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Technological development and clinical validation of a medical device based on electromagnetic fields in inflammatory diseases.

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1 INTRODUCTION

This project has the ambition to realize a new medical device delivering pulsed electromagnetic fields (PEMF) for the treatment of inflammatory joint diseases and to drafting documentation for its validation in the clinical setting. The implementation of such a device would involve a radical breakthrough in the scientific knowledge shared by the medical community to date. The new medical device will target a wide range of people: professional and amateur athletes. The new medical device will be marketed in the "*Sports Medicine*" market for the treatment of minor injuries, for chondro-regeneration, but also as a usual conservative treatment for "*Joint Preservation.*"

Joint Preservation refers to a set of strategies and interventions aimed at maintaining joint health and function in order to delay or avoid the need for invasive surgery, such as joint replacement. Joint preservation has become a topic of great interest in recent years as more and more people seek to maintain the functionality of their joints for as long as possible. This is especially important for active individuals, athletes, and those who wish to avoid or delay joint surgery. The main goal of Joint Preservation is to preserve and restore articular cartilage, improving joint function and reducing pain. This approach is especially relevant for younger, active patients who wish to maintain a healthy, functioning joint over the long term.

The approach to joint preservation is based on several fundamental principles:

1. Early diagnosis: early detection of joint problems is essential to initiate timely and appropriate treatment. The use of advanced diagnostic tools such as high-resolution imaging, such as MRI, can help identify joint injuries or abnormalities accurately.
2. Conservative therapy: conservative therapy is often the first step in the treatment of joint injuries. This may include physical therapy with muscle-strengthening exercises, stretching, joint mobilization, and other nonsurgical treatment modalities to improve

joint stability and function; modifications of physical activity to avoid movements or loads that aggravate the injury; and the use of supportive devices such as braces or orthoses.

3. Intra-articular injections: intra-articular injections can be used to relieve pain and reduce inflammation in the joint. Common injections used in clinical settings include corticosteroids, hyaluronic acid, adipose tissue and PRP (Platelet-Rich Plasma), with the intent to promote healing and tissue regeneration.
4. Cartilage repair or regeneration: articular cartilage has a limited capacity for self-healing. However, various surgical techniques have been developed to stimulate healing and regeneration of damaged cartilage, such as microfracture, autograft, chondrocytes implantation, mesenchymal stem cells, and the use of biocompatible scaffolds.
5. Joint alignment and stabilization: in some cases, correcting joint alignment or instability problems can help reduce stress and strain on joints. This can be achieved through surgical interventions such as osteotomy (bone axis modification) or ligament reconstruction.
6. Lifestyle changes: adopting a healthy and active lifestyle can help maintain joint health. This includes a balanced diet, avoiding overweight or obesity, adequate hydration, avoiding risky behaviors, and adopting good exercise technique.
7. Drug therapy: some drugs may be prescribed to reduce inflammation and control pain associated with joint disorders.

It is important to note that the approach to Joint Preservation may vary depending on the specific condition, age of the patient, individual needs, and available resources. Early diagnosis and treatment is important to maximize the results of joint preservation. Therefore, it is always advisable to consult a sports medicine specialist or orthopedic surgeon

experienced in joint preservation for a comprehensive evaluation and individualized treatment planning (Magee DJ. 2014).

2 SPORTS MEDICINE

Sports medicine is a branch of medicine that focuses on the prevention, diagnosis, and treatment of injuries and illnesses related to physical activity and exercise, as well as health promotion and performance enhancement of athletes and active individuals. It deals with athletes of all levels, from amateurs to professional athletes, and applies to a wide range of sports disciplines (Brukner P. 2017).

Sports medicine was recognized as an official medical specialty by the World Health Organization (WHO) in 1996. Its importance has grown in recent years as more and more people engage in physical activity and sports. Professional athletes, amateurs, young people participating in school sports, and workers in physically demanding jobs are all at risk for sports injuries and activity-related diseases.

Sports medicine has an important role in helping these individuals prevent injuries, recover from injuries, and improve their sports performance as well described in the reference manual for health and fitness professionals, that provides detailed guidelines for exercise prescription and evaluation. ACSM's Guidelines for Exercise Testing and Prescription along with the American Medical Society for Sports Medicine, AMSSM is a medical organization representing sports physicians that provides resources, guidelines, and training for the diagnosis, treatment, and prevention of sports injuries.

Sports injury prevention plays a key role in sports medicine and consists of a number of strategies designed to reduce the risk of injury during physical activity and sport (Bahr R. 2016). These strategies may include warm-up exercises, appropriate training, use of protective equipment, adequate recovery, and assessment of fitness for physical activity (Bahr R. 2005). Adequate training is a key factor in the prevention of sports injuries. Athletes should follow a well-designed training program tailored to their abilities to avoid excessive or repetitive overloads on certain body parts (Bahr R. 2015, Emery CA. 2015). An

experienced coach or physical therapist can help athletes develop a personalized training program to reduce the risk of injury. The use of protective equipment can also help prevent injuries in sports. This can include the use of helmets, appropriate footwear, knee and elbow pads, back suspenders, and safety equipment for contact sports (Hrysomallis, C. 2007; Emery CA 2015). Protective equipment should be selected according to the sport played and the athlete's position. Proper recovery is also important in preventing sports injuries (Frizziero A. 2014). Athletes should take time to rest and recover between training or sporting events in order to avoid overuse fatigue or overuse-related injuries (Gabbett TJ. 2016). Athletes should also eat a balanced diet and hydrate adequately to keep the body healthy and ready for physical activity. Fitness assessment for physical activity is another critical aspect of sports injury prevention (Finch CF. 2016). Athletes should undergo a comprehensive physical examination before beginning athletic activity to identify any health problems that could increase the risk of injury. The evaluation may include blood tests, heart tests, lung function tests, and strength and flexibility assessments (O'Connor KL. 2017).

In summary, sports injury prevention is an integrated approach that requires a combination of strategies, including appropriate training, use of protective equipment, adequate recovery, and assessment of fitness for physical activity (Kirkendall DT. 2010). With proper attention to sports injury prevention, athletes can enjoy physical activity in a safe and healthy way.

Diagnosis of injuries in sports is a critical aspect of sports medicine, as accurate diagnosis can determine the appropriate treatment and accelerate the athlete's recovery (Mahmut Nedim Doral&Jon Karlsson 2015). The diagnosis of injuries in sports is based on the patient's medical history, physical examination, diagnostic imaging, and other diagnostic tests (William HM. Castro 2001). The medical history is the first step in the diagnosis of sports injuries and includes an assessment of symptoms and their development, as well as the nature of the sports activity and events that may have led to the injury. The physician

may also review the patient's medical history, including any previous injuries and preexisting health conditions (Magee DJ. 2014). The physical examination is the second step in diagnosing injuries in sports and focuses on examining the area affected by the injury. This may include assessment of the appearance of the affected area, muscle strength, flexibility, balance, and joint mobility. Diagnostic imaging is often used to confirm the diagnosis and to determine the extent of the injury (Mahmut Nedim Doral&Jon Karlsson 2015). This may include radiographs, magnetic resonance imaging (MRI), computed tomography (CT), or ultrasound. These instrumental assessments can provide information about the severity of the injury, the presence of fractures, lacerations, soft tissue injuries, sprains or ligament injuries (Thacker SB. 2004). Other diagnostic tests may include blood tests, urine tests, or pulmonary function tests (O'Connor KL. 2017).

In general, the diagnosis of sports injuries requires a comprehensive evaluation of the patient, including medical history, physical examination, and other diagnostic tests. Once the diagnosis is established, treatment can be tailored to meet the patient's needs and speed recovery.

The treatment of activity- and exercise-related injuries and illnesses in sports medicine can vary depending on the severity of the injury or illness (William Prentice & Daniel Arnheim 2013). The treatment of sports injuries is a complex process that requires the collaboration of various health and sports professionals, including physicians, physical therapists, coaches, and athletes themselves (Ellenbecker, T. 2009). The treatment of sports injuries focuses on managing pain, function, and the athlete's ability to return to athletic activity (Frontera WR. 2018). The treatment of sports injuries can include several phases, including:

1. Injury assessment: a physician or physical therapist evaluates the injury and determines its severity and cause.

2. Pain and inflammation control: the physician or physical therapist prescribes medication for pain and inflammation, if necessary, and may apply manual therapy techniques, such as massage or joint mobilization, to relieve pain.
3. Restoration of joint function: the physical therapist can use therapeutic exercises and manual therapy techniques to restore joint mobility and movement.
4. Restoring muscle strength: the physical therapist or trainer uses therapeutic exercises to strengthen the muscles that support the injured joint.
5. Sports rehabilitation: the physical therapist or coach works with the athlete to restore the ability to perform sport-specific exercises and movements.
6. Prevention of future injuries: the physical therapist or coach works with the athlete to develop an injury prevention program to prevent recurrence and future injuries.

In general, treatment may include conservative therapies or surgery (Magee DJ. 2014). Conservative therapies are the first approach in treating injuries and illnesses related to physical activity and exercise. These can include pain management, physical therapy, medication use, and rehabilitation (Frontera WR. 2018). Pain management may include the use of pain medications, such as acetaminophen or nonsteroidal anti-inflammatory drugs (NSAIDs), or the application of ice or heat. Physical therapy may include stretching and muscle strengthening exercises, joint mobilization, manual therapy, and therapy with devices that deliver physical stimuli. Rehabilitation may include working with a physical or occupational therapist to restore strength, flexibility and function to the area affected by the injury.

In some cases, surgeries may be necessary to treat injuries and illnesses related to physical activity and exercise. These may include repair of muscle or tendon injuries, ligament reconstruction, repair of fractures, or removal of damaged tissue. After surgery, it is often necessary to follow a rehabilitation program to regain strength and function. The treatment

of sports injuries can vary depending on the type and severity of the injury and the specific needs of the athlete. However, there are some general guidelines that can help ensure proper and safe treatment. For example, it is important that the athlete receives a proper medical evaluation, that treatment is guided by a qualified health care professional, and that the athlete follows a structured and monitored rehabilitation program to avoid recurrence. In summary, the treatment of sports injuries is a complex process that requires the collaboration of various health and sports professionals to ensure proper management of pain and restoration of function and the athlete's ability to return to sports activity.

Thus, sports medicine is a multidisciplinary medical specialty involving several disciplines, including orthopedics, trauma, internal medicine, cardiology, sports physiology, and rehabilitation (Brukner P 2017). As a multidisciplinary medical specialty, sports medicine involves a wide range of health care professionals, including physicians, physical therapists, biomechanists, nutritionists, sports psychologists, and other professionals concerned with the health and well-being of athletes. These professionals work together to provide integrated management of sports conditions, from musculoskeletal problems to nutritional disorders, psychological problems and more. Sports physicians are trained to assess and manage sports injuries, using advanced diagnostic techniques such as MRI, radiography, and ultrasound, as well as conservative therapies or surgery if necessary. Sports physicians may work in various settings, such as professional sports teams, universities, rehabilitation centers, and private clinics (Hardt F. 2020). In addition, they can be involved in managing medical emergencies during sporting events, ensuring the safety of athletes. To become a sports physician, candidates must complete basic medical training and then specialize in sports medicine. Training includes learning the basics of exercise physiology, biomechanics, sports nutrition, rehabilitation and sports injury therapy (Garrett WE& Kirkendall DT. 2000). In addition, sports physicians must be able to assess and diagnose sports injuries and illnesses, as well

as manage and monitor patients during rehabilitation and recovery. Sports physicians work in a variety of settings, including professional sports teams, sports clubs, specialized sports medicine clinics, hospitals and other health care settings. They play a key role in preventing sports injuries by advising on warm-up and cool-down techniques, training in proper technique, assessing physical condition, and prescribing targeted exercises (Domhnall MacAuley&Thomas Best. 2008).

Sports medicine also plays an important role in the rehabilitation of sports injuries. Rehabilitation is a step-by-step process, and the sports physician works with the patient to establish an individualized rehabilitation plan that includes specific exercises, physical therapy, and proper nutrition. During the rehabilitation process, the sports physician monitors the patient's condition and makes necessary changes to the rehabilitation plan to ensure full recovery.

Sports medicine is a constantly evolving field, and sports physicians must continually train and update themselves to provide the best treatment for their patients. Some information about the global sports medicine market. The sports medicine market is growing steadily worldwide. According to a report by ResearchAndMarkets.com and a research report by Grand View Research, the global sports medicine market is expected to reach $9.14 billion by 2026, with a compound annual growth rate of 6.4 percent from 2021 to 2026 (Sports Medicine Market Size). This is attributable to the rise in the popularity of sports, the increase in the number of sports-related injuries, and the increased focus on health and wellness in general.

The report also indicates that North America is the largest market for sports medicine, followed by Europe. This is due to the strong presence of sports infrastructure and professional teams in these regions, as well as high healthcare spending. The growth of the market is stimulated by several factors, including increased awareness about the benefits of

physical activity and exercise, the growing popularity of sports among young people, and the increasing focus on injury prevention programs (Australian Institute of Health and Welfare 2022; Harner CD. 2003). In addition, the increasing media attention on the performance of top athletes has increased the demand for advanced treatments for sports injuries. There are several specializations in the sports medicine market, including trauma, exercise physiology, sports rehabilitation, sports medicine, and sports nutrition (Kolt G&Snyder-Mackler L. 2007). There are also many companies that offer equipment and products to support and rehabilitate athletes.

In general, the sports medicine market is dominated by a few large companies, such as Arthrex, Inc., Smith & Nephew plc, Stryker Corporation, and Zimmer Biomet Holdings, Inc. However, there are also numerous small and medium-sized companies that specialize in specific areas of sports medicine.

In summary, the global sports medicine market is growing significantly and offers numerous opportunities for investors, entrepreneurs, and industry professionals.

The continued evolution of technology and research into the health and well-being of athletes will contribute to the growth and innovation of the market. Sports medicine has a significant impact on the global economy as it contributes to the health and well-being of professional and amateur athletes, as well as to the reduction of costs associated with sports injuries. According to a study published in the British Journal of Sports Medicine, sports injuries account for 10-20 percent of all acute injuries, costing the global economy about $20 billion annually (Collins J. 2021; Mountjoy, M. 2011). The authors pointed out that sports injuries have significant significance in terms of cost and health impact for both professional and amateur athletes. In fact, sports injuries can lead to long-term health consequences, such as the onset of chronic conditions and reduced quality of life. The BJSM also emphasized the importance of sports injury prevention through specific training programs, proper physical

preparation, the use of protective and appropriate equipment, compliance with the rules of the game, and the promotion of healthy lifestyles. In addition, the authors highlighted the importance of prompt and effective intervention in the treatment of sports injuries in order to minimize the negative consequences on the athlete's health. According to the study, the sports at greatest risk of injury are soccer, basketball, ice hockey and rugby. In addition, the most common injuries are muscle and ligament injuries, followed by bone fractures. The cost of sports injuries involves not only the care and rehabilitation of the individual athlete, but also the social cost from lost work productivity and expenses for the health care system. In addition, sports injuries can affect people's lifestyles, limiting their ability to perform daily activities and sports. The management of sports injuries requires a multidisciplinary approach involving physicians, physical therapists, nutritionists, and psychologists. The study is an important source of information for the scientific community and sports professionals, highlighting the importance of sports medicine as a multidisciplinary medical specialty and the investment in sports injury prevention for the well-being of athletes and society as a whole.

The growing interest in the health and well-being of athletes, along with the increasing focus on injury prevention programs and new technologies in the field of sports medicine, have also led to the creation of several specialized organizations and associations worldwide. For example, the Federation International de Medecine du Sport (FIMS) is an international organization that promotes the practice of sports medicine worldwide and provides a platform for sharing knowledge and expertise among sports medicine professionals. The American Medical Society for Sports Medicine (AMSSM) is another specialized organization in the United States dedicated to the promotion of sports medicine and exercise science.

In addition, there are numerous websites and peer-reviewed journals, such as the British Journal of Sports Medicine, the Journal of Science and Medicine in Sport, and the Journal of Athletic Training, that offer advanced information and research on sports medicine and athlete health.

In summary, sports medicine is an evolving multidisciplinary specialty that offers numerous opportunities for growth and innovation. The increasing focus on the health and well-being of athletes, along with continued research and development of new technologies, will help fuel the sports medicine market in the future.

2.1 Joint injuries in athletes

Joint injuries can be classified according to their severity, location, and cause (Junge A. 2105; Finch CF. 2015). Acute joint injuries, such as sprains and tears, can occur suddenly during sports practice, while chronic joint injuries can develop over time due to excessive mechanical stress or repeated use of the joint. Joint injuries are common among professional and amateur athletes and are a major cause of sports discontinuation. The risk of joint injury is influenced by multiple factors, including the type of sport played, duration of sport activity, age of the athlete, gender, fitness level, type of footwear used, type of playing surface, and training technique. Joint injuries can affect several anatomical structures, including cartilage, meniscus, ligaments, tendons, and bones (Engebretsen L. 2012).

There are several types of knee joint injuries, the most important of which are:

1. Articular cartilage injuries: occur when only the surface of the articular cartilage is damaged and can be caused by direct trauma or chronic overuse of the joint. These types of injuries are common in athletes who participate in sports that involve high pressure on the articular surface of the knee, such as soccer, basketball, skiing, and tennis. Symptoms include pain, swelling, and limitation of joint motion. In some cases, the patient may also experience a noise, rattling, or locking sensation in the joint.
2. Anterior cruciate ligament (ACL) injuries are common among athletes and account for about 40 percent of all knee injuries. Sports involving fast movements, changes of direction and jumps, such as soccer, basketball, American football and downhill skiing, have a higher risk of ACL injuries.
3. Meniscus injuries can occur due to sudden movement or direct trauma. These injuries are common in athletes who participate in sports that involve twisting the knee, such as soccer, basketball, field hockey, and skiing. Symptoms include pain, swelling, and unsteady feeling in the joint.

4. Patellofemoral joint injuries can be caused by misalignment of the patella or overuse. These injuries are common in athletes who participate in sports involving excessive pressure on the patellofemoral joint, such as soccer, running, and cycling. Symptoms include pain, swelling, and unsteady feeling in the joint.
5. Osteochondral injuries involve not only cartilage but also the underlying subchondral bone. These injuries can be caused by direct trauma or congenital malformation. Osteochondral injuries are common in athletes who participate in sports that involve high pressure on the articular surface of the knee, such as soccer, basketball, and tennis. Symptoms include pain, swelling, and unsteady feeling in the joint.

In all these injuries, treatment depends on the severity of the injury. For minor injuries, the patient may undergo physical therapy, such as physiotherapy and the use of anti-inflammatory drugs. If the injury is more severe, the patient may need to undergo arthroscopy to repair the injury or, in extreme cases, joint replacement, i.e., prosthesis. Rates of joint injury in professional and amateur athletes can vary widely depending on the sport played and the demographic characteristics of the athletes involved. However, in general, studies have shown that professional and amateur athletes have a significantly higher risk of joint injury than the general population. A study by Darrow et al. in 2014 found that professional athletes had a higher risk of joint injuries than amateur athletes, with joint injury rates on the order of 20-30% (Darrow CJ. 2014). In particular, sports such as soccer, basketball, and American football have particularly high joint injury rates. Another study by Shrier et al. reported that professional athletes had a 10-20% risk of suffering joint injuries each year, with the highest joint injury rates in sports such as rugby and soccer (Shrier I. 2006). A more recent study by van der Worp et al. in 2015 examined joint injury rates in amateur athletes participating in marathons and ultramarathons (van der Worp MP. 2015). The results showed that the risk of joint injury was 30 percent for marathon participants and

42 percent for ultramarathon participants. A study by Poulos et al. examined joint injuries in Australian professional soccer players and reported that the joint injury rate was 33.5 percent (Poulos RG. 2021). This study also showed that older players had a higher risk of suffering joint injuries than younger players. Another study examined joint injuries among athletes participating in the Olympic Games (Engebretsen L. 2012). Their study found that 23 percent of the athletes had sustained a joint injury, with 14.2 percent of these athletes suffering a knee injury. A systematic review by Driban et al. examined joint injury rates in athletes from 29 different sports around the world (Driban JB. 2015). This study found that joint injury rates varied widely by sport played, with the highest joint injury rates in the following sports: soccer (45.2%), wrestling (36.6%), gymnastics (32.8%), ice hockey (32.3%), basketball (31.3%), volleyball (27.9%), and American football (26.5%). Young et al. examined the experiences of professional female tennis players returning to competition from injury and found that the majority of severe injuries were upper limb/shoulder and these were generally treated at tournament sites with some requiring surgery (Young JA 2007).

In general, studies have shown that joint injuries are common in professional and amateur athletes worldwide. However, the nature and extent of joint injuries can vary greatly depending on the sport played and the individual characteristics of the athlete. Professional and amateur athletes should take preventive measures to reduce the risk of joint injuries, including proper warm-up, balanced training, proper use of footwear, adequate nutrition, and effective stress management.

2.2 Cartilage injuries in athletes

Among joint injuries in athletes, an important role is played by cartilage injuries. Cartilage is a connective tissue and has the function of reducing friction and wear during joint movement (Buckwalter JA. 2004). Articular cartilage is a highly specialized tissue that lacks vascularization, which means it has a limited capacity for self-healing and injuries can be difficult to treat. Cartilage injuries can be caused by direct trauma, such as a fall, collision, or sports injury, or by repetitive stresses on the cartilage, as in the case of athletes who engage in activities with jumping and repeated ground impacts, or by a genetic predisposition (Brittberg M. 2003). Professional athletes are particularly at risk for cartilage injuries, as their intensive training and participation in competitions can put enormous pressure on the joints. These types of injuries can include cartilage lesions, tears, or abrasion. Degenerative injuries are caused by a gradual deterioration of articular cartilage, often associated with aging, overuse of the joint, or genetic factors. These injuries can lead to the formation of osteophytes or a loss of cartilage thickness. Injuries can be partial or total and can affect different parts of the cartilage, such as the articular surface or substrate.

Cartilage injuries can cause pain, swelling, and limitation of joint movement. If not treated properly, these injuries can lead to chronic joint problems, such as osteoarthritis (Heinegård D. 2011). Treatment of cartilage injuries depends on the severity of the injury and location (Hunziker EB. 2002). In some cases, rest and physiotherapy may be sufficient for healing, while in other cases surgery, such as cartilage repair or reconstruction, may be necessary (Jackson DW. 1996).

In general, prevention of articular cartilage injuries involves avoiding overuse of joints, maintaining a healthy weight, and doing regular exercises to maintain muscle strength and flexibility. In addition, athletes should use appropriate protective equipment and follow safety guidelines during sports activities.

Diagnosis of cartilage injuries can be difficult as symptoms can be vague and nonspecific. Patients may complain of pain, swelling, peeling or a sensation of locking in the joint. Diagnosis of cartilage lesions can be made through radiological examinations, such as radiography, ultrasound, and MRI, or through arthroscopic examinations, which allow direct visualization of the lesion and, in some cases, treatment during the same surgery. If there is pain or limitation of joint movement, it is important to see a sports medicine specialist for proper diagnosis and treatment.

Treatment of cartilage injuries depends on the severity of the injury, location, and cause of the injury. Minor injuries can be managed with conservative therapies, such as physical therapy, rest, corticosteroid injections, the use of orthopedic prostheses, or drug therapy to relieve pain and inflammation. However, more severe injuries may require surgery to repair or reconstruct the damaged cartilage. Surgical options include microfractures, mosaicplasty, autologous cartilage transplantation, mesenchymal stem cell transplantation, and joint replacement (Mithoefer K. 2009; Bartlett W. 2005; Brittberg M. 1994; Kon E. 2014). Microfracture is a surgical technique in which the bone tissue underlying the injury is drilled to stimulate the growth of new cartilage tissue. Mosaicoplasty is another surgical technique that involves removing a small piece of healthy osteochondral graft from the same joint as the patient to cover the lesion. Autologous cartilage transplantation involves removing a small amount of healthy cartilage from the same joint as the patient to create a patch to cover the lesion. Mesenchymal stem cell transplantation is a new therapeutic approach involving the injection of stem cells taken from the patient's own bone marrow into the cartilage lesion to promote cartilage tissue regeneration. Joint replacement is a surgical option for extensive cartilage injuries that cannot be repaired or reconstructed. This technique involves removal of the joint.

In summary, articular cartilage injuries pose a significant challenge to sports medicine and require a multidisciplinary approach to diagnosis and treatment. Research continues to explore new treatment options and preventive strategies to help athletes avoid or manage these debilitating injuries.

Cartilage injuries are a significant problem for athletes of all levels and can limit their ability to perform their sports activities, causing pain, inflammation, and reduced joint function. Professional athletes are particularly at risk for cartilage injury, as their sporting activities put enormous pressure on their joints and increase the risk of acute or chronic injury. According to a study by Røtterud et al., cartilage injuries are more common in professional athletes than in amateur athletes; but sports medicine is not only limited to professional athletes, but is also applied in amateur and recreational settings (Røtterud JH. 2011). This means that the market is extremely large and offers numerous opportunities for companies in this field. There are numerous companies in the sports medicine industry that offer products and services for athletes and active people. These include sports equipment suppliers, sports nutrition companies, performance monitoring and management software providers, and medical device manufacturers.

3 MDR: EUROPEAN MEDICAL DEVICE REGULATION

A medical device is defined as means any instrument, apparatus, appliance, software, implant, reagent, material or other article intended by the manufacturer to be used, alone or in combination, for human beings for one or more of the following specific medical purposes:

1. diagnosis, prevention, monitoring, prediction, prognosis, treatment or alleviation of disease,
2. diagnosis, monitoring, treatment, alleviation of, or compensation for, an injury or disability,
3. investigation, replacement or modification of the anatomy or of a physiological or pathological process or state,
4. providing information by means of in vitro examination of specimens derived from the human body, including organ, blood and tissue donations,

and which does not achieve its principal intended action by pharmacological, immunological or metabolic means, in or on the human body, but which may be assisted in its function by such means.

The new European Medical Device Regulation (MDR) came into force on May 26, 2021, replacing the previous Medical Device Directive (MDD). The main goal of the MDR is to improve the safety and effectiveness of medical devices used in the European Union (EU). The MDR represents a major change from the previous Medical Device Directive, which was introduced in 1993 and had not undergone significant changes over the years. The MDR reflects the rapid evolution of medical technology and patient needs, as well as ensuring higher levels of safety and security for patients and healthcare professionals. One of the new features of the MDR is the introduction of more stringent requirements for the marketing authorization of medical devices. Among the major changes introduced by the MDR is

increased requirements for high-risk medical devices. Specifically, high-risk medical devices, such as implants, prosthetics, and diagnostic devices, will undergo a more rigorous evaluation process, including risk assessment of adverse events and review of technical documentation. In fact, the MDR includes the introduction of a new classification of medical devices, which is based on the possible risks associated with the use of the device. The new classification takes into account risks to the patient, duration of device use, and invasiveness to the human body. Class III medical devices, for example, will undergo a more rigorous risk assessment than Class I devices.

Medical device evaluation bodies should conduct a comprehensive evaluation of the safety and effectiveness of each device, including identification of possible risks to patients and people involved in their manufacture and distribution.

The regulation also requires special attention to the information provided to patients about medical devices. Manufacturers will have to provide clear and understandable information about devices, including possible side effects and contraindications. Medical devices will have to be labeled in a clear and comprehensive manner that is also understandable to patients. The MDR requires medical device manufacturers to appoint a compliance officer, who will be responsible for ensuring that devices comply with MDR requirements and that technical documentation is available and up-to-date. The compliance officer will also be responsible for handling complaints and incident reports. Another new feature of the MDR is the requirement for manufacturers to continuously monitor their products after they are placed on the market. Manufacturers will have to collect and analyze data on the use of their devices and, if necessary, take corrective measures to ensure the safety and effectiveness of the devices. The MDR provides for the introduction of a mandatory certificate of compliance for all medical devices. The certificate of compliance certifies that the medical device has undergone a comprehensive evaluation process and complies with the requirements of the

MDR. Medical device manufacturers will have to apply for the certificate of compliance from a notified body authorized by the European Union.

In addition, the MDR provides for the establishment of a centralized European database for medical devices, which will make it possible to monitor medical devices on the market, identify any safety problems, and respond quickly to health emergencies.

In summary, the new European Medical Device Regulation (MDR) is a major step forward in protecting patient health and promoting technological innovation in medical devices. Its implementation will require increased attention and preparation by manufacturers, assessing bodies, and regulatory authorities, but its benefits are expected to be significant for all stakeholders involved in health care delivery.

3.1 Clinical investigation of MDR Medical Device

Clinical investigation means any systematic investigation involving one or more human subjects, undertaken to assess the safety or performance of a device.

The clinical investigation involves a Clinical Investigation Plan (CIP) means a document that describes the rationale, objectives, design, methodology, monitoring, statistical considerations, organization and conduct of a clinical investigation.

Clinical data means information concerning safety or performance that is generated from the use of a device and is sourced from the following:

1. clinical investigation(s) of the device concerned,
2. clinical investigation(s) or other studies reported in scientific literature, of a device for which equivalence to the device in question can be demonstrated,
3. reports published in peer reviewed scientific literature on other clinical experience of either the device in question or a device for which equivalence to the device in question can be demonstrated,
4. Clinically relevant information coming from post-market surveillance, in particular the post-market clinical follow-up

This Regulation aims to ensure the smooth functioning of the internal market as regards medical devices, taking as a base a high level of protection of health for patients and users, and taking into account the small- and medium-sized enterprises that are active in this sector. At the same time, this Regulation sets high standards of quality and safety for medical devices in order to meet common safety concerns as regards such products. Both objectives are being pursued simultaneously and are inseparably linked whilst one not being secondary to the other. This Regulation sets high standards of quality and safety for medical devices by ensuring, among other things, that data generated in clinical investigations are reliable and robust and that the safety of the subjects participating in a clinical investigation is protected.

Key elements of the existing regulatory approach, such as the supervision of notified bodies, conformity assessment procedures, clinical investigations and clinical evaluation, vigilance and market surveillance should be significantly reinforced, whilst provisions ensuring transparency and traceability regarding medical devices should be introduced, to improve health and safety.

The rules on clinical investigations should be in line with well-established international guidance in this field, such as the international standard ISO 14155:2011 on good clinical practice for clinical investigations of medical devices for human subjects, so as to make it easier for the results of clinical investigations conducted in the Union to be accepted as documentation outside the Union and to make it easier for the results of clinical investigations conducted outside the Union, in accordance with international guidelines to be accepted within the Union. In addition, the rules should be in line with the most recent version of the World Medical Association Declaration of Helsinki on Ethical Principles for Medical Research Involving Human Subjects.

It should be left to the Member State where a clinical investigation is to be conducted to determine the appropriate authority to be involved in the assessment of the application to conduct a clinical investigation and to organize the involvement of ethics committees within the timelines for the authorization of that clinical investigation as set out in this Regulation. Such decisions are a matter of internal organization for each Member State. In that context, Member States should ensure the involvement of laypersons, in particular patients or patients' organizations. They should also ensure that the necessary expertise is available.

An electronic system should be set up at Union level to ensure that every clinical investigation is recorded and reported in a publicly accessible database.

A clinical evaluation shall follow a defined and methodologically sound procedure based on the following:

(a) a critical evaluation of the relevant scientific literature currently available relating to the safety, performance, design characteristics and intended purpose of the device, where the following conditions are satisfied:

1. it is demonstrated that the device subject to clinical evaluation for the intended purpose is equivalent to the device to which the data relate, and
2. the data adequately demonstrate compliance with the relevant general safety and performance requirements;

(b) a critical evaluation of the results of all available clinical investigations, taking duly into consideration whether the investigations were performed

(c) a consideration of currently available alternative treatment options for that purpose, if any.

The requirement to demonstrate a clinical benefit shall be understood as a requirement to demonstrate the performance of the device. Clinical evaluations of those products shall be based on relevant data concerning safety, including data from post-market surveillance, PMCF, and, where applicable, specific clinical investigation. Clinical investigations shall be performed for those products unless reliance on existing clinical data from an analogous medical device is duly justified.

The clinical evaluation and its documentation shall be updated throughout the life cycle of the device concerned with clinical data obtained from the implementation of the manufacturer's PMCF plan and the post-market surveillance plan.

3.2 Clinical investigation of Medical Devices for human subjects - Good Clinical Practice

General

All parties participating in the design and conduct of the clinical investigation shall be qualified by education, training, or experience to perform their tasks and this shall be documented appropriately. The sponsor shall have access to medical expertise relevant to the clinical investigation. Where the risk management report's conclusions require training on the investigational device, consideration should be made by the sponsor about the extent of the training (e.g. animal model, cadaver training, support to users throughout the clinical investigation) (Hrysomallis C. 2007).

Clinical investigation process

Risk management principles shall be applied to both the planning and the conduct of clinical investigations, in order to ensure the reliability of the clinical data generated and the safety of subjects. The sponsor shall identify, assess and control risks associated with clinical investigation processes to ensure the ethical and scientific conduct of the clinical investigation and the credibility of the clinical investigation results. Clinical risks related to the clinical procedures, including follow-up procedures required by the Clinical Investigation Plan (CIP) other than those related to the medical device, shall be identified from the literature review. Their disclosure in the CIP and if applicable, the informed consent, shall also be determined by the sponsor and managed in the interest of subject safety. Risk control measures should be considered at both the clinical quality management system level (e.g. standard operating procedures, computerized systems, personnel) and clinical investigation planning and conduct (e.g. clinical investigation design, data collection, informed consent process).

Justification for the design of the clinical investigation

The justification for the design of the clinical investigation shall be based on the evaluation of pre-clinical data and the results of a clinical evaluation and shall be aligned with the results of the risk assessment. The clinical evaluation includes an assessment and analysis of clinical data concerning clinical performance, effectiveness or safety of the investigational device or similar devices or therapies. The evaluation shall be relevant to the intended purpose and the proposed method of use of the investigational device or similar devices or therapies. This is a scientific activity that shall be done with rigour and objectivity according to scientific standards.

The results of the clinical evaluation and the risk assessment shall be used to determine the required clinical development stages and justify the optimal design of the clinical investigation. They shall also help identify relevant endpoints and confounding factors to be taken into consideration and serve to justify the choice of control group(s) and if applicable, comparator(s), the use of randomization or blinding, and other methods to minimize bias.

The clinical investigation shall be designed to evaluate whether the investigational device is suitable for the purpose(s) and the population(s) for which it is intended. It shall be designed in such a way as to ensure that the results obtained have clinical relevance and scientific validity and address the clinical investigation objectives, in particular the benefit-risk profile of the investigational device. Several factors are important when designing any medical device clinical investigation, including general considerations of sources of bias and bias minimization, as well as specific considerations related to clinical investigation objectives, subject selection, subject endpoint(s), stratification, investigation site selection, and comparative clinical investigation designs. The clinical investigation should be designed to allow confirmation of the benefit-risk analysis of the investigational device as outlined in the risk management report.

Clinical investigation plan (CIP)

The CIP shall clearly outline the objectives of the clinical investigation. The proposed design shall be adequately justified based on scientific and ethical principles. The objective(s) of the investigation determine(s) whether an exploratory or a confirmatory design is appropriate to ascertain that the objectives of the clinical investigation can be reached.

The CIP and all subsequent amendments to the CIP are prepared by the sponsor in consultation with the biostatistician when relevant, agreed upon between the sponsor and the coordinating investigator and accepted by all principal investigators, and are recorded with a justification for each amendment.

Investigator's brochure (IB)

The purpose of the IB is to provide the principal investigator and the investigation site team with sufficient safety or performance data from pre-clinical investigations or clinical investigations to justify human exposure to the investigational device specified in the CIP. The IB shall be updated throughout the course of the clinical investigation as significant new information becomes available (e.g. a significant change in risk). In case of an investigational device design change that can occur during the course of the clinical investigation, the IB shall be updated and provide a justification for the change including an update of the risk management section of the IB, if required. The principal investigator(s) shall acknowledge receipt of the IB and all subsequent amendments in writing and shall keep all its information confidential.

Case report forms (CRFs)

The CRFs shall be developed to capture the data for each enrolled subject as required by the CIP. The CRFs shall include information on the condition of each subject upon entering, and

during the course of the clinical investigation, exposure to the investigational device and any other therapies. CRFs completion guidelines can also be developed to provide instructions to the investigation site team for accurate completion, correction and signature of CRFs along with expectations on handling clinical investigation deviations and unknown data, thus reducing the need for sponsor data queries. A procedure shall be in place to ensure, that when it is necessary to amend the CIP, the sponsor shall review the CRFs to determine if an amendment of these documents is also necessary.

Monitoring plan

The sponsor shall determine the extent and nature of monitoring appropriate for the clinical investigation based on the risk assessment. The extent and nature of the monitoring, including the strategy for source data verification versus centralized data review (evaluation without visiting the investigation site), subject protection and timely reporting, shall be based on the objective, design, complexity, size, critical data points and endpoints of the clinical investigation and the degree of deviation from normal clinical practice - risk-based monitoring.

In general, there is a need for on-site monitoring throughout the clinical investigation. Centralized monitoring can be performed in addition to complement on-site monitoring. In exceptional circumstances, the sponsor can determine that centralized monitoring in conjunction with procedures such as investigator's documented training, meetings, and extensive written guidance or telephone communication, can ensure appropriate conduct of the clinical investigation. In such circumstances, the sponsor shall provide a justification for omitting the source data verification. In addition, the sponsor shall ensure that the processes and expectations for site record keeping, data entry, reporting are well-defined and ensure timely access to clinical data and supporting documentation. The sponsor shall ensure, through oversight of the clinical investigation and timely adverse event reporting, that

unanticipated adverse device effects are identified and investigated rapidly so that, where necessary, additional risk control measures can be implemented. Results of the risk assessment shall be used to develop a risk-based monitoring plan and a supporting rationale. The monitoring plan shall describe:

(a) the risks associated with the clinical investigation and adequate information on relevant risk control measures;

(b) the processes that need to be monitored including data that is required to be verified in source documents;

(c) the monitoring methods (on-site, a combination of on-site and where justified, centralized monitoring, as appropriate);

(d) the responsibilities;

(e) the procedures and requirements for the investigation's oversight;

(f) the methods for documenting and communicating monitoring results;

(g) the methods for obtaining compliance;

(h) the process for escalation in case of continuous or egregious non-compliance;

(i) those aspects of the clinical investigation which need special attention because if performed incorrectly or inadequately, would compromise the protection of human subjects or the integrity of the data;

(j) the special requirements regarding personal data protection.

The monitoring plan shall be tailored according to the stage of clinical development and the type of clinical investigation.

Investigation site selection

The sponsor shall identify criteria necessary for the successful conduct of the clinical investigation prior to start of the site qualification process, including the facilities required at the clinical investigation site, principal investigator's qualification and the type of

environment (e.g. hospital versus home-based). The investigation site's facilities should be similar to the facilities required for the intended use of the investigational device(s), although additional equipment and capabilities may be needed at investigation sites during the clinical investigation to ensure that the necessary safety precautions are available. Prior to the initiation of the clinical investigation, the qualifications of the principal investigator(s) and adequacy of the investigation site(s) shall be verified and documented in an investigation site selection report. The rationale for selecting an investigation site shall be documented. Investigation site selection rationale can be based on prior experience of the sponsor with the principal investigator or the investigation site.

Agreement(s)

There shall be an agreement between the sponsor and the principal investigator(s)/investigation site(s) and any other relevant parties (e.g. investigators, CRO(s), and core laboratories), which defines the responsibilities of each party in the clinical investigation. All agreements shall be recorded in writing, signed, and dated by all parties involved. The agreement shall identify instances where, by participating in a clinical investigation, the parties share regulatory responsibilities with the sponsor.

Labeling

The investigational device, the instructions for use, or the packaging shall indicate that the investigational device is exclusively for use in a clinical investigation, unless this is not required.

Data monitoring committee (DMC)

The sponsor shall consider establishing a DMC prior to starting the clinical investigation.

The decision to establish a DMC shall be guided by the risk assessment, taking into account both the risks associated with the use of the investigational device and the risks associated with subject's participation in the clinical investigation. The primary function of the DMC shall be described in the CIP. The sponsor or DMC shall establish a Charter to document the following but not limited to:

(a) the responsibilities and scope of activities of the DMC;

(b) the frequency, format, and documentation of meetings;

(c) arrangements for handling emergency situations.

3.3 Clinical investigation conduct

General

The clinical investigation shall be conducted in accordance with the CIP.

The clinical investigation shall not commence until written approval/favourable opinion from the Etical Committee (EC) and, if required, the relevant regulatory authority of the countries where the clinical investigation is taking place has been received.

The sponsor shall ensure ongoing risk management throughout the clinical investigation taking into consideration all aspects related to the investigational device, clinical procedures required by the CIP, and the investigation process.

Investigation site initiation

An initiation visit for each participating investigation site or, alternatively, an investigator meeting shall be conducted and documented by the sponsor or monitor at the beginning of the clinical investigation.

A log shall be initiated identifying names, initials, signatures, functions, and designated authorizations for the principal investigator and members of the investigation site team.

Depending on the type and complexity of the clinical investigation, and its associated risks, site initiation can be performed by a telephone call or other communication, as specified in the risk-based monitoring plan.

Investigation site monitoring

The conduct of the clinical investigation shall be monitored according to the monitoring plan. The results of all monitoring activities shall be documented.

Adverse events and device deficiencies

Signals requiring immediate action

Signals from adverse events or device deficiencies that might indicate a serious health threat can be detected by either the sponsor or principal investigator but are evaluated by the sponsor. Any occurrence of a serious health threat can require a specific reporting process according to regulatory requirements.

Adverse events

All adverse events and any new information concerning these events shall be documented in a timely manner throughout the clinical investigation and shall be reported. This includes adverse events identified in the CIP as critical to the evaluation of the results of the clinical investigation. Adverse events associated with users or other persons can be documented separate from adverse events associated with the subject, taking into account the data privacy regulation. Certain national regulations can apply to reporting of adverse events during post-market clinical investigations. All adverse events shall be reported in an interim or final report of the clinical investigation.

Device deficiencies

All device deficiencies of an investigational device shall be documented throughout the clinical investigation and managed by the sponsor in accordance with written procedures for the control of a nonconforming product. The sponsor shall take, where applicable, appropriate corrective and preventive actions to protect the safety of subjects, users, and other persons. Device deficiencies of the comparator, if applicable, shall be documented.

The sponsor shall arrange for the safe return of the investigational device that is related to the device deficiency. Device deficiencies that did not lead to an adverse event but could have led to a serious adverse device effect

(a) if either suitable action had not been taken,

(b) if intervention had not been made, or

(c) if circumstances had been less fortunate

Where applicable, the analysis of used or explanted investigational devices shall be included as supportive information.

Risk assessment process for potentially unacceptable risks

Risks arising during the course of a clinical investigation shall be managed as follows:

(a) Any person identifying an event or information that could have an impact on subjects', users' or other persons' safety has an obligation to inform the principal investigator and the sponsor of their concerns.

(b) Risks are monitored against established risk acceptability thresholds.

(c) When circumstances of concern have been recognized, a preliminary risk analysis shall be performed by the sponsor in consultation with the principal investigator and, if appropriate, other advisors. The preliminary risk analysis can lead to the following outcomes:

1) The new information is adequately reflected in the existing risk assessment and the individual and overall residual risks to subjects, users, or other persons remain acceptable. The sponsor shall ensure that a rationale for this is recorded in the clinical investigation documentation.

2) Where possible, unacceptable risk or serious health threat has been identified, the sponsor shall suspend the clinical investigation immediately and the preliminary risk analysis shall be documented and notified to the interested parties, while further investigation is conducted.

(d) Where a preliminary risk analysis has resulted in the recognition of the possibility of an unacceptable risk, the sponsor shall make appropriate arrangements for a comprehensive risk assessment in compliance with ISO 14971. Where appropriate, a DMC or expert advisors should provide input into or conduct the risk assessment.

(e) The comprehensive risk assessment can lead to the following outcomes:

1) The new information is adequately reflected in the existing risk assessment and individual and overall residual risks to subjects, users or other persons remain acceptable. The sponsor shall ensure that a rationale for this is recorded in the clinical investigation documentation and necessary activities are performed before resuming the clinical investigation.

2) Corrective actions can be applied, including the following options:

(i) if the corrective actions do not affect the validity of the clinical investigation, the sponsor shall revise the benefit-risk analysis to justify continuation of the clinical investigation; perform necessary activities before resuming the clinical investigation;

(ii) if the corrective actions affect the validity of the clinical investigation, the clinical investigation shall be terminated.

(iii) If corrective actions cannot be applied, the clinical investigation shall be terminated.

Clinical investigation documents and documentation

Amendments

The IB, CIP, CRFs, informed consent form and other subject information, or other clinical investigation documents such as instructions for use shall be amended as needed throughout the clinical investigation in accordance with written procedures for the control of documents and document changes.

Documentation of changes shall include a description of the changes, justification of the changes and their potential impact on the performance, effectiveness, safety or other endpoints, and identification of the affected documents.

Proposed amendments to the CIP shall be reviewed and approved by the same parties, unless specifically designated otherwise. The amendments to the CIP and the subject's informed consent form shall be notified to, or approved by, the EC and regulatory authorities, if required. The version number and date of amendments shall be documented.

If the amendment impacts the integrity of the clinical investigation, the data collected before and after the amendment shall be analyzed statistically to assess the effect of the amendment on performance, effectiveness or safety analysis. This analysis shall be included in the clinical investigation report.

Subject identification log

Each investigation site shall maintain a log of all the subjects enrolled in the clinical investigation, assigning an identification code linked to their names, alternative subject identification or contact information. Depending on the clinical investigation design, a log can be maintained at the investigation site that identifies everyone who has been pre-screened for potential enrolment in the clinical investigation.

Source documents

Source documents shall be created and maintained by the investigation site team throughout the clinical investigation. The type and location of these source documents shall be documented.

Additional members of the investigation site team

New members of the investigation site team may be added from time to time at new or existing sites. New personnel should only start their assignment after receiving adequate training in the clinical investigation requirements and this training shall be documented. The names, initials, signatures, functions, and designated authorisations of new personnel shall be documented. EC approval of new members of the investigation site team can be required before commencement of their responsibilities, in addition to internal site documentation of these responsibilities and new member training.

Subject privacy and confidentiality of data

Confidentiality of data shall be observed by all parties involved at all times throughout the clinical investigation. All data shall be secured against unauthorized access.

The privacy of each subject and confidentiality of his/her information shall be preserved in reports and when publishing any data. The principal investigator or investigation site shall provide direct access to source data during and after the clinical investigation for monitoring, audits, EC review and regulatory authority inspections. As required, the principal investigator or investigation site shall obtain permission for direct access to source documents from the subject, hospital administration and regulatory authorities before starting the clinical investigation.

Document and data control

Traceability of documents and data

All documents and data shall be produced and maintained in a way that ensures reliability, integrity, control and traceability. All documents, and subsequent versions, related to a clinical investigation shall be identifiable, traceable and appropriately stored to provide a complete history of the clinical investigation. Where relevant, the accuracy of translations shall be guaranteed and documented. The investigator shall ensure the accuracy, attribution, completeness, legibility and timeliness of the data reported to the sponsor on the CRFs and in all required reports. All copies of the retained original source documents shall be certified, as indicated by a dated signature by a member of the investigation site team unless generated through a validated process. Special requirements should be applied to the capture, review and retention of electronic source data, to ensure reliability, quality, integrity and traceability.

If assignment to a treatment group is blinded/masked in any way, it shall be safeguarded throughout the clinical investigation, including data entry and processing. Written procedures for decoding blinded/masked clinical investigations shall be followed.

Recording of data

The data reported on the CRFs shall be derived from source documents and be consistent with these source documents, and any discrepancies shall be explained in writing. The CIP shall specify which data can be recorded directly in the CRFs. The acceptance of direct entry of source data into the CRFs can be subject to a hospital's specific documentation requirements. Data that can be directly recorded in the CRFs can also be documented in the monitoring plan. The CRFs shall be signed and dated by the principal investigator or his/her authorized designee(s). Any change or correction to data reported on a CRF shall be dated, initialed and explained if necessary, and shall not obscure the original entry (i.e., an audit trail shall be maintained); this applies to both written and electronic changes or corrections. The sponsor shall:

(a) provide guidance to the principal investigators or his/her authorized designee on making such corrections; the sponsor shall have written procedures to ensure that changes or corrections in CRFs are documented, are necessary, are legible and traceable, and are endorsed by the principal investigator or his/her authorized designee; records of the changes and corrections shall be maintained,

(b) ensure that it is possible to compare the original data and observations with the processed data, if data are transformed during processing;

(c) use an unambiguous subject identification code that allows identification of all the data reported for each subject. The link between the code and each subject shall be retained by the principal investigator in a secure location.

Electronic clinical data systems

Validation of electronic clinical data systems is necessary in order to evaluate the authenticity, accuracy, reliability, and consistent intended performance of the data system from design until decommissioning of the system or transition to a new system. These requirements are applicable to any electronic records, including electronic CRFs, electronic

systems used for entering and processing data from paper CRFs received from sites and other electronic systems required in the clinical investigation.

When electronic clinical databases or electronic clinical data systems are used, written procedures shall be implemented to:

(a) describe system validation and functionality testing, data collection and handling, system maintenance, system security measures, change control, data backup, recovery, contingency planning, and decommissioning,

(b) establish and document requirements for the electronic clinical data system to receive and process data,

(c) verify and validate that the requirements for the electronic clinical data system can be consistently met,

(d) ensure attributability, completeness, reliability, consistency, and logic of the data entered,

(e) ensure accuracy of reports,

(f) ensure that data changes are documented and that there is no deletion of entered data, i.e. maintain an audit trail, data trail and edit trail,

(g) maintain a security system that prevents unauthorized access to the data, both internally and externally,

(h) maintain a list of individuals who have access to the electronic data system as well as the dates of access, privileges granted to each user and removal of access,

(i) ensure the accuracy and completeness of the data reported to the sponsor in the CRFs by implementing a signature by the principal investigator or authorized designee,

(j) maintain adequate backup, retention and retrievability of the data,

(k) train users on the use of the system, and

(l) safeguard the blinding, if any (e.g. maintain blinding during data entry, and processing).

Investigational device accountability

Access to investigational devices shall be controlled and the investigational devices shall be used only in the clinical investigation and according to the CIP.

The sponsor shall keep records to document the physical location of all investigational devices from shipment of investigational devices to the investigation sites until return or disposal. The sponsor shall have instructions in place and make packaging materials available, if applicable, for the safe return or disposal of investigational devices, including potentially hazardous devices. The principal investigator or an authorized designee shall keep records documenting the following:

(a) name(s) of person(s) who received, used, returned, or disposed of the device;

(b) the date of receipt, identification, and quantity of each investigational device (batch number/serial number or unique code);

(c) the expiry date, if applicable;

(d) the date or dates of use;

(e) subject identification;

(f) date on which the investigational device was returned/explanted from subject, if applicable;

(g) the date of return of unused, expired, or malfunctioning investigational devices, if applicable;

(h) the date and documentation of disposal of the investigational devices as per instructions of the sponsor, if applicable.

Written procedures shall be established for the entire process of device accountability.

Accounting for subjects

All subjects enrolled in the clinical investigation (including those withdrawn from the clinical investigation or lost to follow-up) shall be accounted for and documented.

If a subject discontinues participation in the clinical investigation, the reason(s) shall be recorded. The investigator can use existing data and ask for the subject's permission to collect follow-up data about his/her status/condition including information about device clinical performance, effectiveness or safety. If permission is obtained, the relevant data shall be included in the clinical investigation report. The collection of follow-up data from discontinued subjects can be subject to national regulations.

Auditing

Audits of the clinical investigation may be conducted to evaluate compliance with the CIP, written procedures, this document and the applicable regulatory requirements. These audits may cover all involved parties, systems, processes, and facilities, and are independent of, and separate from quality control functions or routine monitoring.

An audit can be used

(a) as a routine part of the sponsor's quality assurance,

(b) to assess the effectiveness of the monitoring activity,

(c) whenever there are serious or repeated CIP deviations or suspicion of fraud,

(d) to bring an investigation site into "inspection readiness" (i.e. to prepare the investigation site for a potential regulatory inspection),

(e) when requested or suggested by a regulatory authority.

The auditors shall be qualified by training and experience to conduct audits and shall be independent of the clinical investigation.

The auditing of clinical investigation systems and processes shall be conducted in accordance with written procedures or specific plan on what to audit, how to audit, the frequency of audits, and the form and content of audit reports and audit certificates.

The audit plan or procedures for a clinical investigation audit shall be guided by the importance of the clinical investigation, the number of subjects in the clinical investigation,

the type and complexity of the clinical investigation, the level of risk to the subjects and any identified problem(s).

The audit results shall be documented and communicated to relevant parties. If applicable, an audit certificate shall be kept in the sponsor files.

3.4 Clinical investigation plan (CIP)

The content of a CIP and any subsequent amendments shall include all the topics together with a justification for each topic if this is not self-explanatory.

Identification of the clinical investigation plan

(a) Title of the clinical investigation.

(b) Reference number identifying the specific clinical investigation, if any.

(c) Version or date of the CIP.

(d) Summary of the revision history in the case of amendments.

(e) Version/issue number and reference number, if any, with the page number and the total number of pages on each page of the CIP.

(f) Abbreviations and acronyms.

Sponsors

Name and address of the sponsor of the clinical investigation and information about funding source.

Certain national or regional regulations can require that if the sponsor is not resident in the country (countries) in which the clinical investigation is to be carried out, the name and address of a local representative who acts as the sponsor fulfilling responsibilities of the sponsor in that country (those countries) are provided.

Principal investigator, coordinating investigator and investigation site(s)

(a) Name, address, contact details and professional position of

1) principal investigator(s),

2) coordinating investigator, if appointed.

(b) Name and address of the investigation site(s) in which the clinical investigation will be conducted.

(c) Name(s) and address(es) of external organizations (such as core laboratories, CROs, consultants or other contractors) involved in the clinical investigation.

The different roles, responsibilities and qualifications of investigators shall be specified.

The sponsor shall maintain an updated list of principal investigators and investigation sites. This list can be kept separately from the CIP. The definitive list shall be provided with the clinical investigation report.

Overall synopsis of the clinical investigation

A summary or overview of the clinical investigation shall include all the relevant information regarding the clinical investigation design such as inclusion/exclusion criteria, number of subjects, duration of the clinical investigation, follow-up, objective(s) and endpoint(s). It can be useful to include a flow chart showing the key stages of the clinical investigation or any other information that can be of value for the conduct of the clinical investigation.

Identification and description of the investigational device

(a) Summary description of the investigational device.

(b) Details concerning the manufacturer of the investigational device.

(c) Name or number of the model/type, including software version and accessories, if any, to permit full identification.

(d) Description as to how traceability shall be achieved during and after the clinical investigation, for example, by assignment of lot numbers, batch numbers, or serial numbers.

(e) Intended purpose of the investigational device in the proposed clinical investigation.

(f) The populations and indications for which the investigational device is intended.

(g) Description of the investigational device, including any materials, that will be in contact with tissues or body fluids. This shall include details of any medicinal substances, human or animal tissues or their derivatives, or other biologically active substances and reference to compliance with applicable national regulations.

(h) Summary of the necessary training and experience needed to use the investigational device based on risk assessment.

(i) Description of the specific medical or surgical procedures involved in the use of the investigational device.

(j) References to the IB and IFU.

The above information shall also be provided as far as available for the comparator, if applicable.

Justification for the design of the clinical investigation

Justification for the design of the clinical investigation, which shall be based on the conclusions of the clinical evaluation and shall comprise

(a) an evaluation of the results of the relevant pre-clinical testing/assessment and prior clinical investigations, if applicable carried out to justify the use of the investigational device in human subjects,

(b) an evaluation of clinical data that are relevant to the proposed clinical investigation,

(c) a description of the clinical development stage (see Annex I), if appropriate.

Benefits and risks of the investigational device, clinical procedure, and clinical investigation

(a) Anticipated clinical benefits.

(b) Anticipated adverse device effects.

(c) Risks associated with participation in the clinical investigation.

(d) Possible interactions with concomitant medical treatments as considered under the risk analysis.

(e) Steps that will be taken to control or mitigate the risks.

(f) Rationale for benefit-risk ratio.

Objectives and hypotheses of the clinical investigation

(a) The purpose of the clinical investigation, claims for clinical performance, effectiveness or safety of the investigational device that are to be verified.

(b) Objectives, primary and secondary, described as 'superiority', 'non-inferiority', or 'equivalence', if applicable.

(c) Scientific justification and clinical relevance for effect sizes, non-inferiority margins or equivalence limits, where applicable.

(d) Primary and secondary hypotheses, if applicable.

(e) Risks and anticipated adverse device effects that are to be assessed.

The objective(s) shall serve the purpose of the clinical investigation and shall relate to the hypotheses (where applicable) and corresponding endpoints relevant to the target population. The objectives of the clinical investigation shall translate directly into the pre-specification and operationalisation of the primary endpoint(s). Claims shall be linked to eligibility criteria for subject and users.

Design of the clinical investigation

General

(a) Description of the design type of clinical investigation to be performed (e.g. randomized, blinded or open-label, parallel groups or crossover, multicenter, international) the control group, (e.g. comparative claim and reversible treatment of a chronic state) and

the comparator with rationale and justification for the choice. Absence of control(s) shall be justified.

(b) Description of the measures to be taken to minimize or avoid bias, such as randomization, concealment of allocation, blinding/masking, and management of potential confounding factors.

(c) Primary and secondary endpoints, with rationale for their selection and measurement. If applicable, composite endpoints, with rationale for their selection and measurement. The primary endpoint shall be appropriate for the investigational device and should be clinically relevant. Composite endpoint is a pre-specified combination of more than one endpoint and can be used cautiously by including only components that have relatively equal clinical importance, frequency, and anticipated response to the presumed mechanism of action.

(d) Methods and timing for assessing, recording, and analyzing variables.

(e) Equipment to be used for assessing the clinical investigation variables and arrangements for monitoring maintenance and calibration.

(f) Any procedures for the replacement of subjects (generally, not applicable to randomized clinical investigations).

(g) Investigation sites: number, location, and, if appropriate, differences in investigation site environment.

(h) Definition of completion of the clinical investigation.

Investigational device(s) and comparator(s)

(a) Description of the exposure to the investigational device(s) or comparator(s), if used.

(b) List of any other medical device or medication to be used during the clinical investigation if not already specified in the instructions for use.

(c) Number of investigational devices to be used, together with a justification.

Subjects

(a) Inclusion criteria for subject selection.

(b) Exclusion criteria for subject selection.

(c) Criteria and procedures for subject withdrawal or lost to follow-up

1) when and how to withdraw a subject from the clinical investigation or stop the use of the investigational device,

2) documentation of efforts to be made to trace subjects that are lost to follow-up and possible reasons,

3) whether and how subjects are to be replaced.

(d) Point of enrolment.

(e) Point of randomization, if applicable.

(f) Total expected duration of the clinical investigation.

(g) Expected duration of each subject's participation.

(h) Number of subjects required to be included in the clinical investigation, and where needed, anticipated distribution of enrolment among the participating investigation sites.

(i) Estimated time needed to select this number (i.e. enrolment period).

(j) Relationship of investigation population to target population.

(k) Information on vulnerable, pregnant, and breastfeeding population, if applicable.

Procedures

(a) Description of all the clinical investigation-related procedures that subjects undergo during the clinical investigation including any deviation from normal clinical practice.

(b) Description of those activities performed by sponsor representatives (excluding monitoring).

(c) Any known or foreseeable factors that can compromise the outcome of the clinical investigation or the interpretation of results.

(d) The methods for addressing these factors in the clinical investigation, for example, by subject selection, clinical investigation design, such as stratified randomization, or by statistical analysis shall be described.

(e) The follow-up period during the clinical investigation shall permit the demonstration of clinical performance, effectiveness or safety over a period of time sufficient to represent a realistic test of the investigational device and allow any risks associated with adverse device effects to be identified and assessed.

(f) Address what specific medical care is appropriate to be provided for the subjects after the clinical investigation has been completed, if applicable.

(g) Address recommended follow-up for the subjects after the clinical investigation has been completed.

(h) Address the final disposition or potential future use of samples obtained from subjects, if applicable.

Monitoring plan

General outline of the monitoring plan to be followed, including access to source data and the extent of source data verification planned.

It is possible to provide a detailed plan for monitoring arrangements separately from the CIP.

Statistical design and analysis

The description of and justification for statistical design and analysis of the clinical investigation shall cover the following:

(a) Analysis population (e.g. intention-to-treat, per-protocol, as-treated) and procedures that take into account all the data.

(b) Descriptive statistics of baseline data, treatments, safety data and where applicable, primary and secondary endpoints.

(c) Analytical procedures including measures of precision such as confidence intervals, if applicable.

(d) The significance level and the power of primary endpoint(s) and the overall statistical testing strategy, if applicable. If a hypothesis is tested, a significance level alpha 0.05 (two-sided) and 0.025 (one-sided) and powers between 0.8 and 1 minus alpha need no justification. Depending on the characteristics of the investigational medical device or the clinical investigation, higher or lower levels of significance can be used. Examples of justifications include but are not limited to: product standards, scientific reasons or discussion with regulatory authorities.

(e) Sample size calculation and justification taking into account:

1) all relevant clinical data on outcome variable and effect size, if applicable;

2) assumptions of expected outcomes across treatment groups, if applicable;

3) adjustments due to any pre-planned interim analyses, if applicable;

4) detectable effect size and non-inferiority margin, which shall be smaller than the detectable effect size and justified with reference to the effect of the comparator, if applicable;

5) randomization allocation ratio (e.g. 1:1, 1:2), if applicable;

6) expected drop-out rate, such as withdrawal, lost to follow-up, death (unless death is an endpoint).

All the statistical parameters and methods used to calculate sample size or the non-inferiority margin shall be clearly provided.

For exploratory and observational clinical investigations in which the sample size is not required to be derived by calculation, the scientific rationale for the chosen sample size shall be provided.

(f) The rationale for the number of procedures to be performed by a single user as part of the learning curve and how these data are to be analyzed, if applicable.

(g) Pass/fail criteria to be applied to the results of the clinical investigation.

(h) The provision for an interim analysis, criteria for the termination of the clinical investigation on statistical grounds, where applicable.

(i) Management of bias and, when randomization, matching, or blinding are applied, plan for assessment of success thereof.

(j) Management of potential confounding factors (e.g. adjustment, stratification, or stratified randomization).

(k) Description of procedures for multiplicity control and adjustment of error probabilities, if applicable.

(l) The specification of subgroups for analysis, if applicable, or if response to treatment is expected to be different in these groups.

(m) Management, justification, and documentation of missing, unused or spurious data, including drop-outs.

n) Exploratory analysis and sensitivity analysis (e.g. to explore robustness of results of primary and secondary analysis with respect to different methods used for handling missing data), if applicable.

(o) Procedures for reporting any deviation(s) from the original statistical analysis plan.

(p) For multicenter clinical investigations, a strategy for handling the potential imbalance of the numbers of subjects across investigation sites.

(q) A strategy for pooling data, if applicable.

Further or more specific information can be found in standards for different types of medical devices or in national regulations or guidance documents.

Data management

(a) Methods (e.g. CRF) for data entry and collection.

(b) Procedures used for CRF tracking, data review, database cleaning, and issuing and resolving data queries. Specifically, timely, and reliable processes for recording data and rectifying errors and omissions, medical coding uniformity, and reconciliation, if applicable, are necessary to ensure delivery of a quality database and the achievement of the clinical investigation objectives through the implementation of the planned analysis.

(c) Procedures for verification, validation, and securing of electronic clinical data systems, if applicable.

(d) Procedures to maintain and protect subject privacy.

(e) Methods for database locking at the start of the analysis and storage upon completion of the clinical investigation.

(f) Procedures for data retention.

(g) Specified retention period.

(h) Other aspects of clinical quality assurance, as appropriate.

Amendments to the CIP

Description of the procedures to amend the CIP.

Deviations from clinical investigation plan

(a) Statement specifying that the investigator is not allowed to deviate from the CIP.

(b) Procedures for recording, reporting, and analyzing CIP deviations.

(c) Notification requirements and time frames.

(d) Corrective and preventive actions and principal investigator disqualification criteria.

Device accountability

(a) Description of the procedures for the accountability of investigational devices;

(b) Procedures and particular materials and instructions for the safe return of investigational devices, including those that are potentially hazardous.

Statements of compliance

(a) Statement specifying that the clinical investigation shall be conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki

(b) Statement specifying compliance with this document and any regional or national regulations, as appropriate.

(c) Statement specifying that the clinical investigation shall not begin until the required approval/favourable opinion from the EC and regulatory authority have been obtained, if appropriate.

(d) Statement specifying that any additional requirements imposed by the EC or regulatory authority shall be followed, if appropriate.

(e) Statement specifying the type of insurance that shall be provided for subjects, if appropriate.

(f) Statement addressing the financing of the clinical investigation including a description of the agreement between the sponsor and investigation site(s), and where applicable with the investigator(s) if not addressed in a separate agreement.

Informed consent process

(a) Description of the general process for obtaining informed consent, including the process for providing subjects with new information and process for incentives for subjects, as needed.

(b) Description of the informed consent process in circumstances where the subject is unable to give it

Adverse events, adverse device effects, and device deficiencies

(a) Definitions of adverse events and adverse device effects.

(b) Definition of device deficiencies.

(c) Definitions of serious adverse events including serious health threat and serious adverse device effects and, where appropriate, unanticipated serious adverse device effects.

(d) List of non-reportable adverse events, if applicable, including rationale.

(e) Time period in which the principal investigator shall report all adverse events and device deficiencies to the sponsor and, where appropriate, to ECs and the regulatory authority.

(f) Details of the process for reporting adverse events (date of the adverse event, treatment, resolution, assessment of both the seriousness and the relationship to the investigational device and the related procedure).

(g) Details of the process for reporting device deficiencies.

(h) List of foreseeable adverse events and anticipated adverse device effects, together with their likely incidence, mitigation, or treatment.

(i) Emergency contact details for reporting serious adverse events and serious adverse device effects.

(j) Information regarding the DMC, if established.

Vulnerable population (if applicable)

(a) Description of the vulnerable population to be included in the clinical investigation.

(b) Description of the screening process to identify and protect the vulnerable population.

(c) Description of the specific informed consent process.

(d) Description of the EC's specific responsibility.

(e) Description of what medical care, if any, will be provided for subjects after the clinical investigation has been completed.

Suspension or premature termination of the clinical investigation

(a) Criteria and arrangements for suspension or premature termination of the whole clinical investigation or of the clinical investigation at one or more investigation sites.

(b) Criteria for access to and breaking the blinding/masking code in the case of suspension or premature termination of the clinical investigation, if the clinical investigation involves a blinding/masking technique.

(c) Requirements for subject follow-up and continued care.

Publication policy

(a) Statement that the clinical investigation will be registered in a publicly accessible database.

(b) Statement indicating that the results of the clinical investigation will be made publicly available.

(c) Statement indicating the conditions and timeframes under which the results of the clinical investigation will be offered for publication including the role of the sponsor and criteria for authorship.

Bibliography

List of bibliographic references pertaining to the clinical investigation.

4 Technological development and validation of a PEMF medical device in sport medicine

4.1 Introduction

As we have seen, articular cartilage promotes movement of the joint and performs a function of absorbing the mechanical stresses to which the joint is subjected. The structure and vascularization of cartilage are such that its regenerative capacities are limited and insufficient to repair damage induced by trauma or the catabolic effects of subsequent inflammatory reactions to it. The cartilage progressively thins and tears, and the subchondral bone tissue is progressively exposed until osteoarthritis or osteochondral damage is established that must be treated by surgical methods. The incidence of cartilage injury is observed not only following sports injury, but also in 65% of patients undergoing knee arthroscopy; 300,000 arthroscopic procedures are performed each year in Italy. The severity and extent of cartilage damage guides the choice of surgical tissue repair treatment.

The most advanced frontier in orthopedics and traumatology is represented by treatment methods that aim to accelerate tissue healing processes, and to restore the functional properties of damaged tissues, in particular, for those involved in inflammatory and degenerative diseases, such as joint tissues. Continuous integration between the knowledge from basic research with orthopedic practice is able to offer great opportunities to solve major clinical issues (Moran CJ. 2010). Modern treatment methods aim at tissue repair and regeneration techniques, promoting strong anabolic tissue activity. Currently, the advanced surgical treatments used in the joint environment consist of several strategies including implantation of engineered tissues at the site of the lesion, mesenchymal stem cells, differentiated cells and growth factors (Getgood A. 2009). However, to date these treatment methods have not yielded reproducible successes over time and comparable to each other (Harris JD. 2010); in particular, the long-term clinical results are unsatisfactory, since in

many cases fibrous tissue formation occurs, with lower mechanical properties and limited durability (Pelttari K. 2009). In addition, it often happens that patients continue to experience a persistence of symptoms over time, such as pain or intra-articular effusion (Brun P. 2008). These observations underline the need to improve the quality of regenerated tissue; however, current treatments are not yet able to control, slow down or inhibit the degeneration of joint tissues, in particular cartilage. International literature shows how great the variability of the results of these modern treatment methods is (Harris JD. 2010). The success or failure of tissue repair surgery is strictly dependent on the type of implant, the manual skills required for the placement of the engineered tissue and the quality of the joint environment in which it is placed. Surgical treatments, although minimally invasive, such as arthroscopy, still cause local damage that generates an inflammatory reaction of variable magnitude but difficult to control over time and that can alter cellular homeostasis. The degeneration process of the cartilage can be described as the alteration of two opposing metabolic activities that, under physiological conditions, such as dynamic stress, are in balance with each other: on one side a catabolic function which tends to damage cartilage tissue, on the other anabolic function which maintains and protects cartilage. Due to its poor restorative capacity, it is of fundamental importance to keep all articular cartilage intact, in all its components, cells and extracellular matrix, also involving the subchondral bone through the stimulation of the functional activities of the chondrocytes and the inhibition of inflammatory damage caused by inflammation.

Inflammation represents a serious harm for joint tissues, cartilage, ligaments, meniscus, and must be controlled in the shortest time and in the most effective and complete way.

The presence of pro-inflammatory cytokines, interleukin-1beta (IL-1β) and tumour necrosis factor-alpha (TNF-α) in the joint environment stimulates the activity of metalloproteinases, the release of prostaglandins E2 (PGE2) and therefore the inhibition of the synthesis of the

extracellular matrix as well as its destruction, strongly orienting the repair activities of cartilage tissue toward the fibrotic tissue (Guilak F. 2004; Kuettner KE. 2005; Ortiz LA. 2007). The activity of inflammatory cells and the release of pro-inflammatory cytokines in the synovial fluid are responsible for the catabolic effects on the cartilage matrix, subsequently leading to the loss of its mechanical function (Schuerwegh AJ. 2003, Goldring SR. 2004).

It is known, in fact, that on the surface of the joint cartilage, during inflammatory processes, neutrophils have been detected. Neutrophils are cells that stimulate enzymatic activities, especially those of metalloproteases, which can degrade the cartilage matrix. To prevent the harmful effect of inflammation on the cartilage, studies have been carried out identifying new molecules or techniques capable of controlling such harmful processes. Physiologically, the human body controls inflammation through the activation of numerous cellular processes, including the involvement of adenosine receptors (ARs), in particular A_{2A} and A_3 ARs (Tesch A. 2002, Borea PA. 2009). A study conducted in an animal model of septic osteoarthritis found that a specific agonist of adenosine A_{2A} receptors significantly reduces cartilage damage, synovial inflammation, and leukocytic infiltration (Cohen SB. 2004, Cohen SB. 2005). However, adenosine-agonist drugs for A_{2A} receptors, although considered chondroprotectors, are not used in clinical settings for possible systemic side effects.

In this regard, the concept of chondroprotection indicates the set of those pharmacological, physical and surgical treatments, alone or combined, which allow to preserve cartilage integrity or which aim to limit the damage due to degenerative, pathological and traumatic processes, and inflammatory reactions.

It is of fundamental importance to develop local treatment methods, which limit and prevent the degeneration of cartilage tissue. Several approaches have been developed in the last decades to resolve this disability cause, including tissue engineering, but to date, there is not

a definitive procedure that is able to promote a repair tissue with the same mechanical and functional characteristics of native cartilage, and to obtain its integration into the subchondral bone.

4.1.1 Clinical background & Current Knowledge on the use of physical energy

The use of physical energy or physical stimuli to modulate a particular cell function and ultimately to promote tissue healing has been the subject of extensive research. In Europe, the relationship between biological systems and electricity dates back to the studies of Galvani and Matteucci, who, as early as the 19th century, had identified the currents of injury and had intuited its role in repair processes.

Clinical biophysics forms the foundation of a new pharmacology, which uses physical stimuli to treat various diseases in human beings. Clinical biophysics is an interdisciplinary science which:

- uses methods and theories from the field of physics to study biological systems
- studies how non-ionizing physical stimuli interact with biological systems

Underlying the new pharmacology is the need to identify the effects of the physical agents in terms of how these modulate a particular cell function, which will then form the basis of its clinical application. The cell membrane has been identified as a target and site of interaction, through which the physical signal activates a cascade of intracellular events; the transduction pathways have been seen to differ depending on the type of energy used (Fig. 1). Each time a physical agent is able to modulate a cell activity, the effect observed will be function-specific, rather than cell- or tissue-specific. This allows all conditions which are positively influenced by the activation or modulation of this cell function to be treated with the same physical agent.

Biophysical stimulation techniques can be used in clinical medicine, either alone, to increase and promote the repair and anabolic activity in tissue, or in association with drug treatment,

to strengthen its activity and lessen side effects. The methods for administrating physical energy to a biological system are known as biophysical stimulation and can be divided into:

- electromagnetic energy applied using coils (pulsed electromagnetic fields, PEMFs)
- electrical energy applied directly to the tissue using adhesive electrodes (capacitively coupled electrical field, CCEF)
- ultrasound energy applied directly to the tissue in the form of mechanical forces (low intensity pulsed ultrasound system, LIPUS).

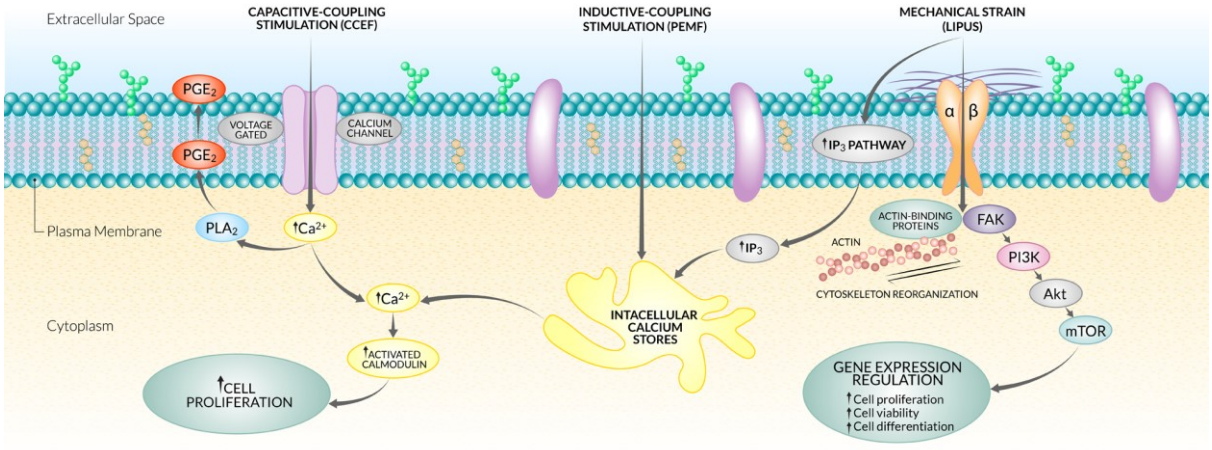


Figure 1. Schematic representation of the biophysical stimuli targets on the cell surface and corresponding metabolic pathways within the cell.

The first use of PEMF was for fracture healing and was reported in Germany by Kraus and Lechner in 1972. In 1977, Bassett and coworkers reported their initial results on the treatment of nonunions (Bassett CA. 1977). Following these reports, extensive clinical experience has been gathered in Italy, Belgium, the UK, and the Netherlands. The first randomized controlled double-blind study was conducted in Italy on femoral intertrochanteric osteotomies stimulated with PEMF (Borsalino G. 1988). In 1979, the US FDA approved PEMF as a safe and effective treatment for nonunions of bone. Since then, the use of PEMF stimulation for bone repair has grown both in the United States and in Europe. In the United States, a survey showed that 72% of hospitals offer bone repair

stimulation treatments for fractures that fail to heal. Moreover, a substantial proportion of Canadian orthopaedic surgeons (45%) currently make use of bone stimulators as part of their management strategy for at least some tibial shaft fractures (for complicated tibial shaft fractures); 80% of respondents felt that a reduction in healing time of six weeks or more, attributed to a bone stimulator, would be clinically important (Busse JW. 2008).

The American Academy of Orthopedic Surgeons (AAOS) hold a symposium on biophysical stimulation in the treatment of bone diseases and from which several manuscripts were published (Cadossi R. 2020). Recently, the Italian orthopedic community has teamed up to create a focus group on the clinical use of electrical stimulation not only on bone healing but also on cartilage chondroprotection, in orthopedics and traumatology clinical indication (Massari L. GIOT 2007). An article was published from the meeting that states that "Therapy with a medical device for the electrical stimulation of osteogenesis and chondoprotection" must ensure:

- the electrical safety of the device;
- biological safety, understood as the absence of side effects;
- the effectiveness of the device in the specific pathology, demonstrated by clinical studies;
- the absence of specific contraindications for the patient who will have to use the medical device."

4.1.2 PEMF Medical device for chondroprotection

A PEMF medical device for chondroprotection and free of side effects already exists on the market. The medical device is made by IGEA SpA and patented in Europe and the USA. The use of PEMF for the treatment of joints must respond to the need to treat articular cartilage in its total extent and thickness, as well as involving joint structures, e.g. meniscus, ligaments, synovial membrane, up to the subchondral bone. These problems to date have been solved exclusively with the use of specific parameters of PEMF (called I-ONE® therapy - the medical device developed by IGEA SpA) as well demonstrated by the CRES (Cartilage Repair and Electromagnetic Stimulation) study group through extensive translational research on the chondroprotective effect of PEMF stimulation (Massari L. JBJS 2007). The experimental and clinical evidence from the CRES study group indicates that I-ONE® therapy is able to inhibit the inflammatory response quickly and effectively, to prevent and / or slow down the degenerative phenomena that accompany surgery. In clinical use, we can position I-ONE® both in the context of the prevention of cartilage degeneration, as a conservative treatment, and in promoting the reparative processes of single or multiple focal cartilage lesions, as a post-surgical treatment. A correct diagnosis and a precise indication for biophysical treatment are the prerequisite for a good clinical outcome. The need for resolutive technologies to obtain "more effective" cartilage tissue substitutes has led the "Functional Tissue Engineering" (FTE) paradigm, whose principles are outlined in a so-called FTE road map. Research results and available clinical studies suggest that I-ONE® therapy can play a fundamental role in the success of tissue regeneration and tissue engineering (Fini M. 2013).

In 2002, Varani et al. observed a significant increase in binding of adenosine to the adenosine receptor subtype A_{2A} in human neutrophils exposed to I-ONE® therapy ($p < 0.05$) (Varani

K 2002). Dose-response studies demonstrated that the effect was detectable after thirty minutes of exposure and saturation of the receptors was achieved with I-ONE® therapy. The effect on adenosine binding with the A_{2A} adenosine receptor was later confirmed in cultures of isolated fibroblast-like bovine and human synoviocytes and chondrocytes by the same group.

(Massari L. 2019). Extensive in vitro data reported in literature shows the effect of I-ONE® therapy on articular cells. In bovine chondrocytes and synoviocytes (Ongaro A. 2012), A_{2A} and A_3 adenosine receptors, endogenous modulator of many biological processes such as inflammation, increased in number in the presence of I-ONE® therapy, reducing the release of PGE_2 , IL-6, IL-8 and COX-2, a result which suggests a reduction in the inflammatory state and in the degradation of cartilage associated with articular diseases. Human synoviocytes treated with I-ONE® therapy reveal a significant increase in A_{2A} and A_3 adenosine receptors, as demonstrated by the mRNA, Western blotting analysis and saturation binding experiments involving ARs, as well as a significant increase in the release of IL-10, a known anti-inflammatory cytokine (Varani K. 2008 2017). The A_{2A} and A_3 receptors exert their anti-inflammatory action by inhibiting the NF-κB transcription factor pathway, which plays a central role in regulating the synthesis and activities of the inflammatory cytokines. Stimulation with I-ONE® therapy further inhibits the activation of NF-κB and is essential for regulating the synthesis and activation of the pro-inflammatory cytokines, including TNF-α and IL-1β, and also of other mediators involved in joint inflammation and bone diseases (Ongaro A. 2011). I-ONE® therapy has been shown to affect the increase of human articular chondrocyte proliferation, based on exposure time, intensity and frequency (De Mattei M. 2007, Veronesi F. 2014). It should be stressed that the effect of I-ONE® therapy on proteoglycan synthesis in human cartilage explants is comparable in all senses to that induced by growth factor IGF-I, the principal cartilage anabolic factor (De Mattei M. 2004).

While the presence of IL-1β inhibits the synthesis of proteoglycans, exposure to I-ONE® therapy can curb the catabolic effect of the cytokine, increasing proteoglycan synthesis even under inflammatory conditions (De Mattei M. 2003). It is interesting to observe that these results regarding the anti-inflammatory role of I-ONE® therapy are also confirmed in the stem cell cultures (Ongaro A. 2015). These results demonstrate the anti-inflammatory role of I-ONE® therapy in chondrocytes and cartilage explants. The authors conclude that I-ONE® therapy plays an important role as prevention treatment against the progression of initial osteoarthritis, exerting a strong anti-inflammatory and chondroprotective effect.

Studies based on *in vitro* and *ex vivo* results have been performed on large and small animal models to evaluate the effect of I-ONE® therapy in preventing osteoarthrosic degeneration and in the repair of tissue damage, as an adjunct to tissue engineering methods. In Dunkin Hartley guinea pigs treatment with I-ONE® therapy was demonstrated as being capable of halting the progression of osteoarthrosis, of limiting cartilage surface clefts and fibrillation, of preserving cartilage thickness and of preventing sclerosis of the subchondral bone (Fini M. 2005-2008). These results are consistent with those of other authors, who have demonstrated an increase in TGF-β1 synthesis and an inhibition of TNF-α synthesis (with a clear anabolic and trophic effect on the articular cartilage) in the animals treated using PEMF. Autologous osteochondral autografts were performed in adult sheep, resulting in a significantly better osteointegration of the graft and a lesser formation of cyst-like resorption areas in the I-ONE® therapy group (Benazzo F. JOR 2008). The synovial liquid in the stimulated animals contained significantly lower levels of proinflammatory cytokines IL-1β and TNF-α and a higher concentration of TGF-β1 compared to the untreated animals. I-ONE® therapy has proved effective in rabbits with osteochondral lesions significantly improving the quality of the regenerated tissue in the osteochondral defects in the presence of collagen scaffold and bone marrow concentrate (Veronesi F. 2015). In adult canine model,

engineered osteochondral constructs were cultured to maturity and implanted into focal defects created in the stifle (knee) joint (Stefani RM. 2020). To assess expedited early repair, animals were assessed after a 3-month recovery period, with microfracture repairs serving as an additional clinical control. I-ONE® therapy leads to a greater likelihood of normal chondrocyte and proteoglycan histological scores in engineered constructs. The studies provided evidence that I-ONE® therapy stimulation enhanced engineered cartilage growth and repair, demonstrating a potential low-cost, low-risk, noninvasive treatment modality for expediting early cartilage repair.

The anti-inflammatory activity of I-ONE® therapy effectively prevented the degenerative effect of IL-1β, significantly improving cartilage regeneration compared to the non-stimulated lesions, thus explaining the anti-degenerative, reparative and anti-inflammatory effects of treatment with I-ONE® therapy in in *vivo* models also.

Together, these findings show that I-ONE® therapy has an A_{2A} adenosine receptor agonist activity, thereby identifying the A_{2A} adenosine receptor as the pharmacological molecular target of therapeutic intervention with I-ONE® therapy in patients with inflammatory joint diseases.

In orthopaedics, Zorzi et al. conducted a randomized prospective and double-blind study in patients with cartilage degeneration and knee pain symptoms treated with microfractures in combination with I-ONE® therapy (Zorzi C. 2007). Patients with Grade I to IV cartilage lesions according to the Outerbridge classification were included in the study. Patients performed I-ONE® therapy for 6 hours a day for 90 days. In the first month after surgery, the percentage of patients using Non Steroidal AntiInflammatory Drugs (NSAIDs) was 26% in the active group and 75% in the control group (p=0.015). The KOOS (Knee Osteoarthritis Outcome Score) clinical evaluation showed higher values (better joint function) in the active group at both 45 days (73.6 ± 10.3 vs. 70.3 ± 14.9, ns) and 90 days (83.6 ± 7.3 vs. 74.7 ±

13.6, $p < 0.05$) from surgery. At 45-days, patients in the active group already showed a level of functional recovery that patients in the placebo group would show at 90 days; demonstrating a halving of recovery time. At 3-years follow up the number of patients showing functional limitation of the knee joint was significantly higher in the control group compared to the active group (87.5% vs. 37.5%).

Similar results were also found in a group of patients undergoing autologous chondrocytes transplantation in the presence of scaffolds (MACI) and treated with I-ONE® therapy (Collarile M. 2018). At the baseline the two groups were perfectly comparable for clinical scores and cartilage injury characteristics. The International Knee Documentation Committee (IKDC) score showed a significant improvement in the treated group compared to the control at 1 ($p=0.01$), 2 ($p=0.041$) and 60 ($p=0.001$) months follow-up. In addition, there was a statistically significant difference between the groups at 1-month ($p=0.023$) and 60-month for SF-36 ($p=0.006$) and at 60 months for EuroQol ($p=0.020$). A significant reduction in pain was observed in the treatment group compared to the control at 1 ($p=0.018$), 2 ($p=0.043$) and 60 ($p=0.011$) months of follow-up.

In a clinical trial on patients with osteochondral lesions of the talus, treated by graft transplantation with the addition of bone marrow concentrate (BMDC) in a single operating session (ONE-STEP Method), Cadossi et al. showed less pain in the experimental group at 2, 6 and 12 months follow-up and significantly higher functional results in the group of I-ONE® therapy patients compared to the controls (Cadossi M. 2014).

Early rehabilitation with less pain can certainly lead to a better clinical outcome even after a long time. Clinical results from all studies show that the cartilage regeneration and/or repair method associated with I-ONE® therapy is an effective solution for the treatment of chondral and osteochondral lesions of the joint in the field of regenerative medicine and tissue engineering.

A clinical experience conducted on patients with initial osteoarthritic degeneration of the knee, treated non-surgically but conservatively with I-ONE® therapy, showed significantly better results in the stimulated group compared to the control ($p < 0.05$) (Gobbi A. 2012, Moretti L. 2021). No adverse reactions or side effects were observed following treatment with I-ONE® therapy. Vicenti et al. showed that I-ONE therapy is a valid option in the conservative management of several knee joint diseases, including early OA, patellofemoral pain syndrome and SONK (Vicenti G. 2018). Viganò et al. in a recent review confirm that I-ONE® therapy is safe and effective treatment for the control of knee OA-related pain and disability at short term (Viganò M. 2021).

Baker states that 19.8% of patients who have undergone total knee replacement (TKA) experience pain 1 year after surgery (Baker PN. 2012). Beswick et al. reports that many patients (10-34%) continue to experience significant pain and functional limitation after TKA even after 3-5 years. The inflammatory response is mainly due to the presence of pro-inflammatory cytokines in the synovium; in fact, it has been shown that there is an inverse relationship between the amount of pro-inflammatory cytokine IL-6 in the joint and the patient's functional recovery.

Two randomized controlled trials, performed in two highly specialized Italian centers for TKA, have in fact confirmed this observation (Moretti B. 2012; Adravanti P. 2014). The purpose of the two studies was to evaluate whether the use of I-ONE® therapy during the rehabilitation phase of the patients had an effect on the reduction of pain, joint swelling and on functional recovery times. The studies were conducted with the same experimental scheme and using I-ONE® therapy 4 hours / day for 60 days. Patients assigned to the experimental group began treatment with I-ONE® therapy within 7 days of surgery, in order to intervene in the early stages of the inflammatory process. No patient treated with I-ONE® therapy has shown adverse events arising from the device, such as redness or skin irritation,

confirming what has already been reported about the maximum tolerability of this treatment. Both studies reported that painful symptoms appeared significantly reduced from the first post-operative month compared to the pre-operative value in both groups of patients (treated and untreated); however, the use of I-ONE® therapy made it possible to obtain a reduction in post-operative pain symptoms more quickly and above all with significantly lower values compared to patients undergoing only the standard rehabilitation protocol, at all evaluation times starting already from the first month. The same observation was found for swelling: in both studies the patients of the I-ONE® therapy showed significantly less swollen knees compared to the control group in the first two months after surgery, while subsequently (6 and 12 months) the results were comparable.

These observations also correlate with a significantly higher score of KSS (Knee Society Score) in terms of functional recovery in the longer term. The study from Adravanti et al., which includes an evaluation 36 months after surgery, showed that at this time-point only 7% of the patients included in the I-ONE® therapy group still complained of persistent pain that required use of anti-inflammatory drugs, compared to 33% of patients in the control group. Furthermore, no patient of the I-ONE® therapy group declared the need, even if occasional, for walking aids, compared to about 20% of the control group. Studies on TKA indicate how the clinical result and ultimately the patient's quality of life, in the short and long term, can be improved with the adoption of a therapy for local control of the joint inflammatory environment, in particular by reducing time and amplitude of the inflammatory response following the prosthetic intervention.

The Reverse Total Shoulder Arthroplasty (rTSA) has in recent years increased in popularity as a treatment option for patients suffering from numerous diseases affecting the glenohumeral joint, first of all the "cuff tear arthropathy" (CTA) (La Verde L. 2019). The definition of "cuff tear arthropathy" is a particular condition which has as primum movens a

massive lesion of the rotator cuff of the shoulder and which progressively leads to the development of an eccentric glenohumeral arthrosis. Clinically, the functionality of the shoulder is considerably reduced, resulting in a "pseudoparalytic shoulder" in the most advanced cases. An early rehabilitation is one of the fundamental elements for an optimal post-surgical recovery. The operation can be burdened by an important rate of postoperative pain, which can compromise the execution of a physiotherapy program and consequently determining results lower than desirable or slow down their achievement. The application of I-ONE® therapy has been studied both for conservative treatment and for post-surgical pain relief by numerous authors. Recently, a prospective randomized study was conducted by recruiting 50 candidate patients for rTSA. Patients were randomized, allocating an equal number in the experimental group and in the control group. Patients in the study group were instructed on the correct use of I-ONE® therapy, which was applied 4 hours a day for 60 days. All patients underwent the same rehabilitation protocol. The two groups were homogeneous in the preoperative period for demographic and pathological characteristics. In the follow-ups at 1, 2 and 3 months after surgery the Constant score, VAS scale, range of motion and the percentage of joint function of the shoulder compared to the contralateral reached significantly higher values in the patients of the study group compared to the control group ($p < 0.05$). No patients in either group reported postoperative complications. At the last postoperative follow-up (6 months), the clinical and functional results improved further in both groups, which however did not show a statistically significant differences between them.

The results of this study showed that the application of I-ONE® therapy after reverse shoulder replacement surgery represents a safe option, capable of reducing postoperative pain and increasing the articular function of the shoulder in the short term.

Recently, a prospective randomized controlled trial (RCT) was performed in which 72 patients undergoing medial UKA were randomized into a control group or an experimental I-ONE® therapy group (D'Ambrosi R. 2022). The patients allocated to the experimental group were instructed to use I-ONE® therapy for 4 hours per day for 60 days. They were evaluated before surgery and then during the time points corresponding to 1, 2, 6, 12, and 36 months after the surgery. The VAS decreased on follow-up visits in both groups, although a statistically significant difference between the groups was observed during the 6 ($p=0.0297$), 12 ($p=0.0003$), and 36-month ($p=0.0333$) follow-ups in favor of the I-ONE® therapy group. One month after UKA, the percentages of patients using NSAIDs in the I-ONE® therapy and control group were 71% and 92%, respectively ($p=0.0320$). At the two months point, 15% of the patients in I-ONE® therapy group used NSAIDs compared to 39% in the control group ($p=0.0317$). The objective knee girth evaluation showed a statistically significant difference at 6 ($p=0.0204$), 12 ($p=0.0005$), and 36 ($p=0.0005$) months with improved values observed in the I-ONE® therapy group. The subjective assessment of the swelling demonstrated a statistically significant difference at 2 ($p=0.0073$), 6 ($p=0.0006$), 12 ($p=0.0001$), and 36 ($p=0.0011$) months with better values noted in the I-ONE® therapy group. Lastly, the OKS result was significantly higher in the experimental group during all the follow-ups (1mth: $p=0.0295$; 2mths: $p=0.0012$; 6mths: $p=0.0001$; 12mths: $p<0.0001$; 36mths: $p=0.0061$).

The use of I-ONE® therapy leads to significant pain relief, better clinical improvement, and lower NSAIDs consumption after medial UKA when compared to the control group.

The effect of I-ONE® therapy has also been studied in patients suffering from spontaneous osteonecrosis of the knee (SONK) in the initial phase (Marcheggiani M. 2013). Twenty-eight patients with symptomatic early phase SONK were enrolled and stimulated with I-ONE® therapy for at least 6 hours a day for 90 days. Results of the study show significantly

reduced pain (mean VAS) after 6 months ($p < 0.0001$), maintained constant after 24 months; a significant clinical improvement (intervals 0, 6 and 24 months): mean KSS from 34.0 ± 13.3 to 76.1 ± 15.9 ($P < 0.0001$) and then 72.46 ± 13.52 ($P = 0.0044$); Tegner Median from 1 (range 1-1) to 3 (range 3-4) ($P < 0.0001$) and then unchanged; EQ-5D averaged from 0.32 ± 0.33 to 0.74 ± 0.23 ($P < 0.0001$) and then 0.86 ± 0.15 ($P = 0.0071$). Significant reduction in areas of bone edema for femoral lesions ($P < 0.005$). The reduction of these areas is strongly correlated to the WORMS (Whole-Organ Magnetic Resonance Imaging Score) grading ($P < 0.005$). Four failures (2 PMG and 2 PTG) at 2 years of follow-up. Stimulation with I-ONE® therapy was able to reduce pain, increase knee function and avoid knee replacement surgery in 85.7% of patients. The treatment with I-ONE® therapy was effective in the early stages of SONK in a similar way to what has already been reported for the femoral head by Massari et al. (Massari L. 2006) Several clinical experiences report the success of I-ONE® therapy in the resorption of edema. The American Academy of Orthopedic Surgeons awarded and published the study conducted by the group of prof. Perugia of La Sapienza in Rome with I-ONE® therapy in patients with knee bone edema (Perugia D. 2015). The authors conclude that I-ONE® therapy represents a valid therapeutic approach for the resolution of bone marrow edema or the healing of localized cartilage lesion.

The problem of treating complex regional pain syndrome (CRPS) is as complex as that of determining the clinical setting of this syndrome for which the control of pain manifestations is a fundamental point. For this purpose, alongside the pharmacological treatment methods, it is certainly appropriate to pay attention to biophysical stimulation methods, whose effectiveness in controlling pain of various kinds is well documented. A recent article shows how the use of I-ONE® therapy in CRPS is based on the A_{2A} anti-inflammatory effect and the anabolic effects on bone tissue with a dual action on both osteoblasts and

osteoclastogenesis (Pagani S. 2017). Control of the inflammatory microenvironment is a prerequisite for successful treatment. Great efforts are needed to reduce the costs of CRPS in terms of patients' suffering and disability. Each improvement in treatment therefore represents a great result. In this sense, the combination of different strategies allows to obtain synergistic or additive effects both on anabolic factors and on the control of the inflammatory microenvironment. The Tuscany Region and the Society of Hand Surgery (Italy) have included the use of biophysical stimulation with I-ONE® therapy in the guidelines for the treatment of CRPS. Several positive clinical experiences on the use of I-ONE® therapy for the treatment of CRPS are published in the journal of Hand Surgery by Dr. Borelli (Borelli PP. 2017).

In a recent study, Notarnicola et al. showed that it seems reasonable to assume that I-ONE® therapy for CRPS-1 should be initiated as soon as possible, in order to act on the inflammatory component of the condition (Notarnicola A. 2021). If I-ONE® therapy and ESWT are shown to be beneficial, these therapies should be developed as the recommended methods to reduce pain and other CRPS-1 symptoms.

Similar results were found after treatment with I-ONE® therapy in patients with patello-femoral pain, a frequent pathology in sportsmen, especially in women, whose etiopathogenesis is controversial and can be traced back to various factors, including malalignment of the joint and dysplasia of the patella or of the femoral trochlea (Servodio Iammarrone C. 2016). Anterior knee pain, which accompanies this pathology, can become chronic over time and become highly limiting for sports practice. I-ONE® therapy has been shown to be effective in promoting joint function, solving pain symptoms and promoting a rapid return to sporting activity, maintaining the result at one year.

The use of I-ONE® therapy allows to act in a specific and targeted way (Varani K . 2021; Moretti L. 2023), focusing the effect exclusively on the joint, on the soft tissues, in the absence of side effects that are frequently associated with drug therapies.

All the studies analyzed reported no adverse effects, and good patient compliance to the treatment. Effects on pain management, swelling and local inflammation can have a positive impact on patient satisfaction and can facilitate a faster recovery, allowing a more intense rehabilitation protocol. In conclusion, the use of I-ONE® therapy in the early control of joint inflammation process during the first days after surgery should be considered an effective completion of the surgical procedure to improve the patient's functional recovery.

Pre-clinical research shows that treatment with I-ONE® therapy is anti-degenerative, helping to control local inflammatory phenomena and supporting cartilage repair processes in the clinical setting.

Clinical trials have been shown that I-ONE® therapy can therefore be used proactively as i) postsurgical treatment with the objective of quickly controlling local inflammatory response due to the surgery and, over the long term, to maintain the mechanical and biological properties of the cartilage or engineered tissue by means of an effective chondroprotective effect; (ii) postarthroplasty treatment to inhibit the inflammatory processes that affect the periarticular tissues and to avoid the development of chronic pain and functional limitations; and (iii) conservative treatment to limit the progression of a degenerative process such as osteoarthritis that comes with age and is accelerated by inflammatory and/or traumatic events.

The above overview clearly shows how the studies conducted with I-ONE® therapy in recent years have made the use of I-ONE® therapy a significant cultural heritage, based on solid scientific foundations. This cultural heritage is the result of a formidable research

activity carried out by the Italian orthopedic community, which has been able to address relevant and highly interdisciplinary scientific questions.

This extensive work carried out in the cartilage field has shown how the biological effects resulting from the exposure of a tissue to I-ONE® therapy are dependent exclusively on the physical characteristics of the signal used. The therapeutic effect on cartilage is also linked to the daily duration of treatment; clinical recommended 4 hours daily.

The preclinical and clinical evidence demonstrates that the A_{2A} adenosine agonist effect of I-ONE® therapy is able to inhibit the inflammatory response quickly and effectively, to prevent and / or slow down the degenerative phenomena occurring at the cartilage surface. I-ONE® therapy is used to prevent cartilage degeneration and to promote focal cartilage lesions repair. I-ONE® therapy is a safe therapeutic treatment that is simple to use, its use is well accepted by patients, without side effect.

All the studies analyzed reported no serious adverse event or complication, no side effects and good patient compliance to the treatment. All the studies analyzed confirm safety and performance of I-ONE® therapy. All known and foreseeable risks, and any undesirable side-effects, are minimized and are acceptable, weighed against the evaluated benefits to the patient and/or user arising from the achieved performance of the device during normal conditions of use.

The effects of I-ONE® therapy on chondroprotection, pain relief, functional recovery, health-related quality of life have a positive impact on patient satisfaction and enable faster recovery.

In conclusion PEMFs (I-ONE® therapy) could be an innovative physiologic alternative to the use of adenosine agonists as they can mediate the tissue-specific agonist effects without any desensitization and downregulation and all the above studies indicate that PEMFs (I-

ONE® therapy) could be a very interesting example of soft pharmacology for joint chondroprotecion (Borea A 2015).

The challenge now remains open not on ccondroprotection, but on chondro-regeneration.

4.2 Purpose

The present project has the ambition to realize a new medical device delivering specific parameters of PEMFs, for the treatment of inflammatory joint pathologies, which cause mild cartilage damage, not to be treated surgically, and aims to drafting the documentation for its validation in the clinical setting. The new medical device will be marketed in the "*Sports Medicine"* market for chondro-regeneration, and also as a usual conservative treatment for "*Joint Preservation*". The new medical device will target a wide range of subjects: professional and amateur sportsmen, who present with painful knee symptoms caused by mild cartilage degeneration.

The implementation of this device would entail a radical breakthrough in the scientific knowledge shared by the medical community to date, since the possibility of regenerating articular cartilage has always been denied (Jakob RP. 2001):

- in 1743 John Hunter stated*: "Cartilage injury is a troublesome thing and once injured is seldom repaired."*
- in 1853, Sir James Paget expressed a similar opinion when he stated 'T*here are, I believe, no instances in which a lost portion of cartilage has been restored or a wounded portion repaired with new and well-formed permanent cartilage in the human subject*"

The irreversibility of articular cartilage damage has thus become a *"dogma"* that the medical community has never questioned in the past. Recently new scientific findings challenged the *"dogma":* regeneration of articular cartilage and restoration of its function are now considered achievable goals. The role of synovial cells (synoviocytes, mesenchymal stem cells residing in the synovium and synovial fluid) and chondrocyte senescence in maintaining and restoring the integrity of articular cartilage has been recognized.

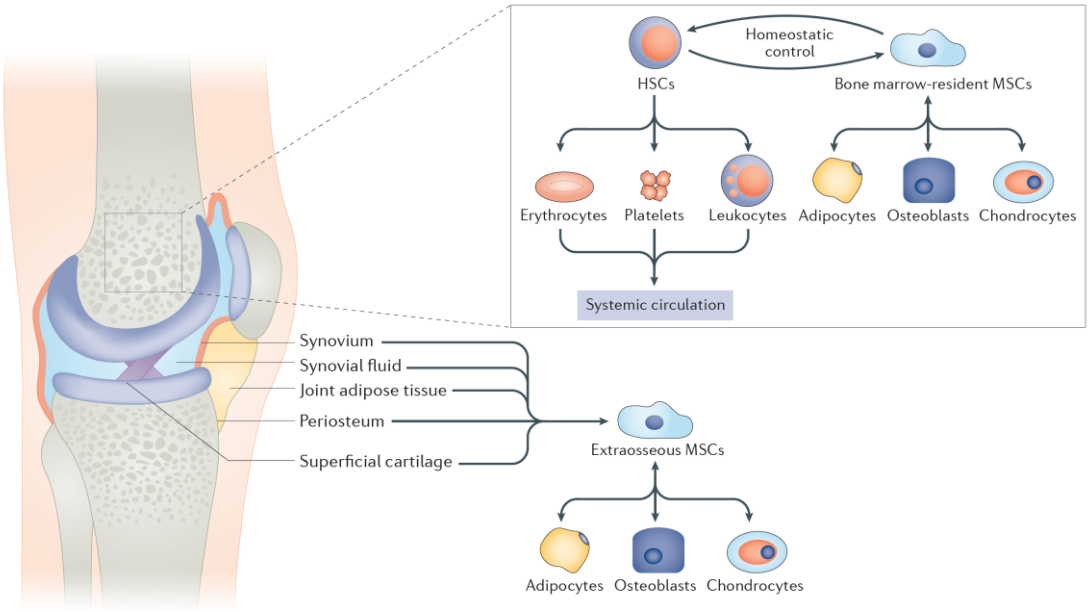


Figure 2a | Stem cells in the joint.

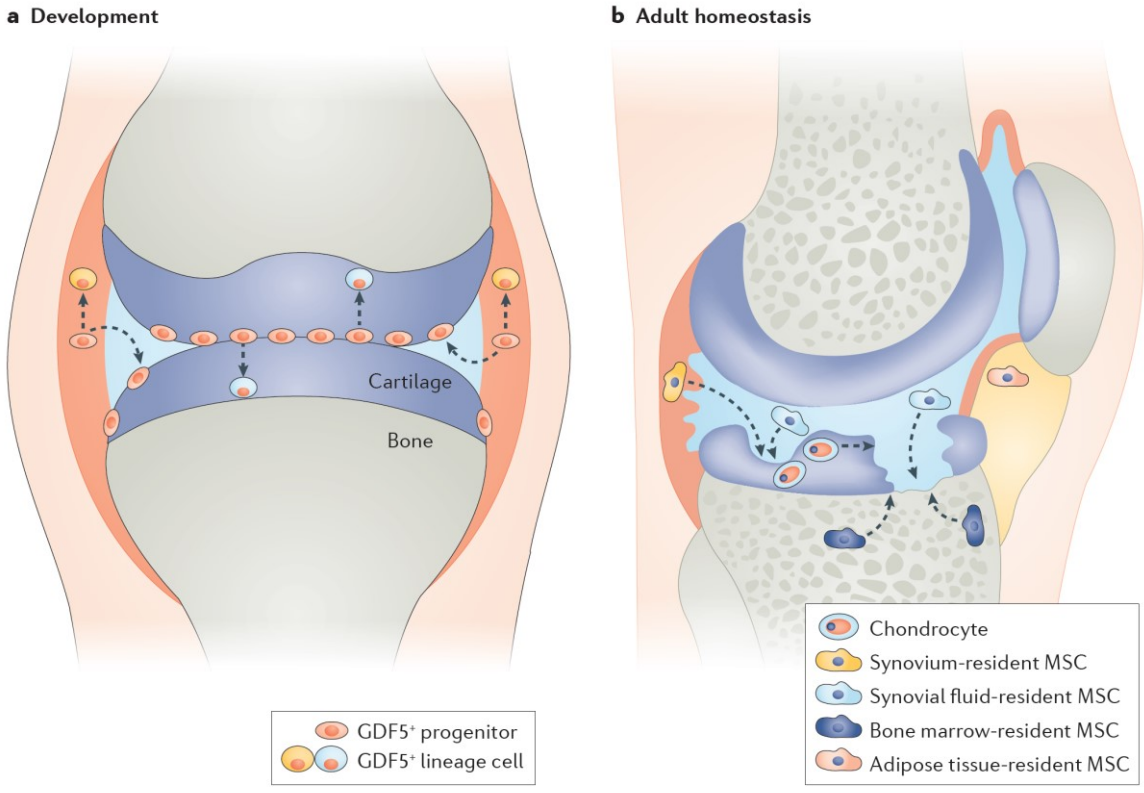


Figure 2b | Progenitor cells in joint development and cartilage repair.

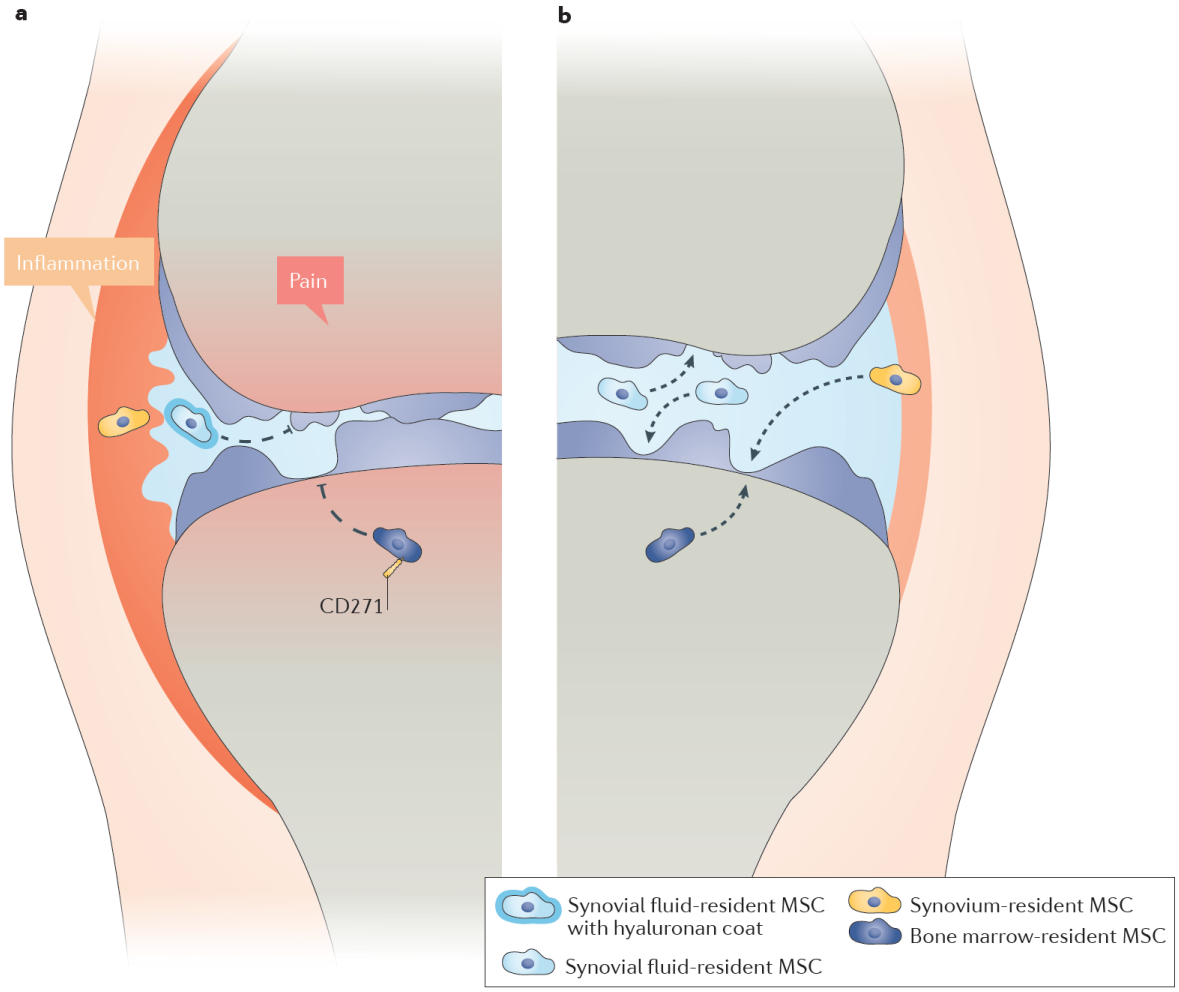


Figure 2c | Endogenous factors influencing mesenchymal stem cells in adult cartilage repair.

Figure 2 shows that limited cartilage damage can be repaired by synovial mesenchymal stem cell activity; more extensive cartilage damage with exposure of subchondral bone tissue also requires migration and contribution of bone marrow cells. These injuries are treated with arthroscopic treatments: microfractures, engineered tissues with or without autologous cell transplants. However, arthroscopic procedures are accompanied by an inflammatory response that, if not controlled, can adversely affect the clinical outcome.

For this goal, pharmacological research has focused on drugs with adenosine agonist action experimentally active on cartilage regeneration. However, they are not yet available for clinical use because the treatment is associated with significant adverse side effects.

The present project has the ambition to create a new medical device capable of promoting articular cartilage regeneration when cartilage damage has not yet deepened into cartilage thickness exposing subchondral bone tissue. My goal finds justification in the latest findings on the mechanisms that control cartilage regeneration, the cell populations involved, and more specifically the role that adenosine receptors may play.

Also of particular interest are the *in vivo* results of research conducted at the Istituti Ortopedici Rizzoli, on the osteoarthritis model in guinea pigs of the Dunkin Hartley strain (Fini M. 2005-2008). PEMF was employed in animals with knee cartilage damage. Histological analysis showed that in animals with advanced cartilage damage it was still possible not only to prevent its progression but also to promote regeneration of the cartilage itself (Figure 3). These results interpreted on the basis of recent insights into the role of joint-resident synovial cells suggest that biophysical stimulation with PEMFs can promote the regeneration of articular cartilage.

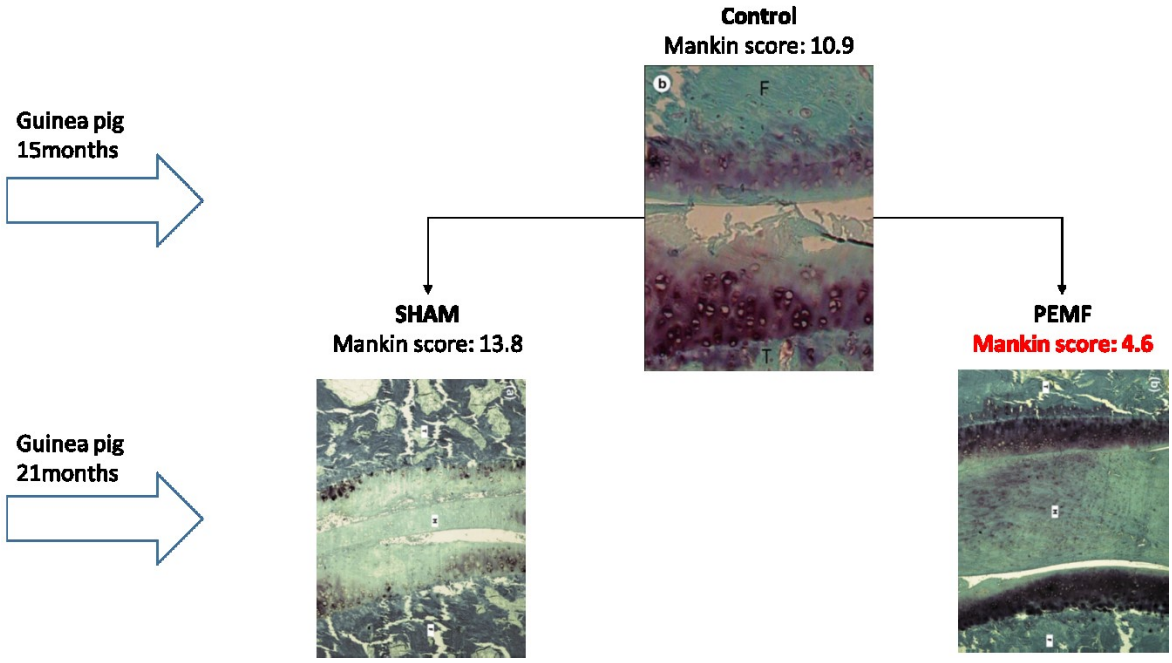


Figure 3. Efficacy of electromagnetic fields in the regeneration of articular cartilage

The action of PEMF on chondrocytes and synoviocytes in the presence of inflammatory cytokines such as IL-1β or TNF-α has been studied, and it has been shown that the chondroprotective effect derives from an inhibitory action on cartilage-induced damage by the inflammatory environment. The new knowledge reported above provides an opportunity to extend the indication for use of PEMF to cellular activities capable of exerting an anabolic action on articular cartilage by promoting its regeneration and reconstituting its integrity.

This change of perspective geared toward the development of a new PEMF device will make it possible to extend the target of treatment to the early stages of articular cartilage suffering, multiplying the number of subjects who will be able to benefit from the therapy. It can be used in the context of "*Sport Medicine"* by professional and amateur sportsmen and women. "*Sport Medicine*" is the medical-orthopedic area with the highest growth rate and the highest added value. Thus, treatment will not be limited, as now, to conservative or post-surgical for a chondroprotective effect, but can be repeated over time and managed directly by the sportsman to preserve joint function and regenerate limited cartilage injury.

The new knowledge developed within this research project will lead to the definition of clinical indications and provide the solid scientific basis necessary for the adoption of the new medical device.

The new medical device will be adopted by professional athletes but also by still active individuals who play noncompetitive sports but have clinical signs of cartilage suffering in its early stages (Figure 4).

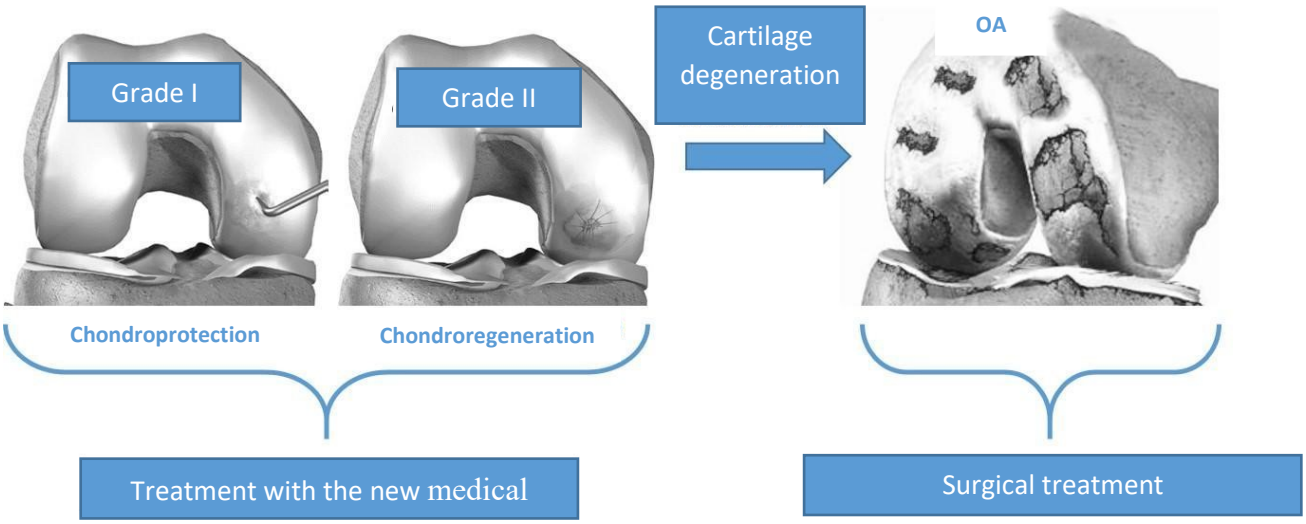


Figure 4. Cartilage injury classification and area of focus of the new medical device.

This condition is not only widely prevalent but still lacks an effective treatment proposal. In this context, an effective, non-invasive, side-effect-free and repeatable treatment has undoubted advantages that will encourage its choice and adoption. The therapeutic effect and expected technological developments will allow for maintaining the bond with the sportsman and foster his loyalty.

Chondropathies, pathologies affecting cartilage, in the sportsman represent a very current topic of great interest to the orthopedic surgeon, consider that in 90% of athletes in the NBA (National Basketball Association) cartilage problems are found during knee MRIs.

Despite the presence of several treatments (conservative or surgical) available, cartilage problems in athletes represent a complex and still partially unresolved question. This is because, the average increase in age poses problems of wear and tear in subjects too young to be targeted for joint replacement surgery, and in sportsmen, a cartilage damage can be repaired, improved with increasingly refined and evolved surgical techniques, but it can never reconstitute the original cartilage, damaged by trauma or wear and tear.

These issues and the new scientific knowledge that has recognized the role of mesenchymal stem cells residing in the synovium and synovial fluid and chondrocyte senescence in maintaining and restoring the integrity of articular cartilage, have allowed a focus on the concept of *'Joint Preservation'*.

'Joint Preservation' should be understood as a therapy that can prevent and/or delay the onset of chondral disease, with a view to broadening the spectrum of intervention to a stage where the joint can still be preserved. Obviously '*clearing'* the notion that articular cartilage possesses limited regenerative capacity due to the absence of blood vessels and the reduced mitogenic potential of chondrocytes will be a challenge. However, through advanced and innovative preclinical studies, which will be carried out in parallel to the present project by the University of Ferrara, Istituto Ortopedico Galeazzi and Istituto Ortopedico Rizzoli, it will be possible to provide the scientific community with evidence of the action of PEMFs on cartilage regeneration. In particular, preclinical research will focus on studying the effect of PEMFs, delivered by prototype devices made in this research project, on different cell populations in the joint environment, such as chondrocytes and synovial cells. The effect of stimulation with PEMFs on metabolic activities and intracellular pathways involved in the processes of proliferation, apoptosis and senescence will be evaluated. In addition, the production of extracellular mediators will be studied by analysis of secreted molecules (or secretome) using broad-spectrum analyses such as proteomics and transcriptomics. An

animal model suitable for the study of cartilage degeneration will be developed, reproducing a condition of cartilage suffering. This model will allow the study of the effects of PEMFs, delivered with appropriate prototype medical devices for animal models, on the aspects of senescence, apoptosis, and inflammation that characterize cartilage degeneration. Overall, the pre-clinical research phase of the project will allow the characterization of the effect of PEMFs on key biological processes that regulate cartilage degeneration and the identification of specific PEMF signal characteristics that can counteract these processes and promote cartilage regeneration. The PEMF signal parameters thus identified will be generated by the new PEMF device, the subject of this research project, and then documentation will be prepared for its use in the clinical setting, according to the new European Medical Device Regulation (MDR), which came into effect on May 26, 2021, replacing the previous Medical Device Directive (MDD).

The goal is to realize a new PEMF-delivery medical device consisting of a portable signal generator powered by a rechargeable battery and equipped with a touch screen display. The study of the usability issues of the product will lead to the identification of the most appropriate size of display, resistive or capacitive, for the intended use. The use of the medical device at home will involve the study of all the technological solutions necessary to comply with the specific regulatory requirements provided for these product categories, such as electrical insulation, protection from electrostatic discharges, limits on leakage currents, limits on electromagnetic emissions, and specific requirements relating to the degree of protection of the casing against the penetration of solid foreign bodies and liquids.

The device will be managed by a state-of-the-art microcontroller equipped with an operating system, which will enable efficient and integrated control of all functions.

The applied part of the device will consist of a copper coil powered to generate the PEMF. Issues related to the study and development of different types of coils, that should be able to

adapt to various anatomical sites and be powered by treatment protocols stored in the microcontroller's nonvolatile memory, will be addressed. The physical construction characteristics of the coils will have to make them sufficiently light and moldable to limit as much as possible potential discomfort during the treatment phase.

The device is planned for individual use and will be equipped with a biometric sensor that will be able to recognize the sportsman by reading the fingerprint. The different technological solutions available will be evaluated both from the point of view of the technology used for secure fingerprint acquisition and management and the interfacing with the microcontroller.

The device will also have the ability to connect in Bluetooth (Bluetooth Low Energy, BLE) mode with a smartphone. An application will be developed on the iOS and Android platforms that the sportsman can download from his or her app store and that will allow for augmentation of the information provided by the device related to the ongoing therapeutic course. The mobile application will also contain evaluation forms that will allow the sportsman to perform an anatomical site-specific clinical self-assessment.

In addition, again through the mobile application, a remote control system of the device will be implemented, which, by connecting to the IGEA server, will be able to verify the proper functioning of the device. Finally, the sportsman will be able to use the developed protocols to schedule treatment with PEMF at different anatomical sites according to the indications identified during clinical trials.

Applications in the device design phase will not be intended only for the professional sportsman, or for preventive health purposes. They will also be able to interact with other applications from the systems hosted by the target smartphones, integrating the data collected and, through the calculation of the "Fitness Index," providing parametric information to resident applications (such as may be "Health" for iOS). Interaction with

information from applications that monitor lifestyle, nutrition, physical activity, mindfulness will be possible, if not directly from other sources, such as could be wearable technologies, gymnastic workout tools, and bitcoin systems deployed in wellness and fitness centers.

The objectives of this research project pursued over the 3 years of the doctoral program can be summarized as:

- **Study, preparation and development of prototype devices for preclinical research**
    - Study, design and development of signal generator and coil prototypes for *in vitro* stimulation.
    - Study, design and development of signal generator and coil prototypes for stimulation of animal models.
    - Molecular dynamics modeling of PEMFs on preclinical models.
- **Study, preparation and development of the new medical device**
    - Study of technological problems and possible solutions at the hardware and software level of the signal generator.
    - Design and development of the final components (hardware, software, and coil coating materials for different joints) of the device.
    - Study and definition for the optimization of the communication protocol between applications, the new device and other applications dedicated to IOT (Internet of Things) devices.
- **Study, preparation and development of documentation in clinical settings**
    - Study and preparation of self-assessment forms.
- **Documentation for clinical study to be submitted to the ethics committee.**
    - CIP study and preparation.

4.3 Objectives achieved

4.3.1 Study, preparation and development of prototype devices for preclinical research

Study, design and development of signal generator and coil prototypes for in vitro stimulation.

Stimulation systems capable of generating a PEMF, with peak magnetic field values between 1 and 3 mT, frequency of 75 Hz, and pulse duration of 1.3 ms have been realized.

Given the nature of the project, which aims to optimize the intensity of PEMF to act in the processes of senescence, apoptosis, and autophagy of the cells that make up joint tissues, it was decided to observe the response of cells *in vitro* to two different intensities of PEMF: 1.5mT and 2.5mT. This will make it possible to evaluate a dose-response effect that will allow the most appropriate signal to be determined for the target patients of the new medical device.

Two different coils were made for this *in vitro* exposure systems:

i) standard double coil with 1400 turns per coil, copper wire diameter equal to 0.2mm, coil size 25 x 17 cm, resistance equal to 300 ± 24 Ohm for exposure at 1.5 mT;

ii) double intermediate coil with 1000 turns per coil, copper wire diameter equal to 0.25 mm, coil size 19.5 x 13.5 cm, resistance equal to 300 ± 24 Ohm for exposure equal to 2.5 mT.

The magnetic field gradient in the different exposure modes was analyzed by FEM analysis with COMSOL software (Figure 5).

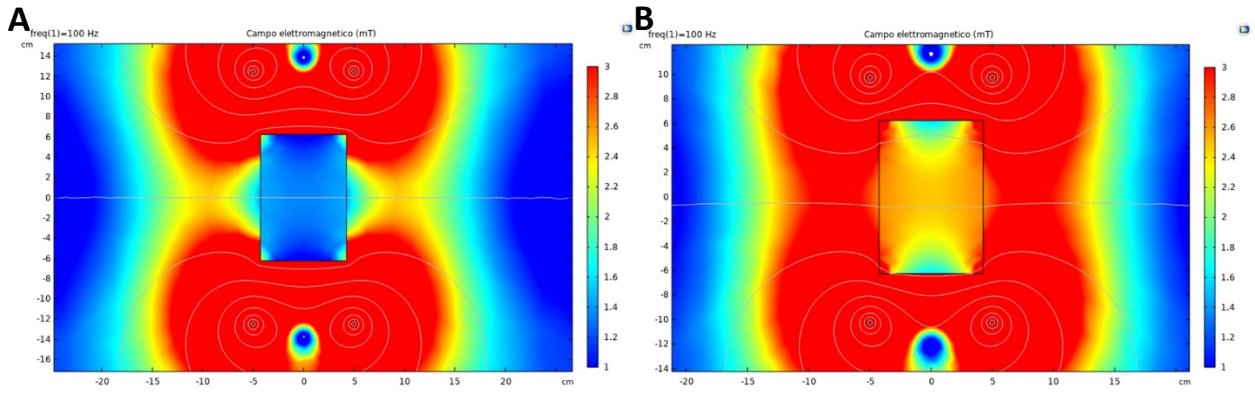


Figure 5. Modeling of the magnetic field gradient in the 1.5 mT configuration (panel A) and the 2.5 mT configuration (panel B).

The homogeneity and characteristics of the generated PEMF were verified using a measurement system consisting of a gaussmeter (Lake Shore mod. 425 Gaussmeter) and corresponding Hall probe effect (Lake Shore probe mod. HMNT-4E04-VR). The corresponding peak amplitude of the induced electrical voltage is detected using a coil probe, and the time course of the signal was displayed using a digital oscilloscope (Rohde & Schwarz - HAMEG mod. HMO3054 - Bandwidth 500 MHz - 4GSa/s).

Figure 6 shows the peak magnetic field strength and corresponding peak amplitude of induced electric voltage for the *in vitro* exposure systems at 1.5 mT (panel A) and 2.5 mT (panel B).

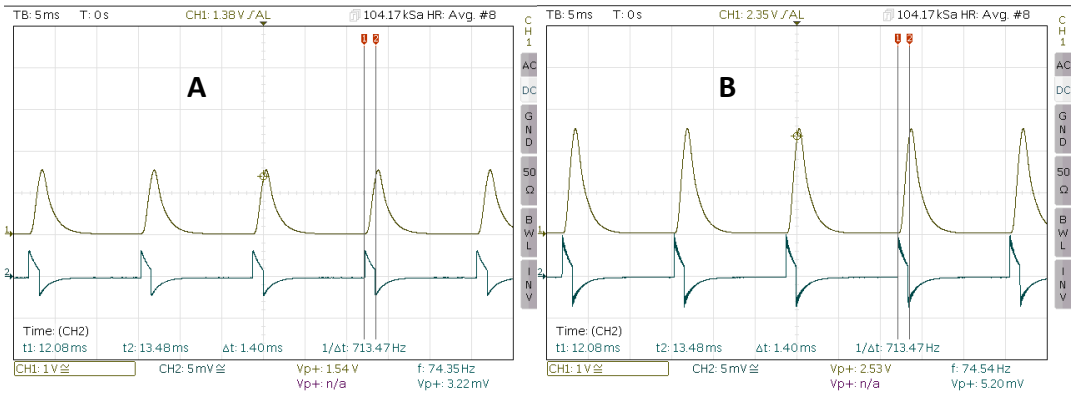


Figure 6. Peak magnetic field strength and peak amplitude of induced electric voltage for *in vitro* exposure systems at 1.5 mT (panel A) and 2.5 mT (panel B).

To ensure the homogeneity of PEMF on the cell cultures, plexiglass supports were used to house the cell cultures within the exposure system. In order to rule out thermal effects on the cell cultures, the temperature inside the plexiglass supports was monitored.

These measurements revealed that within the *in vitro* cell culture exposure systems set at 1.5 mT and 2.5 mT and housed in the respective plexiglass holders, a temperature increase of +0.3° C and +1.5° C occurs, respectively (Figure 7).

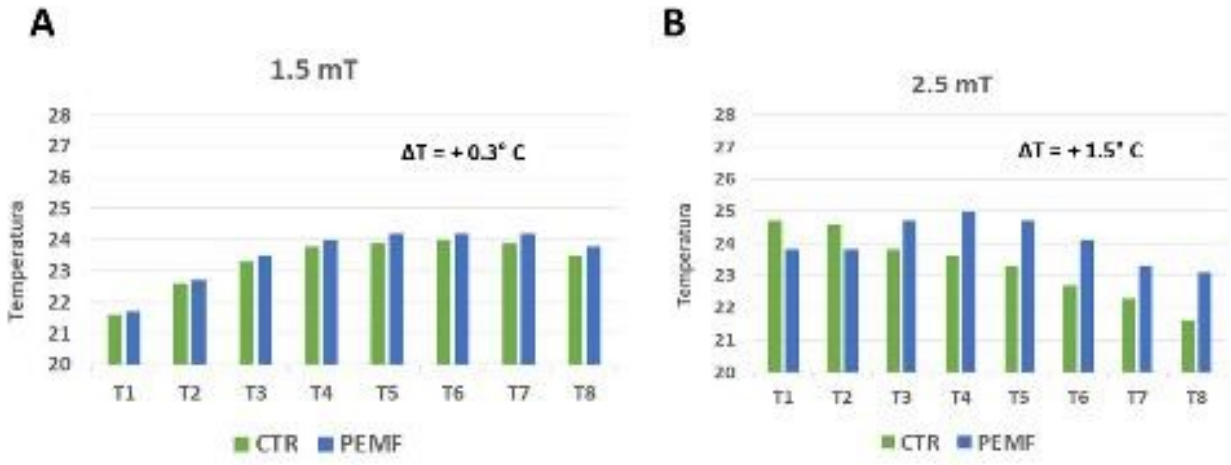


Figure 7. Temperature monitoring within *in vitro* exposure systems at 1.5 mT (panel A) and 2.5 mT (panel B).

For the purpose of controlling the temperature inside the *in vitro* exposure systems, plexiglass holders were made to allow air exchange between the inside and outside of the exposure system and to have a shelf for cell cultures not in direct contact with the coils (Figure 8).

Figure 8. Plexiglass supports for *in vitro* display systems.

Temperature measurement inside the new Plexiglas holders with exposure systems set at 1.5 mT and 2.5 mT revealed a temperature rise of + 0°C and + 0.4°C, respectively (Figure 9). A temperature increase of + 0.4° C is considered acceptable because it is easily compensated by the forced air ventilation and temperature control system inside the cell culture incubator.

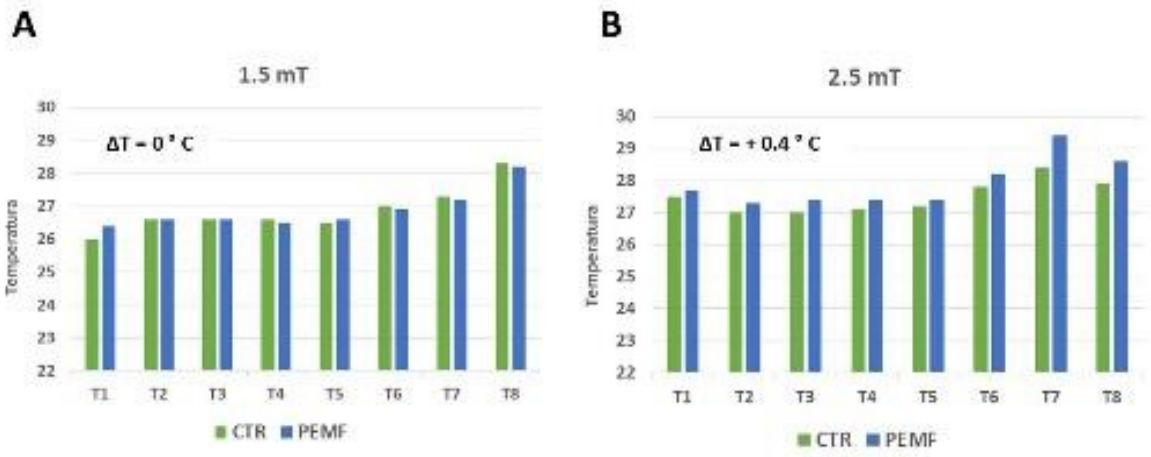


Figure 9. Temperature monitoring within the new *in vitro* exposure systems at 1.5 mT (panel A) and 2.5 mT (panel B).

Therefore, 6 *in vitro* exposure systems (3 set at 1.5 mT and 3 set at 2.5 mT) were set up (Figure 10), and no temperature changes were detected following the installation of the exposure systems inside the cell culture incubator.

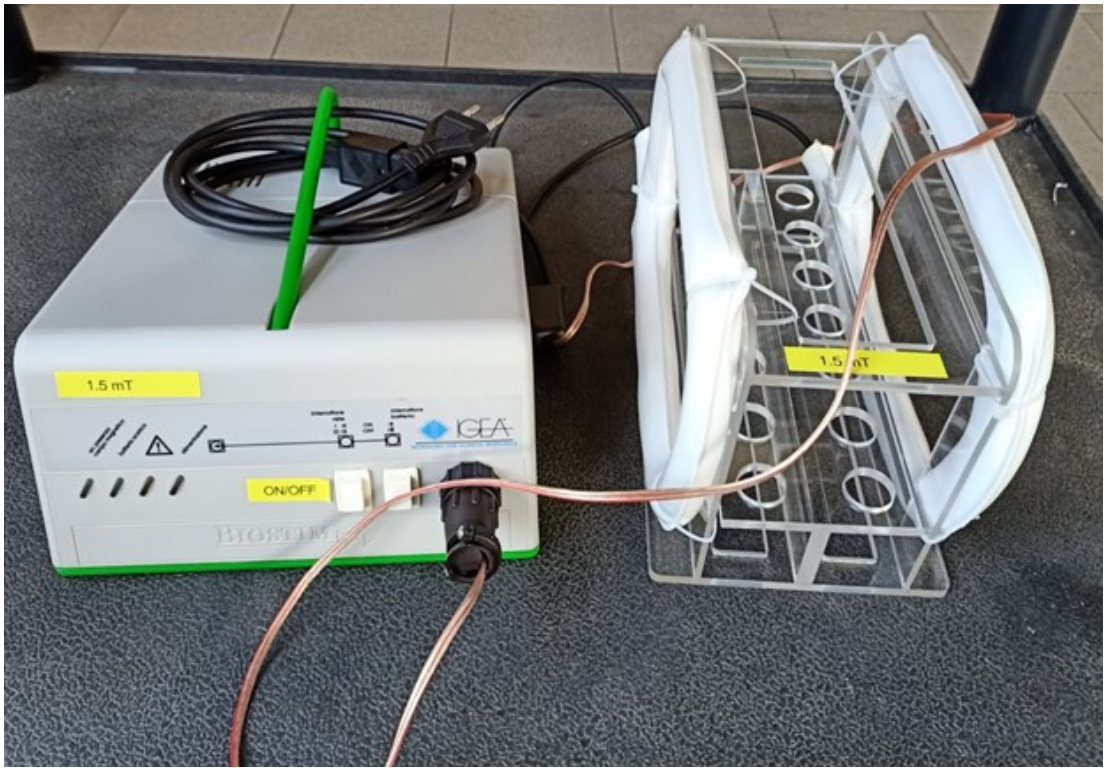


Figure 10. Example of an *in vitro* exposure system.

The final experimental set-up is thus composed as follows:

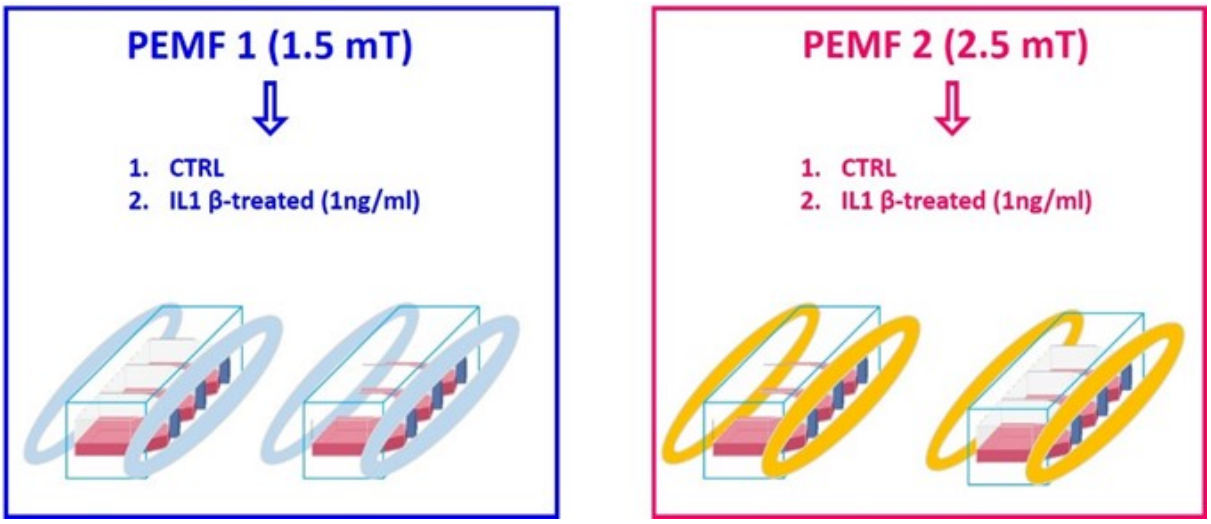


Figure 11. Schematic representation of the experimental set-up

In an effort to optimize the number of treatments/exposures to PEMF of the isolated cells following the set-up outlined above, prototypes were made to properly accommodate the culture systems and expose the cells to uniform PEMF (Figure 12).

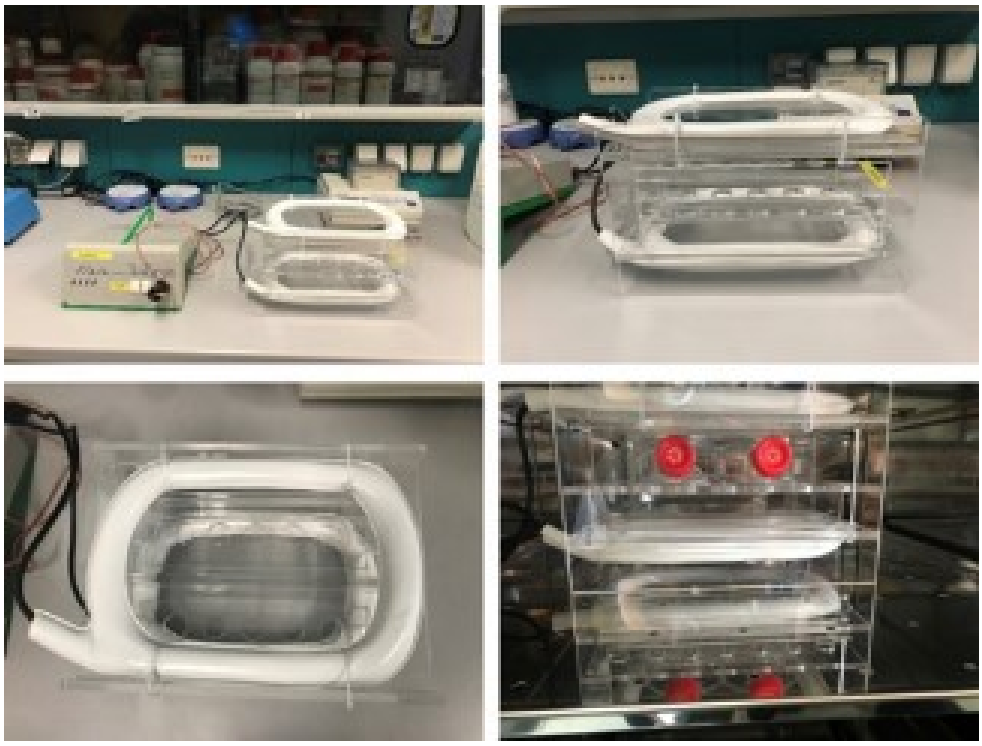


Figure 12. Prototype realization of the experimental set-up

After making some prototypes, the final model setting for treatment was identified. Specifically, the PEMF treatment will involve exposing the three cell types isolated from each donor to the two PEMFs (1.5mT, 2.5mT; 75Hz frequency). Control cells, not exposed to PEMFs, will be represented by the same cell populations maintained under the exact same conditions except for PEMF stimulation. PEMF stimulations were performed by housing the flasks, intended to receive this type of treatment, in the appropriate devices, as shown in Figure 13. *In vitro* exposure systems adapted for the housing of cell culture flasks were studied and implemented in order to enable the recovery of the amounts of culture medium needed to perform cell secretome analyses. Thus, No. 4 *in vitro* exposure systems were set up.

Figure 13. Prototype realization of the experimental set-up

Study, design and development of signal generator and coil prototypes for stimulation of animal models.

The *in vivo* exposure system for carrying out the activities at Istituto Ortopedico Rizzoli was also developed. For the *in vivo* exposure, a custom coil of the size equal to the base of the Guinea Pigs' housing cage was made with the following characteristics: 1300 coils, copper wire cross section equal to 0.25mm, average coil length 80 cm, coil size 23 x 17 cm (Figure 14).

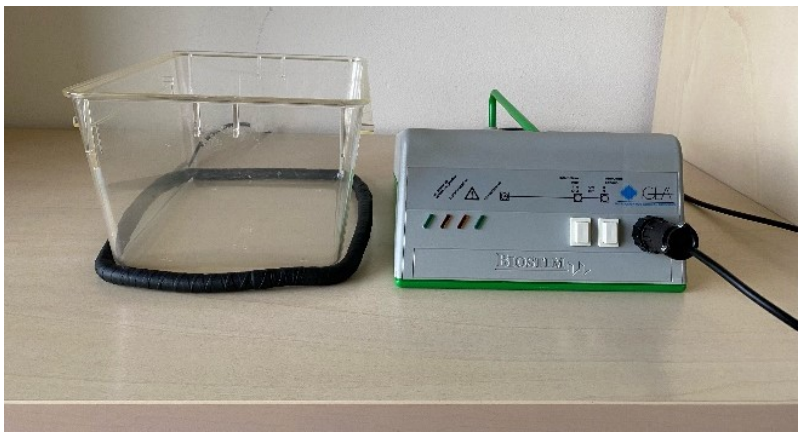


Figure 14. Example of an *in vivo* exposure system.

By means of Hall probe, measurements of the electromagnetic field strength inside the cage were taken; the measurements showed an average magnetic field strength of 2mT inside the guinea pig housing cage (Figure 15).

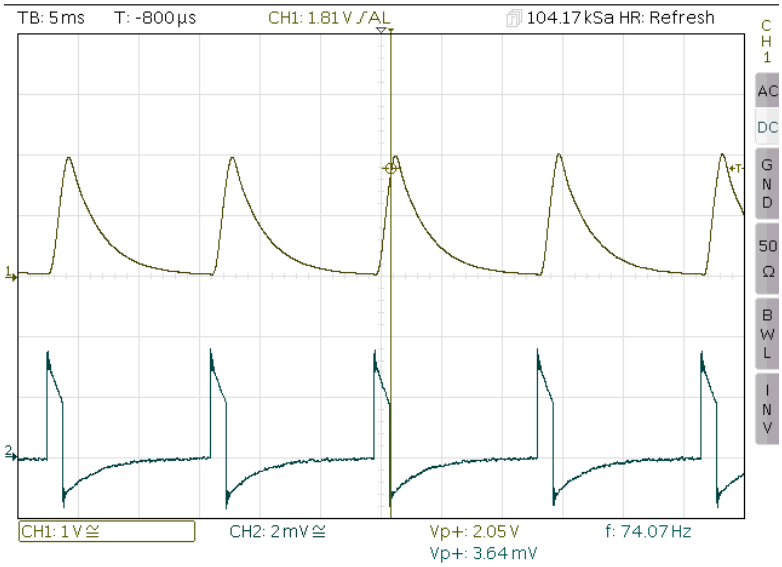


Figure 15. Peak magnetic field strength and peak amplitude of induced electric voltage for *in vivo* exposure systems.

Finally, temperature control was performed inside the cage to ensure the well-being of the guinea pigs during the experiment. Temperature measurements revealed that a temperature increase of + 0.3°C occurs inside the cage in the presence of PEMF of an average intensity of 2 mT (Figure 16). This temperature increase is considered acceptable for animal welfare, as guidelines report a range of 4°C for the ideal rodent housing temperature (ambient temperature between 20 - 24°C).

Therefore, 15 *in vivo* exposure systems were made and delivered to the Rizzoli Orthopaedic Institute's enclosure. These display systems will allow the simultaneous treatment of at least 12 Guinea Pig guinea pigs.

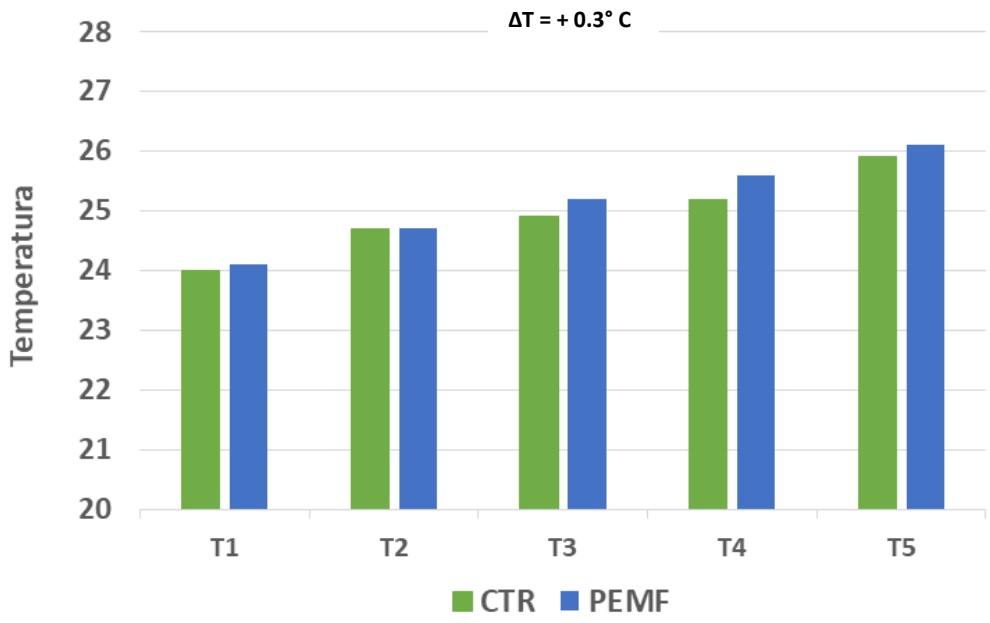


Figure 16. Temperature monitoring inside the housing cage in the presence of PEMF of 2 mT intensity.

Molecular dynamics modeling of PEMFs on preclinical models

The A_{2A} receptor for adenosine belongs to the family of G protein-coupled receptors also known as seven transmembrane domains (7TM) receptors. Recently, by nuclear magnetic resonance spectroscopy studies targeting the TM5 and TM6 transmembrane domains, showed that the adenosine receptor A_{2A} is present in two inactive conformational states (S1 and S2) and two active conformational states S3 and S3' (Ye L. 2016). The presence of a agonist receptor stabilizes the active conformation S3'. This conformation is able to interact with and bind G protein and thus activate receptor A-mediated signal transduction$_{2A}$.

In order to evaluate whether and how PEMF affects the conformation of the adenosine A_{2A} receptor, the following molecular dynamics issues were addressed:

- Identification of the most appropriate molecular model for modeling adenosine receptor binding;
- Identification of the effect of PEMF on the adenosine A_{2A} receptor binding site.

These investigations were carried out in collaboration with the research group directed by Prof. Liberti of "La Sapienza" University of Rome, taking advantage of the computational power and software available at the university laboratories. The investigation focused on the action of PEMF on the A_{2A} receptor binding site: in particular, the effect of the magnetic field B on the geometric fluctuations of six amino acid residues (Ser67, Glu169, Ala265, His264, Pro266, Leu267) crucial for the binding process of adenosine to the receptor was evaluated by the covariance matrix method.

Thus, a region was identified, represented by the yellow ellipse in Figure 17 that approximates the adenosine binding site on the A_{2A} receptor. Within the observation time of 270ns, the magnetic field exerts a significant force on specific amino acid residues.

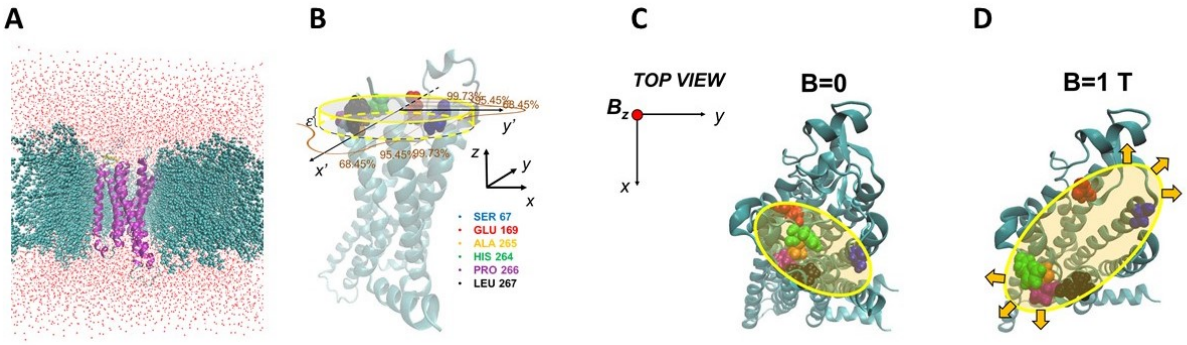


Figure 17. Effect of PEMF on the adenosine receptor binding site. A_{2A} receptor for adenosine (A); amino acid residues critical for the process of adenosine binding to the receptor (B); conformation of the adenosine binding site on A_{2A} receptor in the absence (C) and presence (D) of PEMF.

One of the most affected residues is Glu169, which is important for receptor stabilization through the formation of a salt bridge with the His264 residue. Preliminary molecular modeling data suggest that exposure to PEMF is able to change the geometry of the adenosine binding region at the A_{2A} receptor.

The display systems set up for pre-clinical research activities allow analog-type modulation of the parameters that make up the signal: amplitude (Figure 6), frequency (Figure 18, panels A-C) and pulse duration (Figure 18, panels D-F).

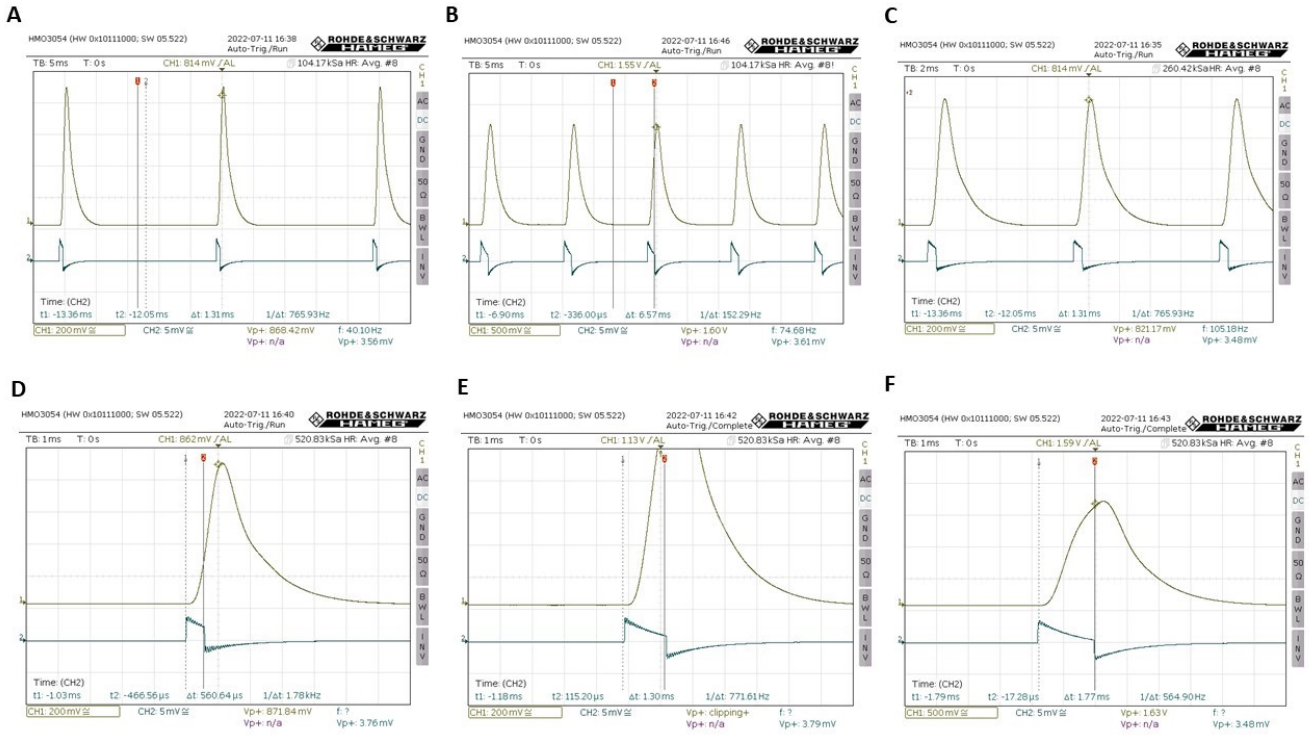


Figure 18. Modulation of signal frequency(A-C), and pulse duration(D-F).

Regarding the molecular dynamics modeling of the effect of PEMFs on the adenosine receptor, molecular modeling data suggest that PEMF exposure is able to change the geometry of the adenosine binding region at the A_{2A} receptor specifically identifying 2 states of the receptor:

- a state with smaller binding site area (which we termed "closed")
- a state with larger binding site area (which we termed "open") (Figure 17, panels C and D).

Using molecular modeling techniques, 10 simulations of A_{2A} adenosine receptor were performed in the presence and absence of magnetic field (Figure 19, panels A and B). These modelings allow measurement of the time that A_{2A} receptor spends in the two different states. The results show that exposure to PEMF results in an increase in the time that A_{2A} receptor spends in the open state (Figure 19 C).

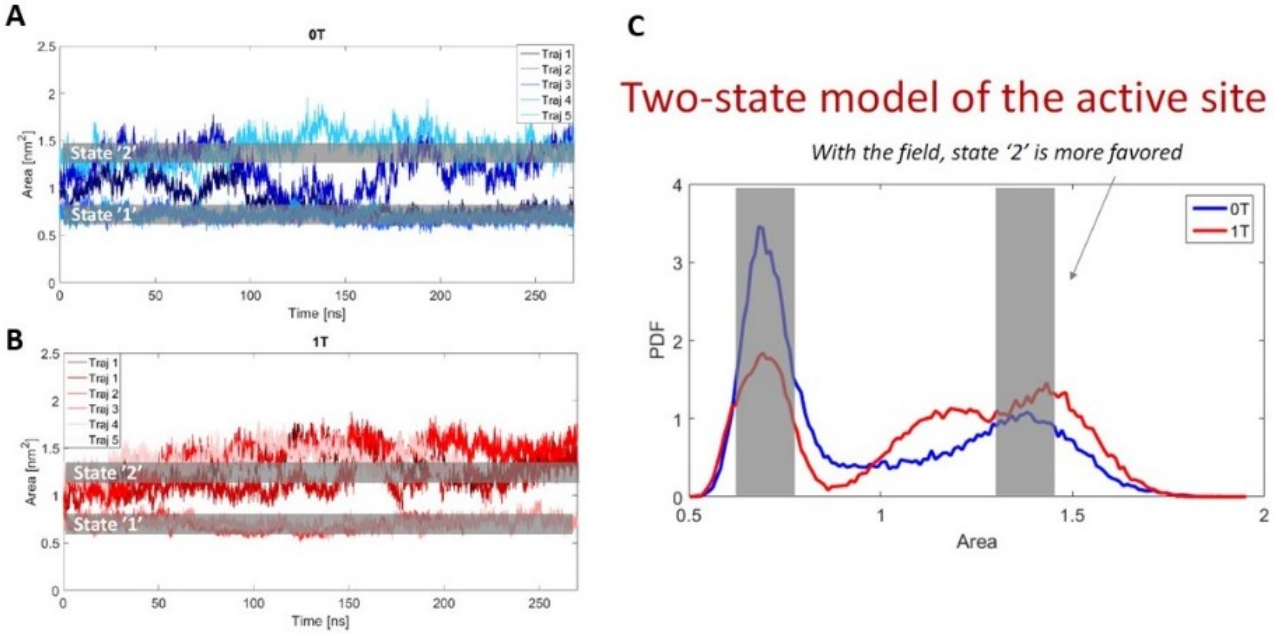


Figure 19. Molecular modeling of A_{2A} receptor in the absence (A) and presence (B) of magnetic field. (C) Two-state model of the binding site of A_{2A} receptor for adenosine.

4.3.2 Study, preparation and development of the new medical device

Study of technological problems and possible solutions at the hardware and software level of the signal generator.

A study was performed to identify the most effective technological solutions to the hardware and software issues of this new medical device for joint cartilage regeneration based on the principle of PEMFs.

After careful analysis, the specifications to be implemented in the new device were confirmed: the generator of the new device will be able to deliver up to 5 treatment protocols and manage 3 different coils per treatment site.

The signal generator will be managed by a state-of-the-art microcontroller with ARM Cortex-M4 architecture capable of running a real-time embedded operating system, will be powered by a rechargeable lithium battery, and will be equipped with a color touch-screen display.

It will also be equipped with a wireless communication module to connect with modern smartphone-type cell phones.

The wireless connection will allow the device to communicate treatment data to an application that will be developed ad-hoc for iOS and Android platforms.

Through the mobile application, it will be possible to implement a remote control system for the device, which, through connection to the server of the company manufacturing the new medical device, will be able to check the proper functioning of the device and update, if necessary, the treatment protocols according to the indications identified during clinical experiences.

This research phase related to the signal generator then focused on the main elements shown in Figure 20.

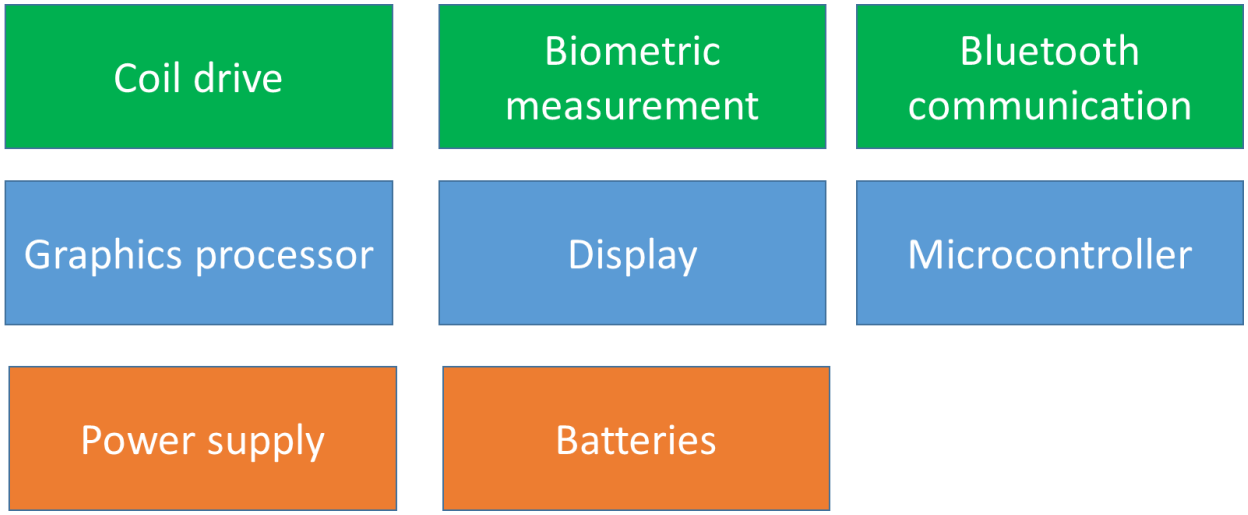


Figure 20. Functional blocks of the generator of the new device

An analysis of the functional blocks is given below:

a) Evaluation of the most suitable microcontroller and graphics processor

Selection of the most suitable microprocessor is critical because the microcontroller is the control center of the entire device. The focus has been on next-generation microcontrollers with ARM Cortex-M4 architecture because they feature low power consumption, low cost, high efficiency, and because they offer advanced digital control performance that makes them ideal for applications where there is a need to generate digital and analog signals. Among the various manufacturers of microcontrollers with ARM Cortex-M4 architecture (to date, we find chips from Freescale, NXP, ST, TI and Silicon labs), a model from ST (STMicroelectronics) and in particular the STM32F429VIT model was selected. Although the various manufacturers offer the same ARM architecture, the internal equipment of each microcontroller model varies in speed of execution, size of flash memory, RAM memory, types of peripherals available (ADC converters, DAC converters, digital outputs, RTCs, timers, counters, PWM outputs, etc.): the ST model was chosen over the others precisely

because of these features. In addition, ST offers developers a rich set of evaluation boards for its microcontrollers (called "evaluation boards") along with software development tools such as the "STM32 Cube MX," which allows one to graphically "draw" the configuration of the microcontroller's internal peripherals and directly translate that configuration into C-type source code that can be directly used in the development of the device's firmware.

Another key element of this phase of the research was the selection of a graphics processor with which to implement the device's graphical user interface ("Graphical User Interface" GUI). The graphics processor selected was a chip from FTDI mod. FT813, which is an "Embedded Video Engine" capable of handling a color TFT display with capacitive touch-screen and also offers audio output for acoustic feedback from the device.

b) Choice of display

The characteristics of displays from different manufacturers (Ampire, Multi-Inno Technology, Topovision Technology, and Riverdi) were analyzed, and different sizes were evaluated, keeping in mind that the ultimate goal is to create a portable device with palm-sized compatible dimensions. Among the various displays analyzed, the Riverdi mod. RVI43ANTNWC03 display was chosen for the first prototypes, a 4.3-inch color TFT display with 480 x 800 resolution, with IPS technology (which allows for a "black" background when the display is not powered) driven directly by the FT813 graphics processor with the 24 RGB signal lines. This display also has a capacitive touch-screen also driven by the FT813 graphics processor.

c) Choice of batteries and power supply

Battery selection was oriented toward lithium batteries (lithium ion or lithium polymer). This choice was followed by the selection of the battery output voltage and internal capacity in terms of mAh available to power the circuit. Based on the voltage required to power the coil drive section, the battery pack output voltage of 11.1V obtained by connecting 3 lithium

cells of 3.7V each in series was chosen. Relative to capacity, a battery pack with a minimum capacity of 1800 mAh was chosen to be selected in order to deliver a treatment of 4-6 consecutive hours. To monitor the efficiency of the batteries, a battery "health status" monitoring section was implemented using the MAXIM mod. MAX17205 integrated unit, which is a "FuelGauge." An external medical grade power supply with wide range input 100-240 VAC 50-60 Hz with output equal to 15VDC - 2A was selected to charge the internal lithium battery. A power supply from SL Power company mod. ME30A1541B01 was selected which is a medical grade power supply complete with CB Test Report internationally recognized by testing laboratories and notified certification bodies.

d) Coil drive

This phase of research focused specifically on the use of a new output voltage generation system applied to the coils by means of a Texas Instruments mod. TPS61175 survoltor working in switching mode.

e) Choice of biometric measurement

The research focused on a biometric measurement made through a fingerprint sensor. The phase of researching possible manufacturers of these biometric sensors led to the identification of the company Fingerprint Cards AB from which samples were purchased for testing on early prototypes of the device. The following figure shows the fingerprint sensor attached to its evaluation card.

Figure 21. Fingerprint sensor of Fingerprint Cards AB

f) Choice of Bluetooth communication module

Among the various technologies available today for wireless communication, the research considered both wi-fi and bluetooth connections, and considering the energy-saving requirements of the device and the multi-connection flexibility offered by smartphones for bluetooth, the research focused in particular on Bluetooth Low Energy (BLE) technology, which has undergone great development in recent years. Several BLE modules from different manufacturers U-BLOX, Panasonic and Microchip were evaluated and tested: the research phase started with the mod. NINA-B112 module from U-BLOX and then landed on the mod. RN4871 module from Microchip.

g) Choice of microcontroller software/firmware development environment

The writing of the microcontroller management software was done using the Keil MDK-ARM development environment, which has the platform called µVision as its user interface. The operating principle of the device is that of a "state machine" working with several concurrent threads managed by means of a real time operating system RTX based on a round-robin algorithm and priority. The following two figures show the structure of the development environment and the tools that the real time operating system provides to

manage the microcontroller's resources and enable data exchange between the various threads.

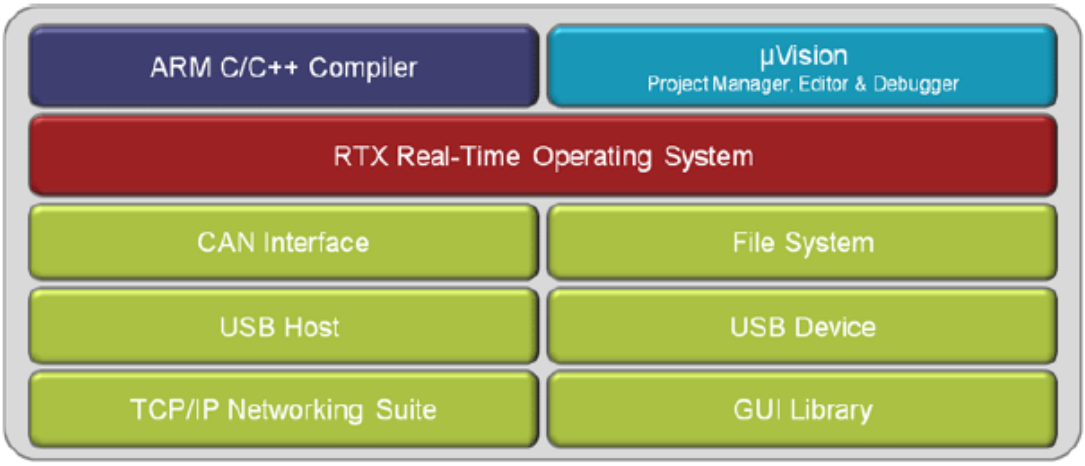


Figure 22. Keil MDK-ARM development environment.

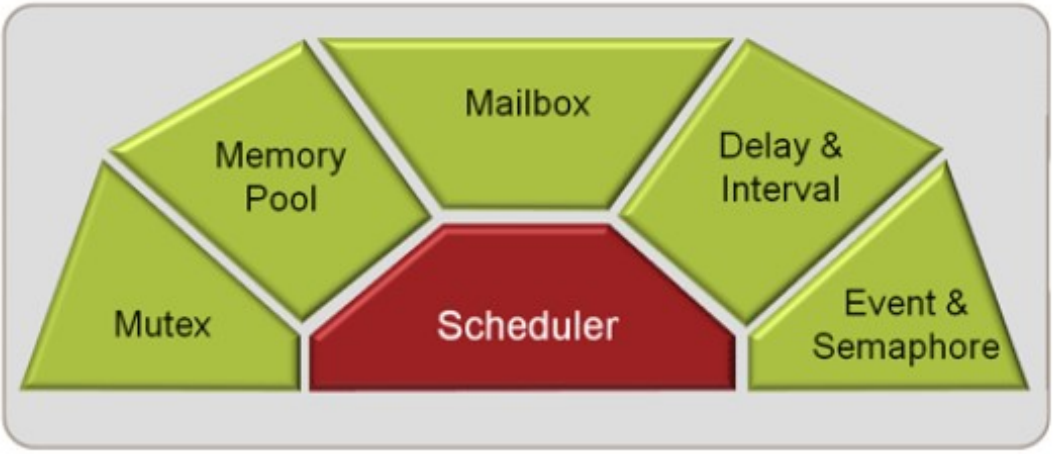


Figure 23. Tools of the Real Time Operative System

The research activity laid the foundation for the development of a first prototype consisting of the evaluation board of the selected microcontroller and a first electronic board with the graphics processor for driving the color capacitive touchscreen display, the coil drive section, and the Bluetooth BLE module (the BLE module of the U-BLOX mod. NINA B112 was used in this first phase).

The following figure shows a photograph of this first working "spun" system in which the switched-on display is already visible.

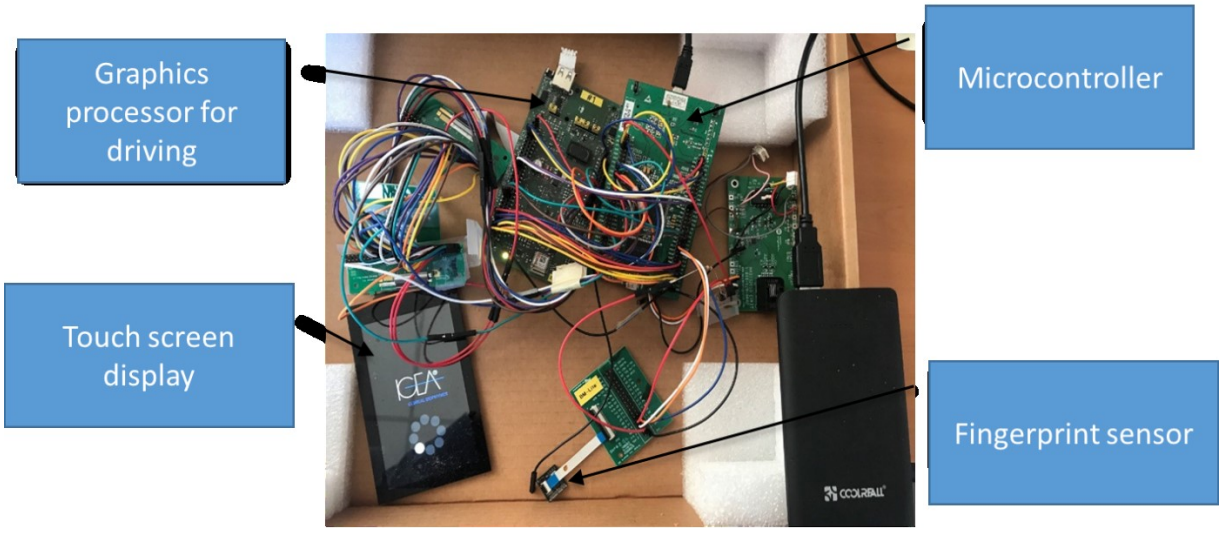


Figure 24. Evaluation board of the microcontroller and circuit board with graphics processor

This first prototype made with the evaluation board allowed us to gain experience with all the new elements of the device. Special attention required the programming of the microcontroller, the graphics processor with touch-screen management, the generation of the coil drive signal, the programming of the bluetooth module (which was used for the first internal tests of wireless communication with smartphones based on the two most common operating systems iOS and Android) and the fingerprint recognition module.

The next step was to make a new electronic board, this time complete with an on-board microcontroller, which became the first real prototype of the new device.

This new electronic board was made according to the following block diagram:

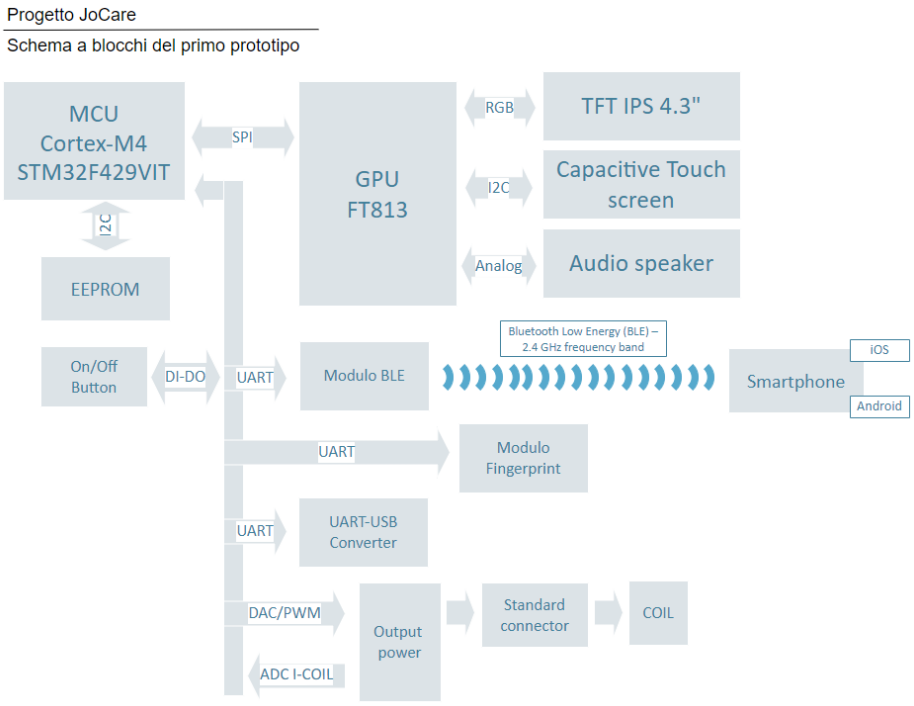


Figure 25. Block diagram of the first prototype

The following photographs show this first working prototype to which a coil of the "medium-single" type among those currently produced has been connected:

Figure 26. First prototype of the new device

The following figures show on the oscilloscope the acquisition of the signal that is generated by the coil connected to the device: the upper trace on the oscilloscope represents the magnetic field generated by the coil while the lower trace represents the induced electric field (both signals have a frequency of 75Hz).

Figure 27. Measurement of magnetic field and induced electric field.

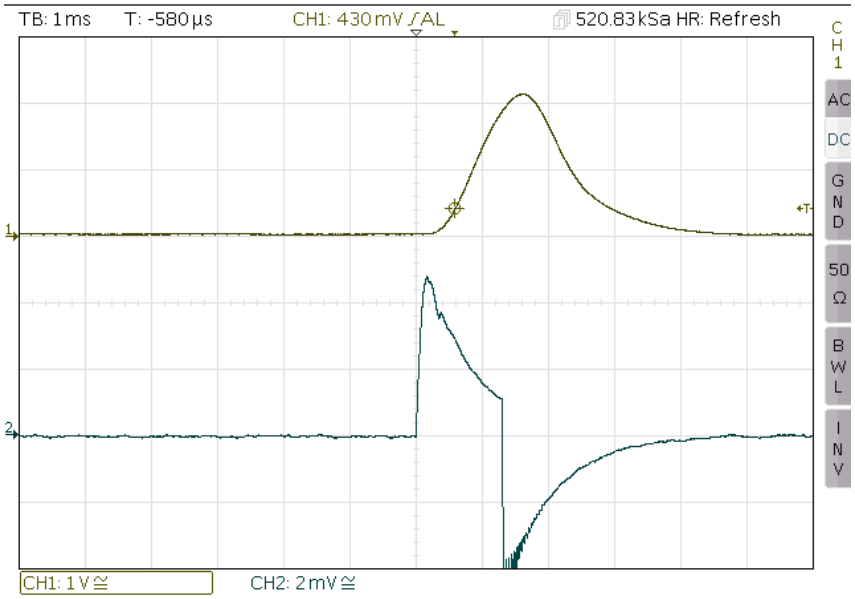


Figure 28. Measurement of magnetic field (upper trace) and induced electric field (lower trace).

The device management software/firmware is multi-threaded and has been organized according to the structure shown in the block diagram below:

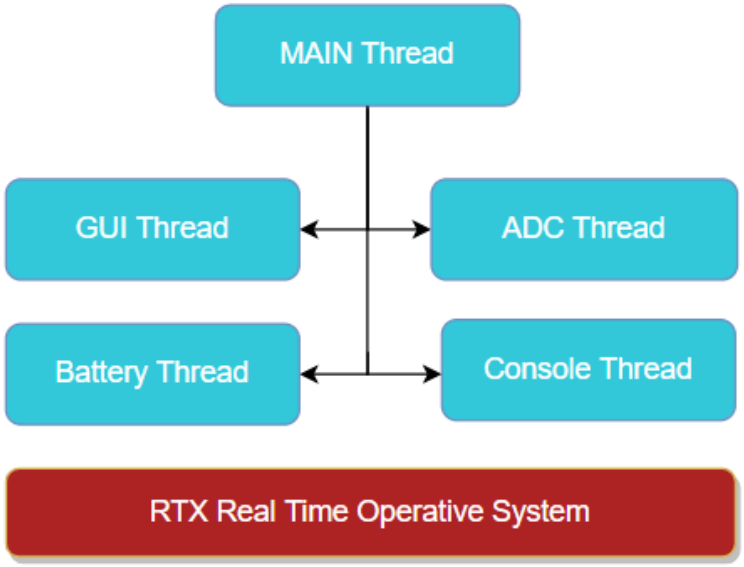


Figure 29. Multi-thread structure of device management

Design and development of the final components (hardware, software, and coil coating materials for different joints) of the device

a) Microcontroller

The choice was made to include the ability to show the user guides in video format for positioning the coils on the treatment site. A larger microcontroller and display was then chosen; the battery charging section was revisited by introducing a type of power supply equipped with a USB Type-C port and the Power Delivery protocol that allows direct communication between the power supply and the microcontroller. In order to meet the demand for multimedia, a microcontroller also produced by ST mod. STM32H747xI Dual core Cortex-M7 + Cortex-M4, which is equipped with two cores within the same chip, was switched. In the microcontroller family, this model from ST is very high-performance and can handle both the deterministic part of signal generation (with the Cortex-M4 core) and the demonstration video playback (with the Cortex-M7 core). The microcontroller already

implements, among the internal peripherals, everything necessary to manage the display and, as a consequence, it is no longer necessary to use the FT813 graphics processor, which is thus removed from the device schematic.

Among the various types of packaging available for this microcontroller, the BGA was chosen and thus the mod. STM32H747XIH6 with the TFBGA240+25 (14x14mm) packaging. In fact, this type of BGA ("Ball Grid Array") packaging makes it possible to obtain a chip with a very small size (only 14x14 mm) (to which the 265 available pins are mapped).

With BGA technology, the chip's pins are arranged on a two-dimensional array of dots called "balls" that are soldered onto the PCB only by automatic systems, thus excluding the possibility of manually soldering the integrated onto the PCB.

Below is a photograph of an integrated with this type of BGA packaging:

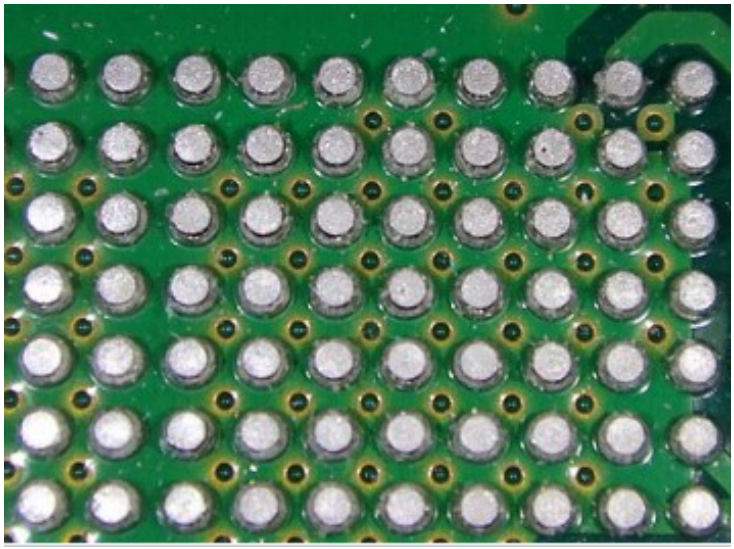


Figure 30: Packaging BGA - Ball Grid Array

b) Display

The 5" TFT IPS display from RAYSTAR 720x1280 with capacitive touch screen and driven directly by the microcontroller via MIPI-DSI protocol was chosen. This display from RAYSTAR is the mod. RF3500D-AYW-MNG1.

This new display will provide the following advantages:

- larger graphics
- images with better resolution
- the MIPI-DSI protocol allows the display to be driven with a significantly reduced number of signal lines compared to 24 RGB lines, and this is a major advantage in reducing electromagnetic emissions and thus in terms of electromagnetic compatibility

c) Battery charging section - battery monitor - power supply unit

The lithium battery management section has been upgraded with a new integrated unit from MAXIM mod. 77960 dedicated to battery charging and a battery monitor also from MAXIM mod. 17205. Also chosen for the power supply is a new technologically advanced model again from SL Power mod. SLE60SPD96B01 with medical grade USB Type-C output differs from its predecessor essentially in the following features:

- USB Type-C output connector
- Power Delivery protocol that makes it capable of delivering programmable power up to 60W. This protocol allows the power supply to exchange messages with the on-board microcontroller, which establishes the output parameters of the power supply in terms of voltage and current delivered.

d) Biometric sensor

The activity related to the biometric sensor for fingerprint reading was successfully completed. From a careful analysis of the performance of the biometric sensors, it was deemed not appropriate to deploy the sensor for the following reasons:

- biometric sensors are devices closely related to the consumer world of portable devices such as smartphone cell phones that, by their nature, are subject to frequent changes and updates. In contrast, it is important that the components of a medical device are more related to the industrial world where a greater guarantee of several years of product availability in the market is offered;
- the use of the user's fingerprint involves legal issues related to the management of personal data;
- the fingerprint recognition process is affected by a certain degree of possible non-recognition, and this could prevent processing for the user who is not recognized. This type of error would result in a very negative impact on the image of the device and not acceptable to the customer.

Personalization of the device will be ensured by writing the client's name into the software: the name will appear on the device's screen when the device is turned on and will not be editable by the user.

e) Microcontroller software/firmware development environment

The switch to the ST mod. STM32H747xI Dual core Cortex-M7 + Cortex-M4 microcontroller was accompanied by the switch to the new IAR Embedded Workbench development environment, which consists of the components shown in the block diagram in Figure 31:

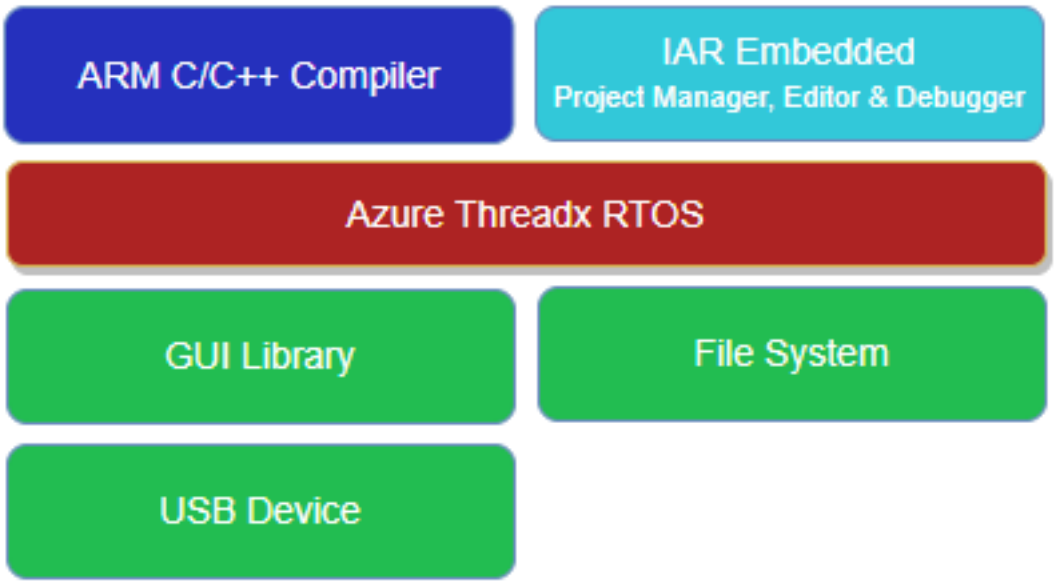


Figure 31: IAR Embedded Workbench

Microsoft's RTOS called Azure Threadx RTOS has been adopted as the Real-Time Operating System, which is an embedded development suite that includes a powerful operating system that provides reliable and ultra-fast performance for devices where resource optimization is essential. It is an easy-to-use RTOS and is a widely deployed system used in more than 10 billion devices worldwide.

The operating principle of the device remains that of a "state machine" working with several concurrent threads managed through the real time operating system based on a round-robin algorithm and priority.

The graphics library developed by ST called TouchGFX was chosen for the realization of the user interface. TouchGFX is a graphical software framework optimized for STM32 microcontrollers. By taking advantage of the STM32 graphics features and architecture, TouchGFX enables the creation of graphical user interfaces quite similar to those of smartphones.

The TouchGFX framework includes TouchGFX Designer (TouchGFXDesigner), which is a PC-based graphics creation tool that simplifies GUI development by combining WYSIWYG (What You See Is What You Get) simulator and automatic code generation in C++ language.

In addition, the TouchGFX framework provides all the necessary support features for playing the video tutorials that are intended to be included in the final device.

Figure 32: Examples of GUIs with the TouchGFX graphics library.

Finally, a new research activity led to a major revision of the device prototype in terms of both design complexity and constructability.

From a design point of view, the use of the new dual-core M4 + M7 microcontroller involves the realization of a PCB that must meet very specific requirements regarding: the length of the signal lines that connect the microcontroller with external peripherals such as, for example, the external memory since these lines must all have the same length in order to guarantee signal transmission times with tolerances that fall within the order of picoseconds. In order to be able to meet this requirement, it is necessary to use PCB design tools that enable automatic implementation of this function and to apply, where necessary, a "serpentine" topology of the signal lines involved. From the point of view of constructive feasibility, the current global situation regarding the availability of electronic components is such that components are often not available except with very long lead times (often 54

weeks and more), and this means that it is not possible to have all the components necessary for the realization of prototype electronic boards. The new STM32H747 family microcontroller currently has a lead time of over a year. We searched for a viable solution to develop the prototypes envisioned in the project while not having the immediate availability of the microcontrollers as individual components.

Therefore, the use of evaluation boards mod. STM32H747I-DISCO (known as "Evaluation Boards") of this microcontroller was defined at this stage. Although these microcontrollers, as individual components, are not available on the world market in a reasonable time and at an acceptable cost, the relevant evaluation boards are available on the channels of large-scale distribution, and this allowed the development of a new prototype of the device based precisely on this Evaluation Board mod. STM32H747I-DISCO, which allowed all the various sections of the device to be tested.

The realization of the final prototype of the device has been divided into two successive phases described in Figure 33 below and referred to as "Prototype 1" of the device, which will be realized with the microcontroller evaluation board, and "Prototype 2" of the device, which will be realized with the microcontroller chip placed inside a single electronic board containing all sections of the final device.

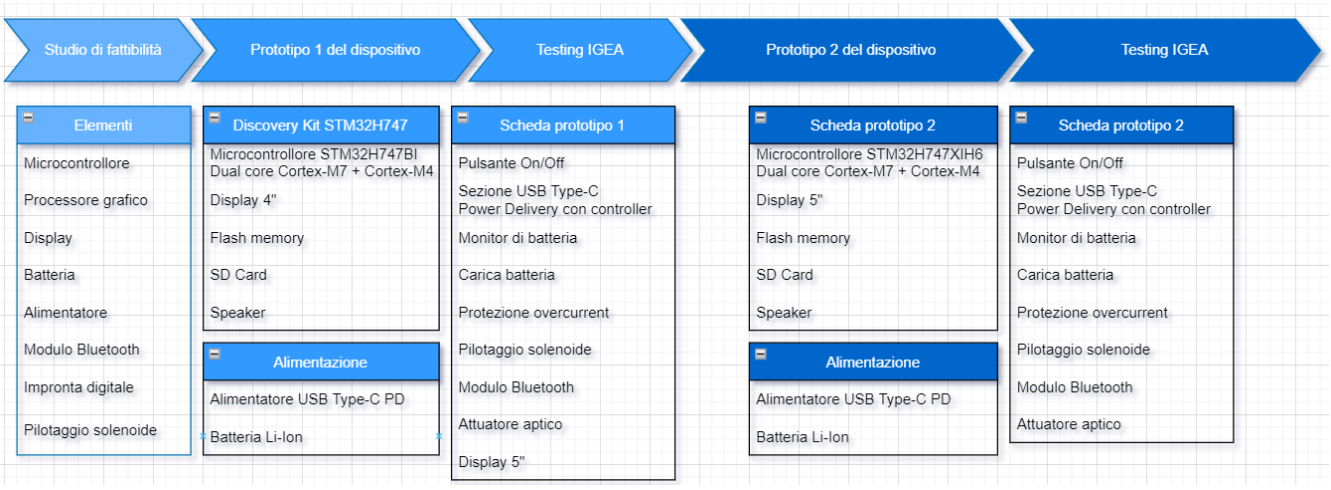


Figure 33: Road map of the final prototype divided into two successive phases "Prototype 1" and "Prototype 2"

The block diagram of "Prototype 1" is shown in the block diagram in Figure 34:

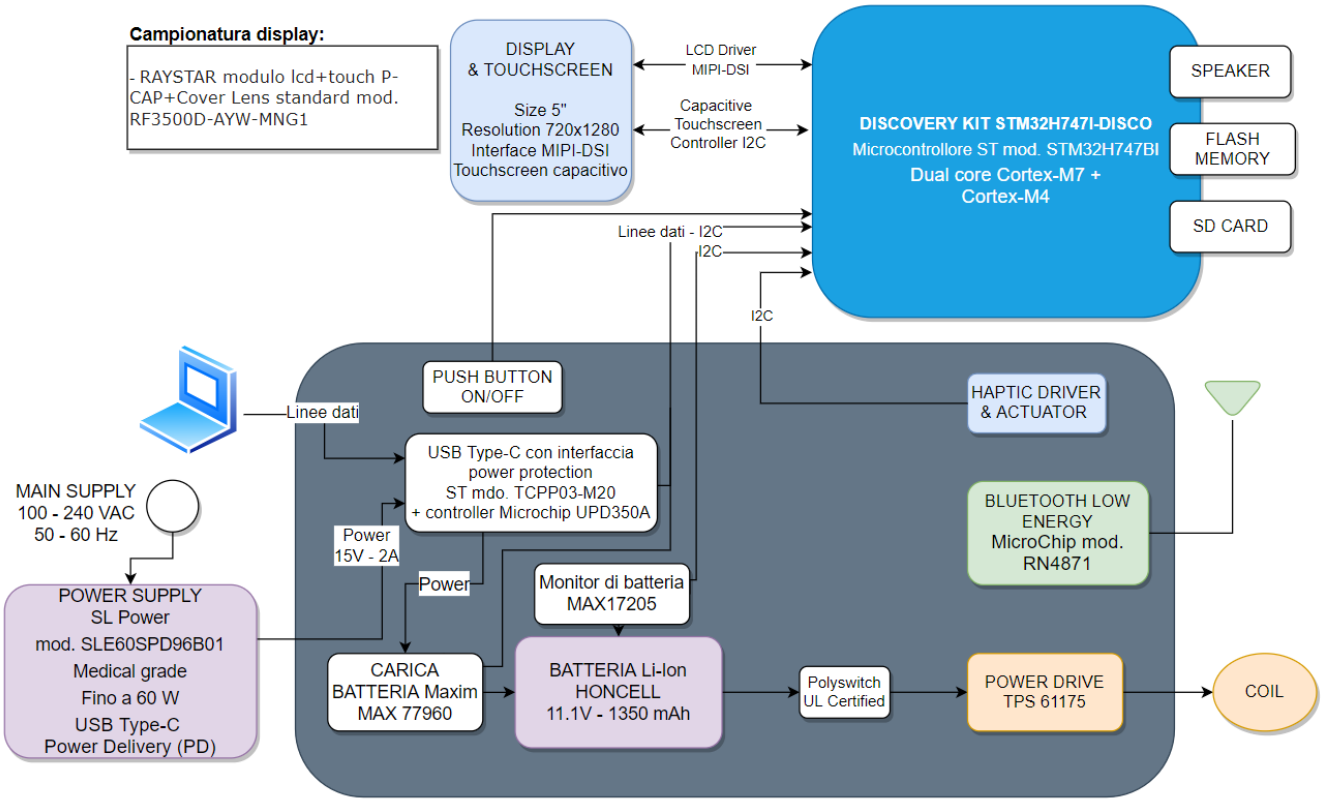


Figure 34: Block diagram of "Prototype 1" device with evaluation board

From a practical point of view, prototype 1 was realized by means of 3 electronic boards, which are shown in the table below:

Evaluation board of the microcontroller mod. STM32H747I-DISCO	Electronic board mod. **Display** cod. 90001	Electronic board mod. **USB** cod. 90005



- The electronic board "Evaluation board mod. STM32H747I-DISCO" contains.
  - the microcontroller STM32H747XIH6
  - all the accompanying components required for its operation
- The electronic board named "Display" contains:
  - the 5" TFT display with capacitive touch-screen
  - the RGB led for visual feedback
  - the acoustic feedback speaker
  - the prototype power button
- The electronic board named "USB" contains:
  - the USB Type-C communication port
  - The charging and "health status" monitoring section of the battery
  - the Bluetooth BLE communication section
  - The haptic actuator section for haptic feedback
  - The coil drive signal generation section for generating the PEMF

The 3 electronic boards were assembled as shown in Figure 35:

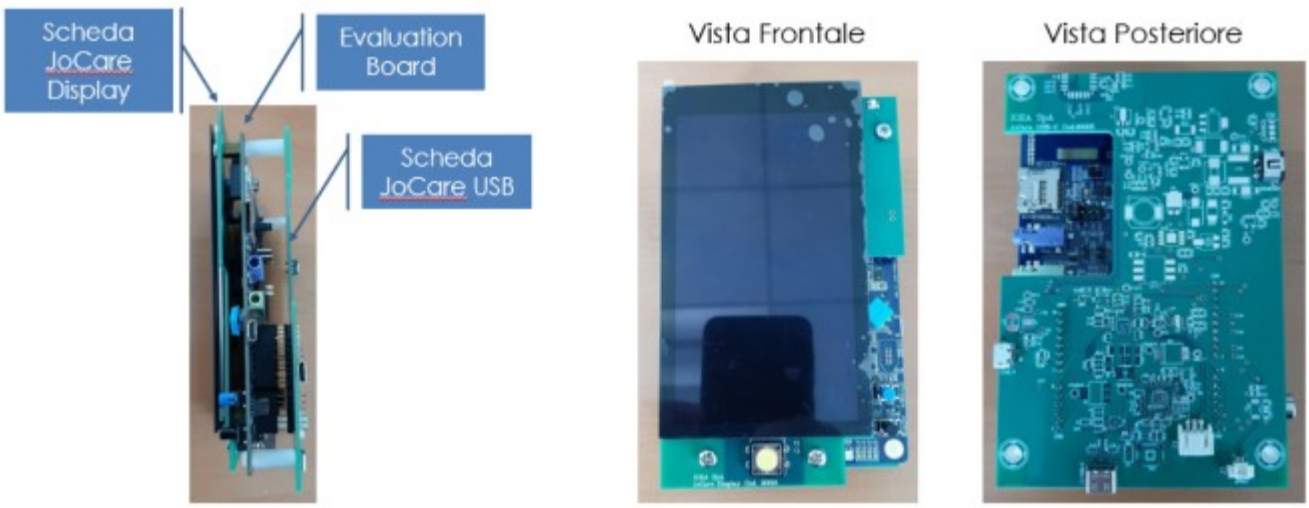


Figure 35: Assembly of the 3 electronic boards of "Prototype 1"

As shown in the block diagram of "prototype 1," Microchip's mod. RN4871 module was selected for the Bluetooth BLE communication section. In parallel with the development of "prototype 1," the development of the Bluetooth BLE communication section was continued. Two simulators of the Bluetooth communication were made (Figure 36) along with a description of the communication protocol used to transfer data from the device to the smartphone.

Figure 36: Bluetooth BLE module mod. RN4871 from Microchip mounted on the circuit board.

The Bluetooth communication protocol that has been defined provides all the commands that allow the smartphone (with iOS or Android operating system) to query the device with the purpose of obtaining data about the treatment sessions that have been performed.

The data is sent from the device according to the standard JSON (JavaScript Object Notation) format and has been organized according to the structure shown in Figure 37 below:

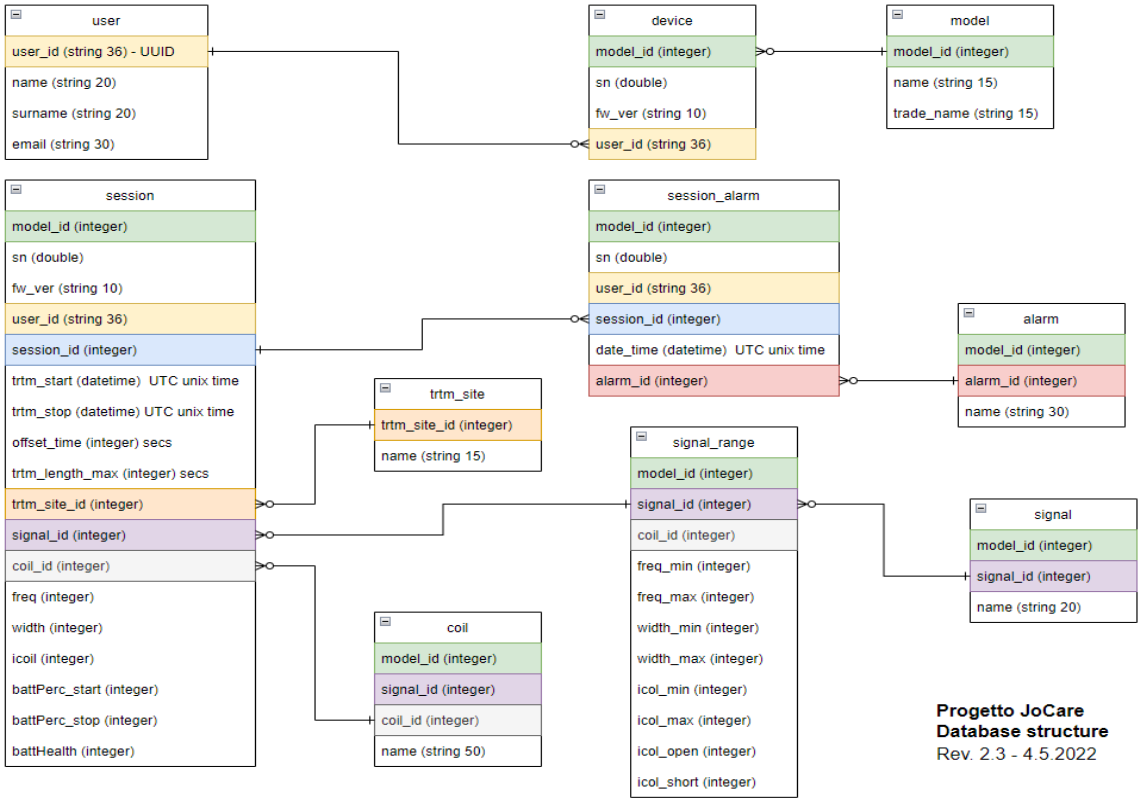


Figure 37: Structure of data transferred via Bluetooth BLE.

Study and definition for the optimization of the communication protocol between applications, the new device and other applications dedicated to IoT (Internet of Things) devices

A study was then performed inherent in the specifications to realize the app\system related to the new device:

- Articulations & Analysis | Insight | Research Articulations

The purpose of the research was to understand which joints are most affected by cartilage degeneration/osteoarthritis. The data on people subject to the diseases and the difficulties they face in their daily activities allow us to learn more about the end user.

- MKT applied to eCommerce

The purpose of the research was to identify what are the main needs from the marketing side of eCommerce (Payment methods, analytics, advertising, cookies, customer support, etc.).

- eCommerce and Cloud

The purpose of the research was to choose the eCommerce tool to be used within the project for the provision of accessories and complementary services to the device. Accordingly, several eCommerce and cloud-based offerings of eCommerce products were investigated in depth and evaluated from a project perspective. They turn out to be compatible with the project, Prestashop, Woocommerce, Shopify, Magento.

- Study of the user profile

The research aimed to study the function and role of the user profile within the design and development of software solutions. It aimed to:

    - Figure out how to precisely delineate who will be the user of the applications
    - Being able to design the experience in such a way that it is user-centred
    - Structure a user profile in which people can identify themselves

- Onboarding and Evaluation Sheets

This research delved into Onboarding techniques in a Mobile Application with a focus on how Applications collect user data and use it to create scorecards or custom scoring. Thus, the ultimate goal of the research is to understand whether an Onboarding process will need to be included in the Application included in the project and, if so, how best to structure it to make the user experience smooth and enjoyable.

- Exercises and training programs in the rehabilitation/prevention app

The purpose of the research was to understand how exercises and training schedules could be managed within fitness or rehabilitation apps. Specifically, there is a need to manage a session calendar to which a possible session schedule could be linked for therapies to be performed. In addition, the app could host exercise programs to strengthen muscles.

- Interfaces to connect App to Wearable Device

The purpose of the research is to analyze how a technology device connects and synchronizes data with a mobile application. A technology device connected via Bluetooth to a mobile application will be offered to users.

- IoT protocol choices

The aim of the research was to arrive at defining the most suitable IoT protocol for communication between the new device and the mobile app and between it and the central data repository, in the Cloud. The final choice also took into account the results that emerged from some practical tests, obtained through the implementation of a prototype.nTaking into account these considerations and the specifics of the project, the compatible and chosen technologies are:

    - Bluetooth for communication between device and mobile app
    - HTTPS and MQTT for mobile app communication and central repository (Cloud)

- IoT and Cloud

The purpose of the research was to evaluate the most suitable communication protocol and Cloud services, which involves having the mobile app communicate with a central repository, built via the Cloud. The final choice fell on the Google Cloud and in particular the services offered by this Cloud platform, namely: IoT Core, Cloud Functions, BigQuery.

- IoT & Edge Computing

The topic of edge computing in the context of IoT and specifically how a smartphone mobile app can be used as edge computing between a Bluetooth device and Cloud services was explored. This research originated within the "IoT Protocol Definition" research to further explore the topic of secure data transmission between devices and the central repository in the Cloud.

- Customer Experience in Device IOT

The purpose of the research was to investigate the user experience in the presence of smart devices in the Health and Wellness market. The research analyzes the user experience of a selection of IoT devices and the tips provided to users in the first weeks of use. Recommendations for device maintenance are also explored in depth. The research results provide us with industry best practices and tips that will be considered when designing app functionality.

- Prediction via machine learning of malfunctions

The study was aimed at identifying possible practical applications of machine learning in the area of device malfunction diagnosis. For the time being, the study focused on methods for estimating battery status. In this area, several machine learning methods are already available and are characterized by good prediction accuracy. The conclusion of the research identified the DNN (Deep Neural Network) solution as the most suitable in this area because of its excellent machine learning capability and good prediction of the remaining battery life.

Therefore, it is desirable to apply this approach within the project to predict the remaining battery life and inform the end user or technicians in a timely manner.

- Digital Health Care and Telemedicine - What it is, potentials and limitations in doctor-patient communication

The purpose of the research was to investigate what the developments of Digital Healthcare in Italy are and what the developments of Telemedicine are. In this research, multiple factors were analyzed that led to the conclusion of not wanting to introduce a direct doctor-patient communication system within the project applications. It must be considered that the introduction of a chat within such a specific application has thorny issues to be addressed:

  - Who would the user communicate with? There should be a dedicated team of clinicians whose job it would be to respond to and manage chat communication;
  - Why should users write to the App Chat if they experience problems or have concerns about their treatment and not directly contact their personal physician who prescribed the device?
  - Why would a physician recommending the device to one of his patients use the app's chat instead of the normal communication tools he already routinely uses to communicate with all of his patients?

A dedicated chat within the App, for this specific context, has more negatives than positives. Sensitive data that could circulate within the App is another aspect to consider. If a user were to send reports, documents related to his or her health status within the chat, this data would pass through the App owner's infrastructure. The latter becomes responsible for the security of this data and is "obliged" to regularize the entire data management as much as possible. In addition, it must be taken into account that the costs of implementing and maintaining a chat service (between infrastructure and personnel dedicated to maintaining and using it),

are high and do not compensate for the little added value that a chat would bring to the project application.

The results of this in-depth study helped to choose the optimal solution for the system, from a protocol and Cloud services perspective.

Activities then took place to set up the environments on Google Cloud Platform (GCP).

An initial mobile prototype for iOS and Android was built in order to evaluate the communication issues and validate the technology choices made, particularly for:

- Verify the correct data exchange between the device prototype and the mobile app prototype
- Verify proper data exchange between mobile app prototype and the cloud

The verifications led to the following conclusions:

- Bluetooth BLE is the most suitable protocol for communicating between devices and mobile apps
- HTTPS is the most suitable protocol to communicate between mobile app and IoT Core service in Google Cloud

Design thinking aims to define the vision of the project, justifying the design choices made downstream. The topics covered were:

- Generation of user personas
- Stakeholders map
- Empathy map
- Vision brainstorming
- Value proposition canvas
- Business model canvas

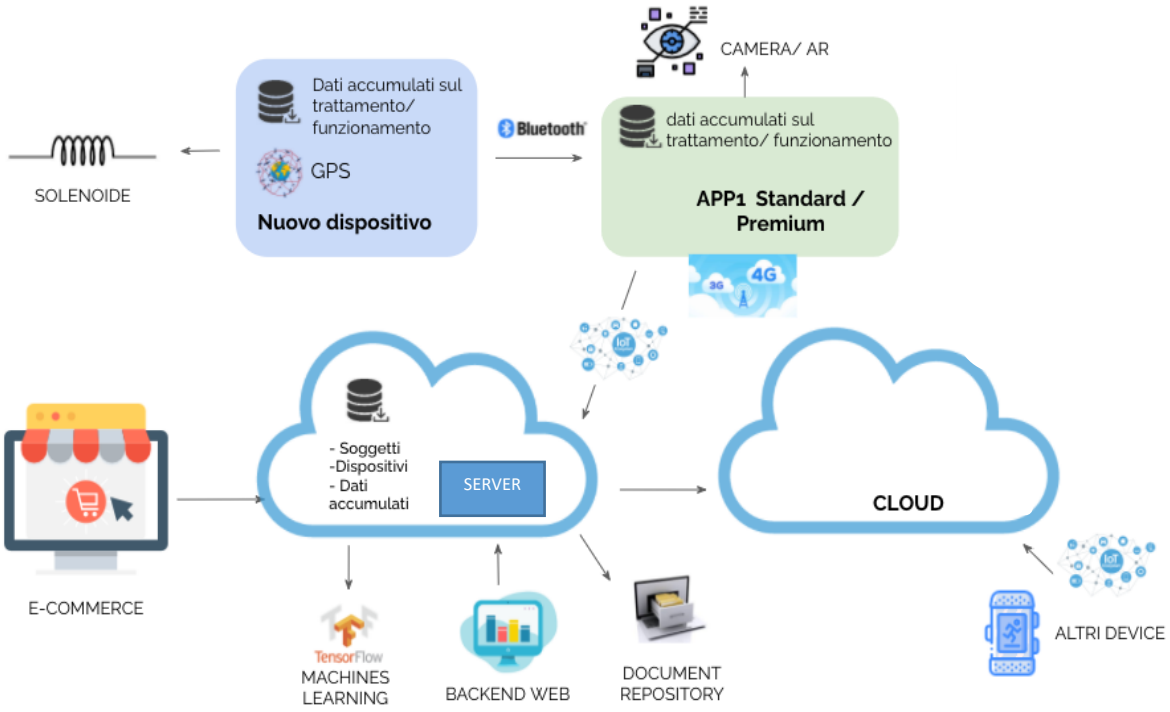


Figure 38. Logical scheme of the project

Directions to be followed during mobile and web app development were drafted.

Commands and related formats for communication between mobile app and new device have been defined.

The techniques and methods used to construct and design interactions that the user uses in relating to a specific "product" were defined.

It has also been defined how the analysis of aspects that condition the way people access the digital tool and its information is to take place.

The User Interface represents the actual graphical user interface, that set of elements that enables interaction between the end user and the digital tool.

It was described how interface design, the final stage of design, is constructed by taking as a reference point the wireframe containing the general structure of the product and creating a library of elements to be used to assemble a user interface suitable for various services.

The above activities then flowed into the design of a first version of the App and specifically:

- Project Brief
- Definition of functionality
- FX design
- Design flow Design sprint
- Mobile App Design
- Backend web design

A new project has been prepared on Google Cloud Platform, dedicated to the web backend development environment with which mobile apps communicate.

Two new mobile apps, representing the second version of the prototype, have also been prepared, developed natively and for the Apple iOS and Google Android platforms, and can be downloaded from a dedicated site.

The new device, mobile app, and web backend are a single system made up of closely related components, in terms of data flows, so functionality design was carried out in increments on both sides through strong co-design activities.

As for the mobile app, the design has covered these issues:

- Onboarding
- User Profile
- Self-Assessment Sheets
- Support
- Sessions
- APP Home Page
- Home Page and Notifications
- Navigation and Settings

As for the backend web, the design has covered these issues:

- Login/Login

- Device management (list+detail)
- Customer management (list+detail)
- CMS Support
- CMS Self-Assessment Sheets
- User/role management
- Navigation (React)
- Dashboard

From the above design activities, the database in the Cloud was then designed to collect user, device, session, and on-boarding data.

For the time being, the database for backend functionality has been modeled:

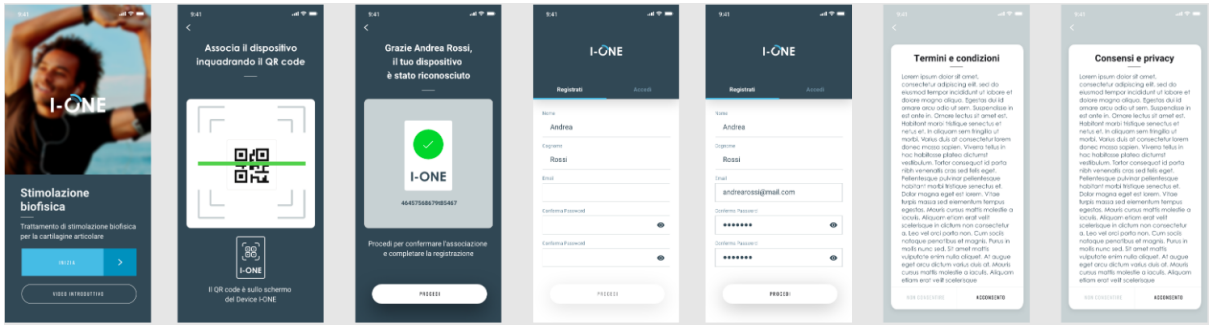
- Login/Login
- Device management (list+detail)
- Customer management (list+detail)
- User/role management

Following the modeling part, the database was then created on the Google BigQuery data warehouse, on which the developed application functions rest.

Implementation activities can be divided into several parts:

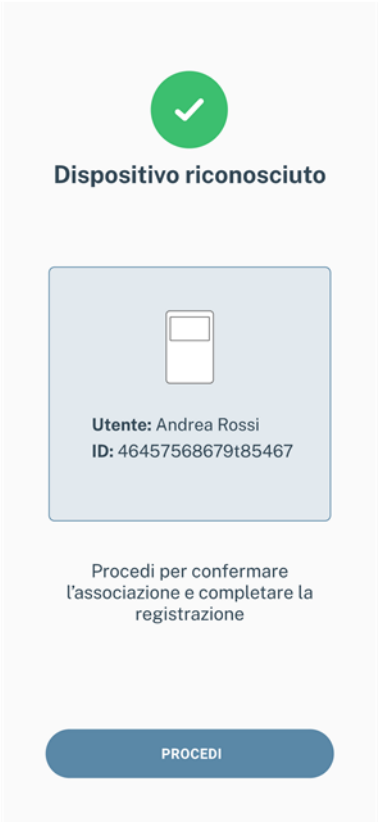
- mobile infrastructure developments, related to certain parts of the mobile app that it is good to realize as reusable components or software layers, so as to then speed up the development phase and at the same time standardize the way these parts work. The infrastructure activities were related to:
    - Development of a generic software layer, for communication via Bluetooth BLE, for both iOS and Android, so as to facilitate data exchange between mobile app and device

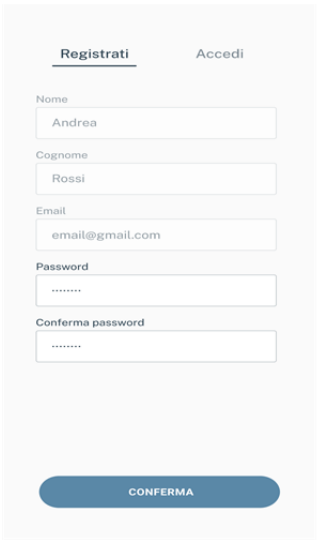
    - Development of the IoT protocol-based software layer for communication between the mobile app and the cloud, so that data can be retrieved from the device via the mobile app and stored in the cloud
- Development of a software layer for the web backend user interface, based on the ReactJs and PrimeReact frameworks. The developed extensions allow for graphical components compatible with what emerged in the co-design phase.
- Data flow development for IoT: The Google Cloud is used not only to host the web backend, but also to make available a set of services listening to data from the mobile apps, via the IoT protocol. Once the communication protocol via Bluetooth BLE (command set) was designed, the next step was the implementation of the services listening to the data collected via the mobile app. These services were implemented as Cloud Functions, to ensure maximum scalability and also offering optimal integration between the IoT protocol and the BigQuery database.
- Development of second mobile prototype for iOS and Android, containing functionalities consistent with what emerged in the co-design phase. More specifically, the functionalities developed to date are those related to on-boarding and retrieval of master data (devices, users, coils, etc.) and of treatment sessions:
    - APP Home Page
    - Onboarding
    - Navigation and Settings

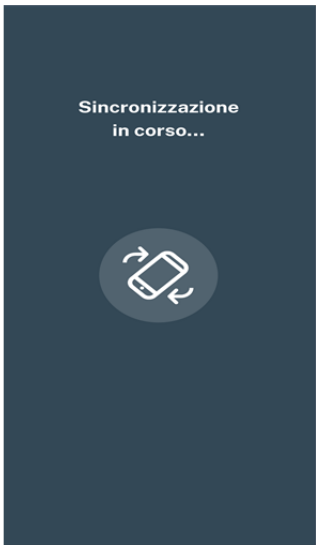

I-ONE
Stimolazione biofisica
Trattamento di stimolazione biofisica per la cartilagine articolare
Associa il dispositivo inquadrando il QR code
Il QR code è sullo schermo del Device I-ONE
Grazie Andrea Rossi, il tuo dispositivo è stato riconosciuto
Procedi per confermare l'associazione e completare la registrazione
Registrati
Accedi
Andrea
Rossi
andrearossi@mail.com
Termini e condizioni
Consensi e privacy

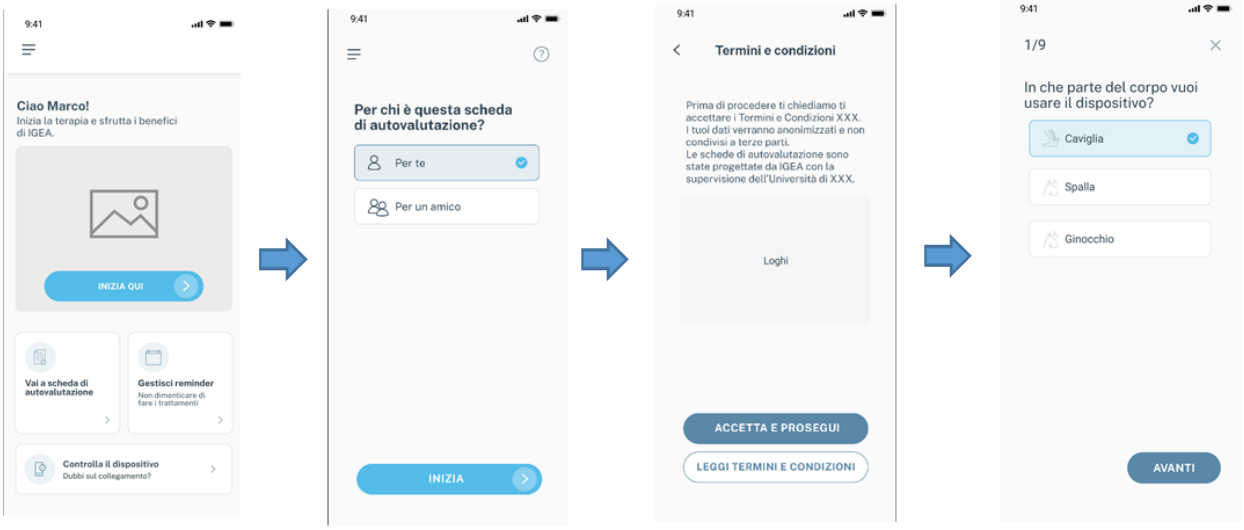

LOGO
Stimolazione biofisica
Trattamento di stimolazione biofisica per la cartilagine articolare
INIZIA
SCOPRI DI PIU'

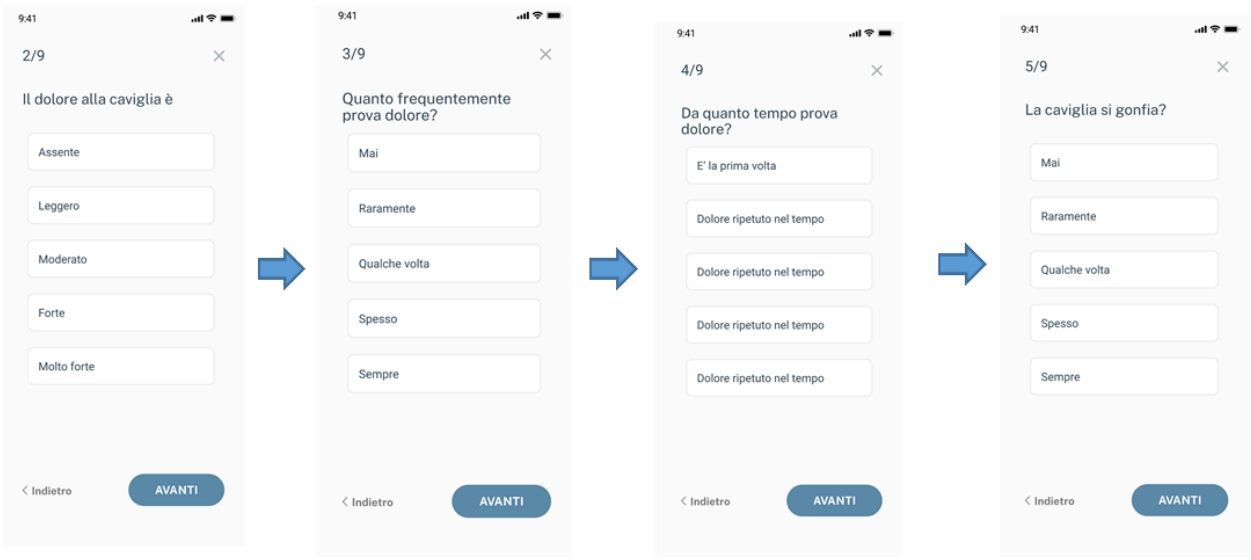

Inquadra il QR code
Avvicina il tuo smartphone al dispositivo e inquadra il QR code che appare sullo schermo.
ANNULLA

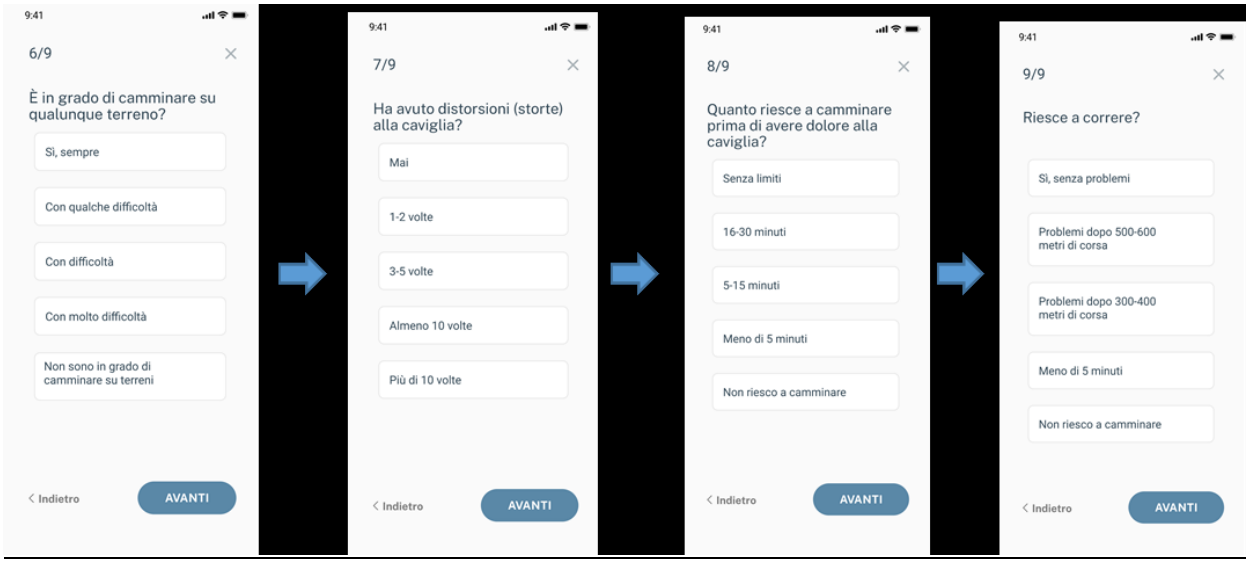

Dispositivo riconosciuto
Utente: Andrea Rossi
ID: 46457568679t85467
Procedi per confermare l'associazione e completare la registrazione
PROCEDI


Registrati
Accedi
Nome
Andrea
Cognome
Rossi
Email
email@gmail.com
Password
Conferma password
CONFERMA


Sincronizzazione
in corso...

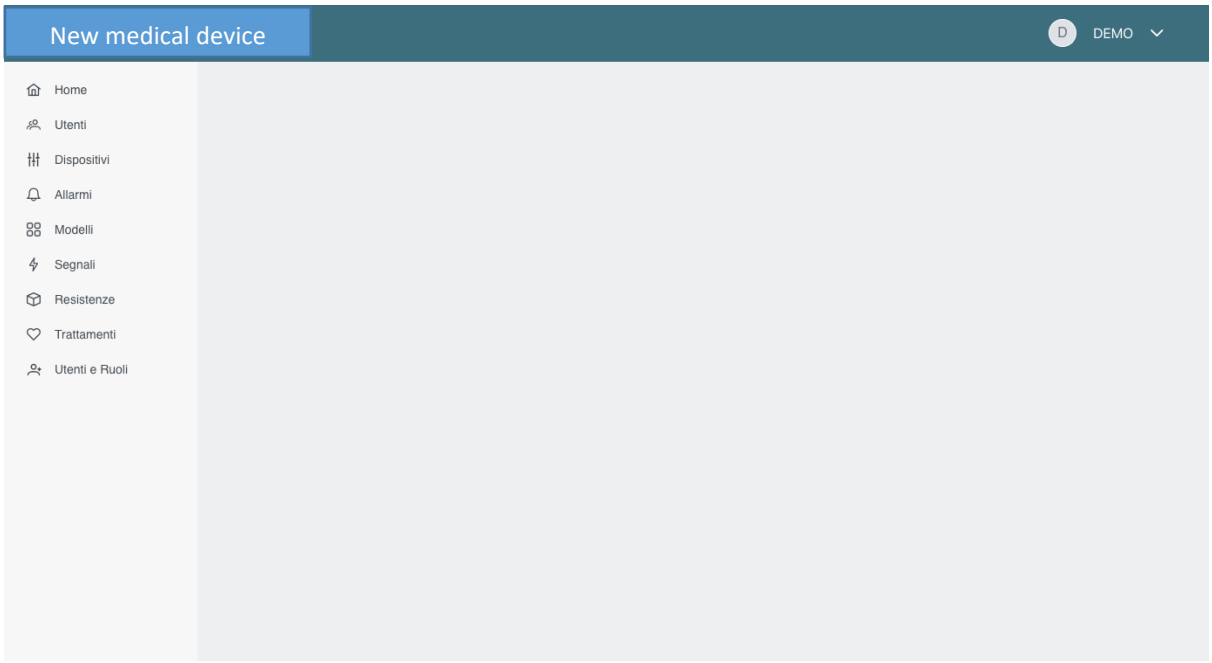

Ciao Marco!
Inizia la terapia e sfrutta i benefici di IGEA.
INIZIA QUI
Vai a scheda di autovalutazione
Gestisci reminder
Non dimenticare di fare i trattamenti
Controlla il dispositivo
Dubbi sul collegamento?
Per chi è questa scheda di autovalutazione?
Per te
Per un amico
INIZIA
Termini e condizioni
Prima di procedere ti chiediamo ti accettare i Termini e Condizioni XXX.
I tuoi dati verranno anonimizzati e non condivisi a terze parti.
Le schede di autovalutazione sono state progettate da IGEA con la supervisione dell'Università di XXX.
Loghi
ACCETTA E PROSEGUI
LEGGI TERMINI E CONDIZIONI
1/9
In che parte del corpo vuoi usare il dispositivo?
Caviglia
Spalla
Ginocchio
AVANTI


2/9
Il dolore alla caviglia è
Assente
Leggero
Moderato
Forte
Molto forte
Indietro
AVANTI
3/9
Quanto frequentemente prova dolore?
Mai
Raramente
Qualche volta
Spesso
Sempre
Indietro
AVANTI
4/9
Da quanto tempo prova dolore?
E' la prima volta
Dolore ripetuto nel tempo
Dolore ripetuto nel tempo
Dolore ripetuto nel tempo
Dolore ripetuto nel tempo
Indietro
AVANTI
5/9
La caviglia si gonfia?
Mai
Raramente
Qualche volta
Spesso
Sempre
Indietro
AVANTI

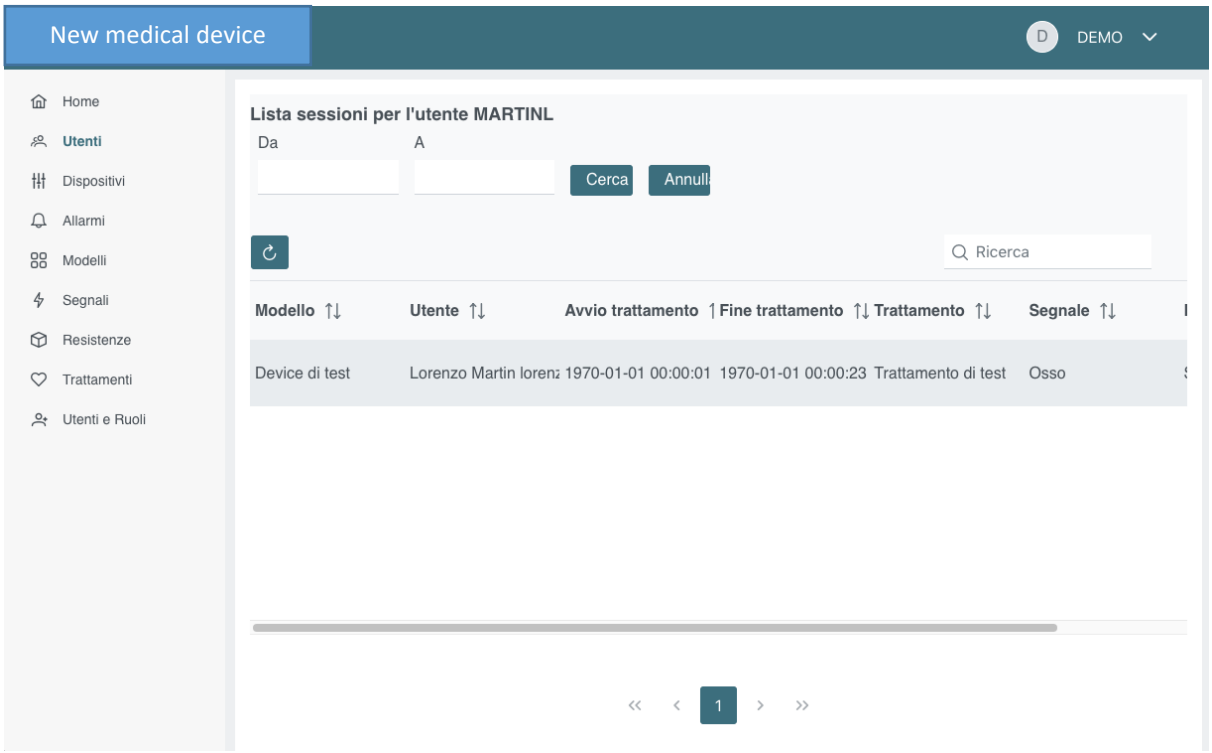

9:41
6/9
È in grado di camminare su qualunque terreno?
Sì, sempre
Con qualche difficoltà
Con difficoltà
Con molto difficoltà
Non sono in grado di camminare su terreni
Indietro
AVANTI
9:41
7/9
Ha avuto distorsioni (storte) alla caviglia?
Mai
1-2 volte
3-5 volte
Almeno 10 volte
Più di 10 volte
Indietro
AVANTI
9:41
8/9
Quanto riesce a camminare prima di avere dolore alla caviglia?
Senza limiti
16-30 minuti
5-15 minuti
Meno di 5 minuti
Non riesco a camminare
Indietro
AVANTI
9:41
9/9
Riesce a correre?
Sì, senza problemi
Problemi dopo 500-600 metri di corsa
Problemi dopo 300-400 metri di corsa
Meno di 5 minuti
Non riesco a camminare
Indietro
AVANTI

This prototype then allows the device to be registered in the cloud and retrieve information about the devices, users and their treatment sessions, which is then searchable in the Web backend.

- Web backend development. Starting from the co-design activity, a web application capable of displaying data collected by the mobile app, related to:
    - Login/Login
    - Device management (list+detail)
    - Customer management (list+detail)
    - User/role management
    - Navigation (React)



Through this web backend, registered devices and related users and treatment sessions and alerts retrieved through the mobile app from the devices can be accessed.



- Prototype development with Augmented Reality

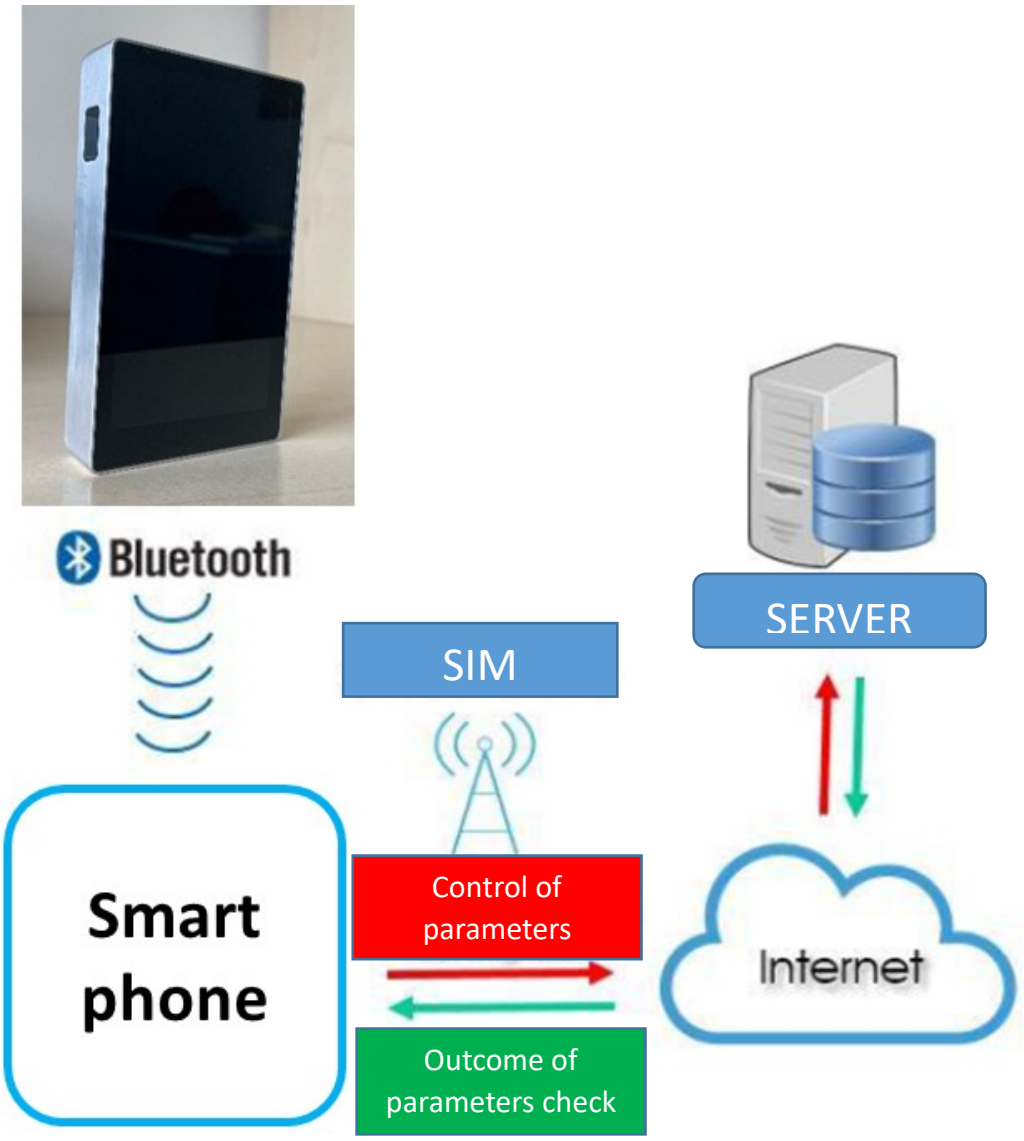
The purpose of this activity is to create an initial mobile prototype related to AR (Augmented Reality).

- Coil recognition, the recognition is done through the smartphone camera and some AR libraries available for iOS and Android (e.g., PIKKART that allows adding digital content such as 3D objects, videos, image galleries, fact sheets, etc.). A possible practical application to be included in the mobile app is a user support feature for assisted positioning, indicating whether the coil has been positioned correctly, for example by displaying the coil and the part of the body to which it is applied in overlay to an image showing the correct positioning.
- Additional information and linkage to services; e.g., showing the identified object (e.g., device) with overlay information (options for color or available models, etc.)

Further practical applications on the mobile app could be:

- Tip on using the device (powering on, checking battery levels...)

- Directions for bluetooth or wifi pairing with the device
- Display in app of a list of model-specific tutorials for user guidance



4.3.3 Study, preparation and development of documentation in clinical settings.

Study and preparation of self-assessment forms

The user-friendly self-assessment forms that will be included in the app will assist the sportsman in using the new device and provide him with guidance on: *i.* appropriateness of treatment, *ii.* use of the device (treatment schedule, hours/day and days of therapy), and *iii.* choice of coil.

The following activities were also carried out during the first year of work:

- Coordination of the clinical research part: this has meant ideation and comparisons with clinical colleagues at the Clinical Institutes to try to identify the most useful and reliable clinical parameters for the purposes of the project, as well as most understandable to the end user of the app;
- Proposal and development of the first clinical self-assessment forms with regard to ankle problems: review of relevant literature and discussion of questions to be included in the questionnaires;
- Meetings, in-person and telematic, to coordinate and evaluate the self-assessment forms related also to knee and shoulder problems; discussion with Clinical Colleagues on the settings of the questionnaires in order to make them as simple, understandable but scientifically correct as possible;
- Design and study of parameters for the creation of the Fitness Index: review and evaluation of the Literature;
- Administration, collection and evaluation of ankle self-assessment forms: identification of the target population for this first phase of setting and review of the self-assessment forms;
- Administration of questionnaires to healthy volunteers to assess whether the questions are understandable and evaluation of responses;

- Presentation of data on self-assessment forms at the various meetings, in-person and telematic, that were held.

During the second year of the project, with a view to developing survey protocols for the clinical use of PEMFs in the context of shoulder disorders, the first two questionnaires were developed dedicated to subjects with high and low function, defined according to the level of activity performed by the individual. Through the study of different scores available in the literature, such as Constant score, Shoulder Pain and Disability Index (SPADI), Disability of the Arm, Shoulder and Hand (DASH), American Shoulder and Elbow Surgeons (ASES), 10 and 8 questions, respectively, were selected for the questionnaires aimed at subjects with low and high functionality.

The questions, developed according to a Likert scale, are devoted to assessing the perception of pain and joint stiffness, as well as the duration and frequency of symptoms. In addition, patients' ability to perform certain daily actions (providing personal hygiene and basic needs) or more intense efforts.

The questionnaires thus constituted were submitted to 87 subjects, 43 males and 43 females with an average age of 38.4±28.7 years. Among those who responded to the questionnaire, 12 reported low function and 75 reported high function. In addition, 5 subjects with overt shoulder pathology emerged after the administration of the questionnaire. This allowed us to note an actual effectiveness of the questionnaire thus developed in identifying subjects with overt pathology, through a profile of responses more shifted toward high values on the Likert scale.

In addition, two main aspects emerged from the response profiles: the duration of symptoms, which was difficult to interpret because it was not posed according to a Likert scale, but with only two possible choices; the high-functionality questionnaire found no response at the

highest level of the Likert scale, even for patients who declared overt shoulder pathology. Following these preliminary evaluations, it was planned to further evaluate the questionnaires and introduce possible modifications.

The results of the questionnaires were discussed with other participants in the clinical research group in order to standardize the questionnaires for different anatomical sites in terms of the number of questions and types of responses. For this, changes were made, which are specified below: 1) a single self-assessment form was defined per anatomical site, without distinction on the subject's level of function; 2) the possible answers related to the duration of symptoms were converted to a 5-point Likert scale, to conform the question to the pattern of the other questions/answers.

During the third year of the project, the CIP and all related documentation was drafted and submitted to the relevant EC to obtain approval before they could be used in the clinical setting.

Below is Clinical Investigastion Plan, Synopsis and Clinical Report Form (CRF) for the multicenter prospective observational spontaneous study for the development of joint wellness self-assessment forms according to Good Clinical Practice (EN ISO 14155:2011) and in compliance with ethical principles in accordance with the Declaration of Helsinki.

Clinical Investigastion Plan

SPONTANEOUS MULTICENTER PROSPECTIVE OBSERVATIONAL STUDY

FOR THE DEVELOPMENT OF SELF-ASSESSMENT FORMS

OF JOINT WELLNESS

Type of study: *Multicenter*

Coordinating and proposing center:

Azienda Ospedaliera-Universitaria Arcispedale S. Anna of Ferrara

O.U. of Orthopedic and Trauma Clinic

University Department of Translational Medicine and for Romagna

Hospital Department: Emergency

Scientific head of the coordinating center: *Prof. Leo Massari*

Short title: Joint Wellness Self-Assessment Sheets

Study Code: JoCare

Version 2

Date 13/01/2023

TITLE

Spontaneous multicenter prospective observational study for the development of joint wellness self-assessment forms.

INTRODUCTION

The recent Covid pandemic has demonstrated the importance of developing telemedicine systems to assist patients remotely. The proposed study is within the scope of telemedicine. With the present study, an initial exploratory phase is conducted aimed at developing anatomical site-specific self-assessment questionnaires (shoulder, knee, and ankle) capable of directing the subject to the need for an orthopedic specialist visit. The questionnaire will allow the subject to understand whether his or her symptomatology indicates the presence of a clinical condition requiring orthopedic specialist examination. In this way, it will be possible to identify patients in need of a specialist visit at an early stage, avoiding chronicity of the painful symptomatology and allowing early diagnosis and potentially faster functional recovery.

To this end, starting from the analysis of clinically validated questionnaires[1-10], orthopedic specialists afferent to the University of Ferrara, the Rizzoli Orthopedic Institute, and the Galeazzi Orthopedic Institute collaboratively developed questionnaires with the following characteristics:

a. Subjectivity: the questions in the questionnaires involve a response that reflects a specific condition of the subject, e.g., ability to perform daily activities or sports.

b. Specificity: the questions in the questionnaires are specific to the anatomical district (shoulder, knee, ankle).

c. Simplicity: since it is a questionnaire filled out by the subject completely independently, the questions are written in simple language and the mode of completion is intuitive.

d. Reliability: the score obtained from the completion of the questionnaires should be able to distinguish subjects who need further diagnostic investigations from subjects who need drug therapy, physical therapy, or no therapy.

The present study represents the pilot phase of the project aimed at developing anatomical site-specific self-assessment questionnaires (shoulder, knee, and ankle) and involves the collection of data from the self-assessment forms in a population of subjects who have requested an orthopedic specialist examination for the anatomical site covered by the questionnaire. As part of the proposed study, patient responses will be compared with the outcome of the orthopedic specialist visit.

The data collected from the questionnaires will be used to: 1. assess the statistical significance (p value) of each question in the questionnaire in relation to the outcome of the orthopedic specialist examination; 2. score each response in relation to the outcome of the orthopedic specialist examination.

The proposed study will allow the development of self-assessment questionnaires that, based on simple questions, can direct patients toward the need for an orthopedic specialist visit.

STUDY

Multicenter, prospective, observational study.

PARTICIPATING CENTERS AND PRINCIPAL INVESTIGATOR

Coordinating and proposing center: Azienda Ospedaliera-Universitaria Arcispedale S. Anna di Ferrara, U.O. of Orthopedic and Traumatology Clinic, University Department of Translational Medicine and for Romagna.

Principal Investigator (P.I.): Prof. Leo Massari

Satellite Center: IRCCS Istituto Ortopedico Rizzoli, Orthopedic and Trauma Clinic II, Bologna

Principal Investigator (P.I.): Prof. Stefano Zaffagnini

Satellite Center: IRCCS Ospedale Galeazzi-Sant'Ambrogio, U.O. Shoulder Surgery, Milano

Principal Investigator (P.I.): Dr. Ettore Taverna

OBJECTIVES OF THE CLINICAL INVESTIGATION

Primary objective

The primary objective of the present study is to collect data from three self-assessment questionnaires specific to shoulder, knee and ankle by observing the distribution of responses for each anatomical site.

Each questionnaire will be specific to one anatomical site: shoulder, knee, and ankle.

The data collected from the questionnaires will be used to: 1. assess the statistical significance (p value) of each question in the questionnaire in relation to the outcome of the orthopedic specialist examination; 2. score each response in relation to the outcome of the orthopedic specialist examination.

Secondary objectives

The collection of the orthopedic specialist's opinion on the therapeutic indication for each subject participating in the study.

STUDY DESIGN

The present study is a pilot study that aims to collect data from anatomical site-specific self-assessment questionnaires. Each questionnaire will be specific to one anatomical site: shoulder, knee, and ankle.

At the same time as an anatomical site-specific orthopedic examination, subjects who have signed the informed consent will be asked to complete the anatomical site-specific self-assessment questionnaire.

Upon completion of the questionnaire and regardless of the answers given, the subject will undergo an orthopedic specialist examination.

At the end of the examination, the orthopedic specialist (blind to the questionnaire) will note the outcome of the specialist examination and the therapeutic indication he or she considers appropriate.

At the end of the study, the collected self-assessment forms will be analyzed in order to: 1. evaluate the statistical significance (p value) of each question in the questionnaire in relation to the outcome of the orthopedic specialist visit; 2. assign a score to each response in relation to the outcome of the orthopedic specialist visit.

Through a multivariate logistic analysis of the responses given by the subject, it will be possible to "weigh" each response in order to obtain a final value that gives an indication for an orthopedic specialist visit.

POPULATION

Inclusion criteria

- Subjects who have requested an orthopedic specialist examination for the anatomical site covered by the self-assessment form;
- Age 18 years or older;
- Understanding of the Italian language.

Exclusion criteria

- Surgery within the previous 12 months at the anatomical site covered by the self-assessment form.

Sample size

The proposed study is a pilot study designed to determine the feasibility of developing self-assessment forms that can direct patients to the need for an orthopedic specialist visit and to determine the sample size of the final study.

Given these assumptions, a power analysis to determine sample size is not applicable to the present study, however, based on clinical experience it is believed that 50 subjects per anatomical site is a sufficient number to allow determination of response weights based on the statistical analysis of the data below.

APPROVAL BY THE ETHICS COMMITTEE

The study will be conducted according to Good Clinical Practice (EN ISO 14155:2011) and in accordance with ethical principles in accordance with the Declaration of Helsinki.

Before the trial begins, the center will obtain approval from the relevant ethics committee for the study protocol, with particular regard to the information sheet and informed consent form.

By signing this protocol, investigators agree to comply with these requirements and to conduct the study diligently and efficiently and in accordance with this protocol. This protocol and any amendments will be submitted to the CE in accordance with legal

requirements for formal approval of the study. The decision of the EC regarding the conduct of the study will be written to the coordinator. Written informed consent is required for all subjects.

Informed consent to the study

The patient's consent to participate in the study will be sought after full information about the study has been provided to the patient, paying special attention to the explanation of the purposes, manner of handling and use of the patient's data. The patient's right to withhold consent or to withdraw it at any time during the study, without explanation and without implications to the proper continuation of treatments to his or her person, will always be respected.

In order to be registered, subjects give consent to the processing of personal data, in accordance with the Privacy Guarantor's Guidelines of 24/07/2008 on the protection of persons and the processing of personal data.

All subjects in this study will receive a Consent Form that describes the study and provides key information to make an informed decision about their participation. (Informed Consent Form). The practitioner will explain all procedures in detail in an individual session. The information should be provided in language and terms that are understandable to the individual and in an appropriate time. The consent form, also signed by the designated investigator and dated, should be kept by the investigator as part of the study records. A copy of the consent, signed and dated, should be given to the subject. The Consent Form must include all the required elements of informed consent according to regulations. In addition, the Consent Form must identify the sponsor or his/her representative. The consent form and explanation will include: detailed information about the methods of investigation, the rationale for conducting the study, potential effects and risks, and emergency and safety

procedures. Information on data collection will be described in detail. Understanding of the consent form will be ascertained with appropriate questions.

Subjects will be reassured that their participation is voluntary and that withdrawal from the study will not affect their treatment or relationship with their caregivers. All subjects will be informed about the potential risks and benefits of being involved in the study. Subjects will be able to request withdrawal from the study, and any assessments conducted will be returned.

The consent form must be approved by the Ethics Committee before the study begins at any site. Any subsequent changes to the approved informed consent form must be reviewed and approved by the Ethics Committee before use.

DURATION OF THE STUDY

Patient enrollment will occur only if the patient meets the inclusion and exclusion criteria of the protocol. The subject is considered enrolled in the study when he or she provides written Informed Consent. No study procedure will be initiated before the Informed Consent is signed.

For each patient, the study will last only as long as it takes to complete the anatomical site-specific self-assessment form and the orthopedic specialist examination.

For each center, the study will last 1 year from the date of approval of the study protocol by its Ethics Committee, subject to reaching the total number of patients to be enrolled.

Study interruption

Patient adherence to this study is completely voluntary. The patient will be able to withdraw from the study at any time without any negative impact on the quality of health care provided to him or her. The date of withdrawal will be recorded along with the reasons for the patient's withdrawal from the study. Similarly, the study may be terminated if the physician finds that

conditions occur that, in the best interest of the patient, make it appropriate to suspend the study.

Patients withdrawn from the study may be replaced with new patients to be recruited.

PROCEDURES FOR MONITORING ANY PROBLEMS, INCIDENTS, COMPLAINTS

As this is an observational study, no related side effects are expected.

EVALUATION OF RESULTS USING STATISTICAL METHODS

The evaluation of the collected data will subsequently receive statistical evaluation by researchers pertaining to the centers participating in the study who were involved neither in defining the questions on the self-assessment forms nor in collecting data from patients.

Each item of the questionnaire will be evaluated in relation to the outcome of the orthopedic specialist visit by dividing the population into subjects (a) for further investigation or (b) for drug therapy, physical therapy, or no therapy. By means of logistic analysis, the statistical significance (p value) of each questionnaire item in relation to the subgroups identified by the specialist visit will be calculated, and if the said item is found to be significantly relevant ($p<0.05$), its weight will be considered on the basis of the coefficient obtained in the resulting multivariate analysis. A mathematical-type algorithm will then be constructed for the normalized combination of the scores obtained in the various items using the weights calculated through the logistic model. The model will be applied for each of the 3 questionnaires under study evaluated separately.

DATA MANAGEMENT

For the purpose of the research, anonymity will be guaranteed through the use of identification codes. The data from the self-assessment forms will be collected through paper

CRFs (Case Report Forms) in compliance with the provisions of the current legislation on personal data protection, including Regulation (EU) 2016/679 (General Data Protection Regulation), Legislative Decree No. 196, June 30, 2003, as amended (Personal Data Protection Code) and in accordance with the safeguards and any other applicable provisions of the Data Protection Authority and will be used exclusively for the purpose of the study. The subject code decoding list will be present only at the centers participating in the study.

The data will be accessible only to the person responsible for the data and to personnel who, because of their role within the study, have operational or data entry needs (Principal Investigator and investigators designated by the Azienda Ospedaliera-Universitaria S. Anna di Ferrara, U.O. of Orthopaedic and Trauma Clinic). It is the responsibility of the Principal Investigator to preserve essential documents and data resulting from the study for at least 10 years after its conclusion.

It is the responsibility of the principal investigator to send the end-of-study report to the appropriate authorities.

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Prof. Leo Massari

Director O.U. of Orthopedic and Trauma Clinic

University Department of Translational Medicine and for Romagna

Azienda Ospedaliera-Universitaria Arcispedale S. Anna Hospital of Ferrara

SYNOPSIS

Title of study	SPONTANEOUS MULTICENTER PROSPECTIVE OBSERVATIONAL STUDY FOR THE DEVELOPMENT OF JOINT WELLNESS SELF-ASSESSMENT FORMS
Study centers	**Center Coordinator/Promoter** STRUCTURE: S. Anna Hospital-University of Ferrara OPERATIONAL UNIT: O.U. of Orthopedic and Trauma Clinic. PRINCIPAL EXPERIMENT: Prof. Leo Massari **Satellite Centers** STRUCTURE: I.R.C.C.S. Rizzoli Orthopedic Institute of Bologna OPERATING UNIT: Orthopedic and Trauma Clinic II PRINCIPAL EXPERIMENT: Prof. Stefano Zaffagnini STRUCTURE: I.R.C.C.S. Hospital Galeazzi-Sant'Ambrogio, Milan OPERATING UNIT: Shoulder Surgery. PRINCIPAL EXPERIMENT: Dr. Ettore Taverna
Principal investigator center coordinator	Prof. Leo Massari Director O.U. of Orthopedic and Trauma Clinic University Department of Specialty Biomedical and Surgical Sciences Azienda Ospedaliera-Universitaria S. Anna di Ferrara
Type of study	Spontaneous multicenter, prospective, observational study.
Purpose of the study	The present study represents the pilot phase of the project aimed at developing anatomical site-specific self-assessment questionnaires (shoulder, knee, and ankle) that can direct the subject to the need for an orthopedic specialist visit. The pilot phase of the project involves the collection of data from questionnaires in a population of subjects who have requested an orthopedic specialist examination for the anatomical site covered by the questionnaire. Each questionnaire consists of questions designed to collect information about the patient's subjective condition. The data collected from the questionnaires will be used to assess the statistical significance (p value) of each question on the questionnaire in relation to the outcome of the orthopedic specialist visit. Through multivariate logistic analysis of the responses given by the subject, it will be possible to "weight" each response in order to obtain a final value that gives an indication for an orthopedic specialist visit.
Goals	**Primary objective**

	The primary objective of the present study is to collect data from three self-assessment questionnaires specific to shoulder, knee and ankle by observing the distribution of responses by anatomical site. Each questionnaire will be specific to one anatomical site: shoulder, knee, and ankle. The data collected from the questionnaires will be used to: 1. assess the statistical significance (p value) of each question in the questionnaire in relation to the outcome of the orthopedic specialist examination and 2. score each response in relation to the outcome of the orthopedic specialist examination. **Secondary objectives** The collection of the orthopedic specialist's opinion on the therapeutic indication for each subject participating in the study.
Sample size in the studio	The proposed study is a pilot study designed to determine the feasibility of developing self-assessment forms that can direct patients to the need for an orthopedic specialist visit and to determine the sample size of the final study. Given these assumptions, a power analysis to determine sample size is not applicable to the present study, however, based on clinical experience it is believed that 50 subjects per anatomical site is a sufficient number to allow determination of response weights based on the statistical analysis of the data below.
Duration of the study	For each patient, the study will last as long as it takes to complete the self-assessment form and orthopedic specialist examination and the. For each center, the study will last 1 year from the date of approval of the study protocol by its Ethics Committee, subject to reaching the total number of patients to be enrolled.
Selection criteria	**Criteria for inclusion of patients** • Subjects who have requested an orthopedic specialist examination for the anatomical site covered by the self-assessment form; • Age 18 years or older; • Understanding of the Italian language. **Exclusion criteria** • Surgery within the previous 12 months at the anatomical site covered by the self-assessment form.
Study design	1. Enrollment concurrently with an orthopedic examination specific to the anatomical site covered by the self-assessment questionnaire. 2. Completion of the anatomical site-specific self-assessment questionnaire. 3. Orthopedic specialist examination (will be performed in all subjects participating in the study, regardless of the answers given in the questionnaire).

	4. At the end of the examination, the orthopedic specialist notes the outcome of the specialist examination with the therapeutic indication he or she deems appropriate. 5. Statistical analysis of collected data.
Clinical evaluations	Orthopedic specialist examination
Statistical analysis	The evaluation of the collected data will subsequently receive statistical evaluation by researchers pertaining to the centers participating in the study who were involved neither in defining the questions on the self-assessment forms nor in collecting data from patients. Each item of the questionnaire will be evaluated in relation to the outcome of the orthopedic specialist visit by dividing the population into subjects (a) for further investigation or (b) for drug therapy, physical therapy, or no therapy. By logistic analysis, the statistical significance (p value) of each item of the questionnaire in relation to the subgroups identified by the specialist visit will be calculated, and if the said item is found to be significantly relevant ($p<0.05$), its weight will be considered on the basis of the coefficient obtained in the resulting multivariate analysis. A mathematical-type algorithm will then be constructed for the normalized combination of the scores obtained in the various items using the weights calculated by means of the logistic model. The model will be applied for each of the 3 questionnaires under study evaluated separately.

Joint Wellness
Self-Assessment Sheets

CRF

GENERAL PATIENT DATA SHEET

To be completed by the orthopedic specialist

Patient code _____

Notes : _____

ANKLE

To be completed by the patient

Please answer each question with an answer that more appropriately describes your condition.

SEX (M/F) __ / __

AGE: ____

Ankle pain is	Very slight	Mild	Moderate	Severo	Unsustainable
How frequently do you experience pain?	This is the first time	Rarely	Sometimes	Often	Always
Does the ankle swell?	Never	Rarely	Sometimes	Often	Always
Is it able to walk on rough or uneven terrain?	Yes Always	With some difficulty	With difficulty	With considerable difficulty	Not able to walk on uneven ground
Have you had sprained (twisted) ankle?	Never	1-2 times	3-5 times	At least 10 times	More than 10
How far can you walk before you have ankle pain?	Limitless	16-30 minutes	5-15 minutes	Less than 5 minutes	I can't walk
Can he run?	Yes, no problem	Problems after 500-600 meters of running	Problems after 300-400 meters of running	Problems after 100-meter run	I can't run
Can you go up and down the stairs?	Yes, always	With some difficulty	With moderate difficulty	With much difficulty	I can't
When you get up from the chair do you have pain in your ankle?	Never	Rarely	Sometimes	Often	Always

CRF

GENERAL PATIENT DATA SHEET

To be completed by the orthopedic specialist

Patient code _____

Notes : _____

KNEEL

To be completed by the patient

Please answer each question with an answer that more appropriately describes your condition.

SEX (M/F) __ / __

AGE: ____

The pain is	Very slight	Mild	Moderate	Severo	Unsustainable
How frequently do you experience pain?	This is the first time	Rarely	Sometimes	Often	Always
Does the knee tend to swell?	Never	Rarely	Sometimes	Often	Always
Does the knee tend to lock/feel stiff?	Never	Rarely	Sometimes	Often	Always
Does the knee tend to give way?	Never	Rarely	Sometimes	Often	Always
How far can he walk?	Limitless	More than 1000 meters	500 to 1000 meters maximum	Less than 500 meters	I can't walk
Can you do the stairs?	Yes, no problem	Problems just getting off	Problems getting down and up	Impossible to get off	I can't do the stairs
Difficulty in running?	No difficulty	A little bit	Moderately	Much difficulty	I can't run

CRF

GENERAL PATIENT DATA SHEET

To be completed by the orthopedic specialist

Patient code _____

Notes : _____

SHOULDER

To be completed by the patient

Please answer each question with an answer that more appropriately describes your condition.

SEX (M/F) __ / __

AGE: ____

Do you have pain in your shoulder?	This is the first time	Rarely	Sometimes	Often	Always
How intense is the pain	Very slight	Mild	Moderate	Severo	Unsustainable
Is the shoulder stiff?	Never	Only in the Morning	Only in Flexion	In Flexion and External Rotation	Always in all planes of space
How many kilograms can you lift above shoulder level	More than 10 kg	5 to 10 kg	5 to 10 kg	Up to 1 kg	I can't lift my arm above shoulder level even without weights
Do you have pain during intense physical activity?	Never	Rarely	Sometimes	Often	Always
Difficulty combing the hair	No difficulty	A little bit	Moderately	Much difficulty	I Can't
Difficulty in washing the back	No difficulty	A little bit	Moderately	Much difficulty	I Can't
Can you hit a ball above shoulder level with your hand?	Yes, no problem	Only after prolonged effort with little force	With little force	Rarely and with little force	I can't

4.3.4 Documentation for clinical study to be submitted to the ethics committee

CIP study and preparation

During the third year of the project, extensive research and development of all the forms to be sent to the relevant ethics committee for the clinical trial with the new MDR medical device, delivering specific PEMF parameters, for the treatment of inflammatory joint diseases, which cause mild cartilage damage, that cannot be treated surgically, was also carried out.

Clinical Investigastion Plan

QUANTITATIVE EVALUATION OF THE EFFECT OF NEW PEMF MEDICAL DEVICE IN PATIENTS WITH INTERMEDIATE-GRADE KNEE OSTEOARTHRITIS:

PILOT STUDY

INTRODUCTION

Exposure of skeletal tissues to PEMF has been shown to result in enhanced extracellular matrix (ECM) synthesis and increased secretion of cytokines resulting in repair of bone and cartilage. The mechanisms of action of PEMF include the synthesis of growth factors, particularly transforming growth factor beta (TGFβ), and suppression of inflammatory mediators. A number of studies, including placebo-controlled double-blinded analyses, have demonstrated enhanced bone formation (Borsalino et al, 1988; Mammi et.al., 1993). Bone remodeling is a feature of OA and the effects of PEMF to increase bone formation are of interest to this study (McLeod and Rubin, 1992). Osteogenic differentiation of mesenchymal stem cells (MSCs) is enhanced by PEMF (Tsai et al., 2009). Aaron and Ciombor (1996) have shown that the progenitor cell pool in endochondral ossification is stimulated by PEMF resulting in increased chondrogenesis and osteogenesis. PEMF also has profound effects on cartilage. Cell proliferation and increased ECM synthesis after PEMF exposure have been observed in chondrocytes *in vitro* (Sakai et al., 1991). A meta-analysis of 6 studies demonstrated that PEMF increases chondrocyte proliferation and aggrecan content (Fini et al., 2005). Anabolic effects of PEMF are synergistic with insulin growth-like factor 1 (IGF-1) and antagonize the catabolic effects of *interleukin 1* (IL-1), restoring ECM loss in bovine explants and human OA chondrocytes (Pezzetti et al, 1999; Fioravanti et al, 2002; DeMattei et al, 2004).

PEMF has been shown to suppress inflammatory cytokines. Among the most striking demonstrations of mechanisms of PEMF action is enhanced binding of adenosine to the adenosine receptor, A_{2A} (Massari et al, 2007). Binding of adenosine, especially to A2A, down-regulates the expression of pro-inflammatory cytokines and cartilage matrix is preserved with A2A agonists (Tesch et al, 2002; Cohen et al, 2004). PEMF has been shown to increase A2A density and the binding of adenosine to A2A and to inhibit

catabolic cytokines (Varani et al, 2002; Varani et al, 2003). In ovine OA, PEMF treatment lowered IL-1 and tumor necrosis factor-alpha (TNF-α) and raised TGFβ levels Benazzo et al, 2008). Aaron et al. (2004) reviewed PEMF effects on inflammatory cytokines and presented 9 studies reporting elevations of the TGFβ/BMP gene family. Aaron et al. (2002) has also demonstrated that PEMF stimulates the synthesis of TGFβ mRNA, and protein in chondrogenesis (Fig. 1).

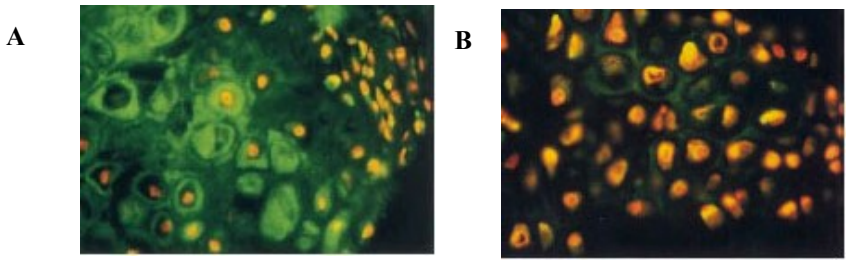

Figure 1: In a model of endochondral bone formation stimulated with PEMF, we showed that TGFβ is synthesized by chondrocytes (a) coincident with chondrogenesis rather than by MSCs (b).

Two independent *in vivo* PEMF studies on OA in guinea pigs have shown significant reduction in cartilage degradation. Ciombor et al. (2003) also demonstrated a reduction in the cartilage neo-epitopes 3B3 and BC-13 as well as matrix metalloproteinase 13 (MMP-3), MMP-13 and IL-1, and an increase in TGFβ. The second study demonstrated preservation of cartilage thickness and subchondral bone structure (Fini et al., 2005). Several clinical studies have shown that PEMF treatment reduced pain after arthroscopic surgery, ACL repair and in OA joints as well as significantly improved Knee Injury and Osteoarthritis Outcome Score (KOOS) compared to placebo treatment (Pipitone and Scott, 2001; Benazzo et al, 2008).

A comprehensive approach to controlling OA should include modulating the cytokine environment in OA joints. There is compelling evidence from in *vitro* and *in vivo* studies

that PEMF has a positive effect on preserving and restoring ECM, based most likely on alteration of cytokines. Studies of the biologic mechanisms of PEMF associated with symptomatic improvement of OA have never been done in humans.

In a seminal study, a reduction in the severity of OA in guinea pigs was demonstrated after exposure to PEMF. This reduction in OA severity was manifested by lower histological/histochemical grades which reflected less cartilage destruction in PEMF-treated knees (Fig 2) (Ciombor et al., 2003). The preservation of the ECM was further demonstrated by a reduction in the neo-epitopes 3B3 (-) and

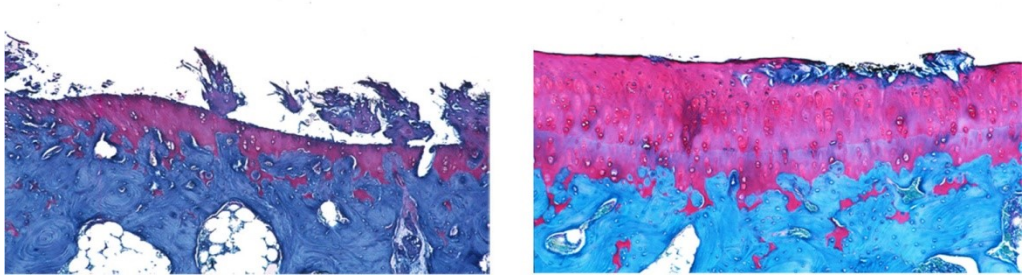

Figure 2: Representative Safranin O stained sections of the medial tibial plateau showing extensive cartilage loss in placebo treated knee (a) and preservation of cartilage in PEMF-treated knees (b).

BC-13, reflecting less severe cleavage of aggrecan, and by decreases in cells immunopositive for the matrix-degrading enzymes, MMP-13 and MMP-3 and the inflammatory cytokine, IL-1. Increases in the number of TGF-β positive cells were observed (Figs 3, 4; Table 3).

	Control	**PEMF**	**% Change**	**P**
MMP-13	7.2±0.8	0.0±0.0	-	0.01
MMP-3	13.8±1.9	8.4±1.0	-39%	0.02

IL-1	13.9±2.3	7.2±0.6	-48%	0.01
IRAP	10.5±1.0	17.3±1.1	+65%	0.003
TGF-β	20.0±3.5	34.4±2.8	+72%	0.006

Table 3: Immunopositive cells (per unit area). PEMF exposure reduced MMP-3, MMP-13 and IL-1 while increasing the protective cytokines, IRAP and TGFβ.

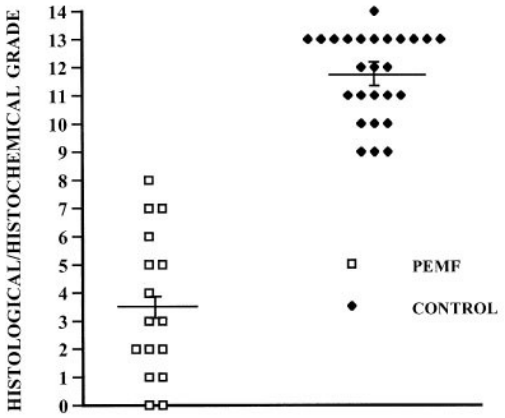

Figure 3: Histological/histochemical grade. Mean grade of control tibias was 11.7±0.3 compared with 3.5±0.7 of PEMF-treated cartilage (p=0.0001).

B

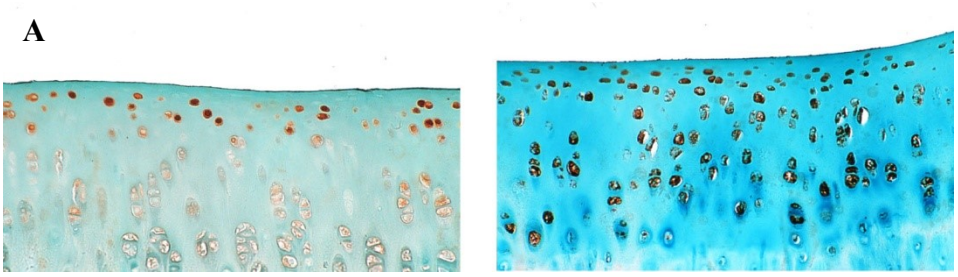

Figure 4: Immunohistochemistry for TGFβ in unexposed (a) and PEMF-treated (b) tibial cartilage showing increased numbers of immunoreactive cells in PEMF-treated sections.

TITLE

Quantitative evaluation of the effect of new PEMF medical device in patients with intermediate-grade knee osteoarthritis: pilot study.

PURPOSE

The present study aims to observe the effect of the new PEMF device on cartilage by high-field MRI (3 Tesla, 3T) investigation that can provide both morphological and biochemical information on joint structures.

Two different treatment regimens of the new PEMF device will be applied in the study: 4 hours per day for 30 days and 1 hour per day for 3 months. The effect of the new PEMF device will be monitored by 3T MRI at the end of therapy and at 6 months after the end of therapy.

OBJECTIVES OF THE STUDY

Primary objective: to evaluate by nuclear magnetic resonance imaging (NMR) the effect of the new PEMF device on cartilage damage present in the medial compartment of the knee in subjects with early-grade osteoarthritis.

Secondary objective: to evaluate the effect of the new PEMF device on pain resolution and clinical scores of knee function in the two different treatment regimens.

STUDY DESIGN

Pilot interventional study with medical device.

The included patients will be divided into two distinct groups by treatment modality.

1. Group 1: new device at PEMF, 1 hour/day for 3 months;
2. Group 2: New PEMF device, 4 hours/day for 1 month.

STUDY SETTING

Patients will perform the treatment at home.

The device consists of a signal generator and an applicator, called a coil. The coil will be placed on the knee, not necessarily in direct contact with the skin. The device operates by a rechargeable battery, has a single on/off button, and is equipped with an hour counter to assess patient compliance. Treatment with the new PEMF therapy device will begin within 3-7 days after recruitment/baseline, lasting 1 hour/day for 3 months (Group 1) or 4 hours/day for 1 month (Group 2).

PATIENT SELECTION CRITERIA

Inclusion criteria :

- Males aged 20-45 years.
- Body mass index, BMI < 35.
- Subjects with Kellgren-Lawrence grade 2 osteoarthritis
- Subjects with VAS at baseline ≥ 2
- Subjects with degree of varus ≤5°
- Subjects with degree of valgus ≤5°
- Absence of ligamentous pathology.
- Subject willing to discontinue all long- and short-acting opioids (pills and/or patches).
- Subjects willing to discontinue corticosteroids administered by any route except intranasal spray and steroids containing ophthalmic solutions and antiasthmatics
- Subjects who did NOT receive infiltration in the target knee

Exclusion criteria:

- Patients with systemic inflammatory or neoplastic diseases
- Arthroscopy of the target knee in the 12 months prior to enrollment.

- Subjects diagnosed with inflammatory arthritis (rheumatoid arthritis, gout, joint infections, Lyme disease, SLE, etc.).
- Subjects diagnosed with secondary arthritis (acromegaly, Charcot arthropathy, hemochromatosis, Wilson's disease, ochronosis, anterior cruciate ligament injury)
- Patients unable to sign informed consent

VARIABLES TO BE COLLECTED AND SOURCE

Primary outcome:

- cartilage status at baseline and at follow-up by T2-mapping quantification system obtained by 3 Tesla MRI.

Secondary outcomes:

- detection at baseline and at follow-ups of clinical scores for pain and joint function that will be made the patient fill in during the follow-up visit.

CLINICAL AND INSTRUMENTAL ASSESSMENTS

To recruitment/baseline

- Medical history
- Objective examination
- Nuclear Magnetic Resonance Imaging (MRI) 3 Tesla.
- MRI analysis of the medial compartment of the knee will be performed with 3Tesla MRI.
- Clinical scales: VAS, KSS, Tegner Activity Score (TAS)
- Registration of NSAID intake, and dietary supplements for cartilage maintenance.
- Registration of other concomitant therapies (Physiotherapy, use of orthopedic footplate)

Upon completion of treatment with the new pemf device and at 6 months after the end of treatment

- Nuclear Magnetic Resonance Imaging (MRI) 3 Tesla.
- MRI analysis of the medial compartment of the knee will be performed with 3Tesla MRI.
- Objective examination
- Clinical scales: VAS, KSS, Tegner Activity Score (TAS)
- Registration of NSAID intake, and dietary supplements for cartilage maintenance.
- Recording of other concomitant therapies (Physiotherapy, anti-inflammatory infiltrative therapy, use of orthopedic footplate)
- Patients' compliance with treatment

SAMPLE SIZE and STATISTICS.

The proposed study is a study to observe the health status of articular cartilage by instrumental measurement 3T MRI with T2 mapping sequences in patients with osteoarthritis of the knee before and after treatment with two different therapeutic regimens of the new PEMF therapy device. Bellisari F. et al. (La Radiologia Medica 2021) data indicated that subjects with grade 2 and 3 osteoarthritis treated with intra-articular injections of platelet-rich plasma (PRP) had a T2 mapping score in the target compartment of 47.8 ± 3.7 before treatment, which decreased to 43.5 ± 3.9 at 12 months after PRP treatment. On this basis, a sample size of 10 subjects per group with pre- and post-treatment T2 mapping score measurements yields a difference of 4.3 points with a power of 83% in a design with 2 repeated measurements with a compound symmetry covariance structure, when the standard deviation is 3.8, the correlation between observations on the same subject is 0.500 and the alpha level is 0.050.

ENLISTMENT PROCEDURE

Patients eligible for the study, will be included in the study after providing written informed consent. No data collection and procedure/analysis will be performed before the consent is signed.

RANDOMIZATION

Patients, upon recruitment into the study protocol, will be divided through a block randomization program (www.randomization.com) into two homogeneous groups of 10 patients each. Patients in group 1 will undergo treatment of the new PEMF device, 1 hour/day for 3 months, patients in group 2 will undergo treatment with the new PEMF device, 4 hours/day for 1 month.

In order to obtain two homogeneous groups, the following patient stratification criteria were defined: age ($20 \leq age \leq 30$ and $30 < age \leq 45$).

CONCEALMENT OF THE RANDOMIZATION LIST

To avoid systematic errors, the randomization center will be external and will exploit an interactive (web) system for allocating patients to the two groups. Clinicians identify patients, obtain consent, decide on enrollment, enter patient characteristics (age, and gender) into a web-based software program, which automatically assigns the patient to the first useful place on the list, in one of the two groups. The program returns a code (A/B) that corresponds to a stimulator that the clinician will use to stimulate that patient.

ENROLLMENT TIMELINE

Enrollment of patients will take place over a 12-month period. Following enrollment, after performing baseline data collection, patients will begin their assigned treatment protocol. The treatment protocols under the study will last between 1 and 3 months, while the last

clinical and instrumental follow-up will be performed at 6 months after the start of treatment. For each patient in Group 1, the study will last 9 months. For each patient in Group 2, the study will last 7 months. For each center, the study will last 2 years from the date of approval of the study protocol by its Ethics Committee, subject to reaching the total number of patients to be enrolled.

DATA COLLECTION.

The patient's clinical data will be collected at the outpatient visit. If the patient has the inclusion criteria and no exclusion criteria, he/she will receive proposal for enrollment. Clinical examination and MRI baseline and at follow-ups will be performed.

A special data collection form will be used to collect the clinical information and the results of the analysis involved in the study.

A data collection form (Case Report Form - CRF) will be completed for each patient entered into the study.

Evaluation	**Screening**	**Day 0** Within 7 days of Screening	**Termination of therapy** ± 15 days	**6 Months from the end of therapy** ± 15 days
Informed Consent	X			
Inclusion/exclusion criteria	X			
Biographical data	X			
Medical history	X			
Concomitant medications	X	X	X	X
Objective examination of the knee	X	X	X	X
SEA		X	X	X
Clinical Scales (KSS, Tegner Activity Score)		X	X	X
MRI		X	X	X
Randomization		X		
EA monitoring		X	X	
Patient compliance with therapy		X	X	
Withdrawal of the device			X	

EVALUATION OF RESULTS USING STATISTICAL METHODS

Evaluation of the collected data will subsequently receive statistical evaluation by investigators belonging to the centers participating in the study who were not involved in enrollment and data collection from patients. The analysis of MRI images will be performed by experienced radiologists blinded to the treatment group and the timing of the examination. The MRI analysis will involve both quantitative analysis through the assessment of relaxation times in T2 (T2 mapping) and qualitative morphological analysis of cartilage. It is also planned to collect information obtained from the VAS, KSS, TAS questionnaires and on the use of NSAIDs for pain control.

A descriptive type analysis is planned on the collected variables: mean, median, standard deviation and range of variability for continuous variables, absolute counts and percentages for categorical variables. Quantitative analysis of the ability of the new PEMF device to change cartilage status will be performed by paired 2-tailed Student's t test on the absolute values of relaxation times at T2 in comparison with values measured at baseline. Further statistical evaluations such as Pearson chi square test and Generalized Linear Mixed effects Model will be applied to scores obtained from VAS, KOSS, TAS questionnaires and on the incidence and amount of NSAID use.

ETHICAL PRINCIPLES.

The research protocol and related documents will be sent before the study begins to the relevant authorities and ethics committee for approval. The responsible investigator will ensure that the study is conducted in accordance with the Declaration of Helsinki in its most current version (Fortaleza, October 2013), as well as with all national and international regulations applicable to clinical research. The protocol has been written and the study will be conducted according to the principles of ICH-GCP (ref: http://www.emea.eu.int/pdfs/human/ich/013595en.pdf).

INFORMED CONSENT

Participation in the study is solely on a voluntary basis. Each potential participant will be given a comprehensive explanation of the conduct of the study at the baseline visit by the orthopedist and will be given the opportunity to ask questions and have any concerns answered. Each subject will be given explicit information regarding the nature of the project, purpose, instrumentation used, and activities required, and will be required to sign a written consent before being included. Sensitive data will be processed according to current regulations by the study leader. In addition, participants may withdraw their consent to participate at any time without any consequences.

The protection of personal information provided by subjects will be carried out in accordance with current legislation regarding the protection of personal data. In line with international regulations regarding data protection, the following measures will be taken:

Paper materials will be stored in dedicated locked cabinets that are not accessible to unauthorized individuals;

At the coordinating center the questionnaires and all paper data will also be stored in digital format at Clinic 2, with access allowed only with a password.

PRIVACY

All patients included in the study will be identified with a numeric code, so that sensitive data will be made pseudo-anonymous and used in accordance with current privacy regulations. Data will be kept by the investigator for as long as necessary for scientific production.

In order to ensure the confidentiality of clinical trial data as stipulated in the applicable national and European regulations, the data will be accessible only to the study sponsor and its designees, for monitoring/auditing procedures, the investigator and collaborators, and the

Ethics Committee of the center where the research is conducted and the relevant health authorities.

The investigator and the Institute will allow access to source data and documentation for monitoring, audit, Ethics Committee review, and Health Authority inspections, but preserving confidentiality of personal data in accordance with current regulations

FINAL REPORT AND PUBLICATION OF RESULTS

The study leader, agrees to produce the final report, publish all data collected as described in the protocol, and ensure that the data are reported responsibly and consistently.

In particular, the publication of data derived from this study will take place regardless of the results obtained.

The transmission or dissemination of data, by way of scientific publications and/or presentation at conferences conventions and seminars, will take place exclusively as a result of the purely statistical processing of the same, or in any case in an absolutely anonymous form.

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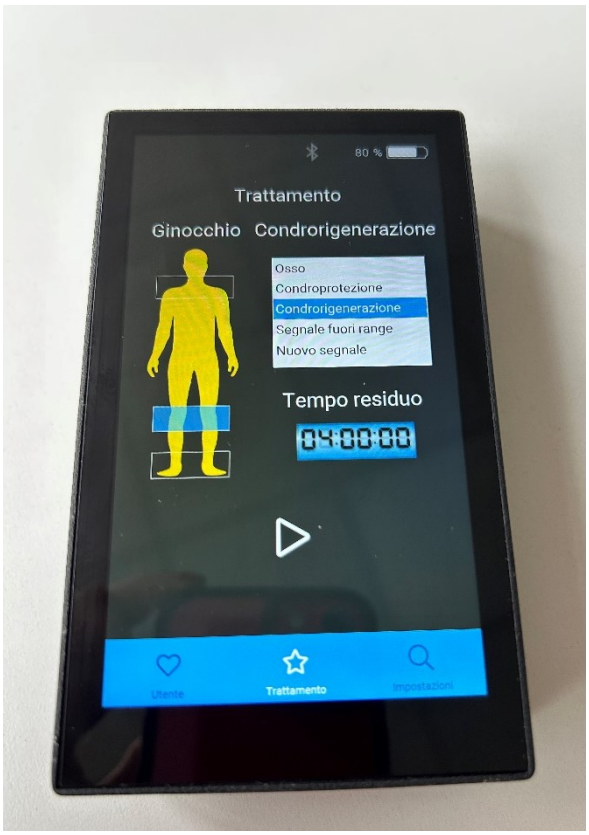
4.4 Conclusions

This project aimed to create a new medical device for home use that can generate a specific PEMF, the signal of which is capable of promoting cartilage regeneration. Most of the scientific literature agrees on a beneficial effect of stimulation with PEMF in chondrocyte proliferation and treatment of joint inflammatory processes. Preclinical studies conducted in parallel with this project allow the identification of specific electromagnetic signal characteristics capable of stimulating cartilage regeneration. These signal parameters have been stored in the new medical device and will also be remotely updatable. In fact, the new medical device is equipped with a Bluetooth communication module for connection with smartphones to remotely control its functionality. The communication between the device and the enterprise server will allow the device to be checked for proper operation and electromagnetic field generation. This communication method will also allow new pulse generation protocols to be transferred to the nonvolatile memory, keeping the device up-to-date. This mode of communication is absolutely innovative compared to devices currently on the market and will allow, not only to control the device, but also to open the possibility of feedback with the sportsman for the application of specific treatments for different anatomical sites. In addition, the self-assessment forms available on the app will guide the sportsman in choosing which treatment protocol to use.

The device is totally innovative, as to date there are various PEMF therapy devices on the market, but none of them have proven effective in regenerating articular cartilage. The device was based on solid scientific results obtained from both *in vitro* and *in vivo* experiments for the choice of PEMF parameters aimed at cartilage regeneration. The solid scientific basis developed in the research centers will unequivocally distinguish the device from the magnetotherapy devices available on the market today, promoting its adoption and widespread use. The technology adopted makes the device specifically for personal use, and

Bluetooth communication, through the smartphone, with the company will keep the device controlled and updated accompanying the sportsman in his activities over time.



5 DEGREE OF INNOVATION OF THE RESEARCH PROJECT

Cartilage injuries mainly due to inflammatory diseases represent the great challenge of the last 20 years. It is a pathology that increasingly affects the young and old due to the increased practice of sports activities in every age group. Such injuries still represent an open challenge for the orthopedic surgeon. More than 20 thousand knee cartilage reconstruction surgeries are performed each year in Italy with a prevalence in the 25-35 age group. Although several therapeutic strategies have been developed in order to repair these injuries, the clinical results are, to date, strongly conflicting. Cartilage injuries if not treated at an early stage lead to the development of degeneration of the entire joint, osteoarthritis.

The articular cartilage for a long time was thought to be a tissue that is difficult to repair once damaged, however, recently, the concept of '*Joint Preservation'* has evolved mainly in response to the limitations of current technologies. Orthopedic surgeons are particularly interested in '*Joint Preservation*' as a technique that can prevent and/or delay the onset of osteoarthritis, with a view to expanding the spectrum of intervention to a stage where the joint can be preserved.

To date, there are no medical devices used for cartilage regeneration on the national and international market. The project involved confirmation of preliminary results already observed following stimulation with PEMF on cellular activities that promote anabolic activity on articular cartilage by reconstituting its integrity.

The results of this project introduce radical changes in character:

- Scientific: The present project enables the identification of cellular mechanisms underlying cartilage regeneration processes and the effects of PEMF stimulation on regenerative activities.
- Technological: The new device is equipped with a remote control system through which it will be possible to check its proper functionality and keep the device up-to-date. In

addition, a Bluetooth mode of communication (BLE) protocol has been developed that will allow the new device to be connected with smartphones.

- Cognitive: the pre-clinical and clinical skills gained in this project will provide a wealth of experience and knowledge that can be used in further studies

The changes brought about through this project led to the development of a new product that features both technological and scientific innovation.

From a scientific point of view, the preclinical results carried out in parallel with the present project has shown how the new device, through intracellular metabolic pathways, can inhibit chondrocyte senescence and apoptosis, promote their proliferative activity, and finally promote cartilage regeneration by joint-resident synovial cell populations. These results will enable the scientific partners in this project to become reference centers for non-surgical cartilage regeneration techniques, to develop innovative investigation techniques that can be offered to other industrial clients for research purposes, and finally to become a clinical reference for sports medicine.

The new device is also supported by major technological innovation, new experimental and clinical application development data. Preclinical activities provide the solid scientific basis necessary and indispensable for the success and acceptance of the new device. The target of treatment with the new medical device is articular cartilage exhibiting fissuring, surface erosion, thickness thinning; basically all conditions framed in the early stages of cartilage distress (initial osteoarthritis). The modalities of application of the new medical device in the main joint sites (knee, ankle, shoulder) have been defined, and the frequency of treatment sessions will be defined soon. Clinical work has led to the development of user-friendly self-assessment forms available through the mobile application that will guide the sportsman in the use of the new medical device.

The World Health Organization (WHO) has defined cartilage injuries as a group of diseases with a high social impact due to their high incidence, large economic costs, and reduced

quality of life. Cartilage injuries are also increasingly common in sports, where they mainly affect athletes who engage in intensive sports. Articular cartilage injuries are found in these athletes with a frequency ranging from 36% to 50%, of which about 15-20% are asymptomatic.

These observations testify to the scale of the problem and the market opportunities that the implementation of the new medical device will offer. The device will be manufactured in Italy and sold through e-commerce to an extremely wide audience of users. The chosen business model, sale of the device, is simplified compared to the current rental model, resulting in lower operating costs per unit and the possibility of addressing foreign markets through a "virtual company" without the need for a complex structure as in Italy today.

The therapeutic solution is configured as a cost-effective solution that increases the value and importance of the product. At the end of this project, CE certification of the medical device, its industrialization, and promotional activities in the field of "Sport Medicine" will be planned with a view to release on the European market.

The introduction of the innovative medical device will enable the company to set a new worldwide benchmark in regenerating articular cartilage damage.

The application methods of the new medical device will give the company a strong competitive advantage in terms of performance, quality and level of service rendered to the customer in the "Sport Medicine" sector. In fact, with this project, the company intends to penetrate/re-position itself in this market, increasing/consolidating its position for the three-year period after the conclusion of the project.

Other interests are based on the acquisition of knowledge and tools to improve the quality of care and reduce the cost of health care spending in a field such as sports medicine-and associated joint disorders-which is increasingly relevant in today's society and set to grow further in the near future.

The new medical device will be used by professional and amateur athletes, as part of "Sport Medicine." CONI estimates report that about 15 million individuals are involved in sports activities in their spare time in Italy. The use of the new medical device will be promoted for regeneration of early cartilage injuries and to preserve joint integrity as a whole. The sportsman will be placed at the center of promotional activities through the personalization of therapy made possible by the new medical device thanks to the web tools made available. This project will enable innovative and effective approaches to foreign markets. In particular, foreign offices will be involved early in the promotion of the new medical device. The development of the foreign market will lead to a radical change in the business model, no longer based on renting the device, but on selling it, monitoring its operation, and constantly updating it via the Web.

The business plan calls for the development of procedures and hardware and software tools necessary for e-commerce activities that will also allow access to foreign markets.

Remote after-sales support and updating of the device will enable its international deployment without having to implant a specific local technical facility in each country. The business model to be implemented will be promoted in both domestic and international markets.

The development of online sales activity will be centered on a specific strategic digital marketing plan that can be implemented through different techniques of positioning and engaging potential targets in the different markets chosen.

Partnerships will be activated with sports clubs to convey and promote the therapeutic proposal on the mass market of professional and amateur sportsmen and women, and collaborative relationships with major top league sports clubs in football and other sports, with their medical managers, staff and athletes successfully treated over the years. The new medical device will make it possible to maintain the functionality of articular cartilage over

time, resulting in less demand for surgical procedures to restore joint function and thus an economic benefit to the National Health System.

The strong scientific background and solid network of relationships with the main Italian and foreign Key Opinion Leaders in the field of "Sport Medicine" will be the basis on which to implement brand building strategies and will allow to significantly expand the target audience of potential users of the new medical device, offering important new business opportunities.

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