Tissue Engineering and Gene Therapy in Orthopaedic Surgery

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Tissue engineering and gene therapy have significantly enhanced patient care in many fields of medicine. Musculoskeletal researchers and investigative orthopaedists are now on the cusp of utilizing these techniques. Bone, nerve and collagenous growth factors are being widely investigated and applied to a multitude of clinical conditions. This ongoing, rapidly advancing pace of scientific discovery and technologic invention challenges physicians as we enter the twenty-first century. It appears that clinical orthopaedics is about to leave the "mechanical age" and enter the "biologic" era where stem cells, chondrocytes, growth factors, genes and gene products will augment and may someday replace the orthopaedic surgeon's operative tools.

Many researchers predict that stem cell transplantation technology will some day be available to persons with advanced rheumatologic joint destruction. Herndon and colleagues have outlined the potential for gene therapy to provide a cure for rheumatoid arthritis using a variety of approaches to produce a biological drug-delivery system for the sustained expression of anti-arthritic proteins such as interleukin-1 receptor antagonist protein (IL-1Ra) (1) . Their approach entailed the delivery of a gene or combination of genes that encode for anti-arthritic proteins. In principle, a stable transferred gene will permit sustained production of the therapeutic protein in-situ, and thus, tissues will have the ability to synthesize their own anti-arthritic factors endogenously. Gene delivery was accomplished through the use of adenoviral vectors that integrate the transferred gene(s) into the host cell's genome so as to offer the best prospect for long-term expression. In a recently completed double-blind trial, Herndon's team demonstrated the successful expression of their trans-gene in the joints of human subjects.

Herndon's colleagues, Evans and Robbins, further eluci-dated the potentialtreatment of osteoarthritis by gene therapy using genes which stimulatechondrogenesis or inhibit breakdown of the cartilaginous matrix via avariety of vectors. (2) Transfer of such genes to chondroprogenitor cells isparticularly attractive. Plasmid injections of interleukin-1 receptor antagonist genes into articular cartilage of rabbits that underwent meniscectomies showed smaller subsequent articular cartilage defects and less osteophyte formation. These results show the immediate application of gene therapy and the potential to change the course of osteoarthritis (3,4) .

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In Toronto, PJ Doherty and associates have genetically modified chondrocytes in vitro for eventual transplantation into

the cartilage of patients with osteoarthritis (5) . They have shown that a beta-galactosidase gene introduced into chondrocytes via infection from an adeno-viral vector both before and after transplantation into cartilage continued to bind to cartilage explants for up to forty-five days. Furthermore, Doherty's team was able to infect the chondrocytes with adenovirus at least thirty-five days after transplantation. This resurfacing of cartilage with genetically modified chondrocytes and the ability to reinfect them well after transplantation may offer an alternative to repeated injections of chondrocytes into the joint space. The potential for single treatment patient injections has obvious advantages.

Gene therapy has been particularly promising in the area of fracture repair. Kitano and colleagues at Children's Hospital in Philadelphia have selected and amplified early cycling adherent cells from murine bone marrow using retroviral transduction (6) . The resulting mesenchymal progenitor cells were cloned and injected subcutaneously into newborn mice which then produced ectopic calcification. Jueren Lou and associates demonstrated that an adenovirus-mediated protein (bone morphogenetic protein-2) could induce mesenchymal progenitor cell differentiation and bone formation (7) . Depending on the dose, mature human bone morphogenetic protein-2 expression, detected by specific antibodies, exhibited differentiation to osteoblasts in vitro. Injection of this cell line (C3H/10T 1/2) into the thighs of nude mice resulted in new ossicle formation having various osseous components such as bone trabeculae, bone marrow, and chondrified tissue. This is further evidence of the usefulness of adeno-associated viral vectors for the delivery of target genes and their ability to mediate gene expression in vivo.

Longaker and colleagues at New York University Medical Center have studied the effects of hypoxia on growth factor expression by osteoblasts (8,9,10,11,12) . They demonstrated hypoxia-induced increases in transforming growth factors such as VEGF and their receptors, as well as collagen types I and II. These are important for collagen synthesis, proliferation of osteoblasts, and endochondral bone repair in the microenvironment of the healing wound. Their data also suggest that osteoblasts may be responsible for angiogenesis. Furthermore, their studies suggest the presence of an oxygen-sensing mechanism that has been implicated in the regulation of gene expression, cellular proliferation and cellular differentiation. Understanding the molecular mechanisms underlying fracture repair may lead to enhanced osteogenesis therapies.

Similarly, researchers at Duke University Medical Center have delivered VEGF to injured skeletal muscle and joints using myoblasts infected with VEGF-adenovirus in a rabbit model 13 . VEGF overexpression in myoblasts resulted in significant (250%) improvement in capillary density and holds promise for new therapies for revascularization procedures.

Just as Longaker et al were able to show evidence that hypoxia stimulated the production of growth factors needed for wound healing, Dudziak and colleagues at Louisiana State University Medical Center demonstrated how ionizing radiation can delay fracture healing by decreasing transforming growth factor (TGF-b) and VEGF production (14) . However, Dudziak et al also effected phenotypic restoration of irradiated osteoblasts using adenovirus-mediated TGF-â 1 gene therapy which caused an increase in VEGF. This data has important implications regarding the potential of gene therapy to reverse impaired fracture healing and osteoradionecrosis.

For upper extremity specialists, Karanus et al at Stanford University has demonstrated that modulation of TGF-â 1 and 3 may potentiate nerve healing by controlling scar formation and improving autogenous nerve regeneration in a rat model (15) . Using genetically engineered primers, these researchers found that TGF-â 1 and TGF-â 3 upregulated in both neural and stro-mal cells, and growth factor levels peaked within the first postoperative week. Finding a method of locally controlling TGF-â levels may have widespread application in successful surgical repair management.

The rapidly expanding field of sports medicine may also benefit from adenoviral vectors. Determining the effects of various factors on meniscal cells may direct research in the amplification and delivery systems of cytokines that will augment healing potential. Hildebrand et al successfully used a retroviral ex vivo technique indicating the possibility of gene transfer to normal and injured knee ligament (16,17) .

In Anthony Radcliffe's presentation at the Cambridge Healthtech Institute's Second Annual Tissue Engineering/Regenerative Healing/Stem Biology Conference (18) , he showed that biocompatible scaffolds seeded with cells allowed for the development of tissues in vitro with an immediate in vivo function of rapid wound repair or replacement of functional tissue. Commercially available tissue-engineered products that can stimulate wound repair are already available (Dermagraft ® , and TransCyte ® ,) and can be applied to chronic ulcers or acute burns. Research is currently underway to develop mature articular cartilage constructs, to be grown in vitro, which can then be transplanted into damaged knees. These constructs have been successfully engineered in the laboratory but they have yet to be integrated into a joint as they inevitably undergo remodeling in vivo. Dr. Radcliffe reminds us that, although cartilage is impossible to replicate because structural characteristics depend on the location and biomechanical forces to which it is exposed, cell transplant technology shows great promise.

Myron Spector of the Brigham & Women's Hospital Orthopaedic Research Laboratory has described tissue engineering as the logical evolution of orthopaedic surgery, since cell-based therapies such as the implantation of exogenous cells, autogenous and allogenic tissue grafts, are merely extensions of accepted orthopaedic practices (19) . Originally coined to describe tissue produced in culture by cells seeded in porous absorbable matrices, "tissue engineering" has now advanced to include the implementation of porous matrices, alone or seeded with cells, as implants to facilitate tissue regeneration in vivo. There is a great deal of excitement over certain cells that have been shown to proliferate and maintain their phenotype when cultured in vitro. Spector and his collaborators have been experimenting with the three basic components of tissue: matrix, cells, and soluble regulators to engineer tissue in vivo or in vitro for eventual implantation into a defect. They are currently investigating the use of an analog to the extracellular matrix (ECM) ñporous collagen-glycosaminoglycan (GAG) copolymers for regeneration of articular cartilage, meniscus, ligament, tendon, intervertebral disc, and gingiva. This ground-breaking work is the result of a multi-disciplinary partnership between orthopaedic surgeons, engineers, and biomedical scientists who, as Dr. Spector so eloquently points out, are critical for advancing the education of the next generation of clinicians and laboratory investigators in tissue engineering approaches to orthopaedic problems.

As orthopaedics and all fields of surgical care continue to further sub-specialize, there will run an underlying and unifying theme of tissue engineering and gene therapy applications. Perhaps someday, the diseases we treat may be cured with these extraordinary efforts. As the new millennium is upon us, so too comes a new age of intellectual and biotechnical promise in orthopaedics. These are indeed very exciting times.

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