Cell Transplantation, Vol. 14, pp. 763–773, 2005
Printed in the USA.

0963-6897/05 $20.00 + .00
E-ISSN 1555-3892
www.cognizantcommunication.com

The Effects of Bone Marrow-Derived Mesenchymal Stem Cells and Fascia Wrap Application to Anterior Cruciate Ligament Tissue Engineering

Zigang Ge,* James Cho Hong Goh,*† and Eng Hin Lee*

*Department of Orthopaedic Surgery, National University of Singapore,10 Kent Ridge Crescent, Singapore
†Division of Bioengineering, National University of Singapore, 10 Kent Ridge Crescent, Singapore

After an anterior cruciate ligament (ACL) injury, surgical reconstructions are necessary in most cases, either with autografts, allografts, or artificial ligaments. Potential tissue-engineered ligaments would circumvent the disadvantages apparent in these methods. While seeding of mesenchymal stem cells (MSCs) and fascia wrap could potentially improve tissue regeneration and mechanical properties, their exact roles were evaluated in the current study. Knitted biodegradable scaffolds of poly-L-lactic acid (PLLA) and poly-glycolic-lactic acid (PGLA) yarns were used to reconstruct ACL in 48 rabbits. These were divided into four equal groups: only knitted scaffolds were used in group I; knitted scaffolds and mesenchymal stem cells were used in group II; knitted scaffolds, MSCs, and fascia lata were used in group III; knitted scaffolds and fascia lata were used in group IV. Carboxyfluorescein diacetate (CFDA)-labeled MSCs were used to trace the fate of seeded cells in groups II and III. Histology, Western blot analysis, and mechanical properties of reconstructed ACL were analyzed after 20 weeks. Fibroblast ingrowths were seen in all four groups while CFDA-labeled MSCs could be found after 8 weeks of implantation in groups II and III. Both the amount of collagen type I and collagen type III in groups III and IV were significantly higher than in group II, which was much higher than in group I. Both maximal tensile loads and stiffness of the reconstructed ACLs in groups I, II, III, and IV were significantly lower than normal controls after 20 weeks of implantation. It is concluded that MSCs could promote synthesis of collagen type I and collagen type III in tissue-engineered ligaments, while fascia wraps have stronger effects. Both MSC seeding and fascia wrap could not enhance ultimate tensile load and stiffness.

Key words: Anterior cruciate ligament; Tissue engineering; Mesenchymal stem cell; Protein synthesis

INTRODUCTION

With increasing emphasis on sporting activities, the incidence of anterior cruciate ligament (ACL) injuries has also increased (11). The poor healing capacity of ACLs has led orthopedic surgeons to perform ACL reconstructions in most cases. In current clinical practice, the most popular and successful surgical replacements for ACL are autografts. This includes the use of the bone–patellar tendon–bone grafts (34,35) and hamstring tendon grafts (36), as they had been shown to have good potential for graft remodeling and integration into the joint (1). However, donor site morbidity is a major concern when utilizing autografts (22). Occasionally autografts are not available for use as a result of repetitive surgery or infection. Use of allograft avoids donor site morbidity, shortens surgical time, and diminishes postoperative pain. However, the decrease in tensile properties as a result of sterilization and preservation as well as risk of inflammatory reaction are of major concerns (11).

The use of synthetic ligament replacements gained some popularity in limited conditions in the late 1980s, but has been seldom used since the 1990s. The use of synthetic ligament replacements, including Gore-Tex™, Stryker Dacron™, and Leeds-Keio™ prosthesis, does not involve the sacrifice of autogenous tissues, thus eliminating the issues of associated morbidity and risk of disease transmission. Artificial ligaments permit an easier reconstructive technique and a more rapid rehabilitation process, because they can maintain their strength at an early stage. However, as they do not have the strength or performance of human ACL and they have no ability to integrate with host tissues, their long-term results will

Accepted June 17, 2005.
Address correspondence to James C. H. Goh, Ph.D., Department of Orthopedic Surgery, National University of Singapore, 10 Kent Ridge Crescent, Singapore, 119260. Tel: (65)-67724423; Fax: (65)-67744082; E-mail: dosgohj@nus.edu.sg

deteriorate due to mechanical shielding (27), high incidence of chronic foreign body inflammation, particulate-induced synovitis, particle shedding into lymph nodes, and complete graft rupture (23).

Research on potential tissue-engineered ACLs has been going on for some time, with the hope of overcoming the above-mentioned problems. This revolutionary approach requires the use of biodegradable scaffolds, as well as biological approaches such as cell seeding, fascia wraps, growth factors, to induce ligament regeneration. Due to harsh anatomic environments (6), functional ACL regeneration is difficult to achieve, especially for collagen bundle formation and optimal mechanical strength matched to the original ACL.

Cell seeding has been proven to further improve the functionality of tissue-engineered constructs (7,12). In a recent in vitro study, bone marrow-derived mesenchymal stem cells (MSCs) was found to be the best cell source for ACL regeneration when compared to fibroblasts derived from ACL and skin, with regards to collagen deposition and proliferation (38). However, in vivo functional state of seeding cells is still unknown. Because a normal ACL is covered by a mesentery-like fold of synovium to protect the ACL from the harsh environment of the knee joint (2), the fascia wrap used in tissue-engineered ligament is to mimic the normal structure. Fascia lata could be helpful for regeneration and functionality by providing cover for tissue-engineered ligaments from harmful cytokines, improving mechanical properties, providing cells, and inducing angiogenesis. Fascia wrapped braided poly-L-lactic acid (PLLA) scaffolds used for sheep ACL reconstruction induced more fibroblast ingrowths, more mature collagen fibers with better orientation, and higher maximal tensile load than nonfascia control at 48 weeks (24,25).

In a previous in vitro study, knitted structures of PLLA and poly-glycolic-lactic acid (PLGA) supported cell attachment and proliferation while providing enough maximal tensile strength for ACL reconstruction in the first 12 weeks (121–158% of essential ultimate tensile strength of 38 MPa) (30), 95% and 87% of it in 16 and 20 weeks, respectively. In consideration of potential tissue regeneration and subsequent functionality after 16 weeks, these structures could be candidates for ACL reconstruction. As type I collagen and type III collagen account for more than 80% of the dry weight of a normal ligament (26), it is important to evaluate their individual amounts in regeneration. On the basis of current knitted scaffolds, we aimed to evaluate the effects of MSC seeding and fascia wrap by examining tissue formation, the relative amount of collagen type I and type III, maximal tensile load, and stiffness as well as tracing the fates of seeding MSCs in different groups.

MATERIALS AND METHODS

Prostheses Fabrication

PLLA yarns (multifilament, nonbraided, 30 filaments, each filament between 15and 20 μm in diameter, denier 83) were purchased from Albany International (USA). PLGA yarns (with ratio of PLA and PGA in the copolymer being 10:9, 12 filaments in one yarn and each filament between 15–35 μm in diameter, denier 50–60) were obtained from Shanghai Tianqing Biomaterial (China). The scaffold was knitted out of two PLLA yarns and one PLGA yarn with four needles in a knitting machine (SK270, Silver Reed, Suzhou Harisa Machinery Co., China). The two ends of each 5-cm-long knitted structure were sealed with heat. The scaffolds were sterilized by immersing in 70% alcohol for 30 min and then washing twice with PBS. Subsequently, the scaffolds were immersed in three changes of PBS for 15 min prior to use.

Isolation and Culture of Mesenchymal Stem Cells

The MSCs were isolated as described by Friedenstein et al. (10). Briefly, the procedure was as follows. The rabbit was put under general anesthesia and 2 ml of bone marrow was aspirated from the iliac crest and subjected to centrifugation. After centrifugation and wash, the cells were cultured in DMEM (Sigma, ph 7.4) supplemented with 10% FBS (Gibco, 10270-106) and 10,000 U/ml penicillin/10,000 μl/ml streptomycin. The cells were cultured in 75-cm^2 flasks (Corning) at 37° with 5% CO_2 until 80% confluence. The medium was changed every three days. Hemopoietic cells were removed with changing of the medium. When reaching 80% confluence, the cells were trypsinized with 1 ml of 0.25% trypsin-EDTA (Gibco) and washed with PBS prior to use.

ACL Reconstruction and Fascia Harvest

Animal experiments were approved by Animal Holding Unit, National University of Singapore. Fifty-two skeletally matured male New Zealand White rabbits weighing 3.0–3.4 kg were used in this study, four of them for the cell survivability study. The remaining 48 rabbits were divided into four groups with 12 rabbits each (Table 1). The anesthesia technique used was: 0.3 ml/kg hyponym was administrated intramuscularly to tranquilize the animal; this was followed by IV 0.3 ml/kg Valium for induction. Subsequently, 1% halothane inhalation was used for maintenance; 0.3 ml per animal of cephacexin was given subcutaneously as postoperation antibiotics.

The operations were performed under aseptic conditions. The right knee joint of the rabbit was exposed using a midline skin incision and lateral parapatellar arthrotomy was performed. The patella was dislocated me-

Table 1. Grouping of Experimental Rabbits

	No. of Rabbits	MSCs	Fascia Wrap
Cell survivability-1	2	Y	—
Cell survivability-2	2	Y	Y
Group I	12	—	—
Group II	12	Y	—
Group III	12	Y	Y
Group IV	12	—	Y

dially with the knee extended. With the knee placed in hyperflexion state, normal ACL was removed by sharp dissection at the tibial and femoral attachment sites, while the fat pad was left intact. Using a 2-mm-diameter drill bit and a gas-driven drill (K100 Air Powered Mini Driver, 3M, USA), bone tunnels were made from the anatomic ACL femur attachment sites to lateral femoral condyles, as well as from the anatomic ACL tibial attachment sites to medial tibia, which were distal and medial to the tibial tubercles. Each end of the sterile knitted scaffold was sutured with 4-0 Vicryl suture (Ethicon, NJ) to facilitate the ease of threading the scaffold through the tibial and femoral bone tunnels. For group I, two scaffolds were combined and passed through the bone tunnel in the femoral bone, into the intra-articular space, and through the tibial bone tunnel separately (normal procedure). For group II, 3 million MSCs in 60 μl of Tisseel fibrin glue (Baxter Healthcare Corporation, Glendale, CA) were loaded on the two scaffolds before normal procedure. For group III, first a 0.5-cm-wide and 5-cm-long fascia lata at the lateral part of the femoral condyle was carefully dissected, while keeping the pedicle near the femur bone tunnel connected. After 3 million MSCs in 60 μl of Tisseel fibrin glue were loaded, the scaffolds were wrapped with pedicle fascia lata and sutured with 4-0 Vicryl suture as in the normal procedure. For group IV, the scaffolds were wrapped and sutured with the same fascia lata as in group III with the prior normal procedure. The two ends of the construct in each group were then sutured to the periosteum with 2-0 Ethibond nonabsorbed sutures (Johnson & Johnson, USA). This was done with the knee at 45° of flexion with the constructs in slight tension. After 20 weeks, the rabbits were euthanized by 1 ml (400 mg)/2.5 kg body weight of pentobabitone sodium (Euthanasia Fort Solution, Apex Laboratories, Australia) and knee joints were dissected for histology and mechanical tests.

Histology

At least two knees from each group were used for histology. For histology, all connective tissues and other ligaments in the knee joints were removed except for the ACL. The harvested specimens were immersed in 10% formalin for 1 week prior to decalcification in 30% formic acid for 3 weeks, followed by normal paraffin embedding for hematoxylin and eosin staining procedure.

Mechanical Test

Five rabbits from each group were used for the mechanical test while five randomly chosen left knees were

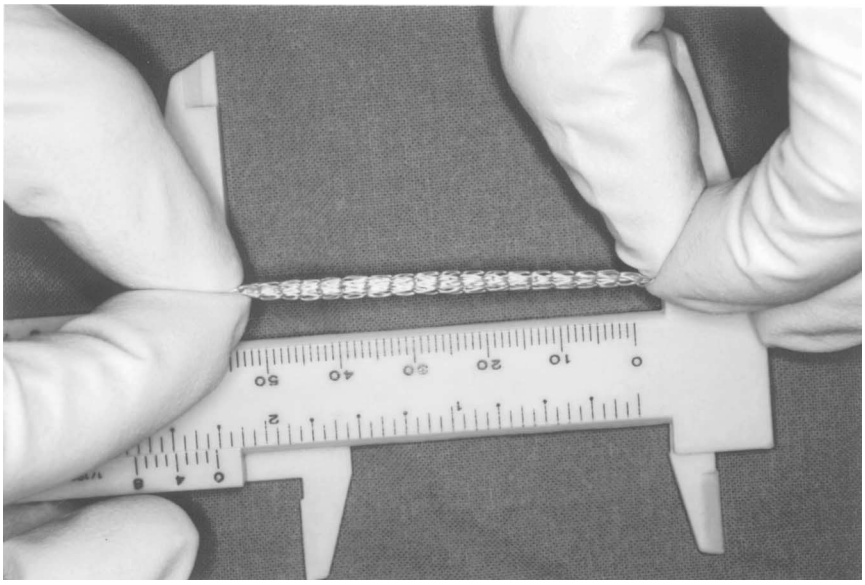

Figure 1. Porous knitted PLLA/PLGA scaffolds.

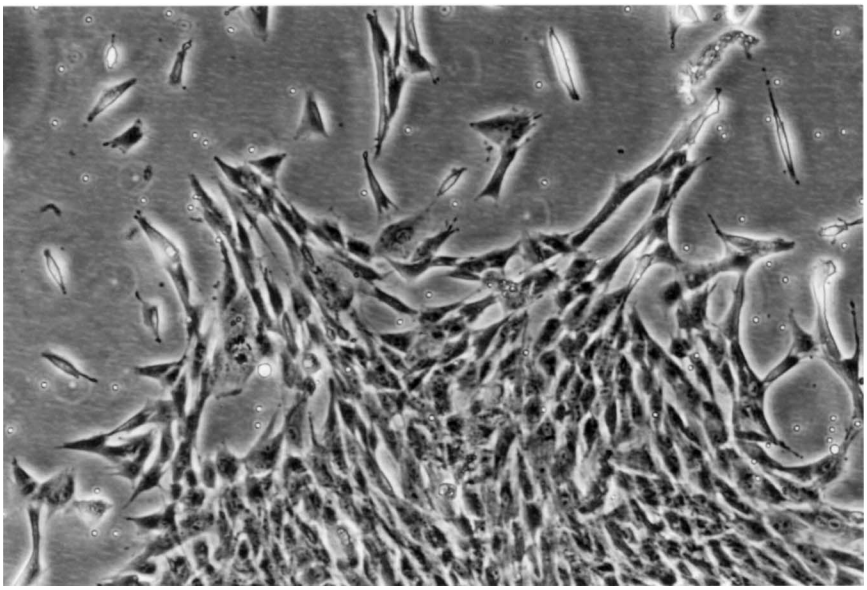

Figure 2. Rabbit primary bone marrow derived MSC (original magnification 60×).

used as control. The knee joints with capsules intact and 4 cm of femurs and tibias intact were harvested. The specimens were sealed and kept at −80°C until tested. The specimens were thawed at room temperature for 24 h and then were dissected to be free from all connectives tissue and other ligaments except for the ACL. The knee joints of the specimens were covered with saline wet gauze to keep the samples moist at all times. The knees were mounted with dental cement (Meliodent, CE 0044, Heraeus Kulzer GmbH, Germany) in the proximal femur and distal tibia, clamped at 45° flexion in an Instron 5543 material testing system (Instron, Canton, MA). The maximal load and elongation were measured at a constant speed of 2 cm/min. Stiffness was determined by calculating the slope of the load versus displacement plot.

Western Blot Analysis

Post mechanical testing or prior histology, proteins in tissue-engineered ligaments were homogenized mechanically, separated, and transferred to 0.45-μm nitrocellulose membrane (Bio-Rad) at 29 V using a Trans-Blot Electrophoretic Transfer Cell (Bio-Rad) overnight. The membrane was probed with monoclonal antibody (mAb) of anti-collagen type I (CP17L, Calbiochem) and anti-collagen type III (Oncogene), followed by horseradish peroxidase-conjugated anti-mouse IgG Ab 6789 (Abcam Ltd). Protein molecular weight marker (Dual color, Bio-Rad) was used on each gel. Bands were visualized using Western Lightening Chemiluminescence Reagent Plus (Perkin Elmer Life Sciences, Inc.) and autoradiograms of the blot were captured by a scanner. Quantification of relative amounts of collagen type I and type III was analyzed with gel pro analyzer (MediaCybernetics) software.

Cell Survival

Four rabbits were used for the cell survivability study, two of which were identical to those in group II and the other two identical to those of group III. Three million MSCs (PI) were labeled with 15 mM of Vybrant™ CFDA SE Cell Tracer Kit (V-12883, Molecular Probes, OR) for 15 min and cultured for 24 h prior to loading onto the scaffolds for ACL reconstruction as in groups II and III. The cell-loaded constructs in the knee joints were dissected and cryosectioned at 8 μm before examination under confocal laser microscope (TCS SP2, Leica, Germany) at 488 nm.

RESULTS

Scaffold Fabrication

The biphasic knitted scaffolds were porous and had soft cord-like structure with a blue color appearance due to the PLGA yarns (Fig. 1).

Isolation and Culture of Mesenchymal Stem Cells

Two to 3 million MSCs were acquired with typical colony-forming property and fibroblast-like morphology after 18–20 days of cell culture. They were also anchorage dependant and grew in monolayers with visible nucleus and nucleolus (Fig. 2).

Gross Morphology and Histology

Five rabbits died before completion of the experiment, two of which were from group I and one each

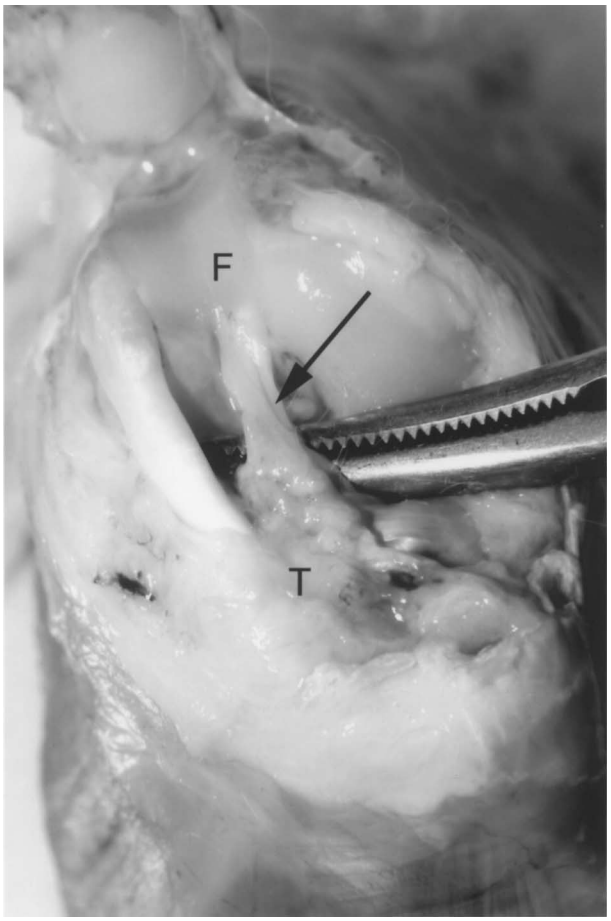

Figure 3. Macroscopic view of regenerated ACL after 20 weeks of implantation in group III. F indicates femur of rabbit and T indicats tibia. The arrow indicates regenerated ACL after 20 weeks.

from groups II, III, and IV. The main cause of death in all five cases was diarrhea. The general condition of all other rabbits in the study throughout the follow-up period was good with no wound infections. After 20 weeks of implantation, one ACL construct each from groups I and IV and two constructs each from groups II and III were found to have ruptured. All other ACL constructs maintained continuity and had fascia cord-like appearance. Furthermore, due to degradation of the blue PGLA yarns, the remaining scaffolds were white in appearance (Fig. 3).

Histologically, fibroblasts were found in all the ACL constructs of the four groups, with few macrophages and lymphocytes present. The cartilaginous surfaces of the joints were intact in all cases. In groups I and II, scaffolds were enveloped and penetrated by large amount of nonparallel fibroblasts in certain parts, whereas relatively paralleled fibroblasts could be found in other areas (Fig. 4). No collagen bundle could be found under higher magnification. There were large amount of extracellular matrix laid down around the scaffolds and fibroblasts. In groups III and IV, fibroblast ingrowths and extracellular matrix were similar with to those in groups I and II. In the outer layer of reconstructed ACL, a thick layer of membrane composed of paralleled fibroblasts and extracellular matrix could be seen (Fig. 4b). This was further surrounded by a thin layer of extracellular matrix with a deeper red. Normal ACL was comprised of paralleled collagen bundles, which were cross-linked to each other. Fibroblasts were attached to individual collagen bundles and elongated longitudinally while cell densities were lower than those in experimental groups (Fig. 5).

Mechanical Test

Of all the specimens tested, the failure mode of two reconstructed ACLs (one from group II and the other from group IV) was dislodgement from the bone tunnels. In all other cases, the ACL constructs failed in the midsubstance inside the knee joint. However, the exact location of rupture was inconsistent, because multiple ruptures occurred at different places simultaneously. Although sometimes certain scaffolds seemed intact, rupture of microfibers may have taken place at various spots, causing a drastic drop in tensile load.

Maximal tensile loads of tissue-engineered ligaments were 9.2%, 9.8%, 13.9%, and 10.4% of normal in groups I, II, III, and IV, respectively (Table 2); however, there was no significant difference between different groups ($p < 0.05$, one-way ANOVA). Stiffness of tissue-engineered ligaments in groups I, II, III, and IV was 9.1%, 18.7%, 16.7%, and 13.1% of normal, respectively (Table 2). All of them were significantly lower than the normal value while there was no significant difference in the data between individual experimental groups ($p < 0.05$, one-way ANOVA).

Western Blot Analysis

In four experimental groups and normal control, two bands of collagen type I were obvious at 210 k and 95 k, respectively, though the densities were different among different groups (Fig. 6a). The amount of collagen I in groups III (146.7%) and IV (163.3%) was higher than in group II (75.1%) and normal control (100%) while there was no significance between groups III and IV, as well as between group II and normal control. Collagen I in group I (20.5%) was significantly lower than that in all other groups (one-way ANOVA, $p < 0.05$) (Fig. 6b).

In all groups, bands of collagen type III at 110 and 290 kDa were obtained with other minor bands, ranging from 63 to 300 kDa (Fig. 7). Collagen III expression in groups III (137.1%) and IV (162.9%) was higher than all other groups, while there was no significance be-

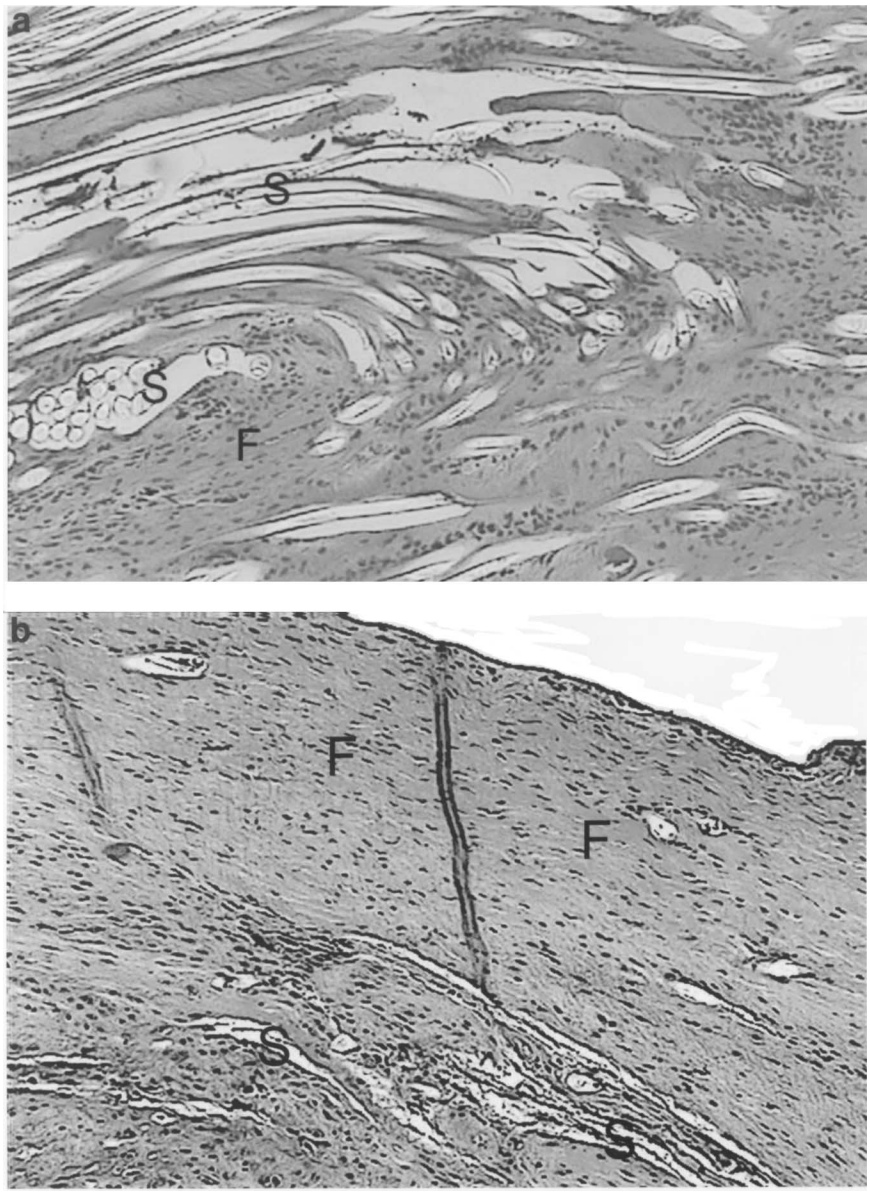

Figure 4. (a) Histology of tissue-engineered ligaments from group I after 20 weeks (H&E, original magnification 100×). S indicates PLLA scaffolds sectioned from different directions and F indicats in-growth fibroblasts. (b) Histology of tissue-engineered ligaments from group III after 20 weeks (H&E, original magnification 100×). S indicates PLLA scaffolds sectioned from different directions and F indicates in-growth fibroblasts.

tween them. Collagen III expression in group II (71.4%) was lower than that in normal control, but higher than group I (20.5%), which was significant (one-way ANOVA, $p < 0.05$) (Fig. 7b).

Cell Survival

After 8 weeks of implantation, fluorescence could still be detected in both groups II and III (Fig. 8). However, it was only present in less than 10% of total area examined. Some clusters of cells attached to fibers could be observed clearly by the fluorescence, but individual cell shape could not be distinguished.

DISCUSSION

Although MSC could be harvested from the periosteum (28), muscle connective tissues (29), and adipose tissues (40), bone marrow is an abundant resource and easily accessible. Because initial isolation of bone mar-

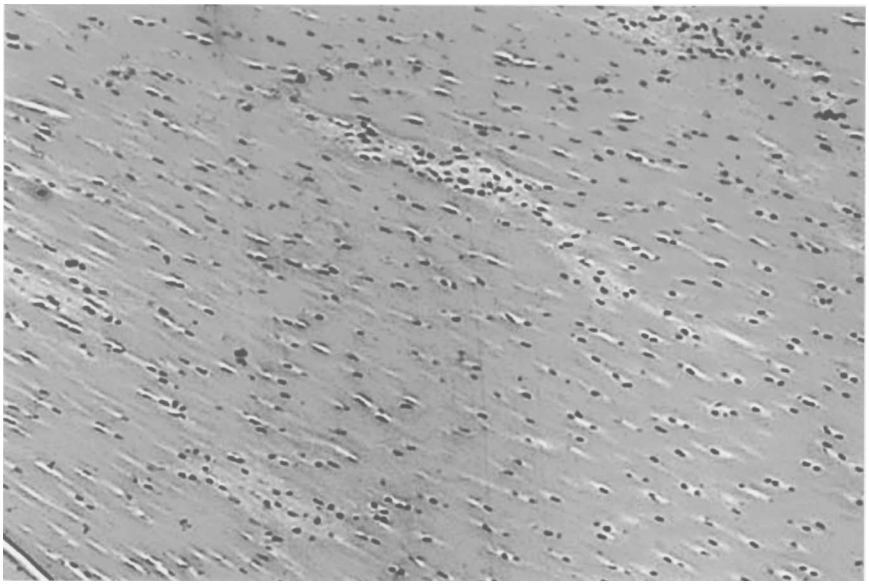

Figure 5. Histology of normal ACL (H&E, 100×). Pink collagen bundles cross-linked to each other with attached fibroblasts.

row MSCs is through their adherence to tissue culture surfaces (10), different protocols have been developed in an attempt to get more homogeneous populations (15, 21,33), but none of these protocols has gained wide acceptance. In this study, the most common protocol, direct plating (10), was used to culture the MSC due to the ease of the procedure and wide acceptance in tissue engineering research. Phenotype of the acquired MSCs has been documented by their potential to differentiate into osteo-lineage, chondro-lineage, and adipo-lineage (31), as well as the typical well-spread morphology (33). Though cell seeding has been proven effective for subsequent regeneration, the optimal number of seeding MSCs is still unknown. Two to 3 million MSCs were used as the loading density from our previous experience in tendon tissue engineering (32).

When compared with ACL reconstruction using cross-linked collagen in a similar rabbit experiment (9), survival rate of the current constructs was higher after 20 weeks of implantation and they incurred less immunoresponses. Though no fully mature collagen bundle has been reported in tissue-engineered ACL research, there are many reports on relatively mature ACL regeneration, such as formation of collagen fibers, orientations, and crimps (9,24). Two reasons could be attributed to inferior collagen fiber formation and orientations in the current study. First, PLLA has inferior ability to encourage fibroblasts to attach and function, mainly due to lack of RGD sequence in collagen (18). Second, the relatively shorter implantation period impedes further maturation of fibroblast in-growth and extracellular matrix, when compared with 48-week (24) or 1-year study (20). On the other hand, polymer-based scaffolds are more suitable to be used to compare the effects from cell seeding and fascia wrap, as they only provide mechanical support while not being involved in subsequent regeneration. It is reasonable that some well-oriented fibroblasts and relatively mature extracellular matrix in fascia wrap groups came from fascia wraps used and not from regeneration, but the exact ratio is still not known.

Collagen is a key component in ligaments, which provides high tensile strength to withstand physiological loads during activities of daily living. It accounts for 70–80% of the tissue dry weight. Collagen type I and type III are major components in normal ligament tissues as well as regeneration process, while other collagens such as types V, VI, and XII occur in small amounts (16). Hence, it is important to evaluate the excretion of collagen I and III. Our results showed that MSC seeding

Table 2. Mechanical Properties of Tissue-Engineered Ligaments After 20 Weeks of Implantation

	Maximal Tensile Load (N)	Stiffness (N/mm)
Group I ($N = 5$)	14.0 ± 7.8	8.6 ± 2.3
Group II ($N = 5$)	14.9 ± 6.6	7.8 ± 3.2
Group III ($N = 5$)	20.9 ± 4.5	8.4 ± 2.2
Group IV ($N = 5$)	15.8 ± 6.8	7.4 ± 3.4
Normal control ($N = 5$)	151.8 ± 20.8	50.4 ± 5.3

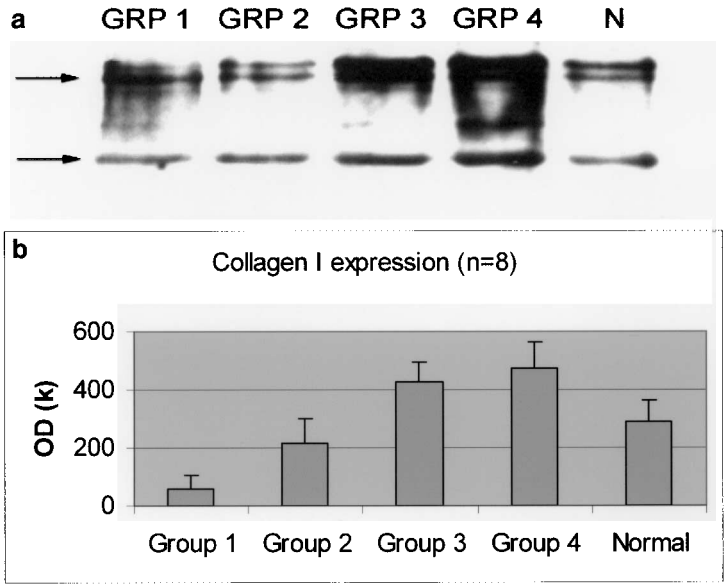

Figure 6. (a) Western blot of collagen I from groups I, II, III, and IV and normal control (N). Arrows indicate collagen type I detected by α-collagen (I) (Calbiochem) significantly at 95 and 210 kDa. (b) Quantitative expression of collagen I expression in different groups.

obviously improved excretion of collagen types I and III. It is postulated that donor cells may help to recruit more progenitor/reparative cells by interacting with host cells, while excreting extracellular matrix (13). To our knowledge, it is the first evidence to quantitatively analyze cell seeding effect on in vivo protein components, though many reports on in vitro protein analysis have been published (38). Fascia wrap has been reported to contribute to the formation of well-oriented fibroblasts and extracellular matrix, as well as to improving mechanical properties (24,25), although it could not provide good mechanical support when used separately

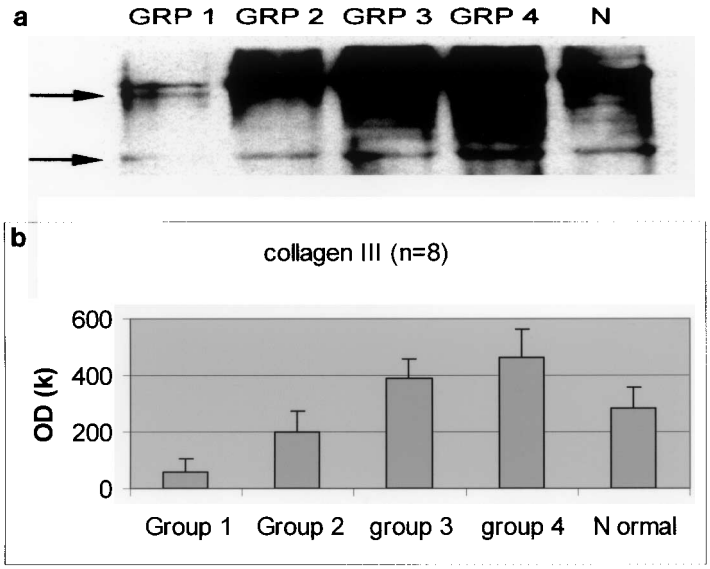

Figure 7. Western blot of collagen III from groups I, II, III, and IV and normal control. Arrows indicate collagen type III detected by α-collagen (III) (Oncogene Research Products) significantly at 110 and 290 kDa. (b) Quantitative expression of collagen III expression in different groups.

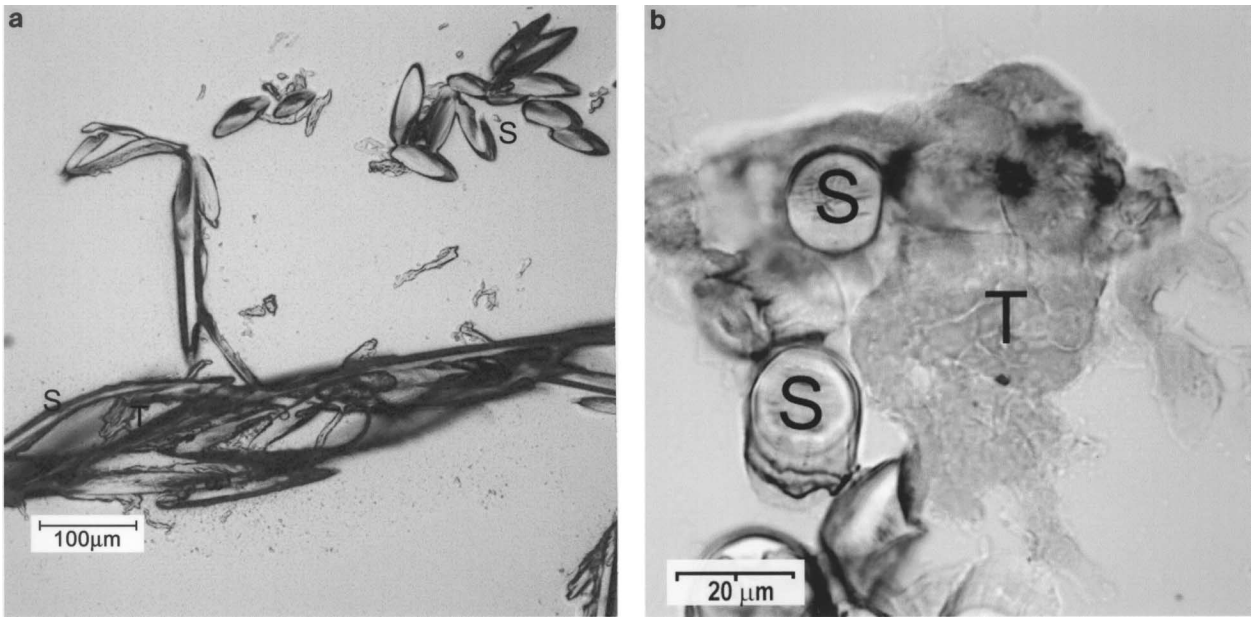

Figure 8. (a) Fluorescence from tissue-engineered ACL in group II after 8 weeks of implantation. S indicates sectioned scaffold and T indicates tissues. (b) Fluorescence from tissue engineered ACL in group III after 8 weeks of implantation. S indicates sectioned scaffold and T indicates tissues.

(14). In the current study, fascia wraps did improve protein synthesis and potential functionality, but it was not clear regarding the exact ratio of protein that fascia wraps brought to protein synthesis from regeneration. Though fascia wraps have shown stronger effects on collagen excretion than MSC seeding in the current study, further study is necessary when the quantity of fascia wraps and number of seeding cells is well controlled. Longer implantation and larger animal models are necessary to evaluate their exact role. The ultimate fibril diameter attained during fibrilogenesis appears to depend on the quantitative ratio in which the type I and type III collagens are secreted during fibril polymerization (17). In the future, it would be worthwhile to determine the ratio of collagen I and III in the same specimen, and establish their roles in the healing process.

Seeding of appropriate cells on scaffold could further improve the functionality of tissue-engineered constructs (3,7,12). However, these potential benefits depend on the sustained viability of seeded cells after implantation into the harsh environment present in knee joints. Eight weeks of viability of loaded MSCs after implantation were consistent with previous reports (4,19,39). Though 8 weeks of survival in the knee joints was not long, it was possibly enough for them to function. As the reparative cells and/or progenitor cells continuously grew, the loaded MSCs would have gradually been overtaken. The regenerated tissue could have taken up the two main roles of seeding MSCs, sending chemotaxis signal and laying down extracellular matrix, which are important in initial stages. There are still many issues to be addressed in the future (e.g., survival rate of seeded cells, percentage of extracellular matrix laid down by seeded cells) for better understanding of seeding cells.

Maximal tensile load and stiffness are important parameters for tissue-engineered ACL (37). Though MSC seeding and fascia wrap were effective to improve collagen excretion and possibly regeneration, neither of them could improve maximal tensile load and stiffness of current tissue-engineered ligaments significantly. The acquired maximal tensile loads and stiffness were mainly from the partially degraded PLLA fibers, while regenerated tissues were not well oriented and far from functional. In activities of daily living, human ACL is only exposed to relatively small tensile forces ranging from 3.87% (for ascending stairs) to 36.42% (for jogging) (8), while its maximal tensile load is 1730 N (30). On the other hand, relatively less stiff scaffolds could transfer more mechanical loads to regenerated tissues, which would promote functional regeneration (5). It would be relatively safe if tissue-engineered ligaments maintained 40–50% of maximal tensile load of normal ACL, together with regenerated functional tissues. Even so, it is still necessary to further improve mechanical properties of the current scaffolds for potential clinical application, either by promoting enough collagen fibril/bundle formation and subsequent orientation and cross-linking, or by adopting stronger scaffolds with controlled degradation.

Although much research has been done on potential tissue-engineered ACLs, progress is rather slow. This is due to several reasons: 1) complex mechanical forces faced; 2) difficulties in fabricating degradable scaffolds with mechanical properties matching that of a normal ACL and with controlled degradation, 3) lack of blood supply, 4) significant changes of cytokine profiles after ACL injuries. However, several methods are promising to facilitate regeneration, including cell seeding, gene therapy, growth factor, collagen ratio control, microenvironment control, etc. More work should be done to understand and further develop these methods before functional tissue-engineered ACL could be fabricated.

CONCLUSION

Both MSC seeding and fascia wrap could increase synthesis of collagen type I and type III while fascia wraps showed stronger effects. None of them could improve maximal tensile load and stiffness of the current tissue-engineered ligaments.

ACKNOWLEDGMENTS: We wish to thank Dominic Tan for helping in the mechanical tests and Ms. Tan Bee Leng for helping with Western blot analysis. This study was support by a grant from the National Medical Research Council, Singapore (Grant No: R-175-000-037-213).

REFERENCES

1. Amiel, D.; Kleiner, J. B.; Roux, R. D. The phenomenon of "ligamentization": Anterior cruciate ligament reconstruction with autogenous patellar tendon. J. Orthop. Res. 4:162–172; 1986.
2. Arnoczky, S. P.; Warren, R. F. Anatomy of the cruciate ligaments. In: Feagin, J. A., ed. The crucial ligaments: Diagnosis and treatment of ligamentous injuries about the knee. New York: Churchill Livingstone; 1994:269–287.
3. Awad, H. A.; Butler, D. L.; Boivin, G. P.; Smith, F. N.; Malaviya, P.; Huibregtse, B.; Caplan, A. I. Autologous mesenchymal stem cell-mediated repair of tendon. Tissue Eng. 5(3):267–277; 1999.
4. Bellincampi, L. D.; Closkey, R. F.; Prasad, R.; Zawadsky, J. P.; Dunn, M. G. Viability of fibroblast-seeded ligament analogs after autogenous implantation. J. Orthop. Res. 16: 414–420; 1998.
5. Butler, D. L.; Shearn, J. T.; Juncosa, N.; Dressler, M. R.; Hunter, S. A. Functional tissue engineering parameters toward designing repair and replacement strategies. Clin. Orthop. Relat. Res. 427(Suppl.):S190–S199; 2004.
6. Cameron, M. L.; Fu, F. H.; Paessler, H. H.; Schneider, M.; Evans, C. H. Synovial fluid cytokine concentrations as possible prognostic indicators in the ACL-deficient knee. Knee Surg. Sports Traumatol. Arthrosc. 2(1):38–44; 1994.
7. Caplan, A. I. Embryonic development and the principles of tissue engineering. Novartis Found. Symp. 249:17–25; 2003.
8. Chen, E. H.; Black, J. Materials design analysis of the prosthetic anterior cruciate ligament. J. Biomed. Mater. Res. 14(5):567–586; 1980.
9. Dunn, M. G.; Tria, A. J.; Kato, P.; Bechler, J. R.; Ochner, R. S.; Zawadsky, J. P.; Silver, F. H. Anterior cruciate ligament reconstruction using a composite collagenous prosthesis. Am. J. Sport Med. 20(5):507–515; 1992.
10. Friedenstein, A. J.; Gorskaja, U.; Kalugina, N. N. Fibroblast precursors in normal and irradiated mouse hematopoietic organs. Exp. Hematol. 4:267–274; 1976.
11. Fu, F. H.; Bennett, C. H.; Lattermann, C.; Ma, C. B. Current trends in anterior cruciate ligament reconstruction. Part 1: Biology and biomechanics of reconstruction. Am. J. Sports Med. 27(6):821–830; 1999.
12. Gao, J.; Caplan, A. I. Mesenchymal stem cells and tissue engineering for orthopaedic surgery. Chir. Organi Mov. 88(3):305–316; 2003.
13. Hilldebrand, K. A.; Jia, F.; Woo, S. L. Response of donor and recipient cells after transplantation of cells to the ligament and tendon. Microsc. Res. Tech. 58:34–38; 2002.
14. Holden, J. P.; Grood, E. S.; Butler, D. L.; Noyes, F. R.; Mendenhall, H. V.; Van Kampen, C. L.; Neidich, R. L. Biomechanics of fascia lata ligament replacements: early postoperative changes in the goat. J. Orthop. Res. 6(5): 639–647; 1988.
15. Huibregtse, A. A.; Johnstone, B.; Goldberg, V. M.; Caplan, A. I. Effect of age and sampling site on the chondro-osteogenic potential of rabbit marrow-derived mesendchymal progenitor cells. J. Orthop. Res. 18:18–24; 2000.
16. Huiskey, R.; Blankevoort, L. Anatomy and biomechanics of the anterior cruciate ligament: A three-dimensional problem. In: Jakob, R. P.; Staubli, H. U., eds. The knee and the cruciate ligaments: Anatomy, biomechanics, clinical aspects, reconstruction, complications, rehabilitation. Berlin: Springer-Verlag; 1992:92–110.
17. Hunziker, E. B. Structural molecules in articular cartilage, tendons, and ligaments. In: Jakob, R. P.; Staubli, H. U., eds. The knee and the cruciate ligaments: Anatomy, biomechanics, clinical aspects, reconstruction, complications, rehabilitation. New York: Springer-Verlag; 1992:62–77.
18. Hynes, R. O. Integrins: A family of cell surface receptors. Cell 48(4):549–554; 1987.
19. Jackson, D. W.; Simon, T. M. Donor cell survival and repopulation after intraarticular transplantation of tendon and ligament allografts. Microsc. Res. Tech. 58:25–33; 2002.
20. Jackson, D. W.; Simon, T. M.; Lowery, W.; Gendler, E. I. Biologic remodeling after anterior cruciate ligament reconstruction using a collagen matrix derived from demineralized bone. Am. J. Sport Med. 24(4):405–414; 1996.
21. Joyner, C. J.; Bennett, A.; Triffitt, J. T. I. Identification and enrichment of human osteoprogenitor by differentiation stage-specific monoclonal antibody. Bone 21:1–6; 1997.
22. Kartus, J.; Movin, T.; Karlsson, J. Donor-site morbidity and anterior knee problems after anterior cruciate ligament reconstruction using autografts. Arthroscopy 17(9):971–980; 2001.
23. Koski, J. A.; Ibarra, C.; Rodeo, S. A. Tissue-engineered ligament. Orthop. Clin. North Am. 31(3):437–451; 2000.
24. Laitinen, O.; Alitalo, I.; Toivonen, T.; Vasenius, J.; Tormala, P.; Vainionpaa, S. Tissue response to a braided poly-L-lactide implant in an experimental reconstruction of anterior cruciate ligament. J. Mater. Sci. Mater. Med. 4:547–554; 1993.

25. Laitinen, O.; Pohjonen, T.; Törmälä, P.; Saarelainen, K.; Vasenius, J.; Rokkanen, P.; Vainionpää, S. Mechanical properties of biodegradable poly-L-lactide ligament augmentation device in experimental anterior cruciate ligament reconstruction. Arch. Orthop. Trauma Surg. 112: 270–274; 1993.
26. McDougall, J. J.; Bray, R. C. Animal models of ligament repair. In: An, H. Y.; Friedman, R. J., eds. Animal models in orthopaedic research. New York: CRC Press; 1999: 461–490.
27. Miller, R. H. Knee injuries. In: Canale, S. T., ed. Campbell's operative orthopaedics. St. Louis: Mosby; 2003: 2274–2275.
28. Nakahara, H.; Goldberg, V. M.; Caplan, A. I. Culture-expanded human periosteal-derived cells exhibit osteochondral potentials in vivo. J. Orthop. Res. 9:465–476; 1991.
29. Nathanson, M. A.; Hay, E. D. Analysis of cartilage differentiation from skeletal muscle grown on bone matrix II. Chondroitin sulfate synthesis and reaction to exogenous glycosaminoglycans. Dev. Biol. 78:332–351; 1980.
30. Noyes, F. R.; Grood, E. S. The strength of the anterior cruciate ligament in humans and Rhesus monkeys. J. Bone Joint Surg. Am. 58(8):1074–1082; 1976.
31. Ouyang, H. W.; Goh, J. C.; Lee, E. H. 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(2):321–327; 2004.
32. Ouyang, H. W.; Goh, J. C.; Thambyah, A.; Teoh, S. H.; Lee, E. H. Knitted poly-lactide-co-glycolide scaffold loaded with bone marrow stromal cells in repair and regeneration of rabbit Achilles tendon. Tissue Eng. 9(3): 431–439; 2003.
33. Pittenger, M. F.; Mackay, A. M.; Eck, S. C.; Jaiswal, R. K.; et al. Multipineage potentials of adult human mesenchymal stem cells. Science 284:143–147; 1999.
34. Shelburne, K. D.; Klootwyk, T. E.; Wilchens, J. H. Ligament stability two to six years after anterior cruciate ligament reconstruction with autogenous patellar tendon graft and participation in accelerated rehabilitation program. Am. J. Sport Med. 23:575–579; 1995.
35. Shelburne, K. D.; Tettig, A. C.; Hardin, G. Miniarthrotomy versus arthroscopic-assisted anterior cruciate ligament reconstruction with autogenous patellar tendon graft. Arthroscopy 9:72–75; 1993.
36. Simonian, P. T.; Harrison, S. D.; Cooley, V. J. Assessment of morbidity of semitendinosus and gracilis tendon harvest for ACL reconstruction. Am. J. Knee Surg. 10: 54–59; 1997.
37. Suggs, J.; Wang, C.; Li, G. The effect of graft stiffness on knee joint biomechanics after ACL reconstruction—a 3D computational simulation. Clin. Biomech. (Bristol, Avon) 18(1):35–43; 2003.
38. Van, E. F.; Saris, D. B.; Riesle, J.; Willems, W. J.; Van Blitterswijk, C. A.; Verbout, A. J.; Dhert, W. J. Tissue engineering of ligaments: A comparison of bone marrow stromal cells, anterior cruciate ligament, and skin fibroblasts as cell source. Tissue Eng. 10(5–6):893–903; 2004.
39. Watanabe, N.; Takai, S.; Morita, N.; Kawata, M.; Hirasawa, Y. A method of tracking donor cells after simulated autologous transplantation: A study using synovial cells of transgenic rats. Cell Tissue Res. 298:519–525; 1999.
40. Zuk, P. A.; Zhu, M.; Mizuno, H.; Huang, J.; Futrell, J. W.; Katz, A. J.; Benhaim, P.; Lorenz, H. P.; Hedrick, M. H. Multilineage cells from human adipose tissue: implications for cell-based therapies. Tissue Eng. 7:211–228; 2001.