Human Adipose Derived Stromal/Stem Cells: Isolation, Characterization, and Applications

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In the last couple of decades, scientist have demonstrated that adult stromal/stem cells are present in multiple organs and tissues, including bone marrow, skeletal muscle, liver, blood vessels, and fat. In particular, fat -commonly known as adipose tissue- has demonstrated to be an easily accessible and rich source of adult stromal/stem cells. Obatala Sciences™ obtains its stromal/stem cells mainly from adipose derived tissue; however, it also offers stromal/stem cells from other human tissues such as, bone marrow and bone, and it is currently developing protocols to isolate and offer stromal/stem cells derived from placental, skeletal muscle, and cord blood tissues. To ensure that Obatala® is providing premium quality stromal/stem cells to its customers, Obatala's scientific team characterizes all manufactured stromal/stem lots with respect to information on the cells' proliferation, differentiation, clonogenicity, and immunophenotypic profile. Additionally, Obatala offers donor demographic information for all of its manufactured stromal/stem cell lots. In this white paper, we demonstrate Obatala's process to characterize stromal/stem cells after being isolated from human-derived tissues.

Donor Demographics:

Obatala Sciences offers information about the donor demographics for each isolated lot of stromal/stem cells. The donor demographics include information about the donor's gender, age, race, and body mass index (BMI; weight in kg divided by height in meters^2). By offering this information, our customers can request stromal/stem cell lots with specific demographic characteristics. Obatala's scientific team will work with the customer to find the cell lot that will be the closest match to the customer's request.

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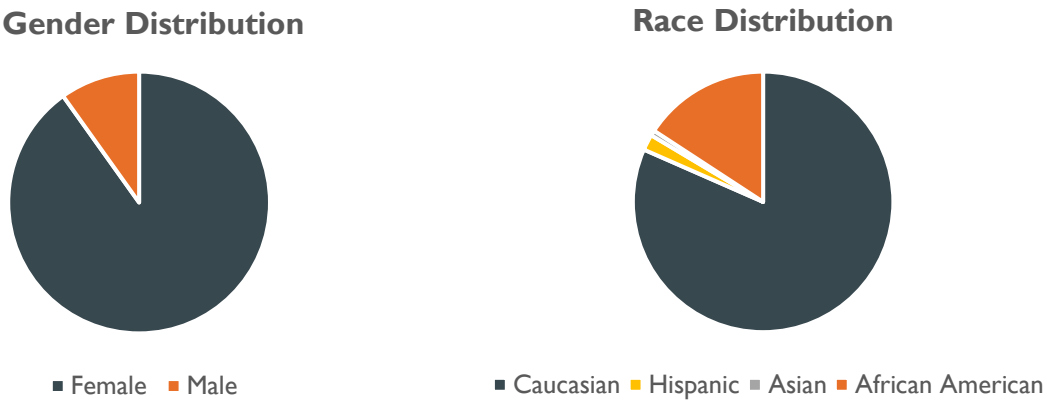

Figure 1: Gender and race distribution of Obatala's hASC inventory
Table 1: Donor demographic information available in Obatala's hASC inventory

Donor Demographics	
Gender	Female, Male
Age Range	25-85
Race	Asian, Caucasian, African American, Hispanic
BMI Range	18.9-51.54
Tissue Site	Information available upon request

Proliferation:

Proliferation of cells is analyzed over a four-day period. Cell counts are performed each day using Trypan Blue Exclusion (follow Obatala's protocol 104) counts and results are reported as population doubling time (PDT).

$$PDT = \frac{(\log_{10} 2) \times 3}{\log_{10} \frac{final\ value}{initial\ value}} = 1.27 \pm 0.33\ days$$

Final Value: number of cells at day 4
Initial Value: number of cells at day 1

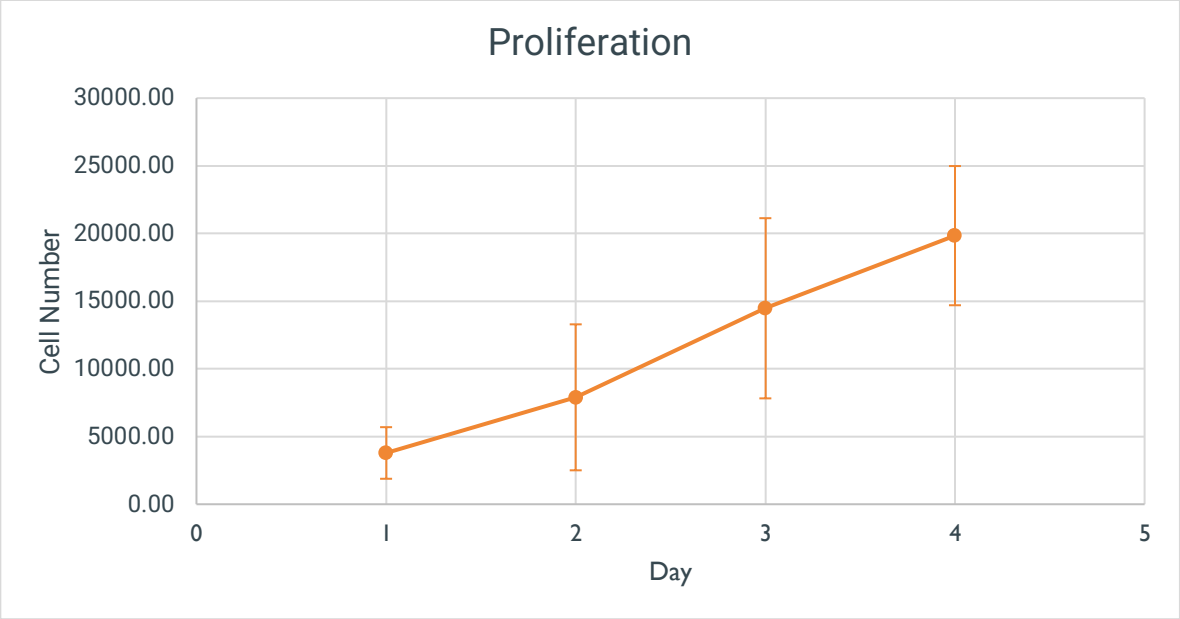

Figure 2: Representative population doubling time and cell proliferation of human-derived adipose stromal/stem cells from various donors (n=15)

Differentiation:

The differentiation potential of Obatala's human derived stromal/stem cells is tested by exposing the cells to adipogenic and osteogenic differentiation cocktails. To induce adipogenesis, cells are treated with AdipoQual™ for up to 14 days and then fixed and stained with Oil Red O (see Obatala Sciences Protocols 201 and 202). To induce osteogenesis, cells are treated with OsteoQual™ for up to 28 days and then fixed and stained with Alizarin Red (see Obatala Sciences Protocols 203 and 204).

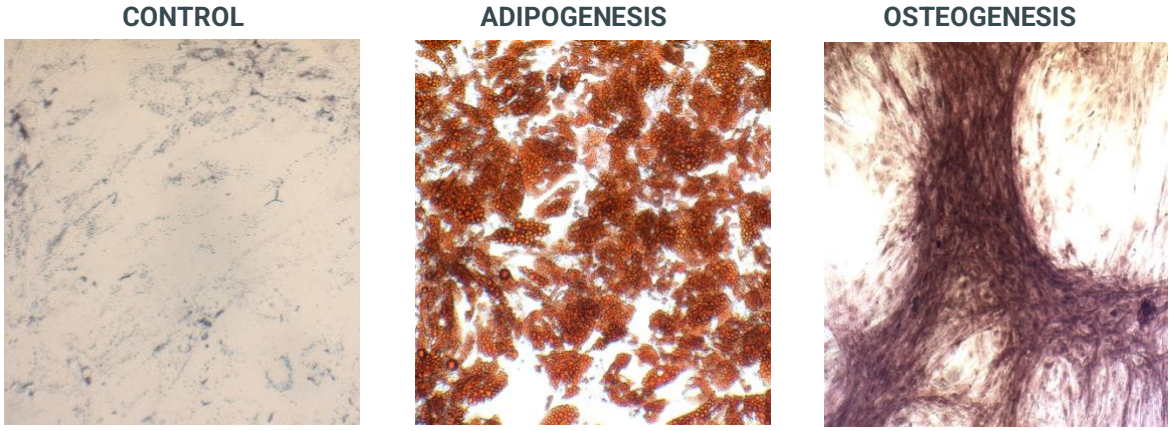

Figure 3: Representative differentiation of human-derived adipose stromal/stem cells from various donors (n=15)

Clonogenicity:

To test the clonogenicity, cells are plated in 6-well plates at low density for a period of two weeks. After maintaining for two weeks, cells are fixed and stained with Toluidine Blue. The number of colonies (defined as those containing 50 adherent cells or more) in each well are counted and results are reported as percent colonies formed.

$$\%\ CFU = \frac{\#\text{ of CFU per well}}{\#\text{ of total cells plated per well}} \times 100 = 53.11\% \pm 26.39\%$$

Table 2: Representative clonogenicity potential of human-derived adipose stromal/stem cells from various donors (n=15)

CFU-F	
PERCENTAGE	53.11% ± 26.39%

Immunophenotype:

The immunophenotype of cells is tested by flow cytometric analysis to ensure that the stromal/stem cells display the immunophenotype profile consistent with the identification of an ASC or MSC. This surface marker profile includes the robust expression of the cell markers CD105+, CD73+, and CD90+. Results are presented as percentage expression for each marker.

$$\%\ Expression = \%\ Positive\ Expression\ of\ Surface\ Mark - \%Expression\ of\ Unstained\ Control$$

Table 3: Representative immunophenotype of human-derived adipose stromal/stem cells from various donors (n=15). Values are represented as mean ± standard deviation.

Surface Marker	Average Expression (%)
CD29 PE	84.6 ± 14.4 %
CD105 PE	86.09 ± 16.02
CD45 PE	0.67 ± 1.94
CD34 PE	5.61 ± 9.35
CD44 FITC	78.5 ± 22.5
CD73 PE	71.23 ± 35.99
CD90 PE	82.92 ± 17.43
IgG PE	0.04 ± 0.12
IgG FITC	3.47 ± 5.08

Applications:

To this date, it has been proven that mesenchymal stem/stromal cells (MSCs) have many different research and clinical applications in the area of tissue engineering and regenerative medicine. For example, specific characteristics of MSCs are conducive for their use in cell-based therapies attempting to repair damage tissues. Specifically, the potential for self-renewal and differentiation of MSCs makes these cells attractive to

such therapies. Thus, MSCs are now being used to investigate their therapeutic potential in injuries/diseases such as, skin wounds, spinal cord injuries, arthritis, liver diseases, etc.[1] Another interesting characteristic of MSCs is their paracrine and immune regulatory functions. These functions allow MSCs to travel to sites where inflammatory responses are activated. Researchers have observed that in these instances, MSCs can intervene in the inflammatory responses by contributing to the repair of wounded tissue. These migratory abilities of MSCs has also served to investigate the delivery of therapeutic agents to tumor microenvironments; thus, allowing for the investigation for cancer treatments[2]. More recently, researchers are modeling diseases in vitro by combining MSCs with 3D hydrogel technology. These three-dimensional culture systems allow researchers to model tissue-like structures more accurately making them suitable for applications that intend to understand different disease models and develop drug treatments. Different disease models can be created by taking advantage of the differentiation potential of these types of cells. The cell-based products that Obatala Sciences offers are versatile and suitable for all of these different applications. Additionally, Obatala not only offers high premium quality mesenchymal stem cells, but also offers supporting products for cell-based research such as, 3D hydrogels and differentiation media. By offering these versatile products we strive to facilitate the investigation for research and clinical applications of mesenchymal stem cells (MSCs).

References:

[1]Kim, N., & Cho, S. G. (2013). Clinical applications of mesenchymal stem cells. *The Korean journal of internal medicine*, *28*(4), 387–402. https://doi.org/10.3904/kjim.2013.28.4.387

[2]Krueger, T.E.G., Thorek, D.L.J., Denmeade, S.R., Isaacs, J.T. and Brennen, W.N. (2018), Concise Review: Mesenchymal Stem Cell-Based Drug Delivery: The Good, the Bad, the Ugly, and the Promise. STEM CELLS Translational Medicine, 7: 651-663. doi:10.1002/sctm.18-0024