibrocartilage and attaches tendon to bone via Sharpey's fibers, which are fibrous connective tissue structures that connect tendons and ligaments to bone providing a strong attachment between these tissues. Both types have been studied in ACL reconstruction (ACLR), but indirect insertion is more commonly seen and provides more favourable outcomes [44]. However, the healing process in ACLR is not uniform due to disruptions in natural mechanical loads caused by placing the tendon graft in a bone tunnel, leading to irregular stress distribution and bone loss. This inconsistency in healing can result in tunnel widening and decreased graft stability. Regeneration of the native ACL insertion site is slow, contributing to unsatisfactory outcomes in graft healing after ACLR.

2.4.2.2 Ligamentization

As the healing progresses after ACL reconstruction, the focus shifts from the connection between the tendon graft and bone tunnel to the middle portion of the graft itself. During this phase, the tendon graft undergoes a transformation known as "ligamentization," where its structure and composition change to resemble that of a natural ligament.

This transformation involves several adjustments. While ligaments and tendons share similarities, they also have distinct characteristics. Ligaments have more active cells and different types of collagen compared to tendons. Regarding the active cells, known as fibroblasts, ligaments normally have more compared to tendons. These fibroblasts are responsible for producing and maintaining the extracellular matrix of the tissue. The higher number of fibroblasts in ligaments reflects the dynamic nature of these tissues, as they are constantly subjected to mechanical stresses and strains. In terms of the type of collagen (main structural protein in connective tissues and provides tensile strength and support), in tendons, collagen fibers are predominantly type I collagen, which is characterized by its high tensile strength and stiffness. In contrast, ligaments contain a higher proportion of type III collagen in addition to type I collagen. Type III collagen is more flexible and elastic compared to type I collagen, which allows ligaments to resist dynamic movements and variable loads in joints.

Additionally, their collagen fibers are less organized and have different cross-links [45]. For the tendon graft to function effectively as a ligament, it needs to undergo metabolic, structural, and compositional changes to mimic the properties of a ligament.

However, this process is not always successful. Issues such as poor blood vessel formation (angiogenesis) and degradation of the graft's extracellular matrix can lead to degeneration of the graft over time, as observed clinically [46] and in animal studies [47].

2.4.2.3 Inflammation as a factor of graft's healing

Shortly after graft implantation during ACL reconstruction surgery, the body initiates an inflammatory response to remove debris (damaged cells, blood cells, and other cellular components, as well as any foreign material introduced during the surgical procedure) and start the tissue repair process. This involves recruiting neutrophils and macrophages (white blood cells that play key roles in the immune system's response to infection and tissue damage) to the site of the tendon-bone interface within a few days. These cells release inflammatory cytokines and growth factors, which help form a fibrous tissue interface between the graft and the host bone [48]. While this fibrous tissue provides initial mechanical support, excessive inflammation can lead to fibrosis, hindering the integration of the graft with the bone. Additionally, excessive inflammation can cause complications such as bone tunnel widening, loss of bone around the tunnel, and degradation of the graft.

Research has shown a clear link between increased inflammation and poor outcomes in ACL graft healing. Excessive inflammation can be triggered by factors such as the initial ACL injury, surgical trauma during ACL reconstruction, and excessive movement of the tendon graft within the bone tunnel. This inflammation causes the release of inflammatory cytokines, which in turn

activate enzymes called matrix metalloproteinases (MMPs) that degrade the extracellular matrix (ECM) and stimulate bone-resorbing cells called osteoclasts.

When the ACL is injured, it leads to an increase in inflammatory substances like cytokines in the synovial fluid [49]. These cytokines can enter the bone tunnel where the graft is placed during ACL reconstruction, leading to inflammation and bone resorption, which slows down the healing of the graft [50].

Research has shown that higher levels of inflammatory cytokines in the synovial fluid are associated with more pain, knee instability, and poorer functional outcomes after ACL reconstruction. Additionally, patients with higher levels of inflammatory substances in their synovial fluid before surgery are more likely to have unsatisfactory outcomes two years after ACL reconstruction [51]. These findings suggest that inflammation can negatively affect the healing of the graft and overall recovery after ACL injury.

2.4.3 ACL reinnervation

The healing process of the ligament and its functions is something that has been studied. However, studies carried out by Ochi et al. confirm the reinnervation process after anterior cruciate ligament reconstruction and even the response to mechanical loads can be restored, and is strongly related to improvement in position sense. In one of them they examined somatosensory evoked potentials (SEPs) in ACL reconstruction that are electrical signals in the nervous system that are generated in response to sensory stimuli, such as touch or movement. The ACL was electrically stimulated using a square wave of 0.2 ms duration at an intensity of 10 mA and rate of 3/s and electrode impedance was maintained between 2 and 10k Ω . They found that SEPs was detected in 15/32 ACL-deficient and 22/23 reconstructed knees and that voltage of SEPs was correlated with joint position sense.

It demonstrated the presence of sensory reinnervation in the reconstructed ACL and the investigators highlight the importance of sensory feedback in knee function that is related to this reinnervation [52].

In another study of the same investigators, they tried to determine whether regenerated nerve fibers and mechanoreceptors in the reconstructed ACL could respond to mechanical loads applied to the ligament. The study aimed to test for afferent impulses and (SEPs to assess functional restoration of axons and mechanoreceptors in the ACL following reconstruction. The mechanical stimulation was administered using a plastic tip (3 x 5 mm) connected to a moveable coil transducer controlled by a feedback circuit. The tip was positioned on the surface of the ACL to produce an indentation with 200 stimuli, each with a force of 3.92 N, delivered at a rate of two per second. They found that this mechanical stimulation of the reconstructed ACL or ACL remnants induced reproducible SEPs, suggesting functional recovery of axons and mechanoreceptors. This indicates that sensory reinnervation in the ACL may be facilitated through the regeneration or preservation of mechanoreceptors, highlighting the potential restoration of sensory function in the ligament [53].

In what concerns the effects of electrical stimulation in tendon healing, a study of Fernandez-Yague et al. found that electromechanical stimulation regulates mechanosensitive ion sensitivity and promotes tendon-specific over non-tenogenic tissue repair processes. They created a self-

powered piezo-bioelectric device that regulates Tendon Repair-Associated Signaling Pathways. In this study the electrical stimulation is applied during tendon stretching to mimic physiological conditions where tendon cells modulate gene expression, protein synthesis, and mitogenesis through mechanotransductive signalling pathways. This stimulation helps in promoting a tendon-specific phenotype characterized by changes in gene and protein expression, biological functions, and cell morphology, ultimately aiding in tendon tissue repair and regeneration [54].

2.5 ELECTROSTIMULATION IN POST-OPERATIVE RECOVERY

As mentioned previously, after an ACL injury and reconstruction, there can be arthrogenic muscle inhibition due to the loss of mechanoreceptors. In other words, the feedback and feedforward mechanisms of the signal transmission between the knee and the brain become altered.

The recovery period following ACL reconstruction surgery is a critical phase, and patients often face certain challenges as mentioned above. In this context, emerging concepts and techniques can be valuable, such as electromyography, electrostimulation, and neuromuscular feedback. Electromyography is a widely used tool for describing nerve and muscle electrical activity, making it essential for diagnosing certain symptoms. Muscle weakness after ACL reconstruction, in this case of the lower limb muscles, is relevant to this study. In order to minimize the disadvantages and setbacks resulting from ACL reconstruction, electrostimulation has emerged as a relevant method. In fact, Neuromuscular and Muscular Electrical Stimulation (NMES) has a number of benefits ranging from pain reduction to increased muscle strength. Within the electrostimulation spectrum, inferential therapy is a method that allows currents to penetrate and stimulate deep tissues easily, combining sinusoidal currents with slightly different frequencies, also bringing some benefits. On the other hand, more recently neuromuscular feedback has been introduced.

In this section, as well as going into more detail about these concepts, we will also look at the origin and electrophysiology of the biosignals produced by the muscles in order to understand the whole picture.

2.5.1 Muscle Electrophysiology

In order to understand the origin of bioelectric signals in muscle, it is necessary to understand the most basic level of organization of the nervous system, the motor units. These are composed of a single alpha motor neuron and all the muscle fibers it innervates. In fact, the term “motor units” highlights the behaviour of all the muscle fibers innervated by each motor unit during the innervation process, since they act “as one” [55].

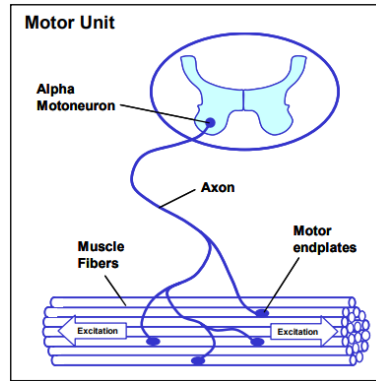


Figure 13: Motor Unit [56].

To detect these signals, it is necessary to acknowledge the phenomenon of excitability of muscle membranes and the action potential. Regarding the first, it is related to the ionic difference between the interior and exterior space of the muscle fibers that allow the formation of a resting potential. This potential difference is achieved through physiological processes that allow a negative charge intracellularly and a positive charge extracellularly.

Briefly, after the activation of an alpha motoneuron and the consequent transmission of biochemical neurotransmitters, an endplate potential is formed in the muscle fibers innervated by this motor unit. The membranes of the muscle fibers are semi-permeable, and during this phenomenon their diffusion characteristics are altered with a marked entry of Na^+ ions into the intracellular medium and an entry of K^+ into the extracellular medium, and this refers to depolarization of the membrane. Then, the resting potential is restored again through repolarization where there is a switch in the ion pump mechanism (marked entry of Na^+ ions into the extracellular medium and entry of K^+ ions into the intracellular medium) [38].

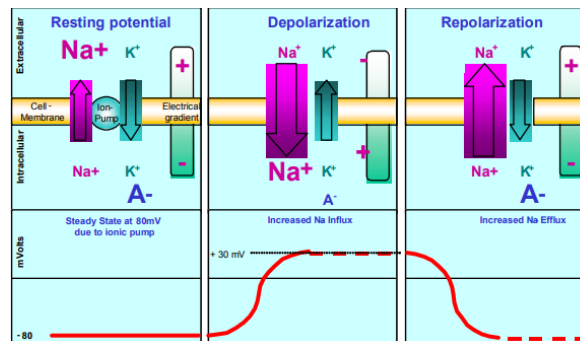


Figure 14: Schematic illustration of depolarization/repolarization cycle [56].

Regarding the action potential, this is fired if the threshold level is reached at the time of depolarization. This threshold is about -55 mV for most neurons in humans. Upon being fired, the action potential spreads along the muscle fiber in both directions through a tubular system.

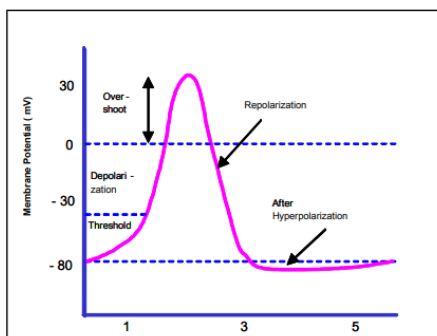


Figure 15: Action Potential [56].

2.5.2 NMES after ACL reconstruction and FES

NMES is a modality that sends electrical impulses to nerves which causes the muscles to contract mimicking the action potential coming from the central nervous system [57].

After an ACL reconstruction surgery, using NMES is recommended to stimulate muscle contractions electrically, which can enhance the activation of the quadriceps muscles voluntarily and lead to increased strength. Additionally, by improving both strength and biomechanics, NMES may also help alleviate pain as a secondary benefit [58].

Actually, some of the benefits of using NMES after ACL reconstruction include [58]:

1. Reduced pain;
2. Improved muscle strength;
3. Reduction in muscle volume or thickness loss;
4. Enhanced self-reported function;
5. Positive effects on gait parameters;
6. Achievement of clinical milestones;
7. Changes in limb circumference;
8. Improved functional performance in activities like lateral step-up and anterior reach.

In addition to NMES, there is another form of electrical stimulation known as Functional Electrical Stimulation (FES), where the stimulation is automatically controlled to convert muscle contractions into functional movements [59]. This approach can be particularly effective in neurological rehabilitation, enhancing proprioception and kinesthetic differentiation. Typically, FES is incorporated into an exercise or bracing device to maximize functionality.

When it comes to electrostimulation, it's essential to understand and manipulate certain parameters, the main ones being pulse duration, pulse frequency, duty cycle, amplitude/intensity, and electrode placement, size and number. In a simple way the pulse duration is the time duration for each electrical pulse sent to activate muscles, the pulse frequency is the rate at which electrical pulses are delivered to muscles, the duty cycle represents the ratio of "on" time (stimulation) to

"off" time (rest) and the amplitude/intensity is the "strength" of electrical stimulation delivered to muscles. In the specific case of NMES, a pulse duration of 400-600 μ s, a pulse frequency of 30-50 Hz, a duty cycle of 1:5 and an intensity of more than 50% of the maximum voluntary contraction, maximizes performance and muscle contraction, minimizing discomfort and fatigue [60].

In a way, the sensitivity of the motoneurons depends on two parameters: the intensity and duration of the stimulus. It is necessary to find a balance and realize that different people have different sensitivities and even that, depending on the conditions, the same person can have different sensitivities at different times. It is therefore necessary to take these aspects into account in order to provide effective and efficient stimulation, without promoting excessive pain and discomfort.

2.5.3 Interferential therapy

Therapists often use transcutaneous electrical stimulation to treat their patients. They can choose between different types of electrical currents, such as alternating current with varying frequencies or direct current, which can be applied continuously or as a series of pulses. Each type of current has its own advantages and disadvantages when used for therapeutic purposes.

Direct current and low-frequency alternating currents, which have a frequency of lower than 1 kHz, face significant resistance when passing through the outer layers of the skin. This can make it painful to treat deeper structures because a large amount of transcutaneous current is needed to ensure that an adequate amount reaches those deep tissues. On the other hand, medium-frequency (between 1 kHz and 10 kHz) and high-frequency (more than 10 kHz) alternating currents encounter less resistance due to a decrease in the effects of skin capacitance on current flow. These currents can easily penetrate tissues, but they often oscillate too rapidly to directly stimulate the tissues [61].

To address these challenges, interferential current therapy was developed in the early 1950s. This equipment generates two alternating currents with slightly different medium frequencies and is widely used to achieve pain relief, induce muscle contractions, regulate the autonomic nervous system, support healing, and reduce swelling [62].

When two or more sinusoidal currents share the same frequency and rise and fall together, they are considered to be "in phase". In-phase waves reinforce each other, resulting in a combined wave with a higher amplitude than the individual waves. On the other hand, when waves are "out of phase," meaning they are half a wavelength out of synchronism, the rising part of one wave coincides with the falling part of another, leading to destructive interference, causing them to cancel each other out [62].

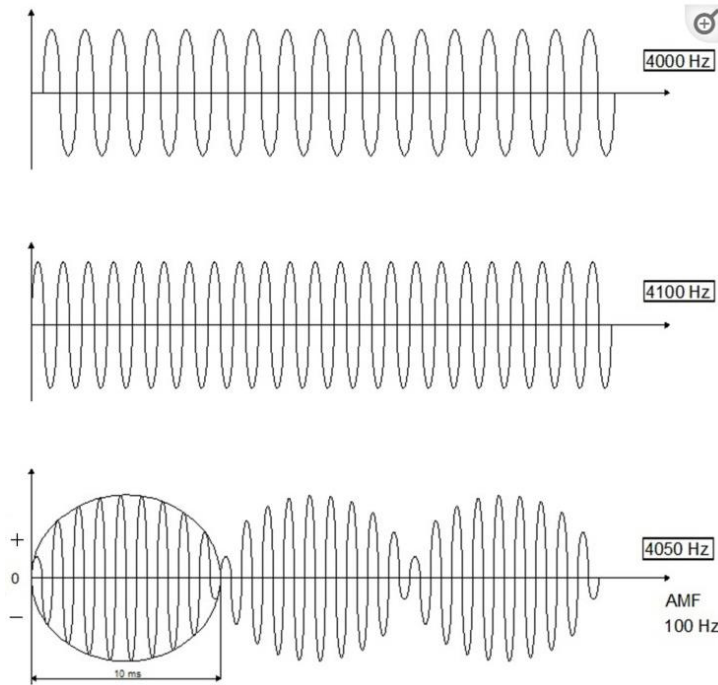


Figure 16: Amplitude modulation [63].

Interference can also occur when waves have slightly different frequencies. When one wave catches up with another, constructive interference briefly boosts the amplitude of the combined wave. However, as the waves gradually move out of sync again, they interfere destructively, causing the amplitude to decrease. The speed at which the combined wave's amplitude rises and falls is determined by the difference in frequency between the original waves ($f_B = |f_1 - f_2|$) and is referred to as the "beat frequency". This process is an example of intermodulation [62]. If for example, one current is at 4000 Hz and its companion current at 3900 Hz, the resulting beat frequency would be at 100 Hz, carried on a medium frequency of 3950 Hz amplitude modulated current.

When two sine waves with different frequencies are multiplied, one gets two new sine waves: one with the difference frequency and the other with the sum frequency.

Giving the example of one sine wave with 1 Hz and another with 100 Hz:

$$x(t) = \cos(2\pi * 1t) * \cos(2\pi * 100t) \quad (1)$$

When applying the Euler expansion, we have:

$$\frac{e^{i2\pi t} + e^{-i2\pi t}}{2} * \frac{e^{i2\pi 100t} + e^{-i2\pi 100t}}{2} \quad (2)$$

$$\frac{1}{4} * (e^{i2\pi 101t} + e^{-i2\pi 99t} + e^{i2\pi 99t} + e^{-i2\pi 101t}) \quad (3)$$

$$\frac{1}{4} * \left(\frac{e^{i2\pi 101t} + e^{-i2\pi 101t}}{2} * \frac{e^{i2\pi 99t} + e^{-i2\pi 99t}}{2} \right) \quad (4)$$

$$\frac{1}{2} * \cos(2\pi * 101t) + \frac{1}{2} * \cos(2\pi * 99t) \quad (5)$$

$$101 = 100 + 1$$

$$99 = 100 - 1$$

We can say that the multiplication of two sine waves with different frequencies is equal to the sum of two sinusoids: one with the frequency equal to the sum of the frequencies of the original waves and other with the frequency equal to the difference.

Interferential current therapy takes advantage of this interference principle to maximize the flow of electrical current through tissues while minimizing unwanted stimulation of superficial nerves in the skin [62].

In fact, studies have indicated that there are five primary approaches to interferential therapy. These approaches are related to pain relief, muscle stimulation, vasodilation, reduction of swelling (edemas), and tissue healing. Each of these approaches necessitates distinct frequencies and intensities to achieve the intended outcomes [62].

- Pain relief: Clinical studies have revealed that pain can be effectively reduced when interferential therapy is used at frequencies between 90 and 130 Hz, especially when targeting acupuncture points on the body. This type of therapy can also help alleviate pain by promoting the circulation of bodily fluids, which, in turn, helps remove substances that cause pain from the injured area.

- Muscle stimulation: When using Interferential Therapy (IFT), it seems that the best range for stimulating motor nerves is between 10 and 20 Hz, or sometimes up to 25 Hz. If the stimulation is set lower than 10 Hz, it can lead to a series of muscle twitches, which can actually be useful in a clinical context. However, the effectiveness of IFT isn't entirely clear and hasn't been definitively proven yet. On the other hand, when IFT is applied at higher frequencies like 20 or 25 Hz, it tends to produce strong and continuous muscle contractions [62].

- Increased local blood flow: Evidence suggests that when using frequencies between 10 and 20 Hz, there's a boost in blood flow in the blood vessels around the area [64]. This increase in blood flow is likely due to the ongoing muscle contractions that occur, which in turn stimulate the production of enzymes that help with metabolism and overall muscle function [65].

- Edema and tissue healing process: The frequencies most effective in reducing edema range from 1 to 30 Hz. Additionally, within this range, 1 to 10 Hz frequencies can also lead to vasodilation, which helps widen blood vessels and further aids in edema reduction [65]. It is important to mention that, interferential therapy appears to influence the levels of certain enzymes and molecules inside cells that play crucial roles in various metabolic processes. Some studies have observed alterations in the amounts of cyclic adenosine monophosphate, acetylcholinesterase, alkaline phosphatase,

and lysosomal enzymes. These effects have been applied to enhance the healing of broken bones, the regeneration of nerves, tendons, and ligaments [62].

Another parameter to take into account in interferential therapy is the type of application. There are currently two types of IFC: "true" IFT and premodulated IFC (Fig. 18). "True" IFT (Interferential Current) involves using four electrodes (Fig. 19) and applying two medium-frequency alternating currents to the skin's surface through separate circuits. The currents are meant to interact inside the tissue. On the other hand, in premodulated IFT, the interference of the two medium-frequency currents happens within the machine, and the resulting wave, an amplitude-modulated current, is delivered to the tissue through a single pair of electrodes (Fig. 20). Some researchers argue that "true" IFC is more comfortable and provides deeper effects with less sensory stimulation. In contrast, others believe that premodulated IFT is easier to use, safer, more effective, and more comfortable compared to "true" IFT [62].

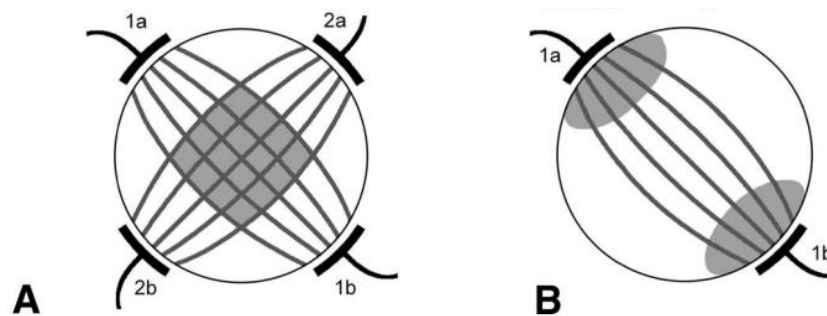


Figure 17: Regions of maximum stimulation (shaded), which are predicted with application of (A) true and (B) premodulated IFTs [66].

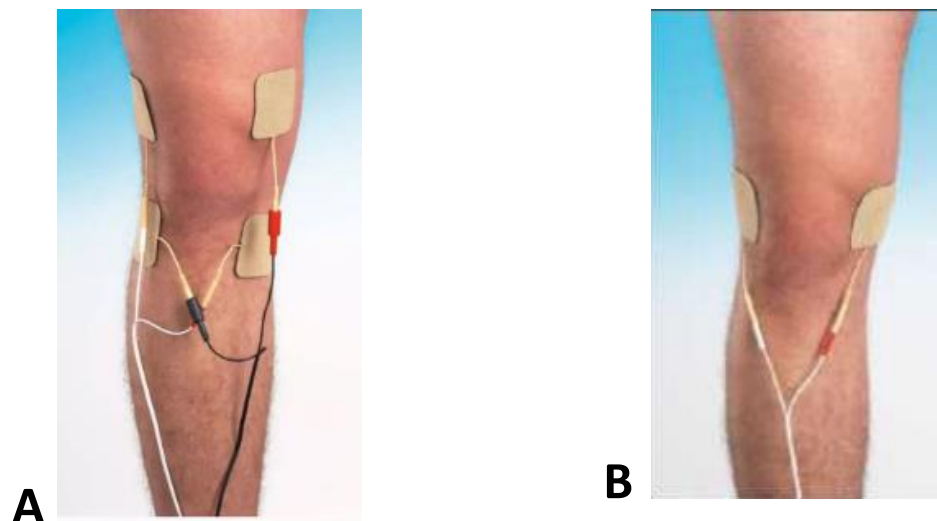


Figure 18: IFT electrodes positioning in "True" IFT knee electrodes placement (A) and- Premodulated IFC knee electrodes placement (B) [67]

According to a study of Beatti et al. (2011) ,“true” IFT showed higher recorded voltage levels compared to premodulated IFC when the goal was to reach deeper tissues, indicating its greater efficiency for this purpose [68].

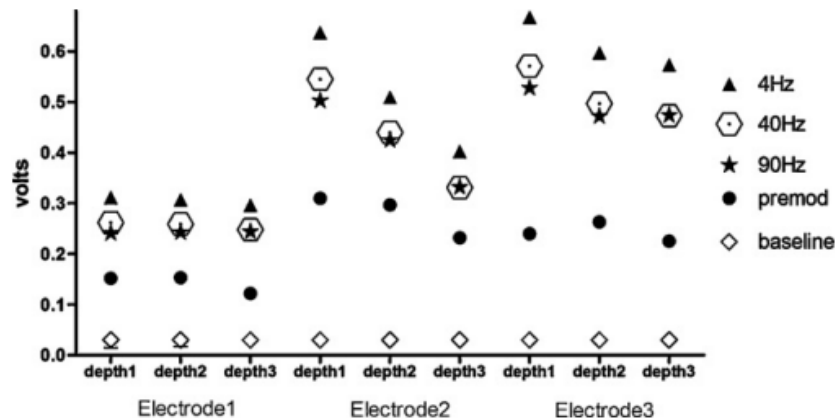


Figure 19: Voltage measurements in three different depths (1 - skin, 2 -subcutaneous tissue, 3 - muscle) and 3 different locations (Electrode 1, recording electrode positioned in the middle of the four IFC electrodes; Electrode 2, recording electrode positioned in line with one circuit of IFC; Electrode 3, recording electrode positioned outside the borders of the four IFC electrodes) [68].

Nevertheless, a study of Ozcan and Robertson (2004) [66] showed us that using “true” IFT didn't provide any benefits over premodulated IFC in terms of motor-sensory threshold or comfort. Additionally, premodulated currents produced more torque and were less uncomfortable than “true” IFC. These results challenge the idea that “true” IFC is superior.

According to the existing literature, it is concluded that no method demonstrates clear superiority over the other, as some authors advocate the superiority of one method while others argue the opposite.

In terms of practical applicability, the electrodes are placed on the skin after cleansing with soap and water, or even alcohol, to reduce resistance. They are positioned so that the two currents meet in the treatment area. The strength of the current is gradually increased until the patient feels it is strong but still comfortable [62]. Knowing the safe levels of electric current applied in the human body is very important for an effective and safe treatment. It can ensure that the current used is within safe limits and could prevent adverse reactions such as burns, pain, or muscle damage. An appropriate current intensity can also ensure the patient comfort and enable adherence to treatment.

Table 2: Safe levels of current in the human body [69].

Current (mA)	Effect	Time Duration
0.2 to 1.0	Threshold of perception	Not critical
10 to 16	Limit of ‘let go’, muscles contract	Minutes
30*	Breathing difficult, ‘safe’ limit	Seconds

50	Irregular heartbeat	About 1 second
60	Respiratory problems, cannot breathe	
>60	Heart fibrillation, electric burns	

Specific electric currents can also translate in different physiological effects in the human body. Knowing the different effects of the current can also help in the personalization of each patient treatment and plan a safe and effective protocol. Certain ranges of current for specific periods of time are known to cause involuntary muscle contractions, breathing difficulties and burns. It is also known that different current intensities can result in different therapeutic effects, such as muscle strengthening, pain relief and increased blood circulation. It is therefore necessary to manipulate the current and the exposure time in order to have a favourable therapeutic effect, but above all one that is safe and comfortable for the patient.

Table 3: Physiological effects of currents of different magnitudes [69].

Current Magnitude	Physiological Effects
From 0 to 0.5mA	Perception possible (10secs)
From 0.5 to 5mA	Perception and involuntary muscular contractions likely but usually no harmful electrical physiological effects (5 secs)
From 5 to 50mA	Strong involuntary muscular contractions. Difficulty in breathing. Reversible disturbances of heart function. Immobilization may occur. Effects increasing with current magnitude. Usually, no organic damage to be expected (2 secs)
From 50 to 100mA	Patho-physiological effects may occur such as cardiac arrest, breathing arrest, and burns or other cellular damage. Probability of ventricular fibrillation increasing with current magnitude and time up to 1 sec. Above 2 secs probability of ventricular fibrillation is approaching 50%.

2.5.3.1 Interferential therapy parameters

The process of natural in vivo vascularization can occur through two distinct processes. One called “vasculogenesis” (Fig. 20) that occurs mainly during embryonic development, where there are formation of new blood vessels from a single cell. And the other called angiogenesis (Fig. 20), where there are the development of new blood vessels from existing ones, by dividing them to expand tissue blood vessels. The second one is the major process in adults [70]. In a research conducted by Beugels, J. et al [71], it was conducted an experiment to see how different electrical

signals affect the behaviour of adipose-derived stem cells (ADSCs) in particular the promotion of growth of new blood vessels (angiogenesis). They tested various parameters like voltage, pulse duration, and frequency to find out what works best in two topics: cell viability, viable cells are those that are alive and functioning properly, while non-viable cells may be dead or damaged, and VEGF-A (Vascular Endothelial Growth Factor A), that is a protein that plays a crucial role in angiogenesis.

Starting with moderate settings, they gradually increased or decreased one parameter at a time. It was tested 2V/cm, 4V/cm and 8V/cm voltages, 3ms, 6ms and 12ms pulse durations, and 2Hz and 25Hz frequencies. It was found that a voltage of 2V/cm, a pulse duration of 6ms and a frequency of 2Hz promoted greater cell viability. Among these 3 parameters, the frequency was the one that had the least impact on cell viability, but a slight decrease in viability was observed when the frequency was greatly increased (2Hz had better results than 25Hz).

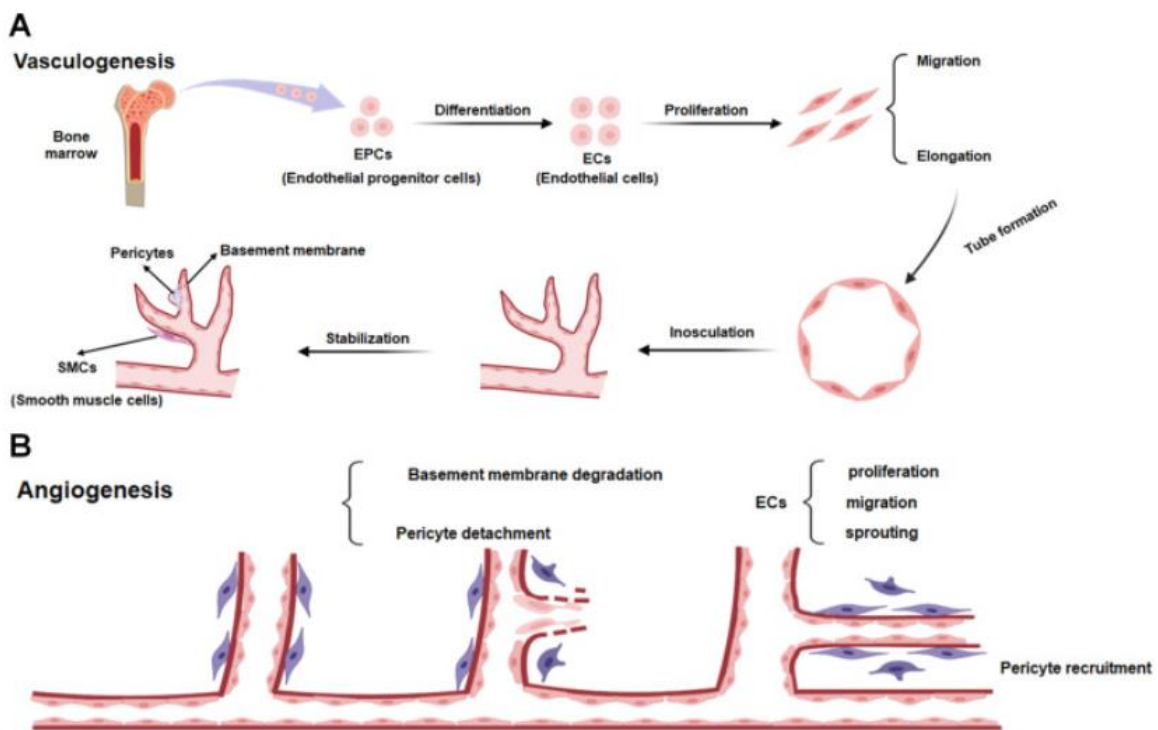


Figure 20: Schematic of vasculogenesis and angiogenesis process [70].

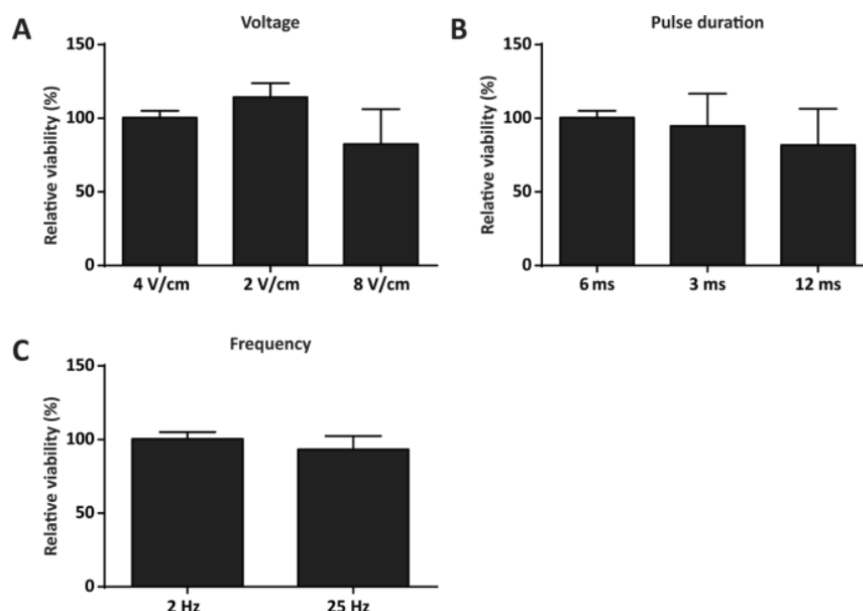


Figure 21: Parameters and its effects on cell's relative viability [71].

In relation to the increase in the concentration of VEGF-A, it was found that higher voltages, pulse durations and frequencies resulted in higher VEGF-A concentrations, but they also resulted in lower cell viability and caused adverse effects on the cells, such as cell detachment and cell death.

It is concluded that appropriate values for angiogenesis that balance the effects of both topics studied (cell viability and concentration of VEGF-A are 4 V/cm, 6 ms and 2 Hz (Fig. 21) [71].

Interferential therapy is a method that has many advantages such as being safe, easy to use, non-pharmacological, non-invasive and effective. According to the literature, it is not only effective for muscle and bone injuries, but also for ligament injuries. However, the five main areas where this type of electrostimulator provides good results are muscle stimulation, oedema reduction, vasodilation, bone regeneration and, above all, pain reduction.

It is known that different interferential therapy parameters translate into different objectives and results, and one important parameter is the frequency used.

Starting with pain reduction, frequencies between 90-130Hz are believed to be beneficial, especially when applied to acupuncture points. Additionally, pain diminishes due to increased blood circulation, which helps remove pain-causing chemicals from the injured area [72].

Vaz carried out a literature review of the effects of different electro-stimulation parameters on tendons and ligaments. In fact, there were positive effects on the regeneration and reinnervation of tendons and ligaments in calves, rabbits, rats and humans [73]. However, it should be noted that in most of the studies analysed, very low currents were applied (ranging from micro amperes to no more than 10mA) for long periods of time (ranging from 1 hour intervals to applying electrostimulation 24 hours a day). The table below, drawn up by Vaz, shows the results obtained using the model, the properties of the current and the stimulation time used.

Table 4: Electrical stimulation wave properties in different models and its results in tendon/ligament tissue [73].

Model	Wave properties	Stimulation Time	Results
Calves	Direct current of 0-0,3 mA and voltages of 0, 2 and 6 V/cm. On/off intervals from 0 to 60 s	Stimulation of calves' ACL ex vivo for 1h or 3 h.	Repair of ACL, through the induction of fibroblasts' migration and biosynthesis.
Rabbits	Direct current of 7 μ A and voltage of 1,4 V	Stimulation of rabbit's tendons ex vivo for 7, 14, 21 or 42 days	Increase of the number and activity of tendon's cells
	Direct current of 0,5 μ A, 1 μ A and 6 μ A	Stimulation of rabbit's tendons ex vivo for 1 or 2 weeks	Activation of tenocytes proliferation and collagen production
	Rectangular wave with a frequency of 4 Hz and impulses duration of 0,2 ms	Stimulation occurred for 1 month, 24 h/day.	Functional reinnervation
Rats	Direct current of 10 nA to 20 μ A	The regiment of stimulations lasted 12 days	The highest currents (1- 20 μ A) were able to repair the medial collateral ligaments
	Current of intensity of 100 μ A, frequency of 10 Hz and pulse width of 50 ms	Negative microcurrent for 1 week followed for positive stimulation for 3 weeks	Myofibroblast attraction
	Asymmetrical square biphasic waveform with a current density of 100 μ A, voltage of 2,5 V and frequency of 10 Hz	Stimulus was applied for 30 min., 6 times/week, until reaching 22 treatment sessions	Improving of the strength of Achilles tendon
	Electric current with an intensity of 10 μ A, frequency of 0,3 Hz and pulse width of 250 ms	Stimulation was applied for 1 h	Activation of protein synthesis, essential to regeneration
Humans	Monophasic waveform with maximum intensity of 50 μ A, trains of eight pulses of duration 1-3 ms, interpulse interval of 5-10 ms, and frequency of 75-160 Hz	The stimulation was applied 99 min/day for 3 weeks	Promotion of the elbow's tendon normalization
	Square wave of 0,2 ms duration, intensity of 10 mA and frequency of 3 Hz	The stimulation was applied during the time the patients were anaesthetised	Reinnervation of the ACL
	Square wave of 0,2 ms duration, intensity of 10 mA and frequency of 3 Hz	The stimulation was applied during the time the patients were anaesthetised	Functional stability and restoration of sensory function of ACL

2.5.4 Modern Stimulators

2.5.4.1 Flexistim IF

The earlier IFT units used analogue circuits, and their capability to generate waveforms was restricted in the waveforms they could produce. Nowadays, there are stimulators such as Flexistim IF that utilizes digital technology to create a genuine sinusoidal bipolar IFT. This process involves a carrier wave at 4000 Hz but delivers a low-frequency signal with amplitude modulation to the entire space between the electrodes, rather than just creating an interference pattern [74].



Figure 22: Stimulator Flexistim IF [74].



Figure 23: Electrode Pads [74].

2.5.4.2 L300 Go stimulator

L300 Go is specifically designed to help individuals with drop foot, a condition often associated with central nervous system disorders like multiple sclerosis, cerebral palsy, or stroke. It achieves this by using functional electrical stimulation, where small electrical pulses are delivered through electrodes on the lower leg. These electrodes, secured with a cuff, ensure proper upward foot movement (dorsiflexion) and, in cases of unstable knees, can also facilitate knee bending and straightening. This innovative approach enhances walking and safety by stimulating nerves the central nervous system can no longer control.

In this system there is no foot sensor required and acceleration sensors that are incorporated monitor movement patterns during walking in all three planes [75].



Figure 24: L300 Go system [75].



Figure 25: SP 8.0 Compex Stimulator [76].

2.5.4.3 Compex SP8.0 IFC Stimulator

This stimulator helps to produce signals originating in the central nervous system, in other words the production of action potentials that translate into simulated muscle contractions. As mentioned above, this stimulation can be useful in conditioning, recovery, pain management, rehabilitation and improving overall fitness.

However, this stimulator stands out due to a technology - Muscle Intelligence (MI) technology. This has features such as [76]:

- “Mi-scan: does a short test scan of the muscle and automatically adjusts the parameters of the stimulation to your muscles.”.
- “Mi-action: combines voluntary contractions and stimulated contractions at your pace, meaning you are in control of your training. Users must actively contract the muscle first to start the stimulation from the device.”.
- “Mi-autorange: a brand-new feature that has the same objective as Mi-range except that in this case it is done automatically by the device.” (COMPEX, 2023).

Mi-scan is a technology that allows physiological parameters of the muscles to be assessed at a given moment and, based on these, a more comfortable, efficient and personalized electrostimulation session to be carried out. This makes it possible not to use a standard stimulation, but to adapt it to the characteristics of each individual, taking into account aspects such as fatigue, dehydration, muscle imbalances and injuries.

Based on the parameters assessed by the MI-scan, MI-Autorange can find the ideal intensity level for the programme to develop automatically.

Mi-action is a technology that leads to the generation and transmission of electrical impulses only when a voluntary contraction on the part of the patient is detected. This has a number of

advantages, including reinforcing the muscle-brain connection and associating the stimulation of certain muscles with the exercise to be performed.

2.5.4.4 Chattanooga Multi-Sessions WIRELESS PROFESSIONAL 4CH

As the Chattanooga website states [77], this electro stimulator has different programmes for rehabilitation, pain reduction, vascular and conditioning.

It has four modules, two standard remotes and a smart docking station that allows you to synchronize modules and remotes to manage several sessions independently at the same time. This also makes it possible to stimulate two parts of the body simultaneously. And since this electro stimulator has the option of choosing TENS and/or NMES, it is possible to combine the two methods.

It also has software that allows you to create a patient history and create personalized programmes.



Figure 26: Chattanooga Multi-Sessions WIRELESS PROFESSIONAL 4CH [77].

2.6 SURFACE ELECTROMYOGRAPHY

The electromyographic signal is a representation of the signal produced by muscle contraction. It can vary depending on the intensity and type of contraction. They can vary from a few microvolts at rest - even at rest a small signal is produced - to tens of microvolts during maximum muscle contractions.

Table 5: Typical signal amplitudes, filter settings and sampling frequency in EMG signals [78].

Recording	Signal amplitude	Typical filters	Sampling frequency (kHz)
Compound muscle action potential	0-50 mV	1 Hz-5 kHz	20
Needle EMG	0-30 mV	2 Hz-10 kHz	50
Single fibre EMG	0-50 mV	500 Hz-10 kHz	50
Surface EMG	0-10 mV	1 Hz-1 kHz	5

Specifically, Surface electromyography (sEMG) is a technique for collecting electromyographic signals captured in the skin surface that measure the electric potentials produced during muscle contractions. In other words, these signals represent the neuromuscular activity. In fact, the EMG signal is based upon action potentials at the muscle fiber membrane resulting from depolarization and repolarization processes as described above. In order to capture the EMG signal, we usually use a bipolar electrode configuration and a differential amplification.

The BITalino (r)evolution® board kit offers a user-friendly data acquisition solution that supports multiple sensors for capturing various biosignals, including sEMG, electrocardiography, and electrodermal activity.

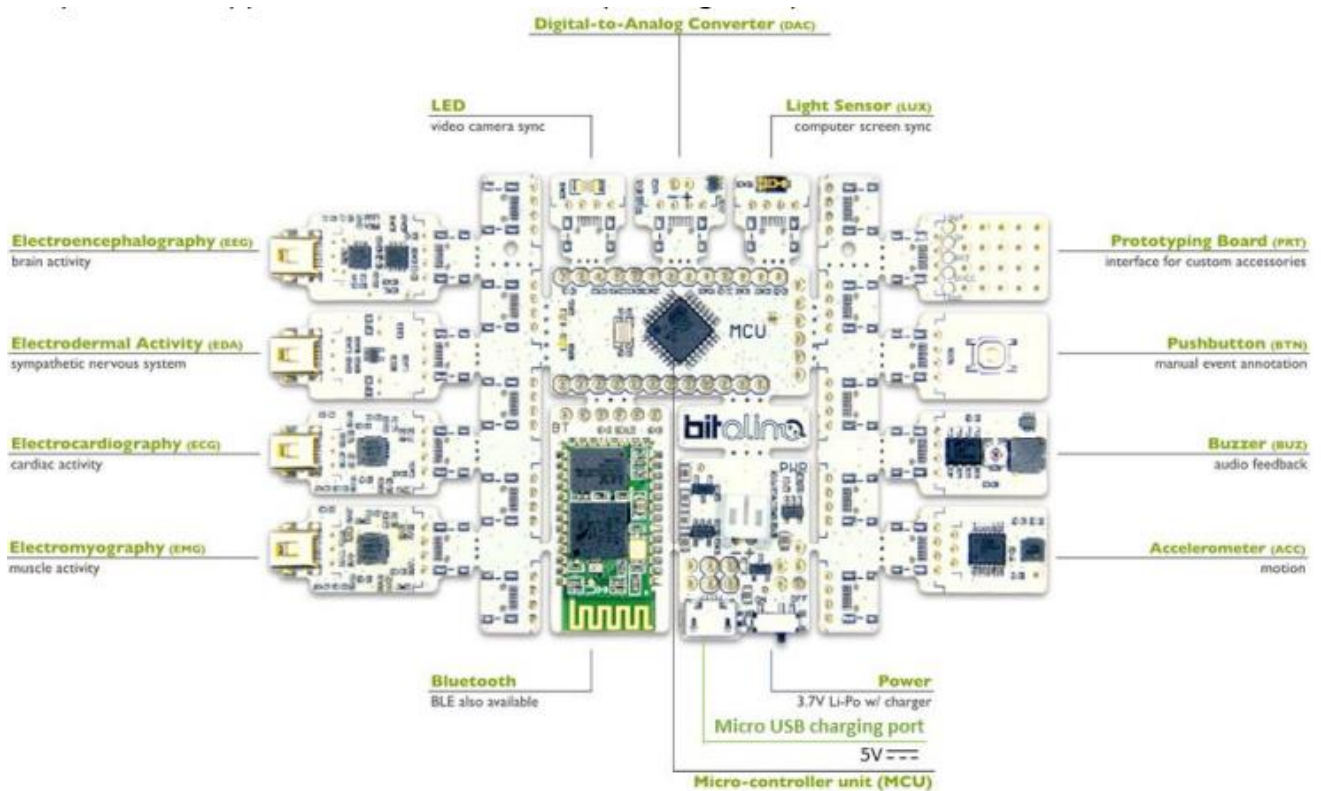


Figure 27: BITalino (r)evolution® board kit [79].

The quality of the collected EMG signal depends on several factors including skin preparation and the type and position of electrodes.

Regarding skin preparation, this is important in order to allow a stable contact of the electrodes and a low impedance of the skin. The first advisable step is to remove the hair, this to increase the adhesion of the electrodes, mainly in humid conditions, sweaty skin types and in dynamic exercises. Then we must clean the skin, namely through special abrasive and conductive cleaning pastes, sand paper, and pure alcohol applied [56].

There are two main types of electrodes the skin surface electrodes and the thin wire electrodes. In this work surface electrodes were used and these are the most common in kinesiological studies, due to their non-invasive character and easy handling. One of the disadvantages of this approach is that the activity of deeper muscles is not detected, being necessary to use fine wire (or needle) electrodes. In fact, the selection of the type of electrode depends on the investigative work in question, and each one of them cannot cover all the problems [56].

There are several guidelines in the application of the electrodes, some of them are:

- Small electrodes show higher impedance values and reduce cross-talk (detection of the EMG signal resulting from the activation of adjacent muscles to that studied).
- The recommended distance between electrodes is 2 cm because it also allows a reduction in cross-talk.
- Wet gel electrodes have the best skin impedance values.
- The electrodes should be applied in the direction of the muscle fibers of the muscle studied.

Regarding the location of the electrodes, the images below show strategic positions for both fine wire electrodes (left) and surface electrodes (right), and the two yellow points show the direction of the muscle fibers.

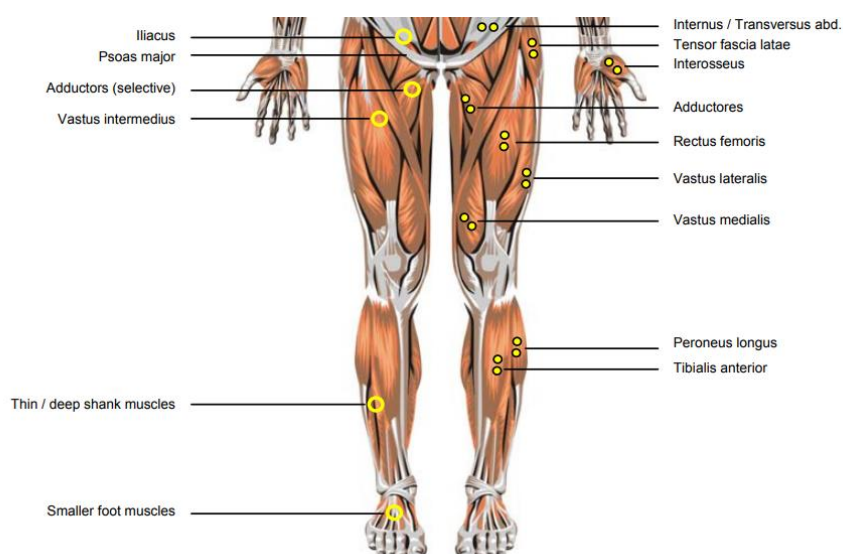


Figure 28: Anatomical positions of selected electrode sites, frontal view [56].

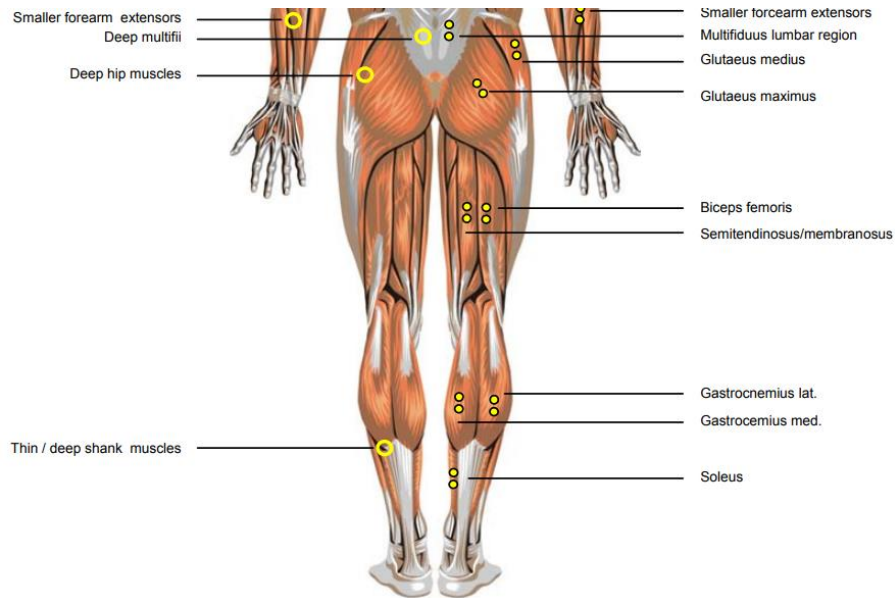


Figure 29: Anatomical positions of selected electrode sites, dorsal view [56]

2.7 EMG SIGNAL PROCESSING

After collecting the EMG signal, it is necessary to process it according to the research objectives. It is important to note that there is no single method for processing the signal, but usually there are 6 main steps: raw signal capture, filtering, rectification, smoothing, root mean square (RMS).

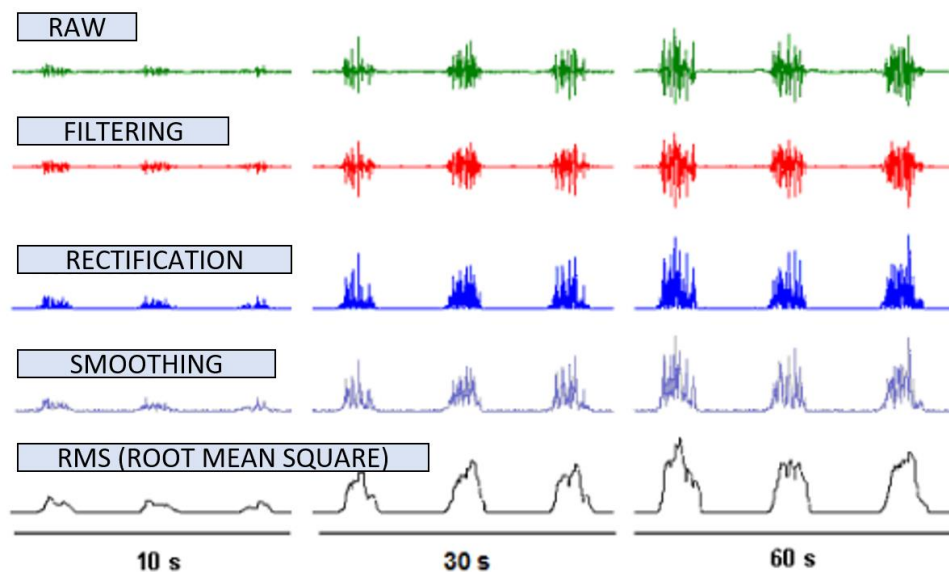


Figure 30: EMG signal process recommended. Green: The raw signal, no treatment was applied; Red: Filtered signal, a limit was created for the signal, excluding everything out of it; Blue: Rectified signal, all negative values were transformed in positive ones and added; Purple: the smoothed signal, a linear enveloped was created and the extreme parts of the signal was excluded; Black: The RMS values after all the treatments [80].

2.7.1 Raw Signal Capture and Filtering

The raw EMG signal is that recorded directly from the electrodes placed on the intended muscle. Despite not being processed it can give us some information such as muscle innervations, amount of electrical activity generated by muscle fibers, changes in the signal size, and timing and duration of muscle activation.

Although this signal can give us some information, this may be limited and inaccurate. One of the main reasons is that this signal is often noisy (unwanted signal that interferes with the desired signal mainly), which makes it a challenge to extract valuable information from it. This could be due to some factors like electrical interference from nearby muscles and electrical equipment, movement artifacts caused by movement of the electrodes, or the underlying tissue.

In order to reduce the impact of noise, it is important to process the signal, and one of the first steps is filtering in different frequency bands.

Low-pass filtering can be used to remove high frequency interference, and allows low frequency components to pass through, with a reference value of cutoff of 400-500 Hz. The high-pass filter works in the opposite way, with reference values of cutoff being 5-30 Hz [81]. The band-pass filtering, which is the most common one, allows the passage of a specific range of frequencies while attenuating the frequencies outside that range. The recommended range of frequencies is 20 Hz to 400-600 Hz [82]. With regard to the order of the filters, in order to manage complexity and performance, filters between the second and fourth order are generally used to filter the electromyographic signal.

The reason for doing this filtering is because frequencies below 10 Hz are associated with slow changes in muscle activity, such as changes in muscle tone or baseline drift, and frequencies above 500 Hz are often associated with electrical interference or noise from sources such as nearby electronics or other muscles, in other words this is the range of frequencies that are typically generated by muscle activity.

2.7.2 Rectification

This phase of the processing aims to make positive all the values of the signal. There are two possible procedures:

1. Cut of all negative values, that means, to delete the values that are under the baseline.
2. Turn all negative values to positive.

To achieve the total signal of the muscle, the second procedure is advisable, because it will allow the negative portion of the signal not to be lost, which will result in more interesting analyses. In fact, this procedure does not affect the noise like other processing steps like filtering, but it is an important step to calculate, for example, the root mean square, as will be discussed later.

2.7.3 Smoothing and RMS

The smoothing process, like filtering, aims to reduce noise by taking out the extremes. The difference between these two is that filtering takes into account the muscle activation range, while

smoothing focuses on the signal itself. Through this noise reduction something called a linear envelope is obtained, leaving only a center part of the signal.

When smoothing it is necessary to select a window to process, which focuses on two characteristics:

- Number of points (single sample or data point in a time series), corresponding to the length of the signal to be processed.
- Value assigned to each point.

After defining these two factors, a representative value will be calculated for each window, forming a new smoothed curve.

It should be noted that the window length can be variable, with some authors advocating time lengths around 10-150 ms, while others use values around 200 ms, 400 ms and 500 ms [83].

The Root-Mean Square (RMS) is a mathematical measure often used to calculate the average power of a signal, by taking the square root of the average of the squared samples of the signal. In other words, this method reflects the level of physiological activation in the motor units during muscle contraction [84].

This analysis is done in a specific period of time, and one study states that a window between 200 ms and 300 ms seems to be ideal, while for dynamic contractions a longer window is recommended [85].

In the same study it is stated that within the available methods for analyzing muscle activity the RMS is one of those that presents the best results, especially in the middle deltoid during abduction and adduction movements.

2.7.4 Maximum Voluntary Contraction

One of the main problems of EMG signal analysis on a micro or millivolt scale is that they are strongly influenced by the detection conditions, which include the position of the electrodes, subjects and even the moment when the detection is performed regardless of whether it is the same muscle being analyzed. As a response to this signal variability, the concept of maximum voluntary contraction (MVC) is relevant. This allows a calibration in order to define a relevant value, which corresponds to the maximum percentage of innervation.

This calibration is usually done in static exercises, because a good stabilization is important to produce a maximum contraction. In this procedure we analyze only one muscle at a time, and define an exercise or position that allows an effective maximum innervation.

It is advisable to warm up briefly and ask subjects to progressively increase strength and repeat at least once with a rest period of 30 to 60 seconds. If more than one exercise is to be performed the order should be randomized to systematic fatigue effects.

The MVC is not calculated with singular peaks as this would lead to a large variability. A more feasible reference would be the mean amplitude of the highest signal portion with 500 ms duration or another time interval depending on conditions and objectives [56].

This measurement among many other uses allows for example, to understand to what capacity the muscles are recruited, the effectiveness of a training for certain muscles or even the ergonomic effort that a person is subjected to at work, in a realistic and unbiased way.

Obviously, this is a concept which should be used with healthy, trained subjects, as an injured person could never be asked to perform an exercise at maximum capacity.

2.8 NEUROMUSCULAR FEEDBACK AND PROPRIOCEPTIVE NEUROFUNCTIONAL FACILITATION

There are some human body systems that operate automatically and, subconsciously, a person is capable of maintaining the body in its homeostatic state. One such system is the sensorimotor system, which controls joint stability during movement through a network of nerve signals.

The objective of this dissertation is to promote a faster and better healing of the reconstructed ACL as well as of the sensorimotor control of the knee and of the whole lower limb. For that purpose, it is proposed to resort to interferential stimulation, in synchronisation with quadriceps activation, to better stimulate the new ACL graft, including the anchoring points, stimulating in particular the mechanoreceptors of the ACL and consequently improve proprioception.

In this context, a method capable of stimulating mechanoreceptors and proprioceptors (muscle spindle and golgi tendon organs) has emerged: Proprioceptive Neuromuscular Facilitation (PNF). It allows to improve the neuromuscular feedback related to knee stability and lower limb muscles' strengthening.

The PNF therapeutic techniques combine proprioceptive input with specific movement patterns to enhance muscle function. It was initially developed by physical therapists to improve strength, flexibility, and coordination, and involve stretching, contracting, and relaxing muscles in specific sequences.

The fact that these patterns of stretching, contracting and relaxing muscles have the ability to promote the stimulation of mechanoreceptors makes us believe that the timing and context in which the electrostimulation is carried out could also make a difference. Certain timing and different contexts could also translate into different results.

Neuromuscular control is defined as an unconscious trained response of a muscle to stimuli regarding dynamic joint stability [86]. For that, neuromuscular feedback is needed that involves the communication between nerves and muscles. This feedback allows the central nervous system to adjust and calibrate the gain of the motor-neuron feedback loop. It allows us to sense tensions more accurately, and to control them more voluntarily. When one performs movements, sensory information from proprioceptors is transmitted to the central nervous system (brain and spinal cord). The nervous system processes this feedback and adjusts muscle activation patterns accordingly. In other words, the main goal of PNF is to stimulate neural afferents from mechanoreceptors, muscles, joints, tendons and deeper tissues, which are transmitted in the form of a neural impulse to the various levels of the central nervous system, so that the information and

position of the segments of the human body on static or dynamic conditions, balance or imbalance can subsequently be verified [87]. For example, if you're lifting a heavy object, neuromuscular feedback ensures that the appropriate muscles contract to support the load.

A study of Blasimann et al. [88], investigated neuromuscular activity in patients undergoing ACL reconstruction after they had completed the rehabilitation process and were theoretically ready to return to normal daily life and unrestricted sports practice, with a time interval of approximately one year.

It was concluded that after that year there were still changes at the neuromuscular level, compared to healthy subjects. In this way, it was realised that standard rehabilitation protocols are not totally effective in neuromuscular rehabilitation after ACLR.

One tool that is gaining relevance is biofeedback, more precisely electromyographic biofeedback. This is a method that allows the subject to monitor their voluntary muscle contraction. You can monitor the EMG signal using visual feedback (for example graphs) or sound. It is believed that this technique can increase the quality of muscle contraction and, in a way, allows the patient to integrate all levels of the nervous system when contracting and relearning to contract the muscle. In fact, there is evidence that in subjects undergoing knee surgery, biofeedback is associated with favourable results in peak torque, higher EMG signal and recovery of knee extension [89].

Treatments combining PNF and FES have already been studied, particularly in traumatic brachial plexus injuries. After a 6-week follow-up of a patient subjected to an injury of this type, positive effects were seen and it was concluded that the combination of PNF and FES is viable [90].

2.9 ACL REHABILITATION EXERCISES

2.9.1 Closed kinetic chain exercises, ACL pressure and co-activation Quadriceps-Hamstring

The rehabilitation process of an ACL injury focuses on reestablishing the range of motion and strengthening leg musculature, in particular the muscles of quadriceps and hamstrings, and simultaneously trying to minimize the forces applied on the ligament [91]. In order to minimize the pressure exerted on the ACL, closed kinetic chain (CKC) exercises are a good option. These are defined as exercises where the feet are fixed against stationary or moving resistance. In fact, these exercises also facilitate co-activation between leg muscles to promote stability at the knee for stance and movement [92].

In order to ascertain which rehabilitation exercises might be beneficial to perform after ACL injury 7 CKC exercises were performed in order to analyze the EMG signal and verify the co-activation balance between the quadriceps (through the rectus femoris muscle) and the hamstrings (through the bicep femoris muscle)

The CKC exercises are (Fig. 31): single-leg squat without weight, single-leg squat with weight, single-leg deadlift without weight, single-leg deadlift with weight, front lunge, side lunge, and step up.

According to a study conducted by Begalle et. al [93], where the co-activation between the quadriceps and the hamstrings muscles in common therapeutic exercises was evaluated, the following conclusions were reached:

1. Exercises that use a quadriceps-dominant activation might negatively affect the knee by increasing strain on the anterior cruciate ligament.
2. The hamstrings muscles can counteract the deleterious effect of the quadriceps except when activation is minimal.
3. Exercises with a more balanced quadriceps-to hamstrings co-activation ratio may benefit anterior cruciate ligament rehabilitation and injury-prevention programs.

To evaluate the co-activation between the two muscles, 5 repetitions of each exercise were performed and the RMS value of each one was noted, for the rectus femoris muscle and for the bicep femoris muscle.

It was calculated the average of the 5 RMS values obtained in the 5 repetitions for each muscle, and it was calculated the relative percentage for each one of them (Fig. 32), taking into account that the total is the sum of the two averages obtained (one for each muscle).

The aim was to try to verify the CKC exercises that provide a more balanced quadriceps/hamstrings co-activation and that can be inserted in ACL rehabilitation protocols.

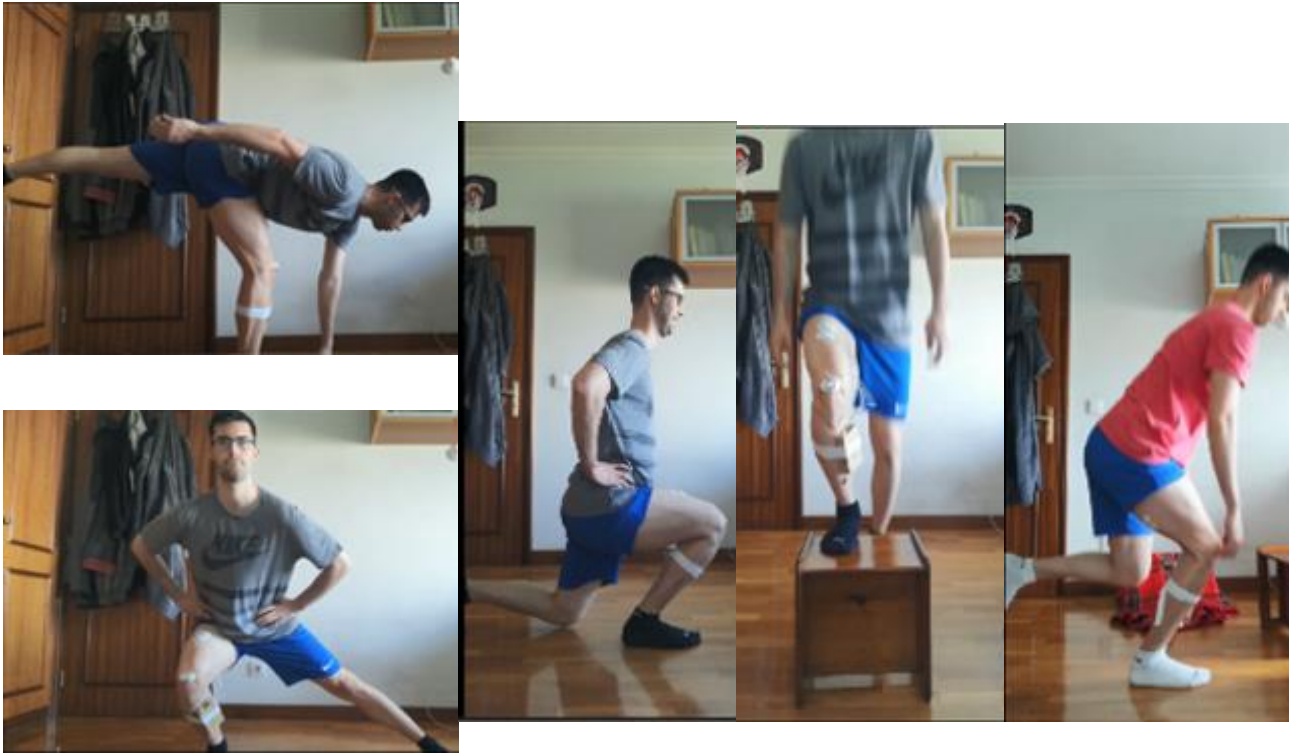


Figure 31: Closed Kinetic Chain exercises (single-leg deadlift, side lunge, front lunge, step up, single-leg squat).

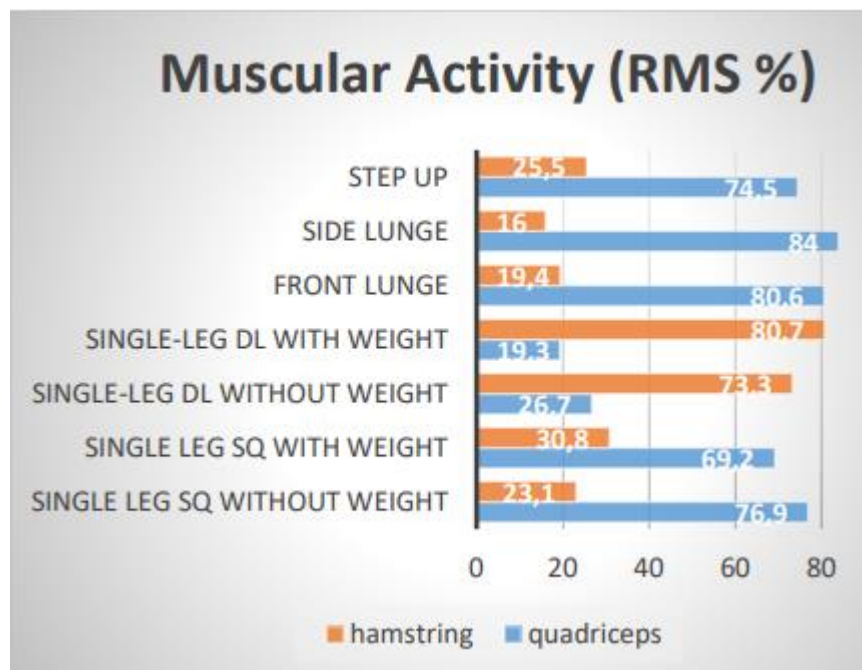


Figure 32: Relative percentage of hamstring and quadriceps.

It could be concluded that adding weight to the squat can facilitate a more balanced co-activation between the quadriceps and hamstrings by adding resistance to the flexion and extension phases. The single leg deadlift is powered by less quadriceps and more hamstring activity. Front and lateral lunges also achieve predominant quadriceps activation, which can result in greater ACL loading, and as in the lunge step up it's clearly a quadriceps predominant exercise.

2.10 . MOTOR LEARNING AFTER ACL RECONSTRUCTION

When we talk about rehabilitation after ACL reconstruction, there are essential processes, one of which is strength training. However, over time other techniques and methods have begun to gain relevance in the rehabilitation of this injury, such as motor learning processes, which translate into the injured person's ability to acquire motor skills with a relatively permanent change in performance as a result of practice or experience [94]. In fact, motor learning has emerged as a tool capable of reducing the risk of recurrence by reducing the risk of a second injury and also reducing the risk of early osteoarthritis [94].

The key concepts of motor learning that make it possible to promote neuroplasticity and consequently improve the patient's functional performance and reduce the risk of relapse are:

1. External focus of attention.
2. Implicit learning.
3. Differential learning.
4. Self-controlled learning and contextual interference.

2.10.1 External focus of attention

External focus of attention involves directing the attention of an individual's movement to its effect on the environment rather than focusing on the movement itself, intrinsically. This approach has been shown to result in more effective and efficient movements, particularly in rehabilitation and motor skill learning contexts [95]. For example, during gait rehabilitation, instead of instructing the patient to straighten their knee more during the stance phase, the therapist instructs the patient to pretend to kick a ball during the end of the swing phase. Saying to straighten the knee is considered internal focus, and instructing to kick a ball is considered external focus. When we orientate the attention outward to the effects of movements on the environment, individuals can engage with automatic processes, leading to smoother, more coordinated movements and facilitating rehabilitation outcomes.

Table 6: Instructions with internal and external focus [94].

Goal: Improve postural stability	Internal focus	External focus
Instructions	Try to keep your knee aligned over your second toe.	Try to keep the bar horizontal.
	Try to minimize movements of your feet.	Try to minimize movement of the bars on the balance board.
	Try to keep your balance by stabilizing your body	Try to keep your balance by stabilizing the platform.

2.10.2 Implicit Learning

Implicit learning involves learning and performing new skills or knowledge without consciously focusing on the learning process itself. Instead of explicit instructions, implicit learning often involves analogies [96], metaphors, or provides limited visual information to help individuals truly perceive a movement or action. One example related to ACL rehabilitation is, instead of focusing on the precise alignment of the knee joint, the therapist might use implicit feedback such as providing visual cues or asking the patient to imagine the sensation of stability while performing dynamic movements. In this case, probably individuals trained with implicit feedback may develop better motor control and coordination, leading to reduced injury risk by improving their ability to anticipate and respond to potential threats to joint stability.

Table 7: Explicit and implicit instructions in different exercises [94].

Task	Explicit instructions	Implicit instructions
Squat	Stand with your feet shoulder-width apart.	Stance: Think about keeping a big ball between your knees.
	Lower down so your thighs are as parallel to the floor as possible, with your knees over your ankles	Imagine you're picking up a heavy box from the floor. Imagine you're going to sit down on a chair.
	Running	
	Bend your knees while landing	Imagine you run like a feather. Land softly Try to make as little noise as possible
Vertical Jump	Bend your knees before you jump	

Explosively extend hips, knees, and ankles, and propel off balls of feet to jump straight up	Imagine you're landing on eggs and you don't want to crack them
Landing: bend knees during landing	Push yourself off the floor as hard as possible
	Pretend you are a rocket that launches

2.10.3 Differential Learning

Differential learning is a methodology based on the theory of dynamical systems. In other words, it encourages intentional variation in movement patterns during practice. Instead of focusing on repeating the same movement with precision, differential learning encourages athletes to explore multiple variations of a movement. The aim is to facilitate a self-organized process of learning, allowing individuals to explore motor solutions that work best for them in different contexts and constraints of the environment and their bodies.

During a rehabilitation session, instead of following a set routine of predetermined exercises for a specific number of repetitions and sets, the therapist should introduce variability by incorporating different exercises and environmental conditions. This can translate into enhanced neuroplasticity and motor learning compared to traditional rehabilitation methods. By engaging in varied movement patterns, they stimulate different neural pathways and adapt more readily to real-world challenges.

Table 8: Differential learning in a double-legged jump task training [94].

Variations of the double-legged jump task	Change of environment	Change of athlete
Jump as far as you can. While you are jumping	Exercises in dark	Perform exercises with fatigue
	Exercises on sand	Perform exercises without fatigue
Make a full turn to left, to right before you jump	With shoes or without shoes	Perform exercises with weighted vest
While landing, one arm in front of and the other arm behind you	In an environment with loud music or noise from audience in stadium	
Land with a very wide stance or with a very narrow stance	In a virtual reality environment	

2.10.4 Self-Controlled Learning

Self-controlled learning gives individuals more authority by giving them some degree of control over their learning process. This control can be given, for example, during feedback or when the individual is performing exercises.

During a rehabilitation process, rather than the therapist telling patients exactly what to do step-by-step, patients are given a choice of exercises to pick from. For example, they might be shown a list of exercises and allowed to choose a few to do during their session. This can result in more interest and motivation of the patients, and they could feel like they are in control of their recovery. They are also more likely to stick to their exercise routine and feel happier with how they are progressing.

2.11 . QUAD SET

Table 9: Quad set execution parameters.

Exercise	Quad Set https://www.youtube.com/watch?v=RaErDb62d90
Goal	A common exercise in the early stages after ACL reconstruction. Isolating, strengthening and 'learning' to contract the quadriceps muscle group with a focus on the rectus femoris
Success Criteria	1. Lie supine with the lower limb that will perform the contraction stretched out and the other lower limb flexed. 2. Contract the quadriceps - so that the back of the knee is close to the surface on which the patient is lying and so that the heel lifts slightly off the surface - a towel or other object can be placed to slightly raise the knee. 3. Contract for approximately 5 seconds.
Mistakes to avoid	Don't arch the back - keep your hands at your sides; Do not flex the neck
Motor Learning	External Focus of Attention: Focus on squeezing the knee on the stretcher and lifting the heel slightly when contracting the quadriceps. Implicit Learning: Completely avoid a gap between the knee and the stretcher Differential Learning: Perform the exercise sitting, lying and/or standing. Self-Controlled Learning: The patient can choose the position.
Illustration	

2.12. REHABILITATION PHASES, GOALS, INTERVENTIONS AND PROGRESS CRITERIA

Recovery time after ACL reconstruction is around 6-12 months. The time frame is so long because it can vary greatly depending on the person, their activity and their future. For example, when an athlete undergoes ACL reconstruction, there is a need to try to find a balance between returning to competition as quickly as possible and avoiding a recurrence. When we're talking about a non-athlete, we simply try to take an approach with the lowest possible risk and the greatest possible benefit. In other words, an athlete usually undergoes a faster recovery period or even skips a few stages and is specific to the sport, while a non-athlete may have a longer-lasting recovery.

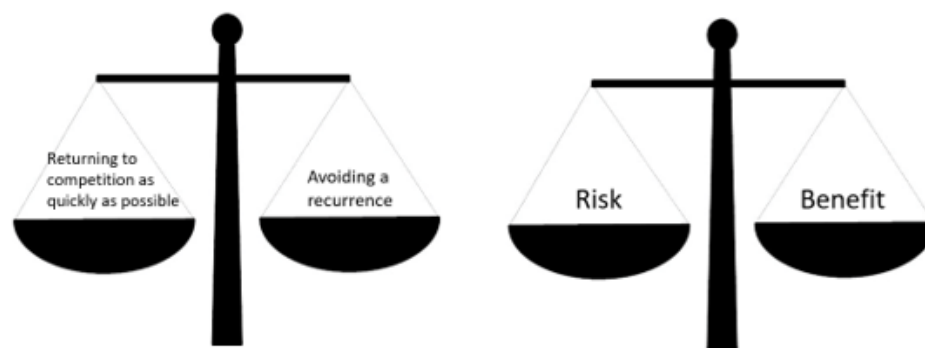


Figure 33: Priorities in the recovery of athletes vs non-athletes.

People of different ages and lifestyles can also have different recovery processes.

When we talk about rehabilitation or sports training protocols, it is important to take into account the individuality of individuals and, depending on their characteristics and response to treatment, the actions of health professionals should be personalised.

There is no doubt that having time guidelines is a reference, but each person reacts differently to the same stimuli and what may be favourable for one individual may not be for another and vice versa.

That's why in this dissertation we advocate the creation of a rehabilitation protocol not only with reference to treatment time, but above all on the basis of criteria that the patient must fulfil in order to progress through the different phases of treatment.

The Massachusetts General Brigham has drawn up a very complete 6-phase protocol [97]. With time-based guidelines and criteria for progressing through these phases (Table 10). It also includes the rehabilitation goals of each phase and interventions.

Table 10: ACL Rehabilitation protocol of Massachusetts General Brigham [97].

	Phase 1: IMMEDIATE POST-OP (0-2 WEEKS AFTER SURGERY)	PHASE II: INTERMEDIATE POST-OP (3-5 WEEKS AFTER SURGERY)	PHASE III: LATE POST-OP (6-8 WEEKS AFTER SURGERY)
Rehabilitation Goals	• Protect graft • Reduce swelling, minimize pain • Restore patellar mobility • Restore full extension, gradually improve flexion • Minimize arthrogenic muscle inhibition, re-establish quad control, regain full active extension • Patient education	• Continue to protect graft • Maintain full extension, restore full flexion (contra lateral side) • Normalize gait	• Continue to protect graft site • Maintain full ROM • Safely progress strengthening • Promote proper movement patterns • Avoid post exercise pain/swelling • Avoid activities that produce pain at graft donor site
Interventions	<i>Swelling Management</i> • Ice, compression, elevation (check with MD re: cold therapy) • Retrograde massage • Ankle pumps <i>Range of motion/Mobility</i> • Patellar mobilizations: superior/inferior and medial/lateral • **Patellar mobilizations are heavily emphasized in the early post-operative phase following patella tendon autograft** • Seated assisted knee flexion extension and heel slides with towel • Low intensity, long duration extension stretches: prone hang, heel prop • Standing gastroc stretch and soleus stretch • Supine active hamstring stretch and supine passive hamstring stretch <i>Strengthening</i> • Calf raises • Quad sets • Straight leg raise • **Do not perform straight leg raise if you have a knee extension lag • Hip abduction • Multi-angle isometrics 90 and 60 deg knee extension	<i>Range of motion/Mobility</i> • Stationary bicycle • Gentle stretching all muscle groups: prone quad stretch, standing quad stretch, kneeling hip flexor stretch <i>Strengthening</i> • Standing hamstring curls • Step ups and step ups with march • Partial squat exercise • Ball squats, wall slides, mini squats from 0-60 deg • Lumbopelvic strengthening: bridge & unilateral bridge, sidelying hip external rotation- clamshell, bridges on physioball, bridge on physioball with roll-in, bridge on physioball alternating, hip hike <i>Balance/proprioception</i> • Single leg standing balance (knee slightly flexed) static progressed to dynamic and level progressed to unsteady surface • Lateral step-overs • Joint position re-training	<i>Range of motion/Mobility</i> • Rotational tibial mobilizations if limited ROM <i>Cardio</i> • 8 weeks: Elliptical, stair climber, flutter kick swimming, pool jogging <i>Strengthening</i> • Gym equipment: leg press machine, seated hamstring curl machine and hamstring curl machine, hip abductor and adductor machine, hip extension machine, roman chair, seated calf machine • Hamstring autograft can begin resisted hamstring strengthening at 12 weeks • Progress intensity (strength) and duration (endurance) of exercises • **The following exercises to focus on proper control with emphasis on good proximal stability • Squat to chair • Lateral lunges • Romanian deadlift • Single leg progression: partial weight bearing single leg press, slide board lunges: retro and lateral, step ups and step ups with march, lateral step-ups, step downs, single leg squats, single leg wall slides • Knee Exercises for additional exercises and descriptions • Seated Leg Extension (avoid anterior knee pain): 90-45 degrees with resistance <i>Balance/proprioception</i> • Progress single limb balance including perturbation training
Criteria to	• Knee extension ROM 0 deg • Quad contraction with superior patella glide and full active extension • Able to perform straight leg raise without lag	• No swelling (Modified Stroke Test) • Flexion ROM within 10 deg contra lateral side • Extension ROM equal to contra lateral side	• No effusion/swelling/pain after exercise • Normal gait • ROM equal to contra lateral side • Symmetrical Joint position sense (<5-degree margin of error)

	PHASE IV: TRANSITIONAL (9-12 WEEKS AFTER SURGERY)	PHASE V: EARLY RETURN TO SPORT (3-5 MONTHS AFTER SURGERY)	PHASE VI: UNRESTRICTED RETURN TO SPORT (6+ MONTHS AFTER SURGERY)
Rehabilitation Goals	• Maintain full ROM • Safely progress strengthening • Promote proper movement patterns • Avoid post exercise pain/swelling • Avoid activities that produce pain at graft donor site	• Safely progress strengthening • Safely initiate sport specific training program • Promote proper movement patterns • Avoid post exercise pain/swelling • Avoid activities that produce pain at graft donor site	• Continue strengthening and proprioceptive exercises • Symmetrical performance with sport specific drills • Safely progress to full sport
Interventions	• Begin sub-max sport specific training in the sagittal plane • Bilateral PWB plyometrics progressed to FWB plyometrics	<i>Interval running program</i> ○ Return to Running Program <i>Progress to plyometric and agility program (with functional brace if prescribed)</i> ○ Agility and Plyometric Program	<i>Multi-plane sport specific plyometrics program</i> <i>Multi-plane sport specific agility program</i> <i>Include hard cutting and pivoting depending on the individuals' goals (~7 mo)</i> • <i>Non-contact practice→ Full practice→ Full play (~9 mo)</i>
Criteria to Progress	• No episodes of instability • Maintain quad strength • 10 repetitions single leg squat proper form through at least 60 deg knee flexion • Drop vertical jump with good control • KOOS-sports questionnaire >70% • Functional Assessment ○ Quadriceps index >80%; HHD or isokinetic testing 60d/s ○ Hamstrings ≥80%; HHD or isokinetic testing 60 d/s ○ Glut med, glut max index ≥80% HHD	• Clearance from MD and ALL milestone criteria below have been met • Completion jog/run program without pain/effusion / swelling • Functional Assessment ○ Quad/HS/glut index ≥90%; HHD mean or isokinetic testing @ 60d/s ○ Hamstring/Quad ratio ≥66% ○ Hop Testing ≥90% compared to contra lateral side, demonstrating good landing mechanics	• Functional Assessment ○ Quad/HS/glut index ≥95%; HHD mean or isokinetic testing @ 60d/s ○ Hamstring/Quad ratio ≥66% ○ Hop Testing ≥95% compared to contra lateral side, demonstrating good landing mechanics • KOOS-sports questionnaire >90% • International Knee Committee Subjective Knee Evaluation >93 • ACL-RSI

Chapter 3 - Methods, Results and Limitations

In the previous chapters, we explored cruciate ligament injury, its reconstruction and sequelae in depth, focusing on arthrogenic muscle inhibition and the consequences at neuromuscular level, as well as some existing solutions. In this way we can use our existing knowledge to define and develop a solution that will enable us to enhance the rehabilitation process after ACL reconstruction with a focus on electrostimulation of sensory cells in order to promote neuromuscular feedback and promote a better connection between nerves and muscles and consequently increase levels of proprioception and the graft reinnervation.

This chapter will describe the procedure for developing a functional electrostimulation interferential therapy device capable of sending electrical stimulation to the knee area when the patient contracts the quadriceps muscle. The construction processes of the developed hardware (electrical circuits and PCB construction) and software (algorithms to detect quadriceps muscle contraction and graphic interface to guide the patient) are explained.

The system developed was applied to four patients between 6 and 18 June who had undergone ACL reconstruction less than two months before the first FES session. The system developed was applied between 4 and 6 sessions in each patient and was applied when performing the quad set exercise, with 3 sets of 8 repetitions performed in each session. After the application of the FES protocol, the patients followed treatment with the therapists at each session.

Patient instruction techniques are also focused on, such as motor learning techniques that allow optimizing the recovery process and achieving better long-term results.

3.1. IFT FUNCTIONAL ELECTROSTIMULATION DEVELOPED SYSTEM

The interferential therapy electrostimulator used was the Sonopuls 992, which generates a electric current stimuli continuously. The objective of this work is to develop a system that would only apply stimuli in the ACL area when contraction of the quadriceps muscles is detected. To accomplish this, an auxiliary circuit was developed to allow current to pass through or not. For the system to work, it was necessary to connect the system (more precisely a BiTalino board) to a laptop to run the muscle contraction detection algorithm, which allows the EMG signal to be collected and processed, where a moving average and a bandpass filter were applied and the RMS was calculated. When the RMS value is higher than a stipulated threshold, it is "understood" as a contraction detection. When this contraction is detected, the auxiliary circuit is switched on for the stimuli to be applied to the electrodes.

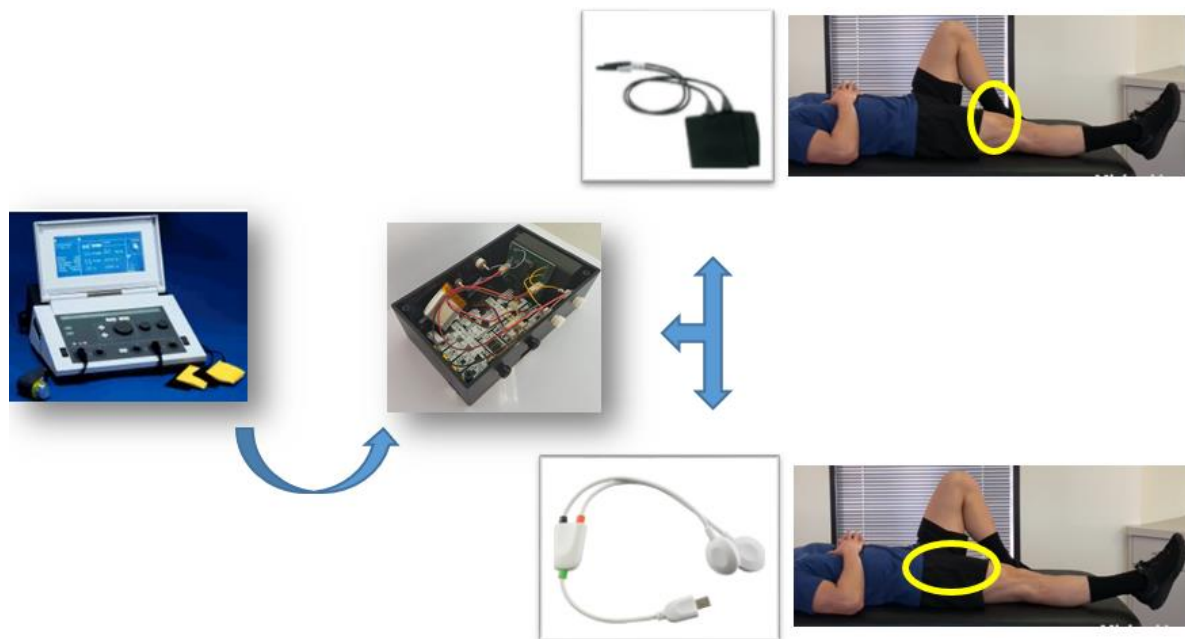


Figure 34: Logic of connection of Sonopuls 992, intermediate circuit, electrodes and its positioning (knee - electrical stimulation), quadriceps - EMG).



Figure 35: System's setup.

3.1.1. Hardware and switching circuit

An auxiliary circuit was developed that allows the current coming from the electrostimulator to be blocked or passed through. This circuit consists of three large parts: a PCB board with the matrix switching circuit, the BITalino and a 4.5V battery power supply. This circuit and the various

components were organized in a box suitable for assembling electronic circuits. This box is made of plastic and has dimensions of 157x95x53 mm³. To establish the connection between the electrostimulator and the intermediate circuit and between the intermediate circuit and the electrodes applied to the patient, female sockets were used to allow the connection of 4 mm banana plugs. The diagram of the organization of the components in the box is shown in the following figures.

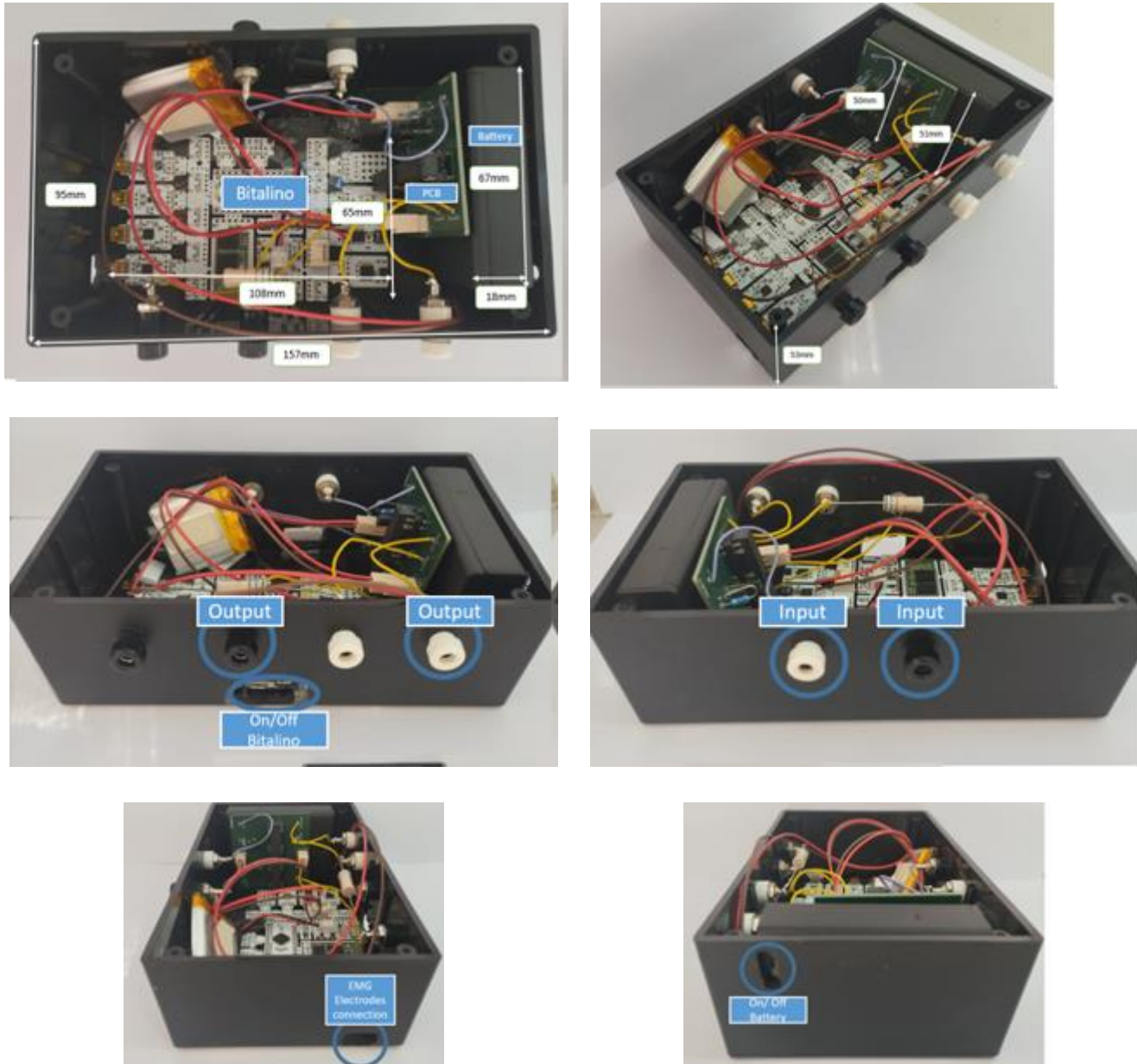


Figure 36: Arrangement of the components in the box, input/output entrances and battery on/off and emg electrodes openings.

3.1.2. Schematic and PCB

The central element of the PCB are two SPDT-CO relays forming a switching matrix which, in short, allow to switch between two different electrical circuits. When the relay is not activated, its common terminal is connected to the normally closed terminal (1) and when it is activated, it connects the common terminal to the normally open terminal (14).

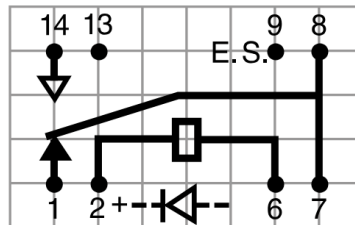


Figure 37: Schematic of SPDT-CO relay.

This is a key aspect in the development of the work because when the electrostimulator used in the hospital, does not detect the passage of current, would reset after three seconds, i.e. the current that was initially set would return to 0. What was done was to create an alternative route for the current to circulate in a loop so that the electrostimulator could detect the passage of current in such a way that it would not reset, when there was no passage of current to the electrodes to be applied to the patient. More specifically, we simply connected the normally closed terminals of the two relays with a resistor so that the current would enter the loop.

Two transistors (P2N2222A) are used to control the current flowing through the relay coil. When a high output from the BITalino buzzer (~3.25V) is applied to the base of the transistor, it switches on and consequently activates the relay. In this way, we can say that the activation of the BITalino buzzer acts as a control to activate the relay.

We know that the voltage required to activate the relay is approximately 5V and that its internal resistance is 500 Ω . So, using Ohm's law, the current needed to activate the relay is 10 mA. Then, knowing that the current in the base is equal to the current in the collector divided by β and that 0.7V is the intrinsic value for the voltage drop of the bipolar transistor used a standard value of 2.2 K Ω was used. It is worth highlighting that the current in the base was increased in the calculation of the base resistor to ensure that the transistor operates in saturation.

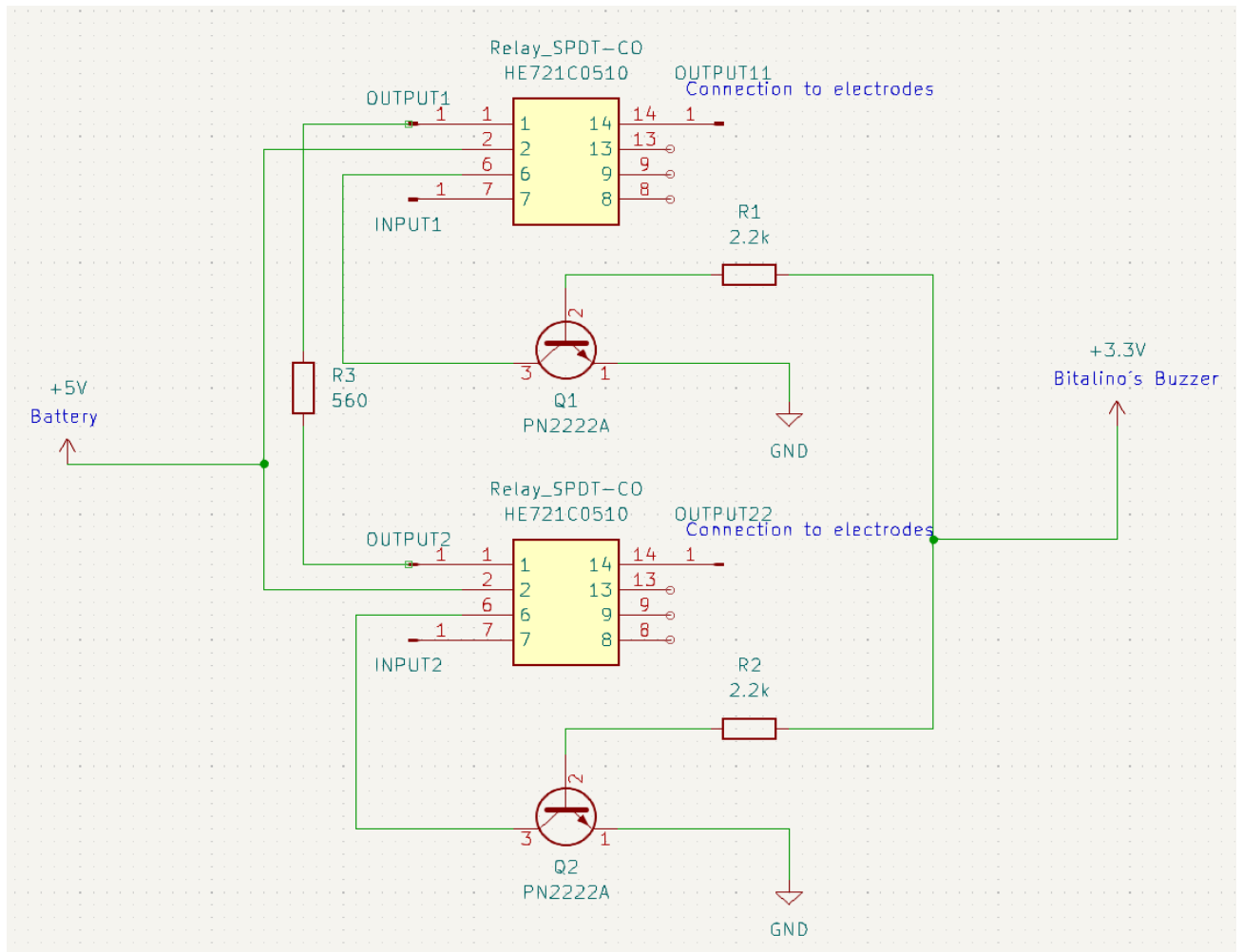


Figure 38: Schematic of intermediate circuit.

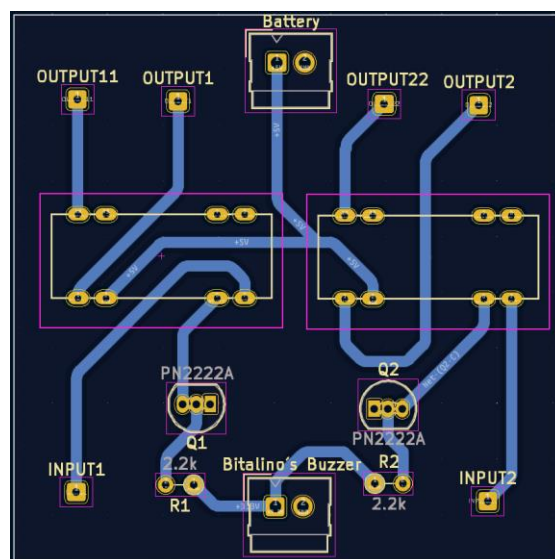


Figure 39: PCB of intermediate circuit.

The activation of the buzzer is triggered by the detection of quadricep muscle contraction. After collecting and processing the EMG signal and calculating the RMS using Pycharm, and after some tests a threshold was set and when the RMS exceeded this threshold an order is given to activate the buzzer, which, as mentioned above, acts as a control to activate the relay and allow current to pass from the electrostimulator to the electrodes applied to the patient's knee.

The base threshold set was a RMS value of 50 mV, which was set by tests in the author. This threshold worked without problems to detect quadriceps contraction, but it is important to emphasise that at the time of the tests the researcher had no injuries or conditions that could directly affect the EMG signal. Since people with recent injuries may have lower EMG signals, it is necessary to adapt this threshold to injured people by reducing it. It should also be emphasised that the EMG signal also varies from individual to individual, even if they are healthy, so it is always necessary to adapt this threshold.

The auxiliary circuit is powered by three 1.5V AA alkaline batteries.

3.2. PATIENT COMMUNICATION INTERFACE

3.2.1. Colored squares guidance

In order to make the process more intuitive for the patient, a graphic interface was developed based on colored squares and countdowns. At an early stage of the process, a gray square appears on the computer screen used with a 30-second countdown to adjust the current so that it is comfortable for the patient. Then a cycle starts until the process ends. A green square appears in the center of which is written "Contract", giving the order for the patient to contract the quadriceps, being the BITalino buzzer activated and current stimuli applied to the electrodes. After muscle contraction is detected, a yellow square appears on the screen with a countdown of 5 seconds and during which the patient must contract the quadriceps as much as possible. Subsequently, a red square appears on the screen with a countdown of 4 seconds, corresponding to the release time when the patient must relax the muscle. This cycle is then repeated, i.e. green square, yellow square and finally red square.

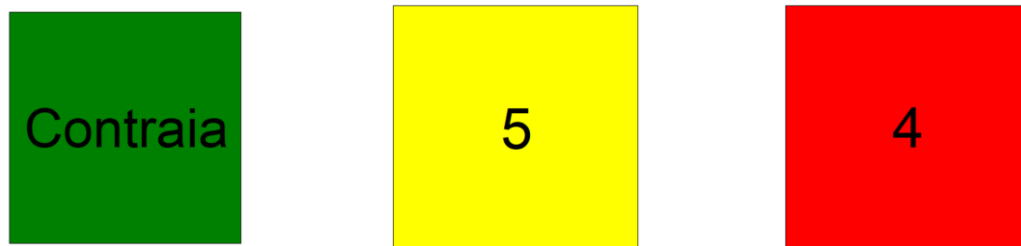


Figure 40: Interface of colored squares for patient guidance.

Process Step-by-step:

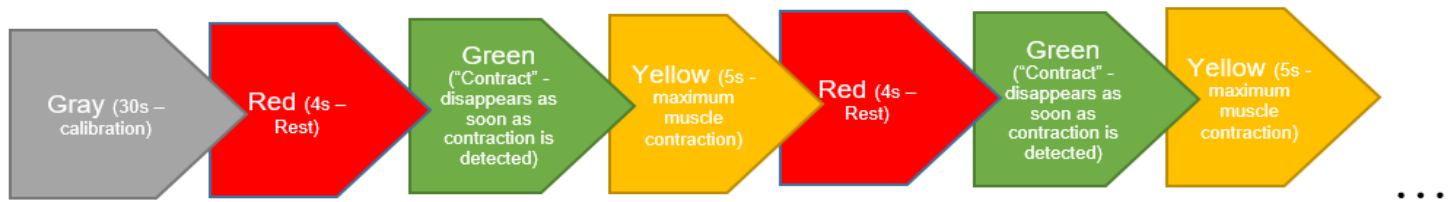


Figure 41: Interface of colored squares logic step-by-step.

In what concerns the algorithm to detect the muscle contraction, the BITalino library was used to facilitate communication with the BITalino board. The numpy library was used to perform the mathematical operations needed to process the EMG signals, such as calculating the RMS, and the tkinter library, that is a standard graphical user interface toolkit included within Python, was used to create the graphic interface for the colored squares.

One variable to note is `nSamples`, which defines the number of samples acquired with each reading of the BITalino's EMG signal.

```
emg = device.read(nSamples)[:, 5]
```

In this line, we are taking the information of column 5 (EMG sensor) and the number of samples that is defined in `nSamples` is read. In a visual way:

$$Emg = [emg\ 1, emg\ 2, emg\ 3 \dots emg\ n] \quad (6)$$

On the one hand, having a high `nSamples` gives you better accuracy, but it can slow down the system's data reading. On the other hand, having lower `nSamples` translates into worse accuracy and the system becomes faster. That's why it's important to find a balance between accuracy and speed.

The value of `nSamples` was set to 300, meaning that 300 samples of the signal are acquired with each reading of the EMG signal. A few tests showed that values below 300 were not very precise and if this value was increased too much, the system would experience significant delays. From this, it was concluded that 300 samples would not slow the system down too much while at the same time making the reading accurate.

```
high = 20/(samplingRate/2)
low = 450/(samplingRate/2)
b, a = sp.signal.butter(4, [high, low], btype='bandpass')
```

One of the fundamental steps in EMG signal processing is filtering. The sampling frequency, i.e. the rate at which we measured the EMG signal, is 1000 Hz.

In relation to the Butterworth filter, it has advantages such as ensuring a gradual and uniform transition between passing and attenuated frequencies, for example reducing peaks in the signal. In this case it was defined as a fourth order bandpass filter, with cutoffs of 20 Hz and 450 Hz.

In order to use the cutoff frequencies in the `sp.signal.butter` function, it was necessary to normalise them in relation to the Nyquist frequency (500 Hz in this case) by dividing these cutoff values by it, obtaining values between 0 and 1, which is the appropriate format for creating a digital filter. This normalisation of the cutoff values makes it possible to provide consistency to the system, ensuring that any filter designed is compatible with the system's sampling rate. It also provides mathematical simplicity, as it is not necessary to manipulate a wide range of absolute frequencies, but rather to manipulate values between 0 and 1. It also makes it possible to avoid errors, as, for example, when using absolute frequency values. For some reason we could define a cutoff frequency higher than the Nyquist frequency, which could consequently cause the filter to fail and promote aliasing.

```
emg_centered = emg - 512
```

The EMG signals captured by the Bitalino are expressed as bipolar values ranging from 1 to 1024, so 512 was defined as the reference midpoint. We know that the EMG signal can be either positive or negative, depending on the muscle activity measured. However, in practice, the EMG signals of interest oscillate around a midpoint when there is no detection of muscle contraction (rest).

By setting the average value to 512 and subtracting this value from the EMG signal read, we are centralising the detected EMG signals. In other words, when the muscle is at rest, the average signal value is expected to be close to (emg-512). So values above (emg-512) indicate that there is muscle activity. In short, we are moving the midpoint of the scale used to zero.

Subsequently, the values are squared, eliminating the negative part and giving us the magnitude of the signal, regardless of the direction (positive or negative). Finally, we calculate the quadratic mean of this magnitude by adding up all the squared values and dividing by the number of samples. Then, to obtain the effective value of the EMG signal, the square root is applied.

Two threads were used to establish real-time communication between the graphical interface and muscle contraction detection, to ensure that the graphical interface remained responsive simultaneously to the collection of EMG signal samples and consequent control of the Bitalino buzzer and delivery of current to the electrodes. In other words, one thread manages the Tkinter event loop and GUI updates, ensuring that the interface remains interactive and the other thread handles the communication with the Bitalino board, including data acquisition and processing, as well as controlling the buzzer and electrode current passage.

```
emg_filtered = sp.signal.filtfilt(b, a, emg_centered)
```

Then we use the `filtfilt` function to apply the filter twice, once forwards and once backwards. This eliminates the phase shift, because when we apply a filter it can cause a delay in the signal and by applying the filter forwards and backwards this phase shift is compensated for. It also allows

for more refined filtering, helping to remove more unwanted noise while maintaining the desired signal shape. In this way we can say that there is the application of an effective eighth-order filter [98].

```
rms = np.sqrt(np.mean(emg_filtered ** 2))
```

This formula was used to calculate the square root of the average of the sum of the squares of EMG values.

$$rms = \sqrt{\frac{1}{n} \sum_{i=1}^n (emg)^2} \quad (7)$$

```
device.trigger([1, 1])
```

Using the `device.trigger` function available in the Bitalino library, it is possible to manipulate both the LED and the Bitalino buzzer. The first value in the list corresponds to the LED and the second to the Buzzer. When these have values of 1 they are activated, when the value is 0 they are deactivated.

```
if rms > rms_threshold:
    # Activate Buzzer
    device.trigger([1, 1])
    # Show Yellow Square for 5 seconds
    update_square("yellow", countdown_from=5)
    time.sleep(5)
    # Deactivate Buzzer
    device.trigger([1, 0])
    # Show Red square for 4 seconds
    update_square("red", countdown_from=4)
    time.sleep(4)
    # Show green square
    update_square("green", countdown_from=0)
```

One of the problems that arose during the testing phase of the system was that, as the tissues of the human body are electrically conductive, the current applied to the knee was detected at a high intensity in the electrodes that collected the EMG signal in the thigh, which meant that it was not possible to differentiate between moments of rest and muscle contraction. The solution we came up with was for the detection of muscle contraction to act as a trigger and activate the buzzer for 5 seconds, during which time the EMG signal would not be read. This was followed by 4 seconds of rest (red square) and after this time (5 seconds of contraction and 4 seconds of rest)

the EMG signal could be captured again. And since after these 9 seconds the buzzer would be turned off and consequently there would be no current passing to the knee electrodes. Therefore, the interference caused by this current is no longer a problem.

3.2.2. Gamification

The interface of colored squares to guide the patient is a simple and effective method, but there may be more appealing methods that can retain patients in treatment and make these moments more enjoyable and therefore more effective. One such method could be gamification, i.e. the application of typical elements of game playing, associating the objectives of a game with the objectives of a treatment.

In this way, a more complex interface than the colored squares was created, where it was possible to control the Dino-Run game (an offline game from Google) using the electromyographic signal, with logistics similar to controlling the BITalino buzzer. On the one hand, in relation to the BITalino buzzer, when the EMG signal exceeded a certain threshold it was activated. On the other hand, in the control of the Dino-Run game, when the EMG signal exceeded a certain threshold, the dinosaur would jump to overcome the obstacles.

In the process of creating the game, a calibration method was tested to determine the reference threshold. This calibration consisted of a maximal contraction of the muscle, which is why it was not included in the protocol applied to the patients at the hospital, since the group of patients who took part in the study had recently undergone surgery and it was not appropriate to perform maximal and submaximal efforts. Calibration can be done in patients at a more advanced stage of recovery.

In more detail, a muscle contraction detection algorithm has already been developed. The first step in building this game was to do a calibration that measured the user's MVC. To do this, a 3-second countdown appears on the console after which the user performs an exercise that promotes maximum muscle contraction for 5 seconds. Then, after collecting and processing the whole signal (rectification and calculation of RMS) the program finds the portion of 300 samples where the RMS was higher, and prints this value in a txt file that will later be used in the main code of the game. According to the game characteristics and after trying different time intervals, 300 ms ended up being a time interval that worked as intended, like the protocol applied in the patients of the Hospital. After registering the MVC, the main code is run, which consists of the game code itself (Dino-Run) and a muscle contraction detection algorithm. Regarding the detection of muscle contraction, the logistics are similar to the calculation of the MVC of the previous code, but the calculation of RMS is performed in real time in portions also of 300 ms through a queue mechanism, where successively the oldest samples are replaced by the most recent. The game has simple controls, where the space bar makes the dinosaur jump above obstacles, so if the RMS is higher than a threshold, in this case 40% of MVC (the main code reads the MVC value from the txt file mentioned above) it is simulated that the space bar was clicked, in case it is lower than a threshold of 20% of MVC, it is simulated that the key is released.

As in the colored squares protocol, exceeding the threshold functioned as a trigger for the dinosaur to jump. However, in future work and studies, systems may be developed that allow for more specificity and that promote greater treatment opportunities, such as jump intensities based on contraction intensity, longer-lasting obstacles with specific timings, biofeedback, performance recording, and rewards and progression.

In addition to the interface itself, effective methods can be developed to reduce noise and interference from the electrical current conducted by the human body, in order to measure the EMG signal without constraints while applying electrical stimulation, and also make the system more efficient and precise, for example, through methods of recognizing movements and body segments to assist in carrying out rehabilitation exercises. Nevertheless, the tests and procedures carried out with patients showed that a stable operating solution was achieved.

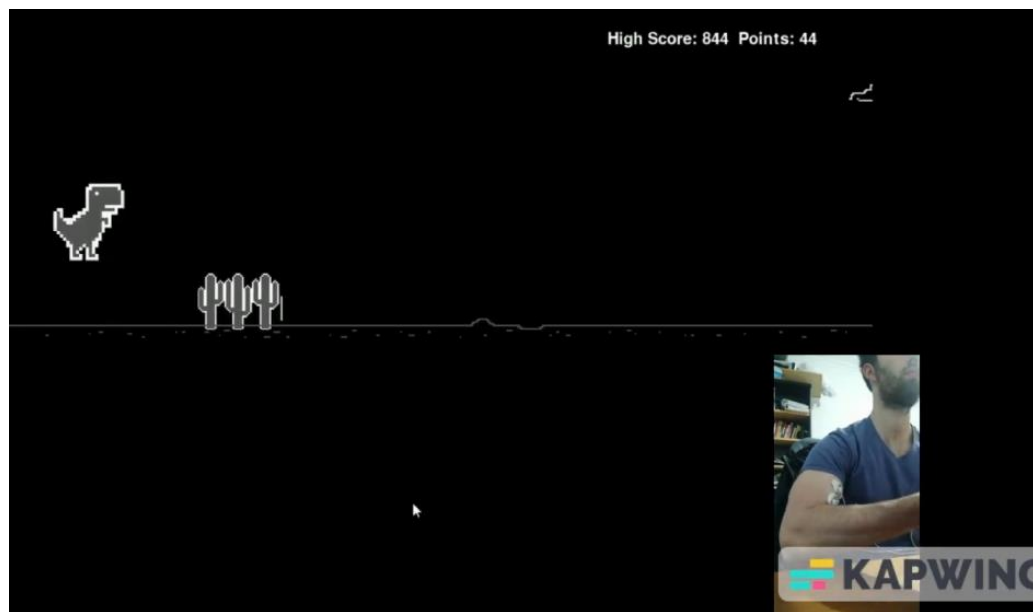


Figure 42: Interface of the game controlled by sEMG.

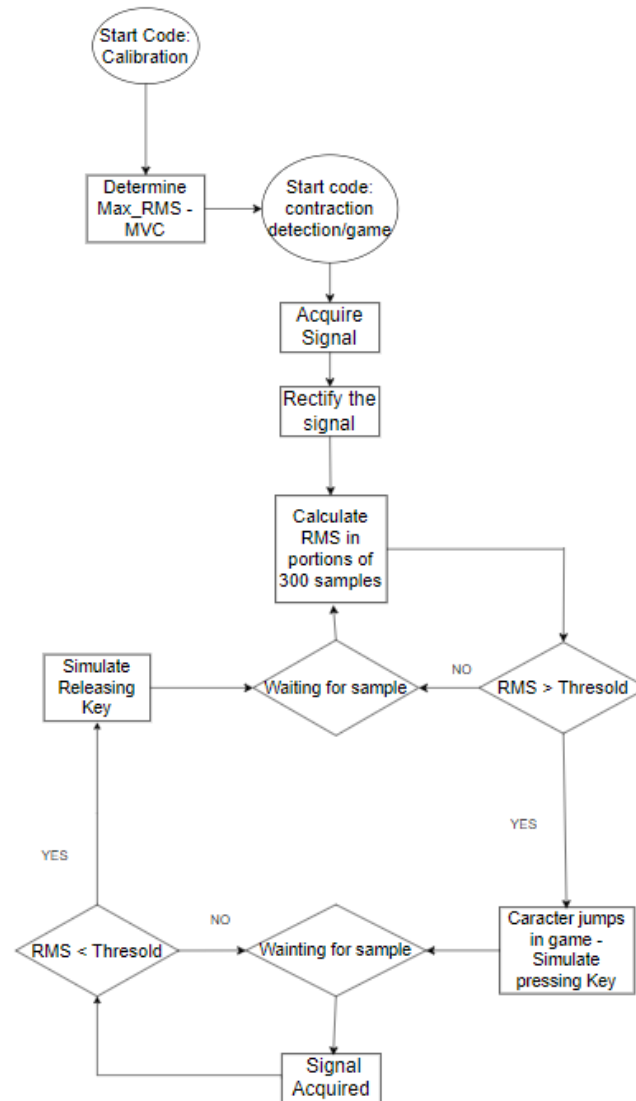


Figure 43: Flowchart of the logic of the game code controlled by sEMG.

3.3. PROTOCOL PREPARATION

In order to capture the EMG signal, we usually use a bipolar electrode configuration and a differential amplification just as within the BiTAlino. This bipolar setup involves placing two electrodes on the muscle being monitored. One electrode acts as the positive, while the other is the negative. These electrodes detect voltage variances relative to a baseline. In addition to the positive and negative electrodes, it is necessary to position a third electrode, the reference. It is

crucial to position this reference electrode in an area with minimal muscle activity. Ideally, it's placed close to a bone, in this case we placed it in the shin to ensure accurate measurement of muscle signals without interference from surrounding muscle activity.

Another option could be to position the reference electrode on the knee, but as the electrostimulator current was applied with electrodes attached to the knee by a bandage, the reference electrode would also be attached by the bandage, which could compromise the signal, not only because of the contact between the bandage and the reference electrode, but also because of excessive interference due to the proximity of the current electrodes to the reference electrode.



Figure 44: sEMG and electrical stimulator electrodes positioning.



Figure 45: Wet gel electrodes for sEMG.

The quality of the EMG signal collection depends on several factors including skin preparation and the type and position of electrodes, aspects that we have taken into account in this work.

Regarding skin preparation, the first step is to remove the hair and then cleaning the skin, namely through special abrasive and conductive cleaning pastes, sandpaper, and pure alcohol applied.

In this work we used surface electrodes. One of the disadvantages of this approach is that the detection of the activity of deeper muscles could be compromised, however since the muscle recruited (rectus femoris) for the study is a superficial muscle, it did not compromise the proper operation of the system when tested in the researcher.

Then we follow several guidelines in the application of the electrodes: small and wet-gel electrodes separated by approximately 2 cm and applied in the direction of the muscle fibers of rectus femoris. The fibers of this muscle descend along the thigh [99].

3.4. IFT PARAMETERS

The electrostimulator used in this work is the Sonopuls 992 and in Two-Pole Interferential Therapy we need to define 4 parameters: Carrier Wave Frequency, Stimulation Frequency, Frequency Modulation (Spectrum) and Frequency Modulation Programs.

The Carrier Wave Frequency is adjustable between 2-10 kHz, allowing customization based on nerve fiber type (motor/sensory). Frequencies like 2.5 kHz for muscle training and 4 kHz for pain control are commonly used, with the standard setting at 4 kHz.

It is known that different carrier frequencies used in interferential therapy translate into different effects. And when we prepare a protocol to apply to a patient, we not only have to take into account the effectiveness, but also the patient's permanence and comfort with the treatment. According to the literature, lower frequencies such as 1kHz and 2kHz are more effective in combating pain symptoms, but these same frequencies are more uncomfortable than higher frequencies such as 8kHz and 10kHz [100].

The Stimulation Frequency is customizable between 0 and 200 Hz and its selection is based on disorder nature, stage, severity, and localization. The standard setting is 50 Hz.

One study argues that low AMF promotes a greater electrical signal in the skin, subcutaneous tissue and muscles. They compared 4 Hz, 40 Hz and 90 Hz, with 4 Hz providing the best results in terms of signal depth in the tissues [68].

The Frequency Modulation (Spectrum) is adjustable between 0 and 200 Hz to prevent accommodation, ensuring consistent efficacy.

In the Frequency Modulation Programs there are three frequency modulation (sweep) programs available: 1/1 s, 6/6 s, and 1/30/1/30 s. Additionally, a "stochastic" program introduces random alterations in frequency modulation, enhancing prevention of accommodation.

Sweep is defined as the fluency in frequency changes between a base value (low frequency value) and the spectrum (maximum value), the most common being triangular, trapezoidal and quadratic in shape. In the case of the electrostimulator used, it offers programmes of 1/1 second, 6/6 seconds and 1/30/1/30 seconds [101].

Since interferential therapy in the context of this study is applied in short time intervals and intermittently, the spectrum used loses relevance and a value between 0-200Hz can be used.

When it comes to the sweep, since these are 5-second stimuli, the 6/6-second option is immediately discarded. The most viable option might be a 1/1s sweep, as a rapid tissue response is desired, helping to control pain and promoting blood circulation through rapid frequency variations.

The electrical stimulation parameters used are defined in the Sonopuls 992 electrical stimulator in image 47.

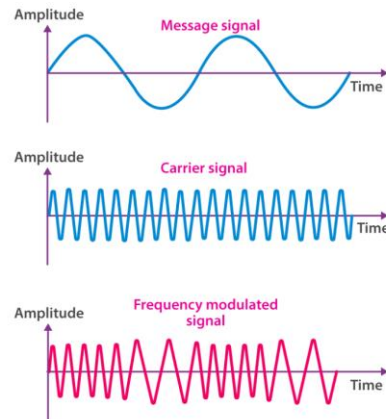


Figure 46: Graphical Representation of Frequency Modulation [102].



Figure 47: IFT Parameters.

3.5. PROPRIOCEPTION

In order to understand the patient's progress after injury and reconstruction of the ACL, it is necessary to carry out tests and measurements in order to realise the stage the patient is at and

the response to treatment. There are parameters such as strength, power, reaction time, among others. However, proprioception, which is an extremely important factor in the patient's return to daily life and sports practice, is a characteristic where it is very difficult to establish functional progression criteria during the rehabilitation process [103].

Being proprioception the body's ability to assess where it is in order to maintain balance when it is stationary, moving or making an effort. One of the most objective ways of assessing knee proprioception can be to evaluate and analyse the angles of the body segments, in this case between the thigh and the leg. In fact, some studies have already assessed proprioception using this method [104, 105].

Nowadays, with software for analysing movement and body position, we end up being able to make a very precise analysis, even more so than using instruments such as goniometers. One such software programme is Kinovea, which allows you to calculate angles based on drawing lines over body segments.

One test could be for the patient to lie on a stretcher with both lower limbs extended. The patient then flexes the injured lower limb and defines a position. The angle formed between the thigh and leg by this flexion is defined as the reference/control angle. The patient then extends the injured lower limb and flexes it again to reproduce the same angle as the reference.

With the help of body segment analysis software, the two angles (reference and test) are calculated and the difference between the two is realised. The smaller the difference, the better the levels of proprioception.

This could be a test used to assess proprioception in future studies, but since the group of patients analysed were at different stages of recovery, with different limitations and even one of them could not flex his knee significantly, it was decided not to use this test, but to use a questionnaire related to the perception of proprioception and knee stability, especially in tasks without load and in line with the patients' stage of recovery.

3.5.1. Proprioception and knee stability questionnaire

The questionnaire was used to check the effect of the protocol on the patients' proprioception and knee stability. It was administered during each patient's third and last sessions. The first two sessions served as a test to see if the system worked and also allowed the patients to do some rehabilitation exercises to serve as a reference for answering the questionnaire. The patients answered 10 questions on a scale of 1 to 10, with 1 representing "Strongly Disagree" and 10 representing "Strongly Agree". Since the time taken to apply the protocol was considerably reduced, such a large scale was used to assess the smallest changes in improvement. The questions centred on assessing aspects such as the ability to perceive the position of the knee without looking at it, the ability to perform the rehabilitation exercises without load, confidence in the stability of the

knee when moving with crutches, confidence in flexion and extension movements and the ability to align the knee.

1.	Consigo perceber a posição do meu joelho lesionado sem olhar para ele enquanto estou sentado ou deitado.									
	1	2	3	4	5	6	7	8	9	10
2.	Consigo realizar os exercícios de reabilitação prescritos para o joelho lesionado, que não exigem carga, com precisão e sem dificuldades.									
	1	2	3	4	5	6	7	8	9	10
3.	Confio na estabilidade do meu joelho lesionado ao mover a minha perna no ar, sem apoio no chão.									
	1	2	3	4	5	6	7	8	9	10
4.	Consigo sentir e controlar bem a posição do meu joelho lesionado ao alternar entre as posições de sentado e deitado.									
	1	2	3	4	5	6	7	8	9	10
5.	Sinto-me confiante na estabilidade do meu joelho lesionado ao usar muletas.									
	1	2	3	4	5	6	7	8	9	10
6.	Consigo sentir e manter o alinhamento correto do meu joelho lesionado durante os exercícios de reabilitação.									
	1	2	3	4	5	6	7	8	9	10
7.	Consigo perceber e manter a posição correta do joelho lesionado durante exercícios isométricos.									
	1	2	3	4	5	6	7	8	9	10
8.	Confio na estabilidade do meu joelho lesionado ao flexionar a perna enquanto estou sentado ou deitado.									
	1	2	3	4	5	6	7	8	9	10
9.	Confio na estabilidade do meu joelho lesionado ao estender a perna enquanto estou sentado ou deitado.									
	1	2	3	4	5	6	7	8	9	10
10.	Sinto que consigo controlar bem o joelho lesionado ao alternar entre movimentos de flexão e extensão.									
	1	2	3	4	5	6	7	8	9	10

Figure 48: Questionnaire of proprioception.

The questionnaire was originally designed to assess two main themes: proprioception capacity and knee stability. Therefore, questions 1, 2, 4, 6 and 7 focus on proprioception and questions 3, 5, 8, 9 and 10 on knee stability.

Table 11: Summary of the Protocol for electrostimulation of the ligament zone during Quad Set exercise to increase proprioception levels.

<u>Type of electrostimulation</u>	- Combination of pre-modulated interferential therapy with functional electrostimulation.
<u>Electrostimulation parameters of the Sonopuls 992 electrostimulator</u>	- Carrier Wave Frequency: 4kHz - Stimulation Frequency (AMF): 50Hz - Frequency Modulation (Spectrum): 100Hz - Frequency Modulation Programs: 1/1s
<u>Intensity</u>	- Maximum intensity tolerable by the patient (15-25mA)
<u>Type of electrodes for EMG signal capture</u>	- Wet gel electrodes preferably as small as possible.
<u>Electrode position</u>	- Positive and negative electrodes spaced approximately 2cm apart in the direction of the muscle fibres of the rectus femoris muscle. - Reference electrode positioned on the shin.
<u>Skin preparation</u>	- Remove the skin in the area where the electrodes are to be applied. - Clean the residue with ethyl alcohol.
<u>Time</u>	- Electrostimulation lasting for the duration of the muscle contractions (approximately 5 seconds).
<u>Frequency (Series/Repetitions)</u>	- 3 sets of 8 repetitions, with 5 seconds of execution and 4 seconds of rest between repetitions and 1 minute between sets. - 3 times a week.
<u>Patient guide</u>	- Graphical interface or game that intuitively indicates when the patient should contract and relax the muscle.
<u>Motor learning techniques to be used</u>	- External Focus of Attention: Focus on squeezing the knee on the stretcher and lifting the heel slightly when contracting the quadriceps. - Implicit Learning: Completely avoid a gap between the knee and the stretcher - Differential Learning: Perform the exercise sitting, lying and/or standing. - Self-Controlled Learning: The patient can choose the position
<u>Evaluating progress</u>	- The patient flexes the injured lower limb and defines a position. - The angle formed between the thigh and leg by this flexion is defined as the reference/control angle. The patient then extends the injured lower limb and flexes it again to reproduce the same angle as the reference. - The two angles (reference and test) are calculated and the difference between the two is realised. - The smaller the difference, the better the levels of proprioception.
<u>*Muscle Contraction Threshold definition</u>	- Specific percentage (for example, 40%) of the MVC.

3.6. LIMITATIONS AND DIFFICULTIES

In the first sessions it was possible to detect some difficulties in the application of the system. The accumulation of excessive fat in the thigh area appeared to be a problem because it made it difficult to capture the EMG signal and consequently detect muscle contraction. In addition to the fat layer, the isolated contraction of the quadriceps muscles was also a problem. One of the patients had difficulty contracting these muscles in isolation, possibly also associated with loss of strength and muscle atrophy. One solution was to resort to motor learning principles, especially the external focus of attention and implicit learning. The patient was instructed to think about bringing the knee to the stretcher and raising the heel slightly, as if kicking a ball, but without ceasing to touch the stretcher with the heel. This motor re-education of the ability to contract the quadriceps allowed the muscle contraction to be visible.

Another problem was that patients had to make submaximal and maximal efforts to detect muscle contraction, which sometimes caused discomfort in the back of the knee and also below it. In order to make the application of the system comfortable and safe for certain patients, the part of detecting the EMG signal was ignored, and the patients guided the moments of contraction/rest through the interface of colored squares, and the green square that instructed to contract appeared for a short time on the screen. This allowed us to minimize discomfort and promote greater safety in the process.

3.7. RESULTS

The group of patients analysed, despite all having undergone ACL reconstruction, was heterogeneous and had different characteristics. For example, some of them were no longer using crutches after the second session, while others still needed them to get around at the end of the study, and some were already doing some rehabilitation exercises in the hospital gym at an early stage, while others were not. It was therefore decided to carry out a descriptive analysis of the answers to the questionnaire for each individual patient, checking for changes from the initial phase of the study to the final one.

Patient 1, who underwent the protocol in 6 treatment sessions, had improvements in 6 of the questions and maintained the same response in 4 others. The ones that had the greatest improvement (3 values) were one aimed at proprioception (performing the prescribed rehabilitation exercises accurately and without difficulty) and another aimed at knee stability (confidence in the stability of the knee when moving the leg in the air, without support on the ground).

There were no differences in knee stability in flexion and extension movements and in the ability to maintain the correct knee position in isometric exercises.

Overall and using the average of the answers, there was an improvement of 1 value from the initial questionnaire to the final one (from 7.4 to 8.4) and with regard to the questions aimed at proprioception and knee stability, there was a greater improvement in terms of proprioception (from 7.2 to 8.4), but there was also an improvement in terms of knee stability (from 7.6 to 8.4).

Patient 2, who underwent 4 sessions of the protocol improved in 5 questions, remained the same in 3 and worsened in 2. It should be noted that in the questions that worsened was only one value and these questions were related to the performance of the prescribed exercises with precision and without difficulty and to the maintenance of knee alignment in isometric exercises. The questions where there was greater improvement were related to the confidence of knee stability when flexing the lower limb and changing between flexion and extension. Overall, there was an improvement from 7.7 to 9.5, and there was a clear evolution with regard to questions on knee stability from 6 to 9.6 and in relation to proprioception questions the average of responses remained unchanged (9.4).

Patient 3, who underwent 5 sessions of the protocol, improved in 2 questions, remained the same in 6 and worsened in 2. Question 2 related to the performance of the prescribed rehabilitation exercises with precision and without difficulties stands out, where it went from the maximum score (10) in the initial questionnaire to 8 at the end, and question 9 related to confidence in the stability of the knee in extension movements with an improvement from 4 to 6 values. Overall, the average of responses remained unchanged (6.8). In terms of proprioception there was a slight decrease from 7.6 to 7.2 and in terms of knee stability there was a slight increase from 6 to 6.4.

Patient 4, who underwent 6 sessions of the protocol, improved in 2 questions in the questionnaire, remained the same in 4 and worsened in 4. The high number of questions in which the patient worsened is noteworthy, two of which were related to proprioception capacity and the other two to knee stability. In fact, there was one question where the patient answered with three

fewer values in the final questionnaire compared to the initial one, relating to realising and maintaining the correct position of the knee during isometric exercises. Overall, this was the only patient who got worse, with a total average of 6.8 for the initial answers and a final average of 6.3. In terms of proprioception responses, the average decreased from 6.4 to 6.6 and in knee stability from 6.6 to 6.2.

There was an interesting finding. Of the two patients who underwent more sessions of the protocol, one showed the greatest overall improvement in proprioception and knee stability, while the other showed the greatest worsening in all three areas (globally, proprioception and knee stability). This could be explained by the fact that the patient with the greatest worsening presented complaints as early as the second session because he had made an uncomfortable movement during sleep and in all the sessions it was necessary to place a cushioned roller under the knee, which meant that the knee was flexed and didn't allow there to be a considerable contraction of the quadriceps muscles, adding that this patient presented complaints when making submaximal efforts. On the other hand, the patient with the greatest improvement was at a more advanced stage of recovery and was able to follow the protocol without constraints.

With regard to the data on proprioception, one patient had similar results and two patients worsened. One of the reasons for this could be that when the initial questionnaire was administered, some of them didn't do any rehabilitation exercises in the gym and those that did were simple, non-invasive movements. Since at the time the questionnaire was provided all the patients were already doing exercises in the gym and the complexity was increased, I think it allowed them to become more aware of their bodies and their movements, including different knee positions.

Regarding knee stability, there was an improvement in three patients, which may translate into ligament regeneration and a favorable ligamentization process, as a result of the protocol applied and the sessions with therapists. Again, the only patient who presented negative results in knee stability was the one with the most recurrent complaints.

CHAPTER 4

4.1. CONCLUSIONS AND FUTURE WORK

With a view to contextualising the more practical work related to the development of a functional interferential therapy electrostimulator and its application in patients who have undergone recent anterior cruciate ligament reconstruction, this work begins with an exploration of topics such as the anatomy of the knee, mechanisms of ACL injury, phases and characteristics of rehabilitation in this type of injury, what already exists in terms of electrostimulation solutions, the focus of this work being interferential therapy, and EMG signal collection and processing.

At the end of this work, we can say that a functional prototype of functional electrostimulation of IFT has been developed, although it clearly needs to be refined, for example by improving it so that it is possible to have true synchronisation between the EMG signal and the electrostimulation, through more refined muscle contraction detection algorithms that allow the quadriceps EMG signal to be isolated by not detecting the noise conducted by the human body coming from the Sonopuls 992 electrostimulator. In this case, a prototype was developed that used the detection of muscle contraction as a trigger for electrostimulation to take place over a certain period of time.

The focus of this work, in addition to the development of a prototype of functional electrostimulation, was also the contact with patients. In this context, an interface of colored squares was developed by which the patients were guided in the moments of muscle contraction and relaxation. In terms of applicability it was positive, but the communication interface with the patient can also be refined through methods such as gamification to make the rehabilitation experience more immersive and retain patients in the process.

During the application of the protocol to the patients, motor learning techniques were applied in order to help patients with motor difficulties who, for example, could not contract the quadriceps in isolation.

Regarding the results, we believe that functional electrostimulation of interferential therapy in the knee area, performed synchronously with the quadriceps contraction and with the moment that ACL is under tension, would help provide more precise sensory feedback to the nervous system regarding the position of the knee and its movement. Therefore, we believe that regular use of FES can promote neuroplastic changes in the brain, helping it to adapt to and better interpret signals from the knee joint. This can lead to improved proprioceptive accuracy over time. This could result in greater reinnervation of the ligament and development of the mechanoreceptors still existing after the reconstruction of the ligament.

The electrostimulation method used was interferential therapy as it is believed that there could be greater penetration of the electrical current into human tissue compared to other methods and since the ligament is a deep structure in the knee, we also believe that it could assist in the regeneration of the knee ligament and result in greater knee stability.

Thus, functional electrostimulation was applied to four patients during the quad set exercise to evaluate the effects on proprioception capacity and perception of knee stability. In the end, a little bit of everything happened. Some patients improved in some aspects, others remained the same, and others worsened. It should be noted that the patients were in different stages of recovery and we cannot say that the group was homogeneous, so it is natural to obtain different results among them. In fact, in terms of applicability of FES, the patients only performed 3 sets of 8 repetitions of the quad set exercise, and in the rest of the sessions they followed treatment with the therapists, so this may also make a difference depending on the protocol followed by the therapists, which may be different between patients.

In future studies, it may be possible to improve the developed prototype, more specifically to develop an algorithm for detecting muscle contraction that allows an electrostimulation in true synchronization with muscle contraction without detecting the current coming from the electrostimulator that was detected by the electrodes that capture the EMG signals. It is undoubtedly necessary to apply an FES protocol over a more extensive period and in a larger group of patients who form a homogeneous group. In addition to the group being homogeneous, it is also necessary that they are subjected to rehabilitation experiences that are as identical as possible and perhaps carry out sessions that focus exclusively on functional electrostimulation, in order to obtain more viable results from the evolution of patients.

APPENDIX A

CODE SCRIPT

```
import tkinter as tk
from threading import Thread
import time
import numpy as np
from bitalino import BITalino
import scipy as sp
from scipy import signal

# Update Square
def update_square(color, countdown_from):
    square.itemconfig(rectangle, fill=color)
    label.config(bg=color)
    countdown(countdown_from)

# Countdown
def countdown(n):
    if n >= 0:
        if n == 0 and square.itemcget(rectangle, "fill") == "green":
            label.config(text="Contraia")
        else:
            label.config(text=str(n))
            label.after(1000, countdown, n-1)

def bitalino_setup(macAddress):

    batteryThreshold = 30

    device = BITalino(macAddress)

    device.battery(batteryThreshold)
    print(device.version())
    return device

# Data acquisition by bitalino
def bitalino_control(device):

    acqChannels = [0] # EMG
    samplingRate = 1000
    nSamples = 300
    digitalOutput = [1, 0]

    # RMS threshold
    rms_threshold = 50
    device.start(samplingRate, acqChannels)

    # Gray square
    update_square("gray", countdown_from=5)
```

```

# Buzzer activation for 5 seconds
device.trigger([1, 1])
time.sleep(5)

# Red Square
update_square("red", countdown_from=3)

# Buzzer Deactivation for 3 seconds
device.trigger([1, 0])
time.sleep(3)

# Green Square
update_square("green", countdown_from=0)

while True:
    emg = device.read(nSamples)[: , 5]

    high = 20/(samplingRate/2)
    low = 450/(samplingRate/2)
    b, a = sp.signal.butter(4, [high,low], btype='bandpass')

    emg_centered = emg - 512
    emg_filtered = sp.signal.filtfilt(b, a, emg_centered)

    rms = np.sqrt(np.mean(emg_filtered ** 2))
    print(rms)

    if rms > rms_threshold:
        # Activate Buzzer
        device.trigger([1, 1])
        # Show Yellow Square for 5 seconds
        update_square("yellow", countdown_from=5)
        time.sleep(5)
        # Deactivate Buzzer
        device.trigger([1, 0])
        # Show Red square for 4 seconds
        update_square("red", countdown_from=4)
        time.sleep(4)
        # Show green square
        update_square("green", countdown_from=0)

macAddress = "20:16:12:22:50:97"

# Starting graphic interface
root = tk.Tk()
root.title("Interface Gráfica")

frame = tk.Frame(root, width=600, height=600)
frame.pack()

square = tk.Canvas(frame, width=600, height=600, bg="white")
square.pack(expand=True)
rectangle = square.create_rectangle(100, 100, 500, 500, fill="red")

```

```
label = tk.Label(square, text="", font=("Arial", 70), bg="green",  
fg="black")  
label.place(relx=0.5, rely=0.5, anchor="center")  
  
device = bitalino_setup(macAddress)  
  
bitalino_thread = Thread(target=bitalino_control, args=(device,))  
bitalino_thread.daemon = True  
bitalino_thread.start()  
  
root.mainloop()
```

APPENDIX B

QUESTIONNAIRE ANSWERS

PATIENT 1 - BEFORE

1. Consigo perceber a posição do meu joelho lesionado sem olhar para ele enquanto estou sentado ou deitado.

1 2 3 4 5 6 7 8 9 10

2. Consigo realizar os exercícios de reabilitação prescritos para o joelho lesionado, que não exigem carga, com precisão e sem dificuldades.

1 2 3 4 5 6 7 8 9 10

3. Confio na estabilidade do meu joelho lesionado ao mover a minha perna no ar, sem apoio no chão.

1 2 3 4 5 6 7 8 9 10

4. Consigo sentir e controlar bem a posição do meu joelho lesionado ao alternar entre as posições de sentado e deitado.

1 2 3 4 5 6 7 8 9 10

5. Sinto-me confiante na estabilidade do meu joelho lesionado ao usar muletas.

1 2 3 4 5 6 7 8 9 10 *m use muletas*

6. Consigo sentir e manter o alinhamento correto do meu joelho lesionado durante os exercícios de reabilitação.

1 2 3 4 5 6 7 8 9 10

7. Consigo perceber e manter a posição correta do joelho lesionado durante exercícios isométricos.

1 2 3 4 5 6 7 8 9 10

8. Confio na estabilidade do meu joelho lesionado ao flexionar a perna enquanto estou sentado ou deitado.

1 2 3 4 5 6 7 8 9 10

9. Confio na estabilidade do meu joelho lesionado ao estender a perna enquanto estou sentado ou deitado.

1 2 3 4 5 6 7 8 9 10

10. Sinto que consigo controlar bem o joelho lesionado ao alternar entre movimentos de flexão e extensão.

1 2 3 4 5 6 7 8 9 10

PATIENT 1 - AFTER

1. Consigo perceber a posição do meu joelho lesionado sem olhar para ele enquanto estou sentado ou deitado.

1 2 3 4 5 6 7 8 9 10

2. Consigo realizar os exercícios de reabilitação prescritos para o joelho lesionado, que não exigem carga, com precisão e sem dificuldades.

1 2 3 4 5 6 7 8 9 10

3. Confio na estabilidade do meu joelho lesionado ao mover a minha perna no ar, sem apoio no chão.

1 2 3 4 5 6 7 8 9 10

4. Consigo sentir e controlar bem a posição do meu joelho lesionado ao alternar entre as posições de sentado e deitado.

1 2 3 4 5 6 7 8 9 10

5. Sinto-me confiante na estabilidade do meu joelho lesionado ao usar muletas.

1 2 3 4 5 6 7 8 9 10

6. Consigo sentir e manter o alinhamento correto do meu joelho lesionado durante os exercícios de reabilitação.

1 2 3 4 5 6 7 8 9 10

7. Consigo perceber e manter a posição correta do joelho lesionado durante exercícios isométricos.

1 2 3 4 5 6 7 8 9 10

8. Confio na estabilidade do meu joelho lesionado ao flexionar a perna enquanto estou sentado ou deitado.

1 2 3 4 5 6 7 8 9 10

9. Confio na estabilidade do meu joelho lesionado ao estender a perna enquanto estou sentado ou deitado.

1 2 3 4 5 6 7 8 9 10

10. Sinto que consigo controlar bem o joelho lesionado ao alternar entre movimentos de flexão e extensão.

1 2 3 4 5 6 7 8 9 10

OTROR
FEUP ENGENHARIA

U. PORTO
FEUP ENGENHARIA

PATIENT 2 - BEFORE

1. Consigo perceber a posição do meu joelho lesionado sem olhar para ele enquanto estou sentado ou deitado.

1 2 3 4 5 6 7 8 9 10

2. Consigo realizar os exercícios de reabilitação prescritos para o joelho lesionado, que não exigem carga, com precisão e sem dificuldades.

1 2 3 4 5 6 7 8 9 10

3. Confio na estabilidade do meu joelho lesionado ao mover a minha perna no ar, sem apoio no chão.

1 2 3 4 5 6 7 8 9 10

4. Consigo sentir e controlar bem a posição do meu joelho lesionado ao alternar entre as posições de sentado e deitado.

1 2 3 4 5 6 7 8 9 10

5. Sinto-me confiante na estabilidade do meu joelho lesionado ao usar muletas.

1 2 3 4 5 6 7 8 9 10

6. Consigo sentir e manter o alinhamento correto do meu joelho lesionado durante os exercícios de reabilitação.

1 2 3 4 5 6 7 8 9 10

7. Consigo perceber e manter a posição correta do joelho lesionado durante exercícios isométricos.

1 2 3 4 5 6 7 8 9 10

8. Confio na estabilidade do meu joelho lesionado ao flexionar a perna enquanto estou sentado ou deitado.

1 2 3 4 5 6 7 8 9 10

U. PORTO
FEUP ENGENHARIA

U. PORTO
FEUP ENGENHARIA

9. Confio na estabilidade do meu joelho lesionado ao estender a perna enquanto estou sentado ou deitado.

1 2 3 4 5 6 7 8 9 10

10. Sinto que consigo controlar bem o joelho lesionado ao alternar entre movimentos de flexão e extensão.

1 2 3 4 5 6 7 8 9 10

PATIENT 2 - AFTER

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1. Consigo perceber a posição do meu joelho lesionado sem olhar para ele enquanto estou sentado ou deitado.

1 2 3 4 5 6 7 8 9 10

2. Consigo realizar os exercícios de reabilitação prescritos para o joelho lesionado, que não exigem carga, com precisão e sem dificuldades.

1 2 3 4 5 6 7 8 9 10

3. Confio na estabilidade do meu joelho lesionado ao mover a minha perna no ar, sem apoio no chão.

1 2 3 4 5 6 7 8 9 10

4. Consigo sentir e controlar bem a posição do meu joelho lesionado ao alternar entre as posições de sentado e deitado.

1 2 3 4 5 6 7 8 9 10

5. Sinto-me confiante na estabilidade do meu joelho lesionado ao usar muletas.

1 2 3 4 5 6 7 8 9 10

6. Consigo sentir e manter o alinhamento correto do meu joelho lesionado durante os exercícios de reabilitação.

1 2 3 4 5 6 7 8 9 10

7. Consigo perceber e manter a posição correta do joelho lesionado durante exercícios isométricos.

1 2 3 4 5 6 7 8 9 10

8. Confio na estabilidade do meu joelho lesionado ao flexionar a perna enquanto estou sentado ou deitado.

1 2 3 4 5 6 7 8 9 10

9. Confio na estabilidade do meu joelho lesionado ao estender a perna enquanto estou sentado ou deitado.

1 2 3 4 5 6 7 8 9 10

10. Sinto que consigo controlar bem o joelho lesionado ao alternar entre movimentos de flexão e extensão.

1 2 3 4 5 6 7 8 9 10

PORTO
FELUP ENGENHARIA

PATIENT 3 -BEFORE

1. Consigo perceber a posição do meu joelho lesionado sem olhar para ele enquanto estou sentado ou deitado.

1 2 3 4 5 6 7 8 9 10

2. Consigo realizar os exercícios de reabilitação prescritos para o joelho lesionado, que não exigem carga, com precisão e sem dificuldades.

1 2 3 4 5 6 7 8 9 10

3. Confio na estabilidade do meu joelho lesionado ao mover a minha perna no ar, sem apoio no chão.

1 2 3 4 5 6 7 8 9 10

4. Consigo sentir e controlar bem a posição do meu joelho lesionado ao alternar entre as posições de sentado e deitado.

1 2 3 4 5 6 7 8 9 10

5. Sinto-me confiante na estabilidade do meu joelho lesionado ao usar muletas.

1 2 3 4 5 6 7 8 9 10

6. Consigo sentir e manter o alinhamento correto do meu joelho lesionado durante os exercícios de reabilitação.

1 2 3 4 5 6 7 8 9 10

7. Consigo perceber e manter a posição correta do joelho lesionado durante exercícios isométricos.

1 2 3 4 5 6 7 8 9 10

8. Confio na estabilidade do meu joelho lesionado ao flexionar a perna enquanto estou sentado ou deitado.

1 2 3 4 5 6 7 8 9 10

9. Confio na estabilidade do meu joelho lesionado ao estender a perna enquanto estou sentado ou deitado.

1 2 3 4 5 6 7 8 9 10

10. Sinto que consigo controlar bem o joelho lesionado ao alternar entre movimentos de flexão e extensão.

1 2 3 4 5 6 7 8 9 10

PATIENT 3 - AFTER

1. Consigo perceber a posição do meu joelho lesionado sem olhar para ele enquanto estou sentado ou deitado.

1 2 3 4 5 6 7 8 9 10

2. Consigo realizar os exercícios de reabilitação prescritos para o joelho lesionado, que não exigem carga, com precisão e sem dificuldades.

1 2 3 4 5 6 7 8 9 10

3. Confio na estabilidade do meu joelho lesionado ao mover a minha perna no ar, sem apoio no chão.

1 2 3 4 5 6 7 8 9 10

4. Consigo sentir e controlar bem a posição do meu joelho lesionado ao alternar entre as posições de sentado e deitado.

1 2 3 4 5 6 7 8 9 10

5. Sinto-me confiante na estabilidade do meu joelho lesionado ao usar muletas.

1 2 3 4 5 6 7 8 9 10

6. Consigo sentir e manter o alinhamento correto do meu joelho lesionado durante os exercícios de reabilitação.

1 2 3 4 5 6 7 8 9 10

7. Consigo perceber e manter a posição correta do joelho lesionado durante exercícios isométricos.

1 2 3 4 5 6 7 8 9 10

8. Confio na estabilidade do meu joelho lesionado ao flexionar a perna enquanto estou sentado ou deitado.

1 2 3 4 5 6 7 8 9 10

9. Confio na estabilidade do meu joelho lesionado ao estender a perna enquanto estou sentado ou deitado.

1 2 3 4 5 6 7 8 9 10

10. Sinto que consigo controlar bem o joelho lesionado ao alternar entre movimentos de flexão e extensão.

1 2 3 4 5 6 7 8 9 10

U. PORTO
FEUP ENGENHARIA

PATIENT 4 - BEFORE

10. Sinto que consigo controlar bem o joelho lesionado ao alternar entre movimentos de

1. Consigo perceber a posição do meu joelho lesionado sem olhar para ele enquanto estou sentado ou deitado.

1 2 3 4 5 6 7 8 9 10

2. Consigo realizar os exercícios de reabilitação prescritos para o joelho lesionado, que não exigem carga, com precisão e sem dificuldades.

1 2 3 4 5 6 7 8 9 10

3. Confio na estabilidade do meu joelho lesionado ao mover a minha perna no ar, sem apoio no chão.

1 2 3 4 5 6 7 8 9 10

4. Consigo sentir e controlar bem a posição do meu joelho lesionado ao alternar entre as posições de sentado e deitado.

1 2 3 4 5 6 7 8 9 10

5. Sinto-me confiante na estabilidade do meu joelho lesionado ao usar muletas.

1 2 3 4 5 6 7 8 9 10

6. Consigo sentir e manter o alinhamento correto do meu joelho lesionado durante os exercícios de reabilitação.

1 2 3 4 5 6 7 8 9 10

7. Consigo perceber e manter a posição correta do joelho lesionado durante exercícios isométricos.

1 2 3 4 5 6 7 8 9 10

8. Confio na estabilidade do meu joelho lesionado ao flexionar a perna enquanto estou sentado ou deitado.

1 2 3 4 5 6 7 8 9 10

9. Confio na estabilidade do meu joelho lesionado ao estender a perna enquanto estou sentado ou deitado.

1 2 3 4 5 6 7 8 9 10

10. Sinto que consigo controlar bem o joelho lesionado ao alternar entre movimentos de flexão e extensão.

1 2 3 4 5 6 7 8 9 10

U-PORTO
FEUP ENGENHARIA

U-PORTO
FEUP ENGENHARIA

PATIENT 4 - AFTER

1. Consigo perceber a posição do meu joelho lesionado sem olhar para ele enquanto estou sentado ou deitado.

1 2 3 4 5 6 7 8 9 10

2. Consigo realizar os exercícios de reabilitação prescritos para o joelho lesionado, que não exigem carga, com precisão e sem dificuldades.

1 2 3 4 5 6 7 8 9 10

3. Confio na estabilidade do meu joelho lesionado ao mover a minha perna no ar, sem apoio no chão.

1 2 3 4 5 6 7 8 9 10

4. Consigo sentir e controlar bem a posição do meu joelho lesionado ao alternar entre as posições de sentado e deitado.

1 2 3 4 5 6 7 8 9 10

5. Sinto-me confiante na estabilidade do meu joelho lesionado ao usar muletas.

1 2 3 4 5 6 7 8 9 10

6. Consigo sentir e manter o alinhamento correto do meu joelho lesionado durante os exercícios de reabilitação.

1 2 3 4 5 6 7 8 9 10

7. Consigo perceber e manter a posição correta do joelho lesionado durante exercícios isométricos.

1 2 3 4 5 6 7 8 9 10

8. Confio na estabilidade do meu joelho lesionado ao flexionar a perna enquanto estou sentado ou deitado.

1 2 3 4 5 6 7 8 9 10

9. Confio na estabilidade do meu joelho lesionado ao estender a perna enquanto estou sentado ou deitado.

1 2 3 4 5 6 7 8 9 10

10. Sinto que consigo controlar bem o joelho lesionado ao alternar entre movimentos de flexão e extensão.

1 2 3 4 5 6 7 8 9 10

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