REVIEW ARTICLE

Regenerative Medicine as a Potential and Future Intervention for Ankle Sprain

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ABSTRACT

Ankle sprain is one of the most common injuries associated with physical activities. Complications including pain and ankle instability are associated with decreased physical activity, reduced sport performance, and increased risk of recurrent ankle injury leading to detrimental effect on activities of daily living. Current management of ankle sprain can be conservative or surgical for serious cases. However, long healing period is required for conservative management in addition to its side effects and the risk of post-operative complications for surgical management. Due to the current challenges and setbacks faced by existing intervention, this paper aims to generate ideas in incorporating regenerative medicine as an intervention for ankle sprain. This review will provide a brief review on the existing management for ankle sprain along with some history, application and the potential of regenerative medicine in speeding up the healing process of ankle sprains.

Keywords: Regenerative medicine, Sport injury, Ankle sprain

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INTRODUCTION

Ankle sprain is one of the most common sports injuries associated with physical activities. Its complications includes decrease physical activity and quality of life (1). The decrease in physical activity and its effects on the quality of life has been proven by both human and animal studies. A study on mice has proven that ankle sprain significantly decreases physical activity across the lifespan in mice which have an impact on the development of numerous chronic diseases (2). This is due to ankle sprain reduces the distance, duration and the running speed of the ankle sprain induced mice (2). Consequently, ankle sprain leads to pain, sensorimotor deficit and ultimately chronic ankle instability which increases the risk of recurrent ankle sprain and imposes an impact on the quality of life (3,4). Studies involving humans with chronic ankle instability reported a reduction in physical activity which is secondary to functional limitations (5). The reduction of physical activity contributes to increase risk of obesity, and cardiovascular disease which in turn increases the non-communicable diseases mortality rate worldwide (6,7). Although the cost for managing isolated ankle sprain includes the follow up care and injury-associate time loss are relatively low, the treatment of ankle sprain in combination with its complications and prolong recovery time frame is a burden to the healthcare sector (1,8). Also, athletes suffering from ankle sprains may have reduced sports performance secondary to chronic ankle instability and prolonged time back to training which could be a major loss for the sports community. In addition to that, ankle sprain requires a relatively long time frame for healing depending on its grade and severity (8). Generally, mechanical stability of the ankle joint occurs only at six weeks to three months after ankle sprain, however moderate percentage of participants experience objective mechanical laxity and subjective ankle instability which last up to one year post ankle sprain. Mechanical laxity of the ankle is define as excessive range of motion of the ankle joint beyond its physiological range which altered the ligamentous tissue and arthrokinematics (9). In view of its complications, slow recovery and high management cost, this paper aims to review the current management and propose the potential used of regenerative medicine in managing ankle sprain.

CURRENT MANAGEMENT FOR ANKLE SPRAIN

Current management of ankle sprain is usually conservative however, surgery is also performed in much severe cases (10). Grade I, II and III ankle sprain can be managed with conservative methods includes the use of ankle brace, neuromuscular and balance training (Table I). In 2018, experts had come to a consensus where ankle sprain can be managed by Rest, Ice, Compression and Elevation (RICE), non-steroidal anti-inflammatory drugs (NSAID), immobilisation, functional support such as the use of ankle brace, exercise, surgery and other therapies which includes physiotherapy modalities and acupuncture (11). However, the time required for healing is still relatively long in addition to the post-op complications such as infection to the wound site and joint stiffness for patients whose ankle sprain were managed surgically.

Table I: Grading of Ankle Sprain

Grades	Description	Signs and Symptoms
Grade 1	• Slight stretch of the ligament fibres • Microscopic tear of the ligament	• Mild tenderness • Swelling
Grade 2	• Partial tearing of the ligament	• Moderate tenderness • Swelling
Grade 3	• Complete tear of the ligament	• Significant tenderness • Swelling • Ankle instability

In order to understand and develop interventions in managing ankle sprain, the knowledge of ligament healing process is crucial. The healing of the sprained ligaments involves three phases namely the acute inflammatory phase, reparative phase and remodelling phase (12). The acute inflammatory phase is an important phase where the permeability of the capillaries surrounding the injured ligament increases resulting in migration of the plasma proteins and leukocytes leading to swelling. This phase also serve as a protective phase to prevent infections and clearance of debris around the injured tissues. During the reparative phase, fibroblastic cells begins to migrate and produce collagen and proteoglycans. Finally at the remodelling phase collagen fibres begin to align themselves longitudinally in a cross linkage form which is similar to the uninjured ligament, and the pre-injured strength of the ligament can be regained within three months.

RICE procedure in managing ankle sprains has been practised in the past. However, a systematic review published in 2012 reported insufficient evidence available from randomized controlled trails regarding its effectiveness in treating acute ankle sprain (13). Similarly, a recent consensus statement by experts also reported where the effectiveness of RICE management for ankle sprain has not been vigorously investigated and the efficacy of such combination is questionable (11). Cryotherapy or ice is an oldest and simplest therapeutic model used in the treating ligament injuries by decreasing tissue temperature leading to diminish in pain and metabolism, minimizing inflammatory process and aids in ligament healing after trauma (14). However, there is no indication where cryotherapy improves function. Also, the reduction in pain during cryotherapy could be via pain gate mechanism. In addition to that since RICE reduces swelling and minimized inflammation while some inflammation is also required for healing process, its role in aiding recovery is questionable.

NSAIDs are commonly prescribed for ankle sprains individuals to relieve pain. However, early administration of NSAIDs at the event of acute ankle sprain may affect its healing due to its inflammation suppression effect (15). This could be explained that administration of NSAIDs during the proliferative phase of healing on the other hand may results in increase scar formation due to its inhibitory effect on Prostaglandin E2 (PGE2) (16). In addition to that, studies had reported where NSAID interferes with the normal process of extracellular matrix remodelling and the cellular control of inflammatory and wound healing gene expression which delay the healing responses (17). This concept is also supported by recent study conducted by Adam et al. (2018).

Although immobilisation of the ankle joint can be an added value in managing ankle sprain by decreasing pain and oedema, prolonged immobilisation with delayed commencement of functional treatment may results in loss of muscle strength and endurance, soft tissue changes, joint stiffness, soft tissue changes, degenerative joint disease, muscle tightness and even contractures leading to reduced ankle range of motion (19). Also based on personal observations, there are patients who are not very receptive to the idea of wearing a plaster cast or rigid support for ankle immobilisation or ankle brace for functional support to the sprained ankle due to aesthetic purpose (personal communication, November 26, 2012). Besides, there are patients who want the easy way out and not very committed to the exercise regime prescribed by the physiotherapist making current available intervention in treating ankle sprain a challenge (personal communication, July 24, 2012).

REGENERATIVE MEDICINE AS POTENTIAL INTERVENTION FOR ANKLE SPRAIN

Due to the challenges and setbacks faced by current intervention in managing ankle sprains and in view of the development and advancement in regenerative medicine, various modern intervention had been proposed and researched on. Hence, future therapy should focus more on a more personalized and holistic approach while reducing the time require for each phase of healing without compromising the physiological mechanism of healing process. Regenerative medicine

utilises knowledge based on a combination of molecular biology, biochemistry and biomechanics to replace, engineer or regenerate human cells, tissues or organs to restore normal function offers a new potential and better treatment in managing ankle sprain (20). Thus, the healing process of ankle sprain is relatively slow in addition to its long term residual symptoms, while existing methods such as RICE procedure and NSAID mainly focuses on the reducing inflammation, pain, and odema in addition to the side effects arises from NSAID, immobilisation and surgical management (21). Regenerative medicine on the other hand have the potential to restore and replace the injured tissues through paracrine effects or differentiation of the injected cells into specialized cells within the injured tissues (22).

Preliminary studies has proven regenerative medicine to be successful in treating various conditions ranging from neurological to musculoskeletal disorders (23–25). Despite many studies identified uses regenerative medicine approach in musculoskeletal disorders, the common focus is in tendon injuries, osteoarthritis and articular cartilage injuries (26). As for ligament disorders such studies are limited to injuries involving the knee ligaments commonly the anterior cruciate ligament (ACL), medial collateral ligaments (MCL), lateral collateral ligament (LCL) and posterior cruciate ligaments (PCL), none of these were done specifically on the ankle ligaments (27–33). The approach for regenerative medicine used for these studies includes cellular therapy where direct injection of stem cells locally on the injured area or systemically, tissue engineering which utilized biological scaffolding and implantation on the injured site and the combination of growth factors with stem cells. As many of the identified studies using regenerative medicine on ligament and tendon healing did not incorporate exercise therapy and mechanical loading in their treatment model, we believed that this standalone intervention could also speed up the healing process of the sprained ligaments (27–29,34–36). However, the effectiveness of this therapy may improve when integrated with exercise regime, speeding up the healing process of the sprained ligament. Empirical studies has reported mechanical loading which can be achieved through exercise may induce extracellular matrix secretion such as collagen by fibroblastic and stem cells (37–41).

CELLULAR THERAPY IN MANAGING ANKLE SPRAIN

Cellular therapy involves the transplantation of cells to the injured area to repair or replace the damaged tissues or cells. Hence, the challenges faced when using this techniques includes the cell source used for the transplantation (27,42), route of cell delivery (43,44), optimal dosage, and safety and effectiveness of the intervention (43). Besides that, although there are a variety of studies relating to the delivery of stem cells via cellular therapy approach, these studies merely provide preclinical evidence while there is limited clinical evidence in this area (45). Also, the ligaments used in such studies are mostly the knee and periodontal ligaments and none of these studies identified are conducted on the ankle ligaments which is commonly involved in the event of ankle sprain.

In addition, there are also articles which generalised the intervention of cellular therapy to both ligaments and tendon (46). Although in general, ligaments and tendons are elastic collagenous tissues that contributes to joint movement besides having similar molecular, cellular and hierarchal structure, the composition of ligaments differs from tendon (20,47). Ligaments composed primarily of water that made up most of its wet weight (~65% to 70%). Its dry weight mainly consist of collagens (~70% to 80%) and type I collagen is the most abundant among other subtypes of collagen, includes type III collagen (3%), type V collagen (12%) and other minor subtypes includes collagen II, IX, X, XI and XII. Tendons on the other hand consists slightly higher type I collagen (~85% dry weight) and less water (~55% wet weight) as compare to ligaments. Despite that, the composition and cell types between different types of ligaments in the body differs depending on their location, function and involvement in weight bearing. This is supported by the study conducted by Zhang and colleagues (2011) which explained the different healing capacity between the ACL and MCL. This is due to the difference of cells presence in both ligaments in which ACL stem cells formed fewer colonies, smaller in size and grow slower as compare to MCL stem cells in vitro. Such differences are explored when the authors extract both the cells from both the ACL and MCL and were cultured in DMEM supplemented with 20% FBS. Although both ACL and MCL stem cells may differentiate into adipocytes, chondrocytes and osteocytes in induction medium, the authors reported significant difference in adipogenesis, chondrogenesis and osteogenesis between these stem cells. As such for in vivo studies, the difference in the cellular properties could also be influenced by the components of the extracellular environment such as the presence of certain growth factors and mechanical stress which depends on the location of the ligaments (30,31,48). However, these studies are predominantly done on animals and findings are being observed by anatomical observations, tissue processing and other laboratory methods. Therefore, although no studies were conducted on the cells of the ankle ligaments, it is believed that the ankle ligaments have different cellular properties and components as compared to the other ligaments which poses a challenge for cellular therapy to treat the injured ligaments to its pre-injured state. However, if the cells were injected in vivo, the in vivo extracellular environment will tune the cells through paracrine effects in promoting healing of the injured ankle ligaments.

TISSUE ENGINEERING IN MANAGING ANKLE SPRAIN

Tissue engineering involves the use of a combination of cells along with a suitable bio-materials which serve as a scaffold for cell seeding to create a new viable tissue to replace the injured tissues. Hence, the challenges faced when using such approach includes the cell source, low survival of implanted cells, and poor revascularization of implanted tissues or organs (49). In addition to that, the properties and bio-scaffold used must be taken into consideration in creating the ankle ligaments (50). As an example, collagen-based scaffold is believed to have low tensile strength and not able to endure fixation with suture. Also, the implantation of the newly created ligaments to replace the injured ligaments requires surgical intervention and therefore poses the risk of post-operative complications.

Despite all those challenges, researchers since the pass two decades had conducted studies on different types of scaffolds such as fabricated acellular collagen scaffolds and synthetic biodegradable polymer fiber scaffolds and reported positive results of these scaffolds (51). A study conducted by Lin et al., (1999) in creating ligament tissues in vitro seeded ACL and MCL cells onto a synthetic biodegradable polymer fiber scaffolds reported the formation of single bundle of ligament tissue bundle by week 5. Similarly, another study by Sahoo et al., (2006) which seeded cells onto a knitted poly(lactide-co-glycolide) (PLGA) scaffolds also reported positive results which is supported by Pan and Ding, (2012). Recent study by Pag6n and colleagues (2019) reported silkworm gut fiber braids to demonstrate excellent biocompatibility in vitro when used as a biomaterials. Such materials promotes adhesion and proliferation of bone marrow mesenchymal stem cells and fibroblasts. However, further in vivo study is required to elucidate all these positive effects. Also, the revascularization of these tissues when implanted in vivo is still unknown.

THE RECOMMENDED TYPES OF CELLS, ITS HISTORY AND MARKERS FOR CELLULAR THERAPY AND TISSUE ENGINEERING

As one of the challenges faced by both cellular therapy and tissue engineering is the cell source and types of cells to be used in achieving the desirable outcomes, several cells types had been identified from previous studies which proves beneficial in ligament healing. These cells include induce pluripotent stem cells (iPSC), embryonic stem cells (ESC) and mesenchymal stem cells derived from different sources such as umbilical cord, adipose tissues and bone marrow. However, the used of iPSC and ESC in cellular therapy face the clinical hurdles of potential tumorigenesis and immunorejection (56). To date, studies on iPSC in reducing the risk of potential tumorigenesis were still on-going on the in vitro phase.
Mesenchymal stem cells (MSC) is one of the most commonly used cells in regenerative medicine due to its self-renewal and multipotent properties (57). These cells was first discovered by Friedenstein, Chailakhjan, & Lalykina (1970) through the presence of fibroblasts in bone and spleen which have the capability to be induced into osteogenesis depending on the location where the cells are obtained.

In the 1990s, a study conducted by Bucala and colleagues (1994) reported where such cells were also presence and circulating in blood which plays an important role in soft tissue healing and contributes to scar formation. As MSC begins to gain popularity in research, different terminology had been used to describe such cells includes mesenchymal stromal cells, fibroblasts, fibrocytes etc. In the past decade, The International Society for Cellular Therapy (ISCT) had defined the criteria for MSC, 1) Plastic adherent when maintained in standard culture conditions, 2) ≥95% of the population must express CD105, CD73 and CD90 while ≤2% of the population expresses CD45, CD34, CD14 and HLA class II as measured by flow cytometry, 3) They must be able to differentiate to osteoblasts, adipocytes and chondroblasts under standard conditions (60). Hence, with such properties described by the ISCT, MSC can be used to induce ligament healing. Empirical studies had reported the expression of CD105, CD73 and CD90 had been proven to improve healing of various soft tissues (61,62).

Despite all that, the choice of the selected MSC do plays an important role in the prognosis of ligament healing. Umbilical cord mesenchymal stem cells (UC-MSC) has been reported to have higher proliferative and differentiation potential as compare to MSC obtained from other sources including those derived from bone marrow, other postnatal and neonatal sources (56,63). In addition, UC-MSC can be harvested painlessly in abundance during child birth without additional invasive procedure, possess stemness properties that last several passages in vitro, multipotent, hypoimmunogenic and do not induce tumorigenesis (56). However, using UC-MSC can be a challenge for those who did not bank their umbilical cord. Although such challenged can be overcome by using other tissue derived MSCs such as bone marrow derived mesenchymal stem cells (BM-MSC) and adipose tissue derived stem cells (A-MSC). However, obtaining such cells requires an invasive procedure which is painful and uncomfortable if done under local anaesthetic, also it tends to increase the risk of infection and the cost of hospital stay and prophylaxis antibiotics (64,65). Another concern about using BM-MSC, A-MSC and MSC not related to birth associated tissues is that it's efficacy to the availability, condition, and age of the donor tissue (64). As an example, cells obtained from younger donor tissues will have higher proliferative potential and are less susceptible to oxidative stress (66). Thus, UC-MSC is still the best choice of cells used for cellular therapy and tissue engineering for

ligament healing. To increase the healing effect of UC-MSC, such cells should be tune to express CD34 markers and matrix metalloproteinase (MMP) which can be done through the use of growth factors, isolation methods and transfection. In addition, to the self-renewal and multipotency of the selected cells, revascularisation is one of the important element required in healing tissues as constant blood supply is required to support ligament healing (67). However, according to the ISCT guidelines, ≤2% of the MSC population expresses CD34+. However such cells can be isolated using various method such as immunomagnetic cell separation and cell sorter (68–70). Suggesting that, the MSC used in cellular therapy and tissue engineering should be CD34+ cells as such cells poses better migration rate to injury site, secrete greater amount of collagen and increases vascularization and vasculogenesis when induce to the ACL reconstruction sites (71). The authors also reported where the biomechanical tensile strength of the reconstructed ACL at 8 weeks post-injection of CD34+ were significantly higher compare to those CD34- and non-sorted cells. Similarly Jiang et al. (2015) also reported where addition of CD34+ MSC produced a greater effect by increasing collagen secretion, collagen fibre alignment score, vascularization as well as failure load when compare MCL derived MSC alone.

In addition, the selected cells for regenerative medicine should also express or should be tune to express Matrix metalloproteinase (MMP). MMP is a group of calcium-dependent zinc-containing enzymes that plays a pivotal role in the regulation of extracellular matrix and hence is important in ligament healing (72). According to the authors, these enzymes is divided into seven groups namely collagenases, gelatinases, stromelysins, matrilysins, metalloelastases, membrane-type MMPs, and other MMPs and each plays a different role in soft tissue healing. Studies had reported that the increase expression of MMP-1 (73) while inhibition of MMP-3 and MMP-13 (29–31) is associated with ligament healing. MMP-1 is a collagenase functions to promote cellular migration and its expression is induced when the cells are in contact with collagen I and its downregulation is important for normal tissue remodelling (72). MMP13 is a collagenase which is responsible for the maturation of granulation tissue by modulating the myofibroblast function, inflammation, angiogenesis and matrix degradation. Hence, during the healing process when fibroblasts secrete collagenous extracellular matrix and begin to align itself on the secreted matrix induces the expression of MMP-1.

COLLAGEN IN LIGAMENT HEALING FRIEND OR FOE

The histology of normal ligament composed of primarily collagen I. However after injury, type III collagen is primarily synthesized by fibroblasts and much lesser extend type I collagen (74). The authors also added where normal ligaments consist of densely packed cross-linked formation of type I collagen which contributes to its strength and stiffness. Scarred ligaments on the other hand, consists of abnormal collagen cross linking and collagen fibril size which contributes to its weakness and decrease in tensile strength.

Collagen is the main component of protein found in the human body making up 25% of its total protein content (75). In addition, ligaments includes the ankle ligaments are dense connective tissue with fibroblasts lying in between the bundles of collagen fibres (76,77). Also, collagen is secreted during the proliferative phase of the healing process (12,32). Hence, collagen is therefore responsible for the healing process and by inducing collagen secretion in cells at the injured soft tissue may promotes soft tissue healing. However, this is not the case when there are many articles published which reported where collagen secretion includes collagen I and collagen III also leads to fibrosis and scar formation leading to the decrease in tensile strength of the tissue (78–84). Thus, we believe that optimal amount and proportion of the collagen secreted may stimulate ligament healing while excess secretion or incorrect proportion of collagen may eventually leads to scar formation.

A study by Lo et al., (2002) compares the cytoarchitecture of ovine ACL and MCL reported where normal ligaments contain of fusiform cells which are arranged in rows and the cytoplasmic processes of these cells are connected via gap junctions to the adjacent cells. Scar tissue on other hand displayed discontinuities in their cellular rows. According to the authors, the discontinuities in MCL are filled with cellular projection and gap junction while ACL scar were devoid of cells and gap junctions. Also it is believed that the significant difference between the scar and normal tissues is the orientation of the fibrous matrix which had been previously mentioned (85). Therefore, cellular therapy to treat ankle sprains should not only have the ability to produce type I collagen but must have the ability to ensure the ligament heals according to the histology of the normal ligament. Suggesting that, future studies should commence by studying the underlying composition of different types of ligaments, followed by the methods in stimulating various cell types to produce the similar composition, will then only proceed to the testing of the stimulated cells in vivo in producing ideal healing which is currently still lacking.

ETHICS, CHALLENGES AND OPPORTUNITIES OF USING STEM CELLS AS THERAPEUTIC AGENT AS INTERVENTION FOR ANKLE SPRAIN

In 1995, Hillard Lazarus was the first to test MSC as a cellular pharmaceutical agent in treating hematologic malignancies involving human patients (86). Gradually such therapy had evoke the interest of worldwide researchers in conducting clinical studies in cell therapy.

Sadly to date, such approach had not gain any approval to market cellular products by the United States (87). One of the reason leading to this could be due to the increase numbers of predatory business activities worldwide calming MSC as a cure to all disease and disorders without any solid evidences (88).

Even though the marketing of MSC products was not being approved in the United States, several countries had taken a step forward as such, Health Canada had issued a provisional approval for a stem cell product (Prochymal) in treating children with acute Graft versus host disease in 17 May 2012 (89). Similarly, Japan had enacted the "Act on Safety of Regenerative Medicine and the Pharmaceuticals, Medical Devices and Other Therapeutic Products Act" in November 2014 creating a framework for regenerative medicine related clinical studies. Also, Korea had tighten its research-ethics standards and regulations relating to stem cells after the Hwang Scandal which damages the reputation of stem cell science (90). In addition to that, recently the European Commission had also approved the Alofisel as the first MSC pharmaceutical agent in treating Crohn's-related enterocutaneous fistula in March 2018 (89).

In par with other developed countries, research on regenerative medicine and cellular therapy in Malaysia had reached the stages of clinical trials and commercialisation (91). In Malaysia, the regulation of cellular therapy is under the purview of the National Pharmaceutical Control Bureau (NPCB), Ministry of Health (MOH) and according to the Ministry of Health, the guidelines for cell and gene therapy products (CGTPs) will be enforced from 2021 (92). Also, the current stem cell research in Malaysia is limited to haemopoietic stem cells (bone marrow, peripheral blood and cord blood) (93). A Malaysian study by Munusamy and his colleagues found that the prevalence of awareness on primary teeth stem cells among adults attending dental clinic is 26.7%. Only 20% of the respondents are aware of its potential therapeutic applications. In view of that, the used of cellular therapy in managing ankle sprain may have a bright future in its clinical application in Malaysia and is needed to increase the public awareness on the potential impact for degenerative diseases (94).
Although the used of cellular therapy in managing ankle sprain may have a bright future in Malaysia, many researches involving stem cells are still in pre-clinical phase with most of the animal studies identified uses mice, rats, and rabbits. Although chimpanzee (Pan troglodytes) and bonobo (Pan paniscus) are the closest relatives to humans which share many similar characteristics includes the genetic build-up, none of the studies on stem cells were conducted on such animals (95) Hence, animal ethics is one a major challenge in stem cells research during its pre-clinical phase in addition to the high cost required for the study.

At laboratory itself, ethical concerns may depend on the types of cells used. The used of human embryonic stem cells which ethically and politically controversial in Malaysia and worldwide as it involves the destruction of human embryo (96). In addition, obtaining umbilical cord mesenchymal stem cells from the Muslim community can be a challenge. This is said as in Islam, the placenta is considered as part of the human body and should be buried as mentioned in the Noble Quran 20:55, 'From the earth We created you, and into it We will return you, and from it We will extract you another time' (97). Also, obtaining other cell types such as bone marrow mesenchymal stem cells and adipose stem cells requires invasive procedure which poses a challenge for stem cell research and therapy.

Despite those ethical concerns and challenges in Malaysia, there are certain stem cells association such as Malaysian Association for Cell Therapy (MACT) and Tissue Engineering and Regenerative Medicine Society of Malaysia (TERMIS) which functions is to provide support and a platform for stem cell researchers as well as to assist in providing scientifically credible and medically appropriate cell therapies to informed patients or consumers. Besides that, stem cells banking companies such as Cryocord Sdn Bhd and Maluha Life Sciences has been established to provide cell banking facilities for individuals who wish to store their cells for future use has been a great opportunity for stem cell development in Malaysia. However, to date there were no written black and white regulation in regards of cellular therapy in Malaysia.

CONCLUSION

Ankle sprain can be disabling depending on its grade while the existing management does not produce promising results. In view of the recent development of regenerative medicine in treating other injured tissues, incorporating studies from other tissues and ligament along with the understanding and integrating the field of molecular biology, biochemistry and biomechanics. We may unlock a new intervention method which have a bright future of clinical application and can effectively treats ankle sprain. In achieving this, several factors such as the healing mechanism of the ankle ligaments, composition of the injured ligaments, cell types, mode of delivery, dosage and timing of intervention should be taken into consideration. After understanding the healing mechanism of ankle ligaments in detailed and its composition, tuning a particular cell types to express the markers and proteins secretion according to the composition of the pre-injured ankle ligaments. With that, we can eventually treat the sprained ligaments effectively while preventing scar tissue formation. As such, this may speed up the healing process at the same time helping the ankle ligament to restore to its pre-injured state. Hence, more studies are required to explore the composition of the ankle ligaments before proceeding into its intervention.

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