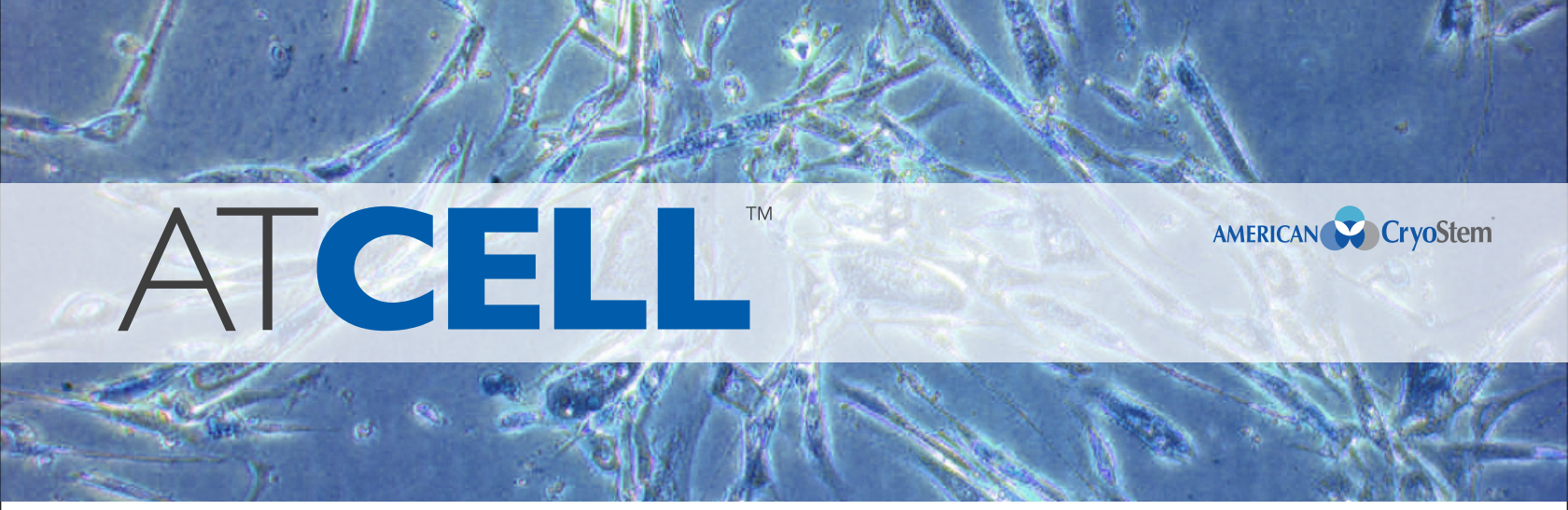


ATCELL™

AMERICAN CryoStem

ATCELL™, a Patient-Specific Stem Cell Product

Cochran, Matthew[1]; Ruth Goldman[2]; Tim Bushnell[1]; Kristen Linderman[2]; David K. Moscatello[2]; and Michael L. Moeller[2]

1. University of Rochester Medical Center Flow Core, 601 Elmwood Ave., Rochester, NY
2. American CryoStem Laboratories, 100 Technology Way, Lab 110, Mount Laurel, NJ

The Promise of Mesenchymal Stem Cells:

A great deal of interest has surfaced in recent years concerning potential therapeutic applications of human mesenchymal stem cells (hMSCs). These are immature cells capable of developing into a defined subset of mature cells: adipocytes, osteocytes, and chondrocytes. Since they were first characterized, interest has grown in the potential uses of these cells in the rebuilding of bone and cartilage, which might provide therapeutic strategies in combating osteoarthritis and disc/joint degeneration. The prospect of being able to create implantable medical devices from a patient's own tissues is an exciting one, and is gaining momentum as is evidenced by the increased scientific literature being published in this field. Currently, hMSCs have been derived from a variety of tissues, including bone marrow, umbilical cord, placenta, and, more recently, adipose tissue. When derived from human adipose tissue, they are referred to as "adipose-derived stem cells", or hADSCs.

hADSCs May Be Identified by the Expression or Absence of Distinct Protein Markers on Their Surfaces:

hADSCs are known to express characteristic sets of surface proteins which may be used to identify them. For example, the International Society for Cellular Therapy defines them as expressing CD73, CD90, and CD1051. To this list, CD44 has been more recently added, as have CD92 and CD293. Specific cross-reactive epitopes of CD14 have also been identified as being expressed on hADSCs, although CD14, itself, is apparently absent (as shown by PCR)4, and CD146 has also been seen to be expressed generally on MSCs derived from multiple sites in the body5-7. Reports have also been published listing CD10 (Lymphocyte Leukemia Antigen), CD13 (Aminopeptidase), CD49d (α4 Integrin), CD54 (ICAM-1), CD55 (Decay-Accelerating Factor), and CD166 (AMCAM), as well8. Certain other protein markers, such as those characteristic of the cells of the immune system (CD14, CD19, CD45) or the endothelial cells lining blood vessels (CD31), are expected to be absent from the surfaces of these cells.

What is ATCELL?

By isolating the cells of the stromal vascular fraction (SVF) of human adipose tissue and expanding them using the patented ACSelerate SF-M™ medium and proprietary culture methods , scientists at American CryoStem are capable of growing cells spindle-shaped, fibroblast-like cells which have the

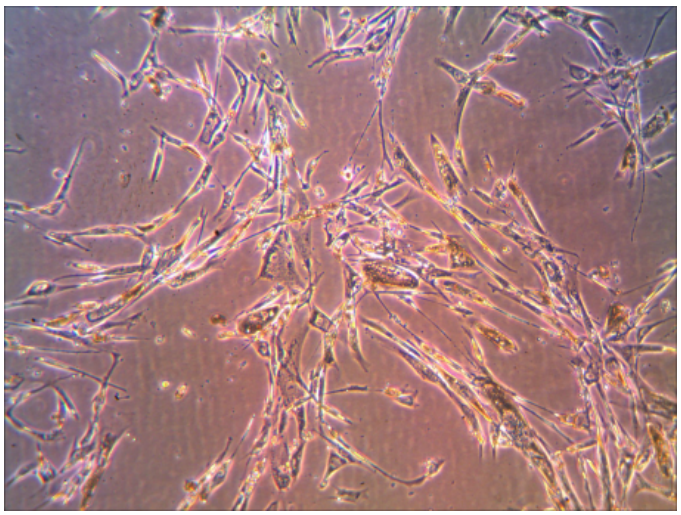


Figure 1

morphological characteristics of hADSCs (Fig. 1). However, to be used reliably in clinical applications, it must be proven that ATCELLs™ are indeed hADSCs. Further it must be proven that the cells maintain their identity as hADSCs as demonstrated by cell surface markers as well as the ability to differentiate into the tissue designated for repair.

Figure 1: ATCELLs™ in culture.

Isolated from the stromal vascular fraction (SVF) of lipoaspirates collected during routine liposuction procedures, ATCELLs™ are grown under proprietary conditions with the patented ACSelerate SF-M™ medium. The resulting cells have morphologies strongly characteristic of hADSCs.

However, to be used reliably in clinical applications, it must be proven that ATCELLs™ are indeed hADSCs. Further it must be proven that the cells maintain their identity as hADSCs as demonstrated by cell surface markers as well as the ability to differentiate into the tissue designated for repair.

ATCELLs™ are Patient-Specific hADSCs:

Flow cytometric analysis was conducted on paraformaldehyde-fixed ATCELLs™ against the antigens CD14, CD19, CD29, CD31, CD34, CD44, CD45, CD49d, CD73, CD90, CD105, and CD146. These antigens were selected primarily based on their reported expressions or exclusions on mesenchymal stem cells. Processing, staining, and analysis of cell samples were performed at the University of Rochester Flow Core Facility (Rochester, NY). As shown (Table 1), cells were found to be negative for the markers CD14, CD19, CD31, CD34, and CD45, while positively expressing the markers CD29, CD44, CD49d, CD73, CD90,CD105, and CD146.

Table 1:

Surface protein markers expressed by ATCELL™ cultured cells. ATCELLs™ were fixed in paraformaldehyde, stained with fluorescent antibodies against specific marker proteins, and subjected to flow cytometric analysis. Ideally, those markers which are known to be associated with hADSCs (CD29, CD44, CD49d, CD 73, CD90, CD105, and CD146) should be expressed, while markers for immune system cells in the bloodstream (CD14, CD19, and CD45) or for blood vessels (CD31) should be absent. As shown, ATCELLs™ meet these criteria, and are, therefore, hADSCs.

Antigenic Marker	Significance	Expressed?
CD14	Innate immune system cells	No
CD19	B-cell marker	No
CD29	Innate immune system cells	Yes
CD31	PECAM-1 (Platelet Endothelial Adhesion Molecule)	No
CD34	BMDSC (Bone Marrow Derived Stem Cells)	No
CD44	Hyaluronic acid receptor	Yes
CD45	Lymphocyte common antigen	No
CD49d	Integrin α4	Yes
CD73	Conversion of AMP to adenosine	Yes
CD90	Thy-1; stem cell marker	Yes
CD105	Endoglin; role in angiogenesis	Yes
CD146	MCAM (Melanoma Adhesion Molecule Marker); MSC marker	Yes

Table 1

ATCELLs™ Maintain Their Cellular Identities Throughout Culture:

To assess the ability of ATCELLs™ to maintain their identities as hADSCs, ATCELLs™ grown in culture (third or fourth passage) were compared with freshly prepared cultures for expression of a select subset of

the marker proteins listed above. As shown (Table 2), ATCELLs™ precisely maintained their identities throughout culture, manifesting the same patterns of proteins expressed over time. Moreover, expression levels were seen to remain at identical levels throughout this time period (data not shown).

Antigenic Marker	Initial Expression	Third Passage (P3)	Fourth Passage (P4)
CD29	Yes	Yes	Yes
CD44	Yes	Yes	Yes
CD49d	Yes	Yes	Yes
CD73	Yes	Yes	Yes
CD90	Yes	Yes	Yes
CD105	Yes	Yes	Yes
CD146	Yes	Yes	Yes

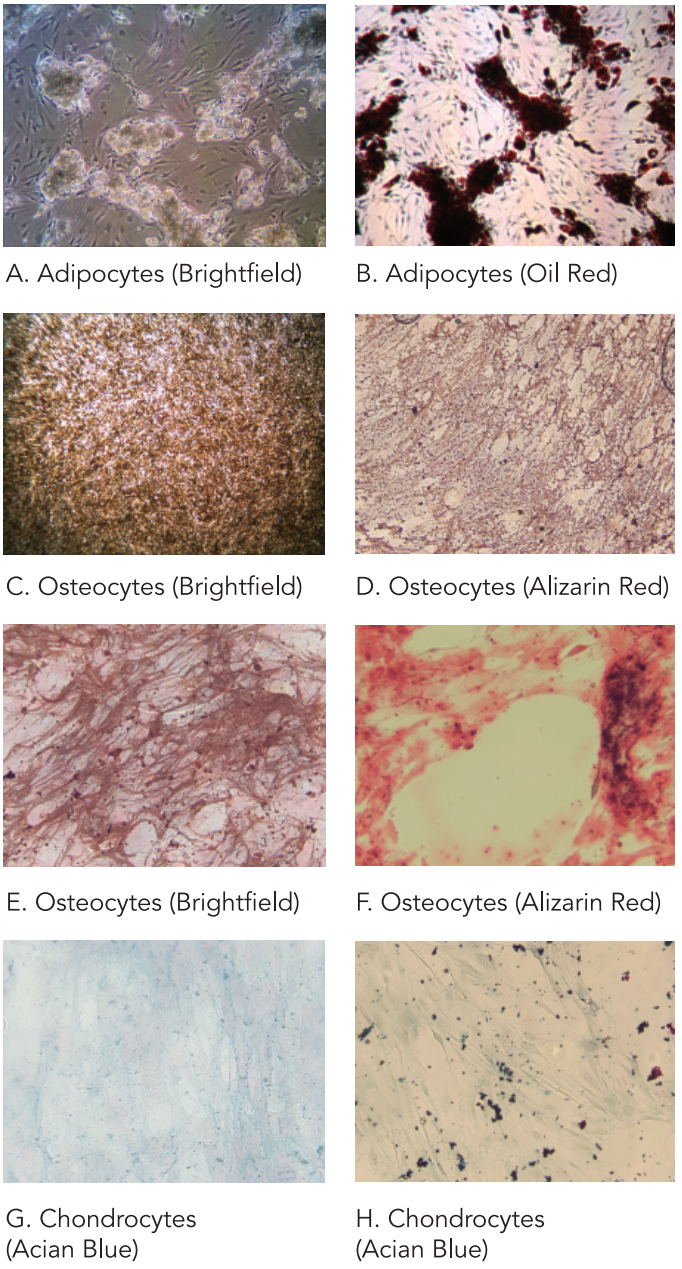
Table 2

Table 2:

ATCELLs™ maintain their cellular identities throughout cell culture. ATCELLs™ were fixed in paraformaldehyde, stained with fluorescent antibodies against specific marker proteins known to be associated with hADSCs, and subjected to flow cytometric analysis.

ATCELLs™ Have The Ability To Become Fat, Bone, or Cartilage Cells:

To assess the ability of these cells to become fat-storing adipocytes, bone-forming osteocytes, and cartilage-forming chondrocytes, cell cultures were incubated with specific proprietary chemical treatments known to induce their differentiation. As shown, the cells readily differentiating into adipocytes (Fig. 2A-B) identifiable by the accumulation of intracellular lipid droplets which stain positive for Oil Red, osteocytes (Fig. 2C-F) identifiable through positive Alizarin Red staining, and chondrocytes (Fig. 2G-H) identifiable through positive Alcian Blue staining. These stains are well-documented methods for demonstrating the presence of fat globules (Oil Red), calcium phosphate crystals (Alizarin Red), or cartilage proteoglycans (Alcian Blue). As hADSCs are known to differentiate into adipocytes, osteocytes, and chondrocytes in the body, this data collectively confirms that ATCELLs™ are, indeed, bona fide hADSCs.



A. Adipocytes (Brightfield)
B. Adipocytes (Oil Red)
C. Osteocytes (Brightfield)
D. Osteocytes (Alizarin Red)
E. Osteocytes (Brightfield)
F. Osteocytes (Alizarin Red)
G. Chondrocytes (Acian Blue)
H. Chondrocytes (Acian Blue)

Figure 2

Figure 2: ATCELLs™ are capable of becoming adipocytes, osteocytes, and chondrocytes when differentiated.

When treated with specific chemical treatments, ATCELLs are observed to differentiate into fat-storing adipocytes which may be identified by the presence of

internal lipid globules (A) which stain positively with Oil Red (B), bone-forming osteocytes which may be identified by calcium phosphate crystals (C) that stain positively with Alizarin Red (D-F), and cartilage-forming chondrocytes which stain positively with Alcian Blue (G-H).

ATCELL™, A Patient-Specific Stem Cell Product:

As the prospects of clinicians being able to use patient-specific hADSCs for the creation of implantable medical devices or the creation of therapeutic strategies for alleviating degenerative conditions improve, there will be a clear need for regulated production strategies of patient-specific cell cultures. Using a proprietary process for isolating and expanding cells derived from patient lipoaspirates collected during routine liposuction, American CryoStem Corporation is able to produce and store populations of patient-specific hADSCs. Moreover, ATCELLs™ maintain their cellular identities as hADSCs throughout culture, creating the possibility of growing large-scale stocks of patient-specific cells which may be cryogenically banked until needed.

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