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Growth Factors and Stem Cells for the Management of Anterior Cruciate Ligament Tears

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Abstract: The anterior cruciate ligament (ACL) is fundamental for the knee joint stability. ACL tears are frequent, especially during sport activities, occurring mainly in young and active patients. Nowadays, the gold standard for the management of ACL tears remains the surgical reconstruction with autografts or allografts. New strategies are being developed to resolve the problems of ligament grafting and promote a physiological healing process of ligamentous tissue without requiring surgical reconstruction. Moreover, these strategies can be applicable in association surgical reconstruction and may be useful to promote and accelerate the healing process. The use of growth factors and stem cells seems to offer a new and fascinating solution for the management of ACL tears. The injection of stem cell and/or growth factors in the site of ligamentous injury can potentially enhance the repair process of the physiological tissue. These procedures are still at their infancy, and more *in vivo* and *in vitro* studies are required to clarify the molecular pathways and effectiveness of growth factors and stem cells therapy for the management of ACL tears. This review aims to summarize the current knowledge in the field of growth factors and stem cells for the management of ACL tears.

Keywords: Anterior cruciate ligament; surgical reconstruction; stem cells; growth factors; repair.

INTRODUCTION

Ligaments are fibrous connective tissue bands that connect two or more bones. Ligaments are fundamental for joint stability and they provide resistance to forces to prevent excessive motion [1]. The ligamentous tissue volume is made of extracellular matrix (ECM) and fibroblasts for the 80 % and 20% respectively. Several proteins compose the dry weight of a ligament, such as collagen (75%), elastin (1%), proteoglycans, and glycoproteins. 90% of the collagen is type I and 10% is type III [2]. The role of fibroblasts is to secrete the ECM and to maintain, repair and regenerate new tissue growth [2]. Ligaments are able to sustain high mechanical loads while they present poor regeneration properties when they are injured [3, 4]. Besides, they are repaired by a weaker and disorganized tissue which is prone to re-injury [5]. Growth factors and stem cells seem to play an important role in the process of ligament healing, but the real molecular setting is not clearly understood [4, 5]. In various studies was hypnotized that the lower cells density and vascularity, the lack of oxygen and the poor concentration of nutrients influence the healing of ligaments [2-4, 6, 7].

The anterior cruciate ligament (ACL) is a fundamental ligament for the knee joint [8]. The ACL consists of two functional bundles: the antero-medial bundle (AMB) and postero-lateral bundle (PLB) [9, 10]. The AMB originates more proximally on the femoral site, and inserts antero-medially on the tibia. The PLB originates more distally on the femoral site, and inserts postero-laterally on the tibia [9-11]. The AMB and PLB are oriented nearly parallel with the knee extended, and twist around each other as the knee flexes [12].

Because the knee joint is subjected to high mechanical loads, especially during numerous sport activities, ACL is frequently injured [6, 13-15]. Despite the medial collateral ligament (MCL) of the knee, the injured ACL rarely heals and usually the surgical reconstruction is required to maintain a similar pre-injury physical activity [3, 16]. Patients with injured ACL, mostly during sport activities, usually present recurrent instability of the knee joint [17], which could determine the development of early

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osteoarthritis [18]. Nowadays, the arthroscopic reconstruction using autografts remains the gold-standard for the management of ACL tears [19-22]. Autografts presents high mechanical strength and compatibility, with high revascularization and remodeling capacities [23]. However, several drawbacks can be associated with autografts, such as donor site morbidity and damage, pain and altered harvest site biomechanics [8, 24-26]. On the other hand, allografts exclude the risks of donor site morbidity and pain. However, they are associated with other important risks such as infection, allergic reactions and disease transmission [27-29]. In addition, according to various studies, allografts present lower early cellularity and less revascularization when compared with autografts [27-31].

Following these findings, several new strategies are investigated for ligament regeneration, such as the use of biomaterials, cell therapies, tissue engineering strategies and gene therapy, to promote a more functional and physiological healing of ligaments without requiring surgical reconstruction [2, 32-37]. The aim of the present review is to summarize the role of growth factors and stem cells therapy for the management of ACL tears.

GROWTH FACTORS

Numerous studies demonstrated the role of growth factors in the healing process of ligaments. These growth factors, including insulin like growth factor I (IGF-I), transforming growth factor-β (TGF-β), vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), epidermal growth factor (EGF), and platelet derived growth factor (PDGF), were proved to be able to regulate the cellular activities, promote ligament cell proliferation and induce ECM to achieve the repair of ligaments tears [5, 38-43]. Moreover, they present the capacity to promote an anabolic state of ligamentous cells, improving the cell proliferation and ECM deposition, and may also influence the differentiation of mesenchymal stem cells (MSCs) into fibroblasts [2, 32, 33, 44-49].

According to previous studies observations, better results with the use of growth factors were obtained in the management of MCL tears [3, 16]. In fact, numerous studies conducted on rabbits showed that MCL fibroblasts are responsive to TGF-β1 and EGF [50]. Moreover, TGF-β1 and IGF-I were able to modify the metabolic and biochemical activity of ligamentous cells [51]. In addition, in another study was reported that the topical application of TGF-β1, with or without the association of EGF, increased the ECM synthesis during ligamentous healing processes [52]. Similar results were obtained using PDGF [40]. Another study demonstrated that PDGF, bFGF, and IGF-I can stimulate cell proliferation in ligaments [43]. Kobayashi *et al*. [53], in a canine study, demonstrated that the application of bFGF enhances neovascularization and the formation of granulation tissue in injured ACL. Other authors, by using a mathematical method to analyze cell densities in a wound model in rabbits, showed that cells were able to migrate into cell-free areas of the ligament [54]. Several authors have observed that the combination of growth factors can have synergistic effects [39-41, 52]. DesRosiers *et al*. [38] found that the combination of EGF and PDGF had a better effect than TGF-β1 and IGF-I concerning cell proliferation, while the proteoglycan production was increased by all four factors. Nevertheless, the TGF-β1 presented the strongest effect regarding proteoglycan production [38]. In other studies, it was demonstrated that ligaments treated with a combination of PDGF plus IGF-I and PDGF plus bFGF presented increased rupture force, stiffness, and breaking energy [41], and that synergistic effect of combination of bFGF, TGF-β1, bovine insulin and PDGF were associated with better outcomes than each individual factor [55]. In the last decades was proved that the use of platelet rich plasma (PRP) was able to improve the healing of ligaments. In fact, Liu *et al*. [56] demonstrated that the platelet concentration had a dose-response relationship with proliferation of MSCs such as fibroblasts. Following these findings, other authors considered the PRP as an effective agent for ligament healing [57-59], while in several studies it was concluded that the application of PRP was not associated with any advantage when performed with ACL reconstruction procedures [60-62].

In conclusion, various findings concerning the growth factors therapy are promoting their application in the human species for the management of ACL lesions. Therefore, many aspects remain to be clarified, such as their use or not in association with surgical reconstruction or in complete ACL tears. More *in vitro* and *in vivo* studies, especially randomized trials conducted in a strict scientific fashion, are required to really understand the role and efficacy of growth factors for the management of ACL tears.

STEM CELLS

Nowadays, the use of stem cells in orthopedics practices and related researches is largely improved [63-66]. Numerous studies focused their attention on MSCs, adipose derived stem cells (ASCs) and primary fibroblasts derived from ligaments (PFLs) for the regeneration of ligamentous lesions [32-35, 44, 45, 67-70].

Adult stem cells are also called non-embryonic stem cells (non-ESC), and are usually obtained from the bone marrow. There are two types of non-ESC available: haemopoietic, which generate the blood cells, and MSCs. The MSCs present the capacity to proliferate, differentiate in several tissues and regenerate tissues in case of lesions. Moreover, they present the capacity to secrete soluble factors which can alter the tissue microenvironment in order to repair tissues. Several cytokines and chemokines guide the MSCs to the zone of tissue injury, completing the also called homing process, to allow tissue repair and regeneration, while the molecular mechanism of the mobilization of MSCs from the bone marrow is not clearly understood. The MSCs of the bone marrow have a greater capacity to differentiate in several tissues when compared with other MSCs of different tissue origin, and the bone marrow aspiration is considered the most useful procedure to acquire MSCs. However, several complications are associated with bone marrow aspiration such as pain, infection and increased risks of morbidity. Following these findings, other sources of MSCs have been investigated such as synovium, adipose tissue and tendon [67, 68], but their differentiation and regenerative capacities are not clearly defined [71, 72].

The MSCs are the most used for ligament tissue engineering. This trend is linked to the capacity of MSCs to

easily differentiate into ligament fibroblasts after few weeks [2, 6, 32-34, 44, 69, 73-75]. Traditionally, MSCs have been extracted from bone marrow and other sources such as adipose tissue and synovial fluid [48, 70]. Moreover, the number of MSCs is known to increase following any ligament injury and in degenerative disorders such as osteoarthritis [76]. In a large animal model study involving pigs, the MSCs demonstrated the exhibition of fibroblast phenotype and the capacity to differentiate at 24 weeks postoperatively with the association of silk-based scaffolds [33]. Lim *et al*. [77] performed ACL reconstructions in adult rabbits using hamstring tendon autografts that were associated with MSC by means of fibrin glue carrier. After 8 weeks, better osteointegration and biomechanical properties were observed in the study group than the control group. On the other hand, in another study conducted on rabbits, the MSC have been shown to have stopped proliferation, increased in size and assumed an irregular morphology at 25–30 days after surgical transplantation [34]. Oe *et al*. [35] conducted a study on rats following intra-articular injection of either fresh bone marrow cells (BMC) or cultured MSCs, 1 week after partial ACL transection. The authors found that, after 4 weeks, donor cells were located within the transected ACL in both the BMC and MSC groups. Moreover, the ACL exhibited almost normal histology and more mature spindle cells, while higher levels of TGF-β were found in the ACL tissue of the BMC group. At the end, they concluded that the direct intra-articular BMC transplantation can be an effective solution for the management of partial ruptures of the ACL [35]. Similar results using intra-articular injections have been reported by other researchers [78]. However, these results were obtained in animal studies performed in partial ACL tears. On the other hand, Cheng *et al*. reported better outcomes from the stem cells derived from the ACL itself compared to bone-marrow derived MSCs [79].

Co-cultures are rapidly becoming popular to promote MSCs differentiation by growing them together with fibroblasts [80, 81]. The molecular mechanism of action is based on the cytokines released within the 3-dimensional environment and on the cell-to-cell interactions between the fibroblasts and MSCs. Nevertheless, in one study was proved that the fascia wrapped around the MSC-seeded ACL tissue construct was useful to promote ECM production while did not enhance the physiological tensile load and stiffness [82].

Recently, the use of several techniques and substances, called bioreactors, were largely used in the orthopedic research in association with MSCs [83-87]. Bioreactors can be chemical, mechanical, electrical, or magnetic, and had demonstrated the ability to promote and accelerate the differentiation process of MSCs into fibroblasts [85]. The chemical stimulation technique consists on the use of growth factors [38-43, 49, 50, 52, 74, 88-90]. The major problem associated with this procedure was the possibility to maintain sufficient quantities of growth factor within the local tissue. To resolve this pitfall, the gene transfer technology has been introduced in association with growth factors injection [91]. Wei *et al*. transfected the bone-marrow-derived MSC with adenovirus vector encoding TGF-β1, VEGF, or TGF-β1/VEGF before surgical implantation into experimental ACL grafts [92], finding an immediate improvement of angiogenesis and excellent mechanical properties 24 weeks after surgery. Mechanical stimulation is another method used to induce the differentiation of MSCs into the fibroblast phenotype. The trigger of the cell surface stretch receptors determines the activation of the anabolic phenotype of the cells, resulting in improving synthesis of ECM proteins [93-95]. Altman *et al*. [94], by using a specialized bioreactor, observed that helically organized collagen fibers were formed in the direction of the load.

In summary, MSCs can be considered a possible solution to regenerate injured ligaments. Numerous advantages are associated with the MSCs injection in ACL tears including: the use of autologous cells, the ability to differentiate into fibroblasts and secrete the ECM, the capacity to regenerate a ligamentous tissue without requiring surgical intervention. Nevertheless, better results were obtained in MCL or in partial ACL tears. Co-cultures, such as bioreactors, can be a possible solution to improve MSCs differentiation and proliferation capacities. Besides, the use of bioreactors, especially growth factors and mechanical stress, in association with MSCs, can be considered an available technique to promote the MSCs differentiation into fibroblasts cells, achieving a physiological ligamentous tissue with good molecular and biomechanical properties.

CONCLUSIONS

The use of growth factors offers a promising solution for the management of ACL tears, especially in young and active patients. According to our literature review, better results were obtained in the treatment of MCL or ACL partial tears. Several advantageous outcomes are linked with the intra-articular injection of growth factors, such as ligamentous cells proliferation, ECM deposition and the MSCs differentiation into ligament fibroblasts cells [2, 32, 33, 44-49]. Furthermore, the use of growth factors in association with MSCs offers several well-known advantages, fully demonstrated in various *in vivo* and *in vitro* studies.

On the other hand, the MSCs can be considered an effective solution for the management of ACL tears, associated with several benefits such as the use of autologous cells, the ability to differentiate into fibroblasts at around 2–4 weeks and the relative ease of procurement. Moreover, the MSCs demonstrated the capacity to secrete the ECM and regenerate ligamentous tissue when injuries occurred. Finally, the use of co-cultures and bioreactors can be useful to accelerate and promote the differentiation process of MSCs into fibroblasts.

The application of growth factors and MSCs for the treatment of ACL tears in the human species seems fascinating such as premature, but also possible in the very near future. Further *in vivo* studies, especially randomized trials on large animal models, are required to clarify the effectiveness of growth factors and MSCs for the management of ACL tears.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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