Large Scale Cell Expansion of Adipose-Derived Stem Cells & Primary Human Fibroblasts

3D Biotek
Enabling Technology for Stem Cell Therapy

Description & Application

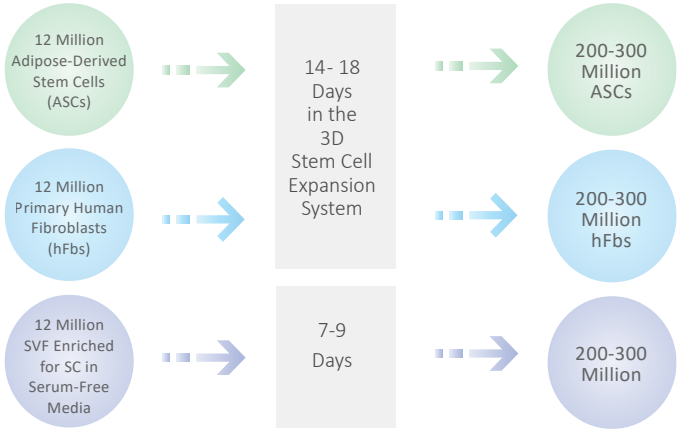
The 3D Cell Expansion System (3D-CES) is an integrated system designed for large scale expansion of anchorage-dependent cells in a 3-dimensional (3D) microenvironment.

Culture and expansion of a large quantity of cells by the traditional two dimensional (2D) method is difficult and known to have many challenges. The 3D-CES is designed to lift this inherent limitation of 2D cell culture and expansion methods. With the 3D-CES, cells are grown and expanded on 3D polystyrene (PS) scaffolds. The unique dynamic system circulates media through out the system using a peristaltic pump providing an efficient exchange of nutrients and waste between media and the cells. With minimum hands-on time, this innovative technology is more efficient and significantly reduces the amount of time, space, and labor needed to achieve the desired number of cells in a shorter amount of time compared to other methods.

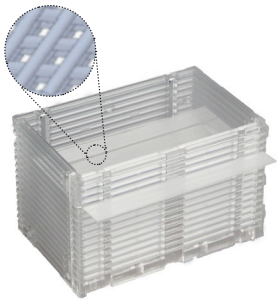
3D Biotek has successfully expanded primary adipose-derived stem cells (ASCs) and primary human fibroblasts (hFb) using 3D-CES from 12 million cells to approximately 200-300 million cells in 14-18 days. Furthermore, we have successfully isolated stromal vascular fraction (SVF) from fat and enriched for mesenchymal stem cells (MSCs) and expanded these cells using 3D-CES in serum free and xeno free media to reach 200-300 million cells in 15 days. Other cell types such as bone marrow-derived stem cells and cancer cells can also be expanded using this system.

In addition to large scale cell expansion applications, the 3D-CES can also be used for mass protein production for clinical treatment and/or cosmetic use.

This new product can be used to greatly increase the ability to expand various cell types and can play an important role in cell therapy, stem cell therapy, and biobanking.

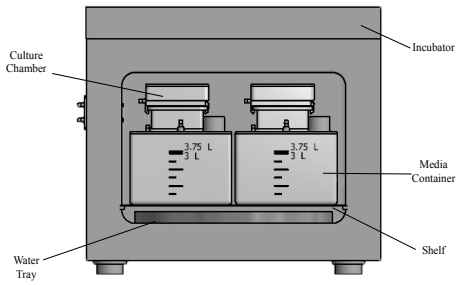


3D Cell Expansion System



Polystyrene scaffold in scaffold holder

Culture Chamber & Media Container



The 3D-CES fits two bioreactors to easily facilitate running two samples simultaneously, or allowing cell growth to double for one sample.

Results

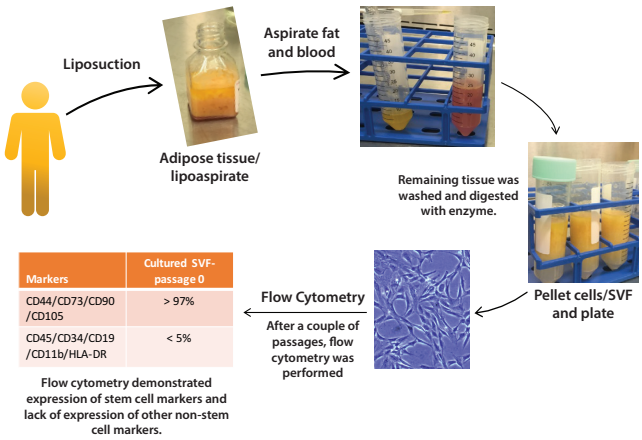


Figure 1. SVF isolation from fat

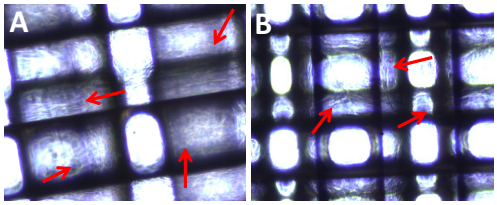


Figure 2. ASC Expansion on Scaffolds
A) Phase contrast image of ASC on 3D PS scaffolds after expansion in the 3D-CES shows confluent PS scaffold.
B) Phase contrast image of SVF in serum free/xeno free media on 3D PS scaffolds afer expansion in the 3D-CES shows confluent PS scaffold.

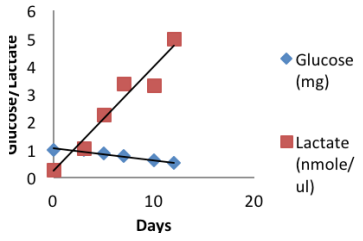


Figure 3. Changes in Glucose and Lactose Levels
Changes in A) glucose and B) lactate levels throughout cell expansion demonstrating that as cells grow lactate levels go up and glucose levels go down.

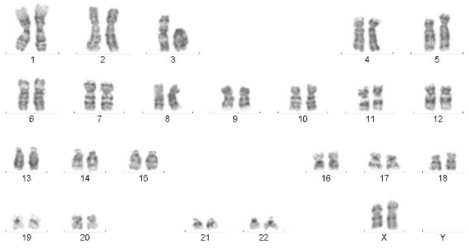


Figure 4. Karyotyping
Karyotype of ASCs after expansion demonstrating a normal karyotype with 46, XX chromosomes.
Procedure: GTG-banding Performed by CGI Laboratories

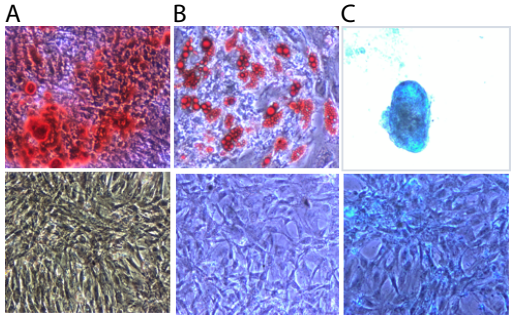


Figure 5. Differentiation of ASCs after expansion
Immunostaining of ASCs induced to differentiated for 21 days after expansion demonstrates that after expansion cells still maintain their multipotency and can differentiate into A) Osteocytes as shown by alizarin red staining, B) Adipocytes as demonstrated by oil red staining and C) Chondrocytes as shown by alcain blue.

Markers	Day 0	Day 18
CD44/CD73/ CD90/CD105	>99%	>95% except CD105

Markers	Day 0	Day 15
CD44/CD73/ CD90/CD105	>90%	>90%
CD11b/CD19/ CD34/CD45/ HLA-DR	<5%	<1%

Figure 6. Stem Cell Markers
Table A. Expression of stem cell markers before (Day 0) and after (Day 18) cell expansion demonstrating that ASCs maintain their stem cell markers after expansion (n=3).
Table B. Expression of SC markers before & after expansion of SVF in serum free/xeno free media (n=1).

CD44, CD90, CD73, and CD105 are positive stem cell makers. CD11b, CD19, CD34, CD45, and HLA-DR are negative stem cell markers

Samples run by the Flow Cytometry facility of Rutgers University

Conclusions

- ASCs & SVF enriched for SCs can attach and grow on scaffolds in 3D-CES
- ASCs & SVF enriched for SCs maintain their stem cell characteristics after expansion
- SVF enriched for SCs can be expanded using 3D-CES using serum-free/xeno-free media
- ASCs maintain a normal karyotype after expansion
- ASCs can differentiate into adipocytes, osteocytes and chondrocytes after expansion
- The 3D-CES is a unique product capable of large-scale cell expansion in 3D with minimal time, labor and space.

3D Biotek LLC.
1031 US 206 - Suite 202
Bridgewater, NJ 08807 USA

Tel: +1.908.801.6138
Fax: +1.908.801.6148
info@3dbiotek.com

www.3DBiotek.com
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