Chapter

Bio-Engineered Meniscus for Tissue Engineering

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Abstract

Meniscus plays fundamental roles in the knee mechanisms and functions. It acts as a shock absorber where it enables even distribution of forces, and also lubricates knee joints. Meniscal injuries could result to the onset of degenerative osteoarthritis if proper treatments are delayed. To date, treatment of meniscal injuries are more towards conservative methods and surgical approach commonly known as meniscectomy. Attempts to develop scaffolds for meniscus implants from synthetic and biological sources have been done in the recent years. This approach involves a multidisciplinary study known as tissue engineering and regenerative medicine. It involves the combination of three crucial aspects; the choice of chondrogenic/stem cells, bioscaffolds and favourable environmental factors such as growth factors. This chapter discusses and highlights on the currently available meniscal scaffolds that have been explored before. Focus is also directed on the potential of decellularized extracellular matrix (ECM), prepared through sonication treatment that produced scaffolds which mimics natural meniscus. The evaluation of decellularized scaffolds was portrayed through recellularization using cells namely chondrocytes, fibrochondrocytes and stem cells in order to regenerate new functional tissue. In short, this chapter serves as a representation of current approaches aiming in bio-engineering the meniscal scaffolds as meniscus tissue replacement.

Keywords: meniscus, bioscaffolds, decellularization, recellularization, implant

1. Introduction

Tissue engineering is deemed as a promising therapeutic tool in treating disease and injuries. There are three crucial aspects in determining the success of this approach which is by combining cells, biomaterial scaffolds and biologically active molecules such as growth factors. Developing three-dimensional scaffolds or constructs that could serve similarly as native tissue is utterly important. A scaffold should provide support and space for cells to grow, migrate and adhere and continually retain their phenotype. Hence, scaffolds should be biocompatible and biodegradable in the sense that it is able to propagate appropriate signals for the seeded cells execute normal cell homeostasis and processes.

Specifically, numerous growth factors have been used on meniscal fibrochondrocytes to evaluate their potential in healing tears or on protein synthesis using cell culture conditions. The most utilized is transforming growth factor-*b* (TGF-*b*). Studies by Marx et al. and Dunsmore et al. showed that TGF-*b* increases

the proteoglycan synthesis of fibrochondrocytes from all different sections of the meniscus. Besides, hepatocyte growth factor (HGF) or bone-morphogenic protein-2 (BMP-2) was also shown to increase DNA synthesis. To add, cell migration rate improved with the usage of HGF and BMP-2 [1, 2] Other growth factor such as interleukin-1 (IL-1) was also reported to stimulate migration of cells taken from the peripheral third of the tissue. The famous fibroblastic growth factor (FGF) was studies by Webber et al. to show proliferation and stimulate the growth of fibrochondrocytes and human platelet lysate (PL) [3].

Next, the key aspect in the success of tissue engineering is the development of effective scaffolds which can serve to replace injured or damaged tissue. Thus for a successful meniscus replacement, consideration in the optimal scaffolds properties such as biomechanical, immunogenicity and potential to recellularize cells are important to be scrutinized. The highlight of this chapter would be discoursing different options in developing novel scaffolds of meniscal tissue replacement. The meniscus primarily functions as a load bearer and shock absorber. Degenerative or traumatic loss of meniscal tissue sometimes requires multiple surgical procedures to be treated. Ideally, treatment of meniscal injury should be focusing on the preservation and restoration of the meniscus function. However, effort of complete replacement of meniscus is deemed warranted in more severe cases. Various types of transplant have been done at the experimental and clinical level for example allogenic and autologous meniscus transplant. Issues such as host reactions towards major histocompatibility complexes of the donor, lead to progressive decellularization and consecutive failure of the transplant in meniscus allograft transplantation. To date, none of the proposed replacement methods could provide long-term chondroprotective effect. Scaffolds are necessary for tissue engineered meniscus replacement and considerations such as biomechanical, cell toxicity and immunological response of the host towards the scaffolds are crucial to be scrutinized. There is evidence suggesting that degenerative tears in older patients without mechanical symptoms can be effectively treated non-operatively with a structured physical therapy programme as a first line. Even if these patients later require meniscectomy they will still achieve similar functional outcomes than if they had initially been treated surgically. While, partial meniscectomy is more suitable for symptomatic tears which is hard to repair but could still preserve meniscal function.

2. Composition and cell characteristics of meniscus

Meniscus possesses a highly heterogeneous extracellular matrix (ECM) and has a wide range of cell distribution [4]. The ECM of meniscus is categorized by region. Collagen type I accounts for >80% of the composition in the red-red region by dry weight, and the remaining content comprises <1%, including collagen types II, III, IV, VI, and XVIII [5]. There is about 70% of collagen from the dry weight in the white-white region. On the other hand, collagen types II and I account for 60 and 40%, respectively. Next, the cell population in meniscus is categorized into 4 types based on where it resides. First, the outer one-third of the meniscal area is comprised of fibroblast-like cells, demonstrated by elongated shapes while outer periphery contain many cell processes like fibroblasts. Second, the inner two-thirds of the meniscal region mainly contain fibrochondrocytes, oval to round in shape. The inner avascular region comprised more rounded and chondrocyte-like cells. Lastly, fusiform cells are positioned parallel to the meniscal surface at the superficial zone [6].

3. Currently available scaffolds for meniscus

Meniscus scaffolds serves as a platform for the ingrowth of cells and provide support for the remodeling of the native tissue. There are two categories of scaffolds available; synthetic and biological types. There is a wide variety of synthetic scaffolds that have been explored. Polymer based scaffolds have been tested in a few experimental animal studies. Fibrocartilage-like tissue was able to grow in about 3 months' time after the seeding/implantation [7]. They also reported that in control group, degeneration of hyaline cartilage proceeded slower but could not be halted. To add, other bioabsorbable synthetic polymers, such as polyurethane (PU), polyglycolic acid (PGA), polylactic acid, and poly (ε- caprolactone) (PCL) are also widely studies to play important part in supporting the development of meniscal scaffolds [8, 9]. The main advantages of using polymer as the main material in scaffolds development is that they provide versatility, comparable biomechanical properties with native tissues and easily available material supply. However, there are some downside of using synthetic polymers which include their hydrophobic properties, non-biocompatibility issues, immunorejection and inflammation. Thus, many attempts have been done to improve polymer based scaffolds. One of them is Koller et al. whom had attempt to enhance the bioactivity of synthetic scaffolds by adding polyethylene terephthalate (PET) to hyaluronic acid/PCL scaffolds and the results were positive [10]. Scaffolds with PET were recorded to express more type II collagen mRNA and secreted more GAGs than without PET. Besides that, Baker and Mauck developed aligned (AL) scaffolds by electrospinning whereby cells in the AL group showed AL morphology whereas those in the control group took a polygonal shape [11]. Koller et al. improved PGA by reinforcing bonding with PLGA at a ratio of 75:25 in order to fabricate meniscus-like scaffolds [10]. Allogenic meniscal cells were seeded into the scaffolds *in vitro* for 1 week to replace the medial meniscus in rabbits. The results showed neomenisci are able to be regenerated which is similar to the native meniscus. However, the newly form neomenisci were not capable to prevent articular cartilage from further degenerating.

They are two main types of natural scaffolds which are tissue derived materials, extra cellular matrix (ECM) components and decellularized tissue. Some of the tissue derived materials that have been studied comprised of small intestine submucosa (SIS), periosteal tissue, and perichondral tissue. Cook et al. have done studies of SIS in dogs and showed promising results. However the study only lasted for 12 week and no mechanical testing was done [12]. One of the significant tests is their major animal trial which consisted of removing 80% of the medial meniscus of the dog and replacing it with this scaffold. The results after 3 years of implantation were promising which showed no degeneration of the articular cartilage. Walsh and co-workers utilized periosteal tissue in rabbits, showed both hyaline cartilage and bone growing in the repair tissue at the end of the 24- week trial. The results from perichondral tissue were not much better; these 12-month sheep tests gave repair tissue that resembled the meniscus grossly, but the tensile modulus of the repair tissue was much lower than native menisci. Besides that, collagen based scaffolds have also been developed from porcine small intestine submucosa (SIS) but however they failed to portray consistent results in experimental animal studies [13, 14].

Next, naturally derived ECM components include collagen, proteoglycans and elastin molecules. These scaffolds were made from collagen retrieved from bovine tendons and then molded into a circumferential orientation. It is now is already in phase II clinical trials. Collagen scaffolds portrayed a more

convincing result whereby Stone, Rodkey, and co-workers used collagen-GAG scaffolds [15].

Attempts in developing decellularized tissues while retaining its ECM properties have been recently studied. Simple tissues as well as complicated organs have been decellularized and decellularization methods have been optimized to completely remove the cellular components while keeping the ECM intact. ECM scaffolds and substrates are very ideal candidates for tissue engineering as it functions in providing supporting materials for cell regulations and functions such as cell survival, proliferation, morphogenesis and differentiation. By comparing these three types of scaffolds, it is undeniable to claim that decellularized based scaffolds which could still contain ECM properties hold the greatest potential in developing ideal scaffolds for meniscus. The next sub topic will be discussed trials to develop decellularized matrices various techniques; biological, chemical and physical methods.

4. Decellularized meniscal scaffolds

Decellularized scaffolds are expected to provide a better alternative for implant development in tissue engineering. Besides of it being a suitable microenvironment for cells, it also preserves appropriate meniscal geometry. Nevertheless, some challenges should be addressed to obtain ideal meniscal scaffolds. Because of meniscus natural shape, it will make it tougher for cells to evenly penetrate a decellularized meniscus. Not only that, an abundance of bone morphogenetic protein-2 (BMP-2), a member of the TGF-β superfamily will directly stimulate MSC differentiation and can affect cell migration [16]. A study by Minehara et al. used recombinant human bone morphogenetic protein-2 (rhBMP-2) loading in solvent-preserved human menisci to induce migration of chondrocytes into decellularized which successfully induces migration of chondrocytes thus improving proteoglycan production *in vitro* [17]. Thus, decellularized scaffolds face a challenge of allowing better cell penetration and migration which depends on variety kinds of exogenous chemokines.

One of the effective detergents used to decellularize menisci is SDS whereby collagen structure is retained [18]. Biomechanical testing using repetitive ball indentation test (stiffness, N/mm; residual force, N; relative compression force, N) on the processed tissue were similar to those of the intact meniscus, and the histological results showed no residual cells. Besides that, Maier et al. used a self-developed enzymatic process to treat ovine menisci whereby results suggested that native cells and immunogenic proteins (MHC-1/MHC-2) are completely removed while retaining significant biomechanical traits [19]. On the other side, Stabile et al. attempted to improve the porosity of decellularized scaffolds by applying concomitant decellularization and oxidation processes [20]. Azhim et al. implemented neoteric sonication decellularization system to produce decellularized bovine meniscal scaffolds [21]. These scaffolds provide similar biomechanical properties of native meniscus, and were able to completely remove the immunogenic cell components. However, the sonication treatment compromised the native ECM components and collagen fiber arrangement. Thus, using decellularized scaffolds are great alternative for implants but more improvising needs to be done in order for successful integration into patients.

5. Decellularization strategies

Biological scaffolds had been widely used in tissue engineering and regenerative medicine field because it virtually resembles native tissue due to the presence

of versatile bioactive nature within the extracellular matrix components [22]. The preparation of natural biological scaffolds involves a process known as decellularization as shown in **Figure 1**. Decellularization is a process that removes whole cellular components within the existing tissue while preserving the composition, integrity and mechanics of the three dimensional extracellular matrix scaffolds to the extent possible [23, 24]. The elimination of the antigens and cellular components from the tissue-derived scaffolds able to reduce the potential immune rejection and inflammation from occurring [25]. The choice of decellularization method varies depending on the characteristics of the particular tissues itself such as geometric considerations, cells and matrix density [26, 18]. An effective and ideal decellularization process supposedly manages to balance the removal of cellular components and preservation of matrix. There are various techniques that had been developed to obtain the most effective outcomes for fabrication of meniscus bioscaffolds using decellularization process. According to Chen & Kawazoe and Gilbert, to obtain an effective decellularization effect, it is encouraged for the method to be applied in combination [22, 26].

Three main strategies had been performed comprised of biological, chemicals and physical methods. For biological method, it is based on treatments using the enzymes such as proteases (trypsin, dispase), nucleases (DNase & RNase), collagenase, lipase and others [23, 27]. Enzymes are known as substances that have high specificity onto biological substrate which able to cleave or hydrolyze the particular bonds within the tissue structure during decellularization. According to Badylak et al. enzymatic decellularization treatment need a long treatment time and has difficulty to achieve complete cellular components removal alone [28]. Moreover, an extensive treatment time up to 2 days will affecting the ECM ultrastructure components, thus weakening the mechanical properties of the tissues. A study done by Maier et al. treated ovine meniscus with trypsin, collagenase and protease enzyme had successfully decellularized the tissue but with GAGs destruction [19].

The second option is the chemical methods which are further expanded into acid & based treatments, alcohols and also surfactants. According to Seiichi et al., chemical detergents treatment was investigated to be the most commonly used for decellularization technique [29]. The mechanism of acid& bases in decellularizing tissues is by catalyzing hydrolytic degradation of the biomolecules that able to dissociate the DNA from the ECM and disrupting nucleic acid [23, 30]. Chen et al. had performed a decellularization of porcine meniscus using five types of acid consist of acetic acid, formic acid, peracetic acid, succinic acid, malic acid and citric acid with different acid immersion incubation time of 2, 4, 6, 8, 10 and 12 hours [31]. The results portrayed that formic acid with 2 h immersion treatment is the most

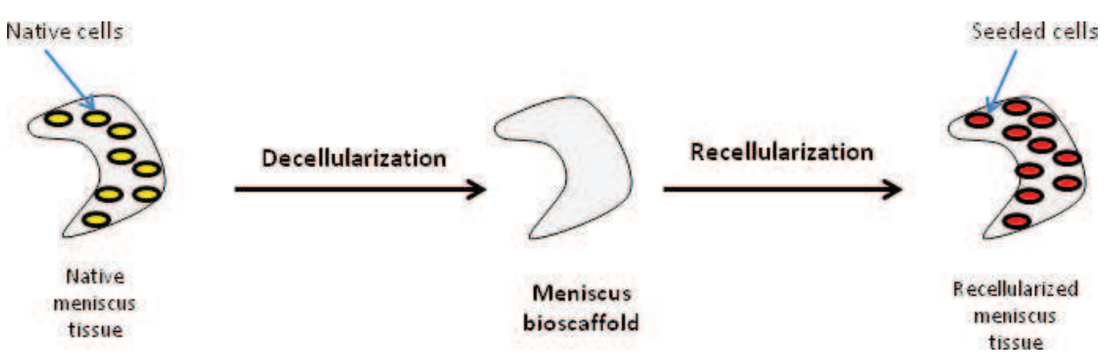

Figure 1.
Decellularization and recellularization of meniscus tissue.

effective because it managed to remove almost 96% of the DNA contents with minor adverse effect on ECM collagens and GAGs [31].

Various types of surfactants widely available for decellularization known as ionic, nonionic and zwitterionic detergents. Ionic detergent is recognized as the strongest acting detergent compared to others which are the most broadly applied in decellularization process [27, 32]. Sodium dodecyl sulfate (SDS), Triton X-200 and sodium deoxycholate are the examples of ionic detergents which are the commonly used for various types of tissue. In 2009, Sandmann et al. investigated and published a study on the effect of 2% SDS with 2 weeks incubation time onto biomechanical strength of human meniscus tissues [33]. It was proven that 2% SDS achieved complete cells removal with minor negative impacts on the biomechanical properties of prepared decellularized human meniscus.

Biological and chemical treatments might result in residual toxicity within the tissues, thus physical treatment for decellularization had been developed. Physical treatments involve freeze–thaw, high hydrostatic pressure, agitation and sonication to disrupt cell membranes and release the cellular components. High hydrostatic pressure system decellularized the tissue by applying pressure from specialized equipment that lead to high cost requirement. This system able to decellularize the tissue in short treatment time but have high risk of extracellular matrix (ECM) ultrastructure disruption due to baric formation of ice crystals throughout the process [34].

In 2010, a novel sonication decellularization with open system had been developed as a new candidate categorized under physical treatment. Sonication system utilizes the ultrasound power assisted by sodium dodecyl sulfate (SDS) to maximize the decellularization efficiency. Researchers and expertise have used ultrasound technology in a wide range of activities such as electrochemistry, food technology, chemical synthesis, material extraction, nanotechnology and surface cleaning [35]. Recently, the application of ultrasound is said to be one of the popular method for cell disruption, emulsification and homogenizing of biological matter [36]. The potential of ultrasound has lead to the development of sonication treatment.

Since 2010, a pilot study had been started by using aorta [21, 38–42] and meniscus tissues [21, 37, 43, 44] as model by testing using different sonication frequency and different percentage of SDS solution concentration that suit with the characteristics of the tissue [21, 37–41]. As for decellularized meniscus tissue preparation, primary study was done using 20 kHz frequency with 2% SDS solution for 10 hours treatment time that resulted in highest cells removal but there was minor presence of cells observed [37]. Thus, further study was done by increasing the sonication to 40 kHz frequency while minimizing the SDS concentration to 0.1% in order to preserve the bioscaffolds properties [21]. This study compared the sonication treated tissue with immersion treatment as control and native tissue. Based on the result of van Gieson staining portrayed in **Figure 2**, it revealed the complete cells removal from meniscus tissue by sonication system (C) where there is no nuclei stained can be observed compared to control (B) and native (A).

In 2014, Azhim et al. had developed a novel closed sonication decellularization system as shown in **Figure 3**. The ultrasonic transducer is the source of sonication that has three different set of frequency of 40, 120 and 170 kHz. The decellularization efficiency of sonication system was contributed mainly by sonication and also SDS detergent. Firstly, sonication influences the process by the disruption of the cell membrane and cell contents release by its phenomenon of acoustic cavitation. Besides that, sonication also assists the flow of SDS solution that thoroughly penetrates

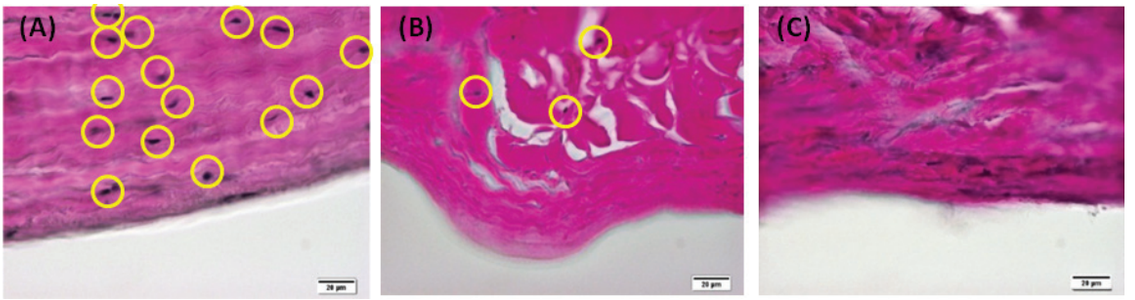

Figure 2.
Photographs of van Gieson staining from surface part of native tissue (A), immersion treated tissue (B), sonication treated tissue (C) with 40× magnification. Yellow circle demonstrated the dark blue nuclei stained [21].

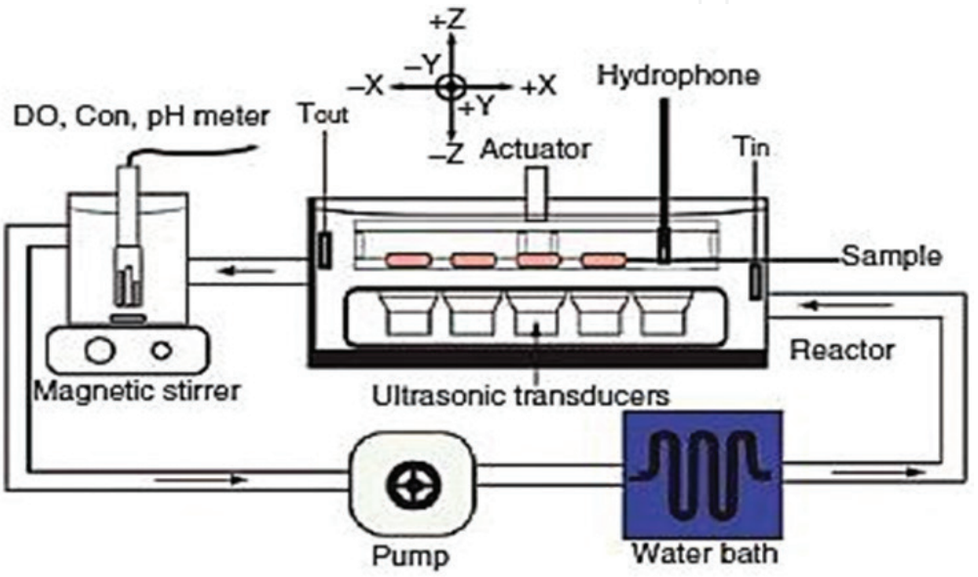

Figure 3.
Sonication decellularization system consists of ultrasonic transducers, pump, cooling water bath, reactor, actuator, temperature monitor, hydrophone, and multiparameter meter that consists of dissolved oxygen (DO), conductivity and pH sensor [39].

within tissue sample. Ionic SDS detergents aid in decellularization by solubilizing nuclear cellular membranes and removing cells residues from the tissue specimens.

6. Recellularization strategies for regeneration of engineered meniscus

In tissue engineering and regenerative medicine, the preparation of engineered meniscus tissue required triad components comprised of scaffolds, cells and growth factors. Basically, the cells will be recellularized onto artificial or natural biological scaffolds with the presence of growth factors for regeneration of tissue. Recellularization using the three dimensional decellularized scaffolds had been one of the attention recently because it resembles natural tissue that have similar biological compositions of the ECM. It is therefore an advantage for the decellularized scaffolds to provide a better environment for the adhesion, differentiation and proliferation of the seeded cells to regenerate functional tissues [45, 46].

According to Chen, recellularization is a crucial part in tissue engineering where cell-seeded constructs can be prepared [31]. This constructs are believed to have many potential advantages for in vitro and in vivo study. First and foremost, it

manages to provide specific microenvironment for cells to proliferate and perform cellular activities for production of ECM. Besides that, cell seeded constructs also ease the integration between scaffolds and native tissues once implanted into the recipient [31].

The advancements of tissue engineering nowadays had the potential to drive meniscus regeneration into clinically relevant strategies and as a promising avenue to improve meniscus repair. For meniscus tissue engineering, various types of cell sources currently being utilized for recellularization process to prepare the cell seeded constructs [47–49]. Different types of cells stimulate different outcome. Ideal cells that suitable for recellularization of meniscus scaffolds should be easy to obtain, low immunogenicity level and able to regenerate the ECM components within the tissue [7]. In this chapter, we will give an overview of promising cell sources that hold great potential meniscus tissue regeneration. There are two main classifications of promising cells available for seeding processes which are stem cells/progenitor cells and mature cells. **Table 1** summarizes the cell sources that are available and broadly applied in meniscus tissue engineering.

6.1 Stem cells/progenitor cells

Stem cells are known as undifferentiated cells that able to proliferate and differentiate into many specialized cell types. Two main characteristics of stem cells that distinguish them with other cells is that stem cells are multipotent where it can be induced into specific tissue with specific functions and it has long term self-renewal [50, 51]. Mesenchymal stem cells are the most studied stem cells that can be harvested from several musculoskeletal tissues such as bone, bone marrow, adipose tissue, synovial membrane and cartilage [47, 52]. Drawbacks of using mesenchymal stem cells lies in the complex understanding of required stimuli to direct the differentiation process to a desired lineage [48]. Besides that, once the microenvironment changes and undergo hypertrophy, the differentiated phenotype can be easily lost [53].

Bone marrow mesenchymal stem cells (BM-MSCs) derived from bone marrow compartment with high proliferative activity is identified as heterogeneous population of stem cells capable to undergo self-renewal [54]. The extraction of BM-MSCs is quit complex because need to undergo bone marrow aspiration procedure which is invasive. BM-MSCs have the ability to differentiate into three lineages of skeletal tissue cells in appropriate in vitro condition; osteoblasts, adipocytes and chondrocytes [55]. Few studies had been attempted in meniscus tissue engineering using BM-MSCs. An in vitro study performed by Yamasaki et al., 2×10^5 cells were seeded onto decellularized rat meniscus in 48 well plate, incubated for 1, 2 and 4 weeks the cell-scaffolds constructs were evaluated with few analyses [56]. The results obtained from the analyses revealed that there was sufficient repopulation of BM-MSCs within the scaffolds. It was noted that there was sufficient generation of ECM compositions such as collagen and GAGs over 4 weeks in culture that resembles the content in control group. Evaluation of mechanical integrity for regenerated tissues portrayed similar stiffness with normal meniscus tissue after incubated 2 weeks in vitro culture. Unfortunately, this study lack of in vivo study which will be done in further study.

Adipose stem cells (ASCs) are considered as alternative cell sources that available in emerging tissue regeneration. This ASCs was discovered in the early 2000 that have high self-renewal capacity and capable to differentiate into three different cell lineages known as adipocytes, osteoblast and chondrocytes if subjected to desired stimuli [47]. It was reported that the isolation of ASCs is easier compared to BM-MSc that are commonly isolated from the intrapatellar fat pad of the knee and is not invasive. [57, 58]. An in vitro study using adipose mesenchymal stem

Classification of cells	Type of cells	Results	References
Stem cells/ progenitor cells	Bone-marrow (BM)-derived MSCs	Extracellular matrices was successfully synthesized at early phase with adequate stiffness observed after 2 weeks culture.	[56]
	Synovium MSCs	Early phase of synovial coverage on injured area was induced and promoted meniscus regeneration	[61]
	Adipose stem cells (ASCs)	Managed to partially formed meniscus-like tissue with no detectable amount of GAG	[59]
	Cartilage progenitor cells (CPCs)	Cells migrated to the tears injury site while promoting bridging across the site	[64]
	Myoblast	High cell yield, rapid proliferation activity with similar biochemical compositions compared to control	[66]
	Articular chondrocytes	Cells proliferated and synthesized ECM production (collagen & gag)	[68]
		High proliferation rates and the tissue generated had notable amount of GAG and collagen type II	[66]
		Cells infiltrated rapidly and distributed evenly in vitro and in vivo at Day 14	[69]
		Chondrocytes able to synthesized meniscal tissue in an *in vivo* situation	[69]
Mature cells	Fibrochondrocytes	Cells survived and proliferated for over 28 days, demonstrating the feasibility of culturing cells within ECM scaffolds	[70]
		The cells manages to self assemble and produced ECM matrix of collagen type I and proteoglycans	[71]
	Fibroblasts	Fibroblastic cell morphology attached and infiltrated into the surface of scaffolds	[75]

Table 1.
Cells sources for meniscus tissues regeneration.

cells seeded on four types of different scaffolds incubated for 3 days had been accomplished by Moradi et al. in 2017 [59]. The cell seeded scaffolds were evaluated for mechanical integrity, biocompatibility and gene expression. The performed real time PCR after 3 weeks culture concluded that ASCs scaffolds had an increase in aggrecan and collagen type II expression compared to control group. For in vivo study, the ASCs scaffolds were implanted into rabbit model for 7 months to discover neomeniscus tissue formation. It was resulted that ASCs scaffolds were found to generate homogenous neomeniscus with poor quality [59].

In 2001, a study conducted by De Bari et al. that characterized the synovial MSCs reported that the cells capable to proliferate extensively and maintained their multilineage differentiation potential in vitro culture [60]. According to Ozeki et al., his study chose synovium MSCs to be seeded onto tendon grafts and assessed

the meniscus regeneration through in vitro and in vivo. It was revealed that the tendon grafts with synovial MSCs succeeded to induce early phase of synovial coverage at the defect site and had better integration with the meniscus defect that promote meniscus regeneration compared to control group [61].

Recently, cartilage progenitor cells (CPCs) represented as new and potential great cell sources available for cartilage and meniscus tissue regeneration. CPCs are basically obtained from the full thickness of mature cartilage. The chondrocytes population was first isolated and need to further undergo differential adhesion to fibronectin process in order to obtain the CPCs [47, 62]. It was identified that CPCs appeared in fibrochondrocytes-like appearance with chondrogenic potential. These CPCs were reviewed to be resistant towards common problems faced by MSCs recognized as terminal differentiation and also hypertrophy [47, 63]. Hypertrophy is a situation where the cells tend to enlarge with increase in cell mass that required more energy [63]**.** According to Williams, CPSs were reported to experience better complex chondrogenesis processes compared to other mesenchymal stem cells [62]. CPCs supplied onto meniscus with tears demonstrated that the cells capable to migrate to the tears site of tears injury while promoting bridging across the site [64]. Unfortunately, the study about CPCS in meniscus tissue engineering is still limited.

Myoblasts are considered as adult stem cells candidate that have multiple differentiation potentials that capable to differentiate mainly into myocytes, adipocytes and osteocytes [65]. Myoblasts were recognized as easily accessed cells with relatively abundant availability with acute donor site morbidity [66, 67]. Chondrogenic differentiation of myoblasts within PLGA scaffolds was concluded to have high cell yields with rapid cells proliferation. Besides that, it was observed that the biochemical compositions and mechanical strength of the implanted myoblast-scaffolds was similar to native tissue [66].

6.2 Mature cells

Mature cells are a nonprogenitor cells including chondrocytes, fibrochondrocytes and fibroblasts able to be derived from cartilage, meniscus and dermal tissues. The application of mature cells somehow managed to overcome major limitation of stem cells that mainly facing the hypertrophy occurrence [47].

As for articular chondrocytes (AC), it is principally derived from articular cartilage and can enzymatically isolate. A recent study was done where chondrocytes were seeded directly onto the layer of decellularized porcine meniscus and incubated for up to 28 days. It was reported that the cells able to proliferate healthily and synthesized the extracellular matrix such as collagen type II and GAG within the scaffolds [68]. Besides that, according to an analysis done by Marsano et al. that seeded the cells onto 3D pellet culture to investigate the growth and post-expansion chondrogenic capacity of chondrocytes [67]. Based on the evaluation done after pellet culture for 2, 4 and 6 weeks, it was indicated that articular chondrocytes resulted in high proliferation rate. This AC generated tissue was found to form abundant GAG and collagen type II in inner avascular region at the same time produced collagen type IV in outer vascular region [67]. There had been two other studies portrayed in **Table 1** that recellularized scaffolds with chondrocytes for meniscus regeneration [69, 70].

Meniscal fibrochondrocytes are investigated as one of the cell sources for meniscus regeneration which can be extracted easily from meniscus [19, 71]. Fibrochondrocytes distributed in all regions of meniscus but dominates more in the vascular outer layer that mainly composed of Type I collagen. The multilineage differentiation particularly favors more towards chondrogenesis and

adipogenesis [72]. A study done by Maier et al. 2007 had successfully seeded the fibrochondrocytes onto the decellularized ovine meniscus. The results showed that the cells managed to infiltrate, survive and proliferate for more than 28 days in the scaffolds [19].

The red-red region of the meniscus that mainly exhibit collagen type II consist of fibroblast-like cells with elongated morphology [73]. This kind of cells frequently derived from reliable sources such as dermal skin that obtained from different body sites and also meniscus tissues. Fibroblasts play important roles in physiological process essentially in ECM production, inflammation and wound healing regulation [74]. Stapleton et al. had constructed decellularized scaffolds using freeze thaw method and Sodium dodecyl sulfate (SDS) detergent [75]. The prepared scaffolds were utilized for recellularization process using dermal fibroblasts cells for 7 days and proceed with implantation in GTKO mice. Based on the evaluation after 7 days of culture in decellularized meniscus, the fibroblasts successfully infiltrate the scaffolds to a depth of 150 mm and the cells were found to appear on the surface with flattened morphology [75].

7. Future prospects for meniscal tissue engineering

There is a dearth in the number of studies done currently in engineering meniscus as compared to other tissues like articular cartilage. The fundamental knowledge on meniscus and its mechanism needs to be fully understood for researchers and clinicians to target a problem. Besides that, knowledge fibrochondrocytes, chondrocytes and other mesenchymal stem cells reactions to a variety of growth factors need to be understood through a variety of tests, both from tissue engineering studies and meniscal repair enhancement studies. Meniscus scaffolds for future application should focus on engineering the entire functional unit which includes meniscal body with anterior and posterior ligaments. Great perception regarding the characteristics of the meniscus constructs in designing for meniscal replacement is important to the overall function. However, an intensive attempt in discovering the appropriate seed cells, biological and mechanical strength stimulation should be given more attention. Broad study on cell sources chose for recellularization should focusing more to in vivo study in animal model. The well performance of in vivo study might depend on several factors such as the quality of neotissue formed during in vitro. More importantly, the fabrication of engineered meniscus tissue with excellent mechanical strength and function that mimic native tissue is the key issue in this field. Good mechanical strength contributes to normal meniscus function. Besides that, three-dimensional construct printing could profit the development of meniscal scaffolds ideally. In short, the development bio-engineering ideal scaffolds for meniscal tissue engineering applications depend on the ability to preserve the biomechanical and biochemical properties of the tissue. The scaffolds should be biocompatible and emit minimum inflammatory effect on to the host.

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