African Journal of Biotechnology Vol. 8 (25), pp. 7182-7189, 29 December, 2009
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2009 Academic Journals

Review

Tissue engineered devices for ligament repair, replacement and regeneration

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Accepted 10 November, 2008

The anterior cruciate ligament (ACL) is one of five ligaments in the knee that are important for stability and kinematics. It is also the most commonly injured ligament of the knee and due to its poor healing potential, severe damage warrants surgical intervention including complete replacement. Ligaments are longitudinally arranged, complex tissues; the mechanical properties of ligaments are a direct result of their components and the arrangement of these components in the tissue. It is these mechanics that have made ligaments so difficult to replace. Past ACL replacements have had many limitations that prevented their extensive use. These limitations range from mechanical fatigue over time to fraying of the device after implantation. In light of these problems, investigators have begun to pursue a host of new techniques to create a range of viable options for the repair, replacement, and/or regeneration of the ACL. These options include tissue engineered scaffolds with novel designs and specially treated transplanted tissues. In this article, the composition, arrangement, and mechanics of the ACL will be discussed in order to elucidate important aspects of ACL repair and past replacements will be described. Afterwards, novel replacement options that look to solve problems faced by older replacement options will be presented. These devices use a wide variety of materials and designs to replicate ligament mechanics and allow for new tissue regeneration.

Key words: Anterior cruciate ligament (ACL), tissue engineering, cells, tensile, stress relaxation, polymer, allograft, xenograft.

INTRODUCTION

The anterior cruciate ligament (ACL) is the major intra-articular ligament of the knee; it is also the most commonly injured knee ligament. Each year in the United States there are between 100,000 and 250,000 ACL injuries, or 1 in 3,000 in the general population; approximately 50,000 ligament reconstructions are performed annually (Cameron et al., 2000; Cooper et al., 2005; Miyasaka et al., 1991). The purpose of this ligament is to support and strengthen the knee while preventing extreme translation of the tibia relative to the femur. ACL injury is a growing problem; in a study of 17,397 people with 19,530 sport injuries, 37% of the patients had knee injuries. Of this 37%, 45.4% of these injuries involved the ACL with 33.9% of them requiring surgery (Majewski et al., 2006). A number of repair techniques are currently available, and the success rates for long term clinical outcome are 85 - 90% (Dandy, 1996; Eriksson, 1997; Fu and Musahl, 2001).

The ACL is a dense, highly organized, cable-like tissue composed of types I, III, and V collagen, elastin, proteoglycans, water, and cells. Ligaments have a hierarchical structure with increasing levels of longitudinal organization; collagen molecules form fibrils, multiple fibrils form fibril bundles, and fibril bundles combine to form fascicles. All of these structures are arranged parallel to the long axis of the ligament (Laurencin and Freeman, 2005). The collagen fibrils also display a crimp pattern, a periodic change in direction. In the ACL, the crimp pattern repeats every 45 – 60 nm (Cabaud et al., 1979; Silver, 1994). The fascicles contain collagen fibrils, proteoglycans, and elastin. The ligament is surrounded by an epiligament sheath (Amiel et al., 1990b). The ACL also has antromedial and posterolateral bands and is twisted approximately 180° from the femoral attachment site to the tibial attachment site (Silver, 1994).

The ACL is a viscoelastic tissue; its behavior is strain rate dependant. The combination of collagens, elastin, proteoglycans (such as decorin, hyaluronan, biglycan, fibromodulin, lumican, epiphycan and keratocan), and water and their arrangement in the tissue give ligaments

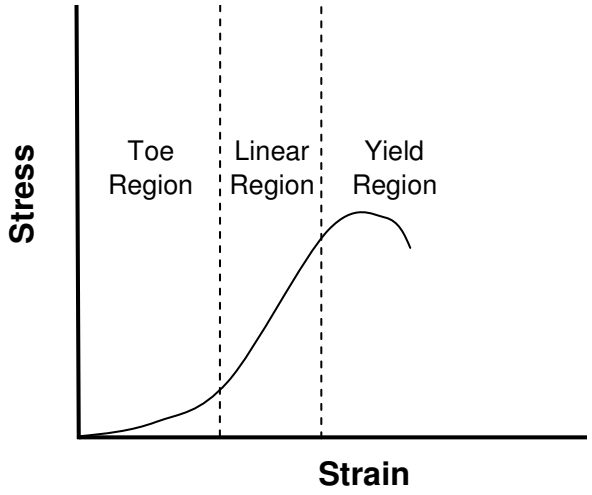

Figure 1. Schematic of a stress-strain curve for ligament or tendon displaying the toe, linear, and yield regions.

their unique properties. Proteoglycans such as decorin organize and align the collagen fibrils during development. The presence of large charged proteoglycans allow ligaments to swell with water, imparting viscoelastic behavior to the tissue. The arrangement of proteoglycans along the collagen fibrils allows the fibrils to slide by each other during the application of strain, leading to viscous dissipation of stress at low strain.

The crimp pattern of the collagen fibers also plays a role in the behavior of the ACL. The presence of the crimp pattern allows ligaments to increase in length under low strains without straining the collagen molecules and plastically deforming the collagen fibers (Figure 1). This enables the tissue to respond to the presence of maintained stress and still recover (up to a certain amount of strain).

Due to the chemistry and arrangement of their components, ligaments display tri-phasic behavior when placed under tensile loads (Figure 1). The first phase is the toe region, an area where the ligament exhibits a low amount of stress per unit strain (low slope). When force is first applied to the tissue it is transferred to the collagen fibrils, causing lateral fibrillar contraction, straightening of the crimp pattern, and stretching of the flexible regions of the collagen molecules and microfibers (Freeman and Silver, 2004). The next phase of tensile behavior is the linear region, which displays an increase in slope. Once the crimp pattern is straightened and flexible regions are stretched, the force is directly translated into collagen molecular strain (Diamant et al., 1972; Silver, 1994). The collagen triple helix is stretched and interfibrillar slippage occurs between crosslinks leading to an increase in stress per unit strain (Amiel et al., 1990a; Mosler et al., 1985). The yield and failure region is the last phase of the tensile behavior. This phase begins with a slight decrease in slope followed by a large drop in slope due to tissue failure. The failure region represents defibrillation of the ligament (Silver, 1994), the collagen fibers in the ligament fail by defibrillation causing a decrease in slope and tissue failure (Amiel et al., 1990a; McBride et al., 1988). In order to successfully restore the functionality of the knee, an ACL replacement must display the same biomechanical behavior as normal ACL tissue.

TRADITIONAL ACL REPAIR OPTIONS AND PAST SYNTHETIC GRAFTS

Traditionally, ACL injuries have been treated with biological based grafts (autografts or allografts) (Amiel et al., 1990a; Amiel et al., 1990b). Both autografts and allografts possess good initial mechanical strength and promote cell proliferation and new tissue growth. However, they suffer from a number of disadvantages. Autografts, tissue from the patient, inherently require additional surgery and which has been known to cause donor site morbidity, increased recovery time, and possible pain at the harvesting site (Cartmell and Dunn, 2004). Autograft material for ACL repair is usually taken from the patient's hamstring, patella, or quadriceps tendons. The patella and hamstring tendon tissues are the most commonly used autografts. The patella tendon graft material is often utilized as a "bone-patellar-bone" graft; this is considered the gold standard of ACL autografts. In this graft the tendon is removed with a piece of bone from the patella and from the insertion point at the tibia. It is then fed through a tunnel drilled through the tibia, drawn across the knee, and anchored into a tunnel drilled through the femur (Laurencin and Freeman, 2005).

Allografts, tissues obtained from cadavers could potentially transmit disease, bacterial infection, and may elicit an unfavorable immunogenic response from the host (Cameron et al., 2000; Cartmell and Dunn, 2004; Vunjak-Novakovic et al., 2004). The benefits of using allografts include the lack of a second surgery for tissue harvest. There is also no limit to the supply of graft tissue as experienced with autografts. Allografts are usually taken from cadaveric patella tendon, hamstring tendon, and achilles tendon (Bell, 1995; Jackson et al., 1993).

Many past attempts have been made to use non-degradable, synthetic materials as ligament replacements (Freeman and Kwansa, 2008). These grafts can be divided into the following categories: permanent replacements (which are not amendable to tissue ingrowth), augmentation devices (which protect biological grafts from loads during the early postoperative period), and scaffolds (which allow tissue ingrowth). Some of the non-degradable synthetic materials that have been used include carbon fibers, polyethylene terephthalate (PET), polypropylene (PP), and polytetrafluoroethylene (PTFE) (Amiel et al., 1990a; Amiel et al., 1990b; Arnoczky, 1983; Silver et al., 1991; Smith et al., 1993; Snook, 1983).

The Leeds-Keio ligament is a woven porous tube composed of PET and attached to woven tapes (Fujikawa, 1988; Silver, 1994). This device is designed to allow the ingrowth of new tissue. The Kennedy Ligament Augmen-

tation Device is a cylindrical PP prosthesis with a diamond-braided construction. This device is implanted in conjunction with biological grafts such as patella tendon tissue (Fujikawa, 1988; Silver, 1994). The Gore-Tex prosthesis is composed of an expanded PTFE fiber that is wound into loops, which are joined together to form a braid (Bolton and Bruchman, 1985; Olson et al., 1988).

Although these synthetic devices initially supply the function of the ligaments that they replace or protect the ligament that they augment, these devices fail over time because they cannot duplicate the mechanical behavior of the ligament over time. Permanent replacements are susceptible to long-term mechanical failure due to creep and fatigue (Freeman and Kwansa, 2008). Augmentation devices may shield the biological graft from stress, which leads to poor long-term neoligament formation. Repeated elongation of these devices leads to permanent deformation at the points of stress (Freeman and Kwansa, 2008). Contact with sharp edges of the bone tunnel can cause abrasions that weaken the implant and create debris that can cause synovitis in the joint (Freeman and Kwansa, 2008). Woven prostheses face the additional problems of axial splitting, low tissue infiltration, low extensibility, and abrasive wear. Eventually, these implants fail due to fragmentation, stress shielding of new tissue, fatigue, creep, and production of wear debris (Laurencin et al., 1999; Smith et al., 1993; Woo et al., 1994). None of these devices have been approved by the FDA for primary ACL repair, but some have been approved for augmentation (McPherson et al., 1985).

A successful ACL device must be designed to successfully support the mechanical loads experienced in the knee. Implanted materials will eventually fail due to fatigue, fragmentation, or other issues. Therefore a successful ACL device must be designed to promote or allow the growth of new tissue and degrade over time to prevent stress shielding. In light of these criteria and past device limitations a number of researchers have looked to tissue engineering as a new direction for ACL replacement and augmentation. Researchers have gone in a many different directions in order to develop devices have that fulfill each of these criteria; they vary in their material composition and structure. These devices can be classified as natural polymer based devices, synthetic polymer based devices, and natural tissue based devices.

NATURAL POLYMER BASED DEVICES

Among the materials used in biodegradable tissue-engineered grafts are type I collagen and silk advantages of collagen are the capability of altering (Altman et al., 2002; Chen et al., 2003; Dunn et al., 1995; Dunn et al., 1992; Freeman and Kwansa, 2008; Vunjak-Novakovic et al., 2004). Some of the resorption rate and mechanical properties of scaffolds through crosslinking and its low antigenicity. These scaffolds experience an early decrease in mechanical strength followed by tissue remodeling between 10 and 20 weeks with a strength gain similar to autografts. Fibrous proteins such as silk or collagen are composed mainly of specific amino acid sequences repeated throughout the primary structure, this creates homo-geneity in the protein's secondary structure (collagens exhibit triple helical structures and most silks display β-sheet conformations) (Altman et al., 2003; Altman et al., 2002; Chen et al., 2003; Vunjak-Novakovic et al., 2004). The rigid, extended structure of these proteins also gives them the mechanical properties necessary for the replacement of load bearing materials making them excellent materials for ACL replacements (Altman et al., 2003; Altman et al., 2002; Vunjak-Novakovic et al., 2004).

Dunn has studied the use of type I collagen fibers in potential ACL scaffolds (Altman, et al., 2002; Smith, et al., 1993). The grafts showed excellent biocompatibility and enhanced cell attachment, proliferation, and production of extracellular matrix. Unfortunately this scaffold did not fare well mechanically; the collagen scaffold is made from type I collagen fibers arranged in parallel (other scaffolds have been made with synthetic polymers coated with a collagen solution). Groups of aligned fibers lack structural reinforcement and do not prevent fatigue. The arrangement of the fibers in parallel with the direction of stress may cause long-term failure due to fatigue, creep, and abrasive wear.

Another natural polymer based tissue engineered structure is a matrix composed of twisted silk fibers developed by Altman et al. (2002), Chen et al. (2003), Vunjak-Novakovic et al. (2004). This structure combines the use of a three-dimensional, porous matrix with the use of isolated cells that have been allowed to proliferate *in vitro*. This matrix is a hierarchical structure where bundles of silk fibers are wound into strands that are wound into cords and arranged to form the matrix. This arrangement is similar to that of collagen fibers in ligaments and tendons. In these tissues collagen fibers arranged to form fascicles and the fascicles join to form the ligament (Silver et al., 2003).

Biocompatibility tests with bone marrow stromal cells (BMSCs) show that the silk matrix is not cytotoxic (Altman et al., 2002; Chen et al., 2003; Vunjak-Novakovic et al., 2004). The twisted fiber architecture gives the scaffold mechanical properties that are similar to ACL. As the matrix is subjected to tensile load the twisting pattern straightens, creating a toe region; this is followed by a linear region. This behavior is important for the prevention of damage due to fatigue and creep. The matrices have a maximum load of 2337 ± 72 N, strain at failure of 38.6 ± 2.4%, and elastic modulus of 354 ± 26 N/mm, which are similar to ACL. The biocompatibility and ability of these matrices to elicit new tissue growth can been increased by coating the surface with RGD sequences (Chen et al., 2003). The addition of RGD sequences has been shown to increase cellular attachment and proliferation. The presence of the RGD sequences on the silk fibers also increased extracellular matrix (ECM) by

BMSCs which could lead to faster tissue regeneration.

SYNTHETIC POLYMER BASED DEVICES

In recent years, a variety of different materials and structures have been investigated for use in tissue engineered ligament replacements. Most of these are synthetic, biodegradable polymers; they include poly glycolic acid (PGA), poly lactic acid (PLA), their copolymers, polyurethane urea (PUU) (Bourke, et al., 2004), poly desaminotyrosyl-tyrosine ethyl carbonate (poly (DTE carbonate)), polydioxanone (PDS) and poly caprolactone (PCL) (Bourke et al., 2004; Freeman and Kwansa, 2008; Laurencin and Freeman, 2005). The use of synthetic biodegradable polymers has several benefits. There is no limit to the supply of grafts (as opposed to autografts) and there is no risk of disease transmission. These polymers are designed to degrade over time and therefore do not cause a long term foreign body response. The mechanical properties of the device may also be controlled by altering the degree of polymer crystallinity, changing the polymer molecular weight, or changing the ratio of each polymer in the copolymer.

A poly (DTE carbonate) scaffold developed by the groups of Dunn and Kohn has shown the ability to support fibroblast growth and display the necessary strength for use as an ACL graft (Bourke et al., 2004). The use of PDS as a potential material for scaffold construction has been slowed. Investigations have shown that the rapid loss of its mechanical strength due to degradation makes it a poor choice for use in ligament tissue engineering (Buma et al., 2004). Once again, structure is a potential flaw in many of these matrices. The (poly (DTE carbonate)) and other PUU grafts were composed of polymeric structures arranged in parallel. The poly (DTE carbonate) matrix is made of parallel fibers and the PUU structure is a woven band. These designs may lead to sudden failure due to lack of structural reinforcement. The arrangement of fibers in parallel with the direction of stress may cause fatigue and creep.

Laurencin and his colleagues have developed a tissue engineered scaffold based on a cell seeded, degradable, three-dimensional (3-D) braided, poly L-lactic acid (PLLA) scaffold (Cooper et al., 2007; Cooper et al., 2005; Lu et al., 2005). PLLA is a degradable polymer which allows load to gradually shift from the implant to the neoligament over time. This scaffold uses 3-D braiding techniques to create a scaffold with controlled pore size, well integrated pores, resistance to wear and rupture, and mechanical properties comparable to the ACL. The fibers in the 3-D braiding technique all reinforce one another increasing scaffold strength. The control of pore size and integration of pores are important for the distribution of nutrients throughout the scaffold, removal of waste from the scaffold, cell motility, and the development of new tissue throughout the scaffold. This braided scaffold also has a hierarchical structure similar to the ligament. It is composed of PLLA fibers (similar in diameter to collagen fibers) which are arranged into bundles and woven throughout the scaffold thickness.

In the past there have been woven or braided ligament replacement structures which performed well in the short term after implantation, but the long-term outcomes of these prostheses have been poor (Arnoczky, 1983; Silver et al., 1991). These braids were limited by poor tissue integration, poor abrasion resistance, and fatigue (Konikoff et al., 1974; Yahia, 1997). The 3-D braided scaffolds have three regions: a femoral tunnel attachment site (bony attachment end), a ligament region (intra-articular zone), and tibial tunnel attachment site (bony attachment end). The attachment sites have a higher fiber braiding angle than the intra-articular zone. These angle differences cause changes in pore size between the areas. Studies have shown that a minimum pore diameter of 150 µm is necessary for bone growth into scaffolds and 200 – 250 µm for soft tissue ingrowth (Konikoff et al., 1974; Yahia, 1997). The different regions of the scaffold contain pore sizes within these ranges to encourage tissue (ligament and bone) ingrowth and capillary supply. The higher braiding angle at the insertion points also provides resistance to wear within bone tunnels, and improves the integration of bone tissue. The pore interconnectivity extending through the implant increases the overall surface area for cell attachment, allowing tissue ingrowth into the interior of the scaffold.

In *in vivo* studies with this scaffold implanted into the knees of adult New Zealand white rabbits, the rabbits were weight-bearing within 24 h after surgery (a display of the scaffold's strength) (Cooper, 2002; Cooper et al., 2007). After 12 weeks of implantation in the knees of adult New Zealand white rabbits, cell seeded scaffolds displayed signs of developing new ACLs. The scaffolds were completely infiltrated by cells and dense connective tissue. The newly synthesized collagen displayed a crimp pattern and was bonded to the PLLA filaments. Histological studies showed the presence of neovasculature in the developing ligament.

Another recent design is the braid-twist scaffold (Freeman et al., 2008; Freeman et al., 2007). It combines two techniques (fiber braiding and fiber twisting) that are commonly used in textiles and have been used separately in previous ligament prostheses. Braiding is a technique that has been used to create products designed to bear axial loads and provide mechanical reinforcement (Kawabata, 1989). Braided structures are shear resistant, conformable, and can transfer large loads while provide extension (Cooper, 2002).

The twisting of fibers is used in the textile industry to form yarns (Joseph et al., 1993). The twisting direction and degree of twisting affect yarn strength, abrasion resistance, and flexibility. A low degree of twist produces weaker yarns that pull apart more easily and can develop protrusions on their surfaces from abrasions. Increasing the amount of twist improves yarn strength and abrasion resistance. Although if the yarns are wound too tightly (and the fibers become increasingly perpendicular to the

long axis of the yarn), the strength and abrasion resistance decrease (Joseph et al., 1993). Combining fiber twisting with fiber braiding yields a mechanically stable structure with a larger load capacity and greater degree of extensibility. The degree of braiding or twisting can be altered in order to match the device mechanics with the mechanics of the tissue being replaced (Freeman et al., 2007).

The structure of this scaffold is also similar to the organization of a native ligament and is designed to mimic the biomechanical behavior of the ACL (display a toe region and linear region when placed under increasing load). It is composed PLLA fibers (which are bundles of microfibers, approximately 20 µm in diameter), a degradable poly α-hydroxyester that has been approved by the FDA for implantation in other biomedical devices (Freeman et al., 2008; Freeman et al., 2007). In previous studies, the braid-twist scaffold with the best degree of twisting and braiding had an ultimate tensile stress (UTS) of 81.6 ± 1.6 MPa, elastic modulus of approximately 750 MPa, toe region length of 4%, and strain at failure of 30% (Freeman et al., 2007). The UTS for human ACL has been measured at 38 ± 9 MPa, which is lower than the UTS of the braid-twist scaffold (Freeman et al., 2007; Pioletti et al., 1999). Mechanical tests on human ligaments have yielded various elastic moduli values, 65 to 111 MPa (Silver, 1994), 180 ± 25 MPa, and 242 ± 28 MPa (Dienst et al., 2002). Previous studies indicate that human ligaments have toe regions that range from 2.0 to 4.8% (Ambrosio et al., 1998; Bonifasi-Lista et al., 2005; Dienst et al., 2002; Moon et al., 2006; Thornton et al., 2002). Higher UTS and modulus values may be necessary to allow the device to degrade while still bearing the appropriate amount of load.

Stress relaxation tests on these scaffolds show that the braid-twist scaffolds have a rapid decrease in stress followed by a plateau region, similar to natural ligament tissue (Freeman et al., 2008). Unfortunately the final relative stresses (0.873 and 0.829) were higher than those seen in ACL and posterior cruciate ligament (PCL) (0.63 and 0.75) (Freeman et al., 2008; Pioletti and Rakotomanana, 2000; Pioletti et al., 1999). Mathematical modeling of the viscoelastic behavior demonstrates that the stress relaxation behavior can be changed by altering the braiding angles and twisting angles (Freeman et al., 2008).

These scaffolds were also evaluated for their ability to sponsor fibroblast proliferation (Freeman et al., 2008). The scaffolds displayed the ability to sponsor the growth of patella tendon fibroblasts. The results of MTS assays showed an increase in the number of viable cells over 28 days. Scanning electron microscope images also showed the presence of ECM material secreted by the cells onto the braid-twist scaffolds (Freeman et al., 2008).

BIOLOGICAL TISSUE BASED DEVICES

Concerns with devices produced from biological tissues are usually based on the source of the material, when using tissues from a source other than the patient. These concerns include the potential of bacterial infection, disease transmission, or unfavorable immunogenic response (Konikoff et al., 1974; Laurencin and Freeman, 2005). Some sterilization methods for these tissues can weaken their mechanical properties. On the other hand, they do not require a second harvest surgery, as in autografts. There is a virtually unlimited supply of graft tissue for these devices and they have the appropriate initial mechanical strength (depending on the tissue source) (Konikoff et al., 1974). They also promote cell proliferation and new tissue growth.

Xenografts (tissues from animals) have been presented as an option for ACL repair. These tissues have the same advantages and disadvantages as allografts, but they may also carry the additional risk of transferring a disease seen in animal populations to a human host. Rejection of the foreign tissue is another very serious risk of xenografts. Recent work by Stone et al. (2007) has looked to reduce these risks; they have shown that specially pretreated xenografts are viable options for tissue repair, including the ACL (Freeman and Kwansa, 2008; Kainer et al., 2004; Laurencin and Freeman, 2005; Stone et al., 2007; Stone et al., 1997; Stone et al., 2007; Yahia, 1997). Stone and colleagues have utilized chemically modified grafts from cloned pigs as ACL replacements. In preparation for implantation, the porcine graft material is immunochemically modified and chemically crosslinked (Kainer et al., 2004; Laurencin and Freeman, 2005; Phelps et al., 2003; Stone et al., 2007; Stone et al., 1997; Stone et al., 2007; Yahia, 1997).

The immunochemical modification involves the enzymatic removal of the the α-gal epitopes in order to prevent graft rejection. This process eliminates the interaction between the natural anti-Gal antibody and α-gal epitopes. This interaction has been a major obstacle in the use of porcine tissues in humans (Stone et al., 1997; Stone et al., 2007). Recently, the cloning of pigs lacking α-gal epitopes has eliminated this obstacle (Galili, 1993; Stone et al., 2007). In addition, the ACL grafts used in these studies have been pulse lavaged to remove cellular components further reducing the risk of rejection. The grafts have also been crosslinked with 0.10% gluteraldehyde for 12 h followed by glycine endcapping to block unreacted gluteraldehyde molecules and sterilization by electron beam irradiation at 17.8 kGy (Stone et al., 1998; Stone et al., 2007). These steps increase mechanical strength and sterilize the graft respectively.

In an *in vivo* study, 20 rhesus monkeys were reconstructed with treated porcine grafts for 2, 6, and 12 months; 3 monkeys were used at the 2 month time point, 5 monkeys were used at the 6 month time point and another 5 monkeys were used at the 12 month time point (Stone et al., 2007). The controls consisted of 1 untreated porcine allograft and 1 rhesus allograft at 2 months along with 5 rhesus allografts at 12 months. Graft material consisted of treated porcine bone–patellar tendon–bone

grafts and fresh frozen rhesus bone–patellar tendon–bone grafts. The devices for implantation were 30 mm long by 4 mm wide (tendon material) grafts with bone plug ends of 5 mm diameter by 7 mm in length. The grafts and corresponding intact ligaments from the opposite leg were mechanically tested at 6 and 12 months. The mechanical testing was followed by histological examination of extracted grafts.

The implants promoted the regeneration of new ligament tissue (Stone et al., 2007) with signs of graft remodeling extending from the periphery of the graft to its center. In addition, after 12 months the porcine grafts displayed comparable ultimate load, yield load, stiffness and ultimate displacement to monkey grafts. The strength of the implanted treated grafts increased from 43 to 58% between 6 and 12 months. The grafts also demonstrated lower values for ultimate strength, yield strength, ultimate strain, and modulus compared to intact ligaments (Stone et al., 2007).

The animals were tested for signs of porcine graft rejection (Stone et al., 1998). Blood samples were taken prior to surgery and after surgery, at days 10, 14, 21, 28, 42, 56 as well as at 3, 6, 9, and 12 months. Blood samples were analyzed for the presence of anti-Gal and anti–non-Gal antibodies, including antibodies to proteins present in the porcine grafts. Serum immunoglobulin (Ig) anti-Gal IgG and IgM activity was determined by ELISA. The analysis showed a greater increase in anti-Gal titers (>200%) in animals with the untreated porcine graft when compared to animals with the treated graft (95% lower than the untreated) within 2 weeks following implantation. The response to the untreated grafts indicates acute rejection which may lead to graft destruction and resorption. It is hypothesized that the smaller increase in anti-Gal titers in animals with the treated grafts may be due to an immune response not to □-gal epitopes on the graft itself, but those on the porcine bone marrow cells in cancellous bone of the bone-ligament-bone graft. The anti-Gal titers reached resolved preimplantation values by 8 to 12 weeks after implantation.

In an additional clinical study by Stone et al. (2007), these porcine grafts were implanted into human subjects for ACL replacement. Western blotting analysis and ELISA showed the presence of anti-non-gal antibodies against multiple pig xenoproteins in the grafts. The antibody levels peaked from 2 to 6 months, but the antibodies were no longer produced 2 years after implantation. No antibodies were produced against human ligament proteins. After 2 years, 5 of the 6 patients displayed no problem with the function of the porcine graft.

CONCLUSION

As the number of incidents of ACL injury increases in increasingly younger populations, additional options for ligament repair that overcome the limitations of current treatments are necessary. The ACL is a complex, highly ordered tissue with mechanical properties that are important for normal knee kinematics. These new options must display the mechanics of the original ligament while sponsoring the growth of new tissue and resisting rejection from the body. In response to these criteria, researchers are turning to a variety of techniques and material sources. Solutions in this new generation of ACL grafts typically fall into one of three categories, natural materials based devices, synthetic materials based devices, and natural tissue based devices. Candidates from each of these categories have a number of benefits and have achieved degrees of success in ACL replacement and regeneration. The devices listed above represent some of the advancements that have been made in ACL tissue engineering in each of these categories. These devices have been designed to fulfill the needs of a tissue engineered replacements for musculoskeletal tissues; these include structural stability, appropriate mechanical strength, promotion of cell and tissue growth, and the ability to slowly degrade and allow the new tissue to bear the load. As research into ACL replacement and regeneration continues, it is expected that tissue engineering techniques will lead the way in the design, production, and testing of next generation scaffolds to will mimic the mechanics of natural ligament and lead to the quick and complete regeneration of a new, mechanically sound, natural tissue.

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