TISSUE ENGINEERING
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Review

Mesenchymal Stem Cells: Cell-Based Reconstructive Therapy in Orthopedics

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ABSTRACT

Adult stem cells provide replacement and repair descendants for normal turnover or injured tissues. These cells have been isolated and expanded in culture, and their use for therapeutic strategies requires technologies not yet perfected. In the 1970s, the embryonic chick limb bud mesenchymal cell culture system provided data on the differentiation of cartilage, bone, and muscle. In the 1980s, we used this limb bud cell system as an assay for the purification of inductive factors in bone. In the 1990s, we used the expertise gained with embryonic mesenchymal progenitor cells in culture to develop the technology for isolating, expanding, and preserving the stem cell capacity of adult bone marrow-derived mesenchymal stem cells (MSCs). The 1990s brought us into the new field of tissue engineering, where we used MSCs with site-specific delivery vehicles to repair cartilage, bone, tendon, marrow stroma, muscle, and other connective tissues. In the beginning of the 21st century, we have made substantial advances: the most important is the development of a cell-coating technology, called painting, that allows us to introduce informational proteins to the outer surface of cells. These paints can serve as targeting addresses to specifically dock MSCs or other reparative cells to unique tissue addresses. The scientific and clinical challenge remains: to perfect cell-based tissue-engineering protocols to utilize the body's own rejuvenation capabilities by managing surgical implantations of scaffolds, bioactive factors, and reparative cells to regenerate damaged or diseased skeletal tissues.

INTRODUCTION

In adults, all the skeletal tissues constantly rejuvenate themselves. This process involves the death of end-stage differentiated cells such as osteoblasts, that is, bone-forming cells, and their replacement by newly differentiated osteoblasts. The new osteoblasts arise in a complex, multistep sequence, called a lineage, from multipotent progenitor cells called mesenchymal stem cells, MSCs, found in bone marrow and other sites.[1,2] The MSCs have the capacity to differentiate into other phenotypes including those that fabricate cartilage, muscle, marrow stroma, tendon/ligament, fat, and other connective tissues (Fig. 1). Thus, adult MSCs *in vivo* function to supply replacement units for the differentiated cells that naturally expire or succumb to injury or disease. This process of stem cell-generated replacement cells decreases with age after reaching its peak in the mid to late 20s in humans. Therefore, past the age of 30 years, supplementation and management of the innate cell-mediated rejuvenation ca-

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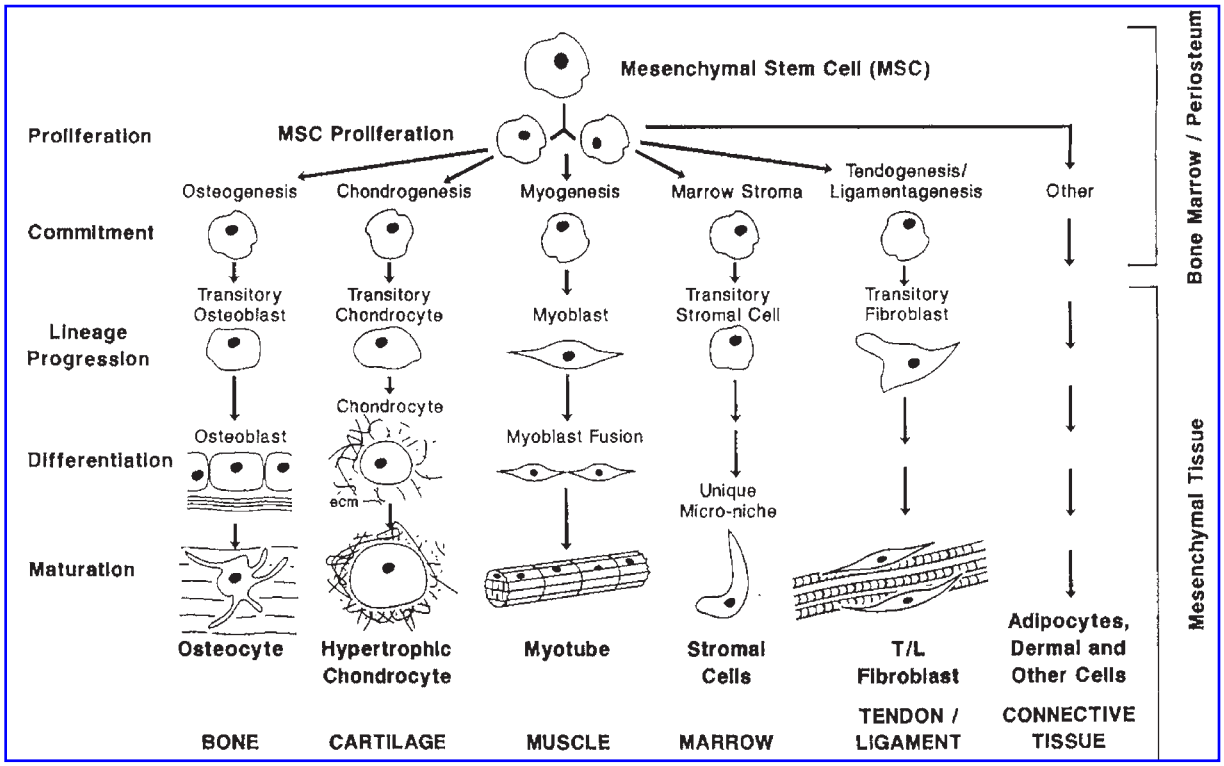

FIG. 1. The mesengenic process. Mesenchymal stem cells (MSCs) have the capacity to differentiate into bone, cartilage, muscle, marrow stroma, tendon/ligament, fat, and other connective tissues.[1,2] The sequence of this differentiation involves multistep lineages controlled by growth factors and cytokines. This figure is structured in a manner comparable to hematopoietic lineage progression and involves well-described lineages for osteogenic differentiation[38] with decreasing information available from left to right.

pacity will enhance skeletal tissue performance and repair.[3]

The cure for many genetic diseases that affect skeletal tissues could be "cell replacement therapy," whereby the host stem cells are replaced by donor cells that do not carry the genetic defect. For example, in the case of osteogenesis imperfecta (OI), host osteoblasts carry a gene lesion in type I collagen. When osteoblasts fabricate the matrix of bone, the type I collagen-rich osteoid is defective and the resulting mineral deposition is likewise defective. The end result is bone stock that is brittle and that fractures at a fraction of the maximum load tolerated by normal bone. If the host MSCs, the source of all osteoblasts, are replaced with genetically normal donor allogeneic MSCs, the newly formed donor-derived osteoblasts will make normal bone stock that naturally replaces the defective stock.[4] Indeed, successful short-term amelioration of OI has been reported by introducing allogeneic MSCs into young, growing OI patients.[5,6]

It follows that the control of MSC number, location, differentiation potential, and rate of differentiation can affect skeletal tissues, their growth and physical properties, and their maintenance and repair capacities. For example, the rate of fracture repair is directly controlled by the rate of fracture callus formation and differentiation; the callus is made up of MSCs and blood vessels. In mechanically unstable breaks, the lack of vasculature causes the bulk of the MSCs to develop into bridging cartilage that eventually spans the defect and then is further stabilized by a surrounding bony bridge.

I outline below how we have developed cell-based and tissue-engineering therapies for skeletal tissues by using MSCs. Because MSCs are present at concentrations of less than 1 in 100,000–500,000 nucleated cells in bone marrow aspirates from adults, the MSCs must be culture expanded to obtain sufficient numbers for clinical use.

The evolution of MSC technology at Case Western Reserve University (Cleveland, OH) and its development into clinical protocols is the focus of this review. The current deficits of this technology provide the goals for future technology. In this regard, perhaps the subtitle for this review should be *Yesterday, Today, and Tomorrow*.

YESTERDAY

In the 1970s, my laboratory reported our studies on the disassociation of embryonic stage 24 chick limb bud mes-

enchymal progenitor cells and their subsequent differentiation into bone, cartilage, muscle, and other mesenchymal tissues[7–9] (Fig. 2). In the early 1980s, we used these embryonic chick limb bud cells in culture as an assay to purify bioactive molecules from demineralized bone matrix.[10–14] Cultures were seeded at sufficiently low density that they did not differentiate into cartilage. Extracts of demineralized bone were exposed to these cultures and this exposure induced chondrogenic differentiation in a dose-dependent manner (Fig. 3A). Schemes to purify the crude extract of such demineralized bone established that the "chondrogenic stimulating activity" (CSA) was a 31-kDa protein on sodium dodecyl sulfate (SDS)–polyacrylamide gel electrophoresis displays (Fig. 3B) that we now know was a heterodimer of bone morphogenetic proteins (BMPs).

The BMPs were cloned in the late 1980s[15] and because of our studies and assays for CSA, I suspected that stem cells comparable to the embryonic chick limb bud mesenchymal cells must reside in adult tissues. This view was principally based on the many studies by Marshall Urist showing that when demineralized bone or extracts from it were implanted into subcutaneous or intramuscular sites, they caused cartilage and bone formation.[16–19]

Furthermore, two important additional facts were known in the mid-1980s. First, orthopedic surgeons routinely used freshly isolated bone marrow to provide rapid and extensive repair of large bone defects or for spinal fusions.[20] This implied that marrow contained reparative or osteogenic cells that contributed to these mesenchymal repair sites. Second, the work of Friedenstein[21,22] and, in particular, of Owen[23,24] indicated that cells isolated and adhered to petri dishes from marrow had osteogenic and adipogenic potential. On the basis of these experiences and facts, Stephen Haynesworth and I developed the technology for isolating and culture expanding adult marrow-derived MSCs.[25–30]

MSC Technology

In standardizing the embryonic chick limb bud mesenchymal progenitor cell culture system, we routinely screened many separate batches of fetal bovine serum (FBS) for its ability to enhance cell attachment and proliferation and to support density-dependent differentiation of cartilage, bone, and muscle.[31] This screening was started in the 1970s when large differences could be seen between individual batches of FBS. In fact, by merely lining up the ten to fifteen 100-mL bottles of different batches of FBS, we could eliminate half of them because we knew that those that had a green hue (hemolysis) would not be suitable for the limb bud cells. Today, unfortunately, large batches of FBS are blended together and greater care is taken in their production that they all look the same.

Thus, when Stephen Haynesworth and I set forth to purify and culture expand human (h) MSCs from fresh bone marrow, we had prescreened batches of FBS that were suitable for embryonic mesenchymal progenitor cells. As this technology evolved, we eventually gained enough experience with hMSCs to develop assays specific to these progenitor cells and, thus, stopped using the

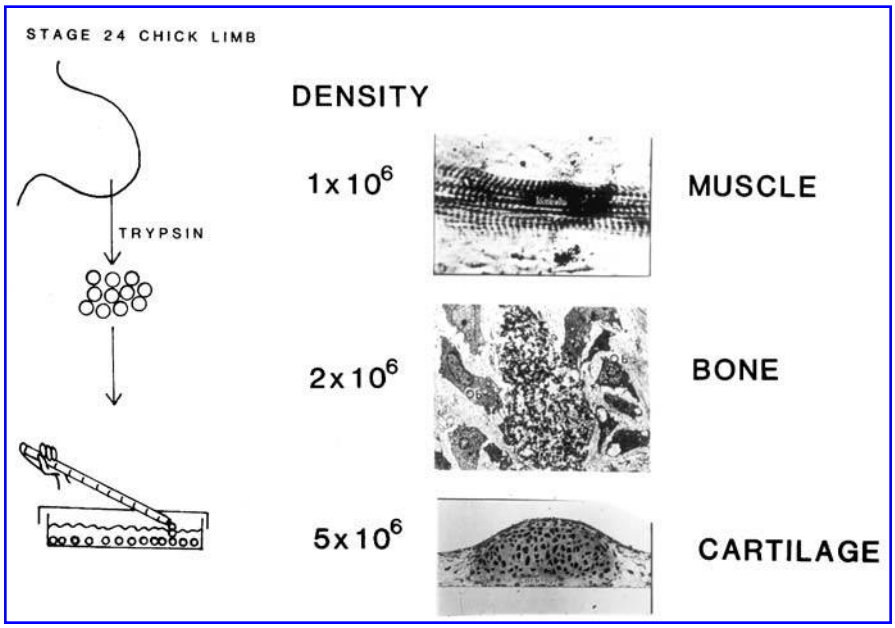

FIG. 2. Stage 24 chick limb bud mesenchymal cell cultures exhibit muscle, bone, and cartilage phenotypes depending on the original plating density in 60-mm petri dishes. The chick limb bud cells are liberated in a trypsin disassociation step after their dissection from the embryos. The cells are plated, and a sequence of differentiation into these phenotypes has been described.[1,7–9]

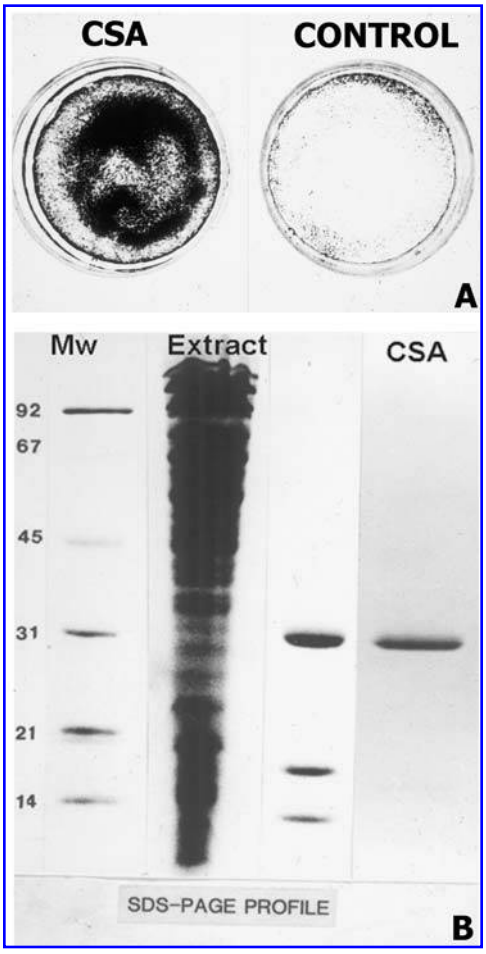

FIG. 3. Chondrogenic stimulating activity (CSA) has been purified by using the embryonic chick limb bud mesenchymal cell culture assay.[12,13] Pictured are petri dishes that have been plated at 2 million cells per 60-mm dish; little chondrogenesis could be expected except at the edges of the control plate as seen in (**A**) on the right. Cultures treated with CSA extracts show a dramatic upregulation of chondrogenesis in these toluidine blue-stained dishes. Pictured in (**B**) is an SDS–polyacrylamide gel molecular weight standards, total extract profile, partially pure preparation, and purified CSA preparation at 31,000 Da, respectively, from left to right.

embryonic chick cells in our serum screen. The current technology selects suitable batches of serum on the basis of hMSC colony counts, cell proliferation (numbers of cells at each passage), and *in vitro* and *in vivo* assays for osteogenesis and chondrogenesis.[31]

The historic and current "gold standard" assay for all MSC preparations is to place the cells onto the pore walls of fibronectin-coated porous calcium phosphate ceramics and, after a brief *in vitro* incubation at 37°C to allow the cells to attach, the cell–ceramic composite is placed in a subcutaneous pocket of immunocompromised rodent or autologous host[32–34] (Fig. 4). These implantation sites are highly vascular and the quantitative estimation of bone (Fig. 5) and/or cartilage in the pores is taken as an indication of the quality of the MSC preparation. By testing 10 to 15 different batches of serum with MSCs from the same donor, the effects of these different serums can be directly compared. Again, currently 1 in 20 or 30 batches of FBS is suitable for hMSCs. It must be firmly stated that the batch of serum suitable for hMSCs is not suitable for rat, rabbit, or mouse marrow-derived MSCs. Because we use a variety of experimental animal preclinical models, we must screen serum batches for the MSCs of each animal, for suitability to support selective attachment to culture dishes and for proliferation with the maintenance of the stem cell properties. More troublesome, with each newly purchased batch of FBS for one species-specific MSC preparation, we expect slight differences in the ability of the MSCs to divide or differentiate. On top of this serum species specificity, every donor or MSC preparation varies even from inbred species. For hMSCs, we have published that the constitutive secretion into the medium of cytokines and growth factors is quantitatively different although the percent increase due to growth factor stimulation is relatively uniform from donor to donor.[35]

Because both whole marrow and purified and expanded MSCs make bone in the calcium phosphate porous ceramics in these *in vivo* incubation sites,[36] these cell delivery vehicle composites have been used in rodent and canine preclinical models for massive bone repair.[37–43] The choice of these calcium phosphate ceramic vehicles is based on their osteoconductive properties, but in addition, these materials support the induction of osteogenesis of MSCs; whether this is due to direct interaction of MSCs with the calcium phosphate surfaces or the binding of specific growth factors to the surface has not been determined. In addition, the MSCs bound to ceramic vehicles can be superinduced *in vitro* to enter the osteogenic lineage before implantation.[36] Thus, MSC-mediated osteogenesis can be jump-started in culture before clinically relevant implantation. MSCs produce bone in the pores of the ceramic faster than is seen with whole marrow, and the jump-started MSCs produce bone faster than uninduced MSCs. These features may be important in bone repair or implant fixation protocols in older patients, whose MSC titers are much lower than in young individuals.[3]

It is important to stress that we have developed *in vitro* assays for MSCs to differentiate into osteoblasts,[44] chondrocytes,[45,46] adipocytes, hematopoietic support[47,48] (both hematopoietic stem cells and monocyte-macrophage development into osteoclasts), and myoblasts.[49] These *in vitro* assays serve as vehicles for studying the control of the pathways of differentiation from MSCs, but they also serve as the starting point for the tissue-engineering strategies of today.

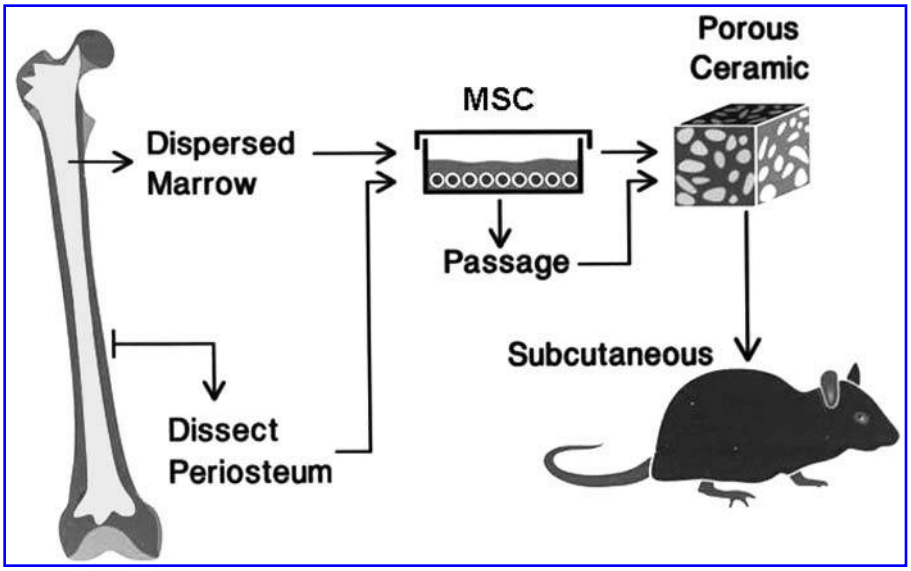

FIG. 4. Whole marrow or dissected periosteum can provide progenitor cells or MSCs. When passaged, the liberated cells can be loaded into fibronectin-coated porous calcium phosphate ceramic 3-mm cubes and implanted subcutaneously in immunounreactive or syngeneic animals.[31,33,34,36]

TODAY

Cell-based clinical therapies using MSCs involve at least three different approaches: First, tissue-engineering strategies in which MSCs are incorporated into three-dimensional (3-D) scaffolds for the replacement of 3-D pieces of *in vivo* tissues; second, cell replacement therapy, in which genetic defects can be cured by replacing the mutant host cells with normal allogeneic donor cells; and third, where MSCs act as cytokine/growth factor pumps to stimulate reparative events or to inhibit degenerative events.

Tissue engineering

We have published studies on the tissue-engineered repair/regeneration of cartilage,[50–57] bone,[39–43] and ten-

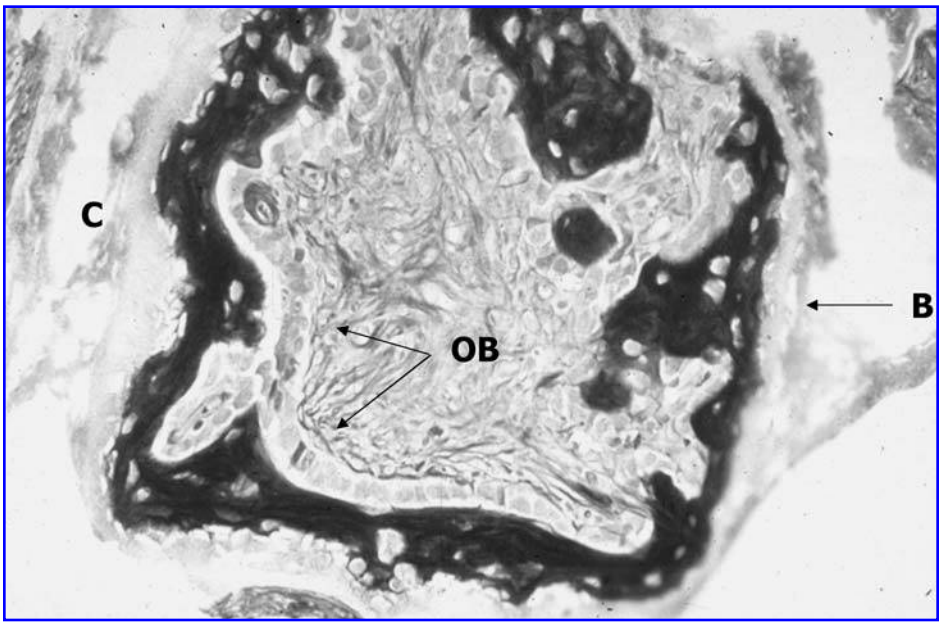

FIG. 5. Calcium phosphate porous ceramic cubes that are implanted in syngeneic or immunounreactive rodents are harvested at 3 or 6 weeks and yield specimens that exhibit bone and cartilage when examined in paraffin sections.[33,34] Shown in a decalcified cube section in which the dark-staining, newly formed bone (B) is being laid down by a layer of osteoblasts (OB) with vasculature at their backs. C, decalcified ceramic residue.

don.[37,58] Each site requires a different 3-D scaffold and a different logic. I emphasize cartilage here because the cell delivery vehicle itself provides cueing both on the initial exposure of the cells and also during the process of tissue filling.[59] The breakdown of the delivery vehicle triggers the final phases of tissue change and the breakdown products add value to the molecular and cellular events related to successful repair.

The important common tissue-engineering issues for all mesenchymally derived tissues with regard to the delivery of MSCs and their appropriate and sequential changes are as follows: the scaffold must

1. Allow for and encourage cell attachment
2. Be porous so the differentiated cells can make abundant and specialized extracellular matrix
3. Allow bioactive molecules to have access to the cells
4. Perfectly integrate into the neotissue or silently disappear
5. Provide some cellular cueing
6. Be mechanically sensitive to the site

Cartilage repair. For cartilage repair, we used a fibronectin-coated sponge formed from hyaluronan (HA) (Fig. 6). With embryonic chick limb bud mesenchymal cells, we showed that high molecular weight HA bonded to the petri dish was chondroinductive.[60,61] Moreover, high molecular weight HA is antiangiogenic. In addition, in porous calcium phosphate ceramic vehicles in subcutaneous sites, MSCs in vascular-excluded pores form cartilage. Thus, MSCs in porous HA sponges provide an avascular and chondroinductive microenvironment. In deep, critical-sized osteochondral defects made in the medial femoral condyle of adult rabbits (Fig. 6), sponges of HA filled with MSCs (Fig. 7) uniformly differentiate into chondrocytes. When the HA of the scaffold is degraded into oligomers, the oligomers trigger the entrance of vasculature into the hypertrophic cartilage at the base of the defect that is followed by replacement by vascularized bone. The cartilage at the top of the defect not only does not undergo replacement, but, more striking, integrates the neocartilage with the host cartilage. We suggest that the HA oligomers facilitate this tissue integration.[50–57] Thus, the 3-D HA delivery vehicle meets all the criteria listed above for a successful tissue-engineering scaffold.

Tendon repair. For tendon repair, the same cells, MSCs, that form cartilage or bone were formatted into a type I collagen gel that formed in a trough around a resorbable suture, the ends of which were fastened to a spring that keep the suture under constant load (Fig. 8). The MSCs contracted the gel and because the suture was loaded, cells oriented with regard to the suture.[37,58] The partially contracted cell–gel–suture composite was sutured into and aligned with the load axis within an Achilles tendon defect in adult rabbits. At 3 months, the neotissue formed was well-integrated tendon tissue. Again, the tissue and site required a specific delivery vehicle to take advantage of the mechanical and chemical microenvironment necessary for the MSCs to develop into functional tenocytes.[37,58]

Cell Replacement Therapy

The bone marrow is a highly differentiated and complex, multicomponent tissue. The major component is the marrow stroma, which is a multicompartment connective

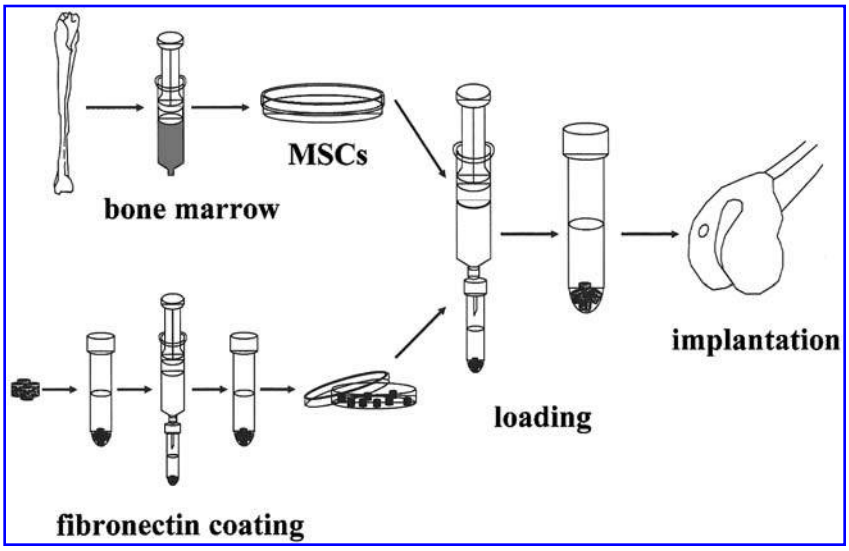

FIG. 6. Cartilage repair protocol in which sponges are coated with fibronectin and left to dry overnight. These sponges are filled with either freshly isolated whole bone marrow, liberated culture-expanded MSCs, or left empty as they are placed into full thickness defects in adult rabbit condyles.[50–52,54–56]

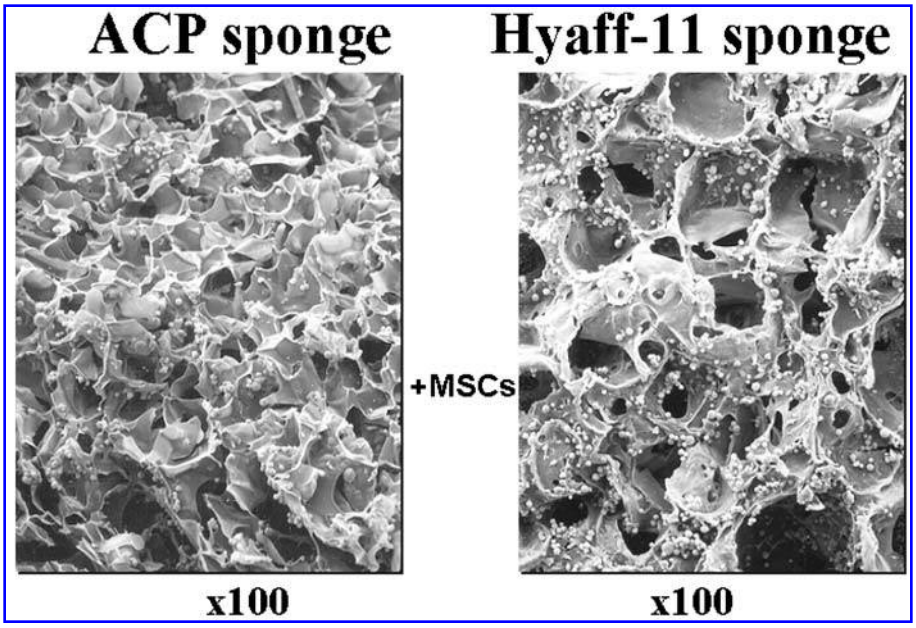

FIG. 7. ACP (auto-cross-linked polysaccharide) and Hyaff-11 sponges are pictured in scanning electron micrographs after coating the pores of these sponges with mesenchymal stem cell preparations from rabbit.[54,55] Original magnification: ×100.

tissue that houses and supports hematopoiesis. This support involves establishing niches that physically house specific arms of the multilineage hematopoietic pathway; both "floor space" and a specific microenvironment of cytokines are provided by the marrow stroma and its stromal cells. Thus, after chemotherapy or radiation, which destroys the hematopoietic progenitors and these pathways, these niches must be reestablished to facilitate hematopoietic engraftment and the production of various blood cells. The other major components of marrow are blood vessels, osteoprogenitor cells, and MSCs. Thus, we developed clinical protocols to establish that autologous

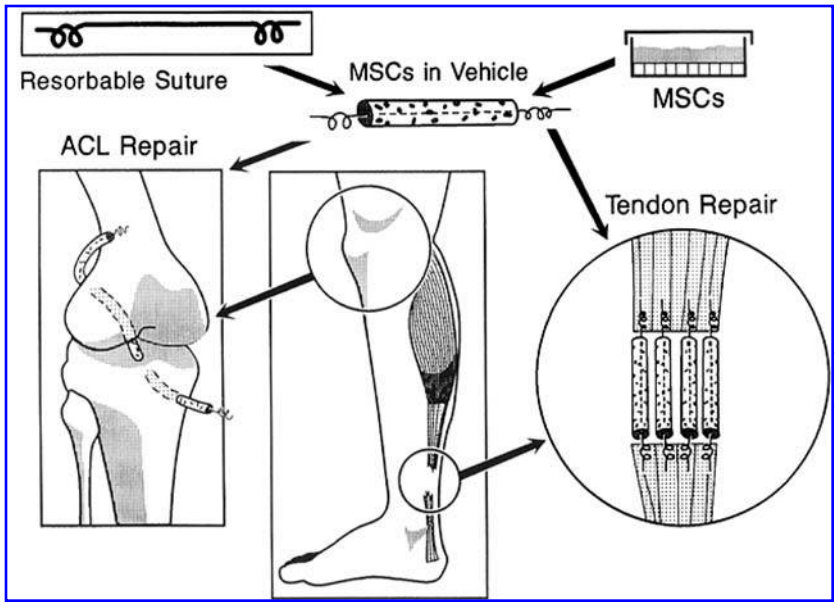

FIG. 8. Ligament/tendon repair is organized by taking a resorbable suture, which is held by a spring under fixed load in a trough that serves to house a collagen gel in which MSCs have been placed. This gel forms in the trough around the resorbable suture and the MSCs contract the gel around this loaded suture. This composite gel–cell–suture is then sutured into place into achilles tendon defects created in adult rabbits.[37,58]

MSCs could be safely and efficaciously delivered back to patients to reestablish this delicate marrow microenvironment.[62,63]

Cell replacement therapy involves eliminating cells with specific genetic defects or mutations and replacing them with allogeneic normal cells or gene therapy-transfected cells[64] (Fig. 9). For the genetic disease osteogenesis imperfecta (OI), there is usually a point mutation in the type I collagen gene. The type I collagen produced by osteoblasts is defective and, thus, the osteoid and subsequent mineral deposition are likewise defective. The resulting bone stock is brittle and multiple fractures occur because these bones cannot withstand normal loads. To cure OI, it would be theoretically possible to use allogeneic MSCs from an immunomatched donor[4] and to destroy some (or preferably all) of the host MSCs and replace them with donor (nonmutant) MSCs (Fig. 9). Indeed, this has been tried with children with OI in a two-step procedure.[5,6] The first step was to do a classic allotransplantation in which whole bone marrow from immunomatched donors was provided after mild chemotherapy. The result was to provide some allotolerance by engraftment of allohematopoietic stem cells and some allo-MSCs. Subsequently, after 18 to 32 months, the children were intravenously given isolated, culture-expanded allo-MSCs matching their original allograft.[5] Most of the children experienced rather substantial increases in skeletal growth. In my view, these therapies were not totally curative because I suspect, on the basis of labeling and imaging studies,[65] that the engraftment of allo-MSCs was low. As addressed below, we must find ways to improve MSC engraftment efficiency to ensure that these cell replacement therapies can provide cures.

Experimentally, we have also tried to cure muscular dystrophy in mice by providing MSCs that do not have the disease-causing mutation.[49,66] A mouse strain called *mdx* has a point mutation in the muscle-specific protein dystrophin (Fig. 10). Using an antibody against dystrophin, we can show that muscle sections are negative for dystrophin.[66] In muscles injected with congenic, normal MSCs, dystrophin-positive myotubes can be seen. The injected MSCs differentiate into myoblasts[66] and fuse with the mutant myotubes and the incorporated nuclei produce normal dystrophin. Control fibroblasts do not show this effect. Again, the limiting factor involves getting enough normal MSCs to the muscles of the *mdx* mouse to effect a cure; injecting every muscle in the body is neither feasible nor prudent.

MSCs as Cytokine/Growth Factor Pumps

In two very different, but in some ways similar, animal models, MSCs have been introduced into infarct (ischemia) lesions in the heart[67–70] and brain[71,72] (stroke model). The MSCs do not act by differentiating into cardiac myocytes or neural elements, respectively, as shown by cell-marking experiments, but rather secrete molecules that increase angiogenesis and decrease scarring or fibrosis. There is no doubt that in the cardiac infarct model (rats and pigs) there is substantial cell death of muscle tissue. However, the contractility and flexibility of the tissue are not compromised and, thus, the heart output is not grossly affected.[67–70] In the case of brain, endogenous neural progenitors respond, migrating and functionally repairing neurological damage so that in functional tests, the animals clearly function.[71,72] In both

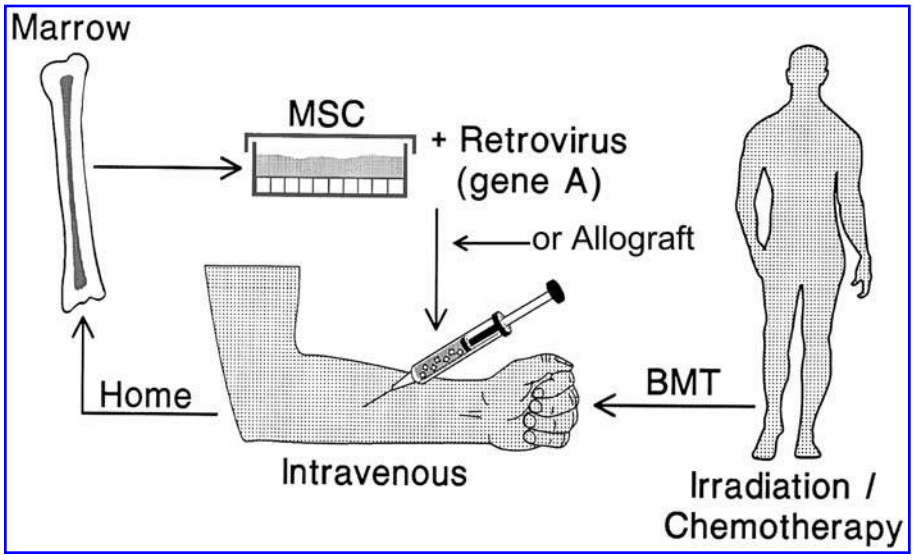

FIG. 9. Allo- or autologous gene therapy. Mesenchymal stem cells can be transfected with retrovirus to house normal genes or allograft preparations can be presented to patients undergoing bone marrow transplantation. Mesenchymal stem cells introduced in this way will home to bone marrow and have the potential to correct gene defects.[4,62,63]

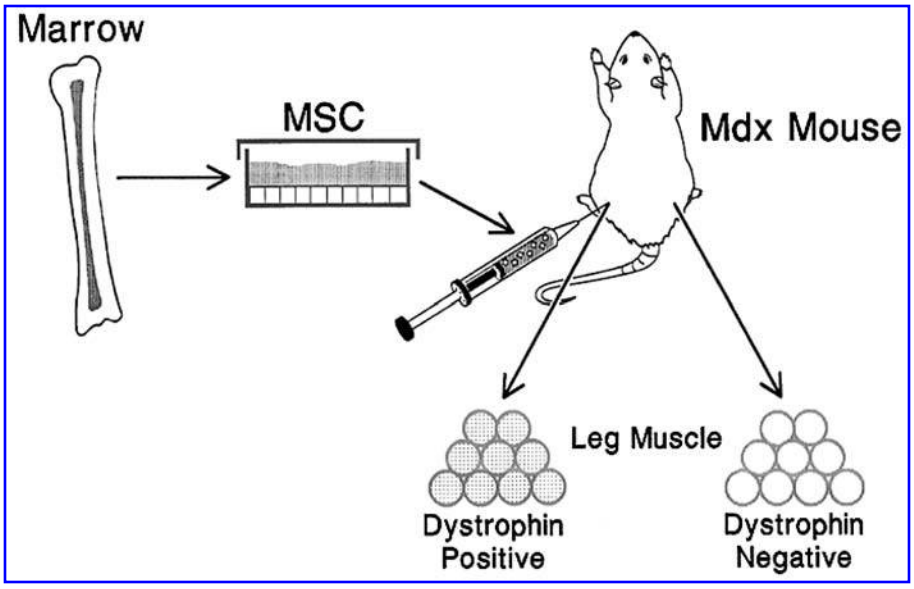

FIG. 10. Congenic bone marrow has been harvested and the MSCs purified. These MSCs have been injected into a specific muscle of the *mdx* mouse on the left side of the animal; on the right side, saline controls were injected. After 8 weeks, individual muscles were harvested, sectioned, and exposed to antibodies for dystrophin. MSC-injected muscles exhibited dystrophin-positive myotubes, which indicated that the injected MSCs differentiated into myoblasts that fused with the host myotubes. The newly fused nuclei, which are normal, produced dystrophin in the myotube and cured the genetic defect in these mice.[66]

of these ischemia models for heart and brain, the predominant mechanism for the improved functional outcomes are the MSC-caused inhibition of fibrosis or scar formation and the increase in vascular elements (angiogenesis). We have reported that human MSCs, as they enter the osteogenic versus stromal lineage, secrete a distinct set of cytokines constitutively.[35] Thus, I imagine that MSCs could exert therapeutic effects by this cytokine secretory activity alone, just as they provide such cytokine support of hematopoiesis in bone marrow.

TOMORROW

Luis Solchaga and others in our group have made unusual and significant gains in learning how to supplement

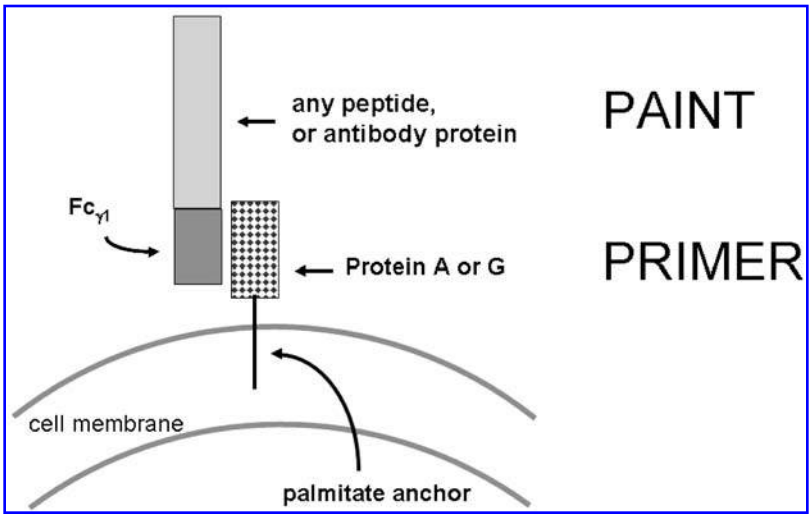

FIG. 11. By using fusion proteins (paints) that have the Fc region of antibodies at one end of the molecule, tight complexes can be assembled on protein A or protein G (primer) molecules, which are noncovalently anchored to cell membranes by palmitic acid. This primer-painting technology forms the basis for cell-targeting strategies that are now being experimentally used.[74]

MSC growth medium (with selected batches of FBS) with extra amounts of specific growth factors to enhance their mitogenic activity and to enhance MSC differentiation potency into specific phenotypes (our unpublished observations). It is premature to fully discuss this new approach here, but it is a "tomorrow" technology that will profoundly affect MSC therapies by structuring their culture expansion to provide maximally efficient and specific differentiation.

In addition, we have been systematically trying to improve MSC engraftment and tissue-targeting efficiency.[65] We have adapted a cell-coating technology initially described by Mark Tykocinski and colleagues originally at our university,[73] but now at the University of Pennsylvania (Philadelphia, PA). This technology, which we have called cell painting,[74] involves attaching a coat of "primer," palmityl fatty acid to which we covalently link the antibody-binding protein, protein G (Fig. 11). The fatty acid quantitatively inserts into the plasma membrane of the cell with the protein G facing out. Protein G binds tightly to the Fc region of antibodies. Thus, we apply a coat of primer and then attach the paint (any antibody or antibody mixture or fusion protein containing the cassette for the Fc region of an antibody). We have shown that the primer and paint do not affect the viability, mitotic activity, or differentiation potential of MSCs or other cells. In an *in vitro* test of the targeting ability of antibody-painted human cells, we created a cartilage defect in an osteochondral plug from a rabbit knee (Fig. 12). The antibodies were against epitopes in deep cartilage matrix and did not bind to the top of cartilage. Not only did the cells specifically bind to the deep cartilage layer of the defect, but after a 2-week incubation in culture, the painted human chondrocytes made human cartilage matrix that started filling the rabbit defect[74] (Fig. 12). Thus, the challenge for us is to develop this targeting technology so that painted reparative cells can be injected directly into the joint space, where they will dock onto

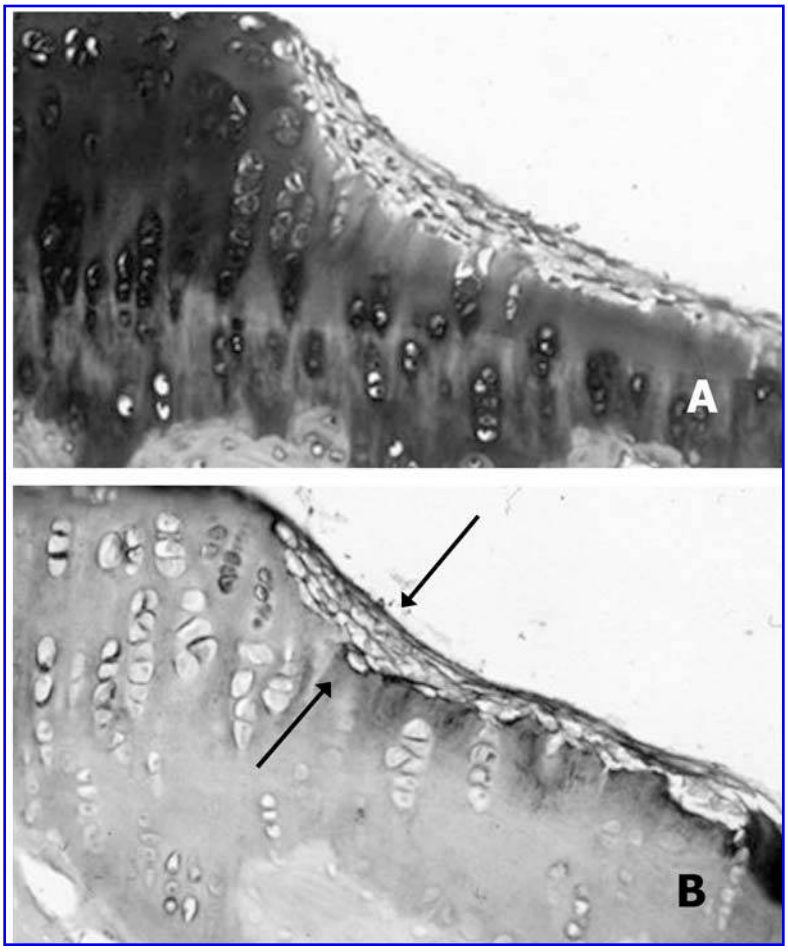

FIG. 12. Human chondrocytes that have been painted with antibodies to type II collagen and glycosaminoglycan epitopes and delivered *in vitro* to a defect in rabbit cartilage. The arrows in (**B**) indicate the human type II collagen-positive cartilage matrix that is being laid down after 2 weeks of *in vitro* incubation. (**A**) Toluidine blue-stained section; and (**B**) human type II collagen immunostaining.[74]

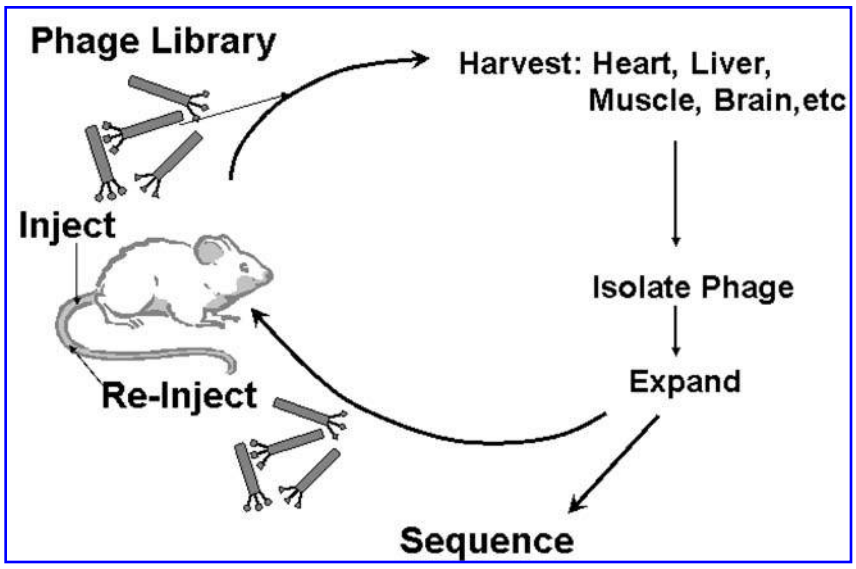

FIG. 13. Phage display library was injected intravenously into mice and individual organs were isolated. From these isolated organs, the associated phage were grown and injected in two more rounds, and the same tissue was isolated. After the third round of exposure the tissue-anchored phage were cloned and the DNA inserts were sequenced.[75,76]

damaged cartilage tissue and start to repair it. Because cartilage is avascular and is in the closed synovial space, it does not have access to reparative cells. By using a targeting strategy, it may be possible to deliver repair cells to the site of damage without opening the joint space: a tomorrow technology.

Likewise, how can we enhance engraftment of MSCs to bone marrow or to muscle to cure genetic diseases? Again, the painting technology can be adapted. On the basis of experiments of Ruoslahti and colleagues[75,76] (Fig. 13), the identity of peptides (Fig. 14) from a phage display library that bind specifically to certain tissues via the vascular tree are known. These vascular and tissue-specific peptide addresses have been shown also to be present in humans[77] (Fig. 14). Thus, a fusion protein of tissue-specific (e.g., muscle or bone marrow) peptide hooked to an Fc cassette will allow us to paint tissue-specific targeting molecules onto MSCs. The peptide region of the paint will bind with its docking site in the specific tissue. By using noninvasive imaging techniques, we intend to perfect this tomorrow technology in the near future.

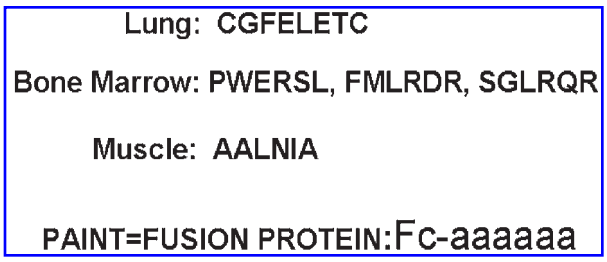

FIG. 14. From the experiments pictured in Fig. 13, targeting addresses for the phage peptide inserts for lung, bone marrow, and muscle have been obtained by sequencing phage clones associated with these tissues.[75,76]

PROSPECTUS

My colleagues and I have followed a basic science pathway that has led us from understanding the formative events in mesenchymal tissue development in the limb to establishing basic science principles of tissue engineering.[78] Orthopedic surgeons have been engineering complex tissue repair and replacement strategies for centuries. In this regard, the dominant principles were mechanical and the implant materials were primary metals. A new era of biologic orthopedics and orthobiologics encompasses new principles of cell and molecular therapies. This new era requires complicated new procedures, new materials, and new talents. The current cell-based, tissue-engineering strategies will set the foundation for learning how to manage the body's intrinsic capacity to repair and rejuvenate skeletal tissues. Ultimately, the expansion of cells outside the body must be replaced by pharmaceutical strategies to bring the reparative cells to the injury site, expand them and differentiate these cells to fill the defect, and induce the neotissue to functionally integrate into the host tissue. It is anticipated that the orthopedic surgeon will be required to transform from a hardware expert into a "mesenchemist" to ensure that the proper bioactive factor is placed in the proper location at the correct time and in the optimal amount to facilitate the body's self-repair by controlling its intrinsic repair-

regeneration capacity. In this regard, new industries must evolve to provide new implant materials that provide morphological boundaries, multiagent release characteristics, and dynamic structural changes to facilitate the proper mechanical and structural properties of the new tissue. Just as power tools have changed orthopedists from carpenters to cabinet makers, so will biologics transform them into conductors of cellular symphonies.

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