國立臺灣大學國際三校農業生技與健康醫療碩士學位學程

主題性統整報告

International Joint Degree Master`s Program in Agro-Biomedical Science in Food and Health (GIP-TRIAD)

National Taiwan University

Comprehensive Report

新穎的急性跟腱斷裂微創手術評估

Evaluation of a Novel Minimally Invasive Surgery for Acute Achilles Tendon Rupture: Tendon Healing and Biomechanical Properties

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中文摘要

急性跟腱斷裂好發於運動員及假日從事休閒運動的中年人。然而隨著年齡的增長，在跟腱斷裂的風險下，老年人的血液循環降低和組織沾黏的機會增加對於病患復原是一大挑戰。微創手術已成為一種趨勢。由於傷口較小，恢復和康復時間較短，因此患者可以在更短的時間內恢復原來的生活方式。透過國際三校學程的引薦，我與骨科醫師進行合作，開發新型跟腱微創手術，其中包含臨床上的應用及市場推廣。在與台灣，日本和法國的 10 多位骨科醫生進行訪談之後，大多數的醫師對於跟腱微創手術肌腱的生物力學特性及復原效果提出疑問。先前的大體研究僅能說明縫合方法的機械強度，而無法對肌腱癒合的過程和恢復後進行有效分析。因此，這份統整性報告將主要側重於新型超音波導引跟腱微創手術的介紹，以及將產品商化、創新創業實務的想法，包括市場規模，商業模型和市場策略。此外，將融合兩年碩士所學，提出有關促進肌腱癒合和生物力學性能測試的相應實驗設計。

關鍵字：急性跟腱斷裂、微創手術

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ABSTRACT

Acute Achilles tendon ruptures often occur in athletes and middle-age people involving in recreational sports. However, with the increase of aging, decreased blood flow and increased tissue stiffness may jeopardize elders under the risk of Achilles tendon rupture. Minimally invasive surgery has become a trend. With less wound size and shorter recovery and rehabilitation time, patients could return to the original lifestyle in a shorter period. With the support of GIP-TRIAD, I had a priceless opportunity to collaborate with Taiwanese orthopedic surgeon and develop a minimally invasive surgery for Achilles tendon rupture. However, after interviewing with more than 10 orthopedic surgeons among Taiwan, Japan, and France, most of them are concerning the biomechanical properties and the rehabilitation procedure regarding minimally invasive surgery. The previous Cadaver studies could only explain the mechanical strength of suture methods but couldn't validate the process of tendon healing and the post-recovery analysis. Therefore, this comprehensive report will be mainly focusing on introducing a novel ultrasound-guidance minimally invasive surgery for Achilles tendon rupture, the idea of creating a new business including the market size, business model, and the market strategy. In addition, a corresponding scientific pre-proposal regarding the acceleration of tendon healing and the biomechanical properties test will be displayed.

Key words: Acute Achilles Tendon Rupture, Minimally invasive surgery

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CONTENTS

doi:10.6342/NTU202003356

doi:10.6342/NTU202003356

LIST OF FIGURES

doi:10.6342/NTU202003356

Chapter 1 Introduction

Achilles Tendon is the largest and thickest tendon in the human's body which can hold up forces up to 9 kN, similar to 12.5 times of the body weight [1]. It is approximately 15 centimeters long and connects the gastrocnemius, soleus and plantaris to the calcaneus. In spite of its strength, acute Achilles tendon rupture is the most frequently sports-related injury occurred in middle-age recreational sports players. Several movements such as violent muscle contraction, direct trauma or long-standing tendonitis may lead to typical tendon rupture [2]. Other factors include aging, the usage of fluoroquinolone antibiotics, corticosteroids use, rheumatoid arthritis and gout [3]. Achilles tendon extracellular matrix have been observed to become weakened by the fluoroquinolone antibiotics, giving rise to less tensile tendon strength [3]. In addition, Corticosteroids, used to decrease tissue inflammation, also impede the formation of collagen and decrease blood supply to an already avascular structure [3]. In terms of aging, chronic degeneration of the tendon and failure of normal inhibitory mechanisms may occur during a person's life time. The Tensile strength of collagen decreases as the reduction of blood flow of the susceptible region with age [4]. While aging, tissue also increase stiffness to decrease the ability to withstand repetitive stress which in return allow forceful and sudden contractions to tear the tendon [5].

The incident number of Achilles tendon rupture vary a lot continentally. The reported incidence in the literature ranges from 6 to 37 per 100,000 person-years [6,7]. In Canada, from 2003 to 2013, the incidence of acute Achilles tendon rupture in Ontario increased from 18.0 to 29.3 per 100 ,000 person-years [8]. In Taiwan, according to the National Health Insurance research databases and the data we acquired from interviewing surgeons, 5,600 patients suffer from acute Achilles tendon rupture annually

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and the compound annual growth rate is approximately 5%. In terms of Japan and France, unfortunately, from the literatures and interview of local surgeons, there's no epidemiology research conducted regarding Achilles tendon rupture. The incident rate in Japan and France are remain unknown.

There are mainly 2 treatment options including surgical and conservative approaches. Compare to conservative methods, operative treatment could significantly reduce the risk of re-rupture rate to 7% [9]. Operative treatments include conventional open wound repair, percutaneous methods and minimally invasive surgery. During open surgery, a 10 to 15 cm incision is made in the back of leg and the Achilles tendon is stitched together with Krackow suture. Although open repair surgery could reduce the re-rupture rate, it may lead to a significant increase in other complications such as poor wound healing, flap necrosis infection, and sural nerve injury, with a reported risk up to 21% [9]. To solve the high complication problems, percutaneous and minimally invasive methods were introduced. These methods vary with more complex repair tools and suture-passing techniques. However, the recent researches indicate a range of 0–27% of sural neuritis after percutaneous procedures [10,11]. For example, the Achillon limited open repair device (Integra Life Sciences Holding Corporation, Plainsboro, NJ) and the Percutaneous Achilles Repair System (PARS) Arthrex Device will create a 3 to 5-centimeter incision to insert the jig system to clip the rupture tendon [11]. This critical procedure may harm the periosteal vessels and cause sural nerve damage during surgeries [12]. In addition, these techniques cannot assure that the sutures are well positioned at the middle portion of the tendon, especially for the farthest suture, which may cause the suture to cut through during knot tying.

A novel ultrasound-guided minimally invasive surgery was invented by Taiwanese Orthopedic surgeons who we are cooperating with [13]. Ultrasound has great

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advantages of no radiation, no tissue damage, and excellent soft tissue visualization. With high-frequency resolution and real time ultrasonography, intraoperative ultrasound could be of assistance during minimally invasive surgery to avoid damaging sural nerves [14]. Comparing to other percutaneous treatments, this novel technique could reduce the wound size from 5 centimeter to approximately 1 centimeter [13]. With less wound size, the complications including re-rupture, wound healing and skin necrosis are 0%. Moreover, this novel surgical treatment could shorten the recovery and rehabilitation time and will be beneficial for elder or diabetes patients with poor healing.

However, few surgeons are aware of this novel technique. During our interview with over 10 orthopedic surgeons from Taiwan, Japan and France, they have a common concern regarding biomechanical strength test and the healing procedure. Plenty of researches have been indicating the biomechanical outcomes and knot strength in the load to failure test of percutaneous surgery have no significantly different comparing with the open Krackow suture [15,16]. However, the minimally invasive repair techniques demonstrated an increased susceptibility to early repair elongation [16]. These are based on the cadaveric study. Further *in vivo* test of postoperative protection for biological healing plays an important role.

Plenty of patients are still suffering from the Achilles tendon repair complications such as re-rupture, infection and worse wound healing situation. Therefore, with the GIP-TRIAD training, I am extremely excited to discover this unmet need and contribute myself to promote this incredible technique. This comprehensive report includes what I have done during 2 years of study and how I combine all the knowledge to propose a scientific pre-proposal and business plan regarding Achilles tendon minimally invasive medical device.

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Chapter 2 Learning Achievements

2.1 Functions of transcription factor MafB in wound healing

2.1.1 Objectives

During the first semester in Japan, I was learning mouse experiments techniques in the Takahashi Sensei lab. What I have done is the training of taking care of mouse, wound healing experiments, FACS analysis and immunostaining.

2.1.2 Introduction

Wound healing is the healing of wounds. The body is equipped with a mechanism that repairs the wound as it is damaged. There are mainly three stages for the wound healing procedure including the inflammatory phase, the cell proliferative phase, and tissue regrowth and remodeling phase. Macrophages play an important role in the wound healing process which by function could be classified M1-type (classically activated macrophage) and M2-type (alternatively activated macrophage) [17]. Initially, M1 macrophage produce inflammatory cytokines during the inflammatory phase, attracting the neutrophils and monocytes and cause inflammation. Subsequently, the proliferative and mature stages, M2 macrophage produce anti-inflammatory cytokines to reduce inflammation. After that, angiogenesis and collagen production are promoted which gradually repairs the wound [18].

Tumor-associated macrophages (TAMs) are M2 type macrophages that can induce angiogenesis, fibrosis, and immunosuppression [19]. TAMs are known to promote cell

doi:10.6342/NTU202003356

proliferation and angiogenesis in tumors and promote cancer which indicate similar action of M2 macrophage in wound healing.

MafB is a transcription factor that regulates genes and is known to be expressed in macrophages, but the detailed function has not been clarified. In the Takahashi Sensei Lab, we are focusing on using LysM-Cre to generate Mafb-deficient mouse only in macrophages. The previous unpublished data showed that Mafb expressed in splenic macrophages and could suppress macrophage differentiation and regulate the number of TAMs supplied by spleen. Moreover, the previous study indicated the repair of wounds is delayed in the mouse from which the spleen has been removed, suggesting that spleen has some function related to wound healing [20]. Above all, these evidences support us to hypothesize that Mafb expressed in macrophages has some effect on wound healing via spleen.

2.1.3 Methods

Animals

Mafb gene GFP knock-in mice (Mafb heterozygous (Mafbgfp/+) and Mafb gene knock-out mice (Mafbf/f) with a C57BL/6J background used in this study. Mouse genotypes were assessed by a PCR on genomic DNA from tail tips. The genotyping primer sequences were reported previously [21].

Wound healing process

First, induce general anesthesia using 5% isoflurane in 100% oxygen (flow rate 1 L/min) and maintain anesthesia using 1-3% isoflurane. Secondly, shave the hair on the

doi:10.6342/NTU202003356

back of mouse with electric shaver. Third, create a five-millimeter wound with a sterile 5 mm biopsy punch on the back of the mouse. Measure the wound size at the 3, 5, 7, 10 days after operation

Flow cytometric analysis of cells isolated from wound tissue

After 3 and 5 days wound operation, wound tissues were harvested. The wound tissues were subsequently placed in PBS and cut into pieces with scissors. The dissected pieces were incubated at 37 °C for 30 min in RPMI medium (Sigma) containing 2% collagenase A (Nitta Gelatin Inc.) and 1% DNase Ⅰ (Roche). Next, separating the cells with gentle trituration and collect cells by passing through a 100-μm cell strainer (BD Bioscience) and immunostained with antibodies against the macrophage markers Mac-2 (e-Bioscience), CD11b (AbD Serotec), F4/80 (AbD Serotec). All cells were analyzed by BD LSR (BD Bioscience).

Immunostaining of wound tissues

Five-micrometer-thick paraffinized sections of wound tissues harvest at the 3 and 5 days after operation. Autoclaving the sections at 121 °C for 10 min in Tris-EDTA (TE) buffer, pH 9.0 to deparaffinize and retrieve antigens. Next, the sections were processed for immunohistochemistry using the rat anti-Macrophage 2 and rabbit anti-Arginine hydroxylase (Arginase I) antibodies. Blocking was performed with 10% donkey serum and 1% BSA in PBST for 30 min at 4 °C, followed by primary antibody incubation (1:400 dilution) in the blocking serum. Finally, the tissue sections were probed with secondary antibodies Alexa fluor 488 donkey anti-rabbit and Alexa fluro 647 donkey anti-rat for 1 hr at nature temperature before visualization.

doi:10.6342/NTU202003356

Statistical analysis

All results are expressed as the means ± S.E.M. Significant differences between two groups were analyzed using Student's t-test. Differences were considered statistically significant at $P < 0.05$.

2.1.4 Results

Wound healing process was delayed in CKO mouse

Wounds were made on the backs of Control and Conditional Knock out mouse (CKO), and the size of the wound was photographed with time on Days 0, 3, 5, 7, and 10. The area of the wound site was measured (figure 1A), and the percentage of the size of the wound was compared with the area on Day 0 (figure 1B). The results showed that wound healing was slower in CKO than in Control throughout, and wound repair was significantly delayed on Day 3 and Day 10.

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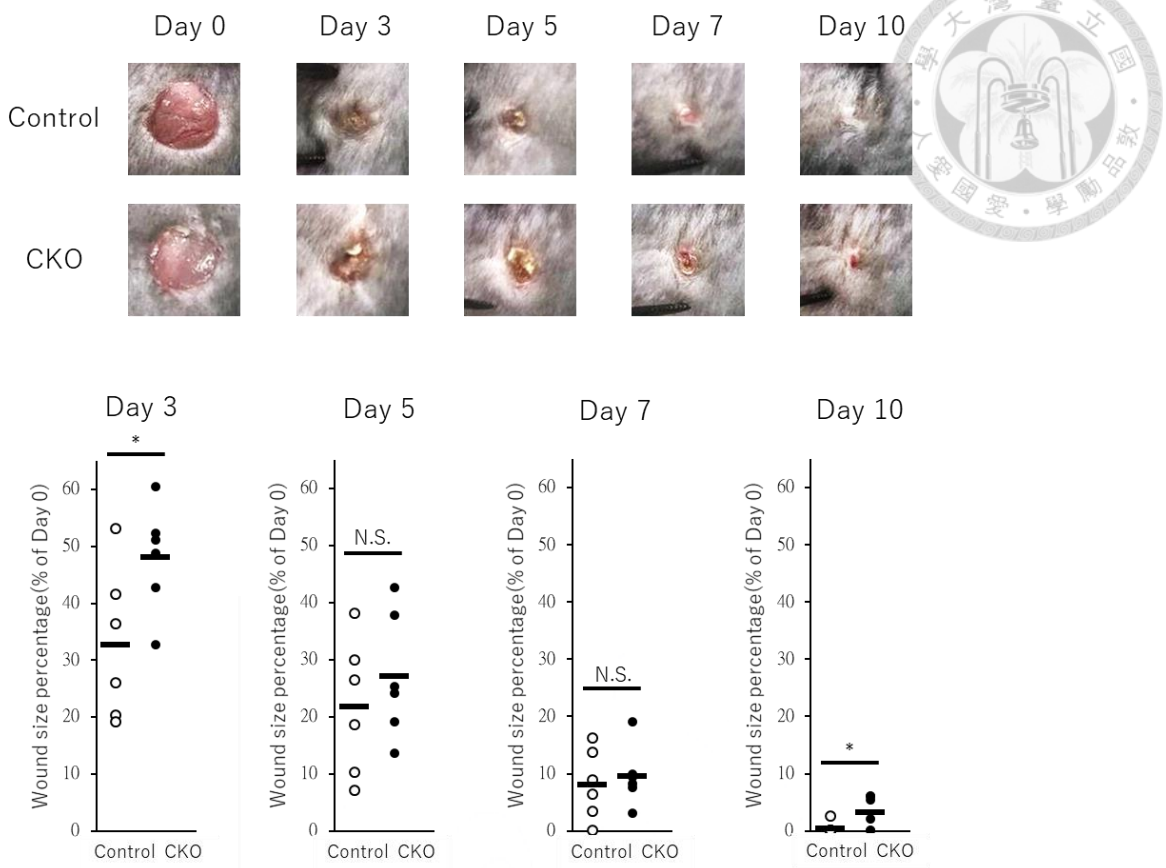

Figure 1. The photo of wound size in Day 0, 3,5, 7.10 after operation and the wound size percentage compare with the wound area of Day 0. （＊：$p<0.05$、 N.S.= not significant (Student's t-test) ）

MafB is expressed in Macrophage at wound site

FACS analysis was performed using single cell suspension of the wound tissue to determine the type of macrophage that exhibits GFP fluorescence. The expression of CD11b, F4/80, and GFP was analyzed following gating of the viable cells. Among all macrophage markers, cells co-expressed with GFP were confirmed. The results indicated that MafB-expressing macrophages 2 were present at the wound site (Figure 2).

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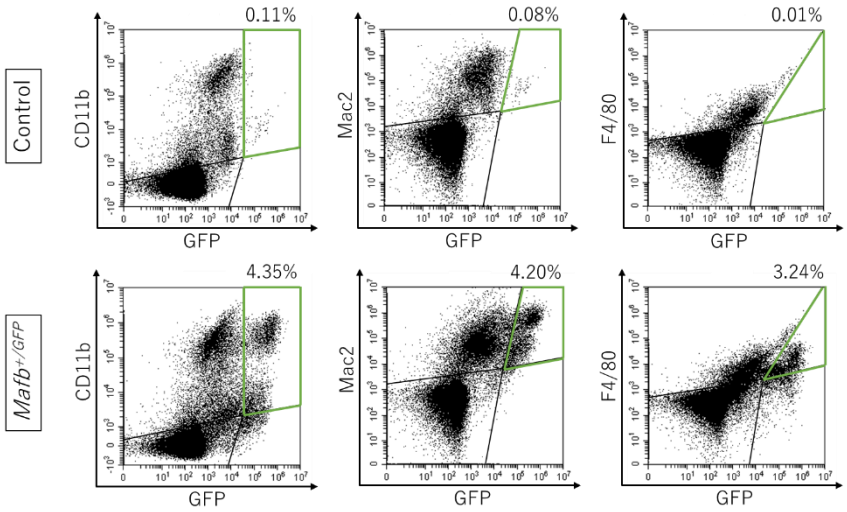

Figure 2. Results of FACS analysis of wound tissue. Macrophage-related Marker CD11b, Mac 2,F4/80 were used. The results indicated that MafB-expressing macrophages were present in the wound.

Arginase I is expressed with GFP, Macrophage2

Arginase I (ArgI) is a ureohydrolase enzyme which will compete with nitric oxide synthase (NOS) for their common substrate L-arginine. In the previous study showed that knocking out ArgI will slow down the process of wound repair, suggesting that differential expression of ArgI is important in wound healing. [22]. A 2017 Scientific Report indicates that MafB directly regulates ArgI transcription *in vitro* and induces it into M2 macrophages [23]. Therefore, immunostaining of GFP and ArgI at the wound of Mafb $^{+/GFP}$ mouse at Day 3 was performed. The results indicated ArgI-positive cells co-express with GFP and Mac2 at the wound site (Figure 3).

doi:10.6342/NTU202003356

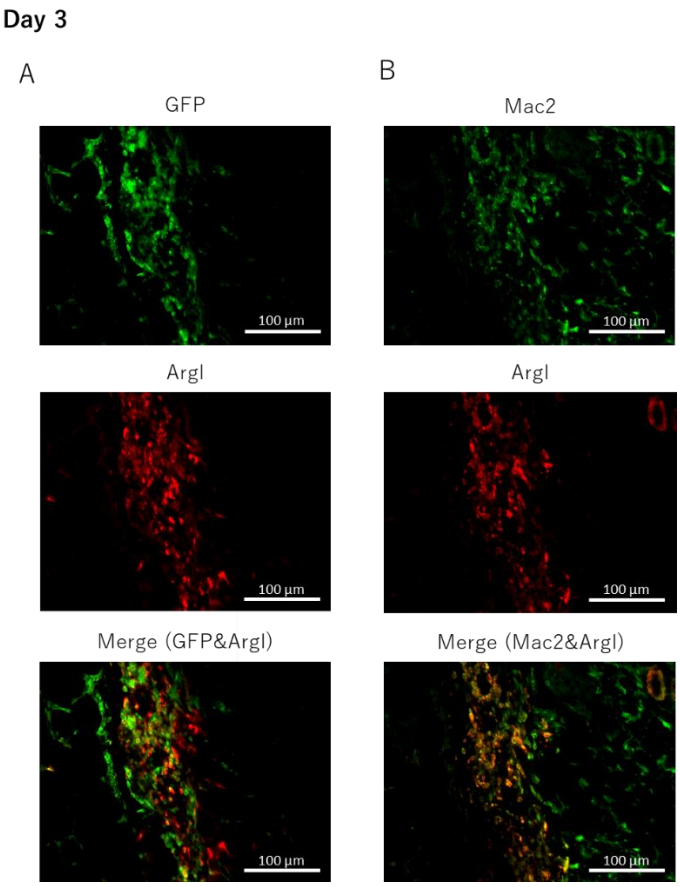

Figure 3. Immunostaining results of ArgI-positive cells at wound site, suggesting they are co-expressed with GFP(Mafb) and Mac2.

2.1.5 Discussion

I was studying microbiology during bachelor degree and had no experience about animal experiments before. During the first semester in Japan, it was a valuable opportunity for me to learn everything new including handling the little mouse, anesthesia methods, FACS analysis and immunostaining. These concrete training support me to think logically on every details of experiment procedure, and broaden my knowledge regarding wound healing and Mafb transcription factor.

doi:10.6342/NTU202003356

2.2 Cyclic economic of Cordyceps militaris

Cordyceps militaris has been used extensively as traditional Chinese herbs and a folk tonic food in Asia. It contains many kinds of active components including cordycepin, polysaccharides, ergosterol and mannitol. Due to multiple physiological activities, *Cordyceps militaris* is now used for multiple medical purposes. The main constituent of C. *militaris* is cordycepin (3′-deoxyadenosine) which is a nucleoside analogue. Due it's similarity to adenosine, some polymerase cannot discriminate between the two. Cordycepin therefore can participate in certain biomechanical reactions such as incorporation into mRNA molecule and leads to the termination of protein synthesis [24]. The previous studies indicated that cordycepin itself acts as an anti-tumour, anti-proliferative, anti-metastatic, insecticidal and anti-bacterial compound [25,26]. This is the point of view based on academic research.

From the industry aspect, *Cordyceps militaris* is cultured by biotechnology company under the sterile rice-based medium and controlled environment. After 55 days of growth until Cordyceps matured, the biotechnology company used 50-degree water to extract cordycepin. About 30% Cordycepin remains in the sporocarp and can be extracted by chemical methods, but it does not meet the cost and economic benefits. Usually, the company will discard the residue after extraction. However, the residue contains plenty of polysaccharides, peptides, ergot alcohol, nucleotides and other components. It's a pity to abandon these valueless residues. Therefore, in Professor TK's lab, I was doing research on some alternative benefits of these *Cordyceps* remaining and try to build up a circulation economy.

doi:10.6342/NTU202003356

2.3 Business development of a novel ultrasound guided minimally invasive medical device

2.3.1 Objective

During the training of GIP-TRIAD, we absorbed lots of entrepreneurship knowledge and experience from every professor. Moreover, the off-campus field trip visits brought us different aspects including business model, technology, regulations and how to become a successful company. The culture and lifestyle are various between Japan, Taiwan and France. Therefore, these valueless experiences have truly broadened my entrepreneurship horizon. During the internship in Taiwan, I teamed up with Ben and a Taiwanese orthopedic surgeon who already developed a novel minimally invasive surgery for tendon rupture. Our team, TendMIN, was accepted to several accelerators to learned more knowledge regarding finance, regulations, intellectual properties, pitching and business development. The objective of this study is to propose a business plan.

2.3.2 Unmet Need

The traditional surgery for Achilles tendon rupture conducts a 10~15 cm open-wound surgery. This may lead to several complications such as deep infection and wound necrosis (15%). Moreover, the recovery and rehabilitation time will be extended up to 6 months. TendMIN developed an ultrasound-guided minimally invasive surgery Kit for Achilles tendon rupture which could reduce infection rates and wound break down [13]. In addition, successfully avoid the iatrogenic nerve injury. We are continuously testing this technique with over 100 cases of clinical trials. This device acquired a 0 % infection rate and shorten the recovery and rehabilitation time to approximately 4 months.

Mainly 3 groups of people will benefit from this technology. Surgeons, patients

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and hospitals. Patients will have a shorter recovery time leading to less Lost Workday Case (LWDC). Low infection and complication rate will release the pressure on doctors and strengthen the doctor-patient relationship. Last but not least, this technology will reduce the waste of medical resources which could be efficiently implemented on those who really needed.

We have interviewed 10+ orthopedic surgeons from Taiwan, Japan and France, most of them indicated that complications from the surgery is mainly a great concern for surgeons. They are highly interested and willing to understand more about our technology.

2.3.3 Our Solution

TendMIN commits to develop a mini-invasive medical device kit for Achilles tendon rupture. The kit is designed for disposable including, specialized suture hook, needle, thread (Ethibond size 2 polyester suture, non-absorbable), thread-carrier, hemostatic forceps. With our unique medical devices and techniques, we can profoundly shorten the wound size to 0.8~1 cm and abridge the recovery time (4 month) with zero infection rate (0%). Our technology is different from other percutaneous methods which usually have a concern of iatrogenic nerve injury. In addition, ultrasound guidance could precisely locate the rupture side and avoid offset suture. This medical device kit has multiple intended use which could be also implemented in rotator cuff tear.

2.3.4 Market Size

The " Orthopedic Soft Tissue Repair Market " in 2020 is estimated to reach 7.35 billion U.S. dollars. With the promotion of health policies and raising awareness of sports, the population of sports-related people has expanded rapidly. However, this may lead to the increase in the cases of sports-associated injuries across prime age-group.

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The Achilles tendon is the thickest and strongest tendon in human body, but acute ruptures are frequent in young athletes and middle-aged subjects who join recreational activities. It is estimated, the incidence rate ranges from 7 to 40 per 100 thousand people per year.

The market size of Taiwan is estimated 560 million USD. We calculated by the annual patients' number (5,600 people/year) multiple estimated surgical fee (1,000 USD). Taiwan is a relatively small market, so the main market we are focusing is United states which is estimated to have 110 million USD with 64,000 cases annually.

2.3.5 Business Model

TendMIN designed a disposable Kit for Achilles tendon rupture. To promote our products, TendMIN will join orthopedic seminar, hold cadaver courses and set up meeting with potential users. Then, our team plan to sell them directly to hospitals for intended usage. In terms of seminar, our advisor, Dr. Wang Chung-Li who specializes in orthopedics can support our research during the seminar. In terms of meeting, at least 5 surgeons in Tsu-chi hospitals system already approached to us and learned the techniques. Additionally, we are actively contacting surgeons from NTU and TMU. We estimated nearly 200 kits will be sold to Tsu-chi, NTU and TMU hospitals per year.

Simultaneously, we are seeking the opportunity to get involved in the US medical device market. In order to negotiate with the insurance company for reimbursement, we are accumulating more clinical data and seeking to collaborate with US medical center.

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2.3.6 Competitor Analysis

There are mainly 3 competitors, Aineng Biotech, Arthrex (PARS jig system) and Integra Life Science (Achillon device) (Figure 4). All of the devices are designed as jig-guide system to insert jig into the ruptured tendon and guide the suture. The jig system may have opportunity to cause sural nerve damage [14]. Moreover, the incision size is about 3 to 5 centimeters. Over all, our device will create a smaller wound size and less complications.

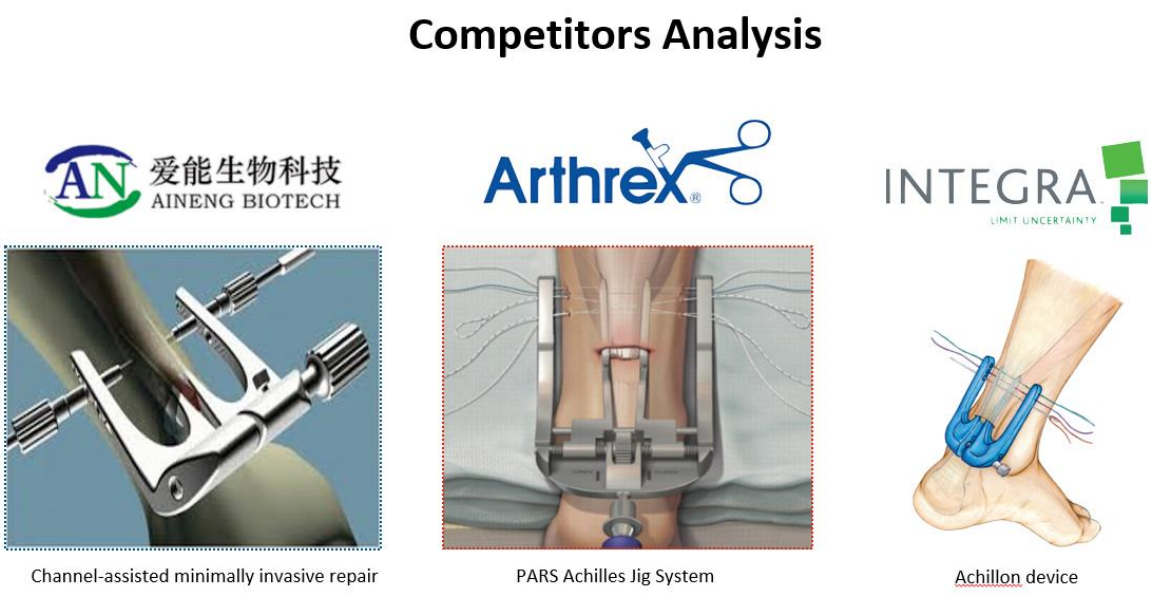

Figure 4 Current Competitors of minimally invasive Achilles tendon repair.

2.3.7 Regulations

Although our specialized hook has less contact with skin and tendon, the device should be sterilized and packaged before selling it. Therefore, by consulting the regulation expert in Taiwan, our specialized design hook is categorized into Class II medical device. However, the final classification should be confirmed from the FDA.

doi:10.6342/NTU202003356

2.3.8 Team

Here are mainly 3 founders in TendMIN. Dr. Wang is an orthopedic surgeon at Tsu-chi hospital who has a PhD on biomedical engineering and works on clinical trials and research development. Ben Huang and I are responsible for market investigation, strategic planning and business development.

2.3.9 Fund Raising Goal

At the beginning of building up the company, the fund will be out of our own pockets with initially 1 million NTD (currently discussing). Simultaneously, we plan to apply for the Entrepreneurship Grant from the Taipei government which is around 0.5 to 1 million NTD. This limited fund will be used for prototyping, patent apply (USA provisional application and Taiwan patent), TFDA regulation and company operation. After that, we are seeking for angel fund raising with 0.9 million USD (Figure 5). We are expecting to develop Taiwan's market to collect more feedbacks from Orthopedic surgeons and accumulate clinical data. The usage of funding is shown in figure 5.

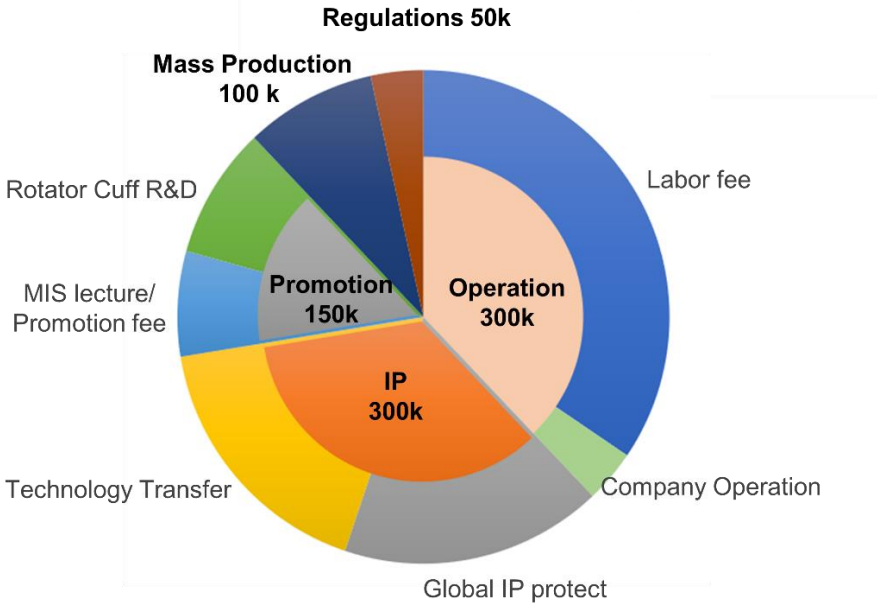

Figure 5. Angel Fund raising 0.9 million USD and the usage details

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2.3.10 Timeline and Financial Forcast

The milestone and financial forecast are shown in figure 6. In 2020, we are prototyping and preparing to apply for TFDA approval. The company will be established in 2021 and expect to acquire TFDA approval. Meanwhile, we will initiate Taiwan's market, apply FDA and start the rotator cuff experiments. We estimate to get FDA approval and initiate US market in 2022. We are strongly confident to break even in early 2024 (Figure 6).

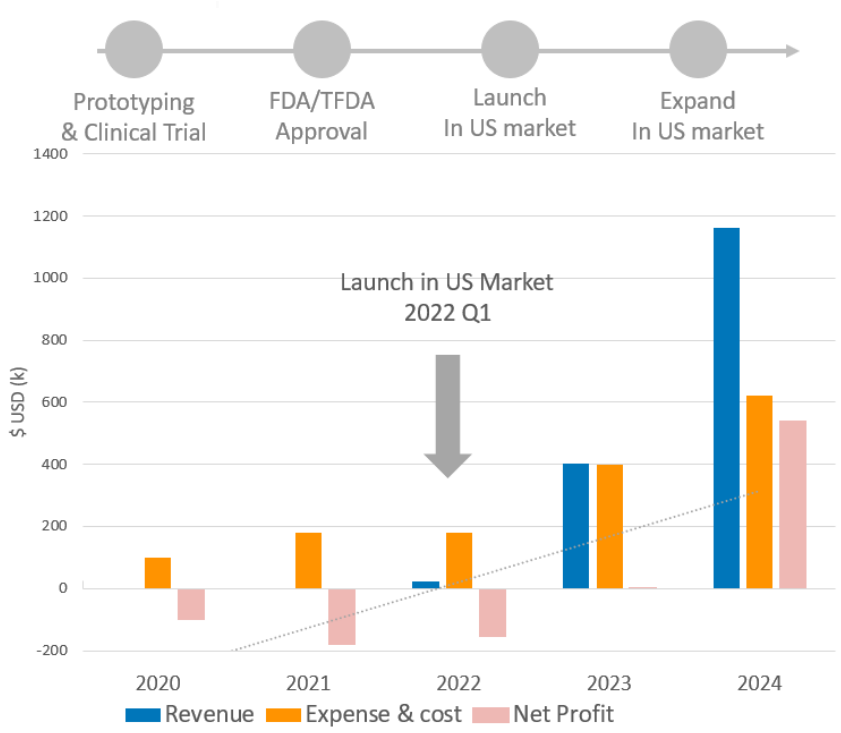

Figure 6. Financial forecast and Timeline

2.4 Conclusion

Through 2 years of GIP-TRIAD training, I had learned animal experiments techniques, the properties and biomedical effects of *Cordyceps militaris*, and the implementation of entrepreneurship. Moreover, to identify the unmet need in any kind of aspects and to effectively utilize the surrounded resources. How to communicate with

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other people and persuasive them to join your project will always be a good training and learning lesson. I am really grateful to have this opportunity to hone myself with not only the scientific knowledge but business-related logical thinking training.

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Chapter 3 Content of Implementation

Through this comprehensive report, the infection and re-rupture are highly concerned by surgeons. These complications are strongly linked with wound and tendon healing respectively. *Cordyceps militaris* is full of functional chemicals including cordycepin, polysaccharides and peptides.

Previous study showed that cordycepin down-regulate the gene expression of Macrophage 1 cytokines (IL-1β, TNF-α) and chemokines and could up-regulate the Macrophage 2 cytokines [27]. Meaning that cordycepin has anti-inflammatory function. In addition, cordycepin has anti-bacterial function [26]. Another literature using a complex physical hydrogel dressing of cordycepin and chitosan that indicated the effect on the wound healing [28]. Above all, *Cordyceps militaris* has so many beneficial compounds such as cordycepin and polysaccharides may be a great candidate for both wound and tendon healing.

Achilles tendon healing mechanism is similar to wound healing, yet the healing time was longer. Tendon after surgical repair generally progresses through a short inflammatory phase, which lasts about a week, followed by a proliferative phase, which lasts a few weeks, followed by a remodeling phase, which lasts many months.

3.1 Application

When people suffer from Achilles tendon rupture, patients can repair it by a novel ultrasound- guided minimally invasive surgery with only 0.8 to 1 centimeter incision. Besides, by intaking cordycepin functional food and apply the hydrogel comprised of cordycepin and polysaccharides on the wound site, patient's healing mechanism will be stimulated. Moreover, patients will have the least chance to get

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infection or re-rupture, giving them a better and shorter post-operative rehabilitation time so that they can go back to their original lifestyle in a shorter time. In terms of cordyceps biotechnology company, they will no longer discard the residue after only extracting cordycepin. They can keep the residue and process them into hydroxyl gels as mentioned above. The circulation economic will not only reduce the waste but could create a multiple win-win between patients, surgeons and the whole community.

3.2 Scientific Aspect

Animal model of Achilles tendon healing with intake and smear of cordyceps product could be conducted. In the molecular level, couple of tendon healing growth factor such as expression of platelet derived growth factor (PDGF-BB), basic fibroblast growth factor (bFGF), transforming growth factor β (TGF-β), and vascular endothelial growth factor (VEGF) or the role of macrophage could be tested. In terms of physiological and histology, immunostaining is a great approach to see the healing condition. Last but not least, harvest the tendons to test the biomechanical strength to see whether the healing effect of cordyceps products will increase the durability of tendon or not.

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