Scar formation and ligament healing

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Ligaments are highly organized, dense, fibrous connective-tissue structures that provide stability to joints and participate in joint proprioception. Injuries to ligaments induce a healing response that is characterized by the formation of a scar. The scar tissue is weaker, larger and creeps more than normal ligament and is associated with an increased amount of minor collagens (types III, V and VI), decreased collagen cross-links and an increased amount of glycosaminoglycans. Studies have shown that certain surgical variables alter the healing of ligaments. Such factors include the size of gap between the healing ligament ends, the use of motion in a stable joint and the presence of multiple ligamentous injuries. Research on ligament healing includes studies on low-load and failure-load properties, alterations in the expression of matrix molecules, cytokine modulation of healing and gene therapy as a method to alter matrix protein and cytokine production.

Les ligaments sont des structures très organisées constituées de tissus conjonctifs fibreux et denses, qui stabilisent les articulations et jouent un rôle dans la capacité proprioceptive des articulations. Les lésions ligamentaires provoquent une réaction de guérison caractérisée par la formation d'une cicatrice. Le tissu cicatriciel est plus faible, plus gros et plus élastique que le ligament normal et associé à une augmentation des collagènes mineurs (types III, V et VI), une réduction des ponts de collagène et une augmentation des mucopolysaccharides. Des études ont démontré que certaines variables chirurgicales altèrent la guérison des ligaments, notamment l'espace entre les extrémités ligamentaires en voie de guérison, le mouvement dans une articulation stable et la présence de multiples lésions ligamentaires. La recherche sur la guérison des ligaments comprend des études sur les caractéristiques à faible charge et à charge de rupture, des altérations de l'expression des molécules de la matrice, la modulation de la guérison par les cytokines et la génothérapie comme moyen d'altérer les protéines matricielles et la production de cytokines.

Ligament healing is characterized by the formation and remodelling of scar tissue that is weaker than normal ligament owing to alterations in biochemical composition and structural organization. The ensuing functional results depend on the particular ligament injured and joint affected. Many studies have identified biological and biomechanical factors that alter ligament healing. However, many questions remain, and new questions have emerged as surgeons and scientists try to improve ligament healing with the ultimate goal of regenerating a new ligament. This review will describe the biologic features of normal ligament, ligament healing and scar formation, as well as clinical variables that affect ligament healing. We review current research, considering mechanisms for the biologic abnormalities and potential therapeutic modalities.

Ligament biology and biomechanics

Ligaments are composed primarily of water (approximately 70%), collagen (approximately 25%), other matrix components such as proteoglycans and fibronectin (approximately 4%) and cells (< 1%). Ligaments are less vascular than visceral organs, but recent work has shown a vascular network with accompanying nerve supply

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in the outer covering of ligaments (epiligament). These neurovascular structures penetrate the ligament substance in a longitudinal pattern.[1] Ligaments are organized into collagen fibres aligned longitudinally, from insertion to insertion, but it is becoming more evident that the fibres are not parallel.[2] As a result, different fibres are recruited depending on the position of the joint (Fig. 1). When forces to the ligament are increased more fibres are recruited, allowing the ligament to accommodate greater physiologic forces. If forces beyond this range are applied, progressive sequential failure of fibres occurs, leading to complete disruption of the ligament.

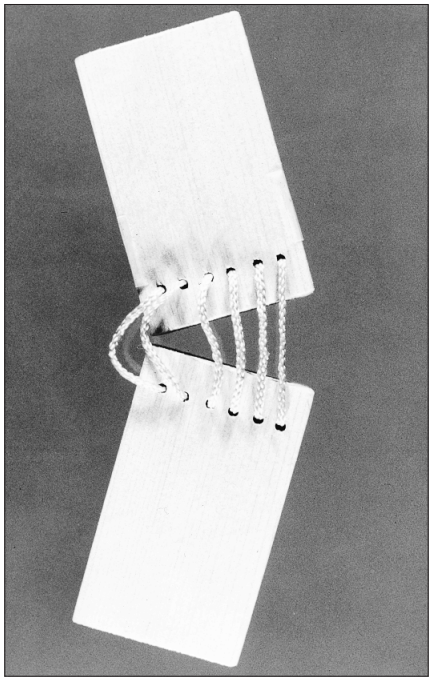

FIG. 1. A simple model of wood and string showing the principle of fibre recruitment in a ligament. The number of fibres under tension depends on a number of factors: where the fibres are attached at the ends and the 3-dimensional positions of the model "bones" are particularly important. This recruitment is crucial to how ligaments function, how they are injured, how their injuries are diagnosed and how they must be treated (reproduced with permission from Frank C, Shrive N. Ligament biology, repair and transplantation. *Curr Opin Orthop* 1996;7[6]:50-6).

Ligaments can be characterized biomechanically in many ways. Tensile testing of bone–ligament–bone complexes yields data about the whole structure. Structural properties recorded during such failure tests include structural strength and stiffness. Material properties of the ligament itself are determined by taking cross-sectional area into account. Stress at the point of failure (force/area) is a material property. Tensile testing can also be performed at tensile forces or stresses that are below those that cause failure. Ligaments and ligament scars that are pulled to a certain length and held there show a decrease in force or stress with time (stress-relaxation). When these structures are exposed to a constant force or stress they increase in length with time (creep). The decrease in force or the increase in length gets smaller with time, and the changes approach zero. Stress-relaxation and creep are examples of viscoelastic behaviour and may represent fine tuning mechanisms to adjust ligament forces and lengths in vivo.

Ligaments are dynamic participants in joint function from biomechanical and physiologic points of view. A joint functions in an equilibrium of compressive and tensile forces (Table I) that are resisted by various structures of the joint.[3] If one of the structures is removed, the balance is disturbed. The ability of the joint to function depends on whether other structures can compensate by taking up more forces. Ligaments are also part of a neurophysiologic mechanism involved with joint function. They contain specialized neurologic receptors that likely play a role in a proprioceptive "ligamentomuscular reflex loop".[4] There is also evidence that autonomic nerve supply may alter blood flow in normal and healing ligaments.[5] Regulation of blood flow could be an important mechanism for inflammation or repair in ligaments and periarticular tissues.

Ligament healing

The pattern of ligament healing is qualitatively very similar to wound healing and culminates in the formation of a scar that bridges the torn ends. The medial collateral ligament (MCL) of the knee is the best characterized model and serves as the basis of this discussion.[4] After an injury to the MCL, hemostasis is activated and a fibrin clot is formed within minutes. An inflammatory response ensues over the next 3 to 5 days, removing debris and attracting large numbers of angiogenic cells and fibroblasts. These cells begin to produce matrix, and formation of new tissue takes over as inflammation lessens over the next several weeks. In ligaments, collagen levels increase rapidly, reaching normal levels by 6 weeks.[4] The collagen types are altered, with greater levels of types III, V and VI collagen ("minor collagens") and less type I collagen than normal (type I is still the most prevalent). Ligaments begin to resist appreciable forces, allowing biomechanical testing as early as 2 to 3 weeks after injury. The healing tissue is remodelled by cells over several months and years, leading to fewer cells and vessels, with better collagen alignment. Collagen types return closer to normal distribution, with type I increasing and most of the minor collagen types decreasing. The structural strength and stiffness, stress and tissue quality continue to improve up to 12 months after injury, but after that time only relatively small increases

Table I

Joint Structures That Resist Compressive and Tensile Forces of Joint Function

Compressive forces	Tensile forces
Menisci	Ligaments
Cartilage	Capsule
Bone	Neuromuscular/ tendon unit

are made.[6] However, the material properties of the ligament scar do not return to normal even after 2 years.[6] Thus, with long-term studies on healing of the MCL in animals, the tissue behaves as a scar with abnormal biomechanical, biochemical and ultrastructural properties (Table II).

Two comments about ligament healing in the context of joint function are necessary. The return of joint function after injury does not mean that the ligament has healed; other structures may compensate for missing ligament. Conversely, a ligament may have a better biomechanical healing environment if other structures can take up "the slack" and protect it. This may be one of the reasons that the anterior cruciate ligament (ACL) has a poorer functional healing response after injury, given that it contributes the most to resistance to anterior tibial translation and there are few structures available to protect it while it heals.[2] Failure of ACL healing in this context would relate to biomechanical factors, not intrinsic biologic failure to mount a healing response.

Surgery to promote ligament healing

Gap versus contact

Suturing is a surgical method designed to improve ligament healing by bringing ruptured edges in contact and closing the gap. When a rabbit MCL model was used to compare an 8-mm gap to a cut ligament with the ends in contact, the structural strength 2 years after injury of the MCLs with a gap was significantly less than the strength of MCLs with the ends in contact.[6] In fact, the structural strength of MCLs in the contact group approached that of normal MCLs. However, the material properties of healing MCLs (scar) for both groups were still significantly less than those of normal MCLs. This is due to the larger cross-sectional area of healing MCLs, indicating that the scars have a greater amount of inferior strength tissue compared with that of normal MCLs. Related studies in rabbits in which MCL ruptures were sutured or not sutured showed no difference in structural or material properties of the MCL.[3] In this model the difference in the size of the gaps between the 2 groups was small, since the torn ends of the nonsutured ligaments were separated by a gap of less than 2 mm. Thus, it appears that suturing may make a difference in higher- or failure-load properties only when there are large gaps between the ends of torn ligaments.

Motion

Motion in stable joints improves the biomechanical properties of healing ligaments compared with immobilization of joints in animal models of MCL healing.[3] Motion leads to an increase in the size of the ligament scar and does not appear to improve the alignment of the collagen fibres.[3] The mechanism presumably involves the application of controlled forces; too little or too much force is detrimental. The key is that in the context of the joint, other structures can take up "the slack" (Table I) and control the forces applied, allowing the joint to function while the MCL heals.

Multiple ligament injuries

Laboratory evidence in animal models indicates that MCL healing is inferior biomechanically when there is an accompanying ACL injury,[3] and in the short-term (less than 12 weeks) some of the detrimental effect of the ACL injury on MCL healing is reversed with ACL reconstruction.[3] This would suggest that the quality of MCL healing is related to the stability provided to the joint. The stability can be altered by the presence of a normal ACL, a reconstructed ACL or no ACL in this animal model. Clinically, it can be difficult to accurately classify ligament injuries as stable or unstable; however, surgical stabilization of some joint injuries is clearly necessary to protect ligaments while they heal.

Autografts versus allografts

The issue of autografts versus allografts focuses on tendon graft sources since there are no "spare" ligaments. Autograft versus allograft ACL reconstructions have been studied clinically and in the laboratory. Both tissue sources experience necrosis and cell death followed by revascularization and

Table II

The Biomechanical, Biochemical and Histologic Changes of Ligament Scars Compared With Normal Ligaments Approximately 1 Year After Injury

Biomechanical changes	Weaker Inferior material quality Larger Greater creep
Biochemical changes	Increased type V collagen Decreased hydroxypyridinium cross-links Increased glycosaminoglycans
Histologic changes	"Flaws" in matrix Abnormal collagen fibril diameter distributions

repopulation with host cells after placement. The process of graft incorporation is somewhat longer for allografts, but in both cases the grafts are weaker and have altered fibril diameters when compared with normal ACLs.[3]

Incorporation of the graft by new cells seems to accelerate early deterioration in the biomechanical properties of the graft. Studies on patellar tendons in rabbits and ACLs in goats where these tissues were frozen in situ to kill the cells (thus simulating an autograft) show a deterioration in biomechanical properties as the "graft" becomes repopulated with cells.[7,8] This deterioration can be blunted by preventing the infiltration of cells, in the short-term at least.[8] The biology of autograft and allograft transplantation for ligament reconstruction is an expanding area of research.

Current studies and future directions

Three properties of ligament biology that have recently been identified to be abnormal in healing ligaments are collagen cross-links, type V collagen, and "flaws" in the scar. Hydroxypyridinium cross-links form mature covalent bonds between adjacent collagen molecules. It has been shown that the levels of these cross-links are decreased up to 1 year after injury and that this decrease correlates with the inferior material properties of scars.[9] Type V collagen alters fibril diameters in collagenous tissues, with increased levels of type V collagen being associated with smaller fibrils.[3] Type V collagen is elevated 1 year after MCL injury,[10] and some investigators have shown that long-term healing of the MCL leads to smaller fibril diameters and poor material quality of the scar.[3,4] "Flaws" in ligament scar tissue are characterized by areas of nontensile bearing material such as blood vessels, fat cells, loose and disorganized collagen, and cellular infiltrates (Fig. 2). The flaws could represent stress-risers within the scar, and it has been shown that these flaws correlate with inferior material properties in ligament scars.[11] Thus, methods that could lessen the number or size of flaws, decrease the levels of type V collagen or increase the number of mature hydroxypyridinium cross-links could improve ligament healing and scar formation.

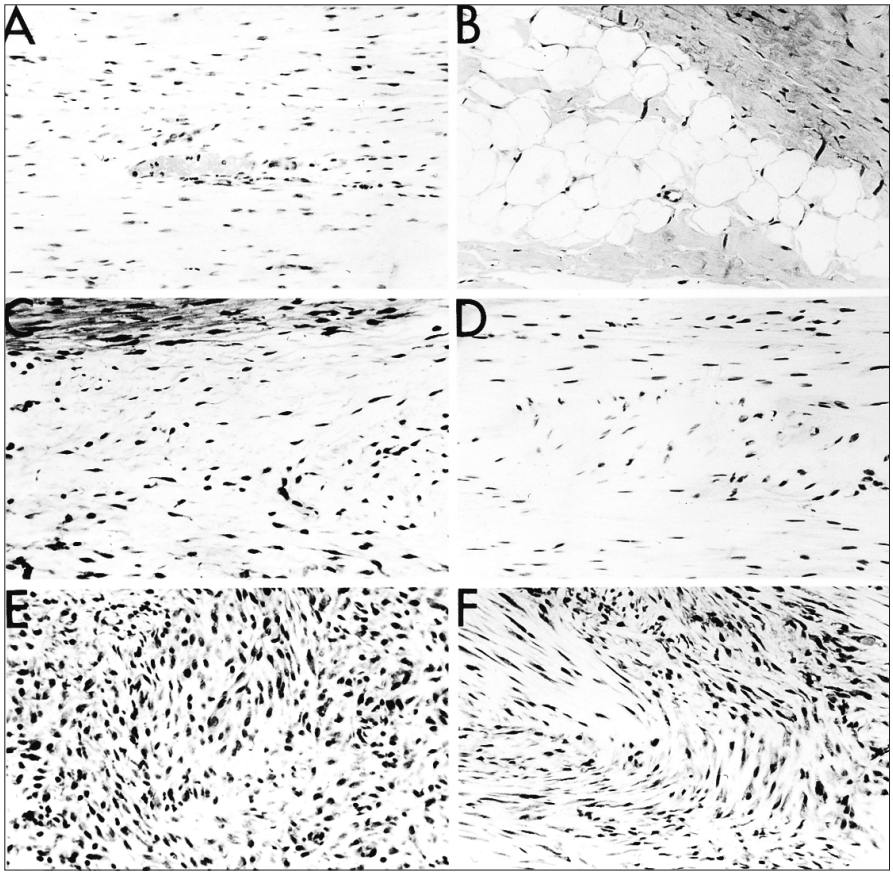

FIG. 2. Photomicrograph of a healing medial collateral ligament showing flaws in the tissue, consisting of blood vessels (panel A), fat cells (panel B), loose collagen (panel C), disorganized collagen (panel D), cellular infiltrate (panel E) and combinations of flaws stained with hematoxylin and eosin (panel F) (reproduced with permission from Hildebrand KA, Frank CB. Biology of ligament healing and repair. In Johnson RJ, Lombardo E, editors. *Current Review of Sports Medicine.* 2nd ed. Philadelphia: Current Medicine; 1998. p. 106).

Recently, 2 potential therapeutic agents for ligament healing have been evaluated: growth factors and gene therapy. Growth factors are molecules that modulate many cellular processes, including proliferation, migration, synthesis and matrix production. Cells from normal ligaments and healing ligament scars are responsive to growth factors in terms of proliferation and collagen synthesis.[3,12] In vivo studies using growth factors have shown that early MCL healing (less than 12 days) was significantly improved with greater structural strength and stiffness using platelet-derived growth factor (PDGF-BB).[3,13] Gene therapy is a method to introduce foreign DNA into cells and alter the endogenous protein synthesis by the cells or induce the expression of therapeutic proteins (e.g., cytokines) by the cells. Preliminary studies have shown that marker genes and growth-

factor genes can be expressed in ligaments and tendons of rabbits.[14,15] Many questions need to be answered before growth factors or gene therapy can be applied to clinical treatment of ligament injuries.

Traditionally, clinical and laboratory studies on ligament healing have used higher- (failure-) load data as end points. Examples in the laboratory include structural strength and stress at failure and, in the clinical setting, the number of graft failures. Recently, various investigators have started to look at lower-load or subfailure data to characterize ligaments and ligament healing. Examples in the laboratory include the use of a robot coupled with a force-moment sensor to look at ligament contributions to specific joint-loading conditions[2] or uniaxial tensile tests of bone–ligament–bone complexes defining the creep properties of healed MCLs.[16] These lower-load properties are important since most activities do not lead to ligament failure, indicating that ligaments function at these lower-load levels in most cases. An additional concept pertains to ligament and ligament graft healing where altered subfailure properties may predispose these structures to damaging forces or stresses that would not be damaging for normal ligaments. This process could lead to gradual stretching of the graft and eventually failure, which would alter joint function. Defining these lower-load or subfailure properties and their underlying mechanisms will be an important step in future research.

Summary

Ligament healing in what may be considered to be the best case scenario (MCL of the knee) is characterized by a scar material with inferior tissue quality, with changes in biochemical and histologic properties, that does not regenerate a normal ligament even after 2 years of healing. The impact on joint function that the ligament scar has depends on the ligament injured and joint affected. Although some surgical interventions may improve ligament healing, such as closure of large gaps, motion and stabilization of joints with multiple ligament injuries, many exciting questions and challenges remain to be addressed with respect to regenerating a new ligament after injury.

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