EFFECTS OF LOW ENERGY EXTRACORPOREAL SHOCKWAVE THERAPY ON GRAFT INCORPORATION, BONE METABOLISM, PAIN LEVEL AND KNEE FUNCTIONS IN INDIVIDUALS WITH POST ANTERIOR CRUCIATE LIGAMENT RECONSTRUCTION

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by

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LIST OF SYMBOLS AND ABBREVIATIONS

µL	Microliter
°C	Degree Celsius
ACL	Anterior cruciate ligament
ACLT	Anterior cruciate ligament transacted
ADSCs	Adipose-derived stem cells
ALP	Alkaline phosphatase
ANOVA	Analysis of variance
AOFAS	American Orthopaedic Foot and Ankle Society Score
BALP/BAP	Bone-specific alkaline phosphatase
bFGF	Basic fibroblast growth factor
BGP	Bone-Gla-protein
BMD	Bone mineral density
BMP	Bone morphogenic proteins
BMSCs	Bone marrow mesenchymal stem cells
BMU	Bone metabolism unit
CDMP	Cartilage derived morphogenetic
Con	Control
CPM	Continuous passive motion
CTX1	Cross Linked C-telopeptide of Type I Collagen
deg/sec	Degree per second
DPD	Deoxypyridinoline
EDTA	Ethylenediamine tetraacetic acid
EFD	Energy flux density
ELISA	Enzyme linked immunosorbent assay
eNOS	Endothelial nitric oxide synthase
ESWL	Extracorporeal shockwave lithotripsy
ESWT	Extracorporeal shockwave therapy

fESWT	Focus extracorporeal shockwave therapy
FFI	Foot function index
GPCR	G protein-coupled receptors
hASCs	Human adipose-derived stem cells
HRPZ II	Hospital Raja Perempuan Zainab II
HUSM	Hospital Universiti Sains Malaysia
Hz	Hertz
IGF	Insulin like growth factor
IKDC	International Knee Documentation Committee
iPTH	Parathyroid hormone
JEPeM	*Jawatankuasa Etika Penyelidikan (Manusia)* / Human Research Ethics Committee of Universiti Sains Malaysia
KOOS	Knee injury and Osteoarthritis Outcome Score
kV	Kilovolt
K-WOMAC	Korean Western Ontario and McMaster Universities Osteoarthritis Index
LMHFV	Low-magnitude high-frequency vibration
LRP5/4	Lipoprotein receptor-related protein 5
mJ	Milijoule
MM	Medial meniscectomy
mm^2	Square milimeter
MMP	Matrix metalloproteinases
MOH	Ministry of health
MOHE	Ministry of higher education
MREC	Medical Research and Ethics Committee
MRI	Magnetic reasonance imaging
MSCs	Mesenchymal stem cells
MVA	Motor vehicle accident
nm	Nanometer

N-MID®	N-terminal/midregion
NMRR	National Medical Research Register
NO	Nitrix oxide
NRS	Numerical rating scale
NSAIDs	Nonsteroidal anti-inflammatory drugs
NTX-1	Type I Collagen N-Terminal Telopeptide
OA	Osteoarthritis
OCN	Osteocalcin
OD	Optical density
OPG	Osteoprotegerin
p-NPP	p-nitrophenyl phosphate
P1CP	Carboxy-terminal propeptide of type 1 procollagen
P1NP	Type I Procollagen N-terminal Peptide
PCL	Posterior cruciate ligament
PCNA	Proliferating cell nuclear antigen
PDGF	Platelet-derived growth factor
PGE2	Prostaglandin E_2
PT	Physiotherapy
PTHrP	Parathyroid hormone-related protein
PTHRs	Parathyroid hormone receptors
PYD	Pyridinoline
RANKL	Receptor activator of nuclear factor kappa-B ligand
rESWT	Radial extracorporeal shockwave therapy
ROM	Range of motion
rpm	Revolutions per minute
RPT	Radial pulse therapy
s	Second
SD	Standard deviation
SLAB	*Skim latihan akademik bumiputera*

SOX9	Protein sex determining region Y-box 9
SPSS	Statistical Package for Social Science
T0	Time 0 – Before ACL reconstruction (week 0)
T1	Time 1 – 2 weeks post ACL reconstruction
T2	Time 2 – 9 weeks post ACL reconstruction
T3	Time 3 – 12 weeks post ACL reconstruction
T4	Time 4 – 6 months post ACL reconstruction
TDSCs	Tendon-derived stem cells
TGF	Transforming growth factor
TRAP5b	Serum band 5 tartrate resistant acid phosphate
UniSZA	Universiti Sultan Zainal Abidin
USA	United States of America
USM	Universiti Sains Malaysia
VAS	Visual analogue scale
VEGF	Vascular endothelial growth factor
VISA-A	Victorian Institute of Sports Assessment- Achilles
X-Ray	X Radiation
3ESWT	Three sessions of extracorporeal shockwave therapy
6ESWT	Six sessions of extracorporeal shockwave therapy
25(OH)D	Serum 25-hydroxyvitamin D

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KESAN TERAPI *SHOCKWAVE EXTRACORPOREAL* YANG BERTENAGA RENDAH TERHADAP PENGGABUNGAN GRAF, METABOLISMA TULANG, TAHAP KESAKITAN DAN FUNGSI LUTUT DI KALANGAN INDIVIDU SELEPAS MENJALANI PEMBEDAHAN REKONSTRUKTIF LIGAMEN KRUSIAT HADAPAN

ABSTRAK

ESWT bertenaga tinggi mempunyai potensi untuk mempercepatkan proses penyembuhan ligamen krusiat hadapan selepas pembedahan rekonstruktif (ACLR). Fakta berkenaan keberkesanan ESWT bertenaga rendah ke atas hasil klinikal di kalangan individu selepas ACLR masih kurang. Tujuan kajian ini adalah untuk menyiasat kesan ESWT bertenaga rendah dengan pelbagai frekuensi terhadap penggabungan graf, metabolisma tulang, tahap kesakitan dan fungsi lutut di kalangan individu selepas ACLR. Tiga puluh orang peserta (berumur 20-36 tahun) telah direkrut dan diagihkan kepada tiga kumpulan, iaitu kumpulan kawalan (tiada sesi terapi *shockwave),* kumpulan 3 kali sesi terapi *shockwave* (3ESWT) dan kumpulan 6 kali sesi terapi *shockwave* (6ESWT) dengan 10 orang peserta bagi setiap kumpulan. Kesemua peserta telah menjalani ACLR dengan menggantikan ligamen krusiat hadapan dengan tendon hamstring dan menerima program rehabilitasi yang sama selepas pembedahan. ESWT telah diaplikasikan sekali seminggu selama 3 dan 6 minggu berturut-turut. Ketumpatan aliran tenaga (EFD) yang digunakan untuk kumpulan 6ESWT dan 3ESWT adalah masing-masing 0.18mJ.mm^{-2} dan 0.09mJ.mm^{-2} dengan 500 kejutan dan 1.5 bar. Parameter-parameter yang diukur termasuk penilaian penggabungan graf, penanda-penanda metabolisma tulang, skala kesakitan dan skor fungsi lutut. Dalam kajian ini, data dikumpulkan sebanyak 5 kali iaitu sebelum ACLR (bacaan awal), 2-, 9-, 12 minggu dan 6 bulan selepas ACLR. Bilangan graf yang bercantum secara separa adalah lebih tinggi secara signifikan

berbanding graf tanpa percantuman dalam kumpulan 6ESWT pada bulan keenam selepas ACLR (p=0.02). Walaubagaimanapun, tiada perbezaan signifikan bagi bilangan graf yang bercantum secara separa di antara semua kumpulan. Tiada perbezaan signifikan bagi kepekatan serum alkaline phosphatase dan osteocalcin di antara kumpulan, di dalam kumpulan dan antara kelima-lima bacaan (p>0.05). Kepekatan serum CTX1 juga tidak berbeza secara signifikan di dalam kumpulan 3ESWT dan 6ESWT bagi kelima-lima bacaan (p>0.05). Kepekatan serum kalsium pada minggu kedua, kesembilan dan kedua belas selepas ACLR adalah lebih tinggi secara signifikan berbanding dengan kepekatan serum kalsium masing-masing pada bacaan awal (p=0.023, p=0.004 dan p=0.001) dengan perbandingan masa bagi kesemua kumpulan. Skor kesakitan adalah lebih rendah secara signifikan pada bulan keenam berbanding minggu kedua selepas pembedahan dengan perbandingan masa bagi kesemua kumpulan (p=0.039). Kesemua kumpulan intervensi dan kawalan menunjukkan peningkatan yang signifikan bagi skor Lysholm pada bulan keenam selepas ACLR berbanding dengan bacaan awal (p=0.002 bagi setiap kumpulan). Enam sesi ESWT menunjukkan kesan signifikan dengan mempercepatkan penyembuhan graf dengan menggalakkan percantuman graf di dalam terowong tibia dan meningkatkan tahap serum kalsium pada minggu kesembilan dan kedua belas selepas pembedahan. ESWT tidak berkesan secara signifikan terhadap penanda pembentukan dan resorpsi tulang selepas pembedahan.

EFFECTS OF LOW ENERGY EXTRACORPOREAL SHOCKWAVE THERAPY ON GRAFT INCORPORATION, BONE METABOLISM, PAIN LEVEL AND KNEE FUNCTIONS IN INDIVIDUALS WITH POST ANTERIOR CRUCIATE LIGAMENT RECONSTRUCTION

ABSTRACT

High energy ESWT has potential to accelerate the healing process of anterior cruciate ligament post reconstruction (ACLR). Evidences regarding the effectiveness of low energy ESWT on clinical outcomes among individuals with post ACLR are scarce. This study aimed to investigate the effects of different frequencies of low energy ESWT on graft incorporation, bone metabolism, pain level and knee functions in individuals with post ACLR. Thirty participants (aged 20-36 years old) being assigned into three groups, i.e. control group (no shockwave therapy sessions), 3 sessions of shockwave therapy (3ESWT group), and 6 sessions of shockwave therapy (6ESWT group) with 10 participants in each group. All participants underwent a single hamstring autograft ACLR and received a similar rehabilitation programme post-operatively. The ESWT was applied once per week for 3 and 6 consecutive weeks. The energy flux density (EFD) used for 6ESWT and 3ESWT groups was 0.18mJ.mm^{-2} and 0.09mJ.mm^{-2} respectively (500 shocks, 1.5 bar). The measured parameters included graft incorporation evaluation, bone metabolism markers, pain score and knee function score. The data were collected 5 times, i.e. before ACLR (baseline), 2-, 9-, 12 weeks and 6 months post ACLR. The number of graft with partial incorporation was significantly higher compared to the number of graft without incorporation in 6ESWT group at 6 months post ACLR (p=0.02). However, no significant differences of number of graft with partial incorporation was found among all the groups. There were no significant differences of serum alkaline phosphatase and osteocalcin concentrations between groups, within group and across the five

measurements (p>0.05). Serum CTX1 concentrations were also not significantly different within the 3ESWT and 6ESWT groups across the five measurements (p>0.05). Serum calcium concentrations at 2-, 9- and 12 weeks post ACLR were significantly higher compared to its baseline value (p=0.023, p=0.004 and p=0.001 respectively) in comparison of time for all the groups. The pain score was significantly lower at 6 months compared to week 2 post-operatively in comparison of time for all the groups (p=0.039). All intervention and control groups showed significant improvement in Lysholm scores at 6 months post ACLR compared with baseline (p=0.002 for respective group). Six sessions of ESWT exhibited significant effect to accelerate the graft healing by inducing graft incorporation in tibial tunnel, and increased the serum calcium level at 9- and 12 weeks post-operatively. ESWT did not significantly affect bone formation and bone resorption markers post-operatively.

CHAPTER 1

INTRODUCTION

During XVII Asian Games in 2014, 30.1% of Malaysian athletes sustained at least one injury. Knee injuries were reported as the highest incident followed by lower back, thighs and ankle injuries. In addition, the three most common injuries were related with strain and tears, ligamentous injuries and contusion or bruises (Hamid *et al.*, 2016; Sazali *et al.*, 2018). According to Hamid *et al.* (2016), 76% from the reported injuries was classified as non-contact injuries. More than half of the total injuries occurred during the training sessions but not during the competition itself. The highest numbers of injuries were diagnosed among badminton players, followed by hockey and rugby. Male athletes were prone to get injured rather than female athletes. As the consequences to the injuries, nineteen injuries prevented the athletes from training or competing. Sazali *et al.* (2018) mentioned that a retrospective study done by a group of researchers from Department of Arthroscopy and Sport Injury of Hospital Kuala Lumpur in 2012 and 2013 found that 66% of the 38 patients who underwent posterior cruciate ligament (PCL) reconstruction surgery suffered from anterior cruciate ligament (ACL) tear.

To date, the true prevalence of the ACL injuries incidence among Malaysian population is unknown due to the lack of population-based studies, and the clinical data obtained from the doctor referrals might be misled as some individuals with knee injuries do not seek treatment. However, the rate of ACL injuries undoubtedly increased with the greater involvement of young adults in sporting activities.

ACL is a ligament in the knee joint. It is attached medially to the anterior intercondylar area of the tibia and partly blending with the anterior of the lateral meniscus; it ascends posterolaterally, twisting on itself and fanning out to attach to the posteromedial aspect of the lateral femoral condyle (Arnoczky, 1983). ACL plays

an important role to prevent anterior translation (Markatos *et al.*, 2013), and rotation of the knee (Suppiah *et al.*, 2013) and helps to stabilize the anterior aspect of the knee (Zaidi and Chan, 2008; Markatos *et al.*, 2013).

The mechanism of ACL injuries reported could probably be due to the rotation of femur and tibia in opposite directions under full body weight. It happens when there is a sudden turning to change direction while running. Common symptoms regarding ACL injuries include pain, swelling, instability and loss of function in severe cases (Suppiah *et al.*, 2013). Acute ACL tears occur less than 6 weeks after injury, whereas chronic tears occur more than 6 months (27 weeks) after injury (Flint *et al.*, 2014).

Conservative or surgical treatment for instability of the knee is indicated for regaining pre-injury function (Trees *et al.*, 2005). ACL reconstruction surgery is required to restore knee functions so that the torn ligament can be replaced with the nearly perfect biological replacement (Dugan, 2005; Shultz *et al.*, 2005). Anatomically, ACL is attached from the tibia bone to the femoral bone. In a reconstruction, the torn ligament will be removed and replaced by autograft or allograft, and it depends on the surgical approach decided by the surgeon. The junction between the graft tissue, i.e. tendon and hard tissue (bone) is also known as the ‘osteotendinous’ junction, ‘enthesis’ or ‘insertion site’ (Benjamin *et al.*, 2006). In long term, an ACL rupture can cause further intraarticular damages like meniscal tears, cartilage defects and osteoarthritis. According to Strehl and Eggli (2007), two-thirds of primarily conservative treated patients decided to have ACL reconstruction after rehabilitation. Ross *et al.* (2001) reported that the younger and more active the patient, the earlier surgical reconstruction is recommended. It has been estimated that 8-10% of ACL reconstructions resulted in recurrent instability and graft failure. Graft selection, tunnel location, initial graft tension, graft fixation, graft tunnel motion, and graft healing rate are all aspects that influence the surgical outcome of ACL reconstruction (Markatos *et al.*, 2013).

One of the factors that could lead to failure of ACL reconstruction is when the graft healing or incorporation is poor. Patients or athletes were previously expected to return to play six months after ACL reconstruction. The current trend, however, suggests that return to play should be guided not only by a time frame, but also by objective clinical criteria and physical testing (Goes *et al*., 2020). Legnani *et al*., (2016) discovered that ACL reconstruction with hamstring autograft takes 7.7 months to recover. The rate of graft healing can be enhanced using external physical stimulus produced by shockwave machine. Shockwave is one of the cost-effective modalities to treat various chronic musculoskeletal disorders which commonly require surgical interventions. Managing chronic diseases could increase the long-term treatment costs (McPhail, 2016). Thus, it is not surprising that research in shockwave have been developed in a wide and new spectrum to explore and optimise the usage of this rehabilitation modality (Olsen *et al.*, 2015; Wang *et al.*, 2015a). Several researches have demonstrated the effectiveness of extracorporeal shockwave therapy to treat various chronic musculoskeletal pathologies (Wang *et al.*, 2005a; Galasso *et al.*, 2012; Aqil *et al.*, 2013; Dingemanse *et al.*, 2014; Louwerens *et al.*, 2014; Romeo *et al.*, 2014; Schaden *et al.*, 2015). Sports injuries related with tendon-bone junction became an interest among the researchers due to its delay in healing for conservative treatment and increasing failure rate in surgical procedures. The complication of tendon and bone to blend together is attributed to the non-homogenous structure in both types of tissues (Leung *et al.*, 2015). From the current literature, shockwaves therapy is one of the rehabilitation modalities that has potential to be applied to tendon-bone junction to accelerate the healing process (Wang *et al.*, 2002; Wang *et al.*, 2003a; Wang *et al.*, 2005b; Furia, 2006; Wang *et al.*, 2008b; Qin *et al.*, 2010; Chow *et al.*, 2012; Notarnicola *et al.*, 2012a; Chow *et al.*, 2014; Wang *et al.*, 2014b). The mechanical stimuli produced by the shockwaves are able to induce physiological responses at the cellular level (Mantila Roosa *et al.*, 2011; Rui *et al.*, 2011; Cheng and Wang, 2015). Several previous studies have reported the effectiveness of

shockwave therapy on bone structure (Cheng and Wang, 2015; Kuo *et al.*, 2015; Schaden *et al.*, 2015; Cheng *et al.*, 2016) and tendon structure alone (Vetrano *et al.*, 2011).

Besides, shockwave therapy is listed as one of the biological therapies for tendon injuries of the knee joint (Demange *et al.*, 2014; Gatewood *et al.*, 2017). Previous studies have shown that extracorporeal shockwave therapy (ESWT) is beneficial to induce the ingrowth of neovascularization and improvement of blood supply at the bone-tendon junction (Wang *et. al*., 2002; Wang *et al.*, 2003a), promote tissue repair (Wang *et al.*, 2005b), up-regulation of angiogenic and osteogenic growth factors (Wang *et al.,* 2008a) and increase cortical bone formation in acute fracture (Wang *et al.*, 2001). Additionally, it was also reported that ESWT is beneficial in promoting tendon-bone healing in the bone tunnel after ACL reconstruction in an animal (Wang *et al.*, 2005b) and human study (Wang *et al.*, 2014b). Nevertheless, studies investigating the effects of ESWT on graft incorporation after ACL reconstruction are scarce.

Detecting early changes to the bone may be crucial for certain cases especially in the pathological conditions. Plain radiography approach requires long follow up time to observe the outcomes of the healing process, and biochemical bone markers provide information regarding the effects of treatment earlier than radiography. In previous researches, diverse methods such as different number of treatment sessions, dosage of shockwave, time of treatment given, sites treated and time to follow up were employed, and varied outcome were observed. However, the exact mechanisms on how shockwave therapy act on bone biochemical markers are still debatable.

The pain experienced by the patients is an important parameter for recovery (Valkering *et al.*, 2015). Pain management after ACL reconstruction involves

monitoring of the pain level by using the visual analog scale (VAS) and following the analgesic postoperative programme (Buescu *et al.*, 2017). Besides, Numerical Rating Scale (NRS) can be used to objectively measure pain level using grades from 0 to 10. NRS has shown high correlations with other pain-assessment tools in several studies (Kremer *et al*., 1981; Jensen *et. al*., 1986). The feasibility of its use and good compliance has also been proven (Farrar *et al.*, 2001; Closs *et al.*, 2004). Valkering *et al.* (2015) showed the pain level trend after close follow up at post-ACL reconstruction using hamstring autograft. The most painful days experienced by the patients were at two days post-operatively. The ACL reconstruction resulted in a higher pain score compared with non-ACL knee arthroscopy at the following days up to one week post-operatively. However, after one year of follow up, it was found that the pain level was reduced for all participants.

The Lysholm score has been reported to be more sensitive for evaluating activities of daily life, and recreational or competitive sports when used as a tool for evaluating knee functions. (Zaidi and Chan, 2008). High Lysholm scores and International Knee Documentation Committee (IKDC) scores are two elements that influence recreational athletes or players to return to sports at the same level (Rodríguez-Roiz *et al.*, 2015). To date, based on a systematic review (Gatewood *et al*., 2017), only Wang *et al.* (2014b) reported the effectiveness of ESWT at post-ACL reconstruction on physical function. Lysholm score was found to be better in participants who received ESWT compared with the participants who did not receive ESWT after one and two years of follow up post hamstring autograft ACL reconstruction. However, in a similar study, the authors reported that the IKDC score was not affected by the intervention prescribed (Wang *et al.*, 2014b).

Athletes are prone to anterior cruciate ligament (ACL) injuries. A previous human study conducted by Wang *et al.*, (2014b) has been carried out to investigate the effects of high energy extracorporeal shockwave therapy on ACL after

reconstruction. In this study, it was found that shockwave therapy with total EFD 0.298mJ.mm^{-2} (20kV), with 1500 impulses reduced the tibial tunnel enlargement, improved knee functions, and reduced joint laxity. The shockwave was applied once immediately after the ACL reconstruction surgery, while the participants were anesthetised. The effectiveness of high energy shockwave therapy on orthopaedic and musculoskeletal area is widely known. According to (Chow *et al.,* 2012; Cheng and Wang, 2015), low energy shockwave therapy has been proved to have similar beneficial effects as high energy shockwave therapy on various musculoskeletal conditions. However, lack of scientific evidences was found related with the application of low energy of shockwave therapy on participants with post ACL reconstruction.

In addition, ligamentous injuries of knee joints are the highest incidence among Malaysian athletes. The injuries prevented the athletes from training or competing (Hamid *et al.,* 2016; Sazali *et al.,* 2018). 67% of the primarily conservative treated patients decided to have ACLR (Strehl and Eggli, 2007). It was found that 8 to 10% of ACLR resulted in recurrent instability and graft failure (Ross *et al.,* 2001). Markatos *et al.* (2013) discovered that graft healing rate is one of the factors that influence the surgical outcome of ACLR. In a previous study, ACLR with hamstring autograft takes 7.7 months to recover (Legnani *et al.,* 2016). As a result, any treatments that might significantly improve graft healing would be extremely useful because the patient would be able to resume their prior level of activity much earlier. A study must be conducted to investigate the effectiveness of a new potential modality on targeted area to accelerate the healing. Thus, the present study was proposed to investigate the effects of different frequencies of low energy extracorporeal shockwave therapy on graft incorporation and bone metabolism post ACL reconstruction. If the present study can find that different frequencies of low energy shockwave therapy could affect graft incorporation and bone metabolism of individuals with ACL reconstruction, the potential mechanism for different frequencies

of low energy shockwave therapy induced graft incorporation and bone metabolism in individuals with ACL reconstruction will be determined. To date, evidences regarding the exact dosage of application and effectiveness of low energy extracorporeal shockwave therapy on tendon-bone junction, bone metabolism markers, pain level and knee functions among individuals with post ACL reconstruction are still lacking. Therefore, the present study was proposed to investigate the effects of different frequencies of low energy extracorporeal shockwave therapy (ESWT) on graft incorporation, bone metabolism, pain level and knee functions in individuals with post anterior cruciate ligament reconstruction.

1.1 Objectives of The Study

1.1.1 General Objective

To investigate the effectiveness of low energy extracorporeal shockwave therapy (ESWT) with different frequencies over a period of time on graft incorporation, bone metabolism, pain level and knee functions in individuals with anterior cruciate ligament reconstruction.

1.1.2 Specific Objectives

1. To determine the effects of three and six sessions of low energy extracorporeal shockwave therapy (ESWT) on graft incorporation in individuals with anterior cruciate ligament reconstruction.

2. To determine the effects of three and six sessions of low energy extracorporeal shockwave therapy (ESWT) on bone metabolism in individuals with anterior cruciate ligament reconstruction.

3. To determine the effects of three and six sessions of low energy extracorporeal shockwave therapy (ESWT) on pain score in individuals with anterior cruciate ligament reconstruction.

4. To determine the effects of three and six sessions of low energy extracorporeal shockwave therapy (ESWT) on knee functions in individuals with anterior cruciate ligament reconstruction.

1.2 Significance of The Study

If positive results from the present study are obtained, it will provide scientific evidence that ESWT intervention with appropriate frequency can be incorporated in the rehabilitation programme for individuals with ACL reconstruction to accelerate the graft incorporation healing, minimised the risk of graft failure and improve bone metabolism. Since anterior cruciate ligament (ACL) injuries occur frequently among athletes, the present study finding can be applied in the management of sports injury and rehabilitation. Additionally, it will add new insight to the field of sports science and sports medicine.

1.3 Hypotheses

H_{O1} : There are no significant differences in graft incorporation treated with three and six sessions of low energy ESWT compared to non-treatment with ESWT in individuals with anterior cruciate ligament reconstruction.

H_{A1} : There are significant differences in graft incorporation treated with three and six sessions of low energy ESWT compared to non-treatment with ESWT in individuals with anterior cruciate ligament reconstruction.

H_{O2} : There are no significant differences in bone metabolism treated with three and six sessions of low energy ESWT compared to non-treatment with ESWT in individuals with anterior cruciate ligament reconstruction.

H_{A2} : There are significant differences in bone metabolism treated with three and six sessions of low energy ESWT compared to non-treatment with ESWT in individuals with anterior cruciate ligament reconstruction.

H_{O3} : There are no significant differences in pain score treated with three and six sessions of low energy ESWT compared to non-treatment with ESWT in individuals with anterior cruciate ligament reconstruction.

H_{A3} : There are significant differences in pain score treated with three and six sessions of low energy ESWT compared to non-treatment with ESWT in individuals with anterior cruciate ligament reconstruction.

H_{O4} : There are no significant differences in knee functions treated with three and six sessions of low energy ESWT compared to non-treatment with ESWT in individuals with anterior cruciate ligament reconstruction.

H_{A4} : There are significant differences in knee functions treated with three and six sessions of low energy ESWT compared to non-treatment with ESWT in individuals with anterior cruciate ligament reconstruction.

1.4 Operational Definitions

Frequency: In this current study, the frequency refers to the number of shockwave therapy sessions, i.e. three sessions for 3ESWT group, and six sessions for 6ESWT group.

Low energy Extracorporeal Shockwave Therapy (ESWT): Extracorporeal shockwave therapy is a new technology using shockwaves to treat painful conditions of the musculoskeletal system. Low energy ESWT generated by radial shockwave machine was used for the treatment groups. Shockwave generated low energy is when concentrated shockwave energy per unit area around 0.08 - 0.27 $mJ.mm^{-2}$.

Graft Incorporation: The blends of new tendon to bone.

Bone metabolism: Measurements of blood parameters such as bone formation marker, i.e. alkaline phosphatase and serum osteocalcin, bone resorption marker, i.e. serum Cross Linked C-telopeptide of Type I Collagen (CTX1), and serum total calcium and phosphorus.

Anterior Cruciate Ligament Reconstruction: It is a surgical procedure where the torn ACL ligament is being replaced by the graft at the original site of the ligament attachment on femur and tibia.

Pain level: Pain level were measured using Numerical Rating Scale (NRS) which has a grade from 0 to 10. The participants were asked to circle the number between 0 and 10 that best fit to their pain intensity.

Knee functions: Functional score of the participants were measured using Lysholm score. Lysholm score is a sheet that contains eight questions. It is designed to give information of how the knee problems have affected the participants' ability to manage everyday life.

CHAPTER 2

LITERATURE REVIEW

2.1 Anterior Cruciate Ligament (ACL)

The ACL is one of the most important ligaments in the knee joint. It is attached medially to the tibia's anterior intercondylar area, which partially blends with the anterior of the lateral meniscus, and ascends posterolaterally, twisting on itself and fanning out to attach to the lateral femoral condyle's posteromedial aspect (Arnoczky, 1983) (Figure 2.1). ACL plays an important role to prevent anterior translation of the tibia on femoral bone (Markatos *et al.*, 2013), rotation of the knee (Suppiah *et al.*, 2013) and helps to stabilize the anterior aspect of the knee (Zaidi and Chan, 2008; Markatos *et al.*, 2013). The mechanism of ACL injuries is reported to be due to the rotation of femur and tibia in opposite directions under full body weight. It happens when there is sudden turning to change direction while running. Common symptoms regarding ACL injuries are pain, swelling, instability and loss of function in severe cases (Suppiah *et al.*, 2013).

2.2 Anterior Cruciate Ligament Reconstruction (ACLR)

Repositioning a free tendon graft from the patella or hamstring tendon into a bone tunnel is a typical procedure for ACLR (Wang *et al.*, 2005b) (Figure 2.2). One of the preferable methods for most ACLR procedure is by arthroscopy using autografts. The standard practise ruled that maximum tension needs to be applied to the autograft as it undergoes final fixation (Dugan, 2005). It is believed that the tension created in the grafts will contribute to the decrease of anterior knee translation thus reducing knee laxity occurrence (Tyler *et al.*, 1999).

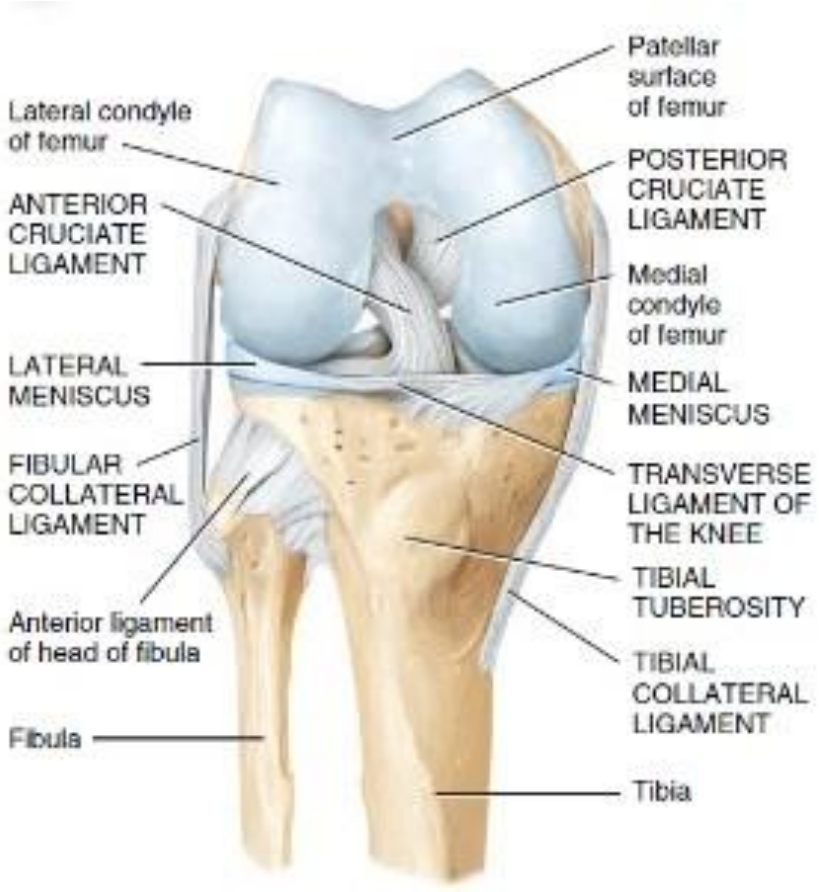


Figure 2.1 Anterior deep view of knee joint (Tortora and Derrickson, 2015)

The main purpose of any ACL reconstruction is to restore knee functions to its pre-injury state by trying to achieve the tightest possible repair with hopes that the surgery of the torn ligament will be the perfect biological replacement (Dugan, 2005; Shultz *et al.*, 2005). A good ACL reconstruction outcome is said to have lesser knee laxity with a firm fixation (Dugan, 2005). However, increased knee laxity is not necessarily an indication of poor knee function. Furthermore, the functional outcome of this surgery depends on the firm healing of graft incorporation with bone in the bone tunnel (Wang *et al.*, 2005b). However, previous studies showed inconsistent results regarding the healing of new tendon to bone. Some of the studies found that the graft incorporated well with the bone after implantation (Muller *et al.*, 2013; Irvine *et al.*, 2014; Arner *et al.*, 2015) but other studies showed contradictory results (Bosch *et al.*,

1989; Bosch and Kasperczyk, 1992). It is known that if the bone and tendon do not heal together, it may lead to future knee pathology (Fleming *et al.*, 2005).

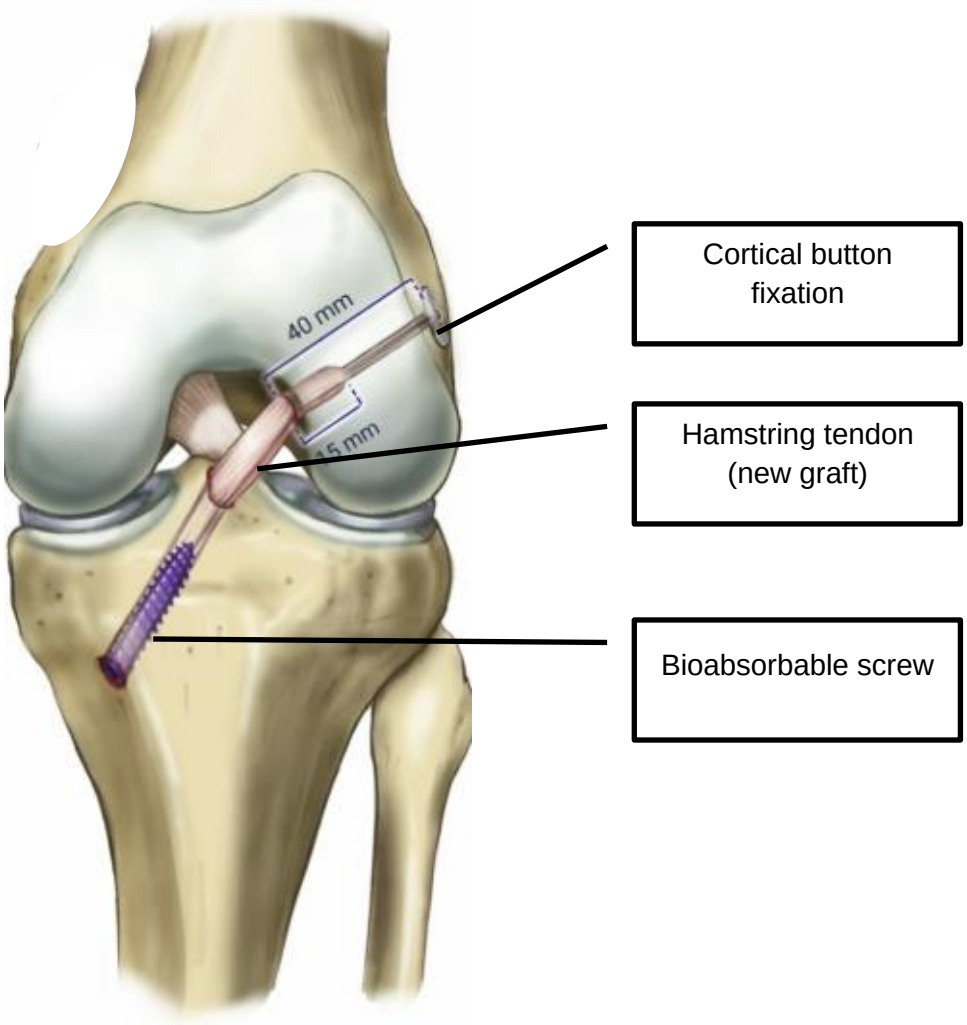


Figure 2.2 Anterior deep view of anterior cruciate ligament reconstruction of knee joint (Martinez-Cano *et al.,* 2020)

Other methods of reconstruction which uses bone-patella tendon-bone approach found that most of the participants' complaint of discomfort and some of them reported anterior knee pain at the donor harvest site which occasionally caused difficulty in kneeling (Zaidi and Chan, 2008). It is well known that difficulty in kneeling may affect some daily activities, occupational, religious and recreational activities. Thus, the alternative graft choice such as hamstring tendon might reduce the number

of patients with these problems. The hamstring tendon graft procedure was claimed to be more expensive while the graft fixation is less secure (Zaidi and Chan, 2008).

2.3 Graft Incorporation

Wang *et al.* (2005b) mentioned that two third of the graft failed as a result of graft pull out at 24 weeks. A study done by Weiler *et al.* (2002) has found that a tendon graft is incorporated into a bone tunnel by ossification or formation of a fibrous sleeve of callus.

Many factors influence tendon-to-bone healing after ACL surgery. Several research employing various procedures and methods have found contradictory results in the healing of new tendon grafted to bone (Lattermann *et al.*, 2004; Tien *et al.*, 2004; Gulotta *et al.*, 2008; Akoto *et al.*, 2015). One of the biological therapies for tendon injuries of the knee joint is shockwave therapy (Demange *et al.*, 2014; Gatewood *et al.*, 2017). Previous studies have demonstrated that extracorporeal shockwave therapy (ESWT) is beneficial to induce the ingrowth of neovascularization and improvement of blood supply at the bone-tendon junction (Wang *et al.*, 2002; Wang *et al.*, 2003a), promote tissue repair (Wang *et al.*, 2005b), up-regulation of angiogenic and osteogenic growth factors (Wang *et al.*, 2008a) and increase cortical bone formation in acute fracture (Wang *et al.*, 2001). Additionally, it was also reported that ESWT is beneficial in promoting tendon-bone healing in bone tunnel after ACL reconstruction in an animal (Wang *et al.*, 2005b) and human (Wang *et al.*, 2014b) study respectively. Nevertheless, to date, the optimal dosage of ESWT is still unknown and studies investigating the effects of ESWT on graft incorporation after ACL reconstruction are scarce.

The healing and remodelling processes of a tendon graft depend on a variety factors including the application of mechanical forces to the grafts (Anderson *et al.*, 2001), addition of osteoinductive growth factors such as bone morphogenetic protein (Rodeo *et al.*, 1999) and method used which give direct contact healing without the

development of a fibrous interzone (Weiler *et al.*, 2002). Additionally, other studies reported that parathyroid hormone and physical factors such as continuous passive motion (CPM) could accelerate tissue healing of the joint including bone and cartilage (O'Driscoll *et al.*, 1986; Andreassen *et al.*, 2001). The healing of graft which is a tendon depends on the blends of the tendon graft to the bone (Dong *et al*., 2012).

2.4 Bone

2.4.1 Bone Structure and Bone Cells

According to the Dorland's Pocket Medical Dictionary by Jacobson (2001), bone can be defined as a hard, rigid form of connective tissue constituting most of the skeleton of vertebrates and composed mainly of calcium salts. Bone is made up of several different tissues working together such as osseous tissue (bone), cartilage, dense connective tissues, epithelium, adipose tissue and nervous tissue. Bone tissue is a complex and dynamic living tissue because it involves continuously growing process, i.e. remodeling with building new bone tissue, breaking down old bone tissue and repairing (Tortora and Derrickson, 2011). It is surrounded by an abundant matrix of intercellular materials which contain 50% of crystallised mineral salts, 25% of water, and 25% of collagen fibers. Bone consists largely of mineralised extracellular matrix which mass and architecture result from a balance between production and resorption. The major components of the organic matrix are type I collagen, osteopontin and osteocalcin (Scott *et al.*, 2008).

Osteocalcin is commonly reported as a bone formation marker. It is vitamin K dependent and the main non-collagenous protein of bone matrix which consists of 49 amino acids. Osteocalcin is synthesised in bone by osteoblasts. It is well known as a marker of the viability, differentiation and osteogenic ability of osteoblast cells. After production, it is partially incorporated into the matrix of the bone and partly delivered to the circulatory system. Circulating level of osteocalcin reflects the rate of bone

formation. Determination of serum osteocalcin has proved to be valuable for monitoring bone metabolism. Osteocalcin is an indicator of osteoblastic activity in human serum and plasma. Its values may vary depending upon the person's age (years after menopause), circadian rhythm, rate of glomerular filtration and duration of treatment (Chapurlat and Confavreux, 2016; Zoch et al., 2016).

The human Cross-Linked C-telopeptide of Type I Collagen which is known as CTX1 is used to evaluate the activity of bone resorption. International Osteoporosis Foundation (IOF), International Federation of Clinical Chemistry and Laboratory Medicine has recommended serum CTX1 to be used as reference bone resorption marker for the evaluation of risk for fracture and monitoring therapy in clinical settings (Vasikaran *et al.*, 2011). The organic matrix of bone mostly consists of collagen type I that is split into its N- and C-terminal telopeptides (CTX) during bone resorption by osteoclasts. The CTX1 is released in the bloodstream and its elevated concentrations are found in patients with increased bone resorption (Christenson, 1997). Serum CTX1 level varies following its circadian variation, with peak in the second half of night and nadir in the afternoon. The peak levels of CTX1 were seen at 05.00 h and of nadir were seen at 14.00 h (Qvist *et al.*, 2002). A previous study investigated the effect of food intake on CTX1 measurement and found that it was 20% lower when compared to the fasting state. Hence, to reduce this discrepancy, it is recommended to collect the sample in the morning after an overnight fast (Clowes *et al.*, 2002).

The three types of bone cells are osteocytes, osteoblast and osteoclasts. Osteoblasts are bone-forming cells which synthesise and secrete collagen fibers and other organic components needed to build the matrix of bone tissue. Matured osteoblasts form osteocytes. Alkaline phosphatase, a serum index of bone formation, is abundant on the plasma membrane of osteoblasts.

Osteocytes are the mature bone cells, and exist as the main cells in bone tissue. These cells are originally osteoblasts that are differentiated and found in bone

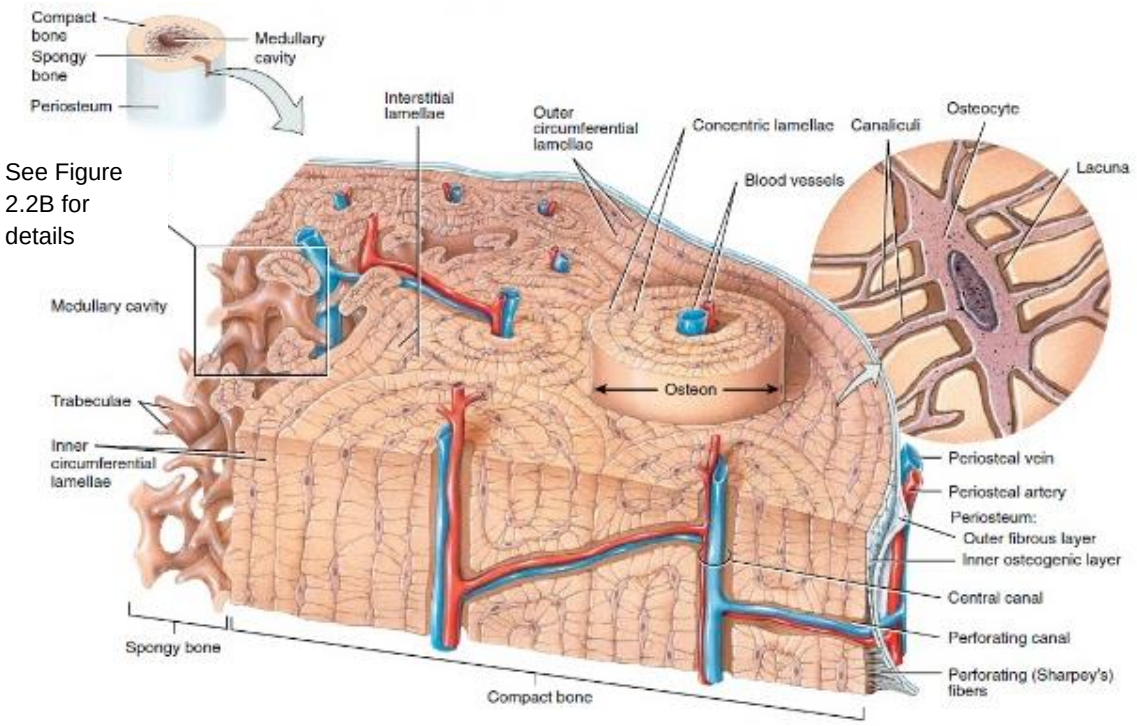
lacunae (Figure 2.3). The main role of osteocytes is the involvement in activation of bone turnover, regulation of extracellular calcium to provide means of communication required in mechanotransduction (Khan, 2001).

Osteoclasts are giant cells and it is derived from the fusion of as many as 50 monocytes and concentrated in the endosteum. It is usually found in contact with a calcified bone surface and within the resorption cavity it created. The cell is able to synthesise powerful lysosomal enzymes and acids, and release them via its ruffled border to digest the protein and mineral components of the underlying bone matrix. Bone resorption process, i.e. breakdown of the bone matrix, is part of the normal development, growth, maintenance and repair of bone (Khan, 2001).

2.4.2 Bone Functions

Besides providing attachment for most of the skeletal muscle, serving as the structural framework for the body by supporting soft tissues and assisting in the body part movements, bones also protect the internal organs from injury. For example, brain is surrounded by several cranial bones, spinal cord in the vertebrae and other vital organs such as heart and lungs are well protected in the rib cage. Bones also store several minerals mainly calcium and phosphorus, and play an important role in minerals homeostasis in the human system. Some part of certain bones in the body which is known as red bone marrow, is involved in hemopoiesis which produces red blood cells, white blood cells and platelets (Tortora and Derrickson, 2011).

A



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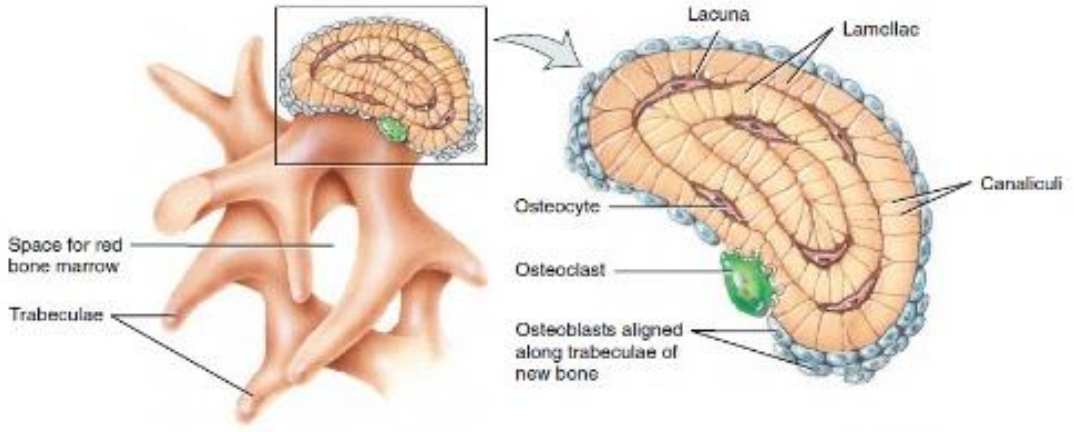

Figure 2.3 Histology of compact and spongy bone. A. The structures and arrangements of bone cells in compact and spongy bone. B. Enlarged aspect of spongy bone trabeculae. C. Details of a section of a tabecula. (Tortora and Derrickson, 2015)

2.4.3 Bone Metabolism Markers

Bone metabolism or bone turnover is a continuous remodeling process that is dynamic and homeostasis is maintained by balancing resorption of old and injured bone and new bone formation. It occurs on the surface of bone at focused sites which also known as bone metabolism unit (BMU) or bone remodeling unit (Christenson, 1997). Bone remodeling is refers to an active process in the skeleton which is vital for homeostasis of calcium. This process also preserves the integrity of the skeleton via the physiological activities of the osteoblasts and osteoclasts (Fazzalari, 2008). Biochemical markers of bone metabolism can provide more real-time assessment and can be used as indicators of bone resorption, formation and turnover (Christenson, 1997).

Markers of bone formation are produced via expression of active osteoblasts during various phases of their development and could reflect different aspects of osteoblast function and bone formation. Bone formation markers can be detected in blood plasma or serum. Bone formation markers are categorised as by-products of collagen synthesis, i.e. propeptides of type 1 collagen such as P1CP and P1NP; osteoblast enzymes, i.e. total alkaline phosphatase (ALP) and bone-specific alkaline phosphatase (BALP/BAP); and matrix proteins, i.e. osteocalcin (OCN) which is also known as bone-Gla-protein (BGP) (Banfi *et al.*, 2010; Shetty *et al.*, 2016).

Markers of bone resorption are formed during the bone resorption phase of bone remodeling, which include by-products of osteoclasts activity released during bone resorption. These markers are categorised as collagen degradation products; i.e. telopeptides of type I collagen (C-terminal such as CTX-1 and CTX-matrix metalloproteinases (MMP)) and NTX-1, hydroxyproline and pyridinium crosslinks such as pyridinoline (PYD) and deoxypyridinoline (DPD); noncollagenous proteins, i.e. bone sialoprotein; osteoclastic enzymes, i.e. tartrate-resistant acid phosphatase and cathepsin K; osteocyte activity markers, i.e. receptor activator of nuclear factor

kappa-B ligand (RANKL), osteoprotegerin (OPG), dickkopf-related protein 1 and sclerostin (Banfi *et al.*, 2010; Shetty *et al.*, 2016). Markers of bone resorption has been used to indicate osteoclast activity and / or collagen degradation (Christenson, 1997). High level of bone resorption results in reduced bone density (Jasim and Al-Saadi, 2019). Markers of bone resorption can be determined in either urine or serum (Banfi *et al.*, 2010). Theoretically, bone turnover can be assessed by comparing the amount of substances that are released during resorption with the amount of substances associated with formation (Christenson, 1997). The increased rate of bone resorption rather than its formation creates an imbalance in bone turnover and causing early stage of osteoporosis (Radhakrishna *et al.*, 2011; Dhahir *et al.*, 2017). In general, biomarkers which can be measured for determining the risk of bone fractures are serum calcium, serum phosphorous and 25(OH) D levels, markers of bone formation, i.e. bone-specific alkaline phosphatase (BAP) and osteocalcin, bone resorption markers, i.e C-telopeptide and urinary hydroxyproline (Singh and Srinivasa, 2013).

Bones contain three major minerals, i.e. calcium, phosphorus and magnesium (Sakat *et al.*, 2018). Thus, calcium and phosphorus are biochemical markers related to bone metabolism (Hussain *et al.*, 2019). It is known that vitamin D is essential for calcium absorption and maintaining adequate serum calcium and phosphate concentrations. It is also needed for bone growth and bone remodelling by osteoblasts and osteoclasts (Shwetha and Priya, 2019). Vitamin D deficiency in children and adolescents can impair bone metabolism regulation (Amanzholkyzy *et al.*, 2019). In addition, bone morphogenic proteins (BMP) regulate various growth factors involved in the healing process after bone fracture and promote endochondral bone formation *in vivo* (Kumar, 2018).

2.4.4 Bone Remodelling

Bones continue to renew themselves even after reaching their shape and size during adulthood. Bone remodeling is an ongoing process, whereby osteoclasts first carve out small tunnels in old bone tissue and then osteoblasts rebuild it. The purposes of remodeling are to renew bone tissue before deterioration sets in, and redistributes bone matrix along lines of mechanical stress which allows the bone to adjust its strength through the strategically placing (Valdés-Flores, 2013).

The breakdown of matrix by osteoclasts is called bone resorption. In this process, an osteoclast attaches tightly to the bone surface at the endosteum or periosteum. Then, it releases protein digesting lysosomal enzymes to digest collagen fibers, and other organic substances and acids to dissolve the bone minerals. Several osteoclasts carve out a small tunnel in the old bone. The degraded bone proteins and matrix minerals mainly calcium and phosphorus are excreted by the osteoclasts to the interstitial fluid. These bone resorption products are then diffused into the nearby blood capillaries. Once a small area of bone has been resorbed, osteoclasts depart and osteoblasts move in to rebuild the bone in that area. In order to achieve homeostasis, bone resorbing actions of osteoclasts must balance the bone making actions of osteoblasts. A loss of too much calcium or inadequate formation of new tissue weakens bone tissue (Tortora and Derrickson, 2011; Valdés-Flores, 2013).

2.5 Tendon

2.5.1 Tendon Structure

Tendon is a cord of dense regular connective tissue composed of 70% parallel bundle of collagen fibres that attach a muscle to the periosteum of a bone (Tortora and Derrickson, 2011; Müller *et al.*, 2015). The parallel orientation of the collagen fibres contribute to the high tensile strength of tendons, i.e. receive the highest tensile strength in the body (Müller *et al.*, 2015).

Tenocytes are the major cellular component of a tendon. It maintains the tendon structural integrity by the synthesis and regulation of extracellular matrix. Tenocytes represent only 5% of the normal tissue volume and the remaining is mainly formed by extracellular matrix which comprised 95% of type I Collagen (Stoll *et al.*, 2010; Müller *et al.*, 2015). The other 5% in the extracellular matrix consists of collagen type III and V, and glycosaminoglycan (Müller *et al.*, 2015). The collagen forms fibrils and the collagen fibrils arrangement forms bundles which include the nerves and blood vessels. The sheet of connective tissues, epitenon (inner layer) and paratenon (outer layer) cover the bundles (Figure 2.4). The synovial fluid between both layers allow smooth gliding motion of the tendons (Müller *et al.*, 2015). This low cellularity of the tendon explains the poor healing tendency of the soft tissue (Schulze-Tanzil *et al.*, 2004).

2.5.2 Tendon Functions

Tendons are the structures that bridge the moving (muscle) and static part (bone) of the musculoskeletal system. It bears loads and transferring forces from muscle to bone (Andarawis-Puri *et al.*, 2015; Nourissat *et al.*, 2015) and thus leading to motion (Müller *et al.*, 2015). The arrangement of its collagen structure and non-fibrillar proteins contributes to its ability to support load with stability (Andarawis-Puri *et al.*, 2015). Tendon injuries such as acute traumatic ruptures, chronic overuse and degenerative tendinopathy will lead to impairment in function (Nourissat *et al.*, 2015) and motion (Müller *et al.*, 2015).

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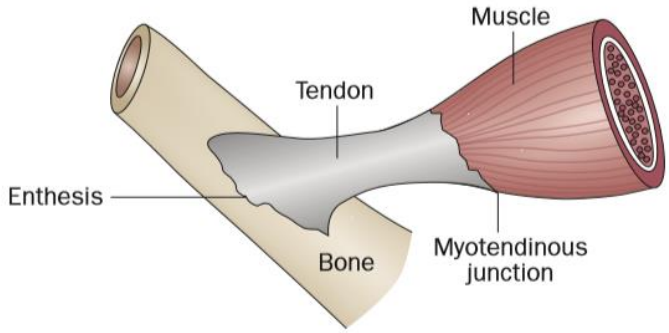

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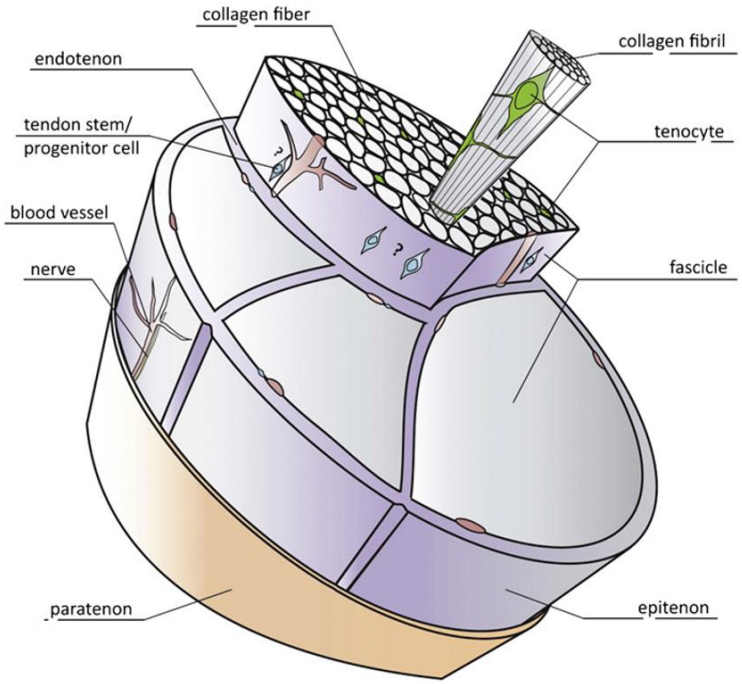

Figure 2.4 Tendon architecture. A. Tendon attaches muscle to bone by the enthesis. Adapted from Nourissat *et al.* (2015). B. A basic tendon structure. Adapted from Docheva *et al.* (2015)

2.5.3 Tendon Healing

Tendon injuries are associated with acute rupture and chronic tendinopathy (Müller *et al.*, 2015). Ligament and tendon are similar in their structures, thus the healing of ligaments is commonly compared and considered to tendon healing (Müller

et al., 2015). Type I collagen is the predominant constituent of a normal tendon, according to Stoll *et al.* (2010), but cells from injured tendons produce more type III collagen (de Mos *et al.*, 2007). Type I collagen possesses tensile strength and stiffness which are crucial during the later stages of healing such as consolidation of tendon following injuries (Berta *et al.*, 2009).

After an ACL reconstruction, the original ligament is replaced by hamstring tendon graft or quadriceps tendon graft. The grafts are secured to the femoral end with an endobutton and the tibial end is secured with a bioabsorbable screw (Suppiah *et al.*, 2013). The healing process involves the new tendon (graft) end and the attached tibia and femoral bone. The tendon healing involves 3 phases, i.e. inflammation, formation and remodelling (Figure 2.5).

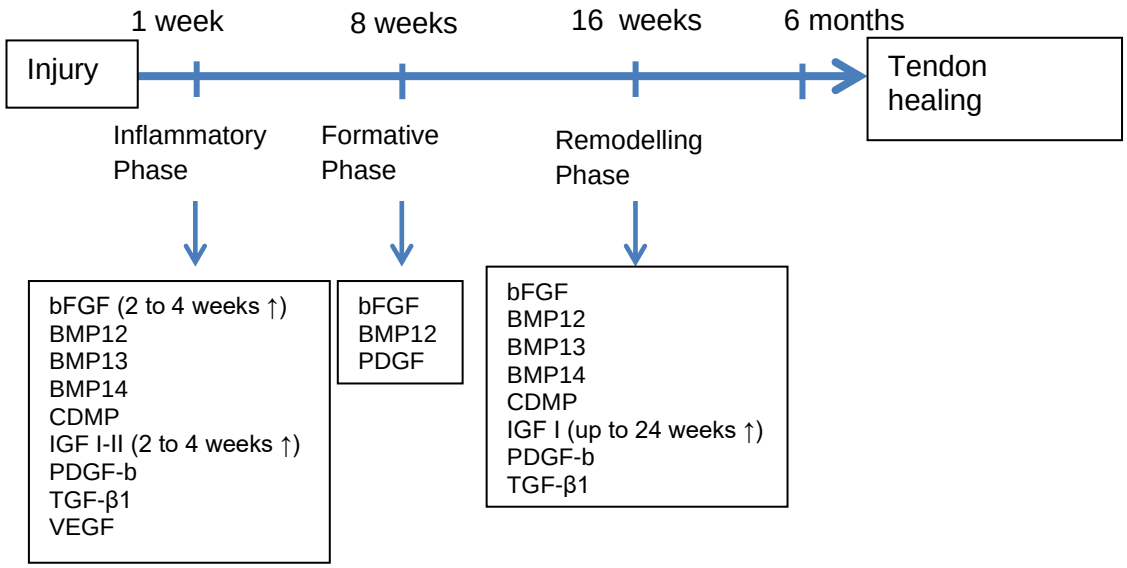

Figure 2.5 The 3 phases of tendon healing process are inflammation phase, formation and remodelling phases. Several growth factors are expressed during the phases. Adapted from Müller *et al.* (2015).

During the haemorragic stage, blood accumulates and clots at the site of injury. Platelets degranulate and simultaenously cytokines and growth factors are released greatly. During the inflammatory phase, neutrophils and macrophages