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Infections of the anterior cruciate ligament: Study of biofilm graft formation and the effect of vancomycin soaking on re-rupture rates

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DOCTORAL THESIS

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“La vida no és un problema a resoldre,

sinó una realitat a experimentar.”

~ Søren Aabye Kierkegaard

LIST OF ABBREVIATIONS

ACL: Anterior cruciate ligament

ACL-r: Anterior cruciate ligament reconstruction

Ht: Hamstrings tendon

4xHt: Quadrupled hamstring tendon

BPTB: Bone-Patellar Tendon-Bone

MRI: Magnetic Resonance Imaging

CNS: Coagulase negative staphylococci

MID: Minimal infective dose

IKDC: International Knee Documentation Committee

SD: Standard deviations

BST: Banc de Sang i Teixits

CFU/ml: Colony-forming units per milliliter

SEM: Scanning electron microscopy

n.s: Non-significant

MCRD: Minimal clinically relevant difference

μW: Microwatt

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ABSTRACT

INTRODUCTION

Anterior cruciate ligament reconstruction (ACL-r) is a common surgical procedure designed to restore knee function and stability following ligament injury. Although significant advances have been made in surgical techniques, postoperative complications, particularly infections, continue to pose a challenge to achieving optimal outcomes. Infections after ACL-r surgeries can lead to graft failure, prolonged recovery, and even additional interventions. This thesis focuses on two critical issues related to ACL-r surgeries. One is the safety and effectiveness of vancomycin presoaking of grafts as a prophylactic measure against infections. The other is the potential role of quadrupled hamstring tendon (4×Ht) grafts, specifically the presence of sutures, as an independent factor that contributes to higher infection rates when compared to bone-patellar-tendon-bone (BPTB) grafts.

SCIENTIFIC JUSTIFICATION

Infections following ACL-r, though relatively uncommon, can significantly impact patient outcomes and increase healthcare costs. The prophylactic use of vancomycin presoaking has emerged as a potential strategy to reduce infection rates. However, concerns about its impact on graft integrity, the re-rupture risk, and patient functional outcomes have limited its widespread adoption. Moreover, the higher infection rates reported with hamstring tendon grafts in comparison to BPTB grafts have raised questions about whether structural differences, particularly the presence of sutures, predispose 4×Ht grafts to greater biofilm formation as well as the risk of infection. This thesis addresses these concerns by means of a two-part investigation aimed at improving patient safety and outcomes in ACL-r surgeries.

HYPOTHESIS AND OBJECTIVES

Hypothesis 1: Vancomycin presoaking of grafts in ACL-r surgeries does not increase the risk of re-rupture or negatively affect functional outcomes.

Hypothesis 2: 4×Ht grafts, when compared to BPTB grafts, exhibit greater biofilm formation *in vitro*.

Primary Objective: To evaluate the safety of the vancomycin presoaking technique by determining the risk of re-rupture and functional outcomes following ACL-r. Additionally, it was to assess and compare biofilm formation on BPTB and 4xHt grafts when contaminated with the same bacterial inoculum.

STUDY DESIGN

The thesis is based on two distinct studies. The first study is a retrospective cohort analysis that compares ACL-r surgeries performed with and without vancomycin presoaking. The primary outcomes evaluated were re-rupture rates and functional recovery in two groups of consecutive patients from historical cohorts. The second study is an *in vitro* investigation examining biofilm formation on 4×Ht and BPTB grafts contaminated with identical bacterial inoculum.

RESULTS

This thesis demonstrated that vancomycin presoaking did not increase the re-rupture risk. Neither did it adversely affect functional outcomes in patients. Re-rupture rates in both the vancomycin and non-vancomycin groups were comparable. Functional outcomes, measured using standardized scoring systems, also showed no significant differences between the two groups. That determination supports the safety of vancomycin presoaking as a prophylactic measure in ACL-r surgeries.

On the other hand, this thesis showed no significant differences in biofilm formation between 4×Ht and BPTB grafts. These findings challenge the hypothesis that sutures in hamstring grafts contribute to higher infection rates.

CONCLUSIONS

Vancomycin presoaking of grafts is a safe prophylactic measure, with no adverse impact on re-rupture rates or functional outcomes. Therefore, this technique is recommended as a standard practice in ACL-r surgeries to reduce the risk of postoperative infections.

Additionally, this thesis refutes the hypothesis that structural differences, particularly the presence of sutures in 4×Ht grafts, predispose them to greater biofilm formation. Therefore, the structural differences between the two graft types are not the basis to justify the predisposition of 4xHt to a higher risk of infection.

RESUM

INTRODUCCIÓ

Les cirurgies de reconstrucció del lligament encreuat anterior (LEA-r) són un procediment quirúrgic comú dissenyat per restaurar la funció i l'estabilitat del genoll després d'una lesió del lligament. Tot i els avenços significatius en les tècniques quirúrgiques, les complicacions postoperatòries, especialment les infeccions, continuen sent un repte per aconseguir resultats òptims. Les infeccions després de les cirurgies de LEA-r poden provocar el fracàs de l'empelt, una recuperació prolongada i intervencions addicionals. Aquesta tesi se centra en dos problemes crítics relacionats amb les cirurgies LEA-r. Una és la seguretat del remull de l'empelt amb vancomicina com a mesura profilàctica contra les infeccions. L'altra és investigar si la presència de sutures en els empelts de tendó isquiotibial quadruplicat (4×Ti) actua com a factor predisposant, independent de majors taxes d'infecció, en les cirurgies amb empelts 4×Ti en comparació a les cirurgies amb empelts de tendó rotulià, os- tendó-os (OTO).

JUSTIFICACIÓ CIENTÍFICA

Les infeccions que segueixen a la cirurgia de LEA-r, tot i que relativament poc comunes, poden condicionar significativament els resultats per al pacient i augmentar els costos de la sanitat. L'ús profilàctic de la immersió prèvia amb vancomicina ha sorgit com una estratègia potencial per reduir les taxes d'infecció. No obstant això, les preocupacions sobre el seu impacte en la integritat de l'empelt, el risc de re-ruptura i els resultats funcionals dels pacients han limitat la seva adopció generalitzada. Per altra banda, les taxes d'infecció més altes reportades amb els empelts 4×Ti en comparació amb els empelts OTO han generat preguntes sobre si les diferències estructurals, particularment la presència de sutures, predisposen els empelts 4×Ti a una major formació de biofilm i risc d'infecció. Aquesta tesi aborda aquestes preocupacions mitjançant una investigació en dues parts destinada a millorar la seguretat i els resultats per als pacients en les cirurgies LEA-r.

HIPÒTESIS I OBJECTIUS

Hipòtesi 1: La immersió prèvia de empelts amb vancomicina en les cirurgies de LEA-r no augmenta el risc de re-ruptura ni afecta negativament els resultats funcionals.

Hipòtesi 2: Els empelts 4×Ti, en comparació amb els empelts OTO, mostren una major formació de biofilm in vitro.

Objectiu Principal: Avaluar la seguretat de la tècnica d'immersió prèvia amb vancomicina mitjançant la determinació del risc de re-ruptura i dels resultats funcionals després de la reconstrucció del LEA; a més d'avaluar i comparar la formació de biofilm en empelts OTO i 4×Ti, quan es contaminen amb el mateix inoculant bacterià.

DISSENY DE L'ESTUDI

La tesi es basa en dos estudis diferents. El primer estudi és una anàlisi retrospectiva de cohort que compara les cirurgies de LEA-r realitzades amb i sense immersió prèvia amb vancomicina. Els resultats principals avaluats van ser les taxes de re-ruptura i la funcionalitat en dos grups de pacients consecutius de cohorts històriques. El segon estudi és una investigació in vitro que examina la formació de biofilm en empelts 4×Ti i OTO contaminats amb inoculants bacterians idèntics.

RESULTATS

Aquesta tesi va mostrar que la immersió prèvia amb vancomicina no va augmentar el risc de re-ruptura, ni va afectar adversament els resultats funcionals dels pacients. Les taxes de re-ruptura en els grups amb i sense vancomicina van ser comparables. Els resultats funcionals, mesurats mitjançant sistemes de puntuació estandarditzats, tampoc no van mostrar diferències significatives entre els dos grups, evidenciant la seguretat de la immersió prèvia amb vancomicina com a mesura profilàctica en les cirurgies de LEA-r.

D'altra banda, aquesta tesi no va mostrar diferències significatives en la formació de biofilm entre els empelts 4×Ti i OTO. Aquests resultats desafien la hipòtesi que els fils de sutura presents en els empelts 4×Ti contribueixen a taxes d'infecció més altes.

CONCLUSIONS

La immersió prèvia d'empelts amb vancomicina és una mesura profilàctica segura, sense impacte advers en les taxes de re-ruptura ni en els resultats funcionals. Per tant, aquesta tècnica es recomana com a pràctica estàndard en les cirurgies de LEA-r per reduir el risc d'infeccions postoperatòries.

A més, aquesta tesi refuta la hipòtesi que les diferències estructurals, particularment la presència de sutures en els empelts 4×Ti, els predisposa a una major formació de biofilm. Per tant, les diferències estructurals entre els dos tipus d'empelts no justifiquen la predisposició dels 4×Ti a un major risc d'infecció.

1. INTRODUCTION

The anterior cruciate ligament (ACL) is one structure within the knee joint that serves as a primary stabilizer during movement and provides support for activities involving running, jumping, pivoting, and other dynamic motions(1). Positioned within the knee, the ACL acts as a key restraint that prevents excessive forward and rotational movement of the tibia in relation to the femur, thus maintaining the integrity of the knee joint during various physical activities (2).

Beyond its mechanical role, the ACL also significantly contributes to proprioception, the body's sense of its position in space. This multi-faceted function underscores the critical nature of the ACL in facilitating smooth and coordinated movement of the knee joint(3).

Injuries to the ACL are common, particularly among athletes. Such injuries can have profound implications for individual quality of life as it leads to pain, instability, and limitations in mobility and participation in sports activities(4).

The management of ACL injuries often involves surgical intervention with the aim to reconstruct the damaged ligament and restore stability to the knee joint. Surgical techniques have evolved over the years with advancements in graft selection, fixation methods, rehabilitation protocols, and risk prevention. All of the are aimed at optimizing outcomes and facilitating the patient’s return to pre-injury levels of function and activity (5).

Given the significance of the ACL in maintaining knee joint stability and function, as well as the increasing incidence of ACL injuries, ongoing research efforts are essential to further enhance our understanding of ACL anatomy, its biomechanics, injury mechanisms, and treatment modalities. Such advancements hold the potential to improve patient outcomes, reduce the risk of reinjury, and enhance the overall management of ACL-related conditions.

1.1. INCIDENCE

The prevalence of ACL injuries has risen significantly in recent years. In the United States alone, ACL injuries affect approximately 200,000 individuals annually with an annual incidence of 68.6 cases per 100,000 population (6,7).

This trend is largely attributed to the growing emphasis on sports and recreational activities, leading to a higher risk of traumatic knee injuries and the increased patient preference for an active lifestyle.(8) Therefore, an increased call for ACL reconstruction surgery has been observed, making anterior cruciate ligament reconstruction (ACL-R) one of the most frequent surgical procedures in the sports medicine field (9,10).

1.2. MECANISM OF INJURY

ACL injuries most commonly occur due to non-contact mechanisms (indirect trauma, 72%) such as sudden deceleration, cutting, landing, or pivoting motions, which subject the knee joint to excessive valgus, external-rotation, or hyperextension forces (11–13). Contact injuries (direct trauma 28%) involving direct blows to the knee or collision with another individual also contribute to ACL tears, particularly in sports like football, basketball and rugby(14). Additionally, biomechanical studies have highlighted the role of anatomical and neuromuscular factors like quadriceps dominance, poor landing mechanics and tibial slope in predisposing individuals to ACL injuries (14,15).

1.3. DIAGNOSIS

Accurate diagnosis is crucial to the management of ACL injuries as it guides treatment decisions and prognostication. Clinical evaluation typically includes a thorough history, physical examination, and adjunctive imaging studies.

In anamnesis, it is common for the patient to report a trauma with a clear injury mechanism. Common findings on physical examination may include joint effusion, ligamentous laxity, and positive provocative tests like the Lachman test

and pivot shift test, indicative of ACL insufficiency (16,17). When an ACL injury is suspected, magnetic resonance imaging (MRI) enables visualization of ACL tears and associated intra-articular injuries and facilitates surgical planning. For ACL lesions, MRI had a sensitivity of 95.8%, specificity of 100% and a diagnostic accuracy of 97.7%.(17,18)

Additionally, other imaging modalities like Magnetic resonance arthrography and dynamic ultrasound offer enhanced sensitivity and specificity in detecting ACL tears, particularly in cases of partial or complex injuries(17).

A comprehensive diagnostic approach that integrates clinical assessment and imaging findings is essential for accurate diagnosis and the optimal management of ACL injuries.

1.4. ANTERIOR CRUCIATE LIGAMENT RECONSTRUCTION

The ACL-R surgical procedure involves “re-building” the damaged ACL with a graft harvested from autologous sources such as the patellar tendon (BPTB), hamstring tendons (Ht), or quadriceps tendon, or using allograft tissue from a donor(19). Nowadays, arthroscopic reconstruction is the gold standard to minimize surgical morbidity and optimize anatomical graft placement and tensioning (20,21). After bone tunnels have been drilled and after positioning the graft, various fixation techniques can be utilized to secure it in place. These include the use of bioresorbable interference screws, staples, and cortical suspensory fixation.

Postoperative rehabilitation plays a crucial role in achieving successful outcomes. It focuses on restoring range of motion, strength, proprioception, and functional stability (5,22). Long-term studies have demonstrated favorable outcomes following ACL-r with patients experiencing improvements in knee function, stability, and return-to-sports participation (5,23). However, challenges such as graft failure, reinjury, infection and persistent functional deficits remain. They highlight the importance of ongoing research and the need to refine surgical techniques to optimize outcomes and reduce the risk of complications.

1.5. GRAFT TYPES INT ACL RECONSTRUCTION

The selection of the graft type for ACL-r is a critical decision. It significantly influences the outcome and success of the surgical procedure. Common graft options include autografts like the BPTB, Ht, and quadriceps tendon, as well as allografts sourced from cadaveric tissue. Each graft type presents unique advantages and disadvantages in terms of biomechanical properties, graft integration, morbidity, and the risk of graft failure (24,25).

For instance, BPTB autografts consist of a strip of tissue harvested from the central portion of the patellar tendon, including bone plugs from the patella and tibia. These grafts offer excellent biomechanical strength and reliable graft fixation but may be associated with anterior knee pain(25). Moreover, some studies suggest that they are less prone to infection(26,27).

In contrast, Ht autografts involve harvesting tendons from the ipsilateral knee, named the semitendinosus and gracilis hamstrings. These tendons are typically doubled or quadrupled (4xHt) and sutured together to enhance graft diameter and strength. These autografts demonstrate less morbidity at the donor site but may have slightly inferior biomechanical properties in comparison to BPTB(28). Additionally, some studies show that they are more prone to infection (26,27). Several hypotheses have been proposed to explain this tendency, but none are confirmed.

Different types of allografts can be used (Ht, quadriceps tendon, Achilles tendon, etc.). They provide the advantage of avoiding donor site morbidity but carry a higher risk of delayed incorporation, higher cost, and potential disease transmission (29). All of this means that many authors reserve their use for revision surgeries(30).

The choice of graft type is influenced by factors such as patient age, activity level, surgeon preference, and the presence of concomitant injuries, highlighting the importance of individualized treatment approaches in ACL-r(25,30).

1.6. IMPLANT RELATED INFECTIONS IN ORTHOPEDICS

Implant-related infections in orthopedics pose significant challenges due to their complex pathophysiology and the formation of a biofilm on implant surfaces.

When bacteria makes contact with the implant surface, they may adhere to the device and produce a protective layer of extracellular polymeric substances, thereby creating biofilms that shield the bacteria from host immune responses and antimicrobial agents(31). This biofilm formation significantly enhances the resilience of bacteria to conventional antibiotic treatments and host defenses, rendering implant-related infections particularly difficult to eradicate if this foreign body is not removed(31,32). For instance, it has been observed that a wound contaminated with the same quantity of bacteria are more likely to develop an infection if there are implants like sutures on it (33,34).

Consequently, surgical intervention is often necessary to remove and debride the infected tissues. Moreover, implant removal followed by implant replacement or revision surgery to achieve infection control may be required in some cases. These types of surgeries have an significant impact on patient quality-of-life (35).

The challenging treatment and weighty consequences of dealing with implant-related infections underscores the importance of preventive measures. Those measures include meticulous surgical techniques, perioperative prophylaxis, and implant surface modifications aimed at reducing bacterial adhesion and biofilm formation(35,36). However, the preventive techniques should be safe and cost-effective.

1.7. ACL-r INFECTIONS: EPYDEMIOLOGY, ETIOLOGY, TREATMENT AND OUTCOMES

Infections following ACL-r surgery represent a significant complication that may lead to a prolonged recovery period, functional impairment, and the need for revision surgery(37). The pathogenesis of ACL-r infections shares similarities

with other orthopedic implant-related infections in that they often involve bacterial adherence to the implant surface and subsequent biofilm formation. The same concepts applies to prosthetic joint infections(38). The epidemiology of ACL-r infections varies but is generally reported to occur in 1% to 2% of cases in different studies(39,40).

Staphylococci are the predominant causative pathogens (90%), especially coagulase negative staphylococci (CNS) and acute infection is the most common presentation for this kind of infection(26,30,39,40). Some studies have shown that the source of this infections may be the contamination that often occurs during graft harvesting and preparation(41). There are also some rare cases that can present as chronic infections(42).

Prompt diagnosis and management are paramount in minimizing the impact of ACL-r infections. The treatment typically involves a combination of surgical debridement, antimicrobial therapy, and potential implant removal or revision surgery to eradicate the infection and restore knee function(23,38).

The outcomes following ACL infections can vary, with some patients experiencing persistent symptoms, functional limitations, and the need for further surgical interventions. Others achieve a satisfactory recovery with minimal long-term sequelae(37,39).

1.8. PROPHILAXIS FOR ACL-r INFECTIONS

The prevention of ACL-r infections is of foremost importance in optimizing patient outcomes and reducing the healthcare financial burden. While general prophylactic measures are always applied (proper skin preparation and preoperative antibiotic prophylaxis), there is one specific measure that has stood out. It is the vancomycin presoaking technique. It consists of soaking the graft in a 5 mg/ml vancomycin solution prior to implantation. The recognition of the usefulness of this technique has increased in the recent years with the publication of multiple studies about its application and results(43–46).

This technique has been proven to reduce the risk of ACL-r infection, down to 0% in some studies(43–45,47,47–50). This is a very reproducible and easy-to-perform technique that acts by eradicating the most frequent bacteria that can contaminate the graft during harvesting and preparation(41).

Vancomycin has been proven to be safe in terms of *in vitro* viability for the tenocytes, chondrocytes, and osteoblasts in the required concentration (51). Moreover, its use in allografts in the orthopedic field is well known for having shown good clinical results and not having a deleterious effect on human cells(52–54). There is also *in vitro* data that proves no harmful effect in terms of the strength and mechanical characteristics of the graft (55). However, clinical data on the effect of the vancomycin soaking technique on the ACL graft in terms of the re-rupture risk and functional outcomes has been poorly studied.

2. RATIONALE OF THE STUDY

2.1. BACKGRAOUND AND MOTIVATION

The genesis of this thesis can be traced back to my training in the Traumatology and Orthopedics Department at Hospital del Mar in Barcelona. Within that esteemed institution, I had the privilege of working in a highly specialized unit dedicated to the treatment of osteoarticular infections. The experience made it possible for me to deepen my understanding of those complex pathologies and sparked my profound interest in the field. During my tenure at Hospital del Mar, I became involved in an ongoing line of investigation of infections associated with ACL-r surgeries. This engagement further fueled my curiosity and commitment to addressing those critical clinical challenges.

Given my growing interest in this area, the idea of pursuing an observership in Berlin originated in the septic unit at Hospital del Mar. They opened up the opportunity for me to join the renowned team led by Professor Andrej Trampuz at Charité Universitätsmedizin Berlin, a world leader in the field of osteoarticular infections. Working alongside Professor Trampuz and his team in their day-to-day activities and research endeavors provided me with invaluable insights into their advanced methodologies and innovative approaches.

It was during this enriching experience in Berlin that the concept for this thesis began to take shape. The collaborative environment between the septic unit at Hospital del Mar and Dr. Trampuz's team was instrumental in the development of this study. While the idea was conceived during my stay in Berlin, it was produced in collaboration with my colleagues from the septic unit at Hospital del Mar. The collaboration within both teams was pivotal, and I am deeply grateful for their generosity and mentorship.

I would also like to extend my sincere gratitude to my mentors at Hospital del Mar, Dr. Monllau and Dr. Perez-Prieto, for their invaluable guidance and support throughout my training. A special thanks to Dr. Perez-Prieto and the dedicated

septic unit. Their expertise and commitment to excellence have been a constant source of inspiration. Moreover, their mentorship has been crucial in shaping my professional journey and in the realization of this research.

This thesis is not only a culmination of my training and experiences but also a testament to the collaborative spirit of the international medical community. It underscores the importance of cross-institutional learning and research in advancing medical knowledge and improving patient outcomes. I wish to express my gratitude to all my mentors and colleagues who have contributed to this journey.

The focus of the present thesis is to delve into the realm of ACL-r infections with the overarching goal of enhancing the outcomes of ACL-r surgeries. With that, two different issues have been addressed.

2.2. THE SAFETY OF THE VANCOMYCIN SOAKING TECHNIQUE

The effectiveness and potential impact of this technique has already been proven(48,50). While this question about its safety has been raised in the literature, there remains a dearth of mid- to long-term *in vivo* data to definitively answer it. Recognizing the potential importance of providing evidence of the technique's safety, a study was designed to determine whether the main outcome related to safety is the rupture of the ACL graft potentially caused by vancomycin induced damage.

The main aim of the study was to evaluate the risk of re-rupture following ACL-r using the vancomycin soaking technique and compare it with the re-rupture risk in patients who did not undergo this technique. Additionally, the study sought to compare the functional outcomes between these two subsets of patients who underwent ACL-r procedures.

The primary hypothesis posits that the vancomycin technique does not significantly impact the risk of re-rupture after ACL-r. The secondary hypothesis

posits that the vancomycin technique does not significantly affect the functional outcomes of patients undergoing ACL-r procedures.

2.3. EVALUATION OF BIOFILM FORMATION ON DIFFERENT ACL GRAFTS

ACL-r surgeries using 4xHt grafts have been associated with higher infection rates when compared to surgeries utilizing BPTB grafts(26,44,45,48). A meta-analysis revealed an overall estimated infection rate of 0.9% in ACL-r surgeries, with 4xHt grafts demonstrating a higher infection rate (1.1%) than BPTB grafts (0.7%) (56). Despite these findings, there is a lack of any definitive explanations for this phenomenon, which has prompted the exploration of potential predisposing factors. Acquiring knowledge about the nature of this phenomenon could help in developing new prophylaxis and treatment strategies to achieve better results in ACL-r surgeries.

One hypothesis posits that sutures used in 4×Ht graft surgeries may serve as a reservoir for bacteria, increasing the risk of infection. Surgical procedures often entail contamination(57–61). However, not all of them lead to infection. This is because a Minimal infective dose (MID) of bacteria is necessary for infection to set in(62). The probability of infection correlates with the bacterial inoculation during contamination(63,64), particularly concerning foreign bodies reducing de MID(33,59,65,66). Research indicates that biofilm growth differs across surfaces(65–68), with sutures recognized as foreign bodies that open the door to biofilm formation(69,70). Some studies observed that a wound contaminated with the same quantity of bacteria is more likely to develop an infection if there are sutures(33,34).

This disparity in biofilm formation suggests that 4×Ht graft surgeries may introduce a greater bacterial load, potentially surpassing the MID more frequently and leading to a higher infection risk in comparison to BPTB graft surgeries. While this hypothesis has been proposed by other researchers(26,27), it remains unexplored. Therefore, a study was designed to investigate this hypothesis by

comparing biofilm formation between BPTB and 4×Ht grafts contaminated with the same bacterial inoculum *in vitro*.

We hypothesized that biofilm growth would be greater in 4×Ht grafts due to increased biofilm formation around the sutures. This is a potential explanation for the higher infection rates observed in ACL-r surgeries utilizing hamstring grafts.

3. HYPOTHESIS

The Vancomycin presoaking technique does not increase the risk of re-rupture after ACL-r. Neither does it affect the functional outcomes of patients that have undergone ACL-r procedures.

In vitro biofilm growth is greater in 4×Ht grafts than in BPTB grafts when comparing them with the same bacterial contamination inoculum.

4. OBJECTIVES

The purpose of this thesis is to increase our knowledge of ACL-r infections with the aim of improving the outcomes of ACL-r surgeries. To achieve this, a main objective has been established.

OBJECTIVE

To evaluate the safety of the vancomycin presoaking technique by determining the risk of re-rupture and functional outcomes following ACL-r. Additionally, it was to assess and compare biofilm formation on BPTB and 4xHt grafts when contaminated with the same bacterial inoculum.

5. COMPENDIUM OF PUBLICATIONS

5.1. ARTICLE 1

Pérez-Prieto D, Perelli S, Corcoll F, Rojas G, Montiel V, Monllau JC. The vancomycin soaking technique: no differences in autograft re-rupture rate. A comparative study. Int Orthop. 2021 Jun;45(6):1407-1411. doi: 10.1007/s00264-020-04805-5. Epub 2020 Sep 17. PMID: 32944802.

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ORIGINAL PAPER

The vancomycin soaking technique: no differences in autograft re-rupture rate. A comparative study

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Abstract

Purpose The main aim of this study was to evaluate the re-rupture risk after an anterior cruciate ligament reconstruction (ACL-R) using the vancomycin soaking technique and to compare it with the re-rupture risk in patients on whom this technique was not utilized. The secondary purpose was to compare the functional outcomes of those two subsets of patients operated on for ACL-R.

The hypotheses are that the vancomycin soaking technique does not affect the re-rupture risk or the functional outcomes.

Material and methods A retrospective historical cohort study was conducted. Two groups were compared in terms of the re-rupture rate (traumatic or atraumatic) and functional outcomes (International Knee Documentation Committee (IKDC), Tegner, and Lysholm). Group 1 consisted of patients that received pre-operative IV antibiotics. In group 2, the patients received pre-operative IV antibiotics along with a graft that had been presoaked in a vancomycin solution. A minimum follow-up of five years was required.

Results There were 17 patients that suffered a re-rupture in group 1 (4.7%) and 15 in group 2 (3.9%) (n.s.). IKDC was 82.0 in group 1 and 83.9 in group 2 ($p = 0.049$); Tegner scored 4 in both groups (n.s.) and Lysholm was 90.3 in group 1 and 92.0 in group 2 ($p = 0.015$).

Conclusion The vancomycin soaking technique for ACL autografts is a safe procedure for the daily clinical practice, in terms of re-ruptures. Moreover, it does not impair functional outcomes after an ACL-R.

Keywords ACL infection · Vancomycin technique · ACL reconstruction · Infection prevention · Orthopaedic infection

Introduction

Anterior cruciate ligament reconstruction (ACL-R) is a common orthopaedic procedure, being one of the most frequent surgical procedures in the sports medicine field [1, 2]. The number of studies published in PubMed rises every year, reaching approximately 7000 studies only in the last five years. However, most of them relate to anatomy, surgical techniques, and rehabilitation. There are very few about ACL-R infections [3–6].

Nevertheless, an infection can turn out to be a serious complication, especially if proper treatment is not applied. The infection rate has been stated to be at up to 1.5%. Interestingly, the economic burden has not yet been established [7]. Owing to the broad spectrum of costs due to ACL-R infection and the sequelae left in its wake, several authors have tried to develop some prevention strategies [8, 9]. One of the most recent is soaking the anterior cruciate ligament (ACL) graft in a 5 mg/ml vancomycin solution. This technique has been proven to dramatically reduce the risk of ACL-R infection, down to 0% in some studies [10–16]. This is a very reproducible and easy-to-perform technique that acts by destroying the most frequent bacteria like *Staphylococcus* spp. and *Cutibacterium* spp., which can contaminate the graft during harvesting and preparation. [17]

Vancomycin has proven to be safe in terms of in vitro viability for the tenocytes, chondrocytes, and osteoblasts in the aforementioned concentrations [18]. Moreover, its use in

Level of evidence: Level III. Historical cohort study

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allografts in the orthopaedic field is well known for showing good clinical results and not having a deleterious effect on human cells [19–21]. There is also in vitro data that proves no harmful effect in terms of the strength and mechanical characteristics of the graft [22]. However, clinical data on the effect of the vancomycin soaking technique on the ACL graft in terms of the re-rupture risk and functional outcomes has been poorly studied.

The main aim of the present study was to evaluate the re-rupture risk after an ACL-R using the vancomycin soaking technique and to compare it with re-rupture risk in patients in which this technique was not applied. The secondary purpose was to compare the functional outcomes of both subsets of these patients operated on for an ACL-R.

The hypotheses are that the vancomycin technique does not affect the re-rupture risk or the functional outcomes.

Material and methods

This is a retrospective historical cohort group. A retrospective review of all the patients that consecutively underwent primary arthroscopic ACL-R with an autograft in two university hospitals was performed. The study was approved by the Ethics Committee (2020-COT-DEX). Those patients who needed an extra-articular procedure had multiligament injuries or those who received an allograft were not included in the study. Infection was also an exclusion criterion. Meniscectomy or meniscal repair was not considered exclusion criteria.

Group 1 consisted of patients consecutively operated on that received preoperative IV antibiotics over a two year period. In group 2, patients received preoperative IV antibiotics along with the graft being presoaked in a vancomycin solution. Group 2 also consisted of patients consecutively operated on over a sequential two year period. All patients were operated on by the same surgical team (4 surgeons) on an outpatient basis. The prophylactic antibiotic protocol consisted of a single 2-g dose of preoperative IV cefazolin or a single 1-g dose of pre-operative IV vancomycin, if a type 1 penicillin allergy was reported. No patient in this series informed of a vancomycin allergy. The types of grafts employed were a quadrupled hamstring and a bone-patellar tendon-bone (BPTB).

The technique of vancomycin saturation was performed in group 2 as previously described [15]. A solution of 100 ml of sterile saline was prepared in a tray and mixed with 500 mg of vancomycin powder. After the graft was procured and prepared, it was immersed in the tray and then wrapped in gauze that had been saturated with this vancomycin solution beforehand. The graft was left there for ten to 15 minutes (until it was to be used for the ACL reconstruction).

At final follow-up, the patients were scheduled for evaluation. A minimum follow-up of five years was needed to include patients. Functional outcomes were evaluated by means of IKDC, Tegner, and Lysholm scores.

Re-rupture was assessed by means of clinical evaluation (Lachmann, anterior drawer, and pivot shift tests) and magnetic resonance imaging (MRI) and all types of injury mechanisms were included (either traumatic or atraumatic). It was assessed by the same surgical team. It was considered for surgery in case either positive MRI, clinical symptoms, or positive tests. Re-rupture was confirmed intra-operatively.

Statistical analysis

Continuous variables were presented as means (with standard deviation in parenthesis, SD) and ranges as percentages. Categorical data were compared between groups with the chi-square test. The Mann-Whitney test was used to assess differences in functional test scores between groups. A p value under 0.05 was considered statistically significant.

The chi-square difference test was used to determine the sample size. A 10% re-rupture rate was assumed. Accepting an alpha risk of 0.05 and a beta risk of 0.2 in a two-sided test, 333 subjects were necessary in each group to recognize a difference consisting of 5% re-rupture rate. A drop-out rate of 5% was anticipated.

The statistical analysis was done using the SPSS Statistics 18.0 software package (SPSS Inc., Chicago, IL).

Results

There were 785 patients included in the study (383 patients in group 1 and 402 in group 2). Twelve of them (7 in group 1, 5 in group 2) could not been assessed for re-rupture (lost to follow-up). There were five infections (all in group 1, $p < 0.001$) that were also excluded. Another 26 patients were excluded because of extra-articular procedures, multiligament injuries, or because they had received an allograft (11 in group 1, 15 in group 2). Therefore, 360 patients were included in group 1 and 382 in group 2 (a total of 43 patients were not included for study). No differences were found between groups in terms of missing and excluded patients (n.s.). Demographic data (gender and age) were similar in both groups (n.s.). No differences in terms of body mass index (BMI) and type of autograft between groups were found either (n.s.). Mean follow-up was 94 months (range 82–105 months) in group 1 and 72 months (range 60–83 months) in group 2 ($p < 0.001$).

In group 1, 17 patients suffered a re-rupture (4.7%) and 15 patients had one in group 2 (3.9%). Although it is a slightly lower rate, it did not reach statistical significance (n.s.).

Time to re-rupture was 51 months (range 48–60 months) in group 1 and 50 months (range 45–56 months) in group 2 (n.s.)

For the second purpose, it was only possible to schedule 182 patients in group 1 for functional assessment and 199 in group 2. The mean IKDC in group 1 was 82.0 (SD 8.0) and 83.9 (SD 8.0) in group 2 ($p = 0.049$). The median Tegner score was 4 points in both groups (n.s.). As for the mean Lysholm, it was 90.3 (SD 6.6) in group 1 and 92.0 (SD 6.1) in group 2 ($p = 0.015$).

All results are summarized in Table 1.

Discussion

The main finding of the present study is that soaking the ACL hamstring and BPTB autograft in a 5 mg/ml vancomycin solution does not increase the risk of re-rupture. Moreover, this vancomycin technique does not affect functional outcomes. In that sense, the hypotheses have been confirmed.

Prevention strategies to avoid infection like antibiotic IV prophylaxis or *Staphylococcus* spp. decolonization have been developed in the orthopaedic field [23–25]. The ideal prevention technique must be effective, reproducible, easy-to-perform, and cost-effective. In that sense, the vancomycin technique has been proven to reduce the risk of infection in several studies [8, 10–16]. It is supposedly cost-effective because of the low price of vancomycin. However, this has not been well studied.

Another key point is the safety of the technique as it should not jeopardize the joint or the structure where it is applied. In that sense, the major concern about the vancomycin technique is the risk of potential harm to the autograft cells [12]. Some antibiotics like cephalosporins and quinolones have been shown to inhibit chondrocyte and tenocyte replication. Therefore, it could also jeopardize the ligamentization process [26, 27]. However, vancomycin has been used for decades for local treatment and prevention in bone cement, bone allograft soaking, and calcium sulphate substitutes with good results in terms of infection control with no effect in terms of bone healing [19, 21, 28]. In vitro studies showed no deleterious consequences when it comes to its effect on tenocytes, especially when it is used in low concentrations as is the case of the vancomycin technique [18]. A study of clinical safety of the vancomycin technique has recently been published for the first time. Höher et al. showed no deleterious effect of the vancomycin soaking in terms of re-rupture [14]. Indeed, they found a lower risk of graft failure in the vancomycin group (3% failure in the vancomycin group versus 10% in the group without vancomycin soaking). In the present study, similar outcomes have been found as there is no more risk of re-rupture by soaking the graft in vancomycin. However, a reduction rate was not observed as Höher et al. This is very interesting as the authors single out low-grade infections (LGI) as a cause for failure. Therefore, the vancomycin technique might reduce these low-grade infections and then re-rupture. This theory will be worth investigating in future studies. In fact, bacterial DNA has been present in widened tunnels and tunnels of failed grafts [29, 30]. There is evidence that LGI can cause pain and loosening, without pus discharge and normal C-reactive protein, as the only clinical feature in prosthetic joint infections (PJI) and fracture-related infections (FRI) [31–34]. These LGI are mostly caused by *Cutibacterium* spp. and coagulase-negative *Staphylococci* for which vancomycin is an active antibiotic [17].

The re-rupture rate after an ACL-R is known to range from 0 to 25% [35]. The vast majority of the studies report graft failure rates around 7%. It is quite similar to the results presented here in both the vancomycin technique group and in the group without it [14, 35, 36].

Functional outcomes are also comparable with the published data [37, 38]. Although significant differences have been found between groups, the clinical relevance is very low as some 2-point difference was the mean observed in the present study. However, it should be noted that the best result was obtained in the vancomycin group. It is in line with the results of Höher et al.

Limitations

The present study has several limitations. The most important is its retrospective design even though the groups were similar and there was no other modification in the surgical protocol except for the vancomycin technique. Another fact of its retrospective design and the sequential groups is the different follow-up. This is an important limitation as the group of patients with longer follow-up may have a higher chance of

Table 1 Results and comparative between groups

	Group 1 (no vancomycin soaking)	Group 2 (vancomycin soaking)	*p* value
n	360	382	
Follow-up (months)	94 (82–105)	72 (60–83)	< 0.001
Re-rupture cases	17 (4.7%)	15 (3.9%)	n.s.
IKDC	82.0 (8.0)	83.9 (8.0)	0.049
Tegner	4	4	n.s.
Lysholm	90.3 (6.6)	92.0 (6.1)	0.015

re-rupture, although time to re-rupture was similar between groups.

The drop-out of patients to assess functional outcomes is also a limitation. However, the number of missing patients in which re-rupture was evaluated is much lower and the re-rupture evaluation was the main objective of the present study.

The final limitation is that vancomycin can also affect an autograft in many other ways that have not been assessed in the present study. They include tunnel widening or radiological ligamentization.

Conclusions

The vancomycin soaking technique for ACL autografts seems to be a safe procedure in terms of re-ruptures. Moreover, it does not impair functional outcomes after an ACL-R.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Andrade R, Pereira R, van Cingel R et al (2019) How should clinicians rehabilitate patients after ACL reconstruction? A systematic review of clinical practice guidelines (CPGs) with a focus on quality appraisal (AGREE II). Br J Sports Med. https://doi.org/10.1136/bjsports-2018-100310
2. Nwachukwu BU, Patel BH, Lu Y et al (2019) Anterior cruciate ligament repair outcomes: an updated systematic review of recent literature. Arthroscopy 35:2233–2247. https://doi.org/10.1016/j.arthro.2019.04.005
3. Alentorn-Geli E, Pelfort X, Mingo F et al (2015) An evaluation of the association between radiographic intercondylar notch narrowing and anterior cruciate ligament injury in men: the notch angle is a better parameter than notch width. Arthroscopy 31:2004–2013. https://doi.org/10.1016/j.arthro.2015.04.088
4. Karlsson J, Hirschmann MT, Becker R, Musahl V (2015) Individualized ACL surgery. Knee Surg Sports Traumatol Arthrosc 23:2143–2144. https://doi.org/10.1007/s00167-015-3690-9
5. Kopf S, Musahl V, Perka C et al (2017) The influence of applied internal and external rotation on the pivot shift phenomenon. Knee Surg Sports Traumatol Arthrosc 25:1106–1110. https://doi.org/10.1007/s00167-017-4429-6
6. Pérez-Prieto D, Trampuz A, Torres-Claramunt R et al (2017) Infections after anterior cruciate ligament reconstruction: which antibiotic after arthroscopic debridement? J Knee Surg 30:309–313. https://doi.org/10.1055/s-0036-1584559
7. Torres-Claramunt R, Gelber P, Pelfort X et al (2016) Managing septic arthritis after knee ligament reconstruction. Int Orthop 40:607–614. https://doi.org/10.1007/s00264-015-2884-6
8. Vertullo CJ, Quick M, Jones A, Grayson JE (2012) A surgical technique using presoaked vancomycin hamstring grafts to decrease the risk of infection after anterior cruciate ligament reconstruction. Arthroscopy 28:337–342. https://doi.org/10.1016/j.arthro.2011.08.301
9. Yazdi H, Yousof Gomrokchi A, Nazarian A et al (2019) The effect of gentamycin in the irrigating solution to prevent joint infection after anterior cruciate ligament (ACL) reconstruction. Arch Bone Jt Surg 7:67–74
10. Baron JE, Shamrock AG, Cates WT et al (2019) Graft preparation with intraoperative vancomycin decreases infection after ACL reconstruction: a review of 1,640 cases. J Bone Joint Surg Am. https://doi.org/10.2106/JBJS.19.00270
11. Figueroa D, Figueroa F, Calvo R et al (2019) Presoaking of hamstring autografts in vancomycin decreases the occurrence of infection following primary anterior cruciate ligament reconstruction. Orthop J Sports Med 7:2325967119871038. https://doi.org/10.1177/2325967119871038
12. Jefferies JG, Aithie JMS, Spencer SJ (2019) Vancomycin-soaked wrapping of harvested hamstring tendons during anterior cruciate ligament reconstruction. A review of the "vancomycin wrap". Knee 26:524–529. https://doi.org/10.1016/j.knee.2019.04.008
13. Naendrup J-H, Marche B, de Sa D et al (2019) Vancomycin-soaking of the graft reduces the incidence of septic arthritis following ACL reconstruction: results of a systematic review and meta-analysis. Knee Surg Sports Traumatol Arthrosc. https://doi.org/10.1007/s00167-019-05353-1
14. Offerhaus C, Balke M, Hente J et al (2019) Vancomycin presoaking of the graft reduces postoperative infection rate without increasing risk of graft failure and arthrofibrosis in ACL reconstruction. Knee Surg Sports Traumatol Arthrosc 27:3014–3021. https://doi.org/10.1007/s00167-018-5323-6
15. Pérez-Prieto D, Torres-Claramunt R, Gelber PE et al (2016) Autograft soaking in vancomycin reduces the risk of infection after anterior cruciate ligament reconstruction. Knee Surg Sports Traumatol Arthrosc 24:2724–2728. https://doi.org/10.1007/s00167-014-3438-y
16. Phegan M, Grayson JE, Vertullo CJ (2016) No infections in 1300 anterior cruciate ligament reconstructions with vancomycin presoaking of hamstring grafts. Knee Surg Sports Traumatol Arthrosc 24:2729–2735. https://doi.org/10.1007/s00167-015-3558-z
17. Pérez-Prieto D, Portillo ME, Torres-Claramunt R et al (2018) Contamination occurs during ACL graft harvesting and manipulation, but it can be easily eradicated. Knee Surg Sports Traumatol Arthrosc 26:558–562. https://doi.org/10.1007/s00167-017-4735-z
18. Grayson JE, Grant GD, Dukie S, Vertullo CJ (2011) The in vitro elution characteristics of vancomycin from tendons. Clin Orthop Relat Res 469:2948–2952. https://doi.org/10.1007/s11999-011-1768-3
19. Ferguson J, Athanasou N, Diefenbeck M, McNally M (2019) Radiographic and histological analysis of a synthetic bone graft substitute eluting gentamicin in the treatment of chronic osteomyelitis. J Bone Jt Infect 4:76–84. https://doi.org/10.7150/jbji.31592
20. Metsemakers W-J, Fragomen AT, Moriarty TF et al (2019) Evidence-based recommendations for local antimicrobial strategies and dead space management in fracture-related infection. J Orthop Trauma. https://doi.org/10.1097/BOT.0000000000001615
21. Olesen UK, Juul R, Bonde CT et al (2015) A review of forty five open tibial fractures covered with free flaps. Analysis of complications, microbiology and prognostic factors. Int Orthop 39:1159–1166. https://doi.org/10.1007/s00264-015-2712-z
22. Schüttler K-F, Scharm A, Stein T et al (2019) Biomechanical and microbiological effects of local vancomycin in anterior cruciate ligament (ACL) reconstruction: a porcine tendon model. Arch Orthop Trauma Surg 139:73–78. https://doi.org/10.1007/s00402-018-3006-x
23. Pelfort X, Romero A, Brugués M et al (2019) Reduction of periprosthetic Staphylococcus aureus infection by preoperative screening and decolonization of nasal carriers undergoing total

knee arthroplasty. Acta Orthop Traumatol Turc. https://doi.org/10.1016/j.aott.2019.08.014
24. Pérez-Prieto D, Portillo ME, Puig-Verdié L et al (2016) Preoperative antibiotic prophylaxis in prosthetic joint infections: not a concern for intraoperative cultures. Diagn Microbiol Infect Dis 86:442–445. https://doi.org/10.1016/j.diagmicrobio.2016.09.014
25. Weber WP, Marti WR, Zwahlen M et al (2008) The timing of surgical antimicrobial prophylaxis. Ann Surg 247:918–926. https://doi.org/10.1097/SLA.0b013e31816c3fec
26. Edin ML, Miclau T, Lester GE Lindsey RW, Dahners LE (1996) Effect of cefazolin and vancomycin on osteoblasts in vitro. Clin Orthop Relat Res (333):245–251
27. Pouzaud F, Bernard-Beaubois K, Thevenin M et al (2004) In vitro discrimination of fluoroquinolones toxicity on tendon cells: involvement of oxidative stress. J Pharmacol Exp Ther 308:394–402. https://doi.org/10.1124/jpet.103.057984
28. Peñalba Arias P, Furustrand Tafin U, Bétrisey B et al (2015) Activity of bone cement loaded with daptomycin alone or in combination with gentamicin or PEG600 against Staphylococcus epidermidis biofilms. Injury 46:249–253. https://doi.org/10.1016/j.injury.2014.11.014
29. Everhart JS, DiBartola AC, Dusane DH et al (2018) Bacterial deoxyribonucleic acid is often present in failed revision anterior cruciate ligament reconstructions. Arthroscopy 34:3046–3052. https://doi.org/10.1016/j.arthro.2018.06.033
30. Flanigan DC, Everhart JS, DiBartola AC et al (2019) Bacterial DNA is associated with tunnel widening in failed ACL reconstructions. Knee Surg Sports Traumatol Arthrosc 27:3490–3497. https://doi.org/10.1007/s00167-019-05405-6
31. Izakovicova P, Borens O, Trampuz A (2019) Periprosthetic joint infection: current concepts and outlook. EFORT Open Rev 4:482–494. https://doi.org/10.1302/2058-5241.4.180092
32. Morgenstern M, Kühl R, Eckardt H et al (2018) Diagnostic challenges and future perspectives in fracture-related infection. Injury 49(Suppl 1):S83–S90. https://doi.org/10.1016/S0020-1383(18)30310-3
33. Pérez-Prieto D, Portillo ME, Puig-Verdié L et al (2017) C-reactive protein may misdiagnose prosthetic joint infections, particularly chronic and low-grade infections. Int Orthop 41:1315–1319. https://doi.org/10.1007/s00264-017-3430-5
34. Steinmetz S, Wernly D, Moerenhout K et al (2019) Infection after fracture fixation. EFORT Open Rev 4:468–475. https://doi.org/10.1302/2058-5241.4.180093
35. Grassi A, Kim C, Marcheggiani Muccioli GM et al (2017) What is the mid-term failure rate of revision ACL reconstruction? A systematic review. Clin Orthop Relat Res 475:2484–2499. https://doi.org/10.1007/s11999-017-5379-5
36. Samuelsen BT, Webster KE, Johnson NR et al (2017) Hamstring autograft versus patellar tendon autograft for ACL reconstruction: is there a difference in graft failure rate? A meta-analysis of 47,613 patients. Clin Orthop Relat Res 475:2459–2468. https://doi.org/10.1007/s11999-017-5278-9
37. Fernandes TL, Fregni F, Weaver K et al (2014) The influence of femoral tunnel position in single-bundle ACL reconstruction on functional outcomes and return to sports. Knee Surg Sports Traumatol Arthrosc 22:97–103. https://doi.org/10.1007/s00167-012-2288-8
38. Mouarbes D, Menetrey J, Marot V et al (2019) Anterior cruciate ligament reconstruction: a systematic review and meta-analysis of outcomes for quadriceps tendon autograft versus bone-patellar tendon-bone and hamstring-tendon autografts. Am J Sports Med 363546518825340. https://doi.org/10.1177/0363546518825340

5.2. ARTICLE 2

Corcoll F, Pérez-Prieto D, Karbysheva S, Trampuz A, Fariñas O, Monllau JC. Are Hamstring Grafts a Predisposing Factor to Infection in R-ACL Surgery? A Comparative In Vitro Study. Pathogens. 2023 May 25;12(6):761. doi: 10.3390/pathogens12060761. PMID: 37375451; PMCID: PMC10301445.

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Article

Are Hamstring Grafts a Predisposing Factor to Infection in R-ACL Surgery? A Comparative In Vitro Study

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Abstract: Background: The objective of the present study was to evaluate the formation of biofilms in bone patellar tendon bone grafts (BPTB grafts), and to compare it to the formation of biofilm formation in quadrupled hamstring anterior cruciate ligament grafts (4×Ht graft). Methods: A descriptive in vitro study was conducted. One 4×Ht graft and one BPTB graft were prepared. They were then contaminated with a strain of *S. epidermidis*. Later, a quantitative analysis was conducted by means of microcalorimetry and sonication with plating. Additionally, a qualitative analysis was conducted by means of electron microscopy. Results: No significant differences were found between the bacterial growth profiles of the 4×Ht graft and the BPTB graft in microcalorimetry and colony counting. In the samples analyzed with electron microscopy, no specific biofilm growth pattern was identified upon comparing the BPTB graft to the 4×Ht graft. Conclusions: There were no significant differences found at either the quantitative or qualitative level when comparing bacterial growth in the BPTB graft to that in the 4×Ht graft. Therefore, the presence of sutures in the 4×Ht graft cannot be established as a predisposing factor for increased biofilm growth in this in vitro study.

Keywords: arthroscopy; sports medicine; anterior cruciate ligament reconstruction; implant-associated infection; biofilm; septic arthritis

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1. Introduction

Although infections after anterior cruciate ligament reconstruction (r-ACL) are not as common as other implant-associated infections, the magnitude of this complication is equally important, since inappropriate treatment could compromise joint function and return to sports activities [1,2].

For this reason, various studies have focused on the study of this pathology in recent years. Most of those studies concluded that aggressive arthroscopic debridement in combination with specific antibiotic therapy should be the treatment of choice for this complication [1,3,4]. Several studies have also focused on the development of infection prophylaxis techniques such as the vancomycin bath, which dramatically reduces the incidence of infection [5–10].

Other studies have focused on the origin of these infections [3,11]. Some authors have been able to demonstrate that the infections arise as the result of contamination by coagulase-negative staphylococci during the preparation of the graft [11]. These microorganisms are part of the normal microbiota of the skin and mucous membranes.

There are data published by several authors in which higher rates of infection in r-ACL were observed when quadruplicate hamstring grafts (4×Ht grafts) were used compared to surgeries performed with patellar tendon grafts (BPTB grafts) [6,12,13]. Data from a meta-analysis showed an overall estimated infection rate in r-ACL of 0.9% (Confidence interval (CI) 95% 0.8% to 1.0%) [14]. There was also a higher infection rate in 4×Ht autografts surgeries (1.1%, CI 95% 0.9% to 1.2%) than with BPTB autografts (0.7%, CI 95% 0.6% to 0.9%), and allografts of any type (0.5%, CI 95% 0.4% to 0.8%) (Q 5 15.58, $p = 0.001$) [14]. Therefore, it has been considered that 4×Ht grafts may be a predisposing factor for infection. However, there are no studies that give a verifiable explanation for this phenomenon. Nevertheless, multiple hypothesis has been proposed.

One of them is that sutures used in 4×Ht grafts surgeries may harbor bacteria, and that this may be a risk factor for the development of an infection [3]. It has been shown that contamination during surgical procedures is frequent [15–19]. However, not all contamination will lead to infection. For an infection to occur, minimal bacterial contamination is required (minimal infective dose (MID)) [20]. The probability of an infection to arise is directly proportional to the amount of bacterial inoculation during contamination [21,22]. This relation is especially relevant in infections related to foreign bodies [17,23,24]. There is strong evidence that biofilm growth does not grow in the same way on different surfaces [23–26], and it has also been shown that as surgical sutures surface are recognized as foreign bodies, it makes way for the growth of the biofilm [27,28].

All this leads one to think that a greater bacterial load is introduced into the body in 4×Ht graft surgeries, reaching the MID more frequently. This may be the reason for the higher rate of infections in r-ACL surgeries performed with 4×Ht grafts, as they require sutures. Those sutures may behave similarly to a foreign body that facilitates the growth of the biofilm. With the same contaminating bacterial inoculum, biofilm formation would be more elevated in 4×Ht grafts than that produced in BPTB grafts, in which sutures are not used. This would allow for the introduction of a greater bacterial load in the subject, raising the risk of infection [20–22]. This hypothesis has also been proposed by other authors, but never explored [3].

A similar observation was noted in the classic studies of Elek et al. that were performed in 1956. In those studies, they observed that a wound contaminated with the same quantity of bacteria is more likely to develop an infection if there are sutures on it [29,30].

The objective of the present study was to evaluate this hypothesis by comparing biofilm formation in BPTB grafts vs. 4×Ht grafts, with both contaminated with the same bacterial inoculum in vitro.

The hypothesis was that biofilm growth will be greater in 4×Ht grafts than in BPTB grafts due to the greater formation of biofilm around the sutures.

2. Materials and Methods

A descriptive in vitro study was conducted. For the production of grafts, a 4×Ht graft and a BPTB graft were prepared from sterilized and frozen cadaveric donor samples.

The grafts were provided by Banc de Sang i Teixits (BST) (Barcelona, Spain). They were prepared by an orthopedic surgeon with specific training and experience in conducting this type of graft. The production of these grafts was performed in a manner analogous to the one used during the usual surgical procedures, in a sterile environment. In the case of the 4×Ht graft, a high-resistance suture of the same type as those used in normal practice was used (FiberWire, Arthrex, Munich, Germany).

For the BPTB graft, a remodeling of the bony parts to achieve a 10mm diameter was performed as in daily clinical practice.

Both of the grafts were divided into 8 representative fragments of each type of graft.

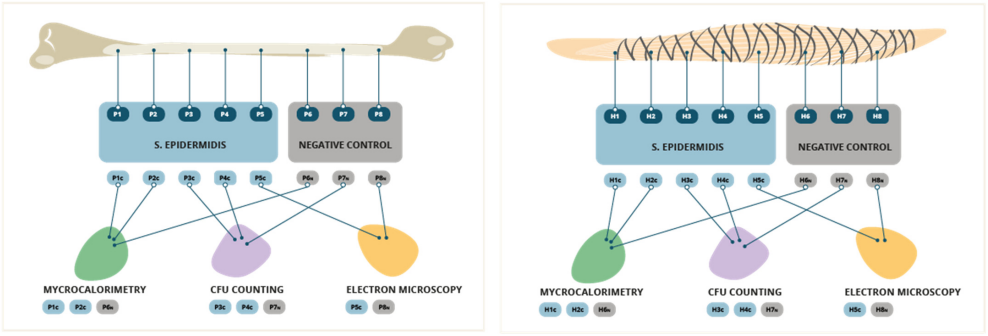
We considered a representative fragment as one that contains the representative elements of the complete graft. In the case of the BPTB graft, eight 5 mm × 5 mm × 1 mm fragments of the patellar tendon were produced using a surgical scalpel. The decision was taken to use these dimensions as they were the maximum dimensions that can be used in

the analysis processes to be conducted later. In the case of the 4×Ht graft, a representative fragment was considered to be one that contained a hamstring tendon fragment and a suture fragment. These fragments were made with the same dimensions and followed the technique as those previously described.

This study was approved by the Parc de Salut Mar Drug Research Ethics Committee with CEIC clinical research project number no. 2018/8269/I on 29 May 2019.

2.1. Contamination and Biofilm Growth Conditions

In 5 fragments from each sample, the formation of the artificial biofilm was brought on. To form the bacterial biofilm, the samples were placed in 2 mL of heart-brain infusion broth culture medium (BHIb, Sigma-Aldrich, St. Louis, MO, USA) contaminated at 1×10^5 CFU/mL by *Staphylococcus epidermidis* (ATCC 35984). They were incubated at 37 °C for 24 h. This bacterium was used, as it is one of the most frequent causes of infection in the context of r-ACL [28]. Moreover, its great capacity for biofilm formation is well known. After the formation of the biofilm, the samples were washed three times with 2 mL of 0.9% NaCl to eliminate the bacteria that were in planktonic form. Three fragments from each sample were left uncontaminated as negative controls. These fragments were then incubated in a 0.9% NaCl solution at 37 °C for 24 h to simulate the same conditions as the contaminated samples, based on the technique previously described in multiple studies [31,32] (Figure 1).



(**a**) (**b**)

Figure 1. Flow chart representing the methodology of the production treatment and analysis of the samples. (**a**) Samples performed with the patellar tendon graft, (**b**) samples performed with the hamstring graft. P1c, patellar tendon contaminated 1; P2c, patellar tendon contaminated 2; P3c, patellar tendon contaminated 3; P4c, patellar tendon contaminated 4; P5c, patellar tendon contaminated 5; P6n, patellar tendon NO-contaminated 6; P7n, patellar tendon NO-contaminated 7; P8n, patellar tendon NO-contaminated 8; H1c, hamstring tendon contaminated 1; H2c, hamstring tendon contaminated 2; H3c, hamstring tendon contaminated 3; H4c, hamstring tendon contaminated 4; H5c, hamstring tendon contaminated 5; H6n, hamstring tendon NO-contaminated 6; H7n, hamstring tendon NO-contaminated 7; and H8n, hamstring tendon NO-contaminated 8.

2.2. Isothermal Microcalorimetry

In 2 contaminated fragments and 1 negative control of each type of graft, the heat produced by the *S. epidermidis* bacterial population was monitored with the microcalorimeter using the isothermal microcalorimetry method (TAM III, TA Instruments, Newcastle, DE, USA). Measurements were conducted through the full vital cycle of the pathogen (48 h). It is the same as the one described by Butini et al. [33].

2.3. Sonication and Plating and CFU Counting

Sonication and seeding analysis was conducted on 2 contaminated fragments and 1 negative control of each type of graft. Each of these was placed in a solution of 1 mL 0.9% NaCl, vortexed for 30 s, and sonicated at an intensity of 40 kHz and 0.1 W cm^2 (BactoSonic, BANDELIN electronic, Berlin, Germany) for 1 min. Then, they were sonicated again for an additional 30 s. Next, 100 µL of the sonication product was seeded in Tryptic Soy Agar (TSA) (Sigma-Aldrich, St. Louis, MO, USA). After 24 h of incubation at 37 °C, the counting of colony-forming units (CFU/mL) was carried out in accordance with the previously described technique [34].

Accepting an alpha risk of 0.05 and a beta risk of less than 0.2 in a bilateral contrast, 2 subjects in the first group and 2 in the second group were required to detect a difference equal to or greater than 5 units. The common standard deviation was assumed to be 1.5. A loss to follow-up rate was estimated at 0%.

2.4. Scanning Electron Microscopy (SEM)

Finally, a qualitative analysis was performed by means of scanning electron microscopy (GeminiSEM 300, Carl Zeiss, OberkochenDSM 982 GEMINI, Zeiss Oberkochen, Germany) to determine the biofilm growth areas in the samples. The pictures taken with this technique were analyzed by a trained technician who was blinded for the aim of our study and had been asked to look for differences in biofilm wroth profiles in the different samples and compare them. This was done in accordance with the previously described technique [34] with a proven validity in biofilm evaluation [35,36]. For this analysis, 1 contaminated fragment and 1 negative control fragment of each type of sample were used.

2.5. Statistical Analysis

The statistical analyzes were performed using the SigmaPlot software package (version 13.0; Systat Software, Chicago, IL, USA) and Prism for the graphics (version 8; GraphPad, La Jolla, CA, USA). Continuous variables are presented as means (with standard deviation in parenthesis, SD) and ranges. The unpaired *t*-test was used to assess the CFU counting. A *p* value under 0.05 was considered statistically significant.

3. Results

3.1. Isothermal Microcalorimetry

Using the isothermal microcalorimetry method, we observed bacterial growth in the contaminated fragments from both the 4×Ht and BPTB grafts. They showed the same growth dynamics of the one typical of this bacterial population. In the beginning, the microcalorimetry curves showed exponential growth rates until they reached a population peak (200 µW) at approximately 8 h after contamination. Then, after a short or non-existent stationary phase, a rapid decline in the bacterial population was detected. Later, there was a phase of senescence or death characteristic of this type of bacterial population (Figure 2), with no significant differences found between them. In the BPTB and 4×Ht graft fragments that were not contaminated (negative controls), no growth profile was observed. Therefore, no significant differences between the bacterial growth profiles of any of the 4×Ht and BPTB grafts were found.

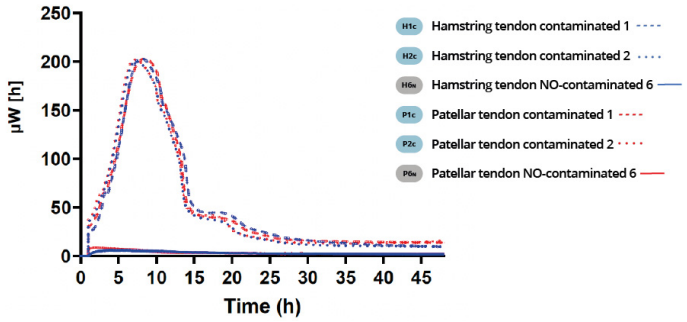


Figure 2. Isothermal microcalorimetry results. No significant differences between the bacterial growth profiles of any of the 4×Ht and BPTB contaminated grafts were found.

3.2. CFU Counting Method

The number of colony-forming units per milliliter (CFU/mL) of the fluid extracted by sonication of the contaminated BPTB and 4×Ht grafts fragments was then determined. The means of the CFU/mL of the different groups were calculated, analyzed, and compared (mean ± SEM, $3.5 \times 10^7 \pm 0.345 \times 10^7$ CFU/mL, and $4.6 \times 10^7 \pm 1.455 \times 10^7$ CFU/mL respectively, $p = 0.6667$) (Figure 3). No significant differences were detected between the 4×Ht graft group and the BPTB graft groups ($p \geq 0.05$). Seeding of the fluid extracted by sonication of the uncontaminated BPTB and 4×Ht grafts fragments (negative controls) did not produce any colony growth.

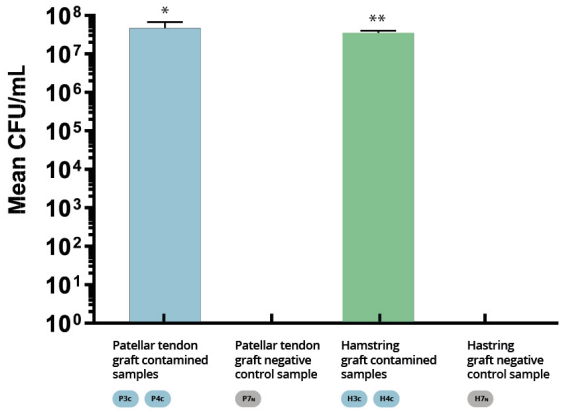
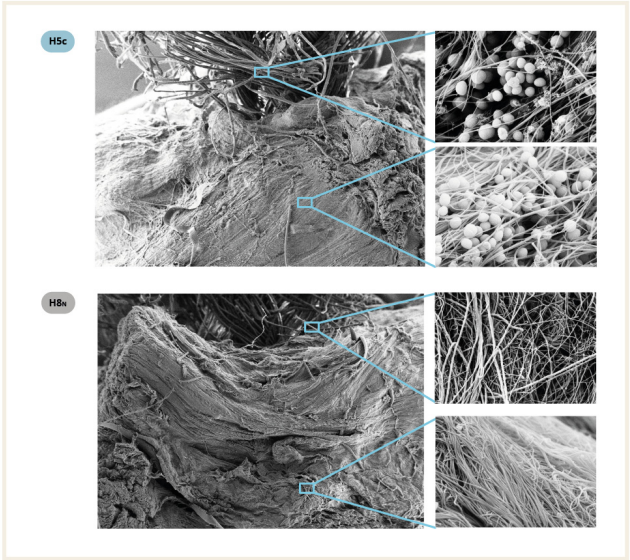


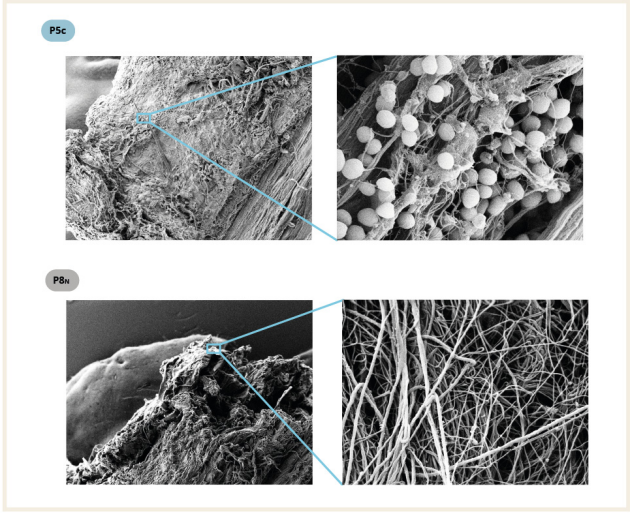
Figure 3. Colony-forming unit counting method. No significant differences were detected between the 4×Ht graft group and the BPTB graft group. * Standard deviation 0.345×10^7, and ** Standard deviation 1.455×10^7, respectively.

3.3. Electron Microscopy

In the electron microscopy analysis, no specific or differential biofilm growth patterns were detected by our technician upon comparing the contaminated BPTB graft fragment to the corresponding 4×Ht graft fragment. There was a homogeneous growth pattern observed regardless of the surface on which the biofilm grew. Of note, an increase in colonization was not observed in the suture areas (Figure 4), and no bacteria was found in the negative control samples.



(**a**)



(**b**)

Figure 4. Electron microscopy at magnifications of 34×, and 10.00 Kx: (**a**) hamstring graft samples, contaminated H5c and negative control H8n, (**b**) patellar tendon graft samples, contaminated P5c and negative controls P8n. Black arrow = hamstring tendon, white arrow = suture, and green arrow = patellar tendon).

4. Discussion

The initial hypothesis of this study equating the BPTB and 4×Ht grafts in their potential for biofilm growth under the contamination of a same bacterial inoculum in vitro has been refuted.

The structural differences between the two graft types do not have any effect on the production of biofilm during r-ACL surgery, at least outside of the body. Therefore, differences in the infection rates in r-ACL surgeries with BPTB and 4×Ht grafts cannot be justified in this way.

Electron microscopy analysis revealed a homogeneous biofilm growth pattern. These findings rule out the sutures present in 4×Ht grafts being a better surface for biofilm growth than the tendon itself (Figure 4a). This suggests that both tissues function as foreign bodies that equally make for biofilm growth. Another study showed no significant differences in infection rates between allografts and autografts, which supports our finding that autografts and allografts behave similarly to foreign bodies [14]. Furthermore, 4×Ht autografts are associated with a higher infection rate when compared to both allografts and BPTBp autografts. This study was performed only with frozen cadaveric donor grafts, and we are aware of the bias that it may produce. Working with fresh donor grafts would have been more difficult to perform for ethical and practical reasons. However, as has been previously stated, we expected no major differences between the use of fresh and cadaveric grafts.

The fact that grafts, by their very nature, are not a predisposing factor for biofilm growth leads us to think that there must be some differences surrounding their processing that changes and conditions the differences in infection rates. Therefore, our study encourages future studies for the development of prophylactic strategies not to focus on the structure or composition of 4×Ht grafts, but in other factors such as the ones discussed below. One possible reason is that there are differences in the contamination of grafts. It is well known that contamination during a surgical procedures is time-dependent [37]. The production of the 4×Ht graft is more time-consuming due to its greater complexity [5,9]. We also know that contamination of grafts occurs during the process of making them [11]. This further leads us to consider that contamination with more bacterial inoculum might occur during the preparation of the 4×Ht grafts, and more frequently reaching the minimal infectious doses (MID). In this way, we come by the higher infection rates seen in r-ACL surgeries performed with 4×Ht grafts. Another hypothesis is that slightly more extensive tissue dissection and morbidity is performed at the 4×Ht autograft harvest site compared to the BPTB autograft harvest site [38,39], thereby justifying the difference in infection rates.

This study has some limitations that should be mentioned. Only *S. epidermidis* was used for contamination and biofilm formation. It would be interesting to perform the same analysis with other types of Gram-positive staphylococci that are frequently associated with this pathology. However, it is unlikely that differences would be found due to their similarity to *S. epidermidis*. On the other hand, we believe that repeating the analysis with pathogens of other strains including anaerobes would be of little relevance due to their low prevalence in r-ACL infections.

Another limitation is the concern around this study being an in vitro study. This does not make for exploring the host–pathogen interaction. However, for the moment, this is the best evidence we can provide relative to this topic for bioethical reasons, as the factors mentioned above cannot be explored experimentally in vivo.

Another factor that was beyond our control in this study were the host characteristics (immune status, comorbidities, etc.). Then again, we know that the infection rate differs according to the type of graft used in studies with a large cohort in which the patients are heterogeneous [6,12,13]. This makes us think that the differences in infection rates must be due to differences that are independent of the individuals themselves. This indicates that regardless of the individual, their circumstances, and their characteristics, infections are more frequent in surgeries performed with hamstring grafts. Therefore, in terms of the host factors, the host–pathogen interaction, and the pathogen itself, the differential factor

should not be looked to as the host factor. We believe this is an argument that favors a positive evaluation of our study.

5. Conclusions

We have found that the amount of biofilm formed on the BPTB and 4×Ht grafts by the same bacterial inoculum is comparable in vitro. Therefore, the 4×Ht graft is not intrinsically a predisposing factor for biofilm growth due to its structure and composition when contaminated outside of the body.

Author Contributions: F.C. wrote the manuscript; F.C. and A.T. contributed in methodology design; O.F. and D.P.-P. contributed to sample acquisition; F.C. and S.K. carried out the experiments and prepared the figures; F.C., D.P.-P. and S.K. contributed in investigation, data curation and formal analysis; F.C., D.P.-P., S.K., A.T. and J.C.M. revised and edited the manuscript; D.P.-P., A.T. and J.C.M. supervised the study; F.C. conceptualized, wrote, and revised the manuscript. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of Parc de Salut Mar Drug Research Ethics Committee with number (CEIC clinical research project no. 2018/8269/I) on 29 May 2019.

Informed Consent Statement: Patient consent was not applicable due to this study being an in vitro study, not including living humans.

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References

1. Boström Windhamre, H.; Mikkelsen, C.; Forssblad, M.; Willberg, L. Postoperative Septic Arthritis after Anterior Cruciate Ligament Reconstruction: Does It Affect the Outcome? A Retrospective Controlled Study. *Arthrosc. J. Arthrosc. Relat. Surg. Off. Publ. Arthrosc. Assoc. N. Am. Int. Arthrosc. Assoc.* **2014**, *30*, 1100–1109. [CrossRef] [PubMed]
2. Zimmerli, W.; Trampuz, A.; Ochsner, P.E. Prosthetic-Joint Infections. *N. Engl. J. Med.* **2004**, *351*, 1645–1654. [CrossRef]
3. Maletis, G.B.; Inacio, M.C.S.; Reynolds, S.; Desmond, J.L.; Maletis, M.M.; Funahashi, T.T. Incidence of Postoperative Anterior Cruciate Ligament Reconstruction Infections: Graft Choice Makes a Difference. *Am. J. Sports Med.* **2013**, *41*, 1780–1785. [CrossRef]
4. Torres-Claramunt, R.; Pelfort, X.; Erquicia, J.; Gil-González, S.; Gelber, P.E.; Puig, L.; Monllau, J.C. Knee Joint Infection after ACL Reconstruction: Prevalence, Management and Functional Outcomes. *Knee Surg. Sports Traumatol. Arthrosc. Off. J. ESSKA* **2013**, *21*, 2844–2849. [CrossRef] [PubMed]
5. Baron, J.E.; Shamrock, A.G.; Cates, W.T.; Cates, R.A.; An, Q.; Wolf, B.R.; Bollier, M.J.; Duchman, K.R.; Westermann, R.W. Graft Preparation with Intraoperative Vancomycin Decreases Infection After ACL Reconstruction: A Review of 1640 Cases. *J. Bone Jt. Surg. Am.* **2019**, *101*, 2187–2193. [CrossRef]
6. Figueroa, D.; Figueroa, F.; Calvo, R.; Lopez, M.; Goñi, I. Presoaking of Hamstring Autografts in Vancomycin Decreases the Occurrence of Infection Following Primary Anterior Cruciate Ligament Reconstruction. *Orthop. J. Sports Med.* **2019**, *7*, 2325967119871038. [CrossRef]
7. Offerhaus, C.; Balke, M.; Hente, J.; Gehling, M.; Blendl, S.; Höher, J. Vancomycin Pre-Soaking of the Graft Reduces Postoperative Infection Rate without Increasing Risk of Graft Failure and Arthrofibrosis in ACL Reconstruction. *Knee Surg. Sports Traumatol. Arthrosc. Off. J. ESSKA* **2019**, *27*, 3014–3021. [CrossRef]
8. Pérez-Prieto, D.; Perelli, S.; Corcoll, F.; Rojas, G.; Montiel, V.; Monllau, J.C. The Vancomycin Soaking Technique: No Differences in Autograft Re-Rupture Rate. A Comparative Study. *Int. Orthop.* **2021**, *45*, 1407–1411. [CrossRef] [PubMed]
9. Pérez-Prieto, D.; Torres-Claramunt, R.; Gelber, P.E.; Shehata, T.M.A.; Pelfort, X.; Monllau, J.C. Autograft Soaking in Vancomycin Reduces the Risk of Infection after Anterior Cruciate Ligament Reconstruction. *Knee Surg. Sports Traumatol. Arthrosc. Off. J. ESSKA* **2016**, *24*, 2724–2728. [CrossRef]
10. Phegan, M.; Grayson, J.E.; Vertullo, C.J. No Infections in 1300 Anterior Cruciate Ligament Reconstructions with Vancomycin Pre-Soaking of Hamstring Grafts. *Knee Surg. Sports Traumatol. Arthrosc. Off. J. ESSKA* **2016**, *24*, 2729–2735. [CrossRef]

11. Pérez-Prieto, D.; Portillo, M.E.; Torres-Claramunt, R.; Pelfort, X.; Hinarejos, P.; Monllau, J.C. Contamination Occurs during ACL Graft Harvesting and Manipulation, but It Can Be Easily Eradicated. *Knee Surg. Sports Traumatol. Arthrosc. Off. J. ESSKA* **2018**, *26*, 558–562. [CrossRef]
12. Jefferies, J.G.; Aithie, J.M.S.; Spencer, S.J. Vancomycin-Soaked Wrapping of Harvested Hamstring Tendons during Anterior Cruciate Ligament Reconstruction. *A Review of the "Vancomycin Wrap." Knee* **2019**, *26*, 524–529. [CrossRef]
13. Naendrup, J.-H.; Marche, B.; de Sa, D.; Koenen, P.; Otchwemah, R.; Wafaisade, A.; Pfeiffer, T.R. Vancomycin-Soaking of the Graft Reduces the Incidence of Septic Arthritis Following ACL Reconstruction: Results of a Systematic Review and Meta-Analysis. *Knee Surg. Sports Traumatol. Arthrosc. Off. J. ESSKA* **2020**, *28*, 1005–1013. [CrossRef] [PubMed]
14. Kuršumović, K.; Charalambous, C.P. Relationship of Graft Type and Vancomycin Presoaking to Rate of Infection in Anterior Cruciate Ligament Reconstruction: A Meta-Analysis of 198 Studies with 68,453 Grafts. *JBJS Rev.* **2020**, *8*, e1900156. [CrossRef]
15. Abdelaziz, H.; Zahar, A.; Lausmann, C.; Gehrke, T.; Fickenscher, H.; Suero, E.M.; Gebauer, M.; Citak, M. High Bacterial Contamination Rate of Electrocautery Tips during Total Hip and Knee Arthroplasty. *Int. Orthop.* **2018**, *42*, 755–760. [CrossRef] [PubMed]
16. Beldame, J.; Lagrave, B.; Lievain, L.; Lefebvre, B.; Frebourg, N.; Dujardin, F. Surgical Glove Bacterial Contamination and Perforation during Total Hip Arthroplasty Implantation: When Gloves Should Be Changed. *Orthop. Traumatol. Surg. Res. OTSR* **2012**, *98*, 432–440. [CrossRef] [PubMed]
17. Davis, N.; Curry, A.; Gambhir, A.K.; Panigrahi, H.; Walker, C.R.; Wilkins, E.G.; Worsley, M.A.; Kay, P.R. Intraoperative Bacterial Contamination in Operations for Joint Replacement. *J. Bone Jt. Surg. Br.* **1999**, *81*, 886–889. [CrossRef]
18. Robinson, A.H.; Drew, S.; Anderson, J.; Bentley, G.; Ridgway, G.L. Suction Tip Contamination in the Ultraclean-Air Operating Theatre. *Ann. R. Coll. Surg. Engl.* **1993**, *75*, 254–256.
19. Schweitzer, D.; Klaber, I.; Fischman, D.; Wozniak, A.; Botello, E.; Amenábar, P.P. Surgical Light Handles: A Source of Contamination in the Surgical Field. *Acta Orthop. Traumatol. Turc.* **2015**, *49*, 421–425. [CrossRef]
20. Southwood, R.T.; Rice, J.L.; McDonald, P.J.; Hakendorf, P.H.; Rozenbilds, M.A. Infection in Experimental Hip Arthroplasties. *J. Bone Jt. Surg. Br.* **1985**, *67*, 229–231. [CrossRef]
21. Johnson, E.N.; Burns, T.C.; Hayda, R.A.; Hospenthal, D.R.; Murray, C.K. Infectious Complications of Open Type III Tibial Fractures among Combat Casualties. *Clin. Infect. Dis. Off. Publ. Infect. Dis. Soc. Am.* **2007**, *45*, 409–415. [CrossRef] [PubMed]
22. Lambert, E.W.; Simpson, R.B.; Marzouk, A.; Unger, D.V. Orthopaedic Injuries among Survivors of USS COLE Attack. *J. Orthop. Trauma* **2003**, *17*, 436–441. [CrossRef] [PubMed]
23. Williams, D.L.; Costerton, J.W. Using Biofilms as Initial Inocula in Animal Models of Biofilm-Related Infections. *J. Biomed. Mater. Res. B Appl. Biomater.* **2012**, *100*, 1163–1169. [CrossRef] [PubMed]
24. Wimpenny, J.; Manz, W.; Szewzyk, U. Heterogeneity in Biofilms. *FEMS Microbiol. Rev.* **2000**, *24*, 661–671. [CrossRef] [PubMed]
25. Costerton, J.W. Cystic Fibrosis Pathogenesis and the Role of Biofilms in Persistent Infection. *Trends Microbiol.* **2001**, *9*, 50–52. [CrossRef]
26. Veerachamy, S.; Yarlagadda, T.; Manivasagam, G.; Yarlagadda, P.K. Bacterial Adherence and Biofilm Formation on Medical Implants: A Review. *Proc. Inst. Mech. Eng. H* **2014**, *228*, 1083–1099. [CrossRef] [PubMed]
27. Franco, A.R.; Fernandes, E.M.; Rodrigues, M.T.; Rodrigues, F.J.; Gomes, M.E.; Leonor, I.B.; Kaplan, D.L.; Reis, R.L. Antimicrobial Coating of Spider Silk to Prevent Bacterial Attachment on Silk Surgical Sutures. *Acta Biomater.* **2019**, *99*, 236–246. [CrossRef]
28. Henry-Stanley, M.J.; Hess, D.J.; Barnes, A.M.T.; Dunny, G.M.; Wells, C.L. Bacterial Contamination of Surgical Suture Resembles a Biofilm. *Surg. Infect.* **2010**, *11*, 433–439. [CrossRef]
29. Elek, S.D. Experimental Staphylococcal Infections in the Skin of Man. *Ann. N. Y. Acad. Sci.* **1956**, *65*, 85–90. [CrossRef]
30. Elek, S.D.; Conen, P.E. The Virulence of Staphylococcus Pyogenes for Man; a Study of the Problems of Wound Infection. *Br. J. Exp. Pathol.* **1957**, *38*, 573–586.
31. Drago, L.; Romanò, C.L.; Mattina, R.; Signori, V.; De Vecchi, E. Does Dithiothreitol Improve Bacterial Detection from Infected Prostheses? A Pilot Study. *Clin. Orthop.* **2012**, *470*, 2915–2925. [CrossRef]
32. Karbysheva, S.; Di Luca, M.; Butini, M.E.; Winkler, T.; Schütz, M.; Trampuz, A. Comparison of Sonication with Chemical Biofilm Dislodgement Methods Using Chelating and Reducing Agents: Implications for the Microbiological Diagnosis of Implant Associated Infection. *PLoS ONE* **2020**, *15*, e0231389. [CrossRef]
33. Butini, M.E.; Gonzalez Moreno, M.; Czuban, M.; Koliszak, A.; Tkhilaishvili, T.; Trampuz, A.; Di Luca, M. Real-Time Antimicrobial Susceptibility Assay of Planktonic and Biofilm Bacteria by Isothermal Microcalorimetry. *Adv. Exp. Med. Biol.* **2019**, *1214*, 61–77. [CrossRef] [PubMed]
34. Trampuz, A.; Piper, K.E.; Jacobson, M.J.; Hanssen, A.D.; Unni, K.K.; Osmon, D.R.; Mandrekar, J.N.; Cockerill, F.R.; Steckelberg, J.M.; Greenleaf, J.F.; et al. Sonication of Removed Hip and Knee Prostheses for Diagnosis of Infection. *N. Engl. J. Med.* **2007**, *357*, 654–663. [CrossRef] [PubMed]
35. Alhede, M.; Qvortrup, K.; Liebrechts, R.; Høiby, N.; Givskov, M.; Bjarnsholt, T. Combination of Microscopic Techniques Reveals a Comprehensive Visual Impression of Biofilm Structure and Composition. *FEMS Immunol. Med. Microbiol.* **2012**, *65*, 335–342. [CrossRef]
36. Pantanella, F.; Valenti, P.; Natalizi, T.; Passeri, D.; Berlutti, F. Analytical Techniques to Study Microbial Biofilm on Abiotic Surfaces: Pros and Cons of the Main Techniques Currently in Use. *Ann. Ig. Med. Prev. E Comunita* **2013**, *25*, 31–42. [CrossRef]

37. Byrne, A.M.; Morris, S.; McCarthy, T.; Quinlan, W.; O'byrne, J.M. Outcome Following Deep Wound Contamination in Cemented Arthroplasty. *Int. Orthop.* **2007**, *31*, 27–31. [CrossRef]
38. Barker, J.U.; Drakos, M.C.; Maak, T.G.; Warren, R.F.; Williams, R.J.; Allen, A.A. Effect of Graft Selection on the Incidence of Postoperative Infection in Anterior Cruciate Ligament Reconstruction. *Am. J. Sports Med.* **2010**, *38*, 281–286. [CrossRef]
39. Katz, L.M.; Battaglia, T.C.; Patino, P.; Reichmann, W.; Hunter, D.J.; Richmond, J.C. A Retrospective Comparison of the Incidence of Bacterial Infection Following Anterior Cruciate Ligament Reconstruction with Autograft versus Allograft. *Arthrosc. J. Arthrosc. Relat. Surg. Off. Publ. Arthrosc. Assoc. N. Am. Int. Arthrosc. Assoc.* **2008**, *24*, 1330–1335. [CrossRef]

6. OVERALL SUMMARY OF RESULTS

The combined analysis of the results of this thesis provides a comprehensive understanding of the vancomycin presoaking technique's safety and the study of the 4xHt grafts as a potential independent risk factor for infection.

A total of 785 patients were enrolled in the study first study, with 383 patients in Group 1 and 402 patients in Group 2. Twelve patients (7 in Group 1 and 5 in Group 2) were lost to follow-up and therefore could not be assessed for re-rupture. Five infections were reported, all of which occurred in Group 1. It resulted in their exclusion from the analysis ($p < 0.001$). Additionally, 26 patients were excluded due to the presence of extra-articular procedures, multiligament injuries, or allograft use (11 in Group 1 and 15 in Group 2). Consequently, a total of 360 patients were included in Group 1, while 382 patients were included in Group 2, resulting in a total of 43 patients being excluded from the study (Figure 2). No significant differences (n.s.) were observed between the two groups regarding missing or excluded patients. Demographic characteristics such as gender and age were comparable between the groups (n.s.). Similarly, there were no significant differences in terms of the body mass index, or the type of autograft utilized for the two groups (n.s.). The mean follow-up was significantly longer in Group 1, with a mean of 94 months (range 82–105 months), compared to 72 months (range 60–83 months) in Group 2 ($p < 0.001$).

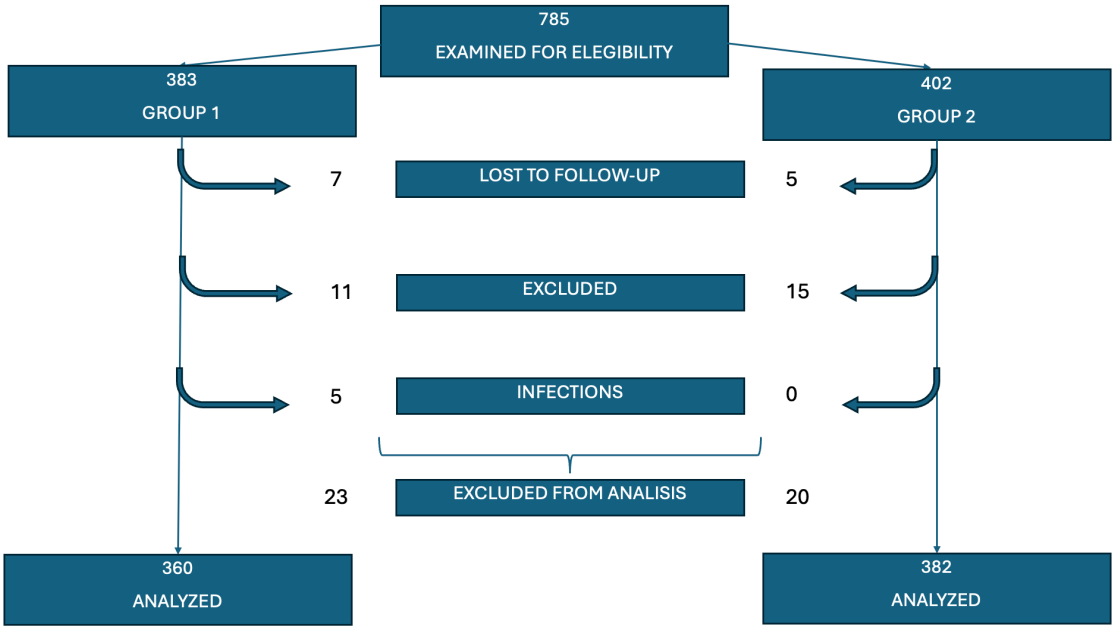


Figure 1. Flow chart illustrating the creation of study cohorts, patient exclusions, and loss of follow-up.

In Group 1, 17 patients experienced a re-rupture, accounting for 4.7% of the total. Group 2 saw 15 patients with re-rupture, representing 3.9% of the cohort. Although Group 2 exhibited a slightly lower re-rupture rate, the difference did not reach statistical significance (n.s.). The time to re-rupture was comparable between the two groups, with a mean of 51 months (range 48–60 months) in Group 1 and 50 months (range 45–56 months) in Group 2 (n.s.).

Regarding the secondary objective, functional assessments could only be scheduled for 182 patients in Group 1 and 199 patients in Group 2. The mean IKDC score was 82.0 (SD 8.0) in Group 1 and 83.9 (SD 8.0) in Group 2 ($p = 0.049$), indicating a statistically significant difference but with no clinically relevant difference (MCRD > 9.8).(71) The median Tegner score remained consistent at 4 points in both groups (n.s.). However, there was a statistically significant difference observed in the mean Lysholm score, with Group 1 scoring 90.3 (SD 6.6) and Group 2 scoring 92.0 (SD 6.1) ($p = 0.015$) but with no clinically relevant difference (MCRD > 25(71).

A summary of all the results can be found in Table 1.

	Group 1 (no vancomycin soaking)	Group 2 (vancomycin soaking)	*p* value
n	360	382	
Follow-up (months)	94 (82–105)	72 (60–83)	< 0.001
Re-rupture cases	17 (4.7%)	15 (3.9%)	n.s.
IKDC	82.0 (8.0)	83.9 (8.0)	0.049
Tegner	4	4	n.s.
Lysholm	90.3 (6.6)	92.0 (6.1)	0.015

Table 1: Results and comparative between groups

Utilizing the isothermal microcalorimetry technique, bacterial growth was observed in the contaminated fragments of both the 4xHt graft and BPTB graft. These fragments exhibited similar growth dynamics, which is consistent with the typical behavior of the bacterial strain utilized. Initially, the microcalorimetry curves displayed exponential growth that culminated in a population peak (200 µW) approximately 8 hours post-contamination. Subsequently, a rapid decrease in bacterial population size, characterized by senescence or bacterial death, was observed. Notably, no significant differences were detected between the growth profiles of the two graft types. In contrast, the negative control fragments of both graft types showed no evidence of bacterial growth, affirming the absence of contamination in these samples. Consequently, no notable disparities in bacterial growth profiles were identified between the 4xHt and BPTB grafts (Figure 3)

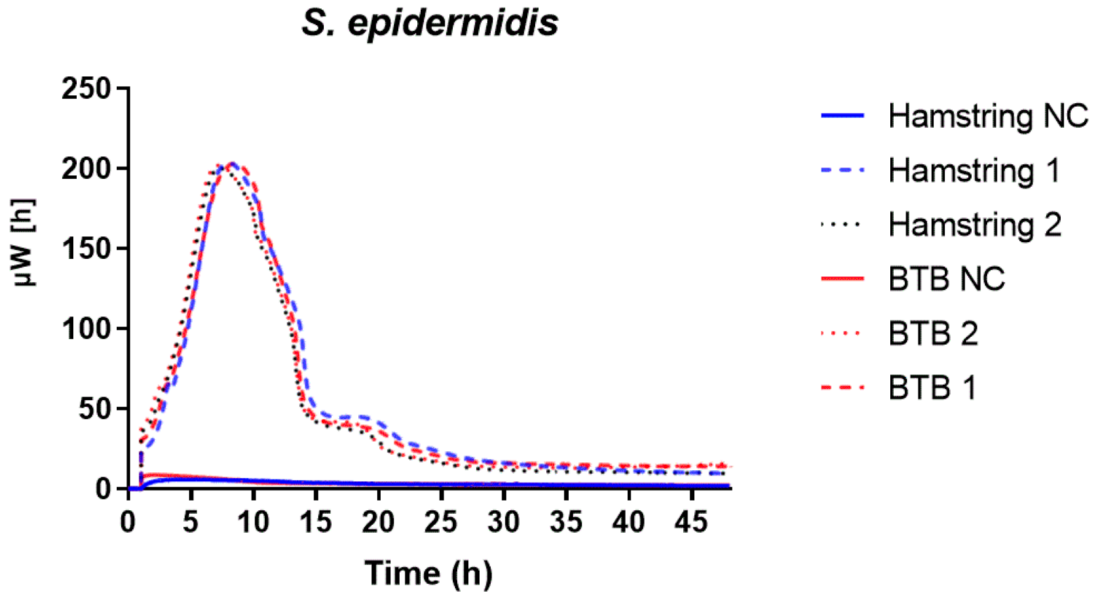


Figure 3. Isothermal microcalorimetry, no significant differences between the bacterial growth profiles of any of the 4xHt and BPTB contaminated grafts were found. (Legend: **H1c** blue discontinuous line - - - -, **H2c** blue spotted line · · · ·, **H6n** blue continuous line ----, **P1c** red discontinuous line - - - -, **P2c** red spotted line · · · ·, **P6n** red continuous line ----. *Sample names explained on **fig 1**)

The CFU/ml count was determined for the fluid obtained through sonication of the contaminated fragments from both the BPTB and 4xHt grafts. The average CFU/ml values for each group were calculated and compared. The mean CFU/ml counts were found to be 3.5x10^7 ± 0.345e^+7 CFU/ml for the BPTB graft group and 4.6 x10^7 ± 1.455e^ +7 CFU/ml for the 4xHt graft group. No significant difference was observed between them (p = 0.6667) (Figure 4). This statistical analysis confirmed that there were no significant disparities in bacterial load between the 4xHt and BPTB graft groups (p > 0.05).

Furthermore, when the fluid extracted from the uncontaminated BPTB and 4xHt graft fragments (negative controls) via sonication was seeded, no colony growth was observed.

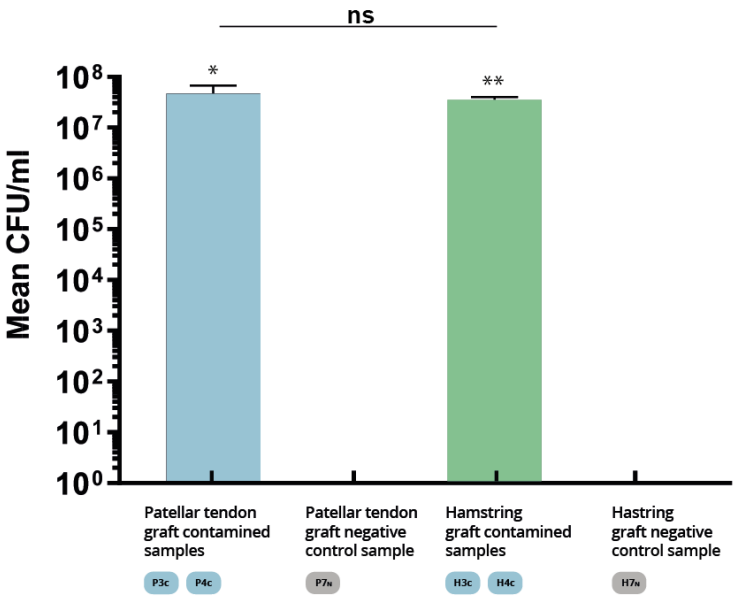
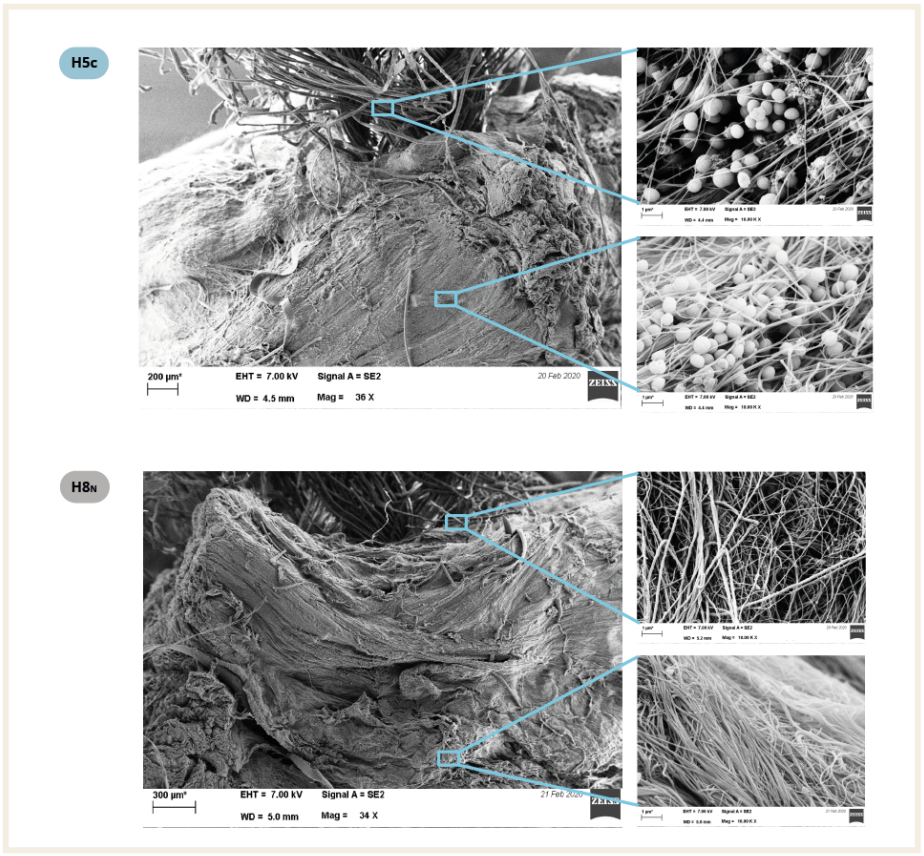


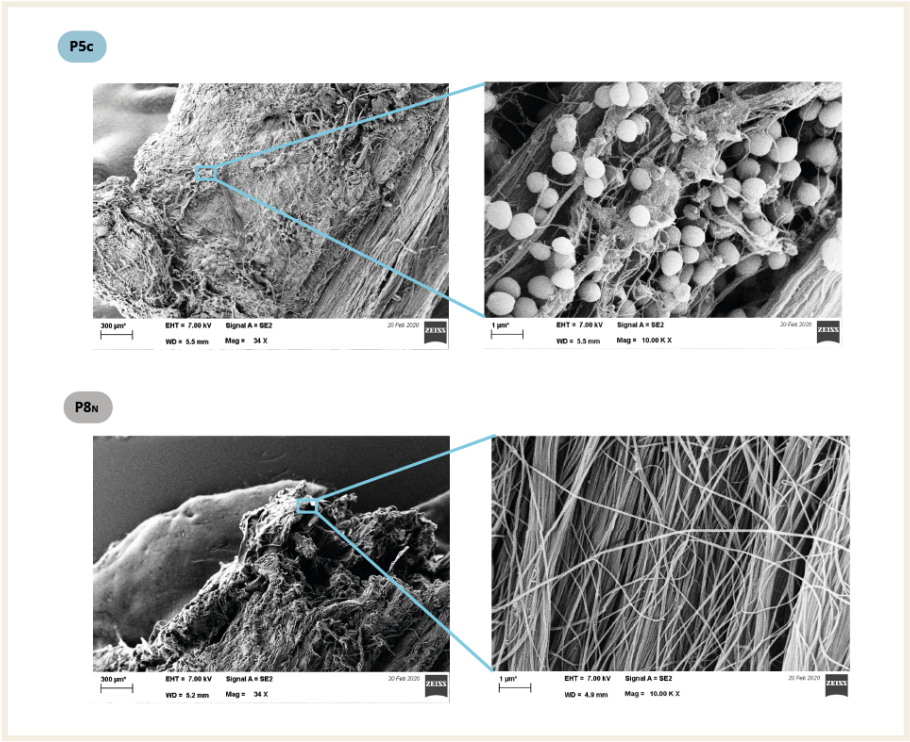
Figure 4. Colony-forming units counting method, no significant differences were detected between the 4xHt graft group and the BPTB graft group. * Standard

deviation 0,345e^+7, **Standard deviation 1.455e^ +7.

During the electron microscopy analysis, the technician observed no discernible or distinctive patterns in biofilm growth upon comparing the contaminated fragments of BPTB grafts to those of 4xHt grafts. Regardless of the graft surface, a uniform growth pattern was observed. Specifically, no noticeable increase in colonization was observed in the suture areas. (Figures 5a and 5b) No bacterial presence was detected in the negative control samples.



(Fig. 5a)



(Fig. 5b)

Figure 5. Electron microscopy at magnification of 34x, and10.00 Kx: **(Fig5a)** Hamstring graft samples, contaminated **H5c** and negative control **H8n**. **(Fig5b)** Patellar tendon graft samples, contaminated **P5c** and negative controls **P8n**. **(Legend: Black arrow = Hamstring tendon, White arrow = suture, green arrow patellar tendon)**

Overall, these findings suggest that the use of the vancomycin presoaking technique does not significantly impact re-rupture rates or functional outcomes post-ACL reconstruction. Moreover, the production of biofilm in 4xHt grafts is much the same as that produced in BPTB grafts contaminated with the same bacterial inoculum.

7. OVERALL SUMMARY OF THE DISCUSSION

The findings from this thesis indicate that soaking 4xHt and BPTB autografts in a 5 mg/ml vancomycin solution concentration does not elevate the risk of re-rupture following ACL-r and does not adversely impact functional outcomes. This corroborates the first hypothesis. Conversely, results from the thesis debunk the second hypothesis, seeing no differences in biofilm growth when BPTB and 4xHt grafts had been exposed to the same bacterial inoculum *in vitro*.

The observed re-rupture rates in this thesis align with existing literature, which reports re-rupture rates ranging from 0% to 25%(72), with the majority of studies reporting rates around 7%. These findings are consistent with the results obtained in both the vancomycin technique group and the group without it. Additionally, the functional outcomes observed in this study are comparable to those reported in previously published data(72,73). This suggests that the vancomycin presoaking technique does not compromise functional recovery post-ACL-r. This further supports its safety and efficacy in clinical practice. However, this study has several limitations. The most important is its retrospective design even though the groups were similar and there was no other modification in the surgical protocol except for the vancomycin technique. Another aspect of its retrospective design is the sequential character of the 2 groups and the resultant different follow-ups. This is an important limitation as the group of patients with longer follow-up may have a greater chance of re-rupture even though the time to re-rupture was similar between groups. Even so and given the consistency of our results with existing evidence, we advocate for the standardization of vancomycin presoaking prophylaxis in ACL-r surgeries. We believe that such standardization will enhance surgical outcomes and mitigate risks.

The results of the second investigation revealed that structural disparities between the two graft types do not influence biofilm production. This suggests that both tissues function as foreign bodies that equally make for biofilm growth. It can be inferred that the 4xHt graft does not inherently predispose to infections due to its structure and composition. However, this study has several limitations.

This study was performed with only frozen cadaveric donor grafts. We are aware of the bias that it may produce. Working with fresh donor grafts would have been more difficult to do for ethical and practical reasons. In any case, a study by Charalambous et al. showed no significant differences in infection rates between allografts and autografts(56), this led us to think that there would be no major differences between the use of fresh and cadaveric grafts. Another factor to mention is that only S. epidermidis (one of the most frequent bacteria infecting ACL-R) was used for contamination and biofilm formation. It would be interesting to perform the same analysis with other types of Gram-positive staphylococci that are frequently associated with this pathology. However, it is unlikely that any differences would be found due to their similarity to S. epidermidis. On the other hand, we believe that repeating the analysis with pathogens of other strains including anaerobes would be of little relevance due to their low prevalence in ACL-r infections. With these limitations laid out, we still consider that this study gives enough evidence to infer that the differences in infection rates observed in ACL-r surgeries utilizing BPTB and 4xHt grafts cannot be attributed to their structural distinctions. This leads us to consider discontinuing further investigation into this avenue and prompts exploration of alternative research directions.

Upon analyzing our results and comparing them with existing literature, we have identified a recently published study by Höher et al. that has also demonstrated the clinical safety of the vancomycin technique. It saw no adverse effects on re-rupture rates(49). Interestingly, they observed a lower risk of graft failure in the vancomycin group compared to those without vancomycin soaking. While our findings align with theirs regarding the absence of increased re-rupture risk, we did not observe a reduction in failure rates. Höher et al. attributed this reduction to a potential decrease in low-grade infections facilitated by the vancomycin technique. This hypothesis warrants further exploration, particularly considering evidence of bacterial DNA presence in widened tunnels and failed grafts, suggesting a link between low-grade infections and re-rupture(74,75).

On the other hand, accepting the results of the study "Are Hamstring Grafts a Predisposing Factor to Infection in ACL-r Surgery? A Comparative *In Vitro* Study,"

we must look for alternative theories to explain why hamstring graft surgeries have higher infection rates. The fact that grafts, by their very nature, are not a predisposing factor for biofilm growth leads us to think that there must be some differences surrounding their processing that changes and conditions the differences in infection rates. One possible reason is that there are differences in the contamination of grafts. It is well known that contamination during a surgical procedures is time-dependent(76). The production of the 4×Ht graft is more time-consuming due to its greater complexity(43,47). We also know that contamination of grafts occurs during the process of making them (41). This further leads us to consider that contamination with more bacterial inoculum might occur during the preparation of the 4×Ht grafts, more frequently reaching the MID. In this way, we could come by the higher infection rates seen in ACL-r surgeries performed with 4×Ht grafts. Another hypothesis is that slightly more extensive tissue dissection and morbidity is performed at the 4×Ht autograft harvest site when compared to the BPTB autograft harvest site(77,78), thereby justifying the difference in infection rates.

8. CONCLUSIONS

With the results of the studies that constitute this thesis, we can conclude that the vancomycin presoaking prophylaxis technique is a safe technique. Moreover, it does not affect the re-rupture rates in the mid- to long-term. Neither does it affect the functional results of ACL-r surgeries. Furthermore, the quantity of biofilm formed on BPTB and 4xHt grafts by the same bacterial inoculum is similar *in vitro*. Therefore, the 4xHt graft is not inherently predisposed to biofilm growth based on its structure and composition.

9. FUTURE LINES OF INVESTIGATION

This thesis is part of an ongoing research initiative at our institution. It has already led to the publication of eleven papers in high-impact journals. Moreover, it has resulted in two clinical practice guidelines as well as contributions to one chapter in two distinct books, and the successful defense of another doctoral thesis. Our efforts to broaden the understanding of ACL-r infections and to improve surgical outcomes remain a priority, and we aim to continue contributing to this field with further studies. The goal is to achieve safer and more effective ACL-r surgeries, thereby reducing the risk of complications and enhancing patient recovery.

Building upon the findings of this thesis, several key areas for future research have already been identified and will now be discussed.

First, while this thesis demonstrates that vancomycin presoaking does not elevate the risk of re-rupture or impair functional outcomes, there are no cost-effectiveness analyses of it. Given the minimal cost of vancomycin, this analysis is expected to be favorable. The cost-factor in and of itself supports the broader adoption of this technique as a standard prophylactic measure in ACL-r surgeries.

Secondly, further exploration of the relationship between low-grade infections and graft failure is in our sights. The hypothesis that vancomycin presoaking may reduce low-grade infections and subsequently lower graft failure rates, as suggested by Höher et al., deserves more in-depth study. An observational investigation of the presence of bacterial DNA in widened tunnels and failed grafts could provide valuable insights into the role of subclinical infections in ACL-r failure. Delving deeper into this phenomenon may well inform the development of targeted strategies to mitigate graft failure.

Finally, alternative theories regarding the higher infection rates observed in hamstring grafts require further investigation. A potential area of focus might be time-dependent contamination during graft preparation, particularly with 4×Ht grafts as they require more time and complex handling. Future studies could

examine the bacterial inoculum present during graft preparation with a focus on minimizing intraoperative contamination. Further research might explore the extent of tissue dissection at the autograft harvest site, particularly in 4×Ht grafts, to assess its role in the risk of infection. Those are some of the future lines of investigation to be considered in relation to ACL-r infections.

With this solid foundation of research and continuous investigation, we aim to broaden our understanding of ACL-r infections and enhance the overall safety and outcomes of these surgeries.

1. BIBLIOGRAPHY

1. Claes S, Vereecke E, Maes M, Victor J, Verdonk P, Bellemans J. Anatomy of the anterolateral ligament of the knee. J Anat. octubre 2013;223(4):321-8.

2. Shelbourne KD, Nitz P. Accelerated rehabilitation after anterior cruciate ligament reconstruction. Am J Sports Med. 1990;18(3):292-9.

3. Georgoulis AD, Pappa L, Moebius U, Malamou-Mitsi V, Pappa S, Papageorgiou CO, et al. The presence of proprioceptive mechanoreceptors in the remnants of the ruptured ACL as a possible source of re-innervation of the ACL autograft. Knee Surg Sports Traumatol Arthrosc Off J ESSKA. novembre 2001;9(6):364-8.

4. Frobell RB, Lohmander LS, Roos EM. The challenge of recruiting patients with anterior cruciate ligament injury of the knee into a randomized clinical trial comparing surgical and non-surgical treatment. Contemp Clin Trials. maig 2007;28(3):295-302.

5. Andernord D, Desai N, Björnsson H, Gillén S, Karlsson J, Samuelsson K. Predictors of contralateral anterior cruciate ligament reconstruction: a cohort study of 9061 patients with 5-year follow-up. Am J Sports Med. febrer 2015;43(2):295-302.

6. Sanders TL, Maradit Kremers H, Bryan AJ, Larson DR, Dahm DL, Levy BA, et al. Incidence of Anterior Cruciate Ligament Tears and Reconstruction: A 21-Year Population-Based Study. Am J Sports Med. juny 2016;44(6):1502-7.

7. Davis BR, Chen J, Inacio MCS, Love R, Prentice HA, Maletis GB. The Incidence of Subsequent Meniscal Surgery Is Higher in the Anterior Cruciate Ligament-Reconstructed Knee Than in the Contralateral Knee. Am J Sports Med. desembre 2017;45(14):3216-22.

8. Zbrojkiewicz D, Vertullo C, Grayson JE. Increasing rates of anterior cruciate ligament reconstruction in young Australians, 2000-2015. Med J Aust. 7 maig 2018;208(8):354-8.

9. Andrade R, Pereira R, Cingel R van, Staal JB, Espregueira-Mendes J. How should clinicians rehabilitate patients after ACL reconstruction? A systematic review of clinical practice guidelines (CPGs) with a focus on quality appraisal (AGREE II). Br J Sports Med. 1 maig 2020;54(9):512-9.

10. Nwachukwu BU, Patel BH, Lu Y, Allen AA, Williams RJ. Anterior Cruciate Ligament Repair Outcomes: An Updated Systematic Review of Recent Literature. Arthroscopy. 1 juliol 2019;35(7):2233-47.

11. Boden BP, Dean GS, Feagin JA, Garrett WE. Mechanisms of anterior cruciate ligament injury. Orthopedics. juny 2000;23(6):573-8.

12. Shin CS, Chaudhari AM, Andriacchi TP. The effect of isolated valgus moments on ACL strain during single-leg landing: a simulation study. J Biomech. 9 febrer 2009;42(3):280-5.

13. Alentorn-Geli E, Mendiguchía J, Samuelsson K, Musahl V, Karlsson J, Cugat R, et al. Prevention of non-contact anterior cruciate ligament injuries in sports. Part II: systematic review of the effectiveness of prevention programmes in male athletes. Knee Surg Sports Traumatol Arthrosc Off J ESSKA. gener 2014;22(1):16-25.

14. Alentorn-Geli E, Mendiguchía J, Samuelsson K, Musahl V, Karlsson J, Cugat R, et al. Prevention of anterior cruciate ligament injuries in sports. Part I: systematic review of risk factors in male athletes. Knee Surg Sports Traumatol Arthrosc Off J ESSKA. gener 2014;22(1):3-15.

15. Hewett TE, Myer GD, Ford KR, Heidt RS, Colosimo AJ, McLean SG, et al. Biomechanical measures of neuromuscular control and valgus loading of the knee predict anterior cruciate ligament injury risk in female athletes: a prospective study. Am J Sports Med. abril 2005;33(4):492-501.

16. Torg JS, Conrad W, Kalen V. Clinical diagnosis of anterior cruciate ligament instability in the athlete. Am J Sports Med. 1976;4(2):84-93.

17. DeFranco MJ, Bach BR. A comprehensive review of partial anterior cruciate ligament tears. J Bone Joint Surg Am. gener 2009;91(1):198-208.

18. Sanchis-Alfonso V, Martinez-Sanjuan V, Gastaldi-Orquin E. The value of MRI in the evaluation of the ACL deficient knee and in the post-operative evaluation after ACL reconstruction. Eur J Radiol. febrer 1993;16(2):126-30.

19. Cheatham SA, Johnson DL. Anatomic revision ACL reconstruction. Sports Med Arthrosc Rev. març 2010;18(1):33-9.

20. Georgoulis AD, Ristanis S, Moraiti CO, Paschos N, Zampeli F, Xergia S, et al. ACL injury and reconstruction: Clinical related in vivo biomechanics. Orthop Traumatol Surg Res OTSR. desembre 2010;96(8 Suppl):S119-128.

21. Provencher MT, Ryu JH, Gaston T, Dewing CB. Technique: bone-patellar tendon-bone autograft ACL reconstruction in the young, active patient. J Knee Surg. juny 2011;24(2):83-92.

22. Kruse LM, Gray B, Wright RW. Rehabilitation after anterior cruciate ligament reconstruction: a systematic review. J Bone Joint Surg Am. 3 octubre 2012;94(19):1737-48.

23. van Melick N, van Cingel REH, Brooijmans F, Neeter C, van Tienen T, Hullegie W, et al. Evidence-based clinical practice update: practice guidelines for anterior cruciate ligament rehabilitation based on a systematic review and multidisciplinary consensus. Br J Sports Med. desembre 2016;50(24):1506-15.

24. Persson A, Fjeldsgaard K, Gjertsen JE, Kjellsen AB, Engebretsen L, Hole RM, et al. Increased risk of revision with hamstring tendon grafts compared with patellar tendon grafts after anterior cruciate ligament reconstruction: a study of 12,643 patients from the Norwegian Cruciate Ligament Registry, 2004-2012. Am J Sports Med. febrer 2014;42(2):285-91.

25. Maletis GB, Cameron SL, Tengan JJ, Burchette RJ. A prospective randomized study of anterior cruciate ligament reconstruction: a comparison of patellar tendon and quadruple-strand semitendinosus/gracilis tendons fixed with bioabsorbable interference screws. Am J Sports Med. març 2007;35(3):384-94.

26. Maletis GB, Inacio MCS, Reynolds S, Desmond JL, Maletis MM, Funahashi TT. Incidence of postoperative anterior cruciate ligament reconstruction infections: graft choice makes a difference. Am J Sports Med. agost 2013;41(8):1780-5.

27. Bansal A, Lamplot JD, VandenBerg J, Brophy RH. Meta-analysis of the Risk of Infections After Anterior Cruciate Ligament Reconstruction by Graft Type. Am J Sports Med. maig 2018;46(6):1500-8.

28. Masuda H, Taketomi S, Inui H, Shimazaki N, Nishihara N, Toyooka S, et al. Bone-to-bone integrations were complete within 5 months after anatomical rectangular tunnel anterior cruciate ligament reconstruction using a bone-patellar tendon-bone graft. Knee Surg Sports Traumatol Arthrosc Off J ESSKA. desembre 2018;26(12):3660-6.

29. Dunn WR, Lyman S, Lincoln AE, Amoroso PJ, Wickiewicz T, Marx RG. The effect of anterior cruciate ligament reconstruction on the risk of knee reinjury. Am J Sports Med. desembre 2004;32(8):1906-14.

30. Prentice HA, Lind M, Mouton C, Persson A, Magnusson H, Gabr A, et al. Patient demographic and surgical characteristics in anterior cruciate ligament reconstruction: a description of registries from six countries. Br J Sports Med. juny 2018;52(11):716-22.

31. Gristina AG, Costerton JW. Bacterial adherence and the glycocalyx and their role in musculoskeletal infection. Orthop Clin North Am. juliol 1984;15(3):517-35.

32. Kapadia BH, Berg RA, Daley JA, Fritz J, Bhave A, Mont MA. Periprosthetic joint infection. Lancet Lond Engl. 23 gener 2016;387(10016):386-94.

33. Elek SD. Experimental staphylococcal infections in the skin of man. Ann N Y Acad Sci. 31 agost 1956;65(3):85-90.

34. Elek SD, Conen PE. The virulence of Staphylococcus pyogenes for man; a study of the problems of wound infection. Br J Exp Pathol. desembre 1957;38(6):573-86.

35. Zimmerli W, Trampuz A, Ochsner PE. Prosthetic-Joint Infections. N Engl J Med. 14 octubre 2004;351(16):1645-54.

36. Insall JN, Thompson FM, Brause BD. Two-stage reimplantation for the salvage of infected total knee arthroplasty. J Bone Joint Surg Am. octubre 1983;65(8):1087-98.

37. Boström Windhamre H, Mikkelsen C, Forssblad M, Willberg L. Postoperative septic arthritis after anterior cruciate ligament reconstruction: does it affect the outcome? A retrospective controlled study. Arthrosc J Arthrosc Relat Surg Off Publ Arthrosc Assoc N Am Int Arthrosc Assoc. setembre 2014;30(9):1100-9.

38. Torres-Claramunt R, Gelber P, Pelfort X, Hinarejos P, Leal-Blanquet J, Pérez-Prieto D, et al. Managing septic arthritis after knee ligament reconstruction. Int Orthop. 1 març 2016;40(3):607-14.

39. Torres-Claramunt R, Pelfort X, Erquicia J, Gil-González S, Gelber PE, Puig L, et al. Knee joint infection after ACL reconstruction: prevalence, management and functional outcomes. Knee Surg Sports Traumatol Arthrosc Off J ESSKA. desembre 2013;21(12):2844-9.

40. Komnos GA, Chalatsis G, Mitrousias V, Hantes ME. Postoperative Infection after Anterior Cruciate Ligament Reconstruction: Prevention and Management. Microorganisms [Internet]. desembre 2022 [citat 26 abril 2024];10(12). Disponible a: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9781783/

41. Pérez-Prieto D, Portillo ME, Torres-Claramunt R, Pelfort X, Hinarejos P, Monllau JC. Contamination occurs during ACL graft harvesting and manipulation, but it can be easily eradicated. Knee Surg Sports Traumatol Arthrosc Off J ESSKA. febrer 2018;26(2):558-62.

42. Muscolo DL, Carbo L, Aponte-Tinao LA, Ayerza MA, Makino A. Massive bone loss from fungal infection after anterior cruciate ligament arthroscopic reconstruction. Clin Orthop. setembre 2009;467(9):2420-5.

43. Pérez-Prieto D, Torres-Claramunt R, Gelber PE, Shehata TMA, Pelfort X, Monllau JC. Autograft soaking in vancomycin reduces the risk of infection after anterior cruciate ligament reconstruction. Knee Surg Sports Traumatol Arthrosc Off J ESSKA. setembre 2016;24(9):2724-8.

44. Jefferies JG, Aithie JMS, Spencer SJ. Vancomycin-soaked wrapping of harvested hamstring tendons during anterior cruciate ligament reconstruction. A review of the «vancomycin wrap». The Knee. juny 2019;26(3):524-9.

45. Figueroa D, Figueroa F, Calvo R, Lopez M, Goñi I. Presoaking of Hamstring Autografts in Vancomycin Decreases the Occurrence of Infection Following Primary Anterior Cruciate Ligament Reconstruction. Orthop J Sports Med. setembre 2019;7(9):2325967119871038.

46. Vertullo CJ, Quick M, Jones A, Grayson JE. A Surgical Technique Using Presoaked Vancomycin Hamstring Grafts to Decrease the Risk of Infection After Anterior Cruciate Ligament Reconstruction. Arthroscopy. 1 març 2012;28(3):337-42.

47. Baron JE, Shamrock AG, Cates WT, Cates RA, An Q, Wolf BR, et al. Graft Preparation with Intraoperative Vancomycin Decreases Infection After ACL Reconstruction: A Review of 1,640 Cases. J Bone Joint Surg Am. 18 desembre 2019;101(24):2187-93.

48. Naendrup JH, Marche B, de Sa D, Koenen P, Otchwemah R, Wafaisade A, et al. Vancomycin-soaking of the graft reduces the incidence of septic arthritis following ACL reconstruction: results of a systematic review and meta-analysis. Knee Surg Sports Traumatol Arthrosc Off J ESSKA. abril 2020;28(4):1005-13.

49. Offerhaus C, Balke M, Hente J, Gehling M, Blendl S, Höher J. Vancomycin pre-soaking of the graft reduces postoperative infection rate without increasing risk of graft failure and arthrofibrosis in ACL reconstruction. Knee Surg Sports Traumatol Arthrosc Off J ESSKA. setembre 2019;27(9):3014-21.

50. Phegan M, Grayson JE, Vertullo CJ. No infections in 1300 anterior cruciate ligament reconstructions with vancomycin pre-soaking of hamstring grafts. Knee Surg Sports Traumatol Arthrosc Off J ESSKA. setembre 2016;24(9):2729-35.

51. Grayson JE, Grant GD, Dukie S, Vertullo CJ. The in vitro elution characteristics of vancomycin from tendons. Clin Orthop. octubre 2011;469(10):2948-52.

52. Ferguson J, Athanasou N, Diefenbeck M, McNally M. Radiographic and Histological Analysis of a Synthetic Bone Graft Substitute Eluting Gentamicin in the Treatment of Chronic Osteomyelitis. J Bone Jt Infect. 2019;4(2):76-84.

53. Metsemakers WJ, Fragomen AT, Moriarty TF, Morgenstern M, Egol KA, Zalavras C, et al. Evidence-Based Recommendations for Local Antimicrobial Strategies and Dead Space Management in Fracture-Related Infection. J Orthop Trauma. gener 2020;34(1):18-29.

54. Olesen UK, Juul R, Bonde CT, Moser C, McNally M, Jensen LT, et al. A review of forty five open tibial fractures covered with free flaps. Analysis of complications, microbiology and prognostic factors. Int Orthop. juny 2015;39(6):1159-66.

55. Schüttler KF, Scharm A, Stein T, Heyse TJ, Lohoff M, Sommer F, et al. Biomechanical and microbiological effects of local vancomycin in anterior cruciate ligament (ACL) reconstruction: a porcine tendon model. Arch Orthop Trauma Surg. gener 2019;139(1):73-8.

56. Kuršumović K, Charalambous CP. Relationship of Graft Type and Vancomycin Presoaking to Rate of Infection in Anterior Cruciate Ligament Reconstruction: A Meta-Analysis of 198 Studies with 68,453 Grafts. JBJS Rev. juliol 2020;8(7):e1900156.

57. Abdelaziz H, Zahar A, Lausmann C, Gehrke T, Fickenscher H, Suero EM, et al. High bacterial contamination rate of electrocautery tips during total hip and knee arthroplasty. Int Orthop. abril 2018;42(4):755-60.

58. Beldame J, Lagrave B, Lievain L, Lefebvre B, Frebourg N, Dujardin F. Surgical glove bacterial contamination and perforation during total hip arthroplasty implantation: when gloves should be changed. Orthop Traumatol Surg Res OTSR. juny 2012;98(4):432-40.

59. Davis N, Curry A, Gambhir AK, Panigrahi H, Walker CR, Wilkins EG, et al. Intraoperative bacterial contamination in operations for joint replacement. J Bone Joint Surg Br. setembre 1999;81(5):886-9.

60. Robinson AH, Drew S, Anderson J, Bentley G, Ridgway GL. Suction tip contamination in the ultraclean-air operating theatre. Ann R Coll Surg Engl. juliol 1993;75(4):254-6.

61. Schweitzer D, Klaber I, Fischman D, Wozniak A, Botello E, Amenábar PP. Surgical light handles: a source of contamination in the surgical field. Acta Orthop Traumatol Turc. 2015;49(4):421-5.

62. Southwood RT, Rice JL, McDonald PJ, Hakendorf PH, Rozenbilds MA. Infection in experimental hip arthroplasties. J Bone Joint Surg Br. març 1985;67(2):229-31.

63. Johnson EN, Burns TC, Hayda RA, Hospenthal DR, Murray CK. Infectious complications of open type III tibial fractures among combat casualties. Clin Infect Dis Off Publ Infect Dis Soc Am. 15 agost 2007;45(4):409-15.

64. Lambert EW, Simpson RB, Marzouk A, Unger DV. Orthopaedic injuries among survivors of USS COLE attack. J Orthop Trauma. juliol 2003;17(6):436-41.

65. Williams DL, Costerton JW. Using biofilms as initial inocula in animal models of biofilm-related infections. J Biomed Mater Res B Appl Biomater. maig 2012;100(4):1163-9.

66. Wimpenny J, Manz W, Szewzyk U. Heterogeneity in biofilms. FEMS Microbiol Rev. desembre 2000;24(5):661-71.

67. Costerton JW. Cystic fibrosis pathogenesis and the role of biofilms in persistent infection. Trends Microbiol. febrer 2001;9(2):50-2.

68. Veerachamy S, Yarlagadda T, Manivasagam G, Yarlagadda PK. Bacterial adherence and biofilm formation on medical implants: a review. Proc Inst Mech Eng [H]. octubre 2014;228(10):1083-99.

69. Franco AR, Fernandes EM, Rodrigues MT, Rodrigues FJ, Gomes ME, Leonor IB, et al. Antimicrobial coating of spider silk to prevent bacterial attachment on silk surgical sutures. Acta Biomater. novembre 2019;99:236-46.

70. Henry-Stanley MJ, Hess DJ, Barnes AMT, Dunny GM, Wells CL. Bacterial contamination of surgical suture resembles a biofilm. Surg Infect. octubre 2010;11(5):433-9.

71. Ogura T, Ackermann J, Mestriner AB, Merkely G, Gomoll AH. The Minimal Clinically Important Difference and Substantial Clinical Benefit in the Patient-Reported Outcome Measures of Patients Undergoing Osteochondral Allograft Transplantation in the Knee. Cartilage. gener 2021;12(1):42-50.

72. Grassi A, Kim C, Muccioli GMM, Zaffagnini S, Amendola A. What Is the Mid-term Failure Rate of Revision ACL Reconstruction? A Systematic Review. Clin Orthop Relat Res. octubre 2017;475(10):2484.

73. Fernandes TL, Fregni F, Weaver K, Pedrinelli A, Camanho GL, Hernandez AJ. The influence of femoral tunnel position in single-bundle ACL reconstruction on functional outcomes and return to sports. Knee Surg Sports Traumatol Arthrosc. 1 gener 2014;22(1):97-103.

74. Everhart JS, DiBartola AC, Dusane DH, Magnussen RA, Kaeding CC, Stoodley P, et al. Bacterial Deoxyribonucleic Acid Is Often Present in Failed Revision Anterior Cruciate Ligament Reconstructions. Arthroscopy. 1 novembre 2018;34(11):3046-52.

75. Flanigan DC, Everhart JS, DiBartola AC, Dusane DH, Abouljoud MM, Magnussen RA, et al. Bacterial DNA is associated with tunnel widening in failed ACL reconstructions. Knee Surg Sports Traumatol Arthrosc. 1 novembre 2019;27(11):3490-7.

76. Byrne AM, Morris S, McCarthy T, Quinlan W, O'byrne JM. Outcome following deep wound contamination in cemented arthroplasty. Int Orthop. febrer 2007;31(1):27-31.

77. Barker JU, Drakos MC, Maak TG, Warren RF, Williams RJ, Allen AA. Effect of graft selection on the incidence of postoperative infection in anterior cruciate ligament reconstruction. Am J Sports Med. febrer 2010;38(2):281-6.

78. Katz LM, Battaglia TC, Patino P, Reichmann W, Hunter DJ, Richmond JC. A retrospective comparison of the incidence of bacterial infection following anterior cruciate ligament reconstruction with autograft versus allograft. Arthrosc J Arthrosc Relat Surg Off Publ Arthrosc Assoc N Am Int Arthrosc Assoc. desembre 2008;24(12):1330-5.