ACSelerate™ Your Stem Cells!

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Introduction

The application of tissue-derived "adult" mesenchymal stem cells (MSCs) to therapeutic interventions has generated much excitement in recent years. The potential to repair and regenerate bone tissue[1,2], cartilage[3,4], cardiac tissue[5-7], and even nervous tissue[8-10] have created a strong belief that regenerative medicine is poised to take a huge leap forward, and much of the current focus has recently fallen on MSCs derived from adipose tissue. Adipose tissue has been shown to be an extremely rich source of MSCs (adipose-derived stem cells, or ADSCs) which may be readily harvested and expanded, and the cells so obtained were initially defined as being capable of differentiating into adipocytes, chondrocytes, and osteocytes under defined conditions[11], with later studies confirming their abilities to become cardiomyocytes[7], skeletal muscle cells[12], neurons[13], and endothelial cells[14]. Such discoveries have fuelled the desire to bring ADSCs into the clinic, but if the suggested therapies are to become realities, laboratories must be capable of maximizing the numbers of ADSCs that can be grown from patient tissue samples. In the case of those patients who are unable to give large adipose tissue volumes, this ability is made even more critical. The ability to grow maximal numbers of cells from potentially minimal quantities of tissue is imperative.

ACSelerate™: A Serum-Free Growth Medium:

Even a cursory perusal of the literature pertaining to ADSCs reveals that many laboratories are currently using fetal bovine serum (FBS)-containing medium, with typically 10% of the volume coming from FBS. Some commercially-available media use lower quantities of serum, some as a low as 2%. However, there is some concern that the use of medium components derived from non-human species may carry the risk of zoonotic infections15, and it has further been demonstrated that human cells cultured in the presence of FBS have animal-derived proteins fixed to their surfaces, rendering them antigenic upon implantation16. Such antigenicity may result in immune responses against the transplanted cells, which may cause tissue fibrosis and potentially life-threatening adverse events. Thus, media free of animal serum and animal products are desirable for the expansion of cells destined for clinical use.

To meet this demand, American CryoStem Corporation presents ACSelerate™ SF-M, a patented serum-free and animal product-free growth medium capable of expanding ADSC populations significantly, even from very minimal tissue samples. Using ACSelerate™ SF-M, human adipose-derived stromal

cells (American CryoStem Corporation's ATCELLs™) showing a pattern of cell surface markers characteristic of ADSCs (CD14-, CD19-, CD29+, CD31-, CD34-, CD44+, CD45-, CD73+, CD90+, CD105+, CD146+) have been expanded to greater than 30 population doublings without losing or declining in their abilities to differentiate into adipocytes, chondrocytes, and osteocytes, the characteristic phenotypes demonstrating mesenchymal stem cell multipotentiality (Table 2).

Surface Marker	Expression in ADSCs	Expression in ATCELLs™
CD14	Negative	Negative
CD19	Negative	Negative
CD29	Positive	Positive
CD31	Negative	Negative
CD34	Negative	Negative
CD44	Positive	Positive
CD45	Negative	Negative
CD49d	Positive	Positive
CD73	Positive	Positive
CD90	Positive	Positive
CD105	Positive	Positive
CD146	Positive	Positive

Table 1

Surface Marker	Initial Expression	Expression after 15 doublings	Expression at 30 doublings
CD14	No	No	No
CD19	No	No	No
CD29	Yes	Yes	Yes
CD31	No	No	No
CD34	No	No	No
CD44	Yes	Yes	Yes
CD45	No	No	No
CD49d	Yes	Yes	Yes
CD73	Yes	Yes	Yes
CD90	Yes	Yes	Yes
CD105	Yes	Yes	Yes
CD146	Yes	Yes	Yes

Table 2

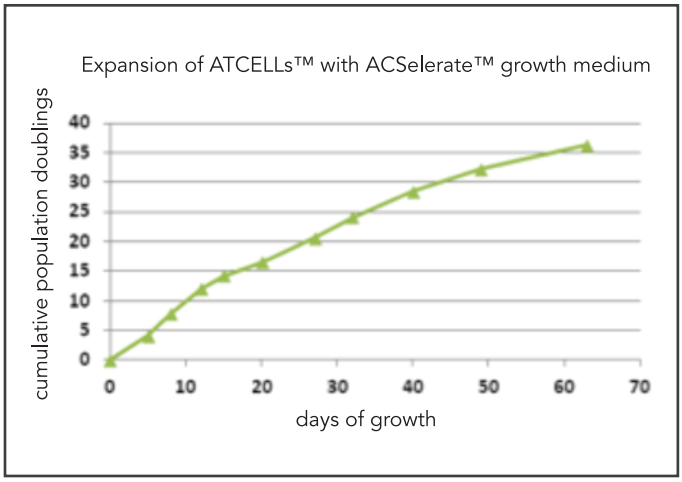
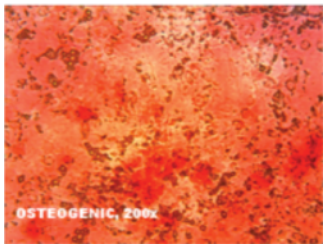
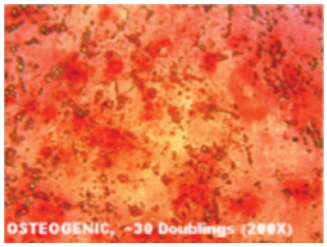


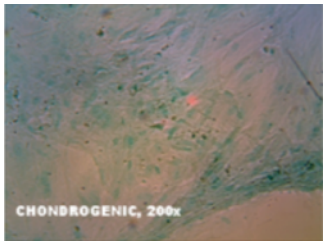
Figure 1



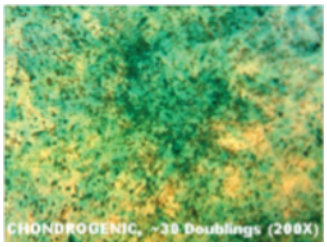
Osteogenic



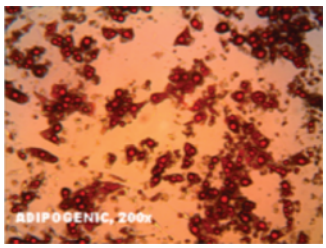
Osteogenic - 30 doublings



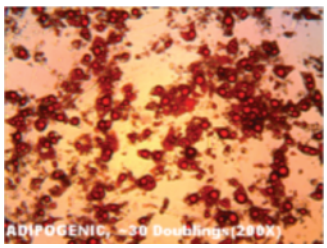
Chondrogenic



Chondrogenic - 30 doublings



Adipogenic



Adipogenic - 30 doublings

Figure 2

ACSelerate™ Serum-Free Growth Medium Drives Robust ADSC Proliferation While Maintaining "Stemness".

Cell lines expanded in ACSelerate growth medium have been expanded routinely to 20+ population doublings at a rate of 0.5-1.0 doublings/day. (Figure 1) Expanded cells show and maintain immunophenotypes consistent with those of

adipose-derived stem cells (Table 1) and may be rapidly differentiated into adipocytes, chondrocytes, and osteocytes (Figure 2) consistent with their status as mesenchymal stem cells. These features are maintained to 30 doublings and beyond (Table 2, Figure 2).

Lifespan studies conducted across a variety of patient-derived cell lines has indicated consistent and reproducible growth regardless of age or gender (Figure 3) and an average growth rate of ~0.65 doublings/day, with an average doubling time of ~37 hours, although some cell lines have been seen to expand at considerably higher rates approaching 1 doubling/day (doubling times of 24 hours). Growth rates observed to date almost always well exceed those reported for serum-containing media, where average doublings times for ADSCs are frequently reported in the 48-72hr. range.

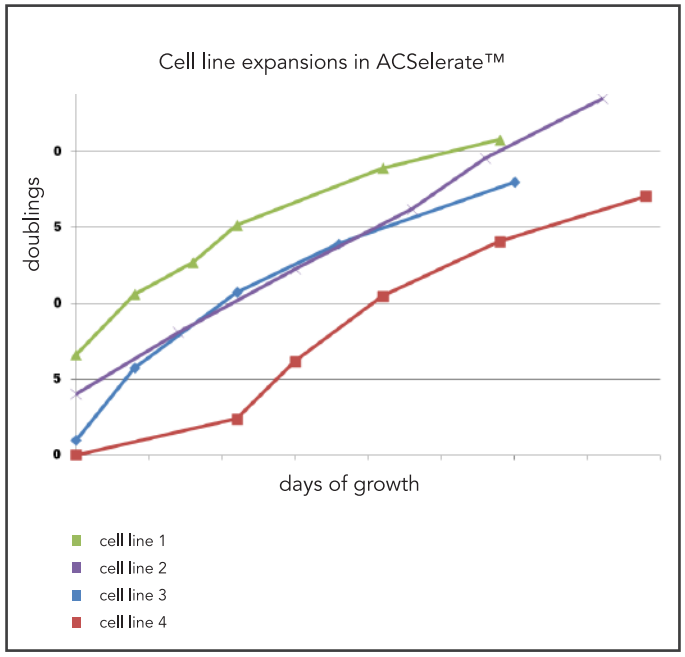


Figure 3

Figure 3: ACSelerate Serum-Free Growth Medium: Lifespans with Multiple Patient-Derived Primary Cell Lines.

Multiple primary cell lines of ADSCs have been generated from patient-specific adipose tissue samples in the American CryoStem Laboratories. Recent lines expanded in ACSelerate medium have demonstrated consistent growth rates regardless regardless of patient age or gender, and have given growth rates ranging from ~0.5 doublings/day to ~1.0 doublings/day, with an average rate of ~0.65 doublings/ day (~37 hour doubling time).

Testing alongside a popular and commonly used commercially available medium has indicated robust performance (Figure 4).

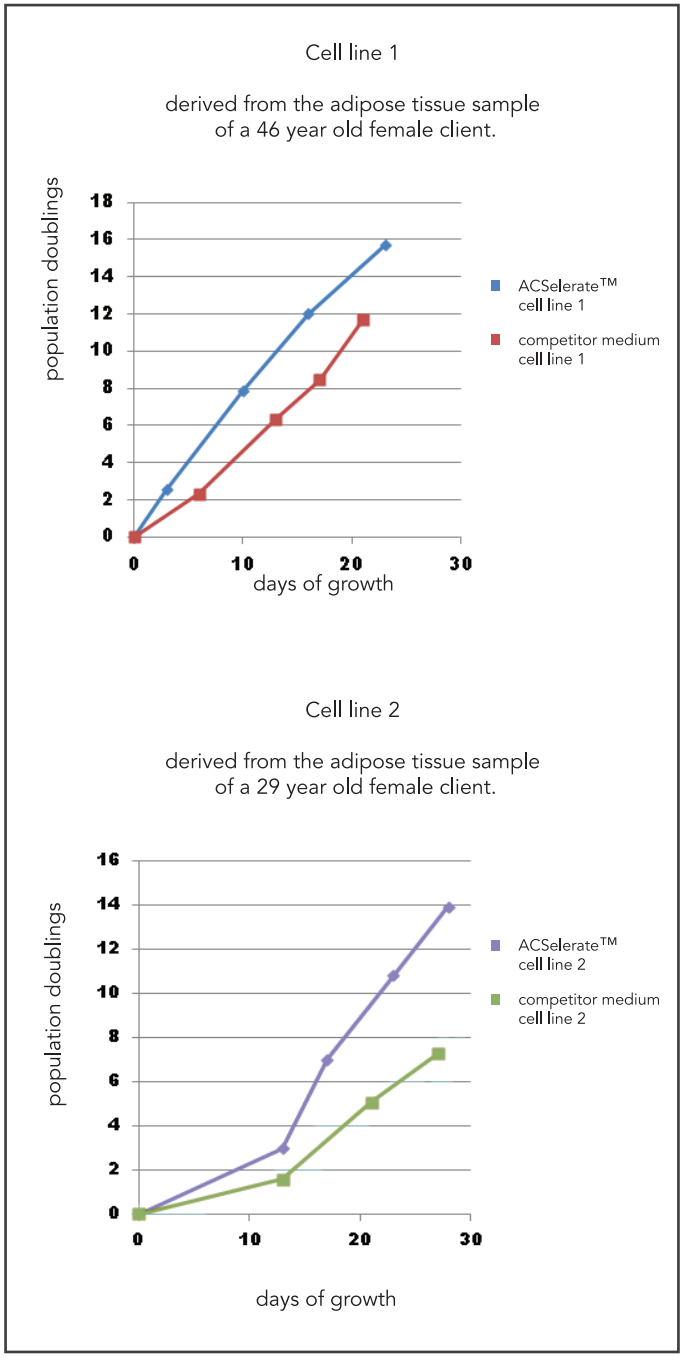


Figure 4

Figure 4: Growth Rates Achieved with ACSelerate SF-M Growth Medium Exceed a Leading Competitor's Medium.

The growth of two client cell lines were assessed using either ACSelerate™ SF-M or a commonly-used

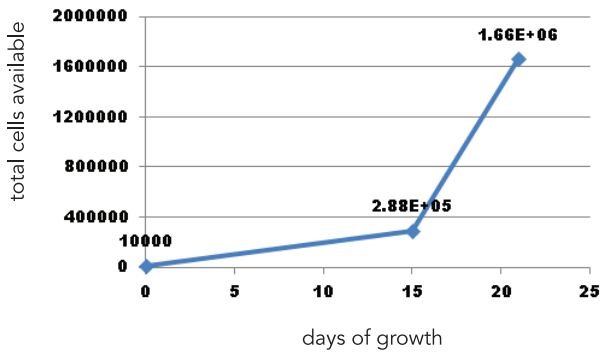
competitor's growth medium. In both instances, cells were seen to expand more robustly when grown with ACSelerate™.

Real World Expansion of Client Cells, Tales from the Bench:

As a service, American CryoStem Corporation is able, on request, to isolate stromal vascular fractions (SVFs) from client adipose tissue samples and to expand and bank ADSCs. These expansions are accomplished in house using ACSelerate™ SF-M medium. As stated previously, a chief need for any cell culture medium that is going to enable and support clinical applications of ADSCs is to be able to obtain usable cell numbers from minimal quantities of harvested tissue. To address this need, we present the following case studies:

Real-World Examples of Customer Cell Expansions Using ACSelerate™ SF-M Growth Medium

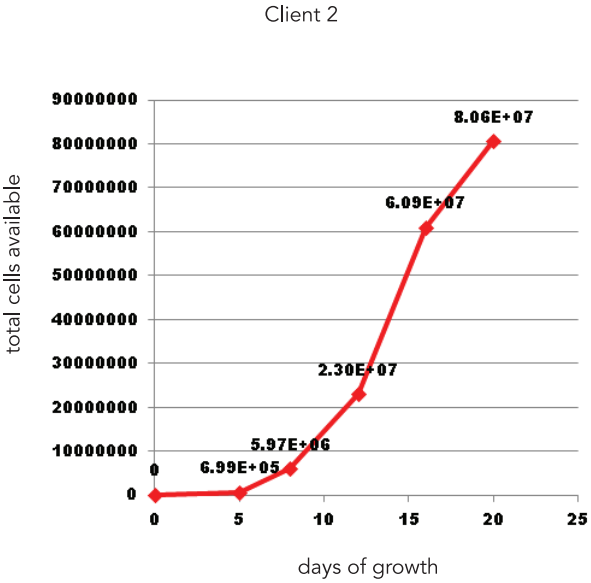
Multiple client samples have been expanded in the American CryoStem Corporation Laboratories. However, the two examples presented here represent extreme cases, where patients were unable to supply robust tissue samples. In both cases, ACSelerate™ SF-M growth medium was able to support the growth of considerable cell numbers from small quantities of tissue.



Client 1: less than 5 g of tissue collected, and roughly 10,000 cells present in the stromal vascular fraction. Adipose-derived stem cells (ADSCs) were expanded to 1.66 million cells in 3 weeks using ACSelerate™-SFM growth medium.

Figure 5

Client #1 (figure 5) was a very lean individual only able to provide roughly 3.5g of fatty tissue. From this sample, only approximately 10,000 cells were able to be harvested as part of the stromal vascular fraction. Current estimates indicate that only 2-10% of the SVF is comprised of ADSCs[17], meaning that only 200-1000 ADSCs would have been present. In approximately 3 weeks, this sample was expanded to 1.66 million cells, all of which were banked. This represents an expansion of 1,660-8,300 fold.



Client 2: an athletic individual was unable to provide a large tissue sample. In only 20 days, 80.6 million ADSCs were expanded and banked using ACSelerate™-SFM growth medium.

Figure 6

Client #2 (figure 6) was a former professional athlete still maintaining a vigorous, athletic lifestyle who was also able to provide only a modest adipose tissue sample. Within the same time frame (roughly 3 weeks), 80.6 million ADSCs were grown and banked from only ½ of the patient tissue sample. Eventually, by rejuvenating and expanding early passages of these cells to add to the collection, over 164 million total ADSCs were banked from ½ of the client's tissue sample.

Summary

To meet the demands of any potential clinical applications for adipose-derived stem cells, a serum-free, animal product-free medium that is able

to the sustain robust growth of ADSCs from even small patient tissue samples while maintaining the "stemness" of the expanded cells. ACSelerate™ SF-M has proven to be more than up to these criteria, consistently supporting robust cellular expansions from even small client tissue samples.

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