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Mesenchymal stem cells and tendon healing

Alphonsus KS Chong[1], James Chang[2], James CH Go[3]

[1]Department of Hand and Reconstructive Microsurgery, National University Hospital, Singapore , [2]Division of Plastic Surgery, Stanford University Medical Center, Stanford, California, USA, [3]Department of Orthopaedic Surgery, Yong Loo Lin School of Medicine, National University of Singapore, Singapore

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1. ABSTRACT

Tendons transmit forces generated by muscle to move the joints they cross. Tendon problems are complicated by slow and incomplete healing as well as re-injury. Mesenchymal stem cell based therapies show promise in improving outcomes. Much of the work has been experimental, although early clinical use in equine strain-induced tendon injury supports the efficacy of this strategy. While much has been studied about the mechanisms of action of implanted MSCs, the relative importance of the various mechanisms is still unknown. Key areas of research that could prove pivotal in the clinical use of MSCs include the use of allogeneic cells, optimization of MSC culture, gene therapy, and mechanical stimulation techniques.

2. INTRODUCTION

Tendon problems are complicated by slow and incomplete healing. Acute flexor tendon injuries and Achilles tendon ruptures are two common tendon problems which require long rehabilitation and prolonged time off work, and are associated with significant complications (1-3). Tissue engineering strategies (4), particularly mesenchymal stem cell based therapies, are promising to improve outcomes in tendon disorders (5, 6). This review outlines the concepts of tendon disorders and mesenchymal stem cell therapy, highlighting significant advances and discussing key areas for further work.

3. TENDONS – STRUCTURE AND FUNCTION

Tendons are dense connective tissue structures connecting muscle to bone. The junction where the tendon

inserts into the bone is the enthesis. Two forms of entheses exist. The first is the fibrous or direct type, where the tendon inserts directly into bone, typically seen on metaphyses and diaphyses of long bones. The second form is the fibrocartilage enthesis, where a transitional zone of fibrocartilage is seen, usually in epiphyses and apophyses. (7) They transmit forces generated by muscle to move the joints they cross. The mechanical behavior of tendon is critical to this function. Tendons have a hierarchical structure which affects their biomechanical properties (8). However, tendons are not merely passive and inert structures conducting forces. Their compliance is important for elastic energy storage and supplies mechanical work for activities such as running (7, 9). In addition, they are capable of biological adaptation following changes to their mechanical environment resulting from injury, disease or following exercise (10-12).

Tendons are often discussed in concert with ligaments, which are related connective tissues to tendon and connect bone to bone. While tendons and ligaments have structural similarities, their functions are different (7). The primary function of ligaments is to maintain stability of joints by preventing excessive or abnormal motion. This function is assisted by their contribution to proprioception. In addition, they may provide attachment to muscles and prevent tendons from bowstringing (7).

Two forms of tendons exist. Intrasynovial tendons have a synovial sheath such as the flexor tendons of the hand and feet. Other tendons are extrasynovial. The synovial sheath of intrasynovial tendons, found in the hand and foot, allows the tendons to glide freely and produces synovial fluid to contribute to tendon nutrition. The synovial sheath resemble the synovium of joints. There is a visceral synovial sheath which covers the tendon which connects to an outer parietal sheath carrying blood vessels, lymphatics and nerves to and from the tendon. The synovial fluid within the sheath plays a vital role in tendon nutrition, contributing 90% of the nutrition (13), as intrasynovial tendons do not have a well developed intratendinous vascular network. Studies of tendon healing have demonstrated significant differences in healing of extrasynovial and intrasynovial tendons. Profound differences have been found in repair responses of intrasynovial and extrasynovial tendon grafts. In general, intrasynovial tendon grafts appear to heal via intrinsic mechanisms. Extrasynovial tendon grafts, on the other hand, tended to show widespread ingrowth of new vessels and cells with adhesion formation (14).

4. TENDON DISORDERS

Two commonly encountered clinical tendon problems are acute injury or chronic tendinopathy (15, 16) . Tendon injuries are common and occur as a result of direct trauma or rupture following sports or other rigorous activities. The direct and indirect costs of tendon injuries are high, especially since these injuries occur in young and economically active individuals (17). Long term complications are also seen. For example, following flexor tendon injury, many patients are left with permanent loss of range-of-motion, thus impacting on quality of life (18). In addition, as the tendon never regains its original strength, the risk of repeat injuries remains (19).

Another common problem is tendinopathy, a condition marked by failed healing response of the tendon (20, 21). This condition causes pain and tenderness, and can be complicated by becoming a chronic condition or tendon rupture. Tendinopathy is most commonly seen in the shoulders, elbows, knees, hips, heels or wrists, and is most commonly caused by injury or overuse due to excessive repetitive movements. Clinical examples include Achilles tenodinopathy, rotator cuff tendinopathy and trigger finger (22-24). Tendinopathy can also be associated with inflammatory diseases such as rheumatoid arthritis. In its early stages, tendinopathy may respond to rest and activity modification or treatment with steroids. However, once chronic or associated with tendon rupture, surgery to the damaged tendon or repair or reconstruction of the tendon is necessary. While there is little published research on the role of MSCs in uncomplicated human tendinopathy, developments on a similar condition affecting horses will be reviewed.

5. MESENCHYMAL STEM CELLS

In the past decade, there has been increasing attempts to modulate tendon healing to improve the outcome in tendon disorders. This has included the using of cytokines or growth factors, as well as gene therapy (25-28). One important strategy has been the use of cells, particularly mesenchymal stem cells, to achieve this. Stem cells have two main features, the ability to differentiate along different lineage pathways and the ability for self-renewal (29). Two major types of stem cells have been described, embryonic stem cells and adult stem cells. Embryonic stem cells are obtained from the inner cell mass of the blastocyst and are associated with ethical issues (30) and the risk of tumorigenesis (31-33). These problems are less pronounced in adult stem cells, which still maintain their multipotency, and make them an attractive choice for clinical applications.

Mesenchymal stem cells (MSCs) are stromal cells that have the ability for self-renewal and exhibit the capability for multi-lineage differentiation (34, 35). While MSCs have been isolated from a variety of tissues including muscle, umbilical cord and synovium (36), most studies utilize MSCs from bone marrow or adipose tissue. This is because the ease of harvest and quantity obtained make these sources most practical for experimental and possible clinical applications.

A clear understanding of the determinants of "stemness" in stem cells is important to fully maximize the potential of these cells, but the search for a molecular code for stemness has been elusive. One possible reason is that the properties of self-renewal and differential potential, although key features of these cells, are not by themselves exclusive to stem cells. Stem cells may be viewed as cells halted in their progression towards differentiation. This state is maintained both by intrinsic cell properties and the environmental niche where the stem cell resides (37).

6. MECHANISMS OF ACTION OF MSCS

Several possible mechanisms of action of MSCs to enhance healing have been suggested. There is *in vivo* and *in vitro* evidence supporting the possibility of these mechanisms. However at this point in time it is still uncertain which of these mechanisms are important in the *in vivo* situation, as well as their relative contributions to the overall healing process. MSCs may act in an autocrine and paracrine fashion to enhance tendon healing (38). Studies using MSCs to treat myocardial infarction support this mechanism of action. MSC-conditioned media injected into infarct sites following myocardial infarction limit the number of apoptotic cells, reduce the infracted area and improve left ventricular function (39, 40).

The ability of mesenchymal stem cells to differentiate into lineage-specific cells is clear. However, it is uncertain if this is the major pathway by which the stem cells produce their effects. Only small numbers of MSC differentiate at all, and they may not fully integrate with the host tissue (41).

Finally, human BMSCs transfer mitochondria to cells with non-functional mitochondria. A recent study showed that adult mesenchymal stem cells can dynamically transfer mitochondria or mtDNA to rescue aerobic respiration (42). Injuries to mitochondria are common early events in animal models, explaining why functional improvements may be seen despite lack of long-term incorporation of implanted cells.

7. ENHANCING TENDON HEALING WITH MSCS

A mesenchymal stem cell-collagen gel construct to bridge a 1 cm rabbit Achilles tendon defect showed better structural and material properties when compared to controls up to 12 weeks following implantation (43). Using a similar experimental model, a tendon construct of knitted poly-lactide-co-glycolide (PLGA) seeded with allogeneic bone marrow stromal cells (bMSCs) showed improved histological and mechanical properties up to 12 weeks after implantation (44). Transgenic rat studies have shown than implanted mesenchymal stem cells could be detected at the site of healing ligaments up to 28 days. The implanted cells were morphologically similar to the surrounding cells, suggesting that they had differentiated (45). Much of the experimental work has been performed using models of tendon defects or gaps. However, in the clinical setting, tendon ruptures or lacerations which can be primarily repaired are much more common, and exhibit slow healing and adhesion formation. Work on the effect of mesenchymal stem cell therapy to modulate healing is much more limited. We studied the use of bone marrow derived mesenchymal stem cells in a fibrin carrier to enhance healing after rabbit Achilles tendon primary healing. Our results showed improved histological, nuclear morphometric and material properties at 3 weeks when compared to controls. However, results were similar at 12 weeks. This suggests that mesenchymal stem cells accelerate but do not change the ultimate result of tendon healing. One concern about the implantation of MSCs within an injury site, particularly with the use of allogeneic cells is whether this will cause increased inflammation and scarring. However, we did not see an increase in inflammatory cells at the repair site (46).

While the work described above have been in laboratory animals, MSCs have been applied clinically in horses, mainly in the treatment of strain-induced tendon injury. This is of major importance among competitive racehorse owners, where superficial digital flexor tendinopathy is a common source of wastage. The condition is associated with a failure to return to competitive performance and may recur (47). Clearly, the results of these trials are of great interest to the medical community interested in translating this technology to human applications. However there are significant differences in the equine strain-induced flexor tendon injury compared to the acute injuries and tendinopathy in humans. In equine digital flexor tendon strain injuries, the lesion is seen in the central core of the tendon with relatively intact surrounding tendon or paratenon in the periphery. In contrast, acute tendon injuries in humans are usually complete ruptures or lacerations. Smith and colleagues re-implanted *in-vitro* expanded autologous MSCs into a damaged superficial digital flexor tendon of a pony who had suffered a strain-induced injury and showed no post-procedural tendon swelling or lameness. Direct bone marrow aspirate without enrichment for MSCs have also been used successfully. In 100 horses with suspensory ligament injuries receiving bone marrow, 84% of them returned to full work compared to 15% of 66 horses managed conservatively (19).

8. KEY AREAS FOR DEVELOPMENT

The work thus far has laid the important foundations necessary for exploring the usefulness of MSC therapy in enhancing tendon healing. To translate the technology to the bedside, much work is still needed. While a better understanding of the basic mechanisms of tendon development and healing is important and will continue, there are four areas that could provide the cornerstones in the quest for successful clinical application of MSCs. These are: the use of allogeneic MSCs, optimal MSC culture conditions, gene therapy and mechanical stimulation.

8.1 Use of Allogeneic MSCs

Much work has been done using autologous cells for tissue engineering applications. This is clearly advantageous from the point of histocompatability (48). Transmission of infectious agents is also eliminated, although the risk of introduction of infectious agents during the e*x vivo* expansion step is still present. Finally, the regulatory hurdles required to bring this technology to clinical use is much less when compared to allogeneic cells.

However the use of allogeneic cells has several benefits. In clinical situations such as acute tendon injuries, repair or reconstruction needs to be within days to maximize outcome potential. In this instance, it is not possible to allow cell harvest and *ex vivo* expansion before

implantation. An "off-the-shelf" solution using allogeneic cells would overcome this problem. In addition, there is individual variability in MSC harvest and detrimental changes to MSCs with age. With increasing age, the number, differentiation potential and lifespan of MSCs decrease (49-52). This has implications in the treatment of degenerative disorders common in the older age groups. Finally, the use of allogeneic cells will obviate the need for harvest, minimizing the risk of complications.

Using MSCs as an allogeneic cell source is particularly advantageous as there is mounting convincing evidence to show that MSCs are able to circumvent the normal immune response associated with the mismatched allogeneic tissue. Several clinical trials utilizing allogeneic MSCs have shown benefit with the use of these cells. Gene-marked allogeneic bone marrow derived mesenchymal stem cells showed incorporation and increased growth velocity in 5 of 6 children undergoing standard bone marrow transplantation for severe osteogenesis imperfecta (53). This occurred despite the lack of pre- or post-procedure chemotherapy. The immunomodulatory effects of mesenchymal stem cells were used to treat severe steroid resistant acute graft-versus-host disease (GVHD) in 8 patients. Resolution of acute GVHD was seen in 6 patients and survival was significantly better compared to a group of 16 patients not receiving MSCs (54). Allogeneic bone marrow derived mesenchymal stem cells also incorporated successfully in patients with metachromatic leukodystrophy and Hurler syndrome (55).

The suggested mechanisms by which bone marrow mesenchymal stem cells avoid immune rejection have been reviewed recently (56). These include hypoimmunogenicity of the MSCs themselves, the prevention of T cell responses by MSCs and the fact that the MSCs can induce a suppressive local environment.

8.2. Optimization of MSC culture

One important step in the development of MSC based clinical applications is *in vitro* expansion of the cells, as the number of stem cells that can be feasibly harvested using current techniques is limited. Standard culture media contain nonhuman serum, commonly obtained from fetal calves. This may be a source of possible contamination (e.g. bovine spongiform encephalopathy from prions) or immune reaction to xenogenic proteins. This problem is more than theoretical as significant reactions such as arthus-like reactions and cellular cardiomyoplasty causing sudden death after fetal calf serum based therapies have been described (57, 58). This issue must be carefully addressed before widespread clinical use of MSC based treatments will reach the bedside.

While efforts have been made to develop human serum and blood derived and serum free alternatives to nonhuman serum, no clear superior alternative has yet to emerge (59). Autologous serum/plasma preserves differential potential and increases cell proliferation but is of limited availability and has been associated with variability. Studies using allogeneic serum and plasma are contradictory. Some have been successful in isolating and expanding MSCs but others have reported senescence and growth arrest. (60-63).

8.3. Gene Therapy

Gene therapy involves transfer of genetic material into individuals for therapeutic purposes by altering cellular function or structure at the molecular level (64). Recently, there has been great interest in using MSCs as the cellular vehicle for these genes, due to the capacity for self-renewal, differentiation potential and homing abilities of the MSCs. Various forms of viral and non-viral approaches to introduce transgenes into MSCs have been used (65). Successful introduction of transgenes *in vivo* has been demonstrated (66, 67). Adenovirus mediated *in vitro* BMP-12 gene transfer into tenocytes increased type I collagen synthesis, and resulted in a two-fold increase of tensile strength and stiffness of repaired flexor tendons (28). Both BMP 14 and GDF 5 transfer have also been shown to improve the quality of healing in animal models of tendon healing (68, 69). The introduction of the appropriate therapeutic genes could further enhance the effectiveness of MSCs for tendon healing.

8.4. The Role of Mechanical Stimulation

Mechanical stress is an important modulator of cell physiology (70). In tendon, biomechanical properties adapt to disuse and exercise (71). This insight has lead to efforts to use mechanical stimulation *ex vivo* in the form of tissue "bioreactors" to enhance the properties of tissue engineered tendon constructs before implantation *in vivo*, given the inferior biomechanical properties of tissue engineered constructs when compared to native tendon (72). Intermittent uniaxial strain has been shown to improve the ultimate tensile stress of engineered tendons by 3 fold (73). Constant strain when applied *in vitro* has also been shown to improve the biomechanical characteristics of tendon constructs (74). Further work utilizing MSCs with mechanical stimulation in the development of tissue engineered tendons could prove this to be a winning combination.

9. NAVIGATING THE REGULATORY FRAMEWORK FOR MSC BASED THERAPIES

In the quest towards a stem-cell based clinical product, a series of regulatory hurdles will need to be crossed. While this is country specific, the process of approval from the American Food and Drug Administration (FDA) will most important, because the market there will be one of the largest for the foreseeable future. The FDA has recently promulgated a comprehensive regulatory framework covering stem-cell based products (75). As there is a high likelihood that stem-cell based products will come from academic laboratories, familiarity with the regulations and how to address the important issues is important. These issues include donor cell screening, cell contamination and damage, cell purity and potency and *in vivo* safety and efficacy of the product (76) .

From the current state of affairs, much work still needs to be performed to address each of these issues before an MSC based product for tendon repair will be available. For MSCs, determining cell purity and potency is particularly challenging because of the lack of specific markers to clearly identify mesenchymal stem cells.

10. PERSPECTIVE

The advances in understanding the biology of tendon healing, stem cell biology and the early work in MSC therapy to enhance tendon healing is opening a new era in the treatment of tendon disorders. Clearly much work is still necessary, but there is much promise that MSCs will have an important role in the management of tendon disorders in the near future.

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Send correspondence to: Alphonsus KS Chong, Department of Hand and Reconstructive Microsurgery, National University Hospital, 5 Lower Kent Ridge Road, Singapore 119074, Tel: 6567725549, Fax: 6567732558, E-mail: alfchong@gmail.com

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