Osteoarthritis (OA) is one of the most economically important diseases facing equine practitioners. The loss of use associated with joint disease is a leading problem in the equine industry. In fact, a recent survey suggested that 60% of lameness issues are related to OA. For these reasons, equine joint disease research remains on the cutting edge of equine research and holds a promising future. In addition, the disease processes seem similar to those in people. The relation to human disease allows the equine researcher to use the human knowledge base regarding pathophysiology more closely as well as funding sources. The launch of the Bone and Joint Decade (2002–2011) has helped to increase awareness, patient education, and resources available for research, with the final goal of improving diagnostics, treatment, and prevention of musculoskeletal disease not only in human beings but in the veterinary field as well. The facts that 300 billion dollars per year is spent on human musculoskeletal conditions and that one in three Americans have joint pain are quite gripping and certainly are compelling for research in this area [1]. Furthermore, many of the current medical and surgical options being developed for people have been pioneered in veterinary medicine, including the horse, because of similarities in pathophysiology. Although OA in all species is believed to be a multifactorial disease that is not well understood, significant advances are being made. This article presents areas of research that are relatively well developed but have not made it to commercialization or routine clinical practice and looks at new applications being investigated for people that may have an equine application.
Biomarkers and the future of joint disease

Protein biomarkers

The term *biomarker* has evolved significantly in the last decade. Ten years ago, it was used to describe a protein or small segment of a protein typically measured in serum, urine, or synovial fluid that could be related to a biologic change. An example of what is referred to as a direct biomarker could be illustrated by examining the synthesis of type II collagen, which is an integral part of articular cartilage. When type II collagen is synthesized and processed into a mature molecule, it is shortened or clipped as it is incorporated into a mature type II collagen fiber, thus releasing a small fragment that can be detected using antibodies. The estimation of this clipped molecule can then be related to type II collagen synthesis. One such antibody is abbreviated as CPII and represents the detection of the carboxy-propeptide, the discarded segment of the mature type II collagen molecule [2]. As well as direct biomarkers, there are indirect biomarkers, such as interleukin-1 (IL-1). The term *indirect biomarker* is used for molecules that are integral in orchestrating the breakdown of key structural components of the tissue but are not a key structural component themselves. Simplistically speaking, the key structural components of articular cartilage are type II collagen and aggrecan. It is well accepted that IL-1 is most likely the key proinflammatory molecule that is involved in joint tissue destruction [3], and when IL-1 is elevated, destruction of the key structural components within the joint likely occurs. Thus, levels of IL-1 are thought of as an indirect biomarker. More recently, molecular biomarkers have been described; this topic is covered elsewhere in this section.

In the horse, the last 10 years have mainly been spent validating protein biomarkers borrowed from human medicine. This has been done through measuring how levels differ based on age, gender, exercise, and disease processes, for example [4–7]. As this information has become better understood, people have become more interested in the specific changes of biomarkers as they relate to the presence and stage of disease. For example, a group of horses presented to Colorado State University for arthroscopic removal of osteochondral fragments was compared with a control population of horses determined to be free of fragments. In this study, biomarkers were able to classify the horse as having a fragment or not 79% of the time based on several biomarkers [8]. Although this was a focused population of horses, it did pave the way for further research using biomarkers in joint disease. Based on these findings, a larger battery of biomarkers was used to explore if normal exercise could be distinguished from joint pathologic findings, because biomarkers are known to rise significantly in both cases. It was determined that although exercise did significantly increase the six biomarkers, measured experimental induction of joint disease through the creation of a solitary osteochondral fragment was
discernible using synovial fluid or serum biomarkers [9,10]. From this work, a clinical prospective biomarker study was undertaken involving Thoroughbred race tracks in southern California in collaboration with the Colorado State University Equine Orthopedic Research Center (EORC). The goal of this study was to enroll 200 horses that were 2 or 3 years of age when they arrived at the track. They would have a musculoskeletal examination and blood drawn monthly by study veterinarians for 10 months or until they sustained an injury that would require them to be out of training for longer than 30 days. Horses had to be enrolled in the study for at least 2 months to be eligible to serve as an injured or control horse. Horses sustaining an injury would be diagnosed by the study veterinarian, and an exit blood sample would be drawn. Only horses sustaining a solitary lesion of one of four types were included in the final population. The four types of lesions examined were intra-articular fragmentation, incomplete and complete nonarticular fracture, tendon or ligamentous injury, and periostitis (bucked shins). Serum samples from this population were analyzed for seven different biomarkers, with the goal of determining what the levels were in these different types of musculoskeletal injury and how these levels compared with those in control or uninjured horses. A second goal of the study was to assess levels of biomarkers in samples taken before the time of injury, with the hope that some predictive values or risk factors could be determined. The data for the first goal have been analyzed, and it does seem that unique patterns of biomarker changes can be identified for each of the studied musculoskeletal injuries. These data are encouraging for determining the usefulness of biomarkers in joint disease as well as other equine injuries. The predictive or second goal of the study is still in the process of evaluation. Other studies have also shown the usefulness of biomarkers in clinical cases of joint disease; some have looked at OA, whereas others have focused on osteochondrosis [11–13]. With significant changes being realized, these studies are paving the way for a greater understanding of the pathophysiology of joint disease using protein-based biomarkers.

Molecular biomarkers

Today, the term biomarker not only reflects measured proteins but molecular markers as well. Thus, a more suitable definition of a biomarker may be any parameter measured in various fluids and tissues that is correlated to a biologic change in that tissue. An example of an indirect molecular biomarker would be an increase in IL-1 mRNA measured in chondrocytes. The ability to characterize and measure RNA and DNA more quickly than proteins has advanced what we know about biomarkers. For example, there are more than 30,000 genes that encode mRNA, and the technology exists to measure the relative levels of all these genes within 48 hours using gene chip or microarray technology. This provides a vastly
increased capacity for analysis at a greater speed than is currently available for measuring proteins. Although a great deal of work has been published in the horse relating to specific up- or downregulation of a handful of genes (before microarray technology), the most exciting potential is in the use of the gene chip technology assessing thousands of genes at once.

The first equine gene chip study was conducted using the human gene chip; this could be done because many of the important gene sequences are relatively conserved between species, although it is not optimal. Cartilage from normal joints was compared with cartilage collected from joints with osteochondrosis dissecans (OCD) [12]. In this study, researchers did see a significant difference in the gene expression pattern when OCD and normal cartilage were compared. More recently, two equine-specific gene chips have been developed and validated. One chip was validated using an in vitro model of synovitis (lipopolysaccharide [LPS] stimulation) and demonstrated significant changes when equine cells were exposed to LPS [14]. Independently, another chip was created that possesses more than 3000 unique equine sequences and was validated using an in vivo model of OA [15]. In this study, the investigators collected peripheral blood samples and assessed the up- and downregulation of gene sequences found in circulating white blood cells (WBCs). After analyzing the data, a unique “gene signature” was observed in horses with experimentally induced OA. The data generated in this study are now being used to plan a clinical trial to see if a similar gene signature exists in horses with clinical OA. This particular gene chip has also been used effectively to identify gene signatures for nonmusculoskeletal disease, such as equine protozoal myelitis, equine herpes virus, and gastric ulceration (Genetraks Holding, personal communication, 2005). It is clear that gene chips could be quite useful in the horse as a diagnostic tool in the near future. Additionally, the potential of exploring this technology for predicting disease and gaining a better understanding of the pathophysiology of joint and other musculoskeletal disease is promising.

Future therapeutics

Future therapies in equine joint disease can realistically take two forms over the next 10 years: surgical options for focal cartilage lesions and medical treatments that can be used in focal cartilage lesions but are more typically applicable for generalized OA. In the last decade, research has led to a greater understanding of joint disease pathophysiology, and the identification of major mediators driving the disease process has fueled great advances in medical treatments. Two of the most prevalent mediators said to be at the “top of the cascade” are IL-1 and tumor necrosis factor (TNF). Equally important discoveries have been the identification of naturally occurring antagonists or synthetic analogues with the capability to block these degenerative mediators.
The treatment of generalized OA using novel methods of administering antiarthritic therapeutic proteins is most likely to be the first advance that changes the future of treating equine joint disease [16–18]. In human medicine, numerous novel medical treatments have successfully undergone clinical trials after demonstrating promising in vivo and in vitro results [16, 18–21]. There are two main issues with medical treatment of OA: first, which specific therapeutic agent to use and, second, how to administer the chosen agent. As previously mentioned, therapies that revolve around administration of natural IL-1 or TNF inhibitors are likely have the greatest impact in the equine field in the short term.

There are two relatively well-defined methods of inhibiting IL-1. The first is through the natural antagonist, interleukin-1 receptor antagonist (IL-1Ra). This molecule binds to the cell membrane IL-1 receptor; however, unlike IL-1, it does not elicit any biologic response from the cell. This then blocks any IL-1 from binding to those receptors. The second method of blocking IL-1 is through the use of soluble receptors, yet another natural method. In this case, IL-1 receptors are released from the cell membrane and bind IL-1, preventing it from activating a cell-bound receptor. Neither IL-1Ra binding to a cell membrane receptor nor the formation of a soluble receptor and IL-1 complex produces a response from the cell. Natural soluble receptors are also used to inhibit TNF. There have also been antibodies specifically designed to bind TNF, thus inhibiting it from activating cell-bound receptors as well.

All the inhibition methods presented to date have been proteins of some fashion, and as such, they can be produced using several methods, including isolation and purification from natural sources, creating recombinant proteins in bacterial or mammalian cell lines, or, alternatively, administering gene sequences that are capable of driving the protein production in situ. The administration of proteins derived from natural or recombinant sources has been tested more extensively as compared with gene transfer methods. In human beings, an anti-TNF monoclonal antibody (Adalimumab; Abbott Laboratories, Abbott Park, Illinois) has shown promising results based on a 52-week randomized placebo-controlled study [18]. This study was conducted on 619 patients with rheumatoid arthritis (RA; a disease more characterized by synovial inflammation driving cartilage degradation) and measured radiographic, clinical, and functional outcomes after subcutaneous administration of anti-TNF monoclonal antibody. Significantly less radiographic progression of disease, reduction in signs and symptoms of disease, and improving physical function of patients receiving treatment were observed in this study compared with the placebo-treated group. Similar improvements in signs, symptoms, and functionality were observed in a study evaluating 277 patients with ankylosing spondylitis treated through subcutaneous administration of a recombinant human TNF
receptor (etanercept) [16]. Finally, a study involving 472 patients with RA demonstrated a significant improvement in the number of swollen joints, investigator disease activity scores, patient assessment scores, and other objective measures of disease activity when the subjects were administered IL-1Ra subcutaneously. Combinations of these therapeutic agents have also shown promise after in vivo testing using animal models of RA. In fact, results of IL-1Ra and a TNF-soluble receptor study concluded that the combination was of more benefit than either agent alone [22].

To date, only one such product has made it to the commercial market for use in horses. A product marketed as Orthokine (autologous conditioned serum; Orthogen AG, Düsseldorf, Germany) in Europe was introduced in the United States during the last quarter of 2004 by Arthrex Biosystems (Germany). This product uses collection of peripheral blood followed by a 24-hour culture period with glass beads soaked in chromium sulfate to stimulate the production of IL-1Ra and other anti-inflammatory mediators without the subsequent stimulation of IL-1 or TNF. In Europe, this product is approved for use in human beings. It is reported to be successful in cases of OA and has been administered to more than 30,000 patients. It has also been anecdotally used in horses in Europe and the United States. It is important to note that the upregulation of beneficial anti-inflammatory mediators has thus far not been confirmed in the horse. Research at the Colorado State University EORC has been conducted evaluating this product in a double-blind, randomized, placebo-controlled model of equine OA, although at the time of this publication, the results were not available. Anecdotal reports from test sites in the United States have been favorable for use of this product, even reporting success in cases that were refractory to corticosteroid administration.

Another method of administering antiarthritic proteins is through the use of gene transfer. In fact, gene therapy has been shown to be an effective treatment in an experimental model of OA [23]. Briefly, the gene sequence for equine IL-1Ra was delivered to the affected joint by direct intra-arterial injection of a nonpathogenic virus. This virus was engineered to deliver the genetic information (equine IL-1Ra gene), thus overproducing equine IL-1Ra protein within joint tissues without causing adverse effects on the cell. In this study, the ability to decrease clinical lameness and to slow the progression of induced joint disease was more profound than with any other medication tested (including corticosteroids, polysulfated glycosaminoglycans, pentosan polysulfate, hyaluronan, or extracorporeal shock wave treatment). Advances in delivering genes or recombinant proteins to specific target cells and the ability to turn on and off the protein production are being realized. The power in this type of technology lies in the ability to insert any gene sequence of potential therapeutic benefit and have it produced in the body. Limitations do exist in the ability to target specific cells and to control the level and duration of gene expression (protein production), but advances are being made. Although we are still years off
from having a commercial gene therapy product, the proof of principle has been completed in the horse.

**Surgical techniques**

In the long term, the field of tissue engineering is burgeoning with the promise of being able to recreate organs systems, and this may provide surgical options for generalized cartilage degeneration that is unresponsive to medical treatments [17]. Realistically, in the short term (next 10 years), the subdiscipline of cartilage engineering is most likely to have the greatest impact on the treatment of focal cartilage lesions in the horse. This is an active area of research at multiple equine orthopedic research facilities throughout the world.

Although subchondral bone microfracture is a recent advance in equine surgery, it is the current preferred therapeutic treatment to stimulate endogenous healing of chondral defects. This is partially attributable to the technical ease of the procedure as well as the low cost. In human patients with focal cartilage lesions, two surgical methods are used: subchondral bone microfracture and autologous chondrocyte transplantation. The latter method is relatively impractical for the horse, based on the need for two surgical procedures. The first procedure involves harvesting chondrocytes, which are then expanded in the laboratory for 1 month; during the second procedure, the cells are implanted under the periosteum, which is sewn over the cartilage defect. This method is obviously more technically difficult compared with subchondral bone microfracture as well as being more expensive.

There have been two basic approaches explored for equine joint resurfacing: stimulated endogenous repair and transplantation or grafting of tissues. Although these techniques are discussed as separate entities, many cutting-edge approaches are combining the techniques as well as augmenting either or both techniques with growth factor supplementation.

**Stimulated endogenous repair**

Because bone marrow has a good supply of stem cells and growth factors that are thought to be integral to cartilage health and repair, direct communication of articular lesions to these elements beneath the subchondral bone plate has been a cornerstone of stimulated cartilage repair. Growth factors believed to be important in cartilage repair include insulin-like growth factor-1 (IGF-1), transforming growth factor-β (TGFβ), and bone morphogenetic proteins 2 and 7 (BMPs 2 and 7). Access to these marrow elements has been facilitated by various different surgical techniques, including abrasion arthroplasty (debridement to the level of the subchondral plate), spongialization (debridement past the subchondral plate into cancellous bone), focal drilling to the depth of cancellous bone
in discrete locations throughout the cartilage lesion (osteostixis), and subchondral bone microfracture (penetration to the level of the subchondral bone in discrete locations). Current literature and clinical practice do not favor spongialization, in part, because it is thought to destabilize the biomechanical stability of the subchondral bone plate.

As mentioned previously, the current recommendations for stimulated endogenous repair of an articular lesion would be debridement of the lesion to the level of the subchondral bone plate (abrasion arthroplasty) alone or in conjunction with subchondral bone microfracture. If the lesion crosses the subchondral bone plate into the cancellous bone, the addition of subchondral bone microfracture is probably not necessary. In the presence of sclerotic bone, the lesion is debrided to a depth that produces petechial bleeding (in the absence of fluid pressure) but does not enter the cancellous bone. In the latter case, subchondral bone microfracture is also used.

Subchondral bone microfracture is thought to allow access to the cells and growth factors beneath the subchondral plate, without destabilization of the subchondral plate’s biomechanical stability. In addition, the penetration of the stainless-steel bone awl causes cracks in the bone as well as spicules of bone protruding from the penetration site, both of which are believed to aid in attachment of the repair tissue. Experimental studies have demonstrated that large articular cartilage defects (1 cm²) debrided to the level of the subchondral bone have a significantly greater volume of healing tissue after subchondral bone microfracture compared with defects that were debrided to the level of the subchondral bone plate but did not undergo microfracture. Biochemical analysis of the repair tissues has also shown a greater type II collagen content in repair tissue of microfractured defects in two experimental studies, although the histologic appearance of the repair tissue was similar [24,25]. Further improvement in the repair tissue obtained after subchondral bone microfracture has been achieved with the supplementation of IGF-1 and IL-1Ra using gene transfer [26]. Experimental work assessing subchondral bone microfracture has also confirmed the poor attachment of repair tissue in areas in which the calcified cartilage layer had been incompletely removed. Confirmation of the level of debridement can be achieved using a microarthroscope or focusing close to the defect margins with a standard arthroscope. A granular appearance to the defect should be evident, differentiating the subchondral bone plate from the glass-like appearance of the calcified cartilage layer. After debridement of the lesion, microfracture holes are spaced 2 to 3 mm apart, ensuring no communication of the sites and penetrating approximately 2 mm into the bone.

To date, no long-term follow-up report on clinical results has been published relating specifically to horses, although anecdotal reports have been promising. Recent human data have compared long-term follow-up of the most commonly used cartilage resurfacing techniques [27], autologous cartilage implantation (ACI), as described by Brittberg and colleagues [28] and subchondral bone microfracture, first described by Steadman and
coworkers [29]. The short- and long-term results of this study show minor significant improvement with subchondral bone microfracture, with no significant differences in the histologic appearance of repair tissue or patient outcome between the two techniques. Both of these techniques are considered better than debridement alone for most human and equine patients. Given that subchondral bone microfracture does not require a second operation and is less technically challenging compared with ACI, it is favored by most equine surgeons.

Articular grafting

Many different tissues have been used to transplant or graft into cartilage defects, including periosteal and perichondrial autografts; osteochondral, chondral or isolated chondrocyte-autografts or allografts; and stem cell transplantation from bone marrow and fat. Periosteal and perichondrial grafts have been studied in laboratory animals with some success, but results in the horse have been disappointing and are no longer a focus of ongoing research [30,31]. Osteochondral grafting procedures have been well developed for use in people but have had limited success in the horse. Early work in the horse demonstrated short-term success but resulted in long-term failures [32–34]. Many of the failures with osteochondral grafting have been attributed to lack of congruity of the recipient and donor tissues as well as to difficulties with surgical technique. Some concern also revolves around morbidity in the donor graft site. Typically, in people, a non–weight-bearing region is used for donor harvest, reducing morbidity issues, but lack of suitable non–weight-bearing donor tissue has been a limitation in horses. More recently, with the advent of specialized surgical tools designed for human osteochondral grafting, a few equine researchers have made advances in the use of this technique in the horse. Surgical technique and donor site selection are the main hurdles yet to be overcome before this technique reaches mainstream practice [35–38].

Chondrocyte transplantation has been an active area of equine research in the last decade. Techniques using allografts and autografts have been reported, but most work has focused on autografts, especially in human beings. As previously mentioned, the ACI technique described by Brittberg and colleagues [28] and marketed by Genzyme (Cambridge, MA) is the most well-studied grafting technique. This technique uses autologous chondrocytes harvested from a non–weight-bearing region, usually the trochlea of the distal femur, followed by a 4-week in vitro expansion of chondrocytes. The expanded cell population is then implanted during a second surgical procedure and held in the defect beneath autologous periosteum, which is sutured to the cartilage bordering the defect to create a watertight seal. Although this technique has been performed in horses with similar outcomes to those seen in people, the cost, laboratory facilities, need for multiple operations, and technical challenges of the procedure have limited its usefulness in clinical cases.

FUTURE DIRECTIONS
Techniques using frozen chondrocytes harvested from neonatal foals, which are implanted in a fibrin glue to help retain the cells in a chondral defect, have had some success in a limited number of chondral defects. The technique is being used more commonly in cystic defects to date [39].

Techniques using autologous chondrocytes harvested from the non-weight-bearing region of the lateral trochlea of the distal femur and implanted into 15-mm diameter defects have been successful in experimental equine trials. One of the tested procedures uses fibrin glue holding minced cartilage to a bioresorbable scaffold, which is subsequently stapled to the subchondral bone of the defect in a one-step surgical procedure [40,41]. In comparable equine experimental trials, this technique has been shown to be superior to the technique of Brittberg and colleagues [28] (known generically as an ACI technique). This technique is now undergoing human clinical trials, and because of the ease, cost, and promising results in equine experimental trials, it is likely to be used in equine clinical cases in the near future.

A considerable amount of research is being directed toward the use of mesenchymal stem cells for implantation in cartilage defects. This cell population has been shown to improve healing in experimental animals, but gaining access to a sufficient number of stem cells without in vitro expansion is a hurdle yet to be resolved in horses [39]. Recent work at the Colorado State University EORC has shown that by increasing the local concentration of fibroblastic growth factor (FGF), there is an increase in the migration of stem cells to a certain location. This work is the beginning of being able to use the horses' own stem cells to direct cartilage repair [42].

Summary

As this article has shown, although the challenges facing the equine practitioner in treating joint disease are numerous, the possibilities for future therapy are vast. Many promising technologies are being developed that should further enable practitioners to treat their equine patients more thoroughly. Although some technologies, such as the recreation of organ systems, are further away on the horizon, many advances are close at hand and should soon become integral components of clinical practice.

References


