Increased participation by the general population in athletic activities leads to increased trauma to bones, joint surfaces, and soft tissues. Management and treatment of these injuries has significantly improved over the past few decades. The application of knowledge gained from basic science research in biology and biomechanics has continuously contributed to that. Biological advances have been made in the field of gene therapy, cell therapy, and tissue engineering. Certainly, the greatest focus is bone and cartilage research that will lead to improved fracture repair in the traumatic injured population, as well as prevention of early osteoarthritic changes in the injured athletic population. In biomechanical research, contributions have been made to further understand kinematic behavior of joints that will lead to improved ligament reconstruction techniques and rehabilitation regimens. Various fixation techniques and several different ligament reconstruction techniques have been studied and validated. In the future, improved understanding of ligament healing, graft incorporation, and revascularization will lead to improved outcome of surgical reconstruction techniques in orthopaedic sports medicine. Exciting research has been performed over the past years and will be reviewed in this article.

With the general population’s increasing participation in athletic activities, the incidence of trauma to bones, joint surfaces, and soft tissues is also increasing. Epidemiologic studies from the early 1990s investigated the incidence of knee ligament injuries in the United States. The average rate of knee ligament injuries was approximately 100/100,000 of the population annually [1] and can be expected to increase. Management of these injuries involves conservative treatment in combination with the sophistication of rehabilitation regimes and operative treatment, extending from diagnostic procedures to combined ligament and transplantation surgeries [2–5]. Treatment of these injuries has significantly improved over the past few decades, with the application of knowledge gained from both basic science and clinical research [6–15]. This article will review biologic and biomechanical aspects in sports traumatology and will summarize the latest basic science knowledge in biology and biomechanics in this field.

**Biology**

In ligament reconstruction surgeries, commonly used tendon grafts undergo biologic modifications before they form the final strong fibrous tissue. In the beginning, the graft undergoes inflammation and (partial) necrosis. The graft then undergoes revascularization and repopulation with fibroblasts. The last stage is marked by a gradual remodeling of the graft and continuous modification of its collagenous structure [16,17]. There is evidence that autograft as well as allograft transplants are repopulated with extrinsic fibroblasts within 4 weeks [18]. After 4 to 6 weeks, the graft is completely repopulated. Donor fibroblasts undergo cell death and are not detectable thereafter. The tendon structure, however, serves as a template for soft tissue remodeling [19,20].

Taking the anterior cruciate ligament (ACL) as an example, normal insertion site anatomy has a specific arrangement of collagen fibers, fibroblasts, fibrochondroblasts, and osteoblasts forming a direct ligament insertion, which consists of four layers. The first layer comprises the ligament, the second layer is characterized as a nonmineralized cartilage zone containing fibrocartilaginous cells, the third layer is the mineralized cartilage zone, where the mineralized cartilage inserts into the subchondral bone plate, the fourth layer, to which the ligament is attached (Fig. 1). The design of this complex insertion site allows for distribution of longitudinal and shear forces from the ligament into the subchondral bone.
plate, thus minimizing stress on single collagen bundles [21]. This complex anatomy, however, is not restored by conventional ACL-transplantations within the first 6 month after graft implantation.

Various growth factors have been identified to affect the healing process in tissues of the musculoskeletal system [22]. Growth factors are small peptides that can be synthesized both by the resident cells at the injury site (eg, fibroblasts, endothelial cells, mesenchymal stem cells) and by the infiltrating reparatory or inflammatory cells (eg, platelets, macrophages, monocytes). They are capable of stimulating cell proliferation, migration, and differentiation as well as the matrix synthesis [23,24]. Meanwhile, the stimulating effect of various growth factors in different tissues has been demonstrated [25–27]. The gene encoding for most of the known growth factors has been determined, and using the recombinants DNA technology, we are now able to produce large quantities of these recombinant proteins for treatment.

Recent literature in growth factor research has focused on enhancing bone healing and improving cartilage regeneration. Boden et al., who are focusing on lumbar spine arthrodesis, have developed an animal model to study nonunion rates in spine fusion. With the discovery of an osteoinductive protein, LIM mineralization protein-1 (LMP-1), adenoviral delivery of LMP-1 complementary DNA (cDNA) to the spine in immunocompetent rabbits could be achieved [28–30]. Several studies on long bone healing have been performed. Bouxsein et al. developed an ulnar osteotomy model in the rabbit and indicated that recombinant human bone morphogenetic protein-2 (BMP-2) is capable of enhancing healing [31]. In a prospective randomized clinical trial, it was shown that recombinant osteogenic protein-1 (BMP-7) that was implanted with a type I collagen layer was a safe and effective treatment for tibial nonunions [32]. In cartilage research, the effect of growth factor application to chondrocytes has been shown to be feasible by several groups. Bonassar et al. recently studied the effect of dynamic compression on the response of cartilage to insulin-like growth factor-I (IGF-I) and found that when applied together, the two stimuli enhanced protein and proteoglycan synthesis by 180% and 290% [33]. Transforming growth factor beta (TGF-β) was found to stimulate the production of elastic cartilage in a porcine model [34].

Despite the fact that these approaches have been capable of improving the local persistence of the growth factor proteins, the results of these delivery techniques remain limited. Gene therapy is a technique that relies on the delivery of therapeutic genes into cells and tissues. Originally, gene therapy was conceived for the manipulation of germ-line cells for the treatment of inheritable genetic disorders, however this method is limited by inefficient technology and considerable ethical concerns. Gene therapy can be applied to orthopaedic surgery by transferring defined genes encoded for growth factors or antibiotics into a target tissue (eg, ligament, cartilage, or bone). Thus, local cells at the injury site can persistently produce therapeutic substances. For gene expression, the transferred DNA material has to enter the nucleus, where it either integrates into the chromosomes of the host cells or remains episomal. After transcription, the generated messenger ribonucleic acid (mRNA) is then transported outside the nucleus, serving as a matrix for the production of proteins (eg, growth factors) in the ribosomes (Fig. 2). Consequently, the transduced cells become a reservoir of secreting growth factors and cytokines capable of improving the healing process. Viral (eg, adenovirus, retrovirus) and nonviral (eg,
liposomes, gene gun) vectors can be used for delivery of generic material into cells [35,36].

The structural and functional relation in healing ligaments is complex; the collagen fiber diameter and orientation is altered, in addition to collagen type ratios and cross-links [15,37–42]. The water content in healing ligaments is higher with increased vascularity. Altering the water content, however, was shown to affect prestress and creep behavior of ligaments [43]. In a study by Nakamura et al., the functional healing of a ligament in a rabbit model could be improved by decorin antisense gene therapy [44•]. It was furthermore shown that the engineering of healing ligaments seems to be possible by supplying mechanical stimuli in vitro, such as cyclic stretching of fibroblasts on grooved surfaces [45], hydrostatic compression, or cyclic tension [46].

Tissue engineering-based approaches aim at using cells to deliver genes. These technologies might offer additional opportunities to improve the healing process [47]. Several different origin tissues have been investigated (eg, mesenchymal stem cells, muscle derived stem cell, dermal fibroblasts, or human adipose tissue) [48–50]. Selecting the appropriate gene delivery procedure depends upon various factors such as the division rate of the target cells, pathophysiology of the disorder, and the accessibility of the target tissues. Preparing muscle-derived stem cells by transduction with an adenovirus containing BMP-2 cDNA, Lee et al. were able to heal a critical sized bone defect in mice [51••]. Scaffolding materials were used to study the in vitro behavior of cells and in vivo they were used to repair tissues. Canine chondrocytes were shown to be viable when seeded on collagen matrices [52]. Murray et al. were able to show that human ACL fibroblasts migrated into a collagen-glycosaminoglycan scaffold to bridge a gap between transected ACL fascicles in vitro [53•]. Small intestinal submucosa (SIS) was used for repair of various musculoskeletal tissues [54,55]. Dejardin et al. replaced a resected infraspinatus tendon with SIS in a dog model and found similar structural properties in a sham operated control [56•].

Biomechanics
The knee is a complex structure that involves the interaction of stabilizing ligaments and muscle-force generating tendons to provide locomotive, static, and dynamic functions. These motions are combinations of 3D translations and rotations that are mediated by ligament forces, joint contact forces, externally applied forces, and musculoskeletal forces. Thereby, the tensile behavior of ligaments is suited to guide joint motion and provide stability over a large range of motion. The disruption of any of the major components of the knee causes abnormal kinematics and therefore osteoarthritis, over time. For disruption of the ACL, anterior tibial translation will be increased [57], internal and valgus rotations will be increased, especially when the medial collateral ligament (MCL) is disrupted [58,59]. Progressing osteoarthritis of the knee joint is a combination of biologic transformations [60] and biomechanical dysfunction, evidenced by meniscal damage, increased ligamentous laxity, and articular cartilage degeneration [61–65].

The kinetic response of a joint to internal and external loads is governed by bone geometry and the anatomic location, morphology, and chemical composition of the tissues within and around the joint. The transfer of load through a ligament must be considered to better understand its contribution to joint kinematics. Physiologically, bone-ligament complexes have been designed to transfer load along the longitudinal direction of the ligament. The structural properties of a bone-ligament-bone
complex include stiffness, ultimate load, ultimate elongation, and energy absorbed at failure. Mechanical properties of the ligament substance are obtained by normalizing the force by the cross-sectional area of the ligament. The change in elongation by the initial length of a defined region of the ligament midsubstance is defined as stress and strain, respectively [66,67]. From the stress-strain curve, the elastic modulus, ultimate tensile strength, ultimate strain, and strain energy density of the ligament substance can be determined [15]. Individual units of the ACL yielded an average modulus and ultimate tensile strength of 278 MPa and 35 MPa, respectively [68].

The in situ force of the knee is an experimentally measured quantity that represents forces existing in the knee at a given position. Various devices, such as buckle transducers [69,70], implantable force and pressure transducers, load cells [71,72], and universal force moment sensors in combination with a 6-degree of freedom robotic

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manipulator [73–75] have been used to determine the in situ forces and in situ strains in ligaments. Forces and distributions in both the anteromedial (AM) and the posterolateral (PL) bundle of the ACL have been quantified during the anterior drawer test, Lachman test and simulated pivot shift test using human cadaveric knee specimens [76,77].

Woo et al. studied two popular grafts for ACL reconstruction, quadruple semitendinosus/gracilis and B-PT-B [78]. Both were found to have little improvement over the ACL deficient knee when rotational loads were applied. An anatomic reconstruction replacing the AM and PL bundles resulted in knee kinematics significantly closer to those of the intact ACL compared with conventional reconstruction procedures [79]. Additionally, the in situ forces in the anatomic reconstruction were substantially closer to those of the intact ACL compared when the knee was subjected to both the Lachman and simulated pivot shift tests [80•]. The forces in the medial meniscus were shown to increase twofold in response to ACL transection in a human cadaveric knee model [81]. Likewise, Hollis et al. studied the meniscal strain in ACL transected human cadaveric knees and reported significant increases. After ACL reconstruction they observed meniscal strain similar to that detected in ACL intact knees [82]. In a long-term follow-up study, Beynnon et al. reported that the elongation of an ACL graft during surgery significantly increases the anterior tibial translation when graft elongation values produced by knee flexion are outside the limits of the native ACL [83•].

For the posterior cruciate ligament (PCL), double-bundle reconstructions were studied and found to be favorable to single bundle reconstructions [84,85]. Bergfeld et al. studied two different PCL reconstruction techniques and found that a posterior inlay technique showed significantly less mechanical degradation than an arthroscopic-assisted tunnel technique [86]. Fixation of the PCL graft at full extension was found to overconstrain the knee and elevate the in situ graft forces, whereas fixation with the knee in flexion and under an anterior tibial load was found to restore the intact knee more closely [87]. The forces in the PCL were found to increase and the forces in the ACL were found to decrease in response to hamstring muscle loads [88,89].

Technical considerations for ACL reconstruction were recently studied in biomechanical investigations. Controversies still exist whether to use grafts with one or two bone plugs (patellar, quadriceps, Achilles tendon) or soft tissue grafts (hamstring tendons). The length and size of (bio)interference screws [90,91] and the insertion torque[92] were found to be indicators of graft fixation strength. Fleming et al. developed a goat model to study the relation between pretension of an ACL graft and anterior tibial translation. A tension of 60 N, applied at a knee flexion angle of 30° was thereby the best combination for restoring anterior tibial translation [93]. In another study on pretensioning grafts, a dog model was developed to study the effect of placing a pretensioned graft across open growth plates. Significant deformity of both the femur and tibia was observed in the treated limbs [94].

Future directions
In the future, gene therapy, cell therapy, and tissue engineering will be the available biologic tools. Therapeutic genes, encoding growth factors such as BMP-2 and TGF-β can be delivered into cells and tissues. Furthermore, improvement of biologic incorporation of replacement grafts will lead to better insertion site healing as well as faster ingrowth of graft materials. Gene-based cell therapy, relying on the ability of mesenchymal stem cells (eg, from blood, fat, bone marrow, muscle) to divide into a variety of cell types, may enable simple biopsies to provide the cell that can restore any kind of defect by growing the local cell line (Fig. 3). However, extensive animal research before application on humans is needed to grant the necessary safety.

Biomechanical research will provide ligament forces that are based on in vivo studies, so that surgical reconstruction procedures can restore the anatomy as closely as possible to normal. Specific knowledge on in vivo forces of ligaments will further enhance rehabilitation to regimens that exercise the knee at forces that do not exceed the normal. Biomechanical research in computer-assisted orthopedic surgery and computed imaging will enhance both surgical precision and preoperative evaluation. Ultimately, biologic and biomechanical research will allow injured athletes to obtain a stable and well-functioning knee that allocates them to go back to original sports at the same level as before injury.

References and recommended reading
Papers of particular interest, published within the annual period of review, have been highlighted as:
• Of special interest
•• Of outstanding interest

7 Clancy WG Jr., Nelson DA, Reider B, et al.: Anterior cruciate ligament recon-
132 Sports-related injuries


50 BMP-2, in a cell-based gene therapy approach, is a powerful agent to enhance bone healing.


53 ACL healing might be enhanced by with tissue engineering approaches.


84 Intraoperative strain values can predict the outcome of ACL reconstruction.


