Best Practice & Research Clinical Rheumatology
Vol. 18, No. 2, pp. 187–202, 2004
doi:10.1016/j.berh.2004.02.007
available online at http://www.sciencedirect.com

6

Tissue repair in rheumatoid arthritis: challenges and opportunities in the face of a systemic inflammatory disease

David A. Hart* PhD

Calgary Foundation-Grace Glaum Professor in Arthritis Research

Departments of Surgery, Medicine, Microbiology and ID, McCaig Centre for Joint Injury and Arthritis Research, University of Calagary, 3330 Hospital Drive NW, Calgary, Alta., Canada T2N 4N1

Alison S. Kydd MD, PhD Student

Leaders in Medicine MD/PhD Program, McCaig Centre for Joint Injury and Arthritis Research, University of Calagary, 3330 Hospital Drive NW, Calgary, Alta., Canada T2N 4N1

Cyril B. Frank MD

McCaig Professor in Joint Injury and Arthritis Research

Kevin A. Hildebrand MD

Health Research Foundation/CIHR Scholar

Department of Surgery, Faculty of Medicine, McCaig Centre for Joint Injury and Arthritis Research, University of Calgary, 3330 Hospital Drive NW, Calgary, Alta. T2N 4N1, Canada

Rheumatoid arthritis (RA) is a systemic inflammatory disease that can elicit a variable disease course, can influence a variable number of joints, and can exhibit a variable response to treatment. All of these factors contribute to the degree and extent of damage to joint components, as well as the potential for repair of other injured joint tissues/components. Furthermore, some of the RA treatments/drugs themselves can influence repair and injury responses, as well as the outcome of surgical interventions for advanced disease. However, as treatments and interventions become more sophisticated and successful in patient populations, the opportunity to initiate the repair/replacement of the damaged joint tissues also becomes more of a reality. This review will address the current clinical findings in the literature, and then discuss the issues and opportunities to initiate repair of damaged or injured joint tissues in order to restore joint function. These include growth factors, gene therapy, and bioengineered tissues, alone or in combination to augment endogenous repair or replace tissue damaged beyond such repair capabilities.

* Corresponding author. Tel.: +403-220-4571; Fax: +403-283-7742.
E-mail address: hartd@ucalgary.ca (D.A. Hart).

Key words: systemic inflammatory disease; gene therapy; bioengineered tissues; connective tissue damage and repair; systemic diseases and tissue repair; combination therapy to enhance tissue repair.

Rheumatoid arthritis affects many connective tissues and healing processes of joints

Rheumatoid Arthritis (RA) is an example of a systemic autoimmune disease that can lead to severe joint changes[1,2], including cartilage and bone erosions[3,4,8,9], damage to other joint structures such as menisci of the knee[5,6] and ligaments[7], and can also involve tendons leading to increased risk of overt disruptions.[10,11] The bone erosions can potentially contribute to fractures and adverse responses post-fracture due to altered bone quality.[12]

In addition to involvement of the synovium of diarthroidal joints, RA can also affect other connective tissues that have a synovium-like structure associated with them or have properties similar to a synovium, such as a tendon. Tendon nodules are a result of inflammation of the paratenon or covering on a tendon, as well as tendon sheaths that surround some tendons and serve to guide the tendon as it glides. Examples of the latter are the flexor and extensor tendons of the fingers and hand. The chronic inflammation of these tendon structures can lead to alterations in the integrity of the tendons with overt tissue rupture or severe loss of function in some cases.[13,14]

These 'bystander' connective tissues (cartilage, bone, ligament, tendon) can also be affected by the inflammation in the synovium and pannus progression. Thus, in the rheumatoid knee the inflammatory milieu with elaboration of a variety of potent mediators such as proinflammatory cytokines can lead to 'bystander' effects on the integrity of tissues such as cartilage, menisci and intraarticular ligaments. Important mediators such as IL-1beta can influence expression of potent modulators of the integrity of these bystander tissues including the matrix metalloproteinases, cyclooxygenases, and others.[15–17]

In addition to direct and indirect involvement of a variety connective tissues in RA, being a systemic inflammatory autoimmune disease, there are other implications due to this systemic nature of the disease. One aspect of connective tissue regulation that could be affected by systemic dysregulation of inflammatory processes is wound healing.[18–20] Normal wound healing in response to an injury results in an orderly and temporally regulated series of events involving both local and systemic elements. Usually this process in a tissue such as skin is viewed as having four overlapping phases: hemostasis, inflammation, fibrosis and formation of granulation tissue, and then a protracted tissue-remodelling phase [discussed in Ref. 21 and 22 and many others]. While the tissue resulting from normal skin healing can never be considered regenerated, it is functional unless some underlying disease process or genetic predisposition leads to development of abnormalities such as hypertrophic or keloid scars[23–26], or it is impacted by environmental factors such as smoking.[27] The initial inflammatory phase is critical to the establishment of an effective healing environment with influx of inflammatory cells such as PMNs and macrophages central to the process.[18–20] Changes or alterations in the ability of such inflammatory cells to contribute to the healing environment due to age and gender[28,29], or the existence of co-morbidities such as diabetes and RA can also potentially compromise the outcome in response to injury. These gender-specific influences are particularly relevant to RA as the majority of patients are females. Such alterations in inflammatory cell function can result in chronic wounds, or wounds that do not heal properly. While this discussion

has focused on skin wound healing, it is likely that the healing of injuries or damage to other connective tissues could also be compromised in the presence of a systemic dysregulation of inflammatory processes since they require many of the same steps in the repair process as does skin wound healing. As the early inflammatory phase is critical to effective wound healing and tissue repair, and some of the same mediators are dysregulated in RA[16,30], this scenario sets the stage for altered repair. As will be discussed later, treatments for RA also target some of the same mediators and thus further compound potential impact on endogenous repair processes.

POTENTIAL IMPACT OF RHEUMATOID ARTHRITIS TREATMENTS ON TISSUE REPAIR AND HEALING OPTIONS

Drugs and combination therapies

A number of the drugs used to control the inflammation associated with a systemic autoimmune disease such as RA were designed to either selectively kill the activated cells involved, disrupt the disease process at the level of the immune cells, or to effect the activity of mediators released by the inflamed tissues which are believed to be directly responsible for some aspects of the joint inflammation. Some of these drugs can impact tissue repair processes and affect healing of surgical wounds[31–34], and in some cases use may lead to alterations in bystander tissues and disruption of connective tissue integrity.[35] As combination therapy with multiple drugs with different targets is the norm in most patients with severe arthritis,[36] the potential opportunity for tissue repair is further complicated. If some treatments can influence surgical wound healing in RA patients, it is likely that such influences may also be felt when one attempts to enhance tissue repair.

Thus, the nonsteroidal anti-inflammatory drugs (NSAIDs), drugs directed at inhibiting the ability of cyclooxygenases to generate subsets of prostaglandins, particularly those of the PG-E2 series which mediate inflammatory processes, are widely used as 'first line' drugs in many patients. While some of the newer NSAIDs are directed primarily at cyclooxygenase -2 (COX-2), others can impact both COX-1and COX-2 leading to unwanted side effects at the level of the intestines and kidney function. NSAIDs can also affect wound healing of soft tissues[37–39], fracture healing[40–42], and likely other basal tissue remodeling processes in tissues such as articular cartilage.[43] Therefore, attempting to initiate tissue repair via some of the mechanisms/approaches discussed in latter sections of this review in patients taking NSAIDS could compromise or alter the effectiveness of these approaches, albeit potentially only at specific stages of the repair. There has been considerable research and industrial effort in the area of NSAIDs development due to their widespread use, and new derivatives such as those with a nitric oxide moiety attached are being developed to eliminate or diminish the side effects. Whether such improvements will impact the ability to distinguish an abnormal pathway from a 'normal' repair pathway is still an open question but it may be unlikely due to reliance on many of the same molecules.

The corticosteroids or glucocorticoids, and their derivatives are also widely used. Some of the side effects of these drugs are believed to occur via 'transactivation' of a subset of genes via glucocorticoid response elements (GRE) in the DNA. The mechanisms that regulate these processes have been the subject of intense investigation over the past several years. Recently, new, rapid pathways for potential regulation of

cellular processes have been identified[44], but it is unclear at the present time how these pathways are involved in the effectiveness of glucocorticoids in the treatment of RA.

Glucocorticoid administration can also have a detrimental effect on wound healing and tissue repair[45,46], as well as detrimental 'bystander' effects on normal joint connective tissues via interference with basal regulatory processes in tissues such as cartilage and others. Recent studies from this laboratory[47] investigating the effect of exogenous glucocorticoids (methylprednisolone and dexamethasone) on mRNA levels for a number of relevant molecules in normal and injured joint tissues, has revealed that even a single injection of a clinically relevant dose of such drugs into an experimental animal can impact the expression of these molecules in a variety of tissues for a prolonged period of time. Interestingly, significant differences in the responsiveness of normal and injured tissue to the corticosteriods were also detected.[48] The tissues examined included synovium, ligaments, tendons, menisci and cartilage. Thus, attempting to initiate repair of tissues in RA patients even on modest maintenance doses of glucocorticoids may present a challenge for both the researcher and the outcome for the patient.

Another drug widely used in RA, is methotrexate.[49,50] In collaboration with Dr Helen Burt's laboratory at the University of British Columbia, we are currently attempting to modify delivery of drugs such as methotrexate to joints in order to lower the doses required and focus the impact of the drugs. Such systems may be required to restrict the effectiveness of the drugs to the affected joints and minimize the impact on other normal tissues or repair processes. Based on the fact that some cells (i.e. inflammatory cells or fibroblasts making the repair matrix) are activated and also potential targets for drugs such as methotrexate, attempting tissue repair in the presence of methotrexate could again compromise the effectiveness of the repair. In addition to previously cited literature in this area, there is also some evidence for such influences by methotrexate in surgical repair in a subset of RA patients.[51,52] Therefore, new approaches are likely needed to minimize such impact in the future.

One of the effects of excess levels of proinflammatory cytokines is the increased expression of matrix metalloproteinases (MMPs).[53] Increased levels of MMPs have been found joint tissues in RA patients.[54,55] Over the past several years, the pharmaceutical industry has had a concerted effort to develop drugs that are specific inhibitors of one or more of the large number of potential targets. Unfortunately, this effort has not yet led to the development of a successful reagent (discussed in Ref. 56), in part due to the fact that MMPs are also important in the normal turnover and remodelling of connective tissues, as well as in wound healing (discussed in 21 and 22). Whether this approach will lead to an effective drug in the future remains unclear, but if applied to RA patients, it would also be a potential problem for initiation and maintenance of adaquate tissue repair.

It should be pointed out again that many, if not most RA patients are on combination drug therapy regimens designed to address different aspects of the disease activity or process in order to more effectively control disease activity. Thus, patients on NSAIDs, glucocorticoids, methotrexate, and a number of other DMARDs may have their RA effectively controlled, but such a regimen of drugs that can also potentially impact various aspects of tissue repair processes poses a severe challenge to overcome.

Biologics

The past several years have seen the implementation of the first generation of RA treatments that are directly focused on regulating levels of cytokines central to

the inflammation associated with the disease. The proinflammatory target of two of these reagents is TNF-alpha, a mediator that is a pleiotrophic cytokine and inhibition of the elevated concentrations found in RA can potentially influence inflammatory processes at multiple levels. As the use of these drugs is an approach to control some aspects of disease activity, and as the treatments have to be administered continually, this could impact attempts to repair tissues since mediators such as TNF-alpha have also implicated in normal wound healing (discussed in Ref. 21, many other references). However, we have not been able to find reports in the literature indicating that patients receiving anti-TNF treatments have overtly compromised wound healing. This may be due to the fact that the goal of the treatments is not to completely abolish the presence of TNF, as complete removal of TNF may be detrimental as shown in animal models.[57] Interestingly, in animal models of RA, it has been reported that polyarthritic transgenic mice treated with anti-TNF undergo some healing of damaged cartilage, bone and synovium[58], possibly indicating that effective treatment may still allow for levels of TNF that are sufficient to participate in healing and repair processes. This is an important concept as we move forward, since in many situations the levels of anti-cytokine biologics used are designed to modify the elevated levels observed in diseases such as RA, but are not sufficiently high as to compromise host defense. Tissue repair and healing likely fall into this category, and the concept applies to anti-TNF, as well as anti-IL-1 discussed below.

Another potential biologic target that has received considerable attention is IL-1beta, another proinflammatory cytokine that is reported to be elevated in RA.[16,59,60] IL-1beta can induce a number of molecules, including MMPs and others, via interaction with specific cell surface receptors, subsequent signal transduction pathway activation, and then induction of alterations in gene expression. Antibodies to IL-1beta, or a natural antagonist called IRAP (IL-1 receptor antagonist protein), should be able to reduce the elevated IL-1beta levels in an inflammatory state by binding the IL-1 and interfering with the IL-1beta-IL-1 receptor interaction, somewhat analogous to the anti-TNF reagents discussed above. Some animal model studies have supported the use of such reagents, and they are currently in clinical trials with approval in some countries[61,62], As IL-1beta is also involved in wound healing[21,63], the same concerns regarding tissue repair apply to this potential target as apply to the TNF modulators discussed above.

It should be pointed out that in RA patients treated with anti-TNF reagents, IL-1beta is still being released and even though the inflammation associated with the disease is diminished, IL-1beta can still have effects both in the synovium and in other joint tissues such as cartilage where it can induce MMPs and contribute to cartilage degeneration.[59,60] This has lead to some support for combination therapy with both anti-TNF and anti-IL-1 reagents.[63,64] However, the potential incidence of side effects due to a compromised host defense system may be another challenge to monitor. Similarly, such combined approaches would potentially impact tissue repair and wound healing as well.

In attempts to limit the side effects of systemic biologics, a number of laboratories have investigated the use of gene therapy approaches to limit local TNF, IL-1beta, or other proinflammatory mediator concentrations in a joint using gene therapy approaches (reviewed in Ref. 65–67). While such approaches may eventually be more effective in limiting systemic side-effects, they would still potentially impact tissue repair and replacement with engineered tissues in joints treated with such reagents, particularly if there was chronic expression of the constructs.

Emerging therapies

There are obviously a number of other biologic therapies that are under development, and anti-TNF and anti-IL-1 likely represent the first generation of such therapies. These include other targets associated with cells of the immune system, or molecules associated with inflammatory pathways. Based on the above discussion, it is likely that many of these will also impact tissue repair and healing processes for some of the same reasons but to varying degrees.

A somewhat different approach to RA is based on the success of cell transplantation in cancer and its extension to autoimmune diseases. Current studies regarding the use of bone marrow transplantation (BMT) to potentially reestablish immunoregulatory circuitry also offers the opportunity to induce remissions of RA.[68–70] Of course, such treatments could impact the availability of mesenchymal stem cells (MSC) for some of the subsequent tissue repair or replacement options that are discussed below, as well as well-controlled inflammatory cell networks that are required to effect healing and repair.

One interesting phenomenon associated with RA that is relevant to the above discussion is the observation that approximately 70% of females with RA experience a remission of disease activity when they are pregnant.[71–73] Studies from a number of laboratories, including our own (Ref. 74, and related publications), have indicated that some aspects of wound healing can also be influenced by pregnancy, so even this approach may have implications for tissue repair and replacement.

APPROACHES TO REPAIR OR REPLACE CONNECTIVE TISSUES DAMAGED BY RA

There are a variety of approaches one could take with regard to repairing tissues damaged by the inflammatory joint disease of RA. Three approaches to enhancing tissue repair have received some recent investigative attention and these include injection of exogenous growth factors, gene therapy, and tissue engineering. The first of these may have limited application, but the latter two of these possibilities to enhance endogenous repair processes and/or autologous replacement of damaged tissues offer some promise. The latter approach will still likely require 'augmentation' using either protein or gene therapy to foster a more complete integration of the tissue, particularly if it requires revascularization and reinnervation from the replacement environment for optimal functioning.

Growth factors

Growth factors are peptide/protein mediators that can influence cells in an autocrine, paracine or endocrine manner. They usually have a short half-life as an active protein, but they can exist as inactive precursors or in inactive complexes bound to other ligands. Furthermore, many of these molecules have redundant activities and are interactive and inter-dependent, features that complicate their use as 'general' mediators of repair. However, as some of these are anabolic in nature, they may offer the opportunity augment compromised tissue repair in specific situations by exposing the damaged tissues to active molecules. In some studies, exogenously supplied growth factors were able to enhance healing/repair of injured ligaments[75], but other growth

factors had no detectable influence on healing.[76] However, in cartilage there is evidence that some of the same growth factors may be beneficial and enhance repair.[77,78] In this latter situation, TGF-beta may not only be acting as an anabolic factor, but also an IL-1 antagonist. Interestingly, NGF has been reported to have efficacy in the treatment of chronic vasculitic ulcers in RA.[79] However, the wide-spread use of exogenous growth factors to enhance repair of damaged tissues compromised by RA may not become an effective modality due to limitations in the half-life of the molecules, the issue of their potential side-effects, and issues related to the temporal and concentration-dependent impact of such molecules on repair.

Gene therapy to enhance the endogenous repair potential of damaged tissues

Many connective tissues have the ability to repair themselves either by endogenous mechanisms that are required to maintain homeostasis in the skeletally mature state, or through initiation of a repair process that requires exogenous cells and influences and usually leads to the development of 'scar-like' material which is not normal but may have sufficient properties to allow the tissue to function in a normal 'physiologic window' (discussed in Ref. 80). However, cartilage, a tissue with no innervation and vascularization, is somewhat different from others regarding the injury response, but it still has some endogenous repair potential. In cartilage, an example of this principle can be derived from results from van den Berg's laboratory, where they showed that injecting a single dose of IL-1beta into a mouse knee leads to changes in cartilage that are largely transitory in nature and somewhat reversible. Repeated injections leads to degeneration of the tissue without obvious repair and an apparent inability to 'marshall' an exogenously derived repair response (discussed in Ref. 78). From such studies, it would appear that repeated insults of cartilage by mediators such as IL-1, can either exhaust the repair potential or alter the ability of the tissue to mount an effective response. This may be analogous to the situation in an RA-joint where there is continual exposure to IL-1. Thus, enhancement of an environment where there is either little endogenous repair activity available, or ineffective repair activity, using gene therapy approaches to 'rescue' the tissue would be the goal of such interventions. In contrast, bone is a tissue with normally a very active turnover and remodeling, and it has been reported that repair of bone erosions can occur in RA patients in sustained remission.[81] Thus, enhancing repair of bone damage with gene therapy in joints impacted by RA may allow for the more rapid and functional repair even in some patients with incomplete remissions.

While gene therapy has received considerable 'hype' over the past decade, the potential of this approach has not yet lived up to expectations for a number of reasons. Some of these include choosing the correct genes to enhance repair, effective delivery of the appropriate gene constructs to very hypocellular connective tissues such as cartilage, menisci, ligaments or tendons; choosing the right vector to avoid unwanted side-effects, and maintaining expression of the construct in the cells at the right level and timing to facilitate repair. Details of some aspects of these issues are beyond the scope of this review, and the reader is referred to recent articles on the subject (Ref. 66,67,82 and many others). However, some aspects of gene therapy will be addressed, as they are relevant to the unique situation of tissue repair in RA.

Probably the most limiting aspect of gene therapy approaches is not knowing which genes would be best to transfer to a damaged tissue to enhance repair. The emphasis thus far has been focused on using gene therapy to control disease activity, and it has

only been recently that the concept of adding an appropriate growth factor or cassette of growth factors that could be a repair stimulus, as well as anti-inflammatory, has started to emerge. This repair versus disease control dichotomy is compounded by the likelihood that the genes that are critical for repair of cartilage may be different than those important for functional meniscus, ligament or tendon repair. Thus, incomplete knowledge of the regulation of normal repair processes, particularly in humans that are genetically diverse, severely limits our abilities in this area. However, some progress has been made in enhancing the repair process in a variety of tissues using gene therapy based on 'intelligent fishing'.

An example of this was a recent study from this laboratory which demonstrated that transfection of cells in vivo in ligament scars with an antisense decorin oligonucleotide lead to a modest improvement in the both the organization of the collagen of the scar and the mechanical function of the scar tissue several weeks post-exposure to the antisense molecules.[83] The selection of decorin as the target was based on literature which suggested this small proteoglycan was important in collagen fibril assembly and it also had biological effects such as binding to the growth factor TGF-beta, as well as binding to the EGF receptor on the surface of cells. However, further analysis of the resultant tissue at the molecular level indicated that simple cause and effect relationships between decorin and the outcome were likely more complex than originally thought.[84]

As the impact of the gene therapy in this example of ligament healing led to significant, but modest improvement in functional outcome, the approach is encouraging but it is still along way from correcting the deficiencies associated with ligament scar tissue. This point raises a number of issues including how to pick the best target for the gene therapy, whether combination therapy with both positive (transgenes) and negative (antisense, siRNA) approaches would be better, and whether there would have to be multiple exposures to different targets at different times in the repair process due to temporal considerations based on the complex interrelationship between the different stages of the repair.

Another interesting advance in tissue repair that could potentially impact the success of the gene therapy approach has been the characterization of mesenchymal stem cells (MSC). MSCs are cells that have pluripotent capabilities to repopulate and differentiate into appropriate cells that can function in a number of connective tissues (reviewed in Ref. 85,86,87). These are bone marrow-derived cells that can circulate in the blood and home to areas of injury. The cells can be purified from blood or bone marrow and transferred to injury sites to enhance the repair process. The circulating levels of MSCs decline with age and this decline may be related to the well-characterized age-dependent alterations in wound healing. However, new approaches for the storage and expansion of such cells may circumvent this problem in the future. Also, it is quite possible to imagine the combination of MSC with gene therapy to enhance repair once such cells are re-implanted into a suitable location.

There are two additional issues that need to be addressed if the gene therapy approach is to be judged 'successful'. The first relates to the question of whether the tissue has to be regenerated for the intervention to be considered successful, or whether success should be based on a return of the tissue to some functional level with the capability to maintain that level. The second issue is related to the first, but addresses the issue of whether initiating repair of one tissue in a damaged joint will lead to a return to function of the whole joint, or whether the tissues in a damaged joint have now reached a new and interdependent 'set point' for function (i.e. the joint as an organ system) (discussed in 88). The goal of the gene therapy should be to impact

the whole joint to make it functional, but not necessarily return it to its 'normal' state prior to the development of RA. These are complex questions that can likely only be answered once we learn more about how the components of a normal joint interact at different stages of life (i.e. sexually mature vs post-menopausal) and in response to different insults.

Finally, joints damaged by RA, particularly those with some residual abnormalities such as pannus or fixed joint deformity, may still require some surgical intervention to eliminate any potentially adverse factors or components that could negatively impact the gene therapy approach to correct damage to salvageable tissues. Thus, for effective gene therapy treatment to be optimized in the RA patient, it will likely require a transdisciplinary team of rheumatologists, orthopaedic surgeons and researchers to implement the strategy and obtain functional outcomes.

Tissue engineering approaches to replacement of tissues damaged by RA

In some cases of RA-associated tissue damage, the damage may be sufficiently severe to warrant tissue replacement rather than enhancement of repair of the residual tissue. Total joint arthroplasty has improved the quality of life for many patients over the past several decades, but this approach has limited longevity due to aseptic or septic loosening of the replacement devices. Optimally, the replacement tissue should be biologic, autologous, and functional. Allografts or xenografts of suitable tissues are in limited supply and pose some infectious disease risk. Autografts derived from other tissues obviously pose wound-healing problems themselves and can also lead to compromising the donor site function. Therefore, autologous cells and scaffolds are likely more optimal than other sources. Advancements in tissue engineering over the past decade, and integration of new findings from molecular and cell biology, plus biomedical engineering into the development of functional tissues, are making this approach more feasible. There are many recent reviews on this subject.[89–91]

Advances in the generation of in vitro tissue engineered constructs have led to their use in replacing damaged cartilage and ligaments either in humans or experimental systems. One form of engineered cartilage that contains autologous cells is commercially available, while engineered ligaments and tendons are still mostly in the experimental stages of development. One advance that has moved the field forward has been the finding that the in vitro engineered tissues are stronger and more functional if they are exposed to mechanical loads in vitro during their development. Thus, mechanobiology is an important aspect of the functioning of connective tissues in vivo, as well as in vitro, and the resulting materials better reflect the in vivo situation (reviewed recently).[92,93] The system developed by Goulet et al for ligaments has led to tissues that have been put into experimental models and these have survived and improved in properties for over a year (F. Goulet, Laval University; personal communication). Recently, a system that has the potential for development of functional bioartificial tendons and ligaments has been described by Garvin et al[94], but the products have not been implanted into experimental models as yet (A. J. Banes, personal communication). Bioartifical tendons are more complicated than cartilage due to the need for an essentially three-part tissue with a myotendinous junction/interface, a tendon proper, and a tendon-bone interface/junction. Bioartificial ligaments require a bone-ligament interface on each end of a ligament proper, a construct somewhat less complex than a tendon. Furthermore, as some tendons exist inside a sheath designed to enhance the gliding of the tendon, there is a need to either reconstruct the sheath as

well as the tendon, or at least develop a method to prevent the bioartificial tendon from developing adhesions with the existing sheath at the implantation site.

In preparing an engineered tissue such as a tendon or ligament, one has to either obtain the appropriate cells from the endogenous tissue, as there is some variability between tendons and ligaments from different locations, or obtain MSCs and differentiate them in an appropriate environment. Furthermore, some tendons glide around a bony prominence and are subjected to compressive loading as well as tensile loading. Such circumstances may require unique propagation strategies for development of the tissue, as well as getting the cells to differentiate not only in response to biological cues, but appropriate mechanical stimuli as well. The use of MSC in such tissue engineering likely will offer the best opportunity to generate cells functioning in diverse mechanical environments since they are pluripotent and would not require access to the tissue to be replaced prior to implantation. If indeed it is found that MSC are the best source of cells to generate replacement tissues in RA, then strategies to obtain a supply of such cells prior to or early after disease onset which can then be frozen until needed will have to be developed. As genetic testing for risk of developing RA later in life becomes more stringent, perhaps the banking of MSC from blood or marrow early in life will be the answer to this possibility.

While autologous in vitro engineered cartilage has been used to repair cartilage defects arising from traumatic events, sport related events, or osteoarthritis, the best results have been with younger people who have defined defects (discussed in[95] and other papers in the cited Novartis Foundation Symposium). The material is functional and maintains its cellularity, but in many instances the replacement tissue never becomes integrated with the remaining endogenous tissue, and it remains to be determined how long such replacements will last in the environment of the knee, for instance. In contrast, studies with tissues that require revascularization and potentially reinnervation once implanted, such as ligaments and tendons, have indicated that implantation of a bioengineered replacement tissue may be more complex than the situation with cartilage. For instance, studies with autografts of rabbit MCLs, where the normal ligament-bone complex is surgically removed and immediately reimplanted, have shown the MCLs become acellular and are then apparently repopulated by exogenous cells. With time, they become essentially 'scar-like'. Similarly, following replacement of a torn ACL in the knee with a biological graft (i.e. autologous patellar tendon), which is initially stronger than the ACL, the graft develops scar-like properties via what is believed to be a similar process.[96] Thus, whatever the mechanical properties of an artificial tendon or ligament that is generated in vitro, and whatever the cell makeup is at the time of implantation, unless it can under go a normal revascularization without loss of the properties that contribute to its success in vivo, it appears that it is destined to become merely a scaffold for an endogenous repair/injury response and effectively become a scar that is in need of enhancement if it is to function properly over the long term. Further understanding is obviously required before it will be possible to modify the endogenous repair/injury response to either prevent the deterioration of the graft and/or to enhance its properties.

It is at this juncture that in vivo gene therapy and tissue engineering may meet. Thus, to prevent reversion of an engineered tissue to become a scar, and to enhance the regulated revascularization without loss of the mechanical properties, the implanted tissue may have to be subjected to gene therapy prior to or after implantation to modify the construct so the effort that went into making the artificial tissue is not compromised. As it is doubtful that one would be able to revascularize an artificial tendon or ligament to circumvent the host response in this regard, at least at

the present state of knowledge, it is likely that regulating the response to the tissue once it is implanted would yield the best short-term results.

Influence of environmental factors

Thus, while tissue engineering and gene therapy offer future promise for the repair and/or replacement of tissues damaged by the disease process in RA, an additional challenge in the RA patient will be the number of joints that will require intervention. In patients with multiple affected joints, one could anticipate that not all joints would be so severely damaged as to require repair or replacement. Among those remaining that do, one would likely have to initially prioritize the joints for intervention based on functional need or some other set of criteria. It is also likely that other variables may also come into play at this juncture. Such variables include age, hormonal status in the females (and RA is more frequent in females than males), and the presence of co-morbidities that could affect the outcome. Endogenous tissue repair is well known to decline with age, and hormones such as estrogen are reported to affect the healing/repair processes[97] The incidence of co-morbidities such as diabetes, which can affect wound healing and repair, increases with age and is not uncommon in RA patients. All of these and other variables may be factors in deciding on an acceptable risk-benefit determination to repair or replace tissues damaged during the RA disease process.

Animal models

One of the key components to moving research from in vitro to patients is the availability and use of appropriate animal models. This is also the case as attempts to initiate repair are undertaken. Current experimental models of RA are somewhat restricted, with the collagen-induced mouse models, rat models, and antigen-induced rabbit models likely the most prevalent or utilized. The mouse models do offer many advantages in developing approaches to control RA-like diseases, and a number of citations derived from the use of such models have been made in this review earlier. Unfortunately, the power of mouse models (genetic, transgenics, etc.) to understand disease processes is undermined by their small size when it comes to repairing the tissues through endogenous or exogenous means or assessing functional outcomes (i.e. motion analysis). The rat models offer a somewhat larger joint size, but they are still limited even though they do exhibit some characteristics of changes occurring in connective tissues such as ligaments[98] that are observed in subsets of RA patients. The rabbit models offer larger sized joints, but many of the reagents likely needed for repair and molecular manipulations are not readily available in this species. Furthermore, some aspects of rabbit joints may not be good approximations for the human case.

Therefore, as we move forward towards the goal of repair of tissues damaged by the disease processes associated with inflammatory arthritis, there is a real need to develop and validate new experimental models that will lead to findings appropriate for future adaptation to patient populations, and are sufficiently large to allow for effective imaging (i.e. MRI) of joint function, and analysis of motion to address functional considerations.

SUMMARY

As RA becomes a more controllable disease in many patients, one can now start to look beyond the damaged joints with a view towards their repair with subsequent increased function and improved quality of life for the patient. Gene therapy for enhancing repair, tissue engineering for replacement of severely damaged tissues, and the combination of gene therapy + bioengineered tissue, offer opportunities to impact the joints of many of the severely affected patients. While there is opportunity in these areas that outweighs many of the alternatives (allografts, xenografts) that have their own sets of problems, there is the need for development of unique strategies to overcome the challenges of dealing with a systemic autoimmune inflammatory disease. Obviously the patient in complete drug-free remission of the disease offers fewer challenges in this regard than the patient whose disease is controlled by a complex drug regimen, and a patient with fewer involved joints offers fewer challenges than one with a large number of joints affected, just as it does presently for the surgeon who is faced with multiple joint replacements. However, as our knowledge and understanding of how normal joints function in a variety of situations increases, and the mechanisms by which the body elaborates and implements repair processes, optimism for these approaches yielding successful interventions increases.

The tone of this review is not meant to convey the impression that the number of obstacles to the repair of joint tissues in RA patients is overwhelming. In fact, a number of international groups are currently assessing evidence that endogenous repair of tissues such as bone can occur in RA patients with controlled disease.[99,100] In addition, there is evidence from some patients, as well as animal models, that some repair can occur in tissues damaged by RA (discussed earlier). However, we have attempted to illustrate and identify the myriad of issues that are potentially to be faced in order to anticipate as many alternatives as possible so as to minimize the 'surprise' factor as we proceed towards this goal. Cautious optimism is the key as we develop new technologies that have up to now been more promise than delivery.

ACKNOWLEDGEMENTS

The authors thank The Arthritis Society (Canada), the Canadian Institutes for Health Research (particularly the Institute for Musculoskeletal Health and Arthritis and the Institute for Gender and Health), and the Canadian Arthritis Network for their support of many of the studies emanating from the authors laboratories. DAH is the Calgary Foundation-Grace Glaum Professor in Arthritis Research, ASK is supported by a CIHR MD/PhD Studentship, CBF is the McCaig Professor in Joint Injury and Arthritis Research, and KH is a Health Research Foundation/CIHR Scholar. The authors apologize to those investigators whose work was not cited due to space limitations and restriction on the number of citations allowed. This is an emerging field that is based on the work of many individuals and groups who have made significant contributions at all levels.

REFERENCES

1. Brown DG, Edwards NL, Geer JM, Longley S, Gillespy T & Panush RS. Magnetic resonance imaging in patients with inflammatory arthritis of the knee. *Clinical Rheumatology* 1990; **9:** 73–83.

2. Gravellese EM. Bone destruction in arthritis. *Annals Rheumatic Diseases* 2002; **2:** 84–84.
3. Salisbury RB & Nottage WM. A new evaluation of gross pathologic changes and concepts of rheumatoid articular cartilage degeneration. *Clinical Orthopaedics* 1985; **199:** 242–247.
4. Gardner DL. Problems and paradigms in joint pathology. *Journal of Anatomy* 1994; **184:** 465–476.
5. Kimura C & Vainio K. The pattern of meniscal damage in the rheumatoid arthritis. *Archives of Orthopaedics Unfallchir* 1975; **83:** 145–151.
6. Jari S & Noble J. Meniscal tearing and rheumatoid arthritis. *Knee* 2001; **8:** 157–158.
7. Fujii M, Tomita T, Nakanishi K, Kaneko M, Hayashida K, Sugamoto K, Ochi T & Yoshikawa H. The value and limitation of gadopentetate-enhanced magnetic resonance imaging in detecting the condition of anterior cruciate ligament in rheumatoid knee: comparative study with histology. *European Radiology* 2003; **13:** 1728–1734.
8. Bogoch ER & Moran EL. Bone abnormalities in the surgical treatment of patients with rheumatoid arthritis. *Clinical Orthopaedics* 1999; **366:** 8–21.
9. Peterfy CG. Magnetic resonance imaging in rheumatoid arthritis: current status and future directions. *Journal of Rheumatology* 2001; **28:** 1134–1142.
10. Simmen BR & Gschwend N. Tendon diseases in chronic rheumatoid arthritis [German]. *Orthopade* 1995; **24:** 224–236.
11. Wilson RL & DeVito MC. Extensor tendon problems in rheumatoid arthritis. *Hand Clinics* 1996; **12:** 551–559.
12. Stromqvist B. Hip fractures in rheumatoid arthritis. *Acta Orthopaedica Scandanavia* 1984; **55:** 624–628.
13. Valeri G, Ferrara C, Ercolani P, De Nigris E & Giovagnoni A. Tendon involvement in rheumatoid arthritis of the wrist: MRI findings. *Skeletal Radiology* 2001; **30:** 138–143.
14. Williamson L, Mowat A & Burge P. Screening for extensor tendon rupture in rheumatoid arthritis. *Rheumaology (Oxford)* 2001; **40:** 420–423.
15. Dayer JM. Interleukin 1 or tumor necrosis factor-alpha: which is the real target in rheumatoid arthritis. *Journal of Rheumatology* 2002; **15**(suppl 1)**:** 10–15.
16. Dayer JM. The pivotal role of interleukin-1 in the clinical manifestations of rheumatoid arthritis. *Rheumatology (Oxford)* 2003; **42**(suppl 2)**:** 3–10.
17. Arend WP & Dayer JM. Cytokines and cytokine inhibitors or antagonists in rheumatoid arthritis. *Arthritis and Rheumatism* 1990; **33:** 305–315.
18. Hart J. Inflammation. 1. Its role in the healing of acute wounds. *Journal of Wound Care* 2002; **11:** 205–209.
19. Steed DL. Wound-healing trajectories. *Surgical Clinics of North America* 2003; **83:** 547–555.
20. Henry G & Garner WL. Inflammatory mediators in wound healing. *Surgical Clinics of North America* 2003; **83:** 483–507.
21. Wang JF, Olson ME, Reno CR, Kulyk W, Wright JB & Hart DA. Molecular and cell biology of skin wound healing in a pig model. *Connective Tissue Research* 2000; **41:** 195–211.
22. Wang JF, Olson ME, Reno CR, Wright JB & Hart DA. The pig as a model for excisional skin wound healing: characterization of the molecular and cell biology, and bacteriology of the healing process. *Comparative Medicine* 2001; **51:** 341–348.
23. Marneros AG, Norris JE, Olson BR & Reichenberger E. Clinical genetics of familial keloids. *Archives of Dermatology* 2001; **137:** 1429–1434.
24. Tredgett EE. Pathophysiology and treatment of fibroproliferative disorders following thermal injury. *Annals of the New York Academy of Sciences* 1999; **888:** 165–182.
25. Scott PG, Ghahary A & Tredgett EE. Molecular and cellular aspects of fibrosis following thermal injury. *Hand Clinics* 2000; **16:** 271–287.
26. Tsou R, Cole JK, Nathens AB, Isik FF, Heimbach DM, Engrav LH & Gibran NS. Analysis of hypertrophic and normal scar gene expression with cDNA microarrays. *Journal of Burn Care and Rehabilitation* 2000; **21:** 541–550.
27. Towler J. Cigarette smoking and wound healing. *Journal of Wound Care* 2000; **9:** 100–104.
28. Ashcroft GS & Mills SJ. Androgen receptor-mediated inhibition of cutaneous wound healing. *Journal of Clinical Investigation* 2002; **110:** 615–624.
29. Ashcroft GS, Mills SJ, Lei K, Gibbons L, jeong MJ, Taniguchi M, Burow M, Horan MA, Wahl SM & Nakayama T. Estrogen modulates cutaneous wound healing by downregulating macrophage inhibitory factor. *Journal of Clinical Investigation* 2003; **111:** 1309–1318.
30. Russell RG, McGuire MK, Meas JE, Ebsworth NM & Beresford J. Intracellular messangers in joint tissues in rheumatoid arthritis. How disturbed control mechanisms may contribute to tissue destruction and repair. *Scandinavian Journal of Rheumatology* 1981; (suppl): 75–87.
31. Peacock Jr EE. Control of wound healing and scar formation in surgical patients. *Archives of Surgery* 1981; **116:** 1325–1329.

32. Perhala RS, Wilke WS, Clough JD & Segal AM. Local infectious complications following large joint replacement in rheumatoid arthritis patients treated with methotrexate versus those not treated with methotrexate. *Arthritis and Rheumatism* 1991; **34:** 146–152.
33. Lyssy KJ & Escalante A. Perioperative management of rheumatoid arthritis. Areas of concern for primary care physicians. *Postgraduate Medicine* 1996; **99:** 191–194.
34. Alster Y, Varssano D, Lowenstein A & Lazar M. Delay in corneal wound healing in patients treated with colchicines. *Ophthalmology* 1997; **104:** 118–119.
35. Madinier I, Berry N & Chichmanian RM. Drug-induced oral ulceration [French]. *Annals of Medicine Interne (Paris)* 2000; **151:** 248–254.
36. Munster T & Furst DE. Pharmacotherapeutic strategies for disease-modifying antirheumatic drug (DMARD) combinations to treat rheumatoid arthritis (RA). *Clinical and Experimental Rheumatology* 1999; **17**(suppl 18)**:** S29–S36.
37. Elder CL, Dahners LE & Weinhold PS. A cyclooxygenase-2 inhibitor impairs ligament healing in the rat. *American Journal of Sports Medicine* 2001; **29:** 801–805.
38. Marsolais D, Cote CH & Frenette J. Nonsteroidal anti-inflammatory drug reduces neutrophil and macrophage accumulation but does not improve tendon regeneration. *Laboratory Investigation* 2003; **83:** 991–999.
39. Wilgus TA, Vodovotz Y, Vittadini E, Clubbs EA & Oberyszyn TM. Reduction of scar formation in full thickness wounds with topical celecoxib treatment. *Wound Repair and Regeneration* 2003; **11:** 25–34.
40. Goodman SB, Ma T, Genovese N & Smith LR. COX-2 selective inhibitors and bone. *International Journal of Immunopathology and Pharmacology* 2003; **16:** 201–205.
41. Gerstenfeld LC, Thiede M, Seibert K, Mielke C, Phippard D, Svagr B, Cullinane D & Einhorn TA. Differential inhibition of fracture healing by non-selective and cyclooxygenase-2 selective non-steriodal anti-inflammatory drugs. *Journal of Orthopedic Research* 2003; **21:** 670–675.
42. Harder AT & An YH. The mechanism of the inhibitory effects of nonsteriodal anti-inflammatory drugs on bone healing: a concise review. *Journal of Clinical Pharmacology* 2003; **43:** 807–815.
43. Dingle JT. The effects of NSAID on the matrix of human articular cartilages. *Zeitschrift Rheumatologie* 1999; **58:** 125–129.
44. Buttgereit F & Scheffold A. Rapid glucocorticoid effects on immune cells. *Steroids* 2002; **67:** 529–534.
45. Beer HD, Fassler R & Werner S. Glucocorticoid-regulated gene expression during cutaneous wound repair. *Vitamins and Hormones* 2000; **59:** 217–239.
46. Schacke H, Docke WD & Asadullah K. Mechanisms involved in the side effects of glucocorticoids. *Pharmacology and Therapeutics* 2002; **96:** 23–43.
47. Kydd, Reno, Sorbetti and Hart, submitted.
48. Kydd and Hart, in preparation.
49. Kremer JM. Methotrexate and lefunomide: biochemical basis for combination therapy in the treatment of rheumatoid arthritis. *Seminars in Arthritis and Rheumatism* 1999; **29:** 14–26.
50. Seitz M. Molecular and cellular effects of methotrexate. *Current Opinion in Rheumatology* 1999; **11:** 226–232.
51. Kasdan ML & June L. Postoperative results of rheumatoid arthritis patients on methotrexate at the time of reconstructive surgery of the hand. *Orthopedics* 1993; **16:** 1233–1235.
52. Bridges Jr SL & Moreland LW. Periopertaive use of methotrexate in patients with rheumatoid arthritis undergoing orthopedic surgery. *Rheumatic Disease Clinics of North America* 1997; **23:** 981–993.
53. Abramson SB & Amin A. Blocking the effects of IL-1 in rheumatoid arthritis protects bone and cartilage. *Rheumatology (Oxford)* 2002; **41:** 972–980.
54. Murphy G, Knauper V, Atkinson S, Butler G, English W, Hutton M, Stracke J & Clark I. Matrix metalloproteinases in arthritic disease. *Arthritis Research* 2002; **4**(suppl 3)**:** S39–S49.
55. Marder G & Greenwald RA. Potential applications of matrix metalloproteinase inhibitors in geriatric medicine. *Israel Medical Association Journal* 2003; **5:** 361–364.
56. Martel-Pelletier J, Welsch DJ & Pelletier JP. Metalloproteinases and inhibitors in arthritis diseases. *Best Practice Research Clinics in Rheumatology* 2001; **15:** 805–829.
57. Douni E, Akassaglou K, Alexopoulou L, Georgopoulos S, Haralambous S, Hill S, Kassiotis G, Kontoyiannis D, Pasparakis M, Plows D, Probert L & Kollias G. Transgenic and knockout analysis of the role of TNF in immune regulation and disease pathogenesis. *Journal of Inflammation* 1995; **47:** 27–38.
58. Shealy DJ, Wooley PH, Emmell E, Volk A, Rosenberg A, Treacy G, Wagneer CL, Mayton L, Griswold DE & Song XY. Anti-TNF-alpha antibody allows for healing of joint damage in polyarthritic transgenic mice. *Arthritis Research* 2002; **2**(R7). [Epub].
59. van den Berg WB. Joint inflammation and cartilage destruction may occur uncoupled. *Springer Seminars in Immunopathology* 1998; **20:** 49–64.
60. van den Berg WB, Joosten LA & van de Loo FA. TNF-alpha and IL-1beta are separate targets in chronic arthritis. *Clinical and Experimental Rheumatology* 1999; **17**(suppl 18)**:** S105–S114.

61. Bresnihan B & Cobby M. Clinical and radiological effects of anakinra in patients with rheumatoid arthritis. *Rheumatology (Oxford)* 2003; **42**(suppl 2)**:** 22–28.
62. Cohen SB & Rubbert A. Bringing the clinical experience with anakinra to the patient. *Rheumatology (Oxford)* 2003; **42**(suppl 2)**:** 36–40.
63. Reno C, Boykiw R, Martinez ML & Hart DA. Temporal alterations in mRNA levels for proteinases and inhibitors and their potential regulators in the healing medial collateral ligament. *Biochemical and Biophysical Research Communications* 1998; **252:** 757–763.
64. Louie SG, Park B & Yoon H. Biological response modifiers in the management of rheumatoid arthritis. *American Journal of Health Systems and Pharmacology* 2003; **60:** 346–355.
65. Ghivizzani SC, Muzzonigro TS, Kang R, Evans CH & Robbins PD. Clinical gene therapy for arthritis. *Drugs Today (Barcelona)* 1999; **35:** 389–396.
66. Robbins PD & Evans CH. Arthritis gene therapy. *Drugs Today (Barcelona)* 2003; **35:** 353–360.
67. Tomita T, Hashimoto H & Yoshikawa H. Gene therapy for arthritis. *Current Drug Targets* 2003; **4:** 609–612.
68. Sullivan KE. The role of bone marrow transplantation in pediatric rheumatic diseases. *Journal of Rheumatology* 2000; **27:** 49–52.
69. Ikehara S. Bone marrow transplantation: a new strategy for intractable diseases. *Drugs Today (Barcelona)* 2002; **38:** 103–111.
70. Van Laar JM & Tyndall A. Intense immunosuppression and stem-cell transplantation for patients with severe rheumatic autoimmune disease: a review. *Cancer Control* 2003; **10:** 57–65.
71. Draca S. Is pregnancy a model how we should control some autoimmune diseases? *Autoimmunity* 2002; **35:** 307–312.
72. Olson NJ & Kovacs WJ. Hormones, pregnancy, and rheumatoid arthritis. *Journal of Gender Specific Medicine* 2002; **5:** 28–37.
73. Ostensen M & Villinger PM. Immunology of pregnancy-pregnancy as a remission inducing agent in rheumatoid arthritis. *Transplantation Immunology* 2002; **9:** 155–160.
74. Hart DA, Reno C, Frank CB & Shrive NG. Pregnancy affects cellular activity, but not tissue mechanical properties, in the healing rabbit medial collateral ligament. *Journal of Orthopedic Research* 2000; **18:** 462–471.
75. Hildebrand KA, Woo SL, Smith DW, Allen CR, Taylor BJ & Schmidt CC. The effects of platelet-derived growth factor-BB on healing of the rabbit medial collateral ligament. An in vivo study. *American Journal of Sports Medicine* 1998; **26:** 549–554.
76. Hildebrand KA, Hiroaka H, Hart DA, Shrive NG & Frank CB. Exogenous transforming growth factor beta1 alone does not improve early healing of the medial collateral ligament in rabbits. *Canadian Journal of Surgery* 2002; **45:** 330–336.
77. Glansbeek HL, van Beuningen HM, Vitters EL, van der Kraan PM & van den Berg WB. Stimulation of articular cartilage repair in established arthritis by local administration of transforming growth factor-beta into murine knee joints. *Laboratory Investigation* 1998; **78:** 133–142.
78. van den Berg WB & van der Kraan PM. Scharstuhl A and van Beuningen HM. Growth factors and cartilage repair. *Clinical Orthopedics* 2001; **391:** S244–S250.
79. Tuveri M, Generini S, Matucci-Cerinic M & Aloe L. NGF, a useful tool in the treatment of chronic vasculitic ulcers in rheumatoid arthritis. *Lancet* 2000; **356:** 1739–1740.
80. Hart DA, Frank CB & Bray R. In Gordon SL, Blair SJ & Fine LJ (eds) *Inflammatory processes in repetitive motion and over-use syndromes: Potential role of neurogenic mechanisms in tendons and ligaments*. Repetitive Motion Disorders of the Upper Extremity, Park Ridge, IL: AAOS Press, 1995, pp 247–262.
81. Sokka T & Hannonen P. Healing of erosions in rheumatoid arthritis. *Annals of the Rheumatic Diseases* 2000; **59:** 647–649.
82. Hildebrand KA, Frank CB & Hart DA. Gene intervention in ligament: current status, challenges, future directions. *Gene Therapy* 2004; . in press.
83. Nakamura N, Hart DA, Boorman RS, Kaneda Y, Shrive NG, Marchuk LL, Shino K, Ochi T & Frank CB. Decorin antisense therapy improves functional healing of early rabbit ligament scar with enhanced collagen fibrillogenesis in vivo. *Journal of Orthopedic Research* 2000; **18:** 517–523.
84. Hart DA, Nakamura N, Marchuk LL, Hiroaka H, Boorman RS, Kaneda Y, Shrive NG & Frank CB. Complexity of determining cause and effect in vivo after antisense gene therapy. *Clinical Orthopedics* 2000; **379:** S242–S251.
85. Caplan AI & Bruder SP. Mesenchymal stem cells: building blocks for molecular medicine in the 21st century. *Trends in Molecular Medicine* 2001; **7:** 259–264.
86. Noel D, Djouad F & Jorgense C. Regenerative medicine through mesenchymal stem cells for bone and cartilage repair. *Current Opinion in Investigative Drugs* 2002; **3:** 1000–1004.

87. Prockop DJ, Gregory CA & Spees JL. One strategy for cell and gene therapy: harnessing the power of adult stem cells to repair tissues. *Proceedings of the National Academy of Sciences USA* 2003; **100**(suppl 1)**:** 11917–11923.
88. Frank CB, Shrive NG, Boorman RS & Hart DA. New perspectives on bioengineering of joint tissues. *Annals of Biomedical Engineering* 2004; . in press.
89. Darling EM & Athannasiou KA. Articular cartilage bioreactors and bioprocessors. *Tissue Engineering* 2003; **9:** 9–26.
90. Brittberg M, Peterson L, Sjogren-Jansson E, Tallheden T & Lindahl A. Articular cartilage engineering with autologous chondrocyte transplantation. A review of recent developments. *Journal of Bone and Joint Surgery (American)* 2003; **85A**(suppl 3)**:** 109–115.
91. Frank CB & Hart DA. *Clinical application of tissue engineered tendon and ligament*. Tissue Engineering, Rosemont, IL: AAOS, 2004. in press.
92. Banes AJ, Tsuzaki M, Yamamoto J, Fischer T, Briman B, Brown T & Miller L. Mechanoreception at the cellular level: the detection, interpretation, and diversity of responses to mechanical signals. *Biochemistry Cell Biology* 1995; **73:** 349–365.
93. Hart DA, Natsu-ume T, Sciore P, Tasveski V, Frank CB & Shrive NG. Mechanobiology: similarities and differences between in vivo and in vitro analysis at the functional and molecular levels. *Recent Research Developments in Biophysics and Biochemistry* 2002; **2:** 153–177.
94. Garvin J, Qi J, Maloney M & Banes AJ. A novel system for engineering bioartificial tendons and application of mechanical load. *Tissue Engineering* 2003; **9:** 967–979.
95. Lohmander S. Tissue engineering of cartilage: do we need it, can we do it, is it good and can we prove it? *Novartis Foundation Symposium* 2003; **249:** 2–10.
96. Frank CB & Jackson DW. The science of reconstruction of the anterior cruciate ligament. *Journal of Bone and Joint Surgery (American)* 1997; **70:** 1556–1576.
97. Ashcroft G & Ashworth J. Potential role of estrogens in wound healing. *American Journal of Clinical Dermatology* 2003; **4:** 737–743.
98. Nawata K, Enokida M, Yamasaki D, Minamizaki T, Hagino H, Morio Y & Teshima R. Tensile properties of rat anterior cruciate ligament in collagen induced arthritis. *Annals of the Rheumatic Diseases* 2001; **60:** 395–398.
99. Sharp JT. and the Subcommittee on Healing of Erosions of the OMERACT Imaging Committee. Repair of erosions in rheumatoid arthritis does occur. Results from 2 studies by the OMERACT subcommittee on healing of erosions. *Journal of Rheumatology* 2003; **30:** 1102–1107.
100. van der Heide DM. and the Subcommittee on Healing of Erosions of the OMERACT Imaging Committee. OMERACT Workshop: repair of structural damage in rheumatoid arthritis. *Journal of Rheumatology* 2003; **30:** 108–109.