lipografter®

THE LIPOGRAFTER® SYSTEM IS EFFECTIVE IN ISOLATING VIABLE AND FUNCTIONAL CELLS

MTF Biologics, Research and Development Department

International Distributor
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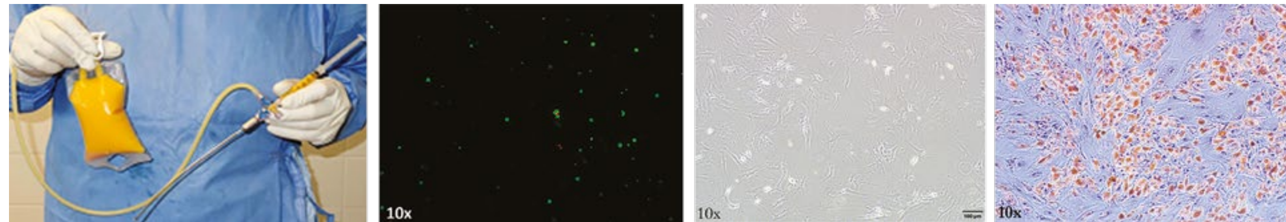
lipografter®



The LipoGrafter® is a closed system, which collects and isolates lipoaspirate through a gentle decantation process to minimize cell damage. The characterization data provides evidence that the resulting lipoaspirate is of high quality. The stromal vascular fraction (SVF) cells are viable and functional as they have the potential to proliferate and differentiate into adipocytes. This high quality lipoaspirate containing these viable and functional cells can be reinjected into the patient to support volume restoration.

INTRODUCTION AND BACKGROUND

Autologous fat grafting (AFG) is a common procedure used in aesthetic and reconstructive plastic surgery to restore volume deficits or improve body contouring. There are several different methods of harvesting fat, including a novel autologous fat harvesting, and grafting device known as the LipoGrafter® system (Figure 1).

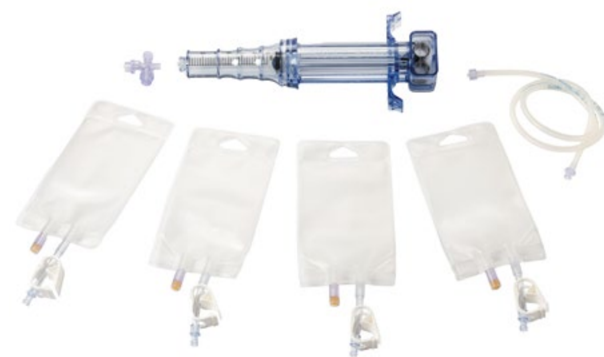


Figure 1: LipoGrafter® kit consisting of an atraumatic tissue valve (AT-Valve®), KVAC® constant vacuum harvesting syringe, tube connection set, and four lipoaspirate collection bags.

The LipoGrafter® is a closed system that can be used to both harvest and re-inject fat into patients, minimizing both fat cell damage and risk of contamination, while allowing for heightened control of suction and volume re-injection.[1] This is made possible by an atraumatic tissue valve, which facilitates a closed system, and the KVAC® syringe, which provides low pressure aspiration shown to decrease adipose trauma.[1] The lipoaspirate is isolated by decantation/sedimentation by gravity to separate the tumescence fluid and blood components. There is less cell damage as there is no shear forces exposed to the cells from centrifugation forces that could lyse and/or damage cell membrane.

The goal of this study was to evaluate cell viability and function (ability to proliferate and differentiate into fat cells) of the stromal vascular fraction (SVF) in the lipoaspirate collected with the LipoGrafter® system.

MATERIALS AND METHODS

LipoGrafter® Procedure

Lipoaspirate was collected from the abdomen from consenting patients, who were undergoing bilateral breast reconstruction revision or bilateral mastopexy (breast lift) procedures. The lipoaspirate was pulled through the cannula into the closed tubing system through the KVAC® constant low pressure vacuum harvesting syringe (which minimizes cell damage), and then collected into the collection bag for sedimentation. After the lipoaspirate was decanted, syringes were used to remove the bottom layer of sediment and excess tumescence fluid (Figure 2). The decantation process is a gentle method of isolating the lipoaspirate, by minimizing cell damage and supporting cell viability, yielding quality AFG for reinjection to restore volume deficits.

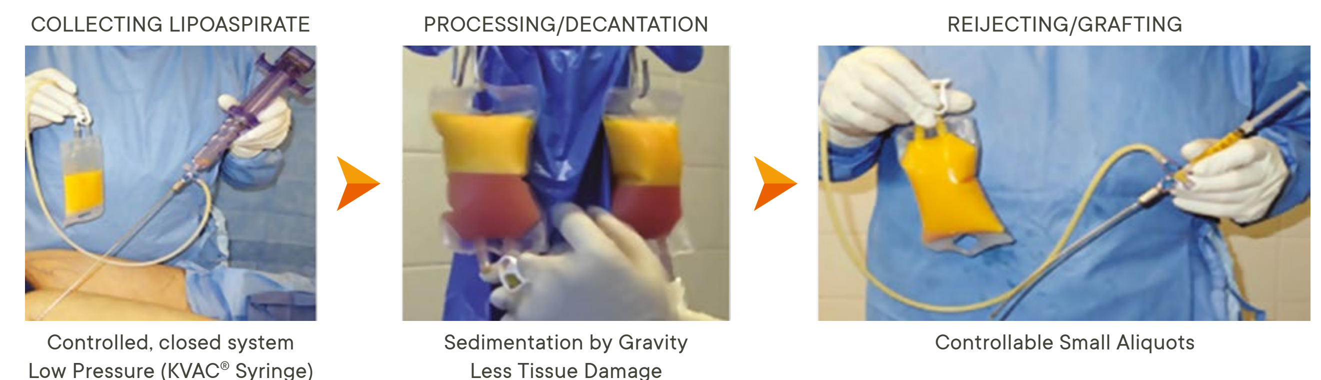


Figure 2: The separation process of lipoaspirate is through sedimentation by gravity and decanting of excess fluid. The collection bags were placed on the IV pole, allowing a clear distinction between the fat layer (top) and excess fluid (bottom). At this point, the bottom layer of excess fluid was drained to isolate the lipoaspirate for reinjection/grafting.

Characterization of Lipoaspirate from the LipoGrafter® System

Excess lipoaspirate was placed into sterile 50mL centrifuge tubes and transported to MTF Biologics for characterization. The lipoaspirate was washed 2–3 times in 1X phosphate buffer saline (PBS), until the tissue appeared yellowish gold in color. After removing the last PBS rinseate, a modified isolation protocol[2] involving a 0.1% collagenase type IA solution (Sigma-Aldrich Inc., GA) was added and incubated for 2 hours in a 37°C water bath (under agitation). Post-digestion, the tissue appeared smooth, and the collagenase digestion was inactivated with an equal volume of maintenance media consisting of DMEM/F12, 10% fetal bovine serum (FBS), and 1% penicillin/streptomycin (antibiotic solution) (Fisher Scientific, PA). The solution was then centrifuged to collect the SVF pellet. The SVF pellet was then resuspended in ammonium chloride solution and incubated at room temperature for 10 minutes to help lyse the red blood cells. Lastly, the solution was centrifuged to isolate the

SVF cells. From this cell solution, 1mL of cells were set aside for cell viability assessment, while the remainder was plated into T25 flasks for cell culture to evaluate cell change function to function (ability for cells to attach, proliferate and differentiate).

Assessing Cell Viability

The cells from the isolated pellet were re-suspended and stained using the Live/Dead Viability Toxicity Kit (Invitrogen, OR) to visualize viable and dead cells. After a 10-minute incubation in the Live/Dead solution at room temperature, the cells were centrifuged and re-suspended in 1mL PBS. Stained cells were imaged under 10x magnification using fluorescent microscope (Leica Microsystems); green staining indicated viable cells and red staining indicated dead cells. Image J software was used to quantify the number of live and dead cells from these images and calculate the average cell viability.

Evaluating Cell Functionality

To verify the viable cells are functional (ability to attach, proliferate and differentiate), the SVF cells from the pellet were cultured in T25 flasks. The maintenance media was exchanged twice a week until cells reached 80% confluency (proliferating cells covered 80% of the surface area in the T25 flask). The confluent cells were then treated with Adipocyte Differentiation Media (Cell Applications, Inc., CA) for up to 2 weeks. Cells were fixed and stained using an Oil Red O Staining Kit (Biovision, CA) on day 14 to assess the ability of cells to produce intracellular lipid droplets, which provides evidence of successful differentiation into mature adipocytes.

RESULTS

Cell Viability

Live/Dead staining on isolated SVF cells was used to establish cell viability of the isolated lipoaspirate from the LipoGrafter® system. The average cell viability was 95.5 ± 3.7% (Figure 3).

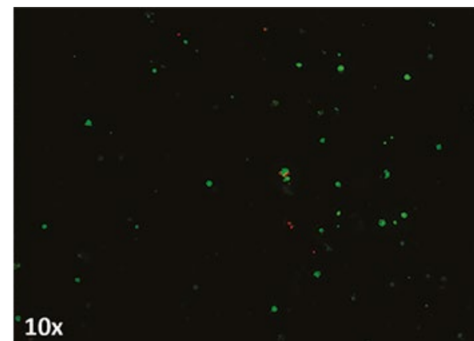


Figure 3: Average cell viability of the SVF cells isolated from the lipoaspirate, from the live/dead analysis was 95.5 ± 3.7% (green cells indicated viable cells, red cells indicated dead cells).

Cell Function

The isolated cells from the SVF pellet were cultured and shown to readily attach, proliferate over time and reach confluency by day 5 (Figure 4). The morphology of the adhered cells were typically fibrotic and elongated in appearance when cultured in maintenance media (pre-differentiation). Once the adherent cells were treated with the Adipocyte Differentiation Media, the cells started differentiating into adipocytes and exhibited the classic round, grape-like appearance. This was observed as early as day 8 post-differentiation and was sustained,

covering the entire dish by day 14. This provided evidence that the viable cells from the SVF were able to proliferate, respond to the external cues provided by the differentiation media and differentiate into adipocytes. Furthermore, to visualize if adipocytes are functioning by depositing intracellular lipid droplets, Oil Red O (orange/red) staining was performed on the differentiated cells on day 14. A strong presence of orange/red staining was observed, which demonstrates an abundance of intracellular lipids present and verify the evidence of functional adipogenic cells.

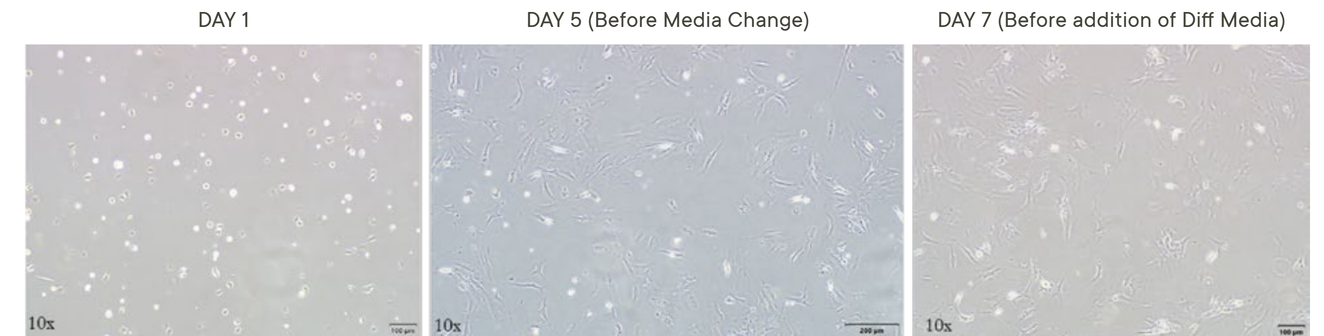


Figure 4: SVF cells readily attached and proliferated to confluency (covering the entire flask surface, 10x magnification).

Furthermore, the presence of orange/red staining (evidence of mature adipocytes) was greater in the differentiated media compared to the maintenance media. In quantifying lipid levels, there was more Oil Red O staining in the adipogenic differentiation media group compared to the maintenance media one. This further confirmed that the SVF cells are healthy, viable and functional in the lipoaspirate collected from the LipoGrafter® system (Figure 5).

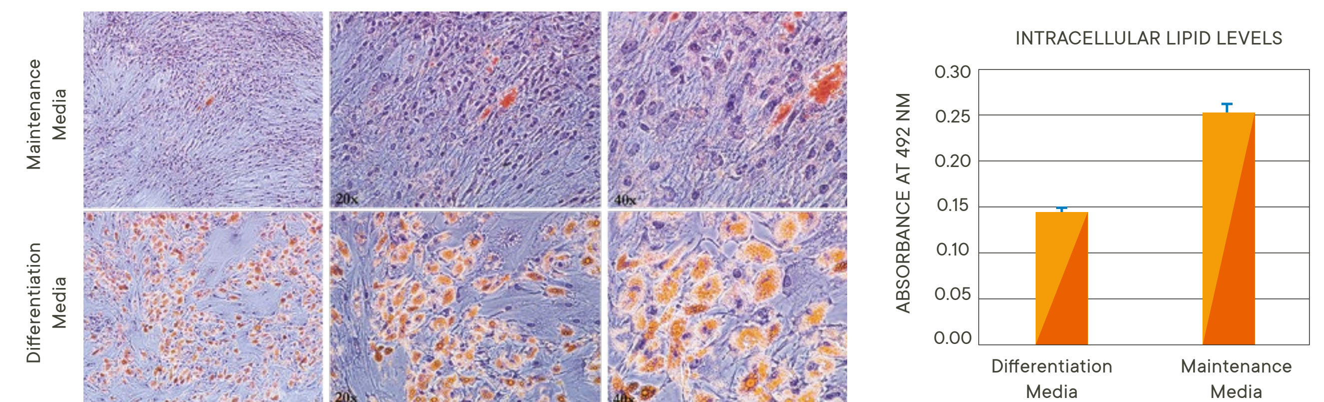


Figure 5: Oil Red O staining of intracellular lipid droplets in differentiated SVF cells (10x, 20x and 40x magnification). Orange/red coloration is indicative of areas with lipids. Classic grape-like appearance was observed. The quantification by the adipogenic staining of Oil Red O revealed higher levels in the differentiated media compared to the maintenance media.

DISCUSSION

The quality of the lipoaspirate from AFG systems is considered important to support volume restoration and body contouring. The LipoGrafter® system provides a closed system to reduce the risk of contamination and cell damage from exposure to air. Furthermore, the low-pressure aspiration to acquire the lipoaspirate together with the gentle sedimentation process ensures the cells are exposed to low shear forces that minimize the cell damage and preserve cell viability. Other AFG systems use centrifugal forces or filtering processes to isolate the lipoaspirate, which can generate shear forces that impact cell viability of the isolated cells in the lipoaspirate.

From the characterization conducted on the isolated lipoaspirate, the data revealed high cell viability in the SVF cells (95.5 ± 3.7%). Other AFG systems report on the quality of the cell viability only, which represents a snap-shot assessment (t=0). An additional angle in this LipoGrafter® characterization was examining cell viability at a longer duration (t=14 days) through cell culture studies and thereby evaluating cell function (the ability of cells to proliferate, deposit functional proteins). From literature, SVF cell function is evaluated by the ability of cells to differentiate into adipocytes and deposit intracellular lipid droplets.[3–5] The characterization data verified that the SVF cells isolated from the lipoaspirate are not only viable over a longer period, but that the cells are functional as they attached to the cell culture surface, proliferated/expanded within 5 days and differentiated into adipocytes (exhibiting typical cell morphology appearance) when exposed to adipogenic culture media. Furthermore, Oil Red O staining confirmed that the differentiated cells produced intracellular lipid droplets indicating the presence of mature adipocytes[3–5]. Lastly, the quantification also corroborated that there were greater levels of lipid droplets in the adipogenic media treated cells than the maintenance media cells, confirming that the viable SVF cells are responding to the specific media cues.

Altogether, this characterization data provides evidence that the resulting lipoaspirate from the LipoGrafter® system is of high quality. The SVF cells are viable and functional (can expand and become fat cells upon exposure to the correct external cues). This viable and functional lipoaspirate can be injected into areas in the human body that need volumization and/or body contouring.

CONCLUSION

The LipoGrafter® system can effectively isolate high quality lipoaspirate, which contains viable and functional cells that can support autologous fat grafting procedures. The decantation process is a gentle method of separation, minimizing damage to fat cells and contributing to cell viability and function.

REFERENCES

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