2010 Nicolas Andry Award: Multipotent Adult Stem Cells from Adipose Tissue for Musculoskeletal Tissue Engineering

Farshid Guilak, Bradley T. Estes, Brian O. Diekman,
Franklin T. Moutos, Jeffrey M. Gimble

Received: 31 October 2009 / Accepted: 18 May 2010 / Published online: 13 July 2010
© The Association of Bone and Joint Surgeons® 2010

Abstract

Background Cell-based therapies such as tissue engineering provide promising therapeutic possibilities to enhance the repair or regeneration of damaged or diseased tissues but are dependent on the availability and controlled manipulation of appropriate cell sources.

Questions/purposes The goal of this study was to test the hypothesis that adult subcutaneous fat contains stem cells with multilineage potential and to determine the influence of specific soluble mediators and biomaterial scaffolds on their differentiation into musculoskeletal phenotypes.

Methods We reviewed recent studies showing the stem-like characteristics and multipotency of adipose-derived

stem cells (ASCs), and their potential application in cell-based therapies in orthopaedics.

Results Under controlled conditions, ASCs show phenotypic characteristics of various cell types, including chondrocytes, osteoblasts, adipocytes, neuronal cells, or muscle cells. In particular, the chondrogenic differentiation of ASCs can be induced by low oxygen tension, growth factors such as bone morphogenetic protein-6 (BMP-6), or biomaterial scaffolds consisting of native tissue matrices derived from cartilage. Finally, focus is given to the development of a functional biomaterial scaffold that can provide ASC-based constructs with mechanical properties similar to native cartilage.

Conclusions Adipose tissue contains an abundant source of multipotent progenitor cells. These cells show cell surface marker profiles and differentiation characteristics that are similar to but distinct from other adult stem cells, such as bone marrow mesenchymal stem cells (MSCs).

Clinical Relevance The availability of an easily accessible and reproducible cell source may greatly facilitate the development of new cell-based therapies for regenerative medicine applications in the musculoskeletal system.

One or more authors have received funding from the Duke Translational Medicine Institute RR24128 (FG), the Coulter Translational Research Partnership (FG), the NIH (Grants AR50245, AG15768, AR48182, and AR48852) (FG), and a NSF Graduate Fellowship (BOD).

The authors (FG, BTE, FTM, JMG) have filed patents on topics related to the contents of this paper. One of the authors (FG) owns equity in Cytex Therapeutics, Inc.

This work was performed at Duke University Medical Center.

F. Guilak, B. T. Estes, B. O. Diekman, F. T. Moutos
Departments of Surgery and Biomedical Engineering,
Duke University Medical Center, Durham, NC 27710, USA

F. Guilak (✉)
Orthopaedic Research Laboratories, Department of Surgery,
Division of Orthopaedic Surgery, Duke University Medical
Center, 375 MSRB, Box 3093, Durham, NC 27710, USA
e-mail: guilak@duke.edu

J. M. Gimble
Stem Cell Laboratory, Pennington Biomedical Research Center,
Louisiana State University System, 6400 Perkins Road,
Baton Rouge, LA 70808, USA

Introduction

Tissue engineering seeks to repair or replace damaged or diseased tissues of the body by implanting combinations of cells, biomaterial scaffolds, biologically active molecules, and genes. An underlying premise of this approach is that exogenously introduced cells will improve the speed and extent of tissue repair. To this end, there is a significant need for potential sources of cells for tissue engineering and other cell-based therapeutic approaches, such as gene therapy.

In recent years, there has been a growing emphasis on the use of undifferentiated progenitor cells for tissue engineering owing to their ability to be expanded in culture and to differentiate into multiple cell types. Although historically there has been controversy regarding the presence of true adult stem cells outside the hematopoietic system, it now is apparent that many adult tissues harbor cells that have the ability to differentiate into multiple cell types, once cultured under specific growth conditions [18]. Depending on their differentiation potential and site of origin, these cells have been described using various terms, such as MSCs, multipotent adult progenitor cells, marrow stromal cells, or mesenchymal progenitors [13, 15, 17, 44, 68, 69, 72–74, 80, 85]. The adult stem cell can be defined as an “undifferentiated (unspecialized) cell that is found in a differentiated (specialized) tissue; it can renew itself and become specialized to yield all of the specialized cell types of the tissue from which it originated” [19]. Because of these characteristics, adult stem cells have been used extensively in various musculoskeletal tissue engineering applications.

We have identified the presence of an abundant source of undifferentiated progenitor cells residing in various adipose tissue sites in the human body. These cells, termed ASCs, or adipose-derived adult stromal (ADAS) cells, show cell surface marker profiles and differentiation characteristics similar to other adult stem cells, such as bone marrow-derived MSCs. Under controlled culture conditions, these cells show phenotypic characteristics of numerous cell types, including chondrocytes, osteoblasts, adipocytes, neuronal cells, or muscle cells. These characteristics are present at the clonal level, suggesting that individual cells have multipotent capabilities. The major advantages of such cells are their ease of availability through standard liposuction procedures and their abundance in normal human fat.

We will review our recent studies that show the stem-like properties and multipotency of ASCs, and their promise for use in cell-based therapies and functional tissue replacement for the musculoskeletal system. In particular, we focus on the application of ASCs for regeneration or repair of cartilaginous tissues, addressing the following questions: (1) Does adult adipose tissue contain multipotent stem cells?; (2) Are these cells capable of chondrogenic differentiation?; (3) What are the influences of various growth factors, biomaterial scaffolds, and environmental cues such as oxygen tension on ASC chondrogenesis?; (4) What effect do these factors have on the functional biomechanical properties of ASC-based constructs?

Background: Articular Cartilage Injury and Repair

Articular cartilage functions as a nearly frictionless load-bearing surface in diarthrodial joints, withstanding loads of

several times body weight for decades [67]. The cartilage extracellular matrix is maintained by a sparse population of cells (chondrocytes) but exhibits little capacity for self-repair owing to the lack of a tissue blood supply or a source of undifferentiated cells that can promote repair. Isolated cartilage lesions may be responsible for significant pain or loss of function, and may lead to degenerative arthritis in the joint with time [42, 43]. To promote cartilage repair, several surgical techniques have been developed to induce bleeding and clot formation, including drilling or microfracture of the subchondral bone [1, 8, 29, 31, 43]. Numerous tissue engineering approaches have been proposed for enhancing cartilage regeneration in vitro or in vivo [2, 12, 14, 30, 34, 41, 55, 56, 70, 77, 79, 81].

However, significant challenges continue to exist in the long-term repair or replacement of cartilage, and there is currently one cell-based therapy available clinically for cartilage repair that involves implantation of autologous isolated chondrocytes (CarticelTM; Genzyme Biosurgery, Cambridge, MA, USA) [10]. Although clinical studies of autologous chondrocyte transplantation generally have reported significant improvements in pain, swelling, and function after surgery [9, 60], one controlled prospective trial showed no benefit of autologous chondrocyte implantation as compared with the standard treatment of microfracture of the subchondral bone, as based on indices of joint function, pain, or characteristics of the cartilage defect [47]. Furthermore, an animal study suggested the harvest procedure may induce donor-site morbidity and iatrogenic damage that may initiate osteoarthritic degeneration in the joint [50]. Thus, such procedures clearly would benefit from the availability of a safe and abundant source of chondrocyte progenitor cells for cartilage repair.

Adipose Tissue as a Source of Multipotent Adult Stem Cells

The isolation procedure of ASCs from adipose tissue has been detailed [23]. In brief, liposuction tissue is washed thoroughly in phosphate buffered saline to remove contaminating erythrocytes, and then digested using type I collagenase. The stromal-vascular fraction of cells is separated from the mature lipid-laden adipocytes by centrifugation. This fraction, which represents a heterogeneous population of cells, contains the ASCs [37, 86]. A large number of ASCs can be harvested in this manner, with yields of approximately 250,000 cells per gram of tissue [3]. This population of cells can be further expanded and purified on tissue culture plastic, yielding approximately 10^9 cells after 2 weeks of expansion. During expansion, our studies show that ASCs do not exhibit markers of hematopoietic stem cells (eg, CD45 and CD14)

Fig. 1A–B (A) Under specific and controlled culture conditions, ASCs can be induced to express the phenotypic characteristics of chondrocytes, osteoblasts, adipocytes, or neurons. (B) Forty-five individual clones were expanded through four passages in culture and then differentiated to adipogenic, osteogenic, chondrogenic, and neurogenic lineages. Fifty-two percent of the cell clones showed stem cell characteristics by displaying bipotent and tripotent differentiation potential. (Reproduced with permission from Guilak F, Awad H, Fermor B, Leddy HA, Gimble JM. Adipose-derived adult stem cells for cartilage tissue engineering. *Biorheology*. 2004;41:389–399.) [33]

but display a similar marker surface profile to marrow derived MSCs [32, 83], although, it generally is recognized there are no specific markers readily available to identify nonhematopoietic stem cells. Nevertheless, ASCs express the stromal markers CD9, CD10, CD29, CD44, CD73, CD90, and CD166, and with increasing passage, the expression of these markers increases while the presence of hematopoietic markers declines [57, 61].

To examine the multipotency of individual cells, ASCs were cultured and ring cloning was performed to select cells derived from one progenitor cell [36]. Forty-five clones were expanded through four passages and then induced for adipogenesis, osteogenesis, chondrogenesis,

and neurogenesis using lineage-specific differentiation media. Quantitative differentiation criteria for each lineage were determined using histologic and biochemical analyses. Our findings showed that 81% of the ASC clones differentiated into at least one of the lineages (Fig. 1). In addition, 52% of the ASC clones differentiated into two or more of the lineages. More clones expressed phenotypes of osteoblasts (48%), chondrocytes (43%), and neuron-like cells (52%) than of adipocytes (12%), possibly owing to the loss of adipogenic ability after repeated subcultures. These findings support the hypothesis that ASCs are a type of multipotent adult stem cell and not simply a mixed population of unipotent progenitor cells.

Chondrogenic Potential of Human ASCs

Our work has focused primarily on the ability of ASCs to produce cartilaginous tissue molecules for potential application in the repair of tissues such as articular cartilage, meniscus, or intervertebral disc (eg, [4, 20, 22, 26, 27, 82, 83]). Through numerous studies, it was shown that under specific conditions, ASCs can express the genes and proteins for several cartilage-specific molecules, including type II collagen and aggrecan, without expression of hypertrophic chondrocyte markers such as type X collagen [26]. Nonetheless, under different defined conditions, ASCs can be induced to synthesize type I and type II collagen, suggesting that a fibrocartilaginous phenotype also is possible [5].

The medium used to induce chondrogenesis was based on that developed by Johnstone et al. for inducing similar differentiation of bone marrow derived MSCs [45]. When maintained in pellet culture or encapsulated in alginate beads and cultured with 10 ng/mL of transforming growth factor beta 1 (TGF- β 1), ascorbate, and dexamethasone, ASCs have been shown to express a chondrocyte-like phenotype and synthesize collagen type II, aggrecan, link protein, and chondroitin sulfate in a time-dependent manner based on mRNA analysis, immunohistochemistry, and radiolabel incorporation [4, 22, 83], with significant enhancement of proteoglycan and protein synthesis under chondrogenic conditions. Winter et al. reported that the gene expression profile of ASCs under chondrogenic conditions is similar to that of MSCs [39]. Following an in vitro differentiation process, Erickson et al. showed that human ASCs retain the chondrocyte phenotype and form cartilaginous tissue when implanted subcutaneously in vivo in immunodeficient mice for up to 12 weeks [22].

The Influence of Culture Conditions on ASC Chondrogenesis

The combinations of growth factors and media supplements used to induce chondrogenic differentiation of progenitor cells such as bone marrow MSCs have been defined empirically, and may depend on numerous factors such as concentration, duration of exposure, or cell type. In a series of studies, it was shown that the chondrogenic potential of ASCs depends on numerous culture conditions such as the expansion/growth media used, the number of passages, and the composition of the extracellular matrix [4, 20, 24, 26, 27].

In one study, more than 27 combinations of growth factors, fetal bovine serum (FBS), and other media supplements were studied to examine the influence of these soluble mediators on ASC chondrogenesis [4]. The findings

showed that these factors may act in an additive or synergistic manner, depending on concentration and time of exposure. For example, the serum substitute ITS + (insulin, transferring, and selenious acid) and TGF- β 1 function to increase ASC proliferation. Similarly, TGF- β 1 and dexamethasone promote protein synthesis rates in an additive manner in the presence of ITS + or FBS. Of particular note was the finding that dexamethasone, which often is used as a media supplement for chondrogenesis, suppresses the ability of TGF- β 1 to stimulate proteoglycan synthesis and accumulation by 1.5- to twofold.

Using further screens of numerous different growth factors, it was found that BMP-6 applied in solution [26] or through genetic transfection of ASCs [20] greatly enhances the chondrogenic potential of ASC cells encapsulated in alginate beads. In particular, soluble BMP-6 upregulated aggrecan and collagen II expression by an average of 205-fold and 38-fold, respectively, over Day 0 controls, while downregulating collagen X expression (as a marker of hypertrophic differentiation) by twofold. These changes in mRNA expression levels were paralleled at the protein level (Fig. 2). In contrast to bone marrow MSCs, which exhibit increased expression of type X collagen in response

Fig. 2A–D Immunohistochemistry of human ASCs in alginate beads after 7 days in culture showed the presence of chondrogenic markers in the presence of TGF- β 1 or BMP-6. (A) The standard growth factor combination of TGF- β 1 + dexamethasone (DEX) used for chondrogenesis of MSCs induced the production of type II collagen by ASCs. (B) Collagen II labeling was increased in the presence of BMP-6 as compared with TGF- β 1 + DEX. (C) TGF- β 1 + DEX increased type X collagen labeling in ASCs, suggestive of a hypertrophic phenotype. (D) Type X collagen expression decreased with BMP-6 treatment. (Reproduced with permission from Estes BT, Wu AW, Guilak F. Potent induction of chondrocytic differentiation of human adipose-derived adult stem cells by bone morphogenetic protein 6. *Arthritis Rheum.* 2006;54:1222–1232.) [26]

to BMP-6, these findings suggest that BMP-6 serves as a potent regulator of ASC chondrogenesis. This finding has been confirmed, and data indicate that BMP-6 may be especially important for ASC chondrogenesis owing to differences in TGF- β receptor expression as compared with MSCs [39].

The Influence of Biomaterial Scaffolds on ASC Chondrogenesis

In other studies, the influence of different biomaterial scaffolds on chondrogenesis of ASCs was investigated. In addition to the biochemical interactions that scaffolds may have with cell surface receptors, there is growing evidence of the important role that physical interactions between stem cells and their extracellular matrix have in regulating stem cell fate [25, 35]. One of the studies showed that different construct materials or structures can significantly influence the differentiation of ASCs and functional properties of the tissue-engineered construct [5]. For example, scaffolds that maintain the cells with a rounded shape and prevent cell-to-cell contact (eg, alginate or agarose) promote a chondrogenic phenotype and prevent the expression of type I collagen. Conversely, porous gelatin scaffolds (Surgifoam®, Johnson and Johnson, New Brunswick, NJ, USA) or fibrin-based scaffolds (Tisseel®, Baxter Bioscience, Westlake Village, CA, USA) also support the chondrogenic differentiation of ASCs but induce type I and type II collagen expression, suggesting the differentiation of ASCs into a fibrocartilaginous phenotype is associated with a more fibroblastic cell shape [48] (Fig. 3).

It also has been shown that a novel genetically engineered biomaterial scaffold can directly influence the chondrogenic potential of ASCs. This material consists of a genetically modified version of native elastin, termed an elastin-like polypeptide (ELP), which is thermally sensitive and undergoes an inverse temperature transition (ie, liquid at room temperature and a solid gel at body temperature) [59]. This property readily allows cellular encapsulation, providing an important advantage for cellular delivery in the context of tissue engineering [7]. Human ASCs were cultured in ELP hydrogels in either chondrogenic or standard media for 2 weeks, at which point, constructs in either medium showed significant increases in sulfated glycosaminoglycans (up to 100%) and collagen content (up to 420%), particularly collagen II with little formation of collagen I. At the mRNA level, ASCs seeded in ELP showed upregulated SOX9 and type II collagen gene expression, whereas type I collagen was downregulated. These findings provide additional evidence of the chondrogenic ability of ASCs and the ability of biomaterial

Fig. 3A–D Cell viability and morphologic features can be seen in different scaffold materials observed using confocal laser scanning microscopy and the Live-Dead fluorescent probes. Cells in (A) agarose and (B) alginate scaffolds had spherical morphologic features that persisted throughout the culture period, regardless of culture conditions. By contrast, the cells in the (C) gelatin scaffolds showed distinct fibroblastic morphologic features at Day 7. (D) By Day 28, the cells in the gelatin scaffolds proliferated and became confluent with significant cell-cell contact as they exerted considerable contraction of the scaffolds. Scale bar = 50 μ m (A, B, C) or 200 μ m (D). (Reproduced with permission from Awad HA, Wickham MQ, Leddy HA, Gimble JM, Guilak F. Chondrogenic differentiation of adipose-derived adult stem cells in agarose, alginate, and gelatin scaffolds. *Biomaterials*. 2004;25:3211–3222.) [5]

scaffolds to directly control stem cell differentiation [35], even in the absence of exogenous growth factors [6].

In other studies, it was shown that biomaterial scaffolds created solely from native extracellular matrix tissues also can control the differentiation of ASCs [16, 21]. Using a porous scaffold derived exclusively from articular cartilage, chondrogenesis of ASCs in the absence of exogenous growth factors was examined. ASCs showed increased gene expression and biosynthesis of cartilage-specific extracellular matrix components, particularly type II collagen (Fig. 4), and mechanical testing showed significant increases in the mechanical properties of the ASC-seeded constructs with time, with a threefold increase in the aggregate modulus during 6 weeks in culture.

The Role of Oxygen Tension in Chondrogenesis

Although it often is acknowledged that cell culture conditions used for tissue engineering fail to mimic the *in vivo* condition, it is unclear whether reproducing *in vivo*

Fig. 4A–D A porous scaffold created purely from native articular cartilage supports chondrogenesis of ASCs. Shown are (A) the gross morphologic features of the cartilage-derived matrix scaffold, (B) a scanning electron micrograph of cartilage-derived matrix scaffold, (C) a microCT showing high porosity of cartilage-derived matrix scaffold, and (D) immunohistochemical samples showing chondrogenesis by ASCs in the porous cartilage derived scaffold. In the first row are blank scaffolds before seeding; in the second row are samples from Day 28; and in the third row are samples from Day 42. Scale bar = 1 mm (A, B, C) and 200 μ m (D). (Reproduced with permission from Cheng NC, Estes BT, Awad HA, Guilak F. Chondrogenic differentiation of adipose-derived adult stem cells by a porous scaffold derived from native articular cartilage extracellular matrix. *Tissue Eng Part A*. 2009;15: 231–241.) [16]

characteristics can influence chondrogenesis. For example, articular cartilage exists in a reduced oxygen (approximately 1%–5%) environment *in vivo*; therefore, oxygen tension has been hypothesized to be an important factor that regulates the metabolism of cartilage [62]. There is significant evidence that oxygen tension can influence the differentiation and biosynthetic activity of primary chondrocytes as they undergo passage in culture [67]. To examine the influence of oxygen on ASC chondrogenesis, human ASCs in alginate beads were cultured in control or chondrogenic media at either low oxygen (5%) or atmospheric oxygen tension (20%) for up to 14 days [82]. Low oxygen tension significantly inhibited the proliferation of ASCs, but induced a twofold increase in the rate of protein synthesis and a threefold increase in total collagen synthesis. Low oxygen tension also increased glycosaminoglycan synthesis at certain times. Immunohistochemical analysis showed significant production of cartilage-associated matrix molecules, including collagen type II and chondroitin-4-sulfate. These findings show that oxygen tension can play an important role in regulating the proliferation and differentiation of human ASCs as they undergo chondrogenesis and suggest that manipulation of the physiochemical culture environment may provide additional means of controlling the activity of undifferentiated progenitor cells in the context of bioreactors [28].

Functional Properties of Tissue-engineered Cartilage Constructs using ASCs

Changes in the biochemical composition of these tissue-engineered cartilage constructs with time also can influence their functional biomechanical and transport properties. For example, increases in the shear and compressive moduli were correlated with increased accumulation of matrix components such as proteoglycan and collagen content. It was shown that the composition and structure of the biomaterial scaffold also affect the diffusion properties of cartilage engineered from ASCs [48]. As cartilage (native or tissue engineered) is avascular, diffusion serves as the primary mechanism for macromolecular transport. The diffusion coefficients of fluorescent, uncharged dextran molecules (ranging from 3 to 500 KDa) were measured using a novel method for fluorescence recovery after photobleaching (FRAP) [49]. The findings showed that independent of the size of the molecule, the diffusion properties of ASC-engineered constructs depended on the biomaterial composition of the construct, presence of cells, growth factor culture conditions, and culture duration [48]. For example, after 4 weeks in culture, construct diffusivity decreased greater than 40% under chondrogenic conditions, and was associated with a net increase in the biosynthesis and retention of matrix macromolecules.

Of note was the finding that the diffusivities of all the different constructs tested in culture were significantly greater than those of native articular cartilage, suggesting that nutrient and metabolite transport to cells in the constructs is not hindered during the early stages of tissue formation, as compared with native tissue [49]. From a functional standpoint, however, constructs created from gel-seeded ASCs or MSCs generally are unable to achieve the mechanical properties of native articular cartilage in a short period of culture time, particularly with respect to tension ([4, 40, 71, 78]). Thus there is an important need to develop scaffold materials that can provide biomechanical function for engineered constructs until the cells can synthesize and assemble a functional matrix [64]. To address this issue, a novel three-dimensionally woven fiber scaffold was developed that replicates the mechanical properties of native cartilage in tension, compression, and shear, while readily allowing consolidation and cell seeding [63]. These porous composite scaffolds can be designed using various combinations of bioresorbable fibers such as poly(glycolic acid) with initial properties that match the anisotropy, viscoelasticity, and tension-compression nonlinearity of native cartilage. Such scaffolds thus provide the potential for load-bearing immediately after cell seeding and implantation, allowing further maturation of the construct in vivo while minimizing in vitro culture times. In one study, a scaffold composed of three-dimensionally woven poly(ϵ -caprolactone) (PCL) was used that was consolidated with human ASCs encapsulated in fibrin gel and cultured for up to 4 weeks [65]. For all times tested, these scaffolds maintained shear, compressive, and frictional biomechanical properties similar to those of native cartilage (Fig. 5),

while exhibiting the synthesis of a collagen-rich extracellular matrix [65]. These findings suggest that ASC-based constructs can be designed and created using biomaterial scaffolds that provide native biomechanical function, allowing for the potential use of these technologies in clinically based applications for cartilage repair and regeneration.

Discussion

Tissue engineering, gene therapy, and other cell-based approaches in regenerative medicine have highlighted the need for an abundant source of undifferentiated progenitor cells. The purpose of this study was to determine the utility of adipose tissue as a source of stem cells for musculoskeletal regeneration and the optimal scaffolds and culture conditions to promote their chondrogenic differentiation. Studies show subcutaneous fat and other adipose tissue depots in the adult human body contain large numbers of stem cells that can be readily isolated from standard liposuction waste, providing an easily accessible source of autologous or allogeneic cells. Under appropriate environmental cues, as detailed herein, these cells can exhibit phenotypic characteristics of multiple cell types, and thus provide a unique and promising cell source for applications in regenerative medicine.

Relative to other adult stem cell sources, ASCs can be harvested with high yields of cells, although some limitations to their use still exist. First, it can be difficult to determine the optimal in vitro culture period required for a specific application. The stromal vascular fraction that

Fig. 5A–D Three-dimensional structures were woven by interlocking multiple layers of two perpendicularly oriented sets of in-plane fibers (x- or warp direction, and y- or weft direction) with a third set of fibers in the z-direction. (A) A surface view of the X-Y plane (SEM), (B) cross-sectional view of the X-Z plane, and (C) cross-sectional view of the Y-Z plane are shown. (D) The structural stiffness of three-dimensional PCL scaffolds was increased when consolidated with fibrin. The aggregate modulus (H_A) and Young's modulus (E) are shown at Day 0 as determined by confined and

unconfined compression, respectively. Three-dimensional PCL/fibrin composite scaffolds had significantly higher H_A and E values than did unconsolidated three-dimensional PCL scaffolds (ANOVA, * $p < 0.05$. ** $p < 0.0001$); data presented as mean $\pm$ SEM; scale bar = 1 mm. (Reproduced with permission from Moutos FT, Guilak F. Functional properties of cell-seeded three-dimensionally woven poly(ϵ -caprolactone) scaffolds for cartilage tissue engineering. *Tissue Eng Part A*. 2010;16:1291–1301.) [65]

initially is isolated represents a heterogeneous mix of cells types that can include endothelial cells, pericytes, and immune cells. Some studies have shown that after controlled induction or serial passaging, ASCs become more uniform in phenotype and differentiation potential [24], suggesting that in vitro expansion preferentially selects for a relatively homogeneous cell population enriched for cells expressing a stromal immunophenotype [61]. However, as extensive expansion in culture may introduce the rare possibility of tumorigenesis, the in vitro culture conditions and selection of early progenitors may strongly influence the phenotype of the cells before differentiation [24, 76], and therefore should be selected carefully before use in a clinical application [75]. Regardless, most applications of ASCs are focusing on minimizing or even eliminating cell expansion in vitro before implantation of the cells [46]. Thus, although limitations exist, ASCs continue to meet many, if not all, of the requisite characteristics for a clinically applicable cell therapeutic.

Our findings and those of others have shown that ASCs exhibit multipotent differentiation capabilities in the mesenchymal lineage, similar to other adult stem cells such as MSCs, with evidence of adipogenic, chondrogenic, myogenic, neurogenic, osteogenic, and tenogenic differentiation [36, 53, 86]. Similarly, the culture conditions used to induce lineage-specific differentiation of ASCs generally are similar to those used for MSCs; although increasing evidence indicates that different adult stem cells possess unique characteristics and properties depending on their site of origin. By measures of cell surface markers, ASCs show expression profiles that are similar but distinct in comparison to bone marrow MSCs. However, the response of ASCs to growth factors and biomaterial scaffolds may differ significantly from MSCs [21, 26]. For example, it has been shown that the addition of BMP-6 significantly enhances ASC chondrogenesis, suggesting that conditions for optimal ASC differentiation may be distinct from those required for MSCs [26, 39]. Thus, based on these data, investigators should be cognizant of such differences between ASC and MSC behavior as they optimize cell-based therapeutics for musculoskeletal disease.

The growth and differentiation of these cells, particularly as they undergo chondrogenesis, is sensitive to multiple environmental factors such as soluble mediators (ie, growth factors and cytokines), physicochemical factors such as mechanical loading, pH, or oxygen tension, and their interactions with biomaterial matrices. In particular, findings suggest that a rounded cell shape (via three-dimensional scaffolds or micromass culture) is a critical factor in the induction of the chondrogenic phenotype, and thus physical interactions with the extracellular matrix may have a significant effect on maintaining ASC phenotype over the long term [35]. This evidence suggests that it will

be possible to exploit such mechanoinductive stimuli to modulate the chondrogenic differentiation and function of ASCs in conjunction with more traditional biochemical growth factors [38, 51, 54].

Ultimately, the ability of ASCs to provide a basis for tissue regeneration in the musculoskeletal system likely will depend on the ability for engineered constructs to provide the appropriate functional properties over the life of the implant [11]. In this respect, the mechanical and biophysical properties of biomaterial scaffolds used in stem cell-based tissue engineering are critical design parameters that will influence the clinical translation of such approaches. To achieve these goals, continued studies involving biomaterial and biomechanical scientists, manufacturing process engineers, orthopaedic clinicians, and stem cell biologists are needed. By fostering collaborative relationships between teams from academia, biotechnology startups, and established biomaterial industry companies, the development of ASC-based regenerative medical therapies for cartilage and bone defects can be accomplished in compliance with existing federal and international regulatory requirements. Evidence already exists suggesting that these goals are feasible since the successful use of autologous ASCs to repair critical-sized craniofacial defects has been published as case reports in the clinical literature [52, 58].

Acknowledgments These studies would not have been possible without the many contributions of Hani Awad, Helawe Betre, Naichen Cheng, Geoffrey Erickson, Beverley Fermor, Lisa Freed, Yuan-Di Halvorsen, Holly Leddy, Dianne Little, Kristen Lott, Henry Rice, Chris Rowland, Lori Setton, Robert Storms, David Wang, Quinn Wickham, William Wilkison, and Art Wu.

References

1. Akizuki S, Yasukawa Y, Takizawa T. Does arthroscopic abrasion arthroplasty promote cartilage regeneration in osteoarthritic knees with eburation? A prospective study of high tibial osteotomy with abrasion arthroplasty versus high tibial osteotomy alone. *Arthroscopy*. 1997;13:9–17.
2. Alsberg E, Anderson KW, Albeiruti A, Rowley JA, Mooney DJ. Engineering growing tissues. *Proc Natl Acad Sci USA*. 2002;99:12025–12030.
3. Aust L, Devlin B, Foster SJ, Halvorsen YD, Hicok K, du Laney T, Sen A, Willingmyre GD, Gimble JM. Yield of human adipose-derived adult stem cells from liposuction aspirates. *Cytotherapy*. 2004;6:7–14.
4. Awad HA, Halvorsen YD, Gimble JM, Guilak F. Effects of transforming growth factor beta1 and dexamethasone on the growth and chondrogenic differentiation of adipose-derived stromal cells. *Tissue Eng*. 2003;9:1301–1312.
5. Awad HA, Wickham MQ, Leddy HA, Gimble JM, Guilak F. Chondrogenic differentiation of adipose-derived adult stem cells in agarose, alginate, and gelatin scaffolds. *Biomaterials*. 2004;25:3211–3222.
6. Betre H, Ong SR, Guilak F, Chilkoti A, Fermor B, Setton LA. Chondrocytic differentiation of human adipose-derived adult

- stem cells in elastin-like polypeptide. *Biomaterials*. 2006;27:91–99.
7. Betre H, Setton LA, Meyer DE, Chilkoti A. Characterization of a genetically engineered elastin-like polypeptide for cartilaginous tissue repair. *Biomacromolecules*. 2002;3:910–916.
 8. Blevins FT, Steadman JR, Rodrigo JJ, Silliman J. Treatment of articular cartilage defects in athletes: an analysis of functional outcome and lesion appearance. *Orthopedics*. 1998;21:761–767; 767–768.
 9. Brittberg M. Autologous chondrocyte transplantation. *Clin Orthop Relat Res*. 1999;367(suppl):S147–S155.
 10. Brittberg M, Lindahl A, Nilsson A, Ohlsson C, Isaksson O, Peterson L. Treatment of deep cartilage defects in the knee with autologous chondrocyte transplantation. *N Engl J Med*. 1994;331:889–895.
 11. Butler DL, Goldstein SA, Guilak F. Functional tissue engineering: the role of biomechanics. *J Biomech Eng*. 2000;122:570–575.
 12. Cao Y, Rodriguez A, Vacanti M, Ibarra C, Arevalo C, Vacanti CA. Comparative study of the use of poly(glycolic acid), calcium alginate and pluronics in the engineering of autologous porcine cartilage. *J Biomater Sci Polym Ed*. 1998;9:475–487.
 13. Caplan AI, Bruder SP. Mesenchymal stem cells: building blocks for molecular medicine in the 21st century. *Trends Mol Med*. 2001;7:259–264.
 14. Caterson EJ, Li WJ, Nesti LJ, Albert T, Danielson K, Tuan RS. Polymer/alginate amalgam for cartilage-tissue engineering. *Ann NY Acad Sci*. 2002;961:134–138.
 15. Caterson EJ, Nesti LJ, Danielson KG, Tuan RS. Human marrow-derived mesenchymal progenitor cells: isolation, culture expansion, and analysis of differentiation. *Mol Biotechnol*. 2002;20:245–256.
 16. Cheng NC, Estes BT, Awad HA, Guilak F. Chondrogenic differentiation of adipose-derived adult stem cells by a porous scaffold derived from native articular cartilage extracellular matrix. *Tissue Eng Part A*. 2009;15:231–241.
 17. Colter DC, Sekiya I, Prockop DJ. Identification of a subpopulation of rapidly self-renewing and multipotential adult stem cells in colonies of human marrow stromal cells. *Proc Natl Acad Sci USA*. 2001;98:7841–7845.
 18. Crisan M, Yap S, Casteilla L, Chen CW, Corselli M, Park TS, Andriolo G, Sun B, Zheng B, Zhang L, Norotte C, Teng PN, Traas J, Schugar R, Deasy BM, Badyal S, Buhning HJ, Giacobino JP, Lazzari L, Huard J, Peault B. A perivascular origin for mesenchymal stem cells in multiple human organs. *Cell Stem Cell*. 2008;3:301–313.
 19. Department of Health and Human Services. *Stem Cells: Scientific Progress and Future Research Directions*. Washington, DC: Department of Health and Human Services; June 2001. </info/scireport/2001report.
 20. Diekmann BO, Estes BT, Guilak F. The effects of BMP6 overexpression on adipose stem cell chondrogenesis: interactions with dexamethasone and exogenous growth factors. *J Biomed Mater Res A*. 2010;93:994–1003.
 21. Diekmann BO, Rowland CR, Caplan AI, Lennon D, Guilak F. Chondrogenesis of adult stem cells from adipose tissue and bone marrow: induction by growth factors and cartilage derived matrix. *Tissue Eng Part A*. 2010;16:523–533.
 22. Erickson GR, Gimple JM, Franklin DM, Rice HE, Awad H, Guilak F. Chondrogenic potential of adipose tissue-derived stromal cells in vitro and in vivo. *Biochem Biophys Res Commun*. 2002;290:763–769.
 23. Estes BT, Diekmann BO, Gimple JM, Guilak F. Isolation of adipose derived stem cells and their induction to a chondrogenic phenotype. *Nature Protocols*. 2010;5(7):1294–1311.
 24. Estes BT, Diekmann BO, Guilak F. Monolayer cell expansion conditions affect the chondrogenic potential of adipose-derived stem cells. *Biotechnol Bioeng*. 2008;99:986–995.
 25. Estes BT, Gimple JM, Guilak F. Mechanical signals as regulators of stem cell fate. *Curr Top Dev Biol*. 2004;60:91–126.
 26. Estes BT, Wu AW, Guilak F. Potent induction of chondrocytic differentiation of human adipose-derived adult stem cells by bone morphogenetic protein 6. *Arthritis Rheum*. 2006;54:1222–1232.
 27. Estes BT, Wu AW, Storms RW, Guilak F. Extended passaging, but not aldehyde dehydrogenase activity, increases the chondrogenic potential of human adipose-derived adult stem cells. *J Cell Physiol*. 2006;209:987–995.
 28. Freed LE, Engelmayr GC Jr, Borenstein JT, Moutos FT, Guilak F. Advanced material strategies for tissue engineering scaffolds. *Advanced Materials*. 2009;21:3410–3418.
 29. Frisbie DD, Trotter GW, Powers BE, Rodkey WG, Steadman JR, Howard RD, Park RD, McIlwraith CW. Arthroscopic subchondral bone plate microfracture technique augments healing of large chondral defects in the radial carpal bone and medial femoral condyle of horses. *Vet Surg*. 1999;28:242–255.
 30. Gao J, Dennis JE, Solchaga LA, Awadallah AS, Goldberg VM, Caplan AI. Tissue-engineered fabrication of an osteochondral composite graft using rat bone marrow-derived mesenchymal stem cells. *Tissue Eng*. 2001;7:363–371.
 31. Ghivizzani SC, Oligino TJ, Robbins PD, Evans CH. Cartilage injury and repair. *Phys Med Rehabil Clin N Am*. 2000;11:289–307, vi.
 32. Gronthos S, Franklin DM, Leddy HA, Robey PG, Storms RW, Gimple JM. Surface protein characterization of human adipose tissue-derived stromal cells. *J Cell Physiol*. 2001;189:54–63.
 33. Guilak F, Awad H, Fermor B, Leddy HA, Gimple JM. Adipose-derived adult stem cells for cartilage tissue engineering. *Biorheology*. 2004;41:389–399.
 34. Guilak F, Butler DL, Goldstein SA. Functional tissue engineering: the role of biomechanics in articular cartilage repair. *Clin Orthop Relat Res*. 2001;391(suppl):S295–S305.
 35. Guilak F, Cohen DM, Estes BT, Gimple JM, Liedtke W, Chen CS. Control of stem cell fate by physical interactions with the extracellular matrix. *Cell Stem Cell*. 2009;5:17–26.
 36. Guilak F, Lott KE, Awad HA, Cao Q, Hicok KC, Fermor B, Gimple JM. Clonal analysis of the differentiation potential of human adipose-derived adult stem cells. *J Cell Physiol*. 2006;206:229–237.
 37. Halvorsen YD, Bond A, Sen A, Franklin DM, Lea-Currie YR, Sujkowski D, Ellis PN, Wilkison WO, Gimple JM. Thiazolidinediones and glucocorticoids synergistically induce differentiation of human adipose tissue stromal cells: biochemical, cellular, and molecular analysis. *Metabolism*. 2001;50:407–413.
 38. Hanson AD, Marvel SW, Bernacki SH, Baner AJ, van Aalst J, Lobo EG. Osteogenic effects of rest inserted and continuous cyclic tensile strain on hASC lines with disparate osteodifferentiation capabilities. *Ann Biomed Eng*. 2009;37:955–965.
 39. Hennig T, Lorenz H, Thiel A, Goetzke K, Dickhut A, Geiger F, Richter W. Reduced chondrogenic potential of adipose tissue derived stromal cells correlates with an altered TGFbeta receptor and BMP profile and is overcome by BMP-6. *J Cell Physiol*. 2007;211:682–691.
 40. Huang AH, Yeager-McKeever M, Stein A, Mauck RL. Tensile properties of engineered cartilage formed from chondrocyte- and MSC-laden hydrogels. *Osteoarthritis Cartilage*. 2008;16:1074–1082.
 41. Huang JI, Beanes SR, Zhu M, Lorenz HP, Hedrick MH, Benhaim P. Rat extramedullary adipose tissue as a source of osteochondrogenic progenitor cells. *Plast. Reconstr. Surg*. 2002;109:1033–1041; discussion 1042–1033.

42. Hunziker EB. Articular cartilage repair: are the intrinsic biological constraints undermining this process insuperable? *Osteoarthritis Cartilage*. 1999;7:15–28.
43. Hunziker EB. Articular cartilage repair: basic science and clinical progress. A review of the current status and prospects. *Osteoarthritis Cartilage*. 2002;10:432–463.
44. Jiang Y, Jahagirdar BN, Reinhardt RL, Schwartz RE, Keene CD, Ortiz-Gonzalez XR, Reyes M, Lenvik T, Lund T, Blackstad M, Du J, Aldrich S, Lisberg A, Low WC, Largaespada DA, Verfaillie CM. Pluripotency of mesenchymal stem cells derived from adult marrow. *Nature*. 2002;418:41–49.
45. Johnstone B, Hering TM, Caplan AI, Goldberg VM, Yoo JU. In vitro chondrogenesis of bone marrow-derived mesenchymal progenitor cells. *Exp Cell Res*. 1998;238:265–272.
46. Jurgens WJ, van Dijk A, Doulabi BZ, Niessen FB, Ritt MJ, van Milligen FJ, Helder MN. Freshly isolated stromal cells from the infrapatellar fat pad are suitable for a one-step surgical procedure to regenerate cartilage tissue. *Cytherapy*. 2009;11:1052–1064.
47. Knutsen G, Drogset JO, Engebretsen L, Grontvedt T, Isaksen V, Ludvigsen TC, Roberts S, Solheim E, Strand T, Johansen O. A randomized trial comparing autologous chondrocyte implantation with microfracture: findings at five years. *J Bone Joint Surg Am*. 2007;89:2105–2112.
48. Leddy HA, Awad HA, Guilak F. Molecular diffusion in tissue-engineered cartilage constructs: effects of scaffold material, time, and culture conditions. *J Biomed Mater Res B Appl Biomater*. 2004;70:397–406.
49. Leddy HA, Guilak F. Site-specific molecular diffusion in articular cartilage measured using fluorescence recovery after photobleaching. *Ann Biomed Eng*. 2003;31:753–760.
50. Lee CR, Grodzinsky AJ, Hsu HP, Martin SD, Spector M. Effects of harvest and selected cartilage repair procedures on the physical and biochemical properties of articular cartilage in the canine knee. *J Orthop Res*. 2000;18:790–799.
51. Lee WC, Maul TM, Vorp DA, Rubin JP, Marra KG. Effects of uniaxial cyclic strain on adipose-derived stem cell morphology, proliferation, and differentiation. *Biomech Model Mechanobiol*. 2007;6:265–273.
52. Lendeckel S, Jodicke A, Christophis P, Heidinger K, Wolff J, Fraser JK, Hedrick MH, Berthold L, Howaldt HP. Autologous stem cells (adipose) and fibrin glue used to treat widespread traumatic calvarial defects: case report. *J Craniomaxillofac Surg*. 2004;32:370–373.
53. Little D, Guilak F, Ruch DS. Ligament-derived matrix stimulates a ligamentous phenotype in human adipose-derived stem cells. *Tissue Eng Part A*. 2010 Apr 20. [Epub ahead of print].
54. Mahmoudifar N, Doran PM. Chondrogenic differentiation of human adipose-derived stem cells in polyglycolic acid mesh scaffolds under dynamic culture conditions. *Biomaterials*. 2010;31:3858–3867.
55. Martin I, Vunjak-Novakovic G, Yang J, Langer R, Freed LE. Mammalian chondrocytes expanded in the presence of fibroblast growth factor 2 maintain the ability to differentiate and regenerate three-dimensional cartilaginous tissue. *Exp Cell Res*. 1999;253:681–688.
56. Masuda K, Sah RL, Hejna MJ, Thonar EJ. A novel two-step method for the formation of tissue-engineered cartilage by mature bovine chondrocytes: the alginate-recovered-chondrocyte (ARC) method. *J Orthop Res*. 2003;21:139–148.
57. McIntosh K, Zvonic S, Garrett S, Mitchell JB, Floyd ZE, Hammill L, Kloster A, Di Halvorsen Y, Ting JP, Storms RW, Goh B, Kilroy G, Wu X, Gimble JM. The immunogenicity of human adipose-derived cells: temporal changes in vitro. *Stem Cells*. 2006;24:1246–1253.
58. Mesimäki K, Lindroos B, Tornwall J, Mauno J, Lindqvist C, Kontio R, Miettinen S, Suuronen R. Novel maxillary reconstruction with ectopic bone formation by GMP adipose stem cells. *Int J Oral Maxillofac Surg*. 2009;38:201–209.
59. Meyer DE, Chilkoti A. Purification of recombinant proteins by fusion with thermally-responsive polypeptides. *Nat Biotechnol*. 1999;17:1112–1115.
60. Minas T, Nehrer S. Current concepts in the treatment of articular cartilage defects. *Orthopedics*. 1997;20:525–538.
61. Mitchell JB, McIntosh K, Zvonic S, Garrett S, Floyd ZE, Kloster A, Di Halvorsen Y, Storms RW, Goh B, Kilroy G, Wu X, Gimble JM. Immunophenotype of human adipose-derived cells: temporal changes in stromal-associated and stem cell-associated markers. *Stem Cells*. 2006;24:376–385.
62. Mobasheri A, Bondy CA, Moley K, Mendes AF, Rosa SC, Richardson SM, Hoyland JA, Barrett-Jolley R, Shakibaei M. Facilitative glucose transporters in articular chondrocytes: expression, distribution and functional regulation of GLUT isoforms by hypoxia, hypoxia mimetics, growth factors and pro-inflammatory cytokines. *Adv Anat Embryol Cell Biol*. 2008;200:1 p following vi, 1–84.
63. Moutos FT, Freed LE, Guilak F. A biomimetic three-dimensional woven composite scaffold for functional tissue engineering of cartilage. *Nat Mater*. 2007;6:162–167.
64. Moutos FT, Guilak F. Composite scaffolds for cartilage tissue engineering. *Biorheology*. 2008;45:501–512.
65. Moutos FT, Guilak F. Functional properties of cell-seeded three-dimensionally woven poly(epsilon-caprolactone) scaffolds for cartilage tissue engineering. *Tissue Eng Part A*. 2010;16:1291–1301.
66. Mow VC, Ratcliffe A, Poole AR. Cartilage and diarthrodial joints as paradigms for hierarchical materials and structures. *Biomaterials*. 1992;13:67–97.
67. Murphy CL, Sambanis A. Effect of oxygen tension and alginate encapsulation on restoration of the differentiated phenotype of passaged chondrocytes. *Tissue Eng*. 2001;7:791–803.
68. Nakahara H, Bruder SP, Haynesworth SE, Holecck JJ, Baber MA, Goldberg VM, Caplan AI. Bone and cartilage formation in diffusion chambers by subcultured cells derived from the periosteum. *Bone*. 1990;11:181–188.
69. Noth U, Osyczka AM, Tuli R, Hickok NJ, Danielson KG, Tuan RS. Multilineage mesenchymal differentiation potential of human trabecular bone-derived cells. *J Orthop Res*. 2002;20:1060–1069.
70. O'Driscoll SW, Fitzsimmons JS. The role of periosteum in cartilage repair. *Clin Orthop Relat Res*. 2001;391(suppl):S190–S207.
71. Pei M, Solchaga LA, Seidel J, Zeng L, Vunjak-Novakovic G, Caplan AI, Freed LE. Bioreactors mediate the effectiveness of tissue engineering scaffolds. *FASEB J*. 2002;16:1691–1694.
72. Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, Moorman MA, Simonetti DW, Craig S, Marshak DR. Multilineage potential of adult human mesenchymal stem cells. *Science*. 1999;284:143–147.
73. Prockop DJ. Adult stem cells gradually come of age. *Nat Biotechnol*. 2002;20:791–792.
74. Prockop DJ, Sekiya I, Colter DC. Isolation and characterization of rapidly self-renewing stem cells from cultures of human marrow stromal cells. *Cytherapy*. 2001;3:393–396.
75. Rubio D, Garcia-Castro J, Martin MC, de la Fuente R, Cigudosa JC, Lloyd AC, Bernad A. Spontaneous human adult stem cell transformation. *Cancer Res*. 2005;65:3035–3039.
76. Sekiya I, Vunjak-Novakovic G, Larson BL, Prockop DJ. In vitro cartilage formation by human adult stem cells from bone marrow stroma defines the sequence of cellular and molecular events during chondrogenesis. *Proc Natl Acad Sci USA*. 2002;99:4397–4402.

77. Solchaga LA, Goldberg VM, Caplan AI. Cartilage regeneration using principles of tissue engineering. *Clin Orthop Relat Res.* 2001;391(suppl):S161–S170.
78. Stok KS, Lisignoli G, Cristino S, Facchini A, Muller R. Mechano-functional assessment of human mesenchymal stem cells grown in three-dimensional hyaluronan-based scaffolds for cartilage tissue engineering. *J Biomed Mater Res A.* 2010;93:37–45.
79. Tognana E, Chen F, Padera RF, Leddy HA, Christensen SE, Guilak F, Vunjak-Novakovic G, Freed LE. Adjacent tissues (cartilage, bone) affect the functional integration of engineered calf cartilage in vitro. *Osteoarthritis Cartilage.* 2005;13:129–138.
80. Verfaillie CM. Adult stem cells: assessing the case for pluripotency. *Trends Cell Biol.* 2002;12:502–508.
81. Waldman SD, Spiteri CG, Gryn timer MD, Pilliar RM, Hong J, Kandel RA. Effect of biomechanical conditioning on cartilaginous tissue formation in vitro. *J Bone Joint Surg Am.* 2003;85(suppl 2):101–105.
82. Wang DW, Fermor B, Gimble JM, Awad HA, Guilak F. Influence of oxygen on the proliferation and metabolism of adipose derived adult stem cells. *J Cell Physiol.* 2005;204:184–191.
83. Wickham MQ, Erickson GR, Gimble JM, Vail TP, Guilak F. Multipotent stromal cells derived from the infrapatellar fat pad of the knee. *Clin Orthop Relat Res.* 2003;412:196–212.
84. Winter A, Breit S, Parsch D, Benz K, Steck E, Hauner H, Weber RM, Ewerbeck V, Richter W. Cartilage-like gene expression in differentiated human stem cell spheroids: a comparison of bone marrow-derived and adipose tissue-derived stromal cells. *Arthritis Rheum.* 2003;48:418–429.
85. Yoo JU, Barthel TS, Nishimura K, Solchaga L, Caplan AI, Goldberg VM, Johnstone B. The chondrogenic potential of human bone-marrow-derived mesenchymal progenitor cells. *J Bone Joint Surg Am.* 1998;80:1745–1757.
86. Zuk PA, Zhu M, Ashjian P, De Ugarte DA, Huang JI, Mizuno H, Alfonso ZC, Fraser JK, Benhaim P, Hedrick MH. Human adipose tissue is a source of multipotent stem cells. *Mol Biol Cell.* 2002;13:4279–4295.