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Tendon tissue engineering with mesenchymal stem cells and biografts: an option for large tendon defects?

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1. ABSTRACT

The most important factors in the tissue engineering approach to tissue repair and regeneration are the use of appropriate cells and scaffolds. Mesenchymal stem cells (MSCs) are one of the most promising seed cells, which can be easily derived and have the potential to differentiate into various mesenchymal cell types as well as tenocytes *in vitro* and *in vivo*. Biological tendon grafts are the most common choice in current clinical practice, as they possess physical structure, strength and biocompatibility. We review the latest research findings on MSC-based tendon tissue engineering and recent advances in biological graft research.

2. INTRODUCTION

It is difficult for tendon to regenerate after injury. This limited capability of tendons to regenerate poses a challenge to tendon tissue engineering, and emphasizes the importance of developing a procedure to do so.

So far, fewer studies have been performed on tendon tissue engineering when compared to the extensive work on bone and cartilage tissue engineering. Moreover, tendon tissue engineering research has not yet undergone translation from the bench to the bedside. The continued development of tendon tissue engineering will depend on

the identification and characterization of appropriate sources of cells as well as the development of practical scaffolds. The identification of an optimal cell source for a particular tissue engineering application will depend on rigorous characterization with regards to plasticity, propagation, and control of differentiation. To guide the organization, growth, and differentiation of cells in tissue engineered constructs, appropriate scaffolds are needed to provide mechanical support and physical, chemical, and mechanical cues in forming functional tissues. Based on the above criteria and the current state of the art, mesenchymal stem cells (MSCs) seem to be the optimal seed cells, and decellularized tendon grafts the most practical scaffolds at present.

3. MSC AS SEED CELLS FOR TENDON REPAIR

Multiple cell types have been seeded within different scaffolds for tendon tissue engineering. As tenocytes are the predominant cell type in tissues, they have been a frequent cell source for active repair (1-17). However, tenocytes are fully differentiated cells with limited lifespan. Moreover, the limitation of donor tissue and morbidity in the donor site prohibit the clinical application of autologous tenocytes (6).

With the rapid development of stem cell biology and technology, clinicians and researchers used stem cells for tendon repair and regeneration. Compared to embryonic stem cells (ESCs), adult stem cells, especially MSCs, have been much more extensively investigated for tissue engineering. MSCs were efficiently expanded and differentiated into cells of a variety of specialized mesenchymal tissues including bone, cartilage, fat, muscle, tendon/ligament and marrow stroma following appropriate stimulation (18-20). They exhibited the potential for a wide range of therapeutic applications through autologous and allogeneic stem cell transplantation (18-20). Moreover, MSCs have been easily harvested and cultured from various types of connective tissue, such as bone marrow (20), periosteum (21), synovium (22), muscle (23), adipose tissue (24), umbilical cord (25), articular cartilage (26), tendon (27, 28) and periodontal ligament (29), amongst others.

Among those sources of MSCs, bone marrow derived MSCs seem to be most appropriate as seed cells for tendon repair. Participatation of MSCs was demonstrated in the long-term remodeling of degradable small intestine submucosa-extracellular matrix scaffold when used as a repair device in the murine model of Achilles tendon repair (30). Furthermore, circulation-derived MSCs where shown to contribute to the rat tendon healing (31). These studies suggest that MSCs from bone marrow source may participate in the natural process of tendon repair. Moreover, bone marrow derived MSCs produced more extracellular matrix with much faster cell proliferation than either anterior cruciate ligament (ACL) cells or dermal fibroblasts. (32)

On the other hand, human bone marrow derived MSCs express low class I human leukocyte antigen (HLA) and do not express class II HLA, which may limit immune recognition (18). In addition to the absence of antigen, MSCs have been shown to exert *in vitro* immunosuppressive activities on activated T cells (33) and inhibition of most B-cell effector functions (34). Also, MSCs were able to secret a variety of cytokines and growth factors with both paracrine and autocrine activities, which could suppress the local immune system, inhibit fibrosis (scar formation) and apoptosis, enhance angiogenesis, and stimulate mitosis and differentiation of tissue-intrinsic reparative procedure (35).

Not only MSCs, but also progenitor cells differentiated from MSC retained their immunoprivilege and immunomodulatory properties *in vitro*, although the later was lost following transplantation (36). To investigate viability and function, allogeneic bone marrow derived MSCs were implanted into the patellar tendon defect of rabbits. The labeled allogeneic MSCs were viable up to eight weeks after implantation, and differentiated into tenocyte-like spindle shape cells. Allogeneic MSCs may therefore be used as “off -the -shelf” seed cells for tendon repair and tissue engineering.

4. MSC BASED TENDON TISSUE ENGINEERING

Many recent studies used bone marrow derived MSCs (2, 37-60) as candidate seed cells for tendon tissue engineering. Most of them compared MSC seeded scaffolds with scaffolds alone (37-60). Table 1 presents a synopsis of the current tendon tissue engineering experiments with cells *in vivo* (Table 1). Researchers have applied undifferentiated autologous bone marrow derived MSC to collagen gels (50, 51, 53, 57) and collagen sponges (58, 59) for both patellar tendon (51, 53-59, 61-64) and Achilles tendon repair (50, 52, 57).

MSC with collagen matrix were able to improve the quality of tendon repair: Several studies showed that autologous MSCs have potential for tendon repair (50, 51, 54). Tissues treated with MSC-collagen gel exhibited faster repair rate and were significantly stronger and stiffer than natural healing tissues. Their maximum force and stiffness were, respectively, 174% and 183% greater than those of natural repairs at 26 weeks (54). Although the grafted repairs were still only 17–25% and 10–19% of normal maximum force and stiffness respectively (54), they were higher than the *in vivo* living peak forces (63, 65, 66) of the tendons measured, suggesting that the MSC repaired tendon were able to fulfill their physical function. Following those experiments, the researchers modified the cell deliver matrix. With the use of collagen-gel-sponge to deliver MSC, the average maximum force and maximum stress of the tendon repairs achieved 50 and 85% of the normal values for the Achilles tendon (57), and 60% and 50% for the patellar tendon (58). Although the failure forces were above the *in vivo* force, the large additional repair displacement (56-60) and moderate crimp pattern compared to the normal tendon (54, 58) indicated that the repair tendon is still inferior to the normal tendon.

The relationship between cell density and the quality of tendon repair was investigated: Gel contraction

Table 1. Tendon tissue engineering *in vivo*

Scaffold structure and cell	Animal and tissue	Outcome	Reference
Knitted PLGA scaffold Allogeneic MSCs	Achilles tendon Rabbit	The repaired tissues composed of bounds of collagen fibers with a crimp pattern .The stiffness and modulus were 87% and 62.6% of normal value.	46, 48
Fibrin gel Allogeneic MSCs	Patellar tendon Rabbit	MSCs survived as long as 8 weeks at tendon reaper site and changed into spindle shape cells	44
Collagen gel Autologous MSCs	Achilles tendon Rabbit	MSC-seeded repairs were twice those for natural healing as early as 4 weeks, more rapid return to normal function	50
Collagen gel Autologous MSCs	Patellar tendon Rabbit	improve its biomechanical properties18% to 33%, at 4 weeks	51
Collagen gel Autologous MSCs	Patellar tendon. Rabbit	MSC-collagen composites significantly improves the biomechanical properties of tendon repair tissues, greater MSC concentrations produced no additional significant histological or biomechanical improvement.	54
Collagen Sponge Autologous MSCs	Patellar tendon window defects. Rabbit	The failure force of biomechanical stimulated tendon repair was up to 150% of the peak in vivo force values recorded	59
Unwoven PGA fibers wrapped with an acellular SIS. Tenocytes	Flexor digitorum tendon Hen	At 14 weeks, the engineered tendons displayed a typical tendon structure hardly distinguishable from that of normal tendon and the breaking strength of the engineered tendons reached 83 percent of normal tendon.	6
Fibrin sealant MSC	Achilles tendon Rabbit	Collagen fibers denser and more organized, increase in modulus	40
Porcine SIS (Restore) and type I/III collagen bioscaffold (ACI-Maix) Tenocytes	Rotator cuff tendon rabbit	Autologous tenocytes on collagen based bioscaffold results in better rotator cuff tendon healing and remodeling	17

Abbreviations: SIS: small intestine submucosa, PLGA: poly(dl-lactide-co-glycolide)

was measured to at about 30% of original diameter when seeded with MSCs, producing elongated cells that aligned along the suture axis (50). Increasing the seeding density (below a threshold value 0.5 million cells/mL (60)) accelerated the rate of collagen gel contraction, improved the alignment and appearance of the cell nuclei up to 72 hours in culture (53). Varying cell-seeding density (1, 4, and 8 million cells/mL) (54) and the lower cell-to-collagen ratio (0.1 million and 1 million cell/mL) at two collagen concentrations (1.3 and 2.6 mg/mL)) (56, 58) did not affect the biomechanical properties of the repair tissues.

MSCs might induce ectopic bone formation at tendon repair site: Ectopic bones were found in 28% of MSC-treated rabbit tendons (54, 62). Lowering cell-to-collagen ratios may have reduced, but did not eliminate ectopic ossification (56, 60, 62). This finding warrants further investigation on selecting the optimal subpopulation of MSCs for tendon repair.

The age of MSCs might relate to the quality of tendon repair: An age-related trend of decline in the strength of tendon repair can be as great as 50%, although this study did not achieve statistical significance (61), likely due to the small sample size (n=5). These results implicated that the age of MSCs and tendon might affect the quality of tendon repair. Further studies with larger samples are needed. Authors have performed tendon/ligament repair studies on the use of allogeneic MSCs (40-49) and various kinds of scaffolds, including biodegradable polymers such as PLGA/PLLA scaffold (42, 48, 67, 68), collagen-coated polymer scaffolds (69), silk-based scaffolds (69) and fibrin (40).

Allogeneic MSCs were able to survive at tendon repair: We examined the fate of allogeneic MSCs delivered by fibrin gel and implanted into patellar tendon defects. The implanted MSCs remained viable at least 8 weeks after surgery. Moreover, the morphology of MSCs changed from round shape to tenocyte-like spindle shape at 5 and 8 weeks after implantation (44). These results illustrated that allogeneic MSCs were able to survive at the tendon wound site after local delivery.

Allogeneic MSCs accelerated and improved tendon repair: Animal studies of tendon defect repair showed that at as early as 4 weeks post-implantation the regenerated tissue comprised bundles of collagen fibers with an apparently mature crimp pattern. The tensile stiffness of allogeneic MSC-knitted PLGA scaffolds treated group reached 87% of normal value, 30% higher than that of natural healing group. As tendon is a structure that transmits force from muscle to bone, the near normal stiffness of MSC treated tendon repair could ensure the restoration of the physical function of the regenerated tendon (46, 48). Also, in the study of primary tendon repair without defect (40), the intratendinous cell therapy with bone marrow-derived mesenchymal stem cells could improve several histological and biomechanical variables in the early stages of tendon-healing.

Knitted scaffold possessed internal connective space and allowed the formation of functional connective collagen fibrous tissues inside the scaffold: PLGA has greater internal connective space as compared to a braided structure, especially when it is under tension. The internal connective space allows enough cells to be seeded initially, and allows bundles of connective tissue to form during the repair process. This was clearly observed from the histology of knitted PLGA scaffold treated tendon repair. Large amounts of cell in-growth and matrix regeneration were observed in the scaffold as early as two weeks post operation. At 4 weeks after implantation, bundles of collagen fibers with proper orientation and crimp pattern

were formed. The potential of knitted structure for tendon tissue ingrowths was well illustrated by those findings (46, 48).

MSCs could restore the fibrocartilage zone at tendon-to-bone insertion: The flexor hallucis longus tendon was transferred through a 2.5 mm diameter tunnel in the calcaneum. The tendon-to-bone insertion was treated with or without MSCs. In the MSCs treated group, the application of a large number of MSCs had the potential to accelerate tissue remodeling, with more perpendicular collagen fibers at the insertion, and promote fibro-cartilage-like tissue formation, confirmed by collagen type II immunostaining. These findings illustrate the added value of MSCs as compared to other cell sources for tendon regeneration (45).

MSCs cell sheet technology could improve the efficiency of cell seeding onto scaffolds with bigger pores or less pores: Porous scaffolds usually have the drawback of poor cell-seeding efficiency, and require a vehicle for cell-delivery. Our *in vitro* study fabricated three-dimensional cell sheets (41, 42) before attaching them to scaffolds. With MSCs sheet techniques, cells were connected by their synthesized matrixes, which avoided the issue of failure of attachment of cells on scaffolds. Fibroblasts and bone marrow cells (41, 42) have been grown into three - dimensional cell sheets, and the combination of cell sheet/PLLA scaffold constructs had transformed into tissue like ligament analogs which consist primarily of collagen type I and small amount of collagen type III and tenascin (42).

Current tendon tissue engineering research proved the efficiency of autologous and allogenic MSCs for tendon repair. However, the scaffolds used are still not practical which impeded the translation of tendon tissue engineering research from the laboratory benches to patients. The collagen gel/sponge is weak, whereas synthetic polymers have several inherent disadvantages, such as acidic degradation products and inferior biocompatibility. Thus, current scaffolds for MSC delivery have not yet fulfilled the requirements of human application. Allogeneic tendon grafts are still the common choice in current clinical practices.

5. ACELLULAR GRAFTS FOR TENDON REPAIR

5.1. Grafts accepted in current clinical practice

Non-degradable synthetic materials used for ligament and tendon repair include carbon fibers, polyethylene terephthalate (Leeds-Keio ligament), polypropylene (Kennedy Ligament Augmentation Device), and polytetrafuoroethylene (Gore-Tex) (70-73). Although these synthetic grafts exhibit excellent short-term results, the long-term clinical outcome is poor, with a failure rate of 40% to 78% from fragmentation, stress shielding of new tissue, fatigue, creep, and wear debris, which can eventually lead to arthritis and synovitis (71, 73, 74, 75). The permanent polymeric prosthesis developed in the 1970s have not gained wide acceptance. Hence, biological grafts are still the main material used in daily clinical practice to repair tendon defects (50, 76, 77).

Biological substitutes include autograft, allograft, and xenograft (71, 76, 78-82). Autologous grafts of patellar tendon and hamstring tendons are considered the "gold standard" in tissue repair (71) and usually preferred to avoid rejection. Both autografts and allografts possess good initial mechanical strength, and promote cell proliferation and new tissue growth, although fresh autologous grafts may be superior to allogeneic grafts in tissue repair and remodeling (41). However, they suffer from a number of disadvantages. For example, autografts inherently require additional surgery which may cause donor site morbidity, increased recovery time, and possible pain at the harvesting site, such as harvest site infection, nerve injury, and patellar fracture.

Allografts include tendon graft, dermal graft and other connect tissue grafts (79). Xenografts are harvested from animal tendons, small intestine submucosa (30, 83), dermis and skin, and pericardium (84). Allografts and xenografts are primarily composed of type I collagen with similar structure and mechanical properties of human tendons. However, allo- and xeno-grafts could potentially transmit disease or infection, and may elicit an unfavorable immunogenic response from the host (70, 71, 85, 86). Therefore, graft processing is most important for safe clinical application of allo- and xeno-grafting (79).

5.2. Current research on the decelullar technology and immunogenicity of biografts

To decrease the bio-burden and the risk of inflammatory or foreign body reactions, all biografts, regardless of their origin, have to be extensively purified to remove proteins, cells, and lipids. The major source of antigenicity in musculoskeletal transplants is the surface histocompatibility complex markers on donor (graft) cells. Removing these intrinsic cells or damaging the histocompatibility markers results in a significant reduction in antigenicity *in vivo*. Common processing techniques such as fresh-freezing or freeze-drying of allografts may also decrease graft antigenicity, but do not remove cells (86). Frozen allografts of tendons/ligaments frequently result in an immunological foreign-body response that hinders tissue remodeling (87). There is no widely used method for removing cells from tendon allografts while maintaining tissue structure, nativity, and mechanical properties (Table 2) (88-92). SDS, TBP and Triton-X treatments are effective at removing most midsubstance cells from tendon tissue while maintaining mechanical properties, crimp characteristics, and glycosaminoglycan content (86, 88).

In addition to cells, alpha-1,3-Gal epitopes can cause major immunogenicity and acute vascular rejection of pig-to-human xenotransplantation (84, 93, 94). To reduce the immunogenicity of xenografts, many studies had investigated to eliminate alpha-1,3-Gal epitopes, producing alpha-gal deficient pigs (94). Pretreatment with anti-Gal antibodies and complement can reduce the immunogenicity of porcine tissue, may be a valuable

Table 2. Decellularisation and recellularisation of tendon grafts

Graft	Methods for decellularisation and recellularisation	Outcome	Recellularisation	Reference
Rabbit PT allografts reseed human fibroblast	1% extraction solutions of TnBP or SDS for various time periods (24–72 h) partial thickness incisions in PT	Removed 70–90% of the intrinsic cells except near the tendon ends. Both SDS and TBP had no effect on mechanical properties (peak force, stiffness).	Fibroblast proliferation was retarded on SDS-treated PTs;, Extrinsic fibroblasts were successfully cultured on the TnBP-treated PTs in vitro,	86
Porcine PT reseed human tenocytes	0.1% (w/v) SDS in hypotonic buffer, and nuclease solution prior to sterilization with 0.1% (w/v) peracetic acid. Ultrasonication treatment Split of fascicular scaffolds	The biochemical constituents (collagen, glycosaminoglycans) and biomechanical characteristics did not effect	Cells seeded onto the splited fascicular scaffolds penetrated throughout the scaffold and remained viable after 3 weeks of culture.	92
Rat tail tendons	Three extraction chemicals TritonX-100, TnBP, and SDS were soaked used	1% SDS for 24 h or 1% TnBP for 48 h removed the intrinsic cells, tendons retained normal structure and mechanical properties.	None	91
Rabbit Semitendinosus tendons autologous dermal fibroblasts	Acellularization by using aqua dest for 24 h, 1% SDS solution for 24 h, aqua dest for 24 h, and 70% ethanol for 24 h.	Tendons became crimped slack; completely cell free without changing their major biomechanical properties.	Cells integrated into the tendons after injection (4, 7, and 14 days), cell-seeded tendon were positive staining for pro-collagen I	90
Porcine bone–ACL–bone	Triton–SDS, Triton–Triton or Triton–TnBP treatments	All treatments had similar ability in extracting cells and preserving the mechanical properties	None	89
Porcine bone–ACL–bone	Triton–SDS, Triton–Triton or Triton–TnBP treatments	None	Triton-X–and TnBP-treated ligaments were more receptive to cellular ingrowth than SDS treated	88

Abbreviations: SDS: sodium dodecyl sulfate, TnBP: tri(*n*-butyl)phosphate,TritonX-100: *t*-octylphenoxypolyethoxyethanol, PT : Patellar tendon, ACL: anterior cruciate ligament

alternative or supplement to immunosuppression in xenotransplantation (95). Recently recombinant alpha-galactosidase was used to remove alpha-1,3-Gal epitopes successfully. The enzymatic treated porcine patellar tendon graft was appropriate for replacing ruptured human ACL (84). Within two years posttransplantation, the pig tissue was replaced by repopulating recipient's fibroblasts which secreted matrix in the process of ligamentization, although low-level inflammation persisted as long as there are pig xenoantigens in xenograft recipients (84).

Porcine small intestine submucosa extracellular matrix xenografts are currently used in clinical practice for tendon repair. They contain a number of growth factor and are rapidly absorbed, with approximately 40–60% of the ECM degenerated within the first 4 weeks, and complete absorption by 3 months after surgery (30, 96). However, xenograft SIS has no recognizable benefit in the repair of large rotator cuff defect (97). In 30 patients, porcine intestinal submucosa patches caused long term severe inflammatory reactions, and did not improve the rate of tendon-healing or clinical outcome scores (98).

Although frozen allografts are already accepted in clinical practice, absence of viable cells in cryopreserved allografts compromises the clinical outcome of tissue repair and regeneration. With the recent development of tissue engineering and stem cell research, decellularized allografts and xenograft serve as high-strength delivery vehicles for a variety of cell types, including differentiated or pluripotential MSCs.

6. MSCS REVITALIZED DECELLULAR GRAFTS

6.1. Current researches on the repopulation of tendon grafts

To be clinically useful, cell-extracted allo- or xeno-grafts of tendons must be recellularized, possibly seeding cells *in vitro* and ingrowing host cells *in vivo* prior to implantation. Current cell-seeding techniques include: 1) delivering cell-gel composites into the scaffold and 2) delivering cell suspension into scaffolds in a static or dynamic situation. However, there are some disadvantages in the above current techniques, such as the low efficiency of cell attachment to dense fibrous matrix or scaffolds and the weak mechanical strength of gel systems. These disadvantages make it very difficult to seed a large number of cells on dense tissue grafts. The limitations of current technology prohibited the use of stem cells to improve the efficiency of large tissue grafts for tissue repair.

So, partial thickness incisions (86) and ultrasonication (92) were developed to allow the seeded cells infiltrate the tendon in culture prior to implantation. Without incisions or ultrasonication, extrinsic cells which were seeded onto the tendon surface have difficulty infiltrating into the tendon. But, with incision and ultrasonication, the grafts decrease their mechanical strength, a priority in clinical applications. Hence, the tissue grafts have to be preserved intact, while cell delivery is limited by the difficulty of cell seeding.

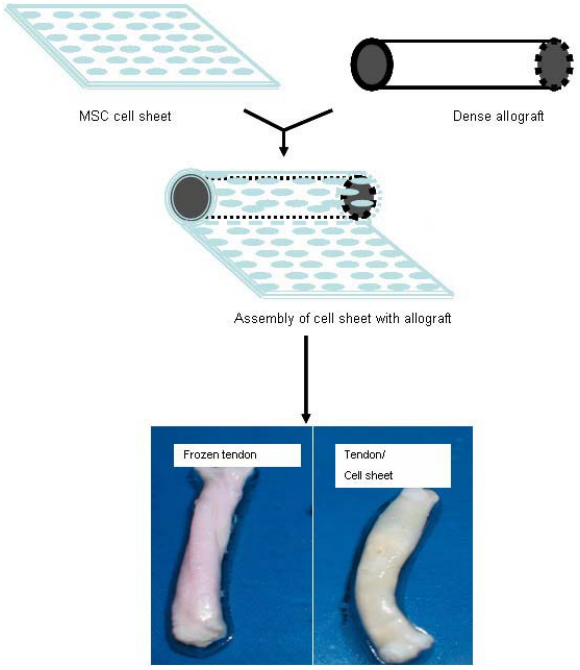

Figure 1. Procedure of obtaining and assembling MSC sheet with frozen tendon grafts.

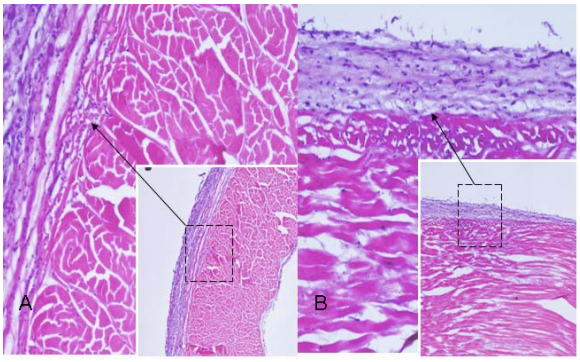

Figure 2. Histology of the (a) tranverse and (b) longitudinal sections of MSCs sheets on tendon grafts were incorporated well within the peritenon around the tendon. (hematoxylin & eosin staining 200X).

6.2. MSCs cell sheet repopulated tendon allograft

Encouraged by the previous studies of MSC sheet, some authors (41) used MSC sheet to seed cells into cryopreserved Achilles tendon graft (Figure 1), and found that MSCs differentiated into spindle-like cells (Figure 2). MSCs proliferated fast, and formed coherent cellular sheets within 2 weeks after attaining confluence. After the cell sheets were formed, these could be detached from the culture substratum, thus yielding a sheet of living cells in a collagen matrix of endogenous origin. When cell sheets were formed, the preserved tendon grafts were put on the cell sheet and rolled up. With this technique, $2.0x10^7$ MSCs were completely and successfully incorporated into the 2.5 cm Achilles tendon graft. This technique overcomes the inherent disadvantages of current tissue engineering techniques of cell seeding. By assembling MSC sheet with dense allogeneic grafts, the advantages of both the cell sheet technique and dense tissue grafts were better utilized. With the MSCs sheet technique, cells were connected by their synthesized matrixes which eased the assembly of cells within dense grafts. Large numbers of MSCs were efficiently seeded onto tendon grafts and MSCs were differentiated into tenocyte-like cells. This novel strategy engineers strong and living tissue grafts to repair large tendon defect, with the possibility of using decellularized grafts as scaffold to deliver large numbers of MSCs. This may revolutionize current technology of tendon tissue engineering and clinical tendon repair and reconstruction (41).

However, the time of MSC culture and MSC cell sheet formation, the immunogenicity of MSC sheets, and the survival of MSC sheets after implantation are still to be optimized (41).

7. FUTURE DIRECTIONS

Cell-based tissue engineering for tendon repair and regeneration hold great promise for future clinical application. Defining the cellular component and appropriate scaffold to generate the tissue engineering paradigm is a complex task. MSC populations may be well suited for this task. Allografts may be the most practical scaffolds to MSCs for tendon repair at present. However, many issues have yet to be investigated.

Future research should be directed toward better characterization of MSCs population, including identifying unique markers and mapping out lineage development. It is hard to fully regenerate tendon. This may due to two reasons. One is that the regeneration ability of tendon tissue is limited. Unlike bone, which can heal by regenerating normal bone in most cases, injured tendon often heals with scar tissue formation. The other is the lack of knowledge about the tissue specific differentiation factors for tendons, with similar function to BMP-2 for bone regeneration. Further studies need to be conducted. Perhaps it may be helpful to obtain some clues from the study of embryonic tendon development.

Allografts and xenograft for tendon reconstruction may be still used. The understanding of biological processes relating allograft and xenograft antigenicity, cellular ingrowth, and tendonization should be investigated to improve the efficacy of tendon grafts. Further studies need to improve the technology of cell extraction and repopulation to reduce the failure of tendon reconstruction with acellular grafts.

With the development of stem cell biology and graft processing technology, the synergic effect of using MSCs and allografts for tendon repair will likely favour the transition from bench top to clinical reality in the next several years.

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9. REFERENCES

1. C. Androjna, R. K. Spragg and K. A. Derwin: Mechanical conditioning of cell-seeded small intestine submucosa: a potential tissue-engineering strategy for tendon repair. *Tissue Eng*, 13, 233-243 (2007)
2. G. S. Kryger, A. K. Chong, M. Costa, H. Pham, S. J. Bates and J. Chang: A Comparison of Tenocytes and Mesenchymal Stem Cells for Use in Flexor Tendon Tissue Engineering. *J Hand Surg (Am)*, 32, 597-605 (2007)
3. S. Calve, R. G. Dennis, P. E. Kosnik, 2nd, K. Baar, K. Grosh and E. M. Arruda: Engineering of functional tendon. *Tissue Eng*, 10, 755-761 (2004)
4. J. A. Cooper, Jr., L. O. Bailey, J. N. Carter, C. E. Castiglioni, M. D. Kofron, F. K. Ko and C. T. Laurencin: Evaluation of the anterior cruciate ligament, medial collateral ligament, achilles tendon and patellar tendon as cell sources for tissue-engineered ligament. *Biomaterials*, 27, 2747-2754 (2006)
5. J. A. Cooper, H. H. Lu, F. K. Ko, J. W. Freeman and C. T. Laurencin: Fiber-based tissue-engineered scaffold for ligament replacement: design considerations and in vitro evaluation. *Biomaterials*, 26, 1523-1532 (2005)
6. Y. Cao, Y. Liu, W. Liu, Q. Shan, S. D. Buonocore and L. Cui: Bridging tendon defects using autologous tenocyte engineered tendon in a hen model. *Plast Reconstr Surg*, 110, 1280-1289 (2002)
7. D. Cao, W. Liu, X. Wei, F. Xu, L. Cui and Y. Cao: In vitro tendon engineering with avian tenocytes and polyglycolic acids: a preliminary report. *Tissue Eng*, 12, 1369-1377 (2006)
8. W. Liu, B. Chen, D. Deng, F. Xu, L. Cui and Y. Cao: Repair of tendon defect with dermal fibroblast engineered tendon in a porcine model. *Tissue Eng*, 12, 775-788 (2006)
9. V. S. Lin, M. C. Lee, S. O'Neal, J. McKean and K. L. Sung: Ligament tissue engineering using synthetic biodegradable fiber scaffolds. *Tissue Eng*, 5, 443-452 (1999)
10. L. D. Bellincampi, R. F. Closkey, R. Prasad, J. P. Zawadsky and M. G. Dunn: Viability of fibroblast-seeded ligament analogs after autogenous implantation. *J Orthop Res*, 16, 414-420 (1998)
11. M. G. Dunn, J. B. Liesch, M. L. Tiku and J. P. Zawadsky: Development of fibroblast-seeded ligament analogs for ACL reconstruction. *J Biomed Mater Res*, 29, 1363-1371 (1995)
12. M. Fini, P. Torricelli, G. Giavaresi, R. Rotini, A. Castagna and R. Giardino: In vitro study comparing two collageneous membranes in view of their clinical application for rotator cuff tendon regeneration. *J Orthop Res*, 25, 98-107 (2007)
13. Y. Cao, J. P. Vacanti, X. Ma, K. T. Paige, J. Upton, Z. Chowanski, B. Schloo, R. Langer and C. A. Vacanti: Generation of neo-tendon using synthetic polymers seeded with tenocytes. *Transplant Proc*, 26, 3390-3392 (1994)
14. M. M. Murray, R. Bennett, X. Zhang and M. Spector: Cell outgrowth from the human ACL in vitro: regional variation and response to TGF-beta1. *J Orthop Res*, 20, 875-880 (2002)
15. T. Funakoshi, T. Majima, N. Iwasaki, N. Suenaga, N. Sawaguchi, K. Shimode, A. Minami, K. Harada and S. Nishimura: Application of tissue engineering techniques for rotator cuff regeneration using a chitosan-based hyaluronan hybrid fiber scaffold. *Am J Sports Med*, 33, 1193-1201 (2005)
16. J. A. Cooper, Jr., J. S. Sahota, W. J. Gorum, 2nd, J. Carter, S. B. Doty and C. T. Laurencin: Biomimetic tissue-engineered anterior cruciate ligament replacement. *Proc Natl Acad Sci U S A*, 104, 3049-3054 (2007)
17. J. M. Chen, C. Willers, J. Xu, A. Wang and M. H. Zheng: Autologous tenocyte therapy using porcine-derived bioscaffolds for massive rotator cuff defect in rabbits. *Tissue Eng*, 13, 1479-1491 (2007)
18. M. F. Pittenger, A. M. Mackay, S. C. Beck, R. K. Jaiswal, R. Douglas, J. D. Mosca, M. A. Moorman, D. W. Simonetti, S. Craig and D. R. Marshak: Multilineage potential of adult human mesenchymal stem cells. *Science*, 284, 143-147 (1999)
19. K. W. Liechty, T. C. MacKenzie, A. F. Shaaban, A. Radu, A. M. Moseley, R. Deans, D. R. Marshak and A. W. Flake: Human mesenchymal stem cells engraft and demonstrate site-specific differentiation after in utero transplantation in sheep. *Nat Med*, 6, 1282-1286 (2000)
20. D. J. Prockop: Marrow stromal cells as stem cells for nonhematopoietic tissues. *Science*, 276, 71-74 (1997)
21. C. De Bari, F. Dell'Accio, J. Vanlauwe, J. Eyckmans, I. M. Khan, C. W. Archer, E. A. Jones, D. McGonagle, T. A. Mitsiadis, C. Pitzalis and F. P. Luyten: Mesenchymal multipotency of adult human periosteal cells demonstrated by single-cell lineage analysis. *Arthritis Rheum*, 54, 1209-1221 (2006)
22. C. De Bari, F. Dell'Accio, P. Tylzanowski and F. P. Luyten: Multipotent mesenchymal stem cells from adult human synovial membrane. *Arthritis Rheum*, 44, 1928-1942 (2001)
23. B. Cao, B. Zheng, R. J. Jankowski, S. Kimura, M. Ikezawa, B. Deasy, J. Cummins, M. Epperly, Z. Qu-Petersen and J. Huard: Muscle stem cells differentiate into haematopoietic lineages but retain myogenic potential. *Nat Cell Biol*, 5, 640-646 (2003)
24. P. A. Zuk, M. Zhu, P. Ashjian, D. A. De Ugarte, J. I. Huang, H. Mizuno, Z. C. Alfonso, J. K. Fraser, P. Benhaim and M. H. Hedrick: Human adipose tissue is a source of multipotent stem cells. *Mol Biol Cell*, 13, 4279-4295 (2002)
25. J. W. Kim, S. Y. Kim, S. Y. Park, Y. M. Kim, J. M. Kim, M. H. Lee and H. M. Ryu: Mesenchymal progenitor cells in the human umbilical cord. *Ann Hematol*, 83, 733-738 (2004)
26. G. P. Dowthwaite, J. C. Bishop, S. N. Redman, I. M. Khan, P. Rooney, D. J. Evans, L. Haughton, Z. Bayram, S. Boyer, B. Thomson, M. S. Wolfe and C. W. Archer: The surface of articular cartilage contains a progenitor cell population. *J Cell Sci*, 117, 889-897 (2004)
27. R. Salingcarnboriboon, H. Yoshitake, K. Tsuji, M. Obinata, T. Amagasa, A. Nifuji and M. Noda: Establishment of tendon-derived cell lines exhibiting

pluripotent mesenchymal stem cell-like property. *Exp Cell Res*, 287, 289-300 (2003)
28. M. de Mos, W. J. Koevoet, H. Jahr, M. M. Verstegen, M. P. Heijboer, N. Kops, J. P. van Leeuwen, H. Weinans, J. A. Verhaar and G. J. van Osch: Intrinsic differentiation potential of adolescent human tendon tissue: an in-vitro cell differentiation study. *BMC Musculoskelet Disord*, 8, 16 (2007)
29. B. M. Seo, M. Miura, S. Gronthos, P. M. Bartold, S. Batouli, J. Brahim, M. Young, P. G. Robey, C. Y. Wang and S. Shi: Investigation of multipotent postnatal stem cells from human periodontal ligament. *Lancet*, 364, 149-155 (2004)
30. T. Zantop, T. W. Gilbert, M. C. Yoder and S. F. Badylak: Extracellular matrix scaffolds are repopulated by bone marrow-derived cells in a mouse model of achilles tendon reconstruction. *J Orthop Res*, 24, 1299-1309 (2006)
31. Y. Kajikawa, T. Morihara, N. Watanabe, H. Sakamoto, K. Matsuda, M. Kobayashi, Y. Oshima, A. Yoshida, M. Kawata and T. Kubo: GFP chimeric models exhibited a biphasic pattern of mesenchymal cell invasion in tendon healing. *J Cell Physiol*, 210, 684-691 (2007)
32. F. Van Eijk, D. B. Saris, J. Riesle, W. J. Willems, C. A. Van Blitterswijk, A. J. Verbout and W. J. Dhert: Tissue engineering of ligaments: a comparison of bone marrow stromal cells, anterior cruciate ligament, and skin fibroblasts as cell source. *Tissue Eng*, 10, 893-903 (2004)
33. M. Krampera, S. Glennie, J. Dyson, D. Scott, R. Laylor, E. Simpson and F. Dazzi: Bone marrow mesenchymal stem cells inhibit the response of naive and memory antigen-specific T cells to their cognate peptide. *Blood*, 101, 3722-3729 (2003)
34. A. Corcione, F. Benvenuto, E. Ferretti, D. Giunti, V. Cappiello, F. Cazzanti, M. Risso, F. Gualandi, G. L. Mancardi, V. Pistoia and A. Uccelli: Human mesenchymal stem cells modulate B-cell functions. *Blood*, 107, 367-372 (2006)
35. A. I. Caplan and J. E. Dennis: Mesenchymal stem cells as trophic mediators. *J Cell Biochem*, 98, 1076-1084 (2006)
36. H. Liu, D. M. Kemeny, B. C. Heng, H. W. Ouyang, A. J. Melendez and T. Cao: The immunogenicity and immunomodulatory function of osteogenic cells differentiated from mesenchymal stem cells. *J Immunol*, 176, 2864-2871 (2006)
37. G. H. Altman, H. H. Lu, R. L. Horan, T. Calabro, D. Ryder, D. L. Kaplan, P. Stark, I. Martin, J. C. Richmond and G. Vunjak-Novakovic: Advanced bioreactor with controlled application of multi-dimensional strain for tissue engineering. *J Biomech Eng*, 124, 742-749 (2002)
38. G. H. Altman, R. L. Horan, I. Martin, J. Farhadi, P. R. Stark, V. Volloch, J. C. Richmond, G. Vunjak-Novakovic and D. L. Kaplan: Cell differentiation by mechanical stress. *FASEB J*, 16, 270-272 (2002)
39. G. H. Altman, R. L. Horan, H. H. Lu, J. Moreau, I. Martin, J. C. Richmond and D. L. Kaplan: Silk matrix for tissue engineered anterior cruciate ligaments. *Biomaterials*, 23, 4131-4141 (2002)
40. A. K. Chong, A. D. Ang, J. C. Goh, J. H. Hui, A. Y. Lim, E. H. Lee and B. H. Lim: Bone marrow-derived mesenchymal stem cells influence early tendon-healing in a rabbit achilles tendon model. *J Bone Joint Surg Am*, 89, 74-81 (2007)
41. H. W. Ouyang, T. Cao, X. H. Zou, B. C. Heng, L. L. Wang, X. H. Song and H. F. Huang: Mesenchymal stem cell sheets revitalize nonviable dense grafts: implications for repair of large-bone and tendon defects. *Transplantation*, 82, 170-174 (2006)
42. H. W. Ouyang, S. L. Toh, J. Goh, T. E. Tay and K. Moe: Assembly of bone marrow stromal cell sheets with knitted poly (L-lactide) scaffold for engineering ligament analogs. *J Biomed Mater Res B Appl Biomater*, 75, 264-271 (2005)
43. Z. Ge, J. C. Goh and E. H. Lee: The effects of bone marrow-derived mesenchymal stem cells and fascia wrap application to anterior cruciate ligament tissue engineering. *Cell Transplant*, 14, 763-773 (2005)
44. H. W. Ouyang, J. C. Goh and E. H. Lee: Viability of allogeneic bone marrow stromal cells following local delivery into patella tendon in rabbit model. *Cell Transplant*, 13, 649-657 (2004)
45. H. W. Ouyang, J. C. Goh and E. H. Lee: Use of bone marrow stromal cells for tendon graft-to-bone healing: histological and immunohistochemical studies in a rabbit model. *Am J Sports Med*, 32, 321-327 (2004)
46. H. W. Ouyang, J. C. Goh, A. Thambyah, S. H. Teoh and E. H. Lee: Knitted poly-lactide-co-glycolide scaffold loaded with bone marrow stromal cells in repair and regeneration of rabbit Achilles tendon. *Tissue Eng*, 9, 431-439 (2003)
47. H. W. Ouyang, J. C. H. Goh, X. M. Mo, S. H. Teoh and E. H. Lee: Characterization of anterior cruciate ligament cells and bone marrow stromal cells on various biodegradable polymeric films. *Mat Sci Eng C-Bio S*, 20, 63-69 (2002)
48. H. W. Ouyang, J. C. Goh, X. M. Mo, S. H. Teoh and E. H. Lee: The efficacy of bone marrow stromal cell-seeded knitted PLGA fiber scaffold for Achilles tendon repair. *Ann N Y Acad Sci*, 961, 126-129 (2002)
49. J. H. Hui, L. Li, Y. H. Teo, H. W. Ouyang and E. H. Lee: Comparative study of the ability of mesenchymal stem cells derived from bone marrow, periosteum, and adipose tissue in treatment of partial growth arrest in rabbit. *Tissue Eng*, 11, 904-912 (2005)
50. R. G. Young, D. L. Butler, W. Weber, A. I. Caplan, S. L. Gordon and D. J. Fink: Use of mesenchymal stem cells in a collagen matrix for Achilles tendon repair. *J Orthop Res*, 16, 406-413 (1998)
51. H. A. Awad, D. L. Butler, G. P. Boivin, F. N. Smith, P. Malaviya, B. Huibregtse and A. I. Caplan: Autologous mesenchymal stem cell-mediated repair of tendon. *Tissue Eng*, 5, 267-277 (1999)
52. D. L. Butler and H. A. Awad: Perspectives on cell and collagen composites for tendon repair. *Clin Orthop Relat Res*, S324-332 (1999)
53. H. A. Awad, D. L. Butler, M. T. Harris, R. E. Ibrahim, Y. Wu, R. G. Young, S. Kadiyala and G. P. Boivin: In vitro characterization of mesenchymal stem cell-seeded collagen scaffolds for tendon repair: effects of initial seeding density on contraction kinetics. *J Biomed Mater Res*, 51, 233-240 (2000)
54. H. A. Awad, G. P. Boivin, M. R. Dressler, F. N. Smith, R. G. Young and D. L. Butler: Repair of patellar tendon injuries using a cell-collagen composite. *J Orthop Res*, 21, 420-431 (2003)

55. M. T. Harris, D. L. Butler, G. P. Boivin, J. B. Florer, E. J. Schantz and R. J. Wenstrup: Mesenchymal stem cells used for rabbit tendon repair can form ectopic bone and express alkaline phosphatase activity in constructs. *J Orthop Res*, 22, 998-1003 (2004)
56. N. Juncosa-Melvin, G. P. Boivin, M. T. Galloway, C. Gooch, J. R. West, A. M. Sklenka and D. L. Butler: Effects of cell-to-collagen ratio in mesenchymal stem cell-seeded implants on tendon repair biomechanics and histology. *Tissue Eng*, 11, 448-457 (2005)
57. N. Juncosa-Melvin, G. P. Boivin, M. T. Galloway, C. Gooch, J. R. West and D. L. Butler: Effects of cell-to-collagen ratio in stem cell-seeded constructs for Achilles tendon repair. *Tissue Eng*, 12, 681-689 (2006)
58. N. Juncosa-Melvin, G. P. Boivin, C. Gooch, M. T. Galloway, J. R. West, M. G. Dunn and D. L. Butler: The effect of autologous mesenchymal stem cells on the biomechanics and histology of gel-collagen sponge constructs used for rabbit patellar tendon repair. *Tissue Eng*, 12, 369-379 (2006)
59. N. Juncosa-Melvin, J. T. Shearn, G. P. Boivin, C. Gooch, M. T. Galloway, J. R. West, V. S. Nirmalanandhan, G. Bradica and D. L. Butler: Effects of mechanical stimulation on the biomechanics and histology of stem cell-collagen sponge constructs for rabbit patellar tendon repair. *Tissue Eng*, 12, 2291-2300 (2006)
60. V. S. Nirmalanandhan, M. S. Levy, A. J. Huth and D. L. Butler: Effects of cell seeding density and collagen concentration on contraction kinetics of mesenchymal stem cell-seeded collagen constructs. *Tissue Eng*, 12, 1865-1872 (2006)
61. M. R. Dressler, D. L. Butler and G. P. Boivin: Age-related changes in the biomechanics of healing patellar tendon. *J Biomech*, 39, 2205-2212 (2006)
62. M. R. Dressler, D. L. Butler and G. P. Boivin: Effects of age on the repair ability of mesenchymal stem cells in rabbit tendon. *J Orthop Res*, 23, 287-293 (2005)
63. N. Juncosa, J. R. West, M. T. Galloway, G. P. Boivin and D. L. Butler: In vivo forces used to develop design parameters for tissue engineered implants for rabbit patellar tendon repair. *J Biomech*, 36, 483-488 (2003)
64. M. R. Dressler, D. L. Butler, R. Wenstrup, H. A. Awad, F. Smith and G. P. Boivin: A potential mechanism for age-related declines in patellar tendon biomechanics. *J Orthop Res*, 20, 1315-1322 (2002)
65. P. Malaviya, D. L. Butler, D. L. Korvick and F. S. Proch: In vivo tendon forces correlate with activity level and remain bounded: evidence in a rabbit flexor tendon model. *J Biomech*, 31, 1043-1049 (1998)
66. D. L. Korvick, J. F. Cummings, E. S. Grood, J. P. Holden, S. M. Feder and D. L. Butler: The use of an implantable force transducer to measure patellar tendon forces in goats. *J Biomech*, 29, 557-561 (1996)
67. S. Sahoo, H. Ouyang, J. C. Goh, T. E. Tay and S. L. Toh: Characterization of a novel polymeric scaffold for potential application in tendon/ligament tissue engineering. *Tissue Eng*, 12, 91-99 (2006)
68. Z. Ge, J. C. Goh, L. Wang, E. P. Tan and E. H. Lee: Characterization of knitted polymeric scaffolds for potential use in ligament tissue engineering. *J Biomater Sci Polym Ed*, 16, 1179-1192 (2005)
69. H. Liu, Z. Ge, Y. Wang, S. L. Toh, V. Sutthikhum and J. C. Goh: Modification of sericin-free silk fibers for ligament tissue engineering application. *J Biomed Mater Res B Appl Biomater* (2007)
70. J. W. Freeman, M. D. Woods and C. T. Laurencin: Tissue engineering of the anterior cruciate ligament using a braid-twist scaffold design. *J Biomech*, 40, 2029-2036 (2007)
71. G. Vunjak-Novakovic, G. Altman, R. Horan and D. L. Kaplan: Tissue engineering of ligaments. *Annu Rev Biomed Eng*, 6, 131-156 (2004)
72. Y. Marois, R. Roy, T. Vidovszky, M. W. King, A. Y. Belanger, C. Chaput and R. Guidoin: Histopathological and immunological investigations of synthetic fibres and structures used in three prosthetic anterior cruciate ligaments: in vivo study in the rat. *Biomaterials*, 14, 255-262 (1993)
73. M. F. Guidoin, Y. Marois, J. Bejui, N. Poddevin, M. W. King and R. Guidoin: Analysis of retrieved polymer fiber based replacements for the ACL. *Biomaterials*, 21, 2461-2474 (2000)
74. M. Bercovy, D. Goutallier, M. C. Voisin, D. Geiger, D. Blanquaert, A. Gaudichet and D. Patte: Carbon-PGLA prostheses for ligament reconstruction. Experimental basis and short-term results in man. *Clin Orthop Relat Res*, 159-168 (1985)
75. E. Pennisi: Tending tender tendons. *Science*, 295, 1011 (2002)
76. N. Juncosa-Melvin, K. S. Matlin, R. W. Holdcraft, V. S. Nirmalanandhan and D. L. Butler: Mechanical stimulation increases collagen type I and collagen type III gene expression of stem cell-collagen sponge constructs for patellar tendon repair. *Tissue Eng*, 13, 1219-1226 (2007)
77. S. F. Badylak, R. Tullius, K. Kokini, K. D. Shelbourne, T. Klootwyk, S. L. Voytik, M. R. Kraine and C. Simmons: The use of xenogeneic small intestinal submucosa as a biomaterial for Achilles tendon repair in a dog model. *J Biomed Mater Res*, 29, 977-985 (1995)
78. E. E. Peacock, Jr. and J. W. Madden: Human composite flexor tendon allografts. *Ann Surg*, 166, 624-629 (1967)
79. D. A. Coons and F. Alan Barber: Tendon graft substitutes-rotator cuff patches. *Sports Med Arthrosc*, 14, 185-190 (2006)
80. R. G. Pulvertaft: Twenty-five years of hand surgery. Personal reflections. *J Bone Joint Surg Br*, 55, 32-55 (1973)
81. S. Badylak, S. Arnoczky, P. Plouhar, R. Haut, V. Mendenhall, R. Clarke and C. Horvath: Naturally occurring extracellular matrix as a scaffold for musculoskeletal repair. *Clin Orthop Relat Res*, S333-343 (1999)
82. L. S. Crossett, R. K. Sinha, V. F. Sechriest and H. E. Rubash: Reconstruction of a ruptured patellar tendon with achilles tendon allograft following total knee arthroplasty. *J Bone Joint Surg Am*, 84-A, 1354-1361 (2002)
83. M. H. Zheng, J. Chen, Y. Kirilak, C. Willers, J. Xu and D. Wood: Porcine small intestine submucosa (SIS) is not an acellular collagenous matrix and contains porcine DNA: possible implications in human implantation. *J Biomed Mater Res B Appl Biomater*, 73, 61-67 (2005)
84. K. R. Stone, U. M. Abdel-Motal, A. W. Walgenbach, T. J. Turek and U. Galili: Replacement of human anterior cruciate ligaments with pig ligaments: a model for anti-

non-gal antibody response in long-term xenotransplantation. *Transplantation*, 83, 211-219 (2007)
85. W. F. Daamen, S. T. Nillesen, T. Hafmans, J. H. Veerkamp, M. J. van Luyn and T. H. van Kuppevelt: Tissue response of defined collagen-elastin scaffolds in young and adult rats with special attention to calcification. *Biomaterials*, 26, 81-92 (2005)
86. J. S. Cartmell and M. G. Dunn: Development of cell-seeded patellar tendon allografts for anterior cruciate ligament reconstruction. *Tissue Eng*, 10, 1065-1075 (2004)
87. D. W. Jackson, E. S. Grood, J. D. Goldstein, M. A. Rosen, P. R. Kurzweil, J. F. Cummings and T. M. Simon: A comparison of patellar tendon autograft and allograft used for anterior cruciate ligament reconstruction in the goat model. *Am J Sports Med*, 21, 176-185 (1993)
88. R. D. Harrison and P. F. Gratzer: Effect of extraction protocols and epidermal growth factor on the cellular repopulation of decellularized anterior cruciate ligament allografts. *J Biomed Mater Res A*, 75, 841-854 (2005)
89. T. Woods and P. F. Gratzer: Effectiveness of three extraction techniques in the development of a decellularized bone-anterior cruciate ligament-bone graft. *Biomaterials*, 26, 7339-7349 (2005)
90. T. Tischer, S. Vogt, S. Aryee, E. Steinhauser, C. Adamczyk, S. Milz, V. Martinek and A. B. Imhoff: Tissue engineering of the anterior cruciate ligament: a new method using acellularized tendon allografts and autologous fibroblasts. *Arch Orthop Trauma Surg* (2007) in press
91. J. S. Cartmell and M. G. Dunn: Effect of chemical treatments on tendon cellularity and mechanical properties. *J Biomed Mater Res*, 49, 134-140 (2000)
92. J. H. Ingram, S. Korossis, G. Howling, J. Fisher and E. Ingham: The use of ultrasonication to aid recellularization of acellular natural tissue scaffolds for use in anterior cruciate ligament reconstruction. *Tissue Eng*, 13, 1561-1572 (2007)
93. U. Galili: Interaction of the natural anti-Gal antibody with alpha-galactosyl epitopes: a major obstacle for xenotransplantation in humans. *Immunol Today*, 14, 480-482 (1993)
94. C. J. Phelps, C. Koike, T. D. Vaught, J. Boone, K. D. Wells, S. H. Chen, S. Ball, S. M. Specht, I. A. Polejaeva, J. A. Monahan, P. M. Jobst, S. B. Sharma, A. E. Lamborn, A. S. Garst, M. Moore, A. J. Demetris, W. A. Rudert, R. Bottino, S. Bertera, M. Trucco, T. E. Starzl, Y. Dai and D. L. Ayares: Production of alpha 1,3-galactosyltransferase-deficient pigs. *Science*, 299, 411-414 (2003)
95. T. Brevig, M. Meyer, T. Kristensen, J. Zimmer and J. Holgersson: Xenotransplantation for brain repair: reduction of porcine donor tissue immunogenicity by treatment with anti-Gal antibodies and complement. *Transplantation*, 72, 190-196 (2001)
96. T. W. Gilbert, A. M. Stewart-Akers, A. Simmons-Byrd and S. F. Badylak: Degradation and remodeling of small intestinal submucosa in canine Achilles tendon repair. *J Bone Joint Surg Am*, 89, 621-630 (2007)
97. J. R. Walton, N. K. Bowman, Y. Khatib, J. Linklater and G. A. Murrell: Restore orthobiologic implant: not recommended for augmentation of rotator cuff repairs. *J Bone Joint Surg Am*, 89, 786-791 (2007)
98. J. P. Iannotti, M. J. Codsi, Y. W. Kwon, K. Derwin, J. Ciccone and J. J. Brems: Porcine small intestine submucosa augmentation of surgical repair of chronic two-tendon rotator cuff tears. A randomized, controlled trial. *J Bone Joint Surg Am*, 88, 1238-1244 (2006)

Abbreviations: MSCs: mesenchymal stem cells, PLGA: poly(dl-lactide-co-glycolide), PLLA: poly-L-lactic acid, SDS: sodium dodecyl sulfate, TnBP: tri(*n*-butyl)phosphate, TritonX-100: *t*-octyl-phenoxypolyethoxyethanol, PT: Patellar tendon, ACL: anterior cruciate ligament, HLA: human leukocyte antigen, ECM: extracellular matrix, BMP: bone morphogenetic protein, ESCs: embryonic stem cells

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