Review

Biomaterials and scaffolds for ligament tissue engineering

Zigang Ge,[1] Fang Yang,[2,3] James C. H. Goh,[1,4] Seeram Ramakrishna,[2,3,4] Eng Hin Lee[1]
[1]*Department of Orthopedic Surgery, National University of Singapore, Singapore 119260*
[2]*NUS Nanoscience and Nanotechnology Initiative, National University of Singapore, Singapore 117576*
[3]*Department of Mechanical Engineering, National University of Singapore, Singapore 117576*
[4]*Division of Bioengineering, National University of Singapore, Singapore 119260*

Received 5 April 2005; revised 14 June 2005; accepted 20 June 2005
Published online 20 March 2006 in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/jbm.a.30578

Abstract: Tissue engineering has achieved much progress in an attempt to improve and recover impaired functions of tissues and organs. Although many studies have been done, progress for tissue-engineered anterior cruciate ligaments (ACLs) has been slow due to their complex structures and mechanical properties. In this review, the ACL anatomical structure, progresses achieved, material selection, structure design, and future direction have been discussed, while the challenges and requirements from materials and scaffolds are highlighted. There is a considerably huge amount work that needs to be carried out; as such, future direction in ligament tissue engineering is proposed in hope that this review will give information on future ligament tissue engineering. © 2006 Wiley Periodicals, Inc. J Biomed Mater Res 77A: 639–652, 2006

Key words: ligament; tissue engineering; biomaterials availability; scaffolds; polymer

INTRODUCTION

About 200,000 Americans required reconstructive surgery of ligaments in 2002, with total expenditure exceeding 5 billion US dollars,[1,2] with even higher costs incurred on loss of working abilities, healthcare, and social benefits. Reconstruction of anterior cruciate ligament (ACL) is the most challenging aspect in all human ligaments. The incidence of ACL injuries is high (about 1 per 3000 Americans in 1999) and has increased over the years.[3]. Hence, our primary focus is on ACL tissue engineering.

ACLs injuries may result in significant joint dysfunction, which may consequently lead to injury of other tissues and the development of degenerative joint disease.[4] The poor healing capacity of the ACL has led orthopedic surgeons to perform ACL reconstructions in most of the cases. In current clinical practice, autografts, including the bone-patellar tendon-bone grafts and hamstring tendons,[5] have been the most popular and successful surgical replacements for the ACL. This is due to their potential for graft remodeling and integration into the joint.[6] Nevertheless, donor site morbidity is a major concern when utilizing autografts. Autografts are occasionally not available for use as a result of repetitive surgery or infection. The use of allograft avoids donor site morbidity, reduces surgical time, and minimizes postoperative pain. However, the decrease in tensile properties during sterilization and preservation, as well as risk of inflammatory reaction, have been a concern.[3]

The use of synthetic ligament replacements have gained some popularity in the late 1980s, but only under limited conditions. They do not involve the sacrifice of autogenous tissues and as such, minimizing the associated morbidity and risk of disease transmission. At the same time, they permit a simpler and easier reconstructive technique, as well as a more rapid rehabilitation, as they do not loose their strength during tissue revascularization and reorganization. Currently, braided polytetrafluoroethylene fibers (Gore-Tex) and woven polyethylene terephthalate (Stryker Dacron) ligament prostheses have received general device release from the Food and Drug Administration (FDA) as permanent prosthetic devices, but limited for use in the salvage of previously failed intra-articular autogenous reconstructions.[7] For both

Correspondence to: J. C. H. Goh, Department of Orthopedic Surgery, National University of Singapore, 10 Kent Ridge Crescent, Singapore 119260; e-mail: dosgohj@nus.edu.sg
Contract grant sponsor: Biomedical Research Council, Singapore

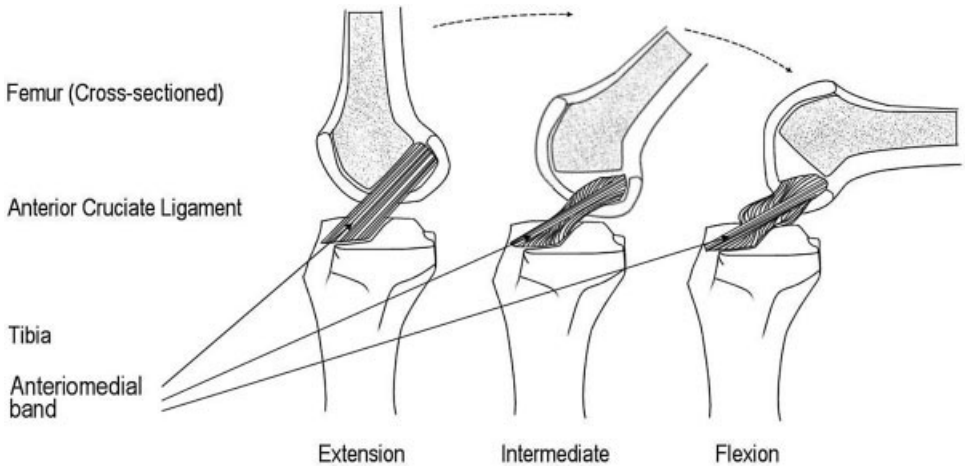


Figure 1. Side view of ACL in extension and flexion.

Gore-Tex and Dacron ligament prostheses, the results of ACL reconstruction deteriorate with time, because of material degradation, foreign body reactions, and related inflammations. Furthermore, ACL prostheses that do not induce tissue ingrowths shield mechanical loading and are prone to fail in the future, because of synovitis, effusions, arthritis, or mechanical deterioration of the prosthesis.[7] Leeds-Keio prosthesis, which is composed of polyester with an open-weave tube to promote fibrous growth, has been popular outside the United States. This device has shown to have host collagenous tissue ingrowths and has improved mechanical properties after implantation.[7] The usage of Leeds-Keio prosthesis is limited because of high incidence of chronic foreign body inflammation, particulate-induced synovitis, some particle shedding into lymph nodes, and complete graft rupture.[8] In 855 prosthetic ligaments tracked for 15 years, 40–78% of them failed owing to wear debris, tissue reactions, and mechanical limitations.[9] As these grafts have yet to display the strength or performance of human ACLs, they are seldom used in current clinical practice.

Research on potential tissue-engineered ACL has been going on for some time, with the hope of overcoming the present problems. Usually, biocompatible and biodegradable scaffolds are necessary to provide structural and mechanical support, which is essential for ligament regeneration. Current research on tissue-engineered ACLs mainly uses biological and synthetic polymers; however, their poor mechanical properties and regeneration are still main concerns.

ANATOMY AND BIOMECHANICS OF ACL

The ACLs are parallel bands of regularly oriented, dense connective tissue that connect the femur and tibia, which are surrounded by a fold of synovium.[10] ACL is made of two bands, the anteromedial band and posterolateral band. The anteromedial band is primarily tight throughout flexion and extension, which makes it even tighter as the knee is flexed. The posterolateral band is tight in extension and becomes quite relaxed, as the knee is flexed (Fig. 1).[11]

Normal ACL comprises of paralleled collagen bundles, which are crosslinked to each other. Fibroblasts are attached to individual collagen bundles and elongated longitudinally while cell densities are low.[12] Fibroblasts not only synthesize fibrillar collagen but also enzymatically break down and remove old collagen as part of a renewal process. The collagen molecule is a glycine-rich triple helix. They assemble sequentially into microfibrils, subfibrils, and fibrils (20–150 nm in diameter) before forming fibers (1–20 μm in diameter) with crosslinks to each others and further make up a subfascicular unit (100–250 μm in diameter). These subfascicular units are surrounded by a loose band of connective tissue known as the endotenon. Three to 20 subfasciculi subsequently form a fasciculus (from 250 μm to several millimeters in diameter), which are surrounded by an epitenon. This interfascicular connective tissue also supports the neurovascular elements of the ligament.[13] These individual fascicles are either oriented in a spiral fashion around the long axis of the ligament or they pass directly from the femur to the tibial attachment. The entire continuum of fascicles is surrounded by the paratenon, a connective tissue cover similar to but much thicker than the epitenon.[10]

ACLs are made up of multiple collagen fibers crosslinked to each other, which give the high tensile strength. As they withstand cyclic loads of ~300 N for about 1.5 million times per year, it is important for fibroblasts to maintain collagen fibers in good conditions dynamically.[12] ACLs attach to the femur and tibia via collagen fibers that join into bone structures.[14] The

TABLE I
Tissue-Engineered ACL Tested *In Vivo*

	Polymer	Structure	*In Vivo* Model/ Duration	Ultimate Tensile Load (N)[a]	Ultimate Tensile Strength (MPa)[a]	Author/Date of Publication
Biological polymers	Collagen fiber	Crosslinked	Rabbit/20 weeks	32 (12.7)	10 (20.4)	Dunn et al., 1992[21]
	Collagen fiber	PLA matrix	Rabbit/4 weeks	40	13 (34.2)	Dunn et al., 1995[22]
	Collagen matrix from bone	Block	Goat/1 year	474 (18.7)	49 (28.7)	Jackson et al., 1996[23]
	Collagen fiber	Braided/Crosslinked	Goat/6 months	102 (6.9)		Chvapil et al., 1993[24]
Synthetic polymers	PLLA fiber	Braided	Sheep/48 weeks	175 (12.3); 295 (20.7, fascia wrap)		Laitinen et al., 1993[25]
	PLLA/PLGA fiber	Knitted	Rabbit/20 weeks	21.1 (13.9)		Ge et al.[26]

[a] Values in parentheses are given in percentages.

abrupt change from flexible ligament tissue to rigid bone is mediated by a transitional zone of fibrocartilage, which allows a gradual change in even distribution of stress.[13] To successfully reconstruct an ACL, it is necessary to understand the anatomy, orientation, and attachment sites of the normal ligament. In the reconstruction of the ACL, the graft must be positioned so as to minimize the change in length within the ligament, as the knee is flexed and extended.[10]

The basic movement between the femur and tibia is a combination of rolling and gliding, as well as rotation. The loss of ACL integrity, as well as three or more knee ligaments following injury, will cause complex pathological instability and disability will probably ensue without surgical intervention.[15] A good understanding about kinematics of the cruciate ligaments is essential not only for surgeons performing the reconstruction but also for scientists to design tissue-engineered structures.

Increasing number of ACL fibers are recruited into action and oriented along force direction (usually longitudinal) under gradually increased tension, from the isometric points to the nonisometric bulk of ligament fibers.[15] The crimp fibers in the ACL allow for 7–16% of creep prior to permanent deformation and ligament damage. These two properties not only allow most of fibers to avoid tension in most of time but also prevent structural damage. The ACL is also regularly exposed to tensile forces ranging from 67 (for ascending stairs) to 630 N (for jogging) during activities of daily living.[16] However, the maximal tensile load for ACL was found to be 1730 N, as well as 182 N/mm for linear stiffness,[17] and 12.8 N m for energy absorbed at failure.[18] Young's modulus of human ACL is 111 MPa and ultimate tensile stress is at least 38 MPa,[17] while ultimate mechanical properties of ligaments generally increase during development and eventually diminish with aging.[19] The maximum strain that a ligament can endure before failure is between 0.12 and 0.15 strains.[20]

CHALLENGES IN LIGAMENT TISSUE ENGINEERING

Current research on tissue-engineered ACLs has focused on the use of biological and synthetic polymers that are biocompatible and degradable. Though there are many reports on tissue-engineered ACLs, only a few of them have been tested *in vivo* for ACL reconstruction (Table I). Collagen and polylactic acid (PLA) are the most often used; however, none of them has achieved more than 20% of ultimate tensile strength of human ACLs. Furthermore, no functional collagen bundle formation has been reported, which could withstand mechanical loads. Fibroin (silk) ACL scaffold have shown promising results,[27] but no further *in vivo* test has been reported. Conceptually, *in vitro* cultured tissue-engineered ligament with two bone ends would be ideal, as what has been reported,[28] but there is no further progress reported. However, it still indicates further direction.

In general, progress in ligament tissue engineering has been rather slow, this is due to several reasons: (1) ACLs have to undergo complex and multidirectional mechanical forces *in situ*. To date, no scaffold has been reported to be able to handle these *in vivo* mechanical loadings properly; (2) the blood supply is disrupted after ACL rupture. It will impede the potential regeneration of ACLs; (3) the transitional fibrocartilage zone between bone and ligament poses a great challenge to reconstitute with current techniques; (4) significant changes of cytokine profiles after ACL injuries lead to the difficulties in ACL regeneration[29]; and (5) inability in current tissue engineering techniques to restore the

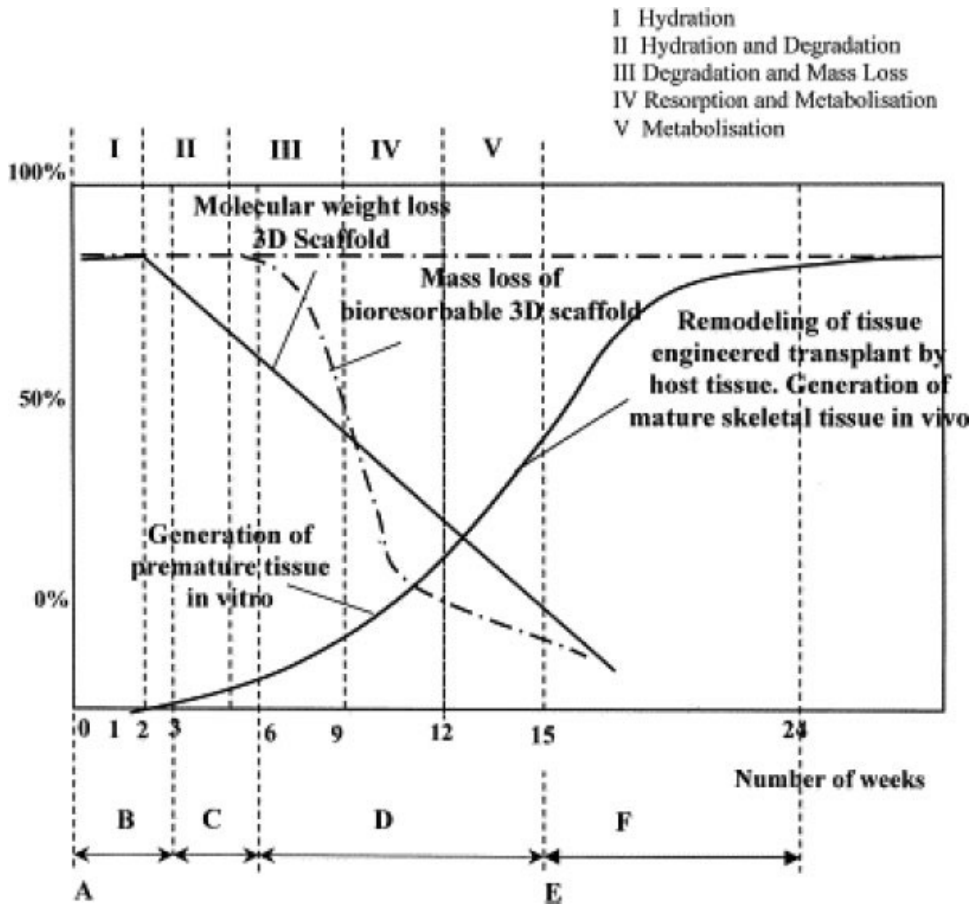


Figure 2. Ideal degradation of materials for tissue engineering. (Reprinted with permission from Dr. Dietmar Hutmacher.[31])

stretch-sensitive mechanoreceptors in ACL that trigger muscle contractions to protect the knee from extremes of motion.[30]

In current stage, most attention is paid to the aforementioned first difficulty to improve the mechanical properties of scaffolds to match ACL's. Except for biocompatibility, there are several technical hurdles before we can get scaffolds with good mechanical properties:

a. Initial mechanical properties of scaffolds should match to ACL, in terms of ultimate tensile load and strength, linear stiffness, viscoelasticity, Young's modulus and so forth.
b. Tissue-engineered ACL structures should withstand multidirectional stresses without deforming *in vivo*, while *in vitro* test can only evaluate in one direction.
c. With *in vivo* tissue ingrowth and material degradation, the mechanical properties of scaffolds will change markedly as the cross-sectional area changes (enlarged after tissue ingrowth).
d. Mechanics of structures usually drop before mass degradation and lead to quick loss of initial properties.
e. Creeping (viscoelasticity) is common for polymers and textile structures, which would lead to catastrophic laxity of scaffolds and loss of their initial functionality.

MATERIALS IN LIGAMENT TISSUE ENGINEERING

All the materials and their degraded products have to be biocompatible when used in tissue engineering. The ideal way is to mimic the normal ACL structures in mechanical properties initially and gradually transfer mechanical strength of scaffolds to regenerated collagen bundles while degrading (Fig. 2).[31]

Biocompatibility, defined as "acceptance of an artificial implant by the surrounding biological tissue and by the body as a whole,"[32] is essential for all materials as well as their potential degraded products when used in ligament tissue engineering. Degradation means the materials could be totally removed by the host with time. Currently, all the materials used in ligament tissue engineering are polymers. A polymer is a large molecule built up by the repetition of small,

simple chemical units. The chains are branched or interconnected to form three-dimensional networks. Except for biocompatibility and degradation, functional feasibility, sterilization, and low cost would further boost their usage. Polymers can be divided into two groups, natural polymers and synthetic polymers. The most often used natural polymer in ligament tissue engineering research is collagen[21–24] as well as the potential silk fibroin (a component of silk).[33] Frequently used synthetic polymers are the most often used synthetic polyesters, especially PLA.[25]

Collagen

Since collagen accounts more than 80% of the dry weight of a normal ligament,[34,35] it is reasonable to reconstruct ACL with it. Many collagen-based constructs have been used in ACL reconstruction.[21–24] Usually, collagen used in laboratories is derived from the bovine submucosa and intestine,[35] as well as from rats' tails in small quantities. The derived collagen has to be processed to remove foreign antigen, improve its mechanical strength, and sometimes to slow down the degradation rate by crosslinking before usage.[36,37] The pure triple helical collagen molecule does not elicit a strong antigenic response, which is mainly from associated cellular debris, ground substance, or the associated nonhelical telopeptide region of the collagen molecule.[34,35] Many methods have been reported to dissociate, purify, and reconstitute collagen to achieve this aim.[36,37]

In normal ligament, crosslinks are essential to both tensile strength characteristics and resistance to chemical or enzymatic breakdown. The most common three crosslink types are hydroxulysinonorleucine (HLNL), dihydroxulysinonorleucine (DHLNL), and histidinohydroxymerodesmosine (HHMD). Their absence causes the collagen fibers to be extremely weak and friable.[38] Crosslinking has been used to slow down the degradation as well as the drop of mechanical properties. As it is difficult to exactly mimic native crosslinking process, the predominant chemical crosslinking agents used in research are glutaraldehyde, formaldehyde, polyepoxy compounds, acyl azide, carbodiimides, and hexmethylene diisocyanate.[39] A drawback is the potential toxic residues. Physical methods include drying, heating, or exposure to ultraviolet or gamma radiation. It does not introduce toxic chemicals, but may incur undefined side effects.[39]

PLA-reinforced collagen also could not improve mechanical strength, as PLA could not integrate well with collagen.[33] Relatively quick *in vivo* degradation and loss of mechanical strength are main concerns when collagen is used in ligament tissue engineering. Di-catechol nordihydroguaiaretic acid (NDGA) crosslinked collagen fibers achieved much improved mechanical properties that matched with normal ACL, but no biocompatibility, structures for ACL reconstruction, and *in vivo* experiment have been reported.[40] However, it was reported that the crosslinking do not arrest the scheduled drop of mechanical strength in ligament tissue engineering as predicted.[24] More research is necessary to optimize the crosslinking conditions and different methods. In general, collagen-based scaffolds are compatible with synthetic polymers, easily modifiable, hemostatic, synergic with bioactive components, and are not toxic. So far, the most matured ACL regeneration is reported from collagen-based ACL regeneration.[21,23] However, high cost, variability, hydrophilicity, complex handling properties, and potential disease transmission are the existing disadvantages.[36]

Silk

Silks are generally defined as protein polymers that are spun into fibers by silkworms, as well as spiders, scorpions, mites, and even flies. Silks from different sources have different amino acid composition and mechanical properties. Similar to collagen with repeated triple helices, silk is characterized by a highly repetitive β-sheet that leads to significant homogeneity in secondary structure.[41–43] Silk from *B. mori* silkworm is the largest and most stable source that has been commercialized for a long time. Silk comprises of a fibroin core and a glue-like sericin cover. Unique mechanical properties, as well as biocompatibility, slow degradation time, and options for genetic control, make fibroin suitable for ligament tissue engineering.[44–46] The extraordinatory mechanical properties and the enhanced environmental stability of silk fibers are due to the high homogeneity in secondary structure (β-sheet), extensive hydrogen bonding, the hydrophobic nature of much of the protein, and the crystallinity. Silk undergoes proteolytic degradation at a variable rate dependent on the environmental conditions. Silk fibers lose the majority of their tensile strength within 1 year *in vivo*, and fail to be recognized in 2 years.[27]. Although the glue-like sericin in silk is the major cause of adverse problems with biocompatibility and hypersensitivity,[47] many processes were used to remove the sericin. It is important to ensure that all sericin has been removed before usage. Fibroin-based ACL tissue engineering constructs have been reported to have similar mechanical properties and good *in vitro* biocompatibility.[47,48] However, no further *in vivo* experiment has been reported.

Polylactic acid

PLA is also an aliphatic polyester and is more hydrophobic and amorphous than polyglycolic acid (PGA).[49] Because of an extra methyl group compared with PGA, PLA usually has two isoform, L- and D-forms, although L-form PLA, also known as PLLA, is most often found. It degrades into lactic acid by de-esterification and degrades completely within a period of between 10 months and 4 years depending on its molecular weight, crystallinity, shape, and implantation site.[50] When used in sheep ACL reconstruction, only 12.3% of ultimate tensile load has been reported after 48 weeks' implantation.[25] There is no encouraging results reported when used as reinforce of collagen fiber.[7]

Poly-caprolactone

Polycaprolactone is also degradable thermoplastic polyester derived from the chemical synthesis of crude oil. As a semicrystalline polymer, its crystallinity tends to decrease with increasing molecular weight. Its high solubility in organic solvents, low melting point (59–64°C), slow degradation rate, and exceptional ability to formulate blends have lead to many biomedical applications. PCL sutures were reported to keep 90% of original strength after 18 weeks' implantation.[51] Though no PCL-based tissue-engineered ligament has been reported, it can be a good candidate for potential ACL prosthesis because of its slow degradation and ability to formulate blends.

Polycarbonate

Polycarbonates are linear thermoplastic polyesters of carbonic acid with aliphatic dehydroxy compounds and degrade by hydrolysis. Fibers from poly(DTE carbonate) have been fabricated to ACL scaffolds with good initial mechanical properties and kept much higher ultimate tensile strength (87% of original) after 30 weeks' *in vitro* degradation, when compared with PLLA (7% of original).[52] They have also shown good biocompatibility *in vitro* and *in vivo*.

Biological polymers, such as polysaccharides, alginate, agarose, chitin, chitosan, and hyaluronan, could be used according to their respective properties due to their good biocompatibility. Synthetic polymers, as well as their composites, have their advantage on ease of processing and controlled degradation.[31] However, the chemically and biologically inert polymeric materials are unlikely to induce cell adhesion and tissue formation. To overcome this drawback of the synthetic materials, natural polymers extracted from the native extracellular matrix (ECM) have been used to modify the synthetic materials to improve the cell adhesion properties.[36]

SCAFFOLD DESIGN IN LIGAMENT TISSUE ENGINEERING

The ACL anatomy structure and its mechanical properties have been described in earlier section in detail. We know that ACL is a load-bearing tissue, which plays an important role in providing stability during the movement of the joints, for example extension and flexion. From mechanical point of view, ACL is an anisotropic structure, which mainly bears the extension force in axial direction. To restore the ACL function, the ACL reconstruction should perfectly mimic all the characteristics of a normal ACL in terms of strength, compliance, elasticity, and durability without any side effects. Therefore, the mechanical properties of the ACL scaffold are of critical importance during the regeneration or remodeling (Table II).

In anatomy, ACL comprises of bands of dense collagen fibers. A fiber is a simple element of anisotropic structure. All the ACL scaffolds and synthetic prostheses are practically composed of fibers. The differences are the way to organize the fibers, that is their textile structures. Parallel structure is the simplest way to organize the fibers. However, the lack of interaction between fibers restricts its application. Twisting fiber bundles is a elegant way to solve the problem.[27] Twisting grafts are morphologically closer copies of the normal ACL and can eventually reduce and fine-tune the peak forces in extension.[51] Because of the complexity of the ACL mechanical environment, researchers are keen to adopt more complex textile structures in ACL reconstruction, including the all four textile categories: woven, knitted, and braided, except for non-woven ones, which are too weak to use in ligament reconstructions.

The properties of textile grafts depend on the characteristics of the constituent yarns or fibers and on the geometry of the formed structure. In general, braided grafts are dimensionally very stable, but less extensible and porous than the other structures. A good example is the Gore-Tex ligament prosthesis composed of braided bundle of polytetrafluroethylene (PTFE).[58] This graft has high strength and fatigue life, but tissue ingrowth due to the low porosity.[2] However, the pore size of the braided structures can be regulated by yarn bundle size and braiding angles.[55] When compared with braided fabrics, knitted structures are highly porous, which supports tissue ingrowth. It has been reported that the Dacron's knitted outlayer promotes good ingrowth of fibrous tissue.[59]

TABLE II
Mechanical Properties of Materials Currently Used in ACL Reconstruction

	Ultimate Tensile Load (N)	Stiffness (N/mm)	Elongation at Break (%)	Young's Modulus (MPa)
Human ACL	2195[53]; 1725–2160[54]; 2160 ± 157[27]	306[53]; 242 ± 28[27]	~33[27]	110[17]
Human hamstring graft	3790–4140[53]	776[53]		
Human patellar-tendon graft		685 ± 86[27]		
Gore-Tex Prosthesis	5300[2]; 4830[54]	322[2,54]	9	
Dacron	3631	420	18.7	
Kennedy ligament augmentation device	1500[54]	36[54]		
Carbon fibers				2100–2350[54]
Twisted silk matrix	2337 ± 72[27]	354 ± 26[27]	38.6 ± 2.4[27]	
Parallel silk matrix	2214[27]	1740[27]	26.5[27]	
Braided PLGA	907 ± 132[55]			
Knitted PLLA–PLGA scaffold	29.4[26]			283[56]

Textile materials for tissue engineering applications typically have specific performance requirements relating to porosity and mechanical properties.

Porosity

It is known that a highly porous scaffold is desirable to allow cell seeding or migration throughout the materials. Pore size is important for tissue ingrowth and determines the internal surface area available for cell attachment. A large surface area is required so that a high number of cells, sufficient to replace or restore organ function, can be cultured.[60] The fabric structures are hierarchically opened porous structures. Their pore porosities can be considered from three aspects.[61] One is the open space inside created by the loops for a knitted fabric or the pores between yarns in other fabric structure, which may range from tens to hundreds of microns. The other is the distance between filaments or fibers, which is about a few microns. A third kind of porosity can be induced by the method of assembling the fabrics by folding or rolling.

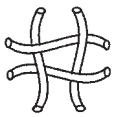
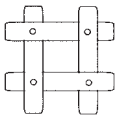
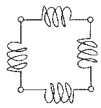
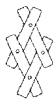
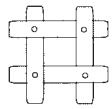
Mechanical properties

The mechanical properties of the scaffold are often of critical importance especially when regenerating hard tissues such as ligament, tendon, cartilage, and bone. The ideal artificial ACL perfectly mimics all the characteristics of a normal ACL in terms of strength, compliance, elasticity, and durability without any side effects. Unfortunately, none of the synthetic grafts have met the qualifications needed for a lasting ACL substitute (Table III). First, synthetic grafts are too rigid and begin to fragment gradually due to repeated cycling of the knee and probably some chafing at the edges of the bone tunnels, which led to particles of prosthetic materials shedding and distributing throughout the joins, even occasionally spreading into the lymphatic system.

Regardless of their initial mechanical properties, a loss of them occurs after transplantation due to the processes of ischemic necrosis and remodeling. Strength and stiffness of the grafts are also lowered by adding fixation.[53]

If the stiffness of the implant greatly exceeds that of the ingrown host tissue, most of the mechanical load will be taken by the implant, and the load-deprived host tissue will not remodel or mature. To address this problem of stress-shielding, a ligament augmentation device (LAD) is needed to protect biological grafts from high loads in the early postoperative period. In addition to being used as a permanent prosthesis, Kenny LAD is a braided polypropylene yarn attached to bone on only one end of the autogenous repair. Because of the low stiffness of the device, stress-shielding of the graft is reduced; thus, normal neoligament remodeling should occur.[54] However, the long-term maintenance of the mechanical properties of the device is not necessary or even desirable. Therefore, an ideal LAD should be biodegradable, gradually transferring mechanical loads completely to the biological graft. The tissue engineering approach provides optimism by using biodegradable scaffolds combining with appropriate cell sources[72] to create mechanically and biologically functional substitute. In contrast to the permanent synthetic prostheses losing strength with time, the mechanical behavior of the tissue engineering grafts should improve with time because of neoligament tissue development and remodeling.

TABLE III
Properties of Textile Structures

	Woven	Knitted	Braided	Nonwoven
Composition[76]	Yarn	Yarn	Yarn	Fiber
Formation [76]	Interlace	Interloop	Interwine	Bond or entangled
Geometry[76]				
Cell model[62]				
Mobility[62]	Limited	Tremendous	Limited	Very slight
Porosity	High	Very high	High	High
Examples	Leeds-Keio PET graft[63,64]	Stryker-Dacron PET graft[65–67]; biodegradable scaffold[56]	Gore-Tex PTFE graft[54,65,68]; biodegradable scaffold[22,55]	Fiber bonding[69]; electrospinning[70,71]

CELL-SURFACE INTERACTION AND SURFACE MODIFICATION

Cells and materials are two essential components in ligament tissue engineering, and so the interactions between them are important. Materials could interfere with cells' adhesion, proliferation, and differentiation,[73] while cell adhesion and subsequent functionality also affect properties of surrounding materials. The materials and subsequent surface modification are supposed to promote reparative cells (fibroblasts) and progenitors to adhere and grow, as well as functionality of regenerated tissues, while repelling inflammation cells, such as macrophages, lymphocytes, neutrophils and so forth. Usually cells could adhere to material surface either by direct adhesion or by preabsorbed proteins.[74] As the most efficient seeding cells for ligament tissue engineering and progenitor cells for ACL fibroblast, mesenchymal stem cells (MSCs) have got much attention[73] and there are many *in vitro* experiment on interaction between MSCs and materials.[4]

Currently, there are two common approaches to modify the materials to improve their biocompatibility, coating with "biocompatible materials," or surface modification. As nearly all interactions between mammalian cells and artificial surfaces are mediated by a layer of adsorbed protein,[75] many proteins have been coated on the surfaces of target materials to promote adhesion, for examples, fibrinogen,[76,77] collagen,[78] hyaluronan,[79–81] and so forth. However, physical surface coating is difficult to be homogeneous at microscopic level as well as only happens on the surface and degrades soon. Collagen can also be grafted chemically on PLLA surface with —COOH groups induced by polymerization of methacrylic acid.[82] Stable collagen layer on 3D PLLA scaffolds was formed by —OOH/Fe^{2+}-induced polymerization.[83]

Integrins are a family of cell surface receptors that medicate the cellular recognition and bind to adhesive proteins of the extracelluar matrix, such as fibronectin. The amino acid sequence Arg-Gly-Asp (RGD) in integrin has been immobilized onto synthetic surfaces to promote cell adhesion in a similar manner to fibronectin.[73]

Furnishing scaffolds with biochemical factors are also promising. Lots of biochemical factors, including growth factors, could promote tissue regeneration, such as Ascorbate-2-phosphate, epidermal growth factor (EGF), platelet-derived growth factor (PDGF), basic fibroblast growth factor (bFGF), insulin-like growth factor II (IGF-II), transforming growth factor-β (TGF-β), and bone morphologic protein (BMP)-12.[84] However, the main concerns to use growth factors are cost and controlled release. Growth factors could be incorporated into the scaffold either in or after scaffold fabrication[2] (or added when cultured *in vitro* sometimes), as well as be cotransplanted through natural growth factor-secreting cells or genetically engineered cells within the tissue engineering constructs.[85]

Vascular endothelial growth factor (VEGF) released from a porous polymer matrix utilized for cell delivery could improve the vascularization and survival rate of seeding cells.[86] Previously, VEGF reversibly bound to the hydrogel carrier could be released on mechanical stimulation.[87] VEGF and platelet-derived growth factor (PDGF) could be released from same polymeric scaffold with distinct kinetics for therapeu-

tic purposes.[88,89] To achieve this aim, VEGF was simply mixed with polymer particles, while PDGF was pre-encapsulated in microspheres in the polymer scaffolds from which it was released by degradation in a delayed fashion. When EGF was covalently coupled to aminosilane-modified glass via poly(ethylene oxide) (PEO), it was as effective as soluble EGF in eliciting DNA synthesis and cell rounding responses.[89,90]

TISSUE REGENERATION AND FUNCTIONALITY

As dense and well-organized connective tissue, ligament attributes its function much to its specific structures, crosslinked collagen bundles, and crimps.[12] Though there are no unique cell markers for ACL, it still can be evaluated and distinguished by its specific expression of different factors and their relative ratios.[2]

- Specific expression of extracellular matrix components of collagen types I, III, V, and their ratios, for example, collagen amount: 80.3 mg collagen/g dry tissue and ratio of collagen types I and III, 7.3 (both lower than tendon)[91];
- Different type and higher amount of reducible crosslinks compared with MCL and tendon[38];
- Different cell morphology and function with distinct regions.[91]
- Special ultrastructure of collagen network (collagen pattern, collagen fibril diameter)[38];
- Higher metabolical rate than tendons[90];
- Specific expression of ground substances, elastin, fibronectin, decorin, and biglycan;
- Specific expression of glycoaminoglycans (GAG), such as hyaluronic acid and chondroitin sulfate[38];
- Furthermore, the composition of individual component changes in regeneration, while some of them promote functionality and the others may not or impede it.[92]

All these characteristics could give clues for future scaffolds for ligament tissue engineering. As degradation of scaffolds is inevitable, it is important to promote rapid functionality of regenerated collagen fibers. Except for seeding cells, enhancing the growth factors that have been implicated in regeneration have all shown promise.[93] Blocking decorin formation by antisense gene therapy could increase diameter and maximal tensile strength of regenerated ligament.[92] Blocking collagen V formation may have similar effect.[94] Scaffolds carrying antisense gene therapy could be helpful.

Collagen fibers and "crimp-like structures" with different maturities have been reported after ACL reconstruction with tissue-engineered ligaments, for examples, from carbodiimide crosslinked collagen fibers after 20 weeks[21] and from hexamethylenediisocyanate (HMDIC) crosslinked collagen after 3 months.[24] So far, the most mature regenerated collagen fibers are from demineralized bone[23] and fascia lata wrapped braided PLLA yarns.[95] However, the unsatisfactory mechanical results showed that the regenerated collagen were unable to demonstrate original mechanical properties. Three reasons could be attributed to (1) the reported relatively mature and well-orientated collagen fibers reside only in some areas of the grafts, but not homogenously; (2) they were still immature collagen with small diameters; and (3) collagen fibers were not well oriented and lacked crosslink.

ANIMAL MODEL OF LIGAMENT

The use of animals in orthopedic research has played a vital role in the numerous medical advances, though public controversy regarding animal experimentation still exists. Most people, however, support animal experimentation, emphasizing the need to pay particular attention to animal welfare and animal rights.[96] As animal research acts as the bridge between *in vitro* studies and human clinical trials, the ideal way is to choose primate models, as the results from them are easily extrapolated to human conditions. However, when ethics, availability, housing requirements, ease of handling, costs as well as susceptibility to disease are considered as a group, the choices of animal species are usually compromised.[97]

To investigate ligament regeneration and reconstruction, it is prudent to choose animal models in which the type and degree of ligament injury are similar to that in humans, and to realize that the results from animal research may be species- and ligament-specific and may not necessarily simulate that in humans.[98] Most often used animal models in ligament research are dogs, rabbits, goats, and sheep. The reasons to use dogs are due to the ease in handling, receptiveness to various exercise regimens as well as well-published information.[98] Goats and sheeps are broadly used for ACL reconstruction using biological grafts and ligament prostheses[99,100] because of larger knee joint size and less degree of flexion. Rabbits are widely used in biomedical research due to their docile nature and relatively inexpensive purchasing cost and maintenance as well as well-documented biochemical and functional properties of rabbit knee ligaments.[97] However, their rather small joint size limits accuracy of ACL operations and obesity during long-term caging leads to high loads in the knee joints.[98] After the consideration of all these factors, the rabbit model was chosen to evaluate the performance of our knitted scaffolds for ACL reconstruction.

COMPOSITE SCAFFOLDS

As mentioned earlier, no satisfactory results have been reported on scaffold with good initial mechanical properties, controlled degradation, and biocompatibility when used for *in vivo* ACL reconstruction. In current stage, a composite structure to meet the basic requirements should be a dual structure. The two ends of the dual structure should be induced for osteogenesis and integrated with host bone after implantation, while the middle part of it should host tissue ingrowth and its subsequent functionality with time [Fig. 3(a)].

From cross-sectional view, it is composed of multiple layers, while individual layer takes different functions as well as degrades at different rates [Fig. 3(b)]. At initial stage (A), the outer layer of the structure, a biomembrane, blocks the inflammation cytokines and other macromolecules from knee joints while allowing free nutrient ion exchange. The middle layer, a loose structure, provides good microenvironment for tissue ingrowth as well as subsequent functionality. The growth factors kept in the middle layer will be released to promote faster tissue ingrowth. The core of the composite structure is made of multiple intact layers, which takes the necessary mechanical strength for ACL reconstruction and degrades layer by layer. The changes of mechanical properties will be moderate and be fully offset by regeneration and functionality in middle layer. Thus, the general mechanical properties of the structure will keep stable during degradation and always match that of the ACL. The growth factors kept inside the core of the composite structure, mainly to promote blood supply and tissue functionality, will be released at later stage at a stable rate.

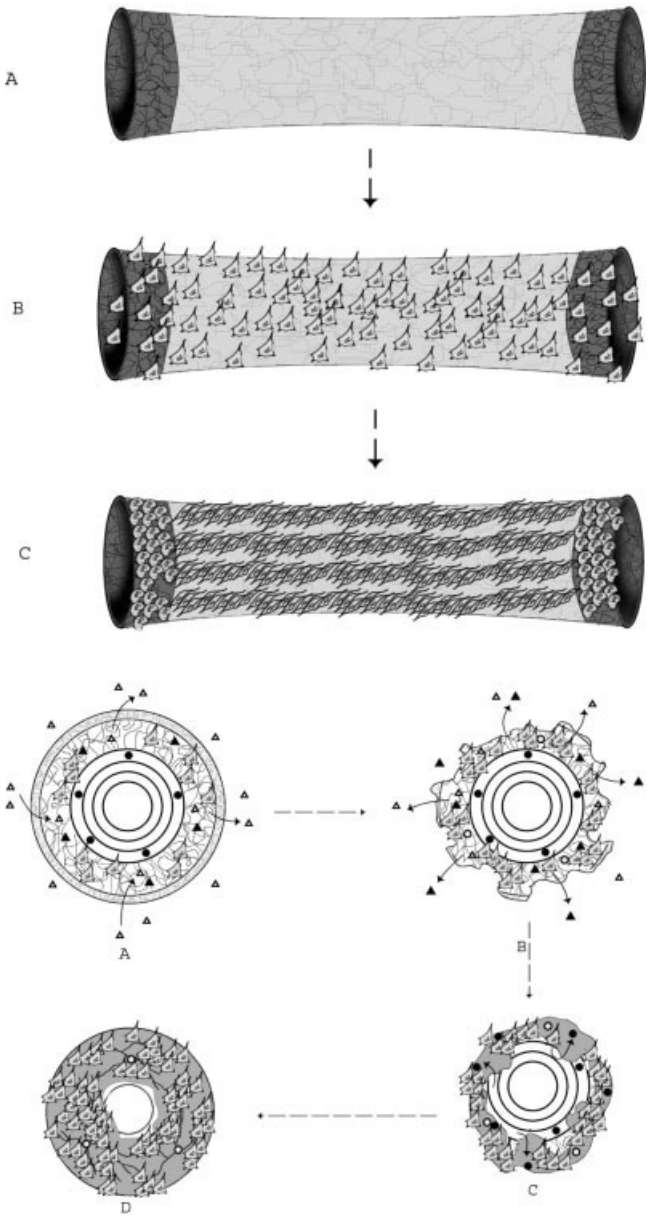


Figure 3. (a) Schematic representation of the progression of composite scaffold in ligament regeneration. A: Composite structure biodegradable scaffold, with both ends being osteo-inductive and the middle portion having potential for ligament/tendon tissue formation/regeneration. B: Cell-seeded composite scaffold with proliferative potential or reparative cells recruited into the scaffold with regenerative potential. C: Cells in two ends will be induced to form bone and integrate with host bone, while cells in middle part will express ECM. (b) Cross-sectional view of composite structure for ACL reconstruction. The cross-sectional view shows the three individual layers in the composite scaffold. They are (1) protective cover (outer layer); (2) porous nanostructures; and (3) strong internal cord. The irregularly shaped structures found within the porous nanostructural region are the transplanted or reparative cells. Growth factor molecules shown here in "open triangle" on porous nanostructures will be released at early stage. Growth factor molecules (closed circle) encapsulated in the internal cord will be released, while internal cord degrades in later stage.

FUTURE DIRECTION

Bioreactor

Potential tissue-engineered ACLs would not only face harsh environments, such as complex mechanical and enzymatic attacks, but also the lack of blood supply at the initial stage.[13] A relatively functional tissue-engineered ACL is needed before reconstruction, which absolutely needs usage of bioreactors. Bioreactor is supposed to provide a controlled environment to direct cells to special tissue structure.[101] While knowledge about embryo development and adult regeneration develops, bioreactors could employ more controlled conditions to fabricate desired tissues. Many progresses have been reported on bioreactors,[23,102,103] with some of them specially designed for ligament tissue engineering. Ligament-like tissue have been

made in some general bioreactors, as well as in ligament designed bioreactors.[101] In the future, tissue-engineered ligaments could be incubated in bioreactor prior to usage. The materials and structures used will have to be adapted to the bioreactors.

Blood supply

As the lack of blood supply after ACL ruptures is one of the main reasons to impede subsequent ligament regeneration,[104] controlled angiogenesis should be beneficial. As VEGF has long been regarded to have strong influence on blood supply, it would be beneficial to introduce it at both two bone tunnels as well as mid-substance of tissue-engineered ligaments.[104,105] Another potential solution is to reconstruct blood vessel inside the tissue-engineered ligaments before implantation, possibly after *in vitro* incubation in bioreactor.

Protective cover

There were significant changes of cytokine profiles after ACL injuries, which could lead to the difficulties of ACL regeneration.[30] Unfortunately, until now the exact roles and pathway of each cytokine are not clear. It has been hypothesized that ACL regeneration could be easier if these cytokines are handled in proper ways. It will be beneficial for tissue-engineered ligaments to have a protective cover, just like normal synovium.

Mechanoreceptors

Stretch-sensitive mechanoreceptors in the ligaments of the knee trigger muscle contractions that protect the knee from extremes of motion.[30] Current reconstructive procedures may restore the structural role of ligaments, but traumatic loss of proprioception is most likely permanent under current therapy. A tissue-engineered mechanoreceptor in ligament will be good for its function.

SUMMARY

Tissue engineering has achieved much progress in recent years while a lot of potential tissue-engineered devices used for ACL reconstructions have been reported, but most of them are only *in vitro* preliminary reports. The main difficulties are complex mechanical stress faced by tissue-engineered ACLs, harsh environment, and poor blood supply after ACL ruptures. Because scaffolds are necessary for ACL reconstructions, here we have discussed ACL anatomy, kinematics, progresses reported, difficulties faced, requirements for materials and structures, functionality, ideal structures as well as future direction in ligament tissue engineering, while focusing on requirement for selection and modification of biomaterials and structures. An ideal model of scaffold is given to overcome technical difficulties in current stages. In addition, functionality and nerve supply are also discussed.

The authors thank Dr. Rong Guowei and William Tan for their valuable contributions during the preparation of this article as well as schematic drawings. Funding for the author's studies of ligament tissue engineering has been provided by Biomedical Research Council (Singapore).

References

1. Pennisi E. Tending tender tendons. Science 2002;295:1011.
2. Vunjak-Novakovic G, Altman G, Horan R, Kaplan DL. Tissue engineering of ligaments. Annu Rev Biomed Eng 2004;6:131–156.
3. Fu FH, Bennett CH, Lattermann C, Ma CB. Current trends in anterior cruciate ligament reconstruction. Part 1: Biology and biomechanics of reconstruction. Am J Sports Med 1999;27: 821–830.
4. Woo SL, Hildebrand K, Watanabe N, Fenwck JA, Papageorgiou CD, Wang JHC. Tissue engineering of ligament and tendon healing. Clin Orthop 1999;367(Suppl):312–321.
5. Simonian PT, Harriso SD, Cooley VJ. Assessment of morbidity of semitendinosus and gracilis tendon harvest for ACL reconstruction. Am J Knee Surg 1997;10:54–59.
6. Amiel D, Kleiner JB, Roux RD. The phenomenon of "ligamentization": Anterior cruciate ligament reconstruction with autogenous patellar tendon. J Orthop Res 1986;4:162–172.
7. Miller RH. Knee injuries. In Canale ST, editor. Campbell's Operative Orthopaedics. St. Louis, MO: CV Mosby; 2003. p 2274–2275.
8. Koski JA, Ibarra C, Rodeo SA. Tissue-engineered ligament. Orthop Clin North Am 2000;31:437–451.
9. Frank CB, Jackson DW. The science of reconstruction of the anterior cruciate ligament. J Bone Joint Surg Am 1997;79: 1556–1576.
10. Arnoczky SP, Warren RF. Anatomy of the cruciate ligaments. In: Feagin JA, editor. The Crucial Ligaments: Diagnosis and Treatment of Ligamentous Injuries about the Knee. New York: Churchill Livingstone; 1994. p 269–287.
11. Jackson RW. The torn ACL: Natural history of untreated lesions and rationale of selective treatment. In: Feagin JA, editor. The Crucial Ligaments: Diagnosis and Treatment of Ligamentous Injuries about the Knee. New York: Churchill Livingstone; 1994. p 485–493.
12. Danylchuk KD, Finlay JB, Krcek JP. Microstructural organization of human and bovine cruciate ligaments. Clin Orthop 1978;131:294–298.
13. Akeson WH, Woo SLY, Amiel D, Frank CB. The chemical basis of tissue repair: Ligament biology. In: Hunter LY, Funk FJ, editors. Rehabilitation of the Injured Knee. St. Louis, MO: CV Mosby; 2004. p 92.

14. Cooper RR, Misol S. Tendon and ligament insertion. A light and electron microscopic study. J Bone Joint Surg Am 1970; 52:1–20.
15. Muller W. Kinematics of the cruciate ligaments. In: Feagin JA, editor. The Crucial Ligaments: Diagnosis and Treatment of Ligamentous Injuries about the Knee. New York: Churchill Livingstone; 1994. p 289–305.
16. Chen EH, Black J. Materials design analysis of the prosthetic anterior cruciate ligament. J Biomed Mater Res 1980;14:567–586.
17. Noyes FR, Grood ES. The strength of the anterior cruciate ligament in humans and Rhesus monkeys. J Bone Joint Surg Am 1976;58:1074–1082.
18. Woo SL, Adams DJ. The tensile properties of human anterior cruciate ligament (ACL) and ACL graft tissues. In: Daniel D, Akeson W, O'Connor J, editors. Knee Ligaments: Structure, Function, Injury, and Repair. New York: Raven; 1990.
19. Woo SL, Hollis JM, Adams DJ. Tensile properties of the human femur-anterior cruciate ligament-tibia complex. The effects of specimen age and orientation. Am J Sports Med 1991;19:217–234.
20. Martin RB, Burr DB, Sharkey NA. Mechanical Properties of Ligament and Tendon; Skeletal Tissue Mechanics. New York: Springer-Verlag; 1998. p 309–349.
21. Dunn MG, Tria AJ, Kato P, Bechler JR, Ochner RS, Zawadsky JP, Silver FH. Anterior cruciate ligament reconstruction using a composite collagenous prosthesis. Am J Sports Med 1992; 20:507–515.
22. Dunn MG, Bellincampi LD, Tria AJ, Zawadsky JP. Preliminary development of a collagen-PLA composite for ACL reconstruction. J Appl Polym Sci 1995;63:1423–1428.
23. Jackson DW, Simon TM, Lowery W, Gendler EI. Biologic remodeling after anterior cruciate ligament reconstruction using a collagen matrix derived from demineralized bone. Am J Sports Med 1996;24:405–414.
24. Chvapil M, Speer DP, Holubec H, Chvapil TA, King DH. Collagen fibers as a temporary scaffold for replacement of ACL in goats. J Biomed Mater Res 1993;27:313–325.
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 1993;112:270–274.
26. Ge Z, Goh J, Lee EH. The effects of bone marrow-derived mesenchymal stem cells and fascia wrap application to anterior cruciate ligament tissue engineering. Cell Transplantation 2006;14(10):763–773.
27. Altman GH, Horan RL, Lu HH, Moreau J, Martin I, Richmond JC, Kaplan DL. Silk matrix for tissue engineered anterior cruciate ligaments. Biomaterials 2002;23:4131–4141.
28. Ibarra C, Cao Y, Vacanti JP. Tissue engineered bone-ligament-bone structures: Potential new grafts for knee ligament reconstruction. In Proceedings of SICOT 20th World Congress, 1996.
29. Cameron ML, Fu FH, Paessler HH, Schneider M, Evans CH. Synovial fluid cytokine concentrations as possible prognostic indicators in the ACL-deficient knee. Knee Surg Sports Traumatol Arthrosc 1994;2:38–44.
30. Borsa PA, Lephart SM, Irrgang JJ, Safran MR, Fu FH. The effects of joint position and direction of joint motion on proprioceptive sensibility in anterior cruciate ligament-deficient athletes. Am J Sports Med 1997;25:336–340.
31. Hutmacher DW. Scaffolds in tissue engineering bone and cartilage. Biomaterials 2000;21:2529–2543.
32. Park JB, Lakes RS. Biomaterials: An Introduction. New York: Plenum; 1992. p 6.
33. Altman GH, Diaz F, Jakuba C, Calabro T, Horan RL, Chen J, Lu H, Richmond J, Kaplan DL. Silk-based biomaterials. Biomaterials 2003;24:401–416.
34. Haut RC. The mechanical and viscoelastic properties of the anterior cruciate ligement and of ACLfascicles. In: Jackson DW, Arnoczky SP, Frank CB, Woo, SLY, Simon TM. The Anterior Cruciate Ligament: Current and Future Concepts. New York: Raven; 1993. p 63.
35. Hutmacher DW, Goh JC, Teoh SH. An introduction to biodegradable materials for tissue engineering applications. Ann Acad Med Singapore 2001;30:183–191.
36. Lee CH, Singla A, Lee Y. Biomedical applications of collagen. Int J Pharm 2001;221(1/2):1–22.
37. Friess W. Collagen—Biomaterial for drug delivery. Eur J Pharm Biopharm 1998;45:113–136.
38. Amiel D, Billings E, Aksson WH. Ligament structure, chemistry, and physiology. In: Daniel D, Akeson W, O'Connor J, editors. Knee Ligaments: Structure, Function, Injury, and Repair. New York: Raven; 1990. p 77–91.
39. Khor E. Methods for the treatment of collagenous tissues for bioprostheses. Biomaterials 1997;18:95–105.
40. Koob TJ, Hernandez DJ. Material properties of polymerized NDGA-collagen composite fibers: Development of biologically based tendon constructs. Biomaterials 2002;23:203–212.
41. Bucknall TE, Teare L, Ellis H. The choice of a suture to close abdominal incisions. Eur Surg Res 1983;15:59–66.
42. Greenwald D, Shumway S, Albear P, Gottlieb L. Mechanical comparison of 10 suture materials before and after in vivo incubation. J Surg Res 1994;56:372–377.
43. Salthouse TN, Matlaga BF, Wykoff MH. Comparative tissue response to six suture materials in rabbit cornea, sclera, and ocular muscle. Am J Ophthalmol 1977;84:224–233.
44. Wen CM, Ye ST, Zhou LX, Yu Y. Silk-induced asthma in children: A report of 64 cases. Ann Allergy 1990;65:375–378.
45. Soong HK, Kenyon KR. Adverse reactions to virgin silk sutures in cataract surgery. Ophthalmology 1984;91:479–483.
46. Dewair M, Baur X, Ziegler K. Use of immunoblot technique for detection of human IgE and IgG antibodies to individual silk proteins. J Allergy Clin Immunol 1985;76:537–542.
47. Engelberg I, Kohn J. Physico-mechanical properties of degradable polymers used in medical applications: A comparative study. Biomaterials 1991;12:292–304.
48. Freed LE, Vunjak-Novakovic G, Biron RJ, Eagles DB, Lesnoy DC, Barlow SK, Langer R. Biodegradable polymer scaffolds for tissue engineering. Biotechnology (NY) 1994; 12:689–693.
49. Chaignaud BE, Langer RS, Vacanti JP. The history of tissue engineering using synthetic biodegradable polymer scaffolds and cells. In: Atala A, Mooney D, editors. Synthetic Biodegradable Polymer Scaffolds. Boston: Birkhauser; 1997. p 1–14.
50. Migliaresi C, Fambri L, Cohn D. A study on the in vitro degradation of poly(lactic acid). J Biomater Sci Polym Ed 1994;5:591–606.
51. Barber FA, Click JN. The effect of inflammatory synovial fluid on the breaking strength of new "long lasting" absorbable sutures. Arthroscopy 1992;8:437–441.
52. Bourke SL, Kohn J, Dunn MG. Preliminary development of a novel resorbable synthetic polymer fiber scaffold for anterior cruciate ligament reconstruction. Tissue Eng 2004;10(1/2):43–52.
53. Rittmeister M, Noble PC, Lintner DM, Alexander JW, Conditt M, Kohl HW III. The effect of strand configuration on the tensile properties of quadrupled tendon grafts. Arthroscopy 2002;18:194–200.
54. Dunn MG. Anterior cruciate ligament prostheses. In: Fahey T, editor. Encyclopedia of Sports Medicine and Science. 2004. Available at http://www.sportsci.org/encyc/index.html.

55. Cooper JA, Lu HH, Ko FK, Freeman JW, Laurencin CT. Fiber-based tissue-engineered scaffold for ligament replacement: Design considerations and in vitro evaluation. Biomaterials 2005;26:1523–1532.
56. Ge Z, Goh JC, Wang L, Tan EP, Lee EH. Characterization of knitted polymeric scaffolds for potential use in ligament tissue engineering. J Biomater Sci Polym Ed 2005;16:1179–1192.
57. Arnold MP, Blankevoort L, ten Ham A, Verdonschot N, van Kampen A. Twist and its effect on ACL graft force. J Orthop Res 2004;22:963–969.
58. Miller RH. Knee injuries. In: Canale ST, editor. Campbell's Operative Orthopaedics. Mosby; 2003, p 2274–2275.
59. Barrett GR, Line LL Jr, Shelton WR, Manning JO, Phelps R. The Dacron ligament prosthesis in anterior cruciate ligament reconstruction. A four-year review. Am J Sports Med 1993;21: 367–373.
60. Thomson RC, Shung AK, Yaszemski MJ, Mikos AG. Polymer scaffold processing In: Lanza RP, Langer R, Vacanti JP, editors. Principles of Tissue Engineering. San Diego: Academic Press; 2000. p 251–261.
61. Wintermantel E, Mayer J, Blum J, Eckert K-L, Luscher P, Mathey M. Tissue engineering scaffolds using superstructures. Biomaterials 1996;17:83–91.
62. Scardino F. An introduction to textile structures and their behavior. In: Chou TW, Ko FK, editors. Textile Structure Composites. Composite Materials Series, Vol. 3. Amsterdam: Elsevier; 1989. p 1–26.
63. Rowland JRJ, Tsukazaki S, Kikuchi T, Fujikawa K, Kearney J, Lomas R, Wood E, Seedhom BB. Radiofrequency-generated glow discharge treatment: Potential benefits for polyester ligaments. J Orthop Sci 2003;8:198–206.
64. Murray AW, Macnicol MF. 10–16 year results of Leeds-Keio anterior cruciate ligament reconstruction. Knee 2004;11:9–14.
65. Cameron M, Fu FH. Prosthetic ligaments in ACL reconstruction. Bull Rheum Dis 1994;43:4–6.
66. Kumar K, Maffulli N. The ligament augmentation device: An historical perspective. Arthroscopy 1999;15:422–432.
67. van Heerwaarden RJ, Stellinga D, Frudiger AJ. Effect of pretension in reconstructions of the anterior cruciate ligament with a Dacron prosthesis. A retrospective study. Knee Surg Sports Traumatol Arthrosc: Official Journal Of The ESSKA. 1996;3:202–208.
68. Roolker W, Patt TW, van Dijk CN, Vegter M, Marti RK. The Gore-Tex prosthetic ligament as a salvage procedure in deficient knees. Knee Surg, Sports Traumatol Arthrosc: Official Journal Of The ESSKA 2000;8:20–25.
69. Mikos AG, Bao Y, Cima LG, Ingber DE, Vacanti JP, Langer R. Preparation of poly(glycolic acid) bonded fiber structures for cell attachment and transplantation. J Biomed Mater Res 1993; 27:183–189.
70. Laurencin CT, Ambrosio AMA, Borden MD, Cooper Jr, JA. Tissue engineering: orthopedic applications. Annu Rev Biomed Eng 1999;1:19–46.
71. Lee CH, Shin HJ, Cho IH, Kang Y-M, Kim IA, Park K-D, Shin J-W. Nanofiber alignment and direction of mechanical strain affect the ECM production of human ACL fibroblast. Biomaterials 2005;26:1261–1270.
72. Ge Z, Goh J, Lee EH. Selection of cell sources for ligament tissue engineering. Cell Transplantation 2005;14(8):573–583.
73. Massia SP, Hubbell JA. Covalently attached GRGD on polymer surfaces promotes biospecific adhesion of mammalian cells. Ann NY Acad Sci 1990;589:261–270.
74. Park KD, Mosher DF, Cooper SL. Acute surface-induced thrombosis in the canine ex vivo model: Importance of protein concentration of the initial monolayer and platelet activation. J Biomed Mater Res 1986;20:589–612.
75. Domotor E, Sipos I, Kittel A, Abbott NJ, Adam-Vizi V. Improved growth of cultured brain microvascular endothelial cells on glass coated with a biological matrix. Neurochem Int 1998;33:473–478.
76. Zhang M, James SP, King R, Beauregard G. Surface modification of UHMWPE for use in total joint replacements. Biomed Sci Instrum 2004;40:13–17.
77. Pavesio A, Renier D, Cassinelli C, Morra M. Anti-adhesive surfaces through hyaluronan coatings. Med Device Technol 1997;8:20–27.
78. Hautanen A, Gailit J, Mann DM, Ruoslahti E. Effects of modifications of the RGD sequence and its context on recognition by the fibronectin receptor. J Biol Chem 1989; 264:1437–1442.
79. Vogel Z, Daniels MP, Chen T, Xi ZY, Bachar E, Ben-David L, Rosenberg N, Krause M, Duksin D, Kalcheim C. Ascorbate-like factor from embryonic brain. Role in collagen formation, basement membrane deposition, and acetylcholine receptor aggregation by muscle cells. Ann NY Acad Sci 1987;498:13–27.
80. Marui T, Niyibizi C, Georgescu HI, Cao M, Kavalkovich KW, Levine RE, Woo SL. Effect of growth factors on matrix synthesis by ligament fibroblasts. J Orthop Res 1997;15:18–23.
81. Fu SC, Wong YP, Chan BP, Pau HM, Cheuk YC, Lee KM, Chan KM. The roles of bone morphogenetic protein (BMP) 12 in stimulating the proliferation and matrix production of human patellar tendon fibroblasts. Life Sci 2003;72:2965–2974.
82. Ma Z, Gao C, Gong Y, Ji J, Shen J. Immobilization of natural macromolecules on poly-L-lactic acid membrane surface in order to improve its cytocompatibility. J Biomed Mater Res 2002;63:838–847.
83. Ma Z, Gao C, Gong Y, Shen J. Cartilage tissue engineering PLLA scaffold with surface immobilized collagen and basic fibroblast growth factor. Biomaterials 2005;26:1253–1259.
84. Fermor B, Urban J, Murray D, Pocock A, Lim E, Francis M, Gage J. Proliferation and collagen synthesis of human anterior cruciate ligament cells in vitro: effects of ascorbate-2-phosphate, dexamethasone and oxygen tension. Cell Biol Int 1998; 22(9/10):635–640.
85. Goh JC, Ouyang HW, Teoh SH, Chan CK, Lee EH. Tissue-engineering approach to the repair and regeneration of tendons and ligaments. Tissue Eng 2003;9(Suppl 1):S31–S44.
86. Smith MK, Peters MC, Richardson TP, Garbern JC, Mooney DJ. Locally enhanced angiogenesis promotes transplanted cell survival. Tissue Eng 2004;10(1/2):63–71.
87. Lee KY, Peters MC, Anderson KW, Mooney DJ. Controlled growth factor release from synthetic extracellular matrices. Nature 2000;408:998–1000.
88. Carmeliet P, Conway EM. Growing better blood vessels. Nat Biotechnol 2001;19:1019–1020.
89. Richardson TP, Peters MC, Ennett AB, Mooney DJ. Polymeric system for dual growth factor delivery. Nat Biotechnol 2001; 19:1029–1034.
90. Kuhl PR, Griffith-Cima LG. Tethered epidermal growth factor as a paradigm for growth factor-induced stimulation from the solid phase. Nat Med 1996;2:1022–1027.
91. Amiel D, Frank C, Harwood F, Fronek J, Akeson W. Tendons and ligaments: A morphological and biochemical comparison. J Orthop Res 1984;1:257–265.
92. Nakamura N, Hart DA, Boorman RS, Kaneda Y, Shrive NG, Marchuk LL, Shino K, Ochi T, Frank CB. Decorin antisense gene therapy improves functional healing of early rabbit ligament scar with enhanced collagen fibrillogenesis in vivo. J Orthop Res 2000;18:517–523.
93. Frank C, Shrive N, Hiraoka H, Nakamura N, Kaneda Y, Hart D. Optimisation of the biology of soft tissue repair. J Sci Med Sport 1999;2:190–210.
94. Shimomura T, Jia F, Niyibizi C, Woo SL. Antisense oligonucleotides reduce synthesis of procollagen α1 (V) chain in

human patellar tendon fibroblasts: Potential application in healing ligaments and tendons. Connect Tissue Res 2003; 44(3/4):167–172.
95. Laitinen O, Alitalo I, Toivonen T, Vasenius J, Törmälä P, Vainionpää S. Tissue response to a braided poly-L-lactide implant in an experimental reconstruction of anterior cruciate ligament. J Mater Sci Mater Med 1993;4:547–554.
96. Orlans FB. Animal rights and animal welfare. Hastings Cent Rep 1979;9(5):45.
97. An HY, Friedman RJ. Animal selections in orthopaedic research. In: An HY, Friedman RJ, editors. Animal Models in Orthopaedic Research. New York: CRC; 1999. p 39–58.
98. McDougall JJ, Bray RC. Animal models of ligament repair. In: An HY, Friedman RJ. Animal Models in Orthopaedic Research. New York: CRC; 1999. p 461–490.
99. Jackson DW, Grood ES, Arnoczky SP, Butler DL, Simon TM. Cruciate reconstruction using freeze dried anterior cruciate ligament allograft and a ligament augmentation device (LAD). An experimental study in a goat model. Am J Sports Med 1987;15:528–538.
100. Jackson DW, Grood ES, Arnoczky SP, Butler DL, Simon TM. Freeze dried anterior cruciate ligament allografts. Preliminary studies in a goat model. Am J Sports Med 1987; 15:295–303.
101. Altman GH, Lu HH, Horan RL, Calabro T, Ryder D, Kaplan DL, Stark P, Martin I, Richmond JC, Vunjak-Novakovic G. Advanced bioreactor with controlled application of multi-dimensional strain for tissue engineering. J Biomech Eng 2002;124:742–749.
102. Goulet F, Rancourt R, Cloutier R. Tendon and ligaments. In: Lanza R, Langer R, Chick W, editors. Principles of Tissue Engineering. San Diego: Academic Press; 2000. p 711–723.
103. Toyoda T, Matsumoto H, Fujikawa K, Saito S, Inoue K. Tensile load and the metabolism of anterior cruciate ligament cells. Clin Orthop 1998;353:247–255.
104. Sckell A, Leunig M, Fraitzl CR, Ganz R, Ballmer FT. The connective-tissue envelope in revascularisation of patellar tendon grafts. J Bone Joint Surg Br 1999;81:915–920.
105. Petersen W, Unterhauser F, Pufe T, Zantop T, Sudkamp NP, Weiler A. The angiogenic peptide vascular endothelial growth factor (VEGF) is expressed during the remodeling of free tendon grafts in sheep. Arch Orthop Trauma Surg 2003; 123:168–174.
106. Molloy T, Wang Y, Murrell G. The roles of growth factors in tendon and ligament healing. Sports Med 2003;33:381–394.