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# What's new in orthopaedic research

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## SPECIALTY UPDATE

# WHAT'S NEW IN ORTHOPAEDIC RESEARCH

BY MATTHEW J. SILVA, PHD, AND LINDA J. SANDELL, PHD

The last year has brought substantial advances in many areas of orthopaedic research. Probably the most impressive is the shift from a focus on implant design, obviously the most successful treatment of end-stage degenerative joint disease, to the potential for earlier interventions, or “biological solutions,” that could increase the life of the cartilage, bone, tendons, and ligaments of the joint. The search for biological solutions touches on all aspects of orthopaedic research: response to implant materials, development of tissue-engineered cartilage and bone, and discovery of biomarkers for joint disease. We will review some of the latest advances made and questions raised in this new age of orthopaedic research.

### Implant Wear

This year's combined Orthopaedic Research Society/American Academy of Orthopaedic Surgeons symposium dealt with clinical, engineering, and biological issues related to implant wear. This symposium complemented a recent publication by the Academy and the National Institutes of Health on implant wear<sup>1</sup>. The manifestations of wear of total hip and knee replacements have been well documented in the past decade. Most commonly, osteolysis progresses in a slow, “linear” fashion and is not detectable radiographically until five years or more postoperatively, when periprosthetic radiolucent zones may be observed. Rates of osteolysis as high as 60% have been reported in association with certain hip implants<sup>2</sup>. In addition to wear of hip and knee replacements, wear of total shoulder and elbow replacements is an emerging problem that should be carefully monitored in the future.

### Biological Aspects of Wear—Osteolysis

Particle-induced osteolysis is a primary cause of aseptic loosening. A consensus has emerged that the predominant process is one of cytokine production in response to phagocytosis of implant wear particles resulting in increased proliferation and differentiation of osteoclast precursors into mature osteo-

clasts. Several cell types, including macrophages, fibroblasts, and osteoblasts, observed in periprosthetic tissues are believed to play a role in the osteolytic process. Nevertheless, because osteolysis ultimately involves bone resorption, investigators have focused on understanding the role of the osteoclast. Recent breakthroughs in the understanding of osteoclast biology—namely, the roles of the OPG/RANKL/RANK system in mediating osteoclast formation—have been directly relevant to the current focus of osteolysis research.

The OPG/RANKL/RANK cytokine system is the predominant, final mediator of osteoclast formation<sup>3,4</sup>. This system coordinates the interaction between osteoblasts and osteoclasts that is necessary for differentiation of pre-osteoclasts (monocytes and macrophages) into mature, bone-resorbing osteoclasts. RANK (receptor activator of nuclear factor kappa B) is a transmembrane protein expressed on the surface of pre-osteoclasts. RANKL (receptor activator of nuclear factor kappa B ligand, also known as TRANCE [tumor necrosis factor {TNF}-related activation-induced cytokine]) is produced by pre-osteoblastic stromal cells and immune cells. When RANKL binds to RANK it initiates an intracellular signaling cascade that activates, among others, the NF- $\kappa$ B (nuclear factor kappa B) pathway and leads to osteoclast differentiation. RANKL, in the presence of macrophage colony-stimulating factor (M-CSF), is sufficient and necessary for osteoclast differentiation *in vitro*. The critical role of RANKL-RANK binding *in vivo* is demonstrated by the fact that deletion of either gene results in severely osteopetrotic mice devoid of osteoclasts. OPG (osteoprotegerin) is the third member of the OPG/RANKL/RANK system. It is a secreted decoy receptor (it lacks a transmembrane domain) that is synthesized by pre-osteoblastic stromal cells and binds to RANKL. OPG-RANKL binding blunts osteoclast differentiation; the critical role of OPG in regulating RANKL-induced osteoclastogenesis is demonstrated by the fact that mice lacking OPG have severe osteoporosis. In summary, pre-

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osteoblast/stromal cells can mediate osteoclast formation by controlling the levels of available RANKL on the basis of the ratio of OPG/RANKL. An increase in this ratio leads to increased OPG-RANKL binding and reduces the bioavailable RANKL that is able to bind to RANK, thereby decreasing osteoclast differentiation. A decrease in this ratio leads to decreased OPG-RANKL binding and increased RANKL available to bind to RANK and activate the critical pathway for formation of mature osteoclasts. Preliminary evidence that the OPG/RANKL/RANK pathway is a critical factor in the development of clinical osteolysis has come from reports that detectable levels of messenger RNA for RANKL, RANK, and OPG have been found in tissue adjacent to loose implants; additional studies with suitable control tissue are needed to confirm these observations.

Many upstream factors can influence osteolysis. Of these factors, TNF- $\alpha$  is recognized as a critical cytokine in mediating osteoclastogenesis following exposure to implant particles. Using a model in which the calvariae of mice were exposed to particles of implant materials (titanium, polymethylmethacrylate, etc.), several investigators demonstrated that rapid osteoclastogenesis and bone erosion result as TNF- $\alpha$  levels are increased. Recently, this process was shown to occur through activation of the NF- $\kappa$ B intracellular pathway<sup>5</sup>, indicating a link between the OPG/RANKL/RANK system, TNF- $\alpha$ , and particle-induced osteolysis.

#### *Therapeutic Targets in Osteolysis*

Will the breakthroughs in the understanding of the biological mechanisms of particle-induced osteolysis lead to drug treatments that can prevent periprosthetic bone loss? Anti-TNF therapies may be effective for treating particle-induced osteolysis, as suggested by their success in treating rheumatoid arthritis<sup>6</sup>. Anti-TNF treatment in the mouse calvarial model of particle-induced osteolysis was shown to be only partially effective in blocking bone loss, a finding that was related to the confounding effects of the adenovirus gene-delivery system that was used<sup>7</sup>. Additional studies with nonviral delivery methods are needed to determine the efficacy of anti-TNF therapy. Promising early findings have been reported with use of agents that target the OPG/RANKL/RANK system. Using the mouse calvarial model, investigators from the University of Rochester reported two approaches that were effective in reducing osteoclastogenesis and bone resorption: OPG gene therapy and RANK blockade by a synthetic RANK fusion protein (RANK:Fc), both of which should work by increasing the ratio of OPG/RANKL<sup>8</sup>. The same research group showed that COX-2 (cyclooxygenase-2) plays a role in particle-induced osteolysis and that COX-2 inhibitors are therefore potential therapeutic agents.

While agents to inhibit TNF- $\alpha$  or to alter the OPG/RANKL ratio may ultimately prove to be beneficial for the treatment of osteolysis, the use of bisphosphonates (which already have been established to be extremely effective in halting

osteoporotic bone loss) may provide a more timely option. Several animal studies have demonstrated that not only can alendronate prevent particle-induced osteolysis but it can also reverse bone loss in established osteolysis<sup>9</sup>. A recent clinical trial demonstrated that administration of bisphosphonate in the first six months after total hip replacement prevented periprosthetic bone loss<sup>10</sup>. However, the bone loss observed in the control group in this study was probably related to acute effects of altered stress distribution or to surgical insult rather than to particle-induced osteolysis. Nevertheless, taken together, these studies illustrate the potential of bisphosphonates for the treatment of osteolytic bone loss.

#### *Engineering and Material Aspects of Wear*

Submicrometer-sized polyethylene particles make up the bulk of particulate wear debris found in periprosthetic tissues<sup>11</sup>. Moreover, the incidence and severity of osteolysis have been correlated with the amount of polyethylene wear. Thus, the major research focus with regard to engineering has been to understand the mechanical and material factors that influence polyethylene wear and to develop alternative processes to improve the resistance of polyethylene to wear. In parallel, there are continuing efforts to characterize and develop alternatives to the predominant metal-polyethylene bearing, including metal-metal, ceramic-polyethylene, and ceramic-ceramic bearings.

Osteolysis is rare in patients with rates of acetabular polyethylene wear of less than about 0.1 mm/yr, which is at the lower end of the average reported range of wear rates, 0.1 to 0.2 mm/yr<sup>12</sup>. Thus, the "Holy Grail" of implant materials research and development has been to develop alternative polyethylenes that will reduce the average wear rate to below the "osteolytic threshold." Extensive work in the past five to ten years has led to the widespread adoption of new processing and sterilization protocols for polyethylene used in acetabular components for total hip replacement. (It is likely that the same or similar processes will soon be available for tibial components of total knee replacements.) While the details of the processes differ among manufacturers<sup>12</sup>, the common goal is to introduce a controlled, modest level of cross-linking while avoiding the detrimental effects of oxidation. Radiation dose determines the degree of cross-linking and in turn the resistance to wear. For the enhanced cross-linked polyethylenes now available for acetabular components, manufacturers are using radiation doses that have been reported to reduce the rate of in vitro wear by  $\geq 85\%$  compared with that of nonirradiated polyethylene. The magnitude of wear reduction may be lower for conditions of third-body wear, although cross-linking should still be beneficial.

A concern raised by some investigators is that the improvement in wear resistance resulting from elevated cross-linking comes at the expense of material strength and fracture toughness. This concern has particular relevance to knee components, which historically have been more susceptible to

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fracture and fatigue-related damage such as delamination or pitting. However, fatigue damage of polyethylene may be largely attributable to reduced toughness caused by oxidative damage that historically occurred because polyethylene components were sterilized by irradiation in air. Because oxidative damage is minimized in the new polyethylenes, the reduction in toughness due to cross-linking alone may be small enough for fracture-related damage not to be an important clinical issue. However, only long-term clinical results will provide answers to the questions of whether elevated cross-linking of polyethylenes will lower wear rates in vivo and, most importantly, whether it will reduce the incidence of osteolysis and aseptic loosening.

### Mouse Models in Orthopaedic Research

The increasingly routine use of murine models in musculoskeletal research represents a revolution that has taken place in the last decade. Mouse studies are generally of two types. In the first, the consequences of targeted mutations that delete (knock out) or enhance (overexpress) the function of a particular gene are examined. The aim of these studies is to determine relationships between genetic structure and function, and they may be useful for identifying the genetic basis of diseases linked to a single (or a few) genes. In the second type of study, inbred strains of mice are examined in an attempt to map quantitative phenotypic traits (e.g., obesity or bone density) to particular chromosomal regions<sup>13</sup>. Genetic analysis of mother-daughter pairs and twins has indicated that bone mineral density (the clinical standard for diagnosis of osteoporosis) is 50% to 70% heritable, and thus researchers have been searching in mice for the gene (or, more likely, the genes) that control phenotypic traits that might affect bone mineral density (and other biomechanically relevant traits such as bone size and strength). In either type of study, the choice of a relevant phenotype (i.e., which traits will be examined) and the techniques for assessing the phenotype are critical and will be discussed.

### Quantitative Genetics

While some discrete orthopaedic disorders can be linked to a single genetic mutation, diseases in which risk is a continuous variable (such as osteoporosis) are likely to be influenced by multiple genes. Because osteoporosis is clinically diagnosed on the basis of bone mineral density, studies in which the aim is to find "osteoporosis genes" typically are based on the assessment of bone mineral density. One method for finding genes that control bone mineral density is quantitative trait loci mapping in mice derived by crossbreeding inbred mouse strains. Mice within a given inbred strain (e.g., C57Bl/6J) are essentially genetically identical and have a relatively narrow distribution of quantitative traits like bone mineral density. By crossbreeding two inbred strains that have different traits, an F2 (second-generation) population can be created that has a random mixture of alleles and a broader distribution of bone

mineral density. With the use of genetic markers, regions of the chromosomes (i.e., quantitative trait loci) that are statistically associated with higher bone mineral densities in the F2 mice can be identified. A variation on this approach is to generate recombinant inbred strains by inbreeding a number of the F2 mice; these mice can then be used for refined mapping of the chromosomal regions of interest<sup>14</sup>. Most recently, congenic strains in which the quantitative trait loci from a donor strain (e.g., a mouse with high bone mineral density) are transferred to a recipient strain (e.g., a mouse with low bone mineral density) have been developed to further assess whether given quantitative trait loci contain a gene or genes that regulate bone mineral density<sup>15</sup>. Together, these quantitative genetics approaches have been used to identify at least twenty-eight quantitative trait loci for genes that control bone mineral density in mice<sup>13</sup>. The large number of quantitative trait loci identified to date may reflect the fact that bone mineral density is under the control of many interacting genes as well as the fact that different investigators have used different methods for assessing bone mineral density (e.g., assessment of volumetric bone mineral density with peripheral quantitative computed tomography compared with assessment of areal bone mineral density with dual-energy x-ray absorptiometry) and have focused on different skeletal sites (the whole body, the spine, the femur, etc.). The fact that different genes may control bone mineral density at different skeletal sites highlights the complexity of the problem.

### Functional Skeletal Phenotype

In recent years, there has been considerable debate about what is the most relevant functional phenotype for gene-mapping studies related to osteoporosis. As noted above, investigators have focused on whole-bone or whole-body bone mineral density because of the strong association between bone mineral density and fracture risk in humans. However, whole-bone or whole-body bone mineral density in mice does not necessarily relate to femoral neck or lumbar bone mineral density in humans. Mouse bones, for example, contain a lower proportion of trabecular bone than do human bones. More recently, investigators have begun to focus on the "bottom line" of skeletal function—i.e., whole-bone strength. To date, most biomechanical phenotype studies have focused on testing long bones (e.g., femora) and thus have primarily assessed cortical bone. Acceptable techniques for testing long bones include three-point and four-point bending and torsion<sup>16</sup>. Efforts to assess sites containing trabecular bone (e.g., vertebral bodies and the femoral neck) have also been reported. Because of the small size of mouse bones it is not possible to isolate trabecular bone, and tests on specimens that contain both trabecular and cortical bone are likely to be the only practical option. To date, no particular testing technique has gained widespread acceptance.

When functional skeletal phenotype is assessed, basic principles of structural mechanics dictate that two types of

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data be obtained: whole-bone (structural) mechanical properties and bone geometric properties. Relevant whole-bone mechanical properties (for either bending or torsion of long bones) include stiffness, yield load, ultimate load, post-yield displacement, and energy to fracture. Relevant geometric properties include cortical thickness, endosteal and periosteal width, bone area, and bone moment of inertia. Ideally, a third type of data—bone material properties (e.g., Young modulus and ultimate tensile stress)—would be obtained from independent tests. In practice, it is difficult to directly assess material properties of mouse bones, and investigators typically infer the “effective” material properties by using engineering equations along with the whole-bone mechanical properties and geometric properties. Armed with data on whole-bone strength, size, and estimated material properties, investigators are able to synthesize a relatively complete picture of the functional skeletal phenotype. However, numerous examples of incomplete phenotype assessment can be found in the literature. In some cases, geometric data have been presented and used to draw conclusions about skeletal strength in the absence of mechanical property data. The drawbacks of such an approach are becoming more recognized<sup>17</sup>.

### Functional Tissue-Engineering— the Importance of Mechanical Loading

The development of tissue-engineered products and processes in the next decade will likely present an unprecedented opportunity for clinicians to expand their treatment options for such problems as repair of articular cartilage. Nevertheless, many challenges must be overcome before successful long-term treatments can be made available. A workshop at this year's meeting of the Orthopaedic Research Society focused attention on the need to better understand cellular mechanotransduction (i.e., how cells respond to physical stimuli) in the context of both native and engineered tissues.

With regard to engineered tissues, *in vitro* physical stimuli are relevant as a means to enhance biosynthetic activity and to promote the formation of a stiffer construct. Both fluid flow and dynamic compression have been shown to increase matrix synthesis and enhance stiffness of cartilage constructs<sup>18</sup>. Moreover, mechanical loading can enhance the positive effects of biochemical stimuli. In engineered agarose-chondrocyte constructs, dynamic loading over a five-week period in conjunction with treatment with either transforming growth factor- $\beta$ 1 (TGF- $\beta$ 1) or insulin-like growth factor-1 (IGF-1) resulted in a synergistic enhancement of tissue stiffness that was greater than the sum of the effects of the two stimuli applied separately<sup>19</sup>. This synergistic effect is consistent with a similar effect observed in native cartilage explants and suggests that the optimal process for producing functionally competent constructs will require the coordinated delivery of appropriate biochemical and biomechanical stimuli. At the time of implantation, cartilage constructs should ideally have mechanical properties approximating those of healthy native

tissue. In particular, the dynamic compressive modulus may be the most important functional property because it will determine whether the tissue can sustain cyclic *in vivo* loading without undergoing excessive strain leading to structural degradation and construct failure<sup>20</sup>. The concept of “functional tissue-engineering” has been advocated to highlight that synthetic constructs must replace the mechanical function of the native tissues<sup>21</sup>.

In addition to the use of mechanical loading to enhance construct properties *in vitro*, the cells in the constructs should be able to modulate their synthetic activity to reflect the *in vivo* loading environment after implantation. One important feature of native tissues (cartilage and bone) is that the cells (chondrocytes and osteocytes) are surrounded by a pericellular matrix that differs from the bulk matrix. (Preliminary data indicate that this is also true for tendon fibroblasts.) This pericellular matrix controls cell-matrix interactions and determines the microenvironment of the cell, including how the bulk mechanical stresses and strains are transferred to the cell surface. The peak strains at the chondrocyte-matrix interface may be twice as high as the average tissue strain, whereas in bone the cell strains may be an order of magnitude higher than the average tissue strain. These relationships between the cell and its surrounding matrix may be difficult to replicate in engineered constructs. Cells seeded in artificial matrices may not be as responsive to mechanical stimuli as are cells in native tissue until they have synthesized a new pericellular matrix<sup>18</sup>. The long-term success of engineered constructs will depend largely on their ability to replicate cell-matrix interactions that enable cells to respond appropriately to biophysical stimuli.

### Development of Cartilage and Bone

Members of the TGF- $\beta$  superfamily include the bone morphogenetic proteins (BMPs) and growth and differentiation factors (GDFs). These potent signaling factors reside in the extracellular matrix, can form gradients of morphogenetic activity, or can signal to the cell to differentiate, proliferate, or die. These growth factors stimulate the differentiation of cartilage and bone from uncommitted “stem” cells during organogenesis in the embryo and in fracture repair. They are also important for the maintenance of cartilage and bone structure. These tissues have a great deal of extracellular matrix that participates in the delivery and storage of growth factors. New extracellular matrix molecules like chordin, noggin, and a specific domain of type-II procollagen bind to and consequently participate in localizing and regulating the availability of the growth factors.

Insights into the mechanism of bone and cartilage differentiation have been provided by a number of investigators studying signal transduction and gene transcription. Rik Derynk, a developmental biologist from the University of California, San Francisco, reported new findings on the regulation of cartilage and bone development by TGF- $\beta$  signaling. Once free from binding proteins, TGF- $\beta$  stimulates the ex-

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pression of cartilage-specific factors and at the same time represses the bone-controlling transcription factor *Cbfa1*, through control of a signal transduction protein, *Smad3*. The differentiation of bone and that of cartilage appear to be mutually exclusive. This was also demonstrated with the transcription factor *C/EBP*. In independent studies, Jane Lian and Gary Stein (University of Massachusetts Medical School, Worcester, Massachusetts), investigating bone development, and Ken Okazaki and one of us (L.J.S.) (Washington University School of Medicine, St. Louis, Missouri), investigating cartilage development, discovered roles for *C/EBP* in the regulation of bone-specific osteocalcin and cartilage-specific CD-RAP, respectively. In bone, *C/EBP* acts as a positive factor increasing expression of osteocalcin<sup>22</sup>, whereas in cartilage, *C/EBP* acts as a negative regulatory factor for CD-RAP and type-II collagen<sup>23</sup>. In fact, studies of transgenic mice have shown that *C/EBP* is highly expressed in muscle and bone and is responsible for inhibition of CD-RAP expression in those tissues. Therefore, cells express a certain set of regulatory factors (e.g., the transcription factor *Cbfa1* or *C/EBP*) that work together to suppress one phenotype and to enhance another. In this case, the same factor, *Cbfa1*, a positive regulator of bone gene expression, is turned off during cartilage differentiation, and high levels of *C/EBP* enhance bone gene expression and repress cartilage gene expression.

Yet another new molecule has been found to be necessary for bone differentiation: *Osterix*<sup>24</sup>. This molecule was found during a screen for bone-specific proteins and, when "knocked out" in mice, no bone was formed. The transcription factor *Osterix* appears to act downstream of the transcription factor *Cbfa1*.

### Molecular Biology of Fracture Repair

For the last few years, investigators in this area have begun to take a molecular view of fracture-healing. Taking advantage of the ability to induce fractures in small animals that was pioneered by Thomas Einhorn (Boston Medical Center, Boston, Massachusetts), researchers have used a combination of histology, in situ hybridization to mRNA, and immunohistochemistry to provide a molecular description of events taking place within the tissue. Currently, the focus is on the use of these models to study factors that may affect bone-healing. Various methods to augment bone-healing with use of BMPs, VEGF (vascular endothelial growth factor), and bisphosphonates have been tested and have shown various capacities to improve the rate of healing. Of critical importance is the effect of the new selective nonsteroidal anti-inflammatory drugs, the COX-2 inhibitors, on bone-healing. These inhibitors are widely used to control pain in a variety of musculoskeletal conditions, and they account for forty-five million prescriptions in the United States alone<sup>25</sup>. Leonelli et al.<sup>25</sup> reported that rats given the COX-2 inhibitor rofecoxib were more likely to have a nonunion, a malunion, and a larger callus than were controls and rats treated with ibuprofen.

Goodman et al.<sup>26</sup> supported these findings by showing that a COX-2 inhibitor also inhibited bone ingrowth in an experimental model.

### The Genetic Basis for Orthopaedic Diseases is Beginning to Emerge: Lessons from Fruit Flies and Nematodes

While the study of fruit flies and nematodes will never be popular in orthopaedic research laboratories, a growing appreciation of the basic mechanisms of development has arisen from the study of more primitive organisms. Research on multiple hereditary exostosis is a good example of how developmental biologists and scientists interested in human disease can complement each others' investigations. The primary insight regarding the mechanism of this human disease came from studying a disease in the fruit fly (*Drosophila*) called *tout-velu*, in which there is a defect in signaling of the hedgehog protein. Signaling of the hedgehog protein is disrupted because of a mutation in the enzyme necessary for synthesis of a proteoglycan that binds to the hedgehog protein for distribution. In a workshop entitled *Genetics of Orthopaedic Disorders*, Chair Chris Evans stressed the recent headway made in understanding the genes involved in controlling some musculoskeletal diseases. Multiple hereditary exostosis is characterized by osseous outgrowths at the margins of the growth plate. Jacqueline Hecht (University of Texas Medical School, Houston, Texas) presented data showing that the genes that cause multiple hereditary exostosis, called *EXT1* and *EXT2*, encode for polymerase enzymes that form the heparin sulfate carbohydrates on proteoglycans. The heparin sulfate proteoglycans are involved in the control of chondrocyte differentiation by regulation of growth-factor stimulation. In this case, the likely protein that is misregulated, and causes the growth of exostoses, is the hedgehog protein. Abnormal signaling of the hedgehog protein, known to be an important regulator of growth-plate function, causes further differentiation of growth-plate chondrocytes into hypertrophic chondrocytes, which then can be mineralized and replaced by bone generated from osteoblasts in the vasculature.

Another unforeseen molecular player, the heparin sulfate proteoglycan perlecan, is now known to participate in skeletal dysplasia of the Silverman-Handmaker type<sup>27</sup>. In fact, while 60% of mice missing this proteoglycan have the dysplasia, 40% die during embryogenesis because of defective cephalic development. It is very possible that the responsible signaling events are the same as those in multiple hereditary exostosis, as described above.

The understanding of rare diseases will help to provide information that can be applied to all musculoskeletal diseases. Although individually rare, the many different forms, taken together, result in a substantial number of affected individuals with major morbidity and mortality. The rapid advances in the understanding of the molecular basis for skeletal dysplasias have made the classification of diseases difficult. For

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example, the clinical symptoms of diseases caused by type-II-collagen mutations can be quite varied; they cannot be classified only according to the molecular defect. Therefore, a new classification of genetic disorders of the skeleton has been proposed<sup>28</sup>. In this system, there is a nosology—a catalog of defined entities. This includes a clinical classification, focused on age-specific presentations and clinical signs, to be of help in the diagnostic approach, and a molecular-pathogenetic classification based on the genes and pathogenetic mechanisms involved. These two classifications will be cross-correlated in an electronic database.

### Stem Cells

The use of stem cells in research has become a household discussion. Stem cells have a pluripotent ability to commit to specific cell fates. Such flexibility is apparent in the embryonic stem cell, which by definition is a precursor of all cells in the developed organism. It is less apparent that many other tissues have a reserve of “stem” cells with limited but distinct potential, such as periosteal and perichondrial cells, pericytes from blood vessels, stem cells from muscle and fat, and, potentially the richest adult source, bone-marrow stem cells. The use of these cells and the conditions under which they can differentiate have been intensively investigated recently. Muscle, fat, and mesenchymal stem cells from bone marrow can be induced to form bone. While it has been known for a while that BMPs stimulate bone differentiation, Majumdar et al.<sup>29</sup>, from Genetics Institute, showed that BMP-2 or BMP-9 together with a relatively new cytokine, IL-11 (interleukin-11), function to promote chondrogenesis in these cells. IL-11 is a member of the IL-6 family and has been found to be effective in prevention of inflammatory bowel disease.

### Gene Therapy

#### Bone

The advantage of gene therapy is that when the gene is resident in the cell, the products of gene expression can be made and distributed in situ. This therapy may be applied to cells while they are in the body (in vivo) or to cells or pieces of tissue that have been removed from the body (ex vivo). More than fifty papers on this valuable technique were presented at the meeting of the Orthopaedic Research Society in 2002. Although none are used yet in the clinical setting, the induction of bone formation through gene therapy may be on the horizon. In addition to studies involving the growth factors BMP-2 and BMP-7 (OP-1), the induction of bone with use of LIM mineralization protein-1 appears to be feasible<sup>30</sup>. LIM mineralization protein-1 is a transcription factor that may function through stimulation of endogenous BMPs. While the methods of gene delivery have not been well defined, the exogenous delivery of genes known to stimulate repair or replacement of bone will likely become a common method for stimulation of cell metabolism. In a novel technique, muscle stem cells were used to deliver BMP-4 in a nonhealing skull defect. The gene

for BMP-4 was cloned into a retrovirus and transduced into the muscle cells ex vivo, after which the cells were implanted into the defect<sup>31</sup>. Use of the retrovirus holds promise as a safe method in humans as it is not antigenic and therefore provides a low immunological risk to the patient.

#### Other Skeletal Tissues

Adenoassociated virus vectors are being used to transduce human meniscal cells and even meniscal explants in vitro. The efficiency of transduction has been quite high, and future experiments will likely focus on the delivery of specific genes. IGF-1 gene therapy has proved productive for inducing healing of cartilage in horses and, in conjunction with dynamic loading, in bovine cartilage discs. The use of BMP-2 and BMP-4 for induction of cartilage-healing has been examined as has gene expression of BMP-2 and other growth factor genes in nucleus pulposus.

#### Osteoarthritis and Biomarkers

Great headway continues to be made in the understanding of matrix degradation in osteoarthritis. The recent discovery of specific “aggrecanases” and “versicanases” has revealed the participation of an entire group of enzymes that cleave proteoglycans in tissue. These enzymes, in addition to the matrix metalloproteinases that have been studied for decades, may be responsible for the initial cleavages in the important proteoglycans. Recently, the attention of investigators has turned to the factors that are responsible for activation of these destructive enzymes and how they might function in vivo. Controversial at this point is the timing of degradation by specific enzymes during the course of progression of osteoarthritis and which molecules are initially targeted. Resolution of the control of these pathways will be the focus of the next few years of research as headway is made in understanding the progression of osteoarthritis.

Recognition that osteoarthritis progresses slowly over a long period has stimulated research into methods of detection of the stage of osteoarthritis and the potential for prediction of short and long-term outcomes. Certainly, if the disease can be better defined, interventional therapies short of joint replacement could be developed and implemented. Various methods are being used to determine the state of cartilage and the rate of disease progression. Mechanical, imaging, and biological parameters are under investigation. Camacho et al.<sup>32</sup> are using a fiberoptic probe for detection of degenerative cartilage, while others are using pressure probes to detect mechanical changes in the cartilage, or serum markers for collagen and aggrecan metabolism. With one promising method, changes in the rate of synthesis are compared with the rate of degradation so that when repair slows down and degradation increases, the molecular changes can be monitored in urine and serum over time, predicting the rate of cartilage loss<sup>33</sup>.

Finally, as there have been so many advances in orthopaedic research recently, the reader is referred for further in-

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depth study to the *Transactions of the Orthopaedic Research Society*, which are available on the web site [www.ors.org](http://www.ors.org), and as a CD-ROM containing the abstracts, available from the Society by telephone (847-698-1625).

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