Adipose Tissue Cell Viability of Four Fat Harvesting Modalities

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Adipose tissue cell viability of four different fat harvesting modalities was measured by analyzing fresh tissue samples taken from one live human subject using the Vision Cell Analyzer from Nexcelom, Inc. of Lawrence, Massachusetts. The four fat harvesting techniques were: 1. Coleman syringe, 2. standard Suction Assisted Lipoplasty, 3. Vaser-Ultrasonic Assisted Lipoplasty and 4. HydraSolve Lipoplasty System.

Participating in the study were Tamara Tchkonia, PhD and Nino Giorgadze, PhD, adipose tissue cell biologists, both from The Mayo Clinic, Rochester, Minnesota. Also present, Bruce Kuo, PhD from Jefferson Medical College of Thomas Jefferson University, a cell biologist specializing in hepatocytes. Also present, was Ning Lai, PhD a representative of Nexcelom Corporation from Lawrence, MA (a cell biologist trained at Tufts University).

Dr. Lai brought the Vision Cell Analyzer to use in the testing the cellular samples. Mark Andrew, M.D. served as coordinator, observer and recorder. Dr. Tchkonia served as chief science director of activities, and Drs. Giorgadze and Kuo conducted the actual bench top analysis. Dr. Lai completed the fluorescent dye staining, to prepare the cellular samples for reading in the automated cell counter, and Dr. Lai performed the actual cell readings in the cell counter with observation and direction by Dr. Tchkonia.

A total of 7 samples, taken from 7 different waste containers containing fat aspirates, were read in the automated cell counter. The waste containers containing the fat aspirates were brought from the third floor operating suite to the first floor lab. Upon arrival in the lab, the material in the containers had settled into an obvious supernatant layer consisting primarily of fat tissue and an infranatant layer consisting mainly of a fluidic mixture of blood and saline. The difference between the HydraSolve container and the others was marked: the HydraSolve

supernatant was light yellow in color, was clearly a homogeneous liquid, and was devoid of connective tissue chunks as well as blood. The HydraSolve infranatant consisted of a thin, light salmon/pink colored liquid. The other liposuction waste containers did not look similar to HydraSolve, but they did look similar to each other: the supernatant was reddish-orange in color, the Suction Assisted and Ultrasound Assisted supernatants contained obvious chunks of connective tissue and the Coleman supernatant appeared thick and clumpy (chunks of connective tissue were not discernible); all the non-HydraSolve infranatants consisted of a dark red, thick and blood-like fluid (see image 1 below, C2, U2 and S2).

Image 1
C – Coleman, U – Ultrasound (UAL), S – Suction Assisted (SAL), A – HydraSolve

The 7 aspirate samples arrived in the lab between 12:53 pm to 3:06 pm.; each one arriving ~15-20 minutes after the previous one. As the samples arrived they were allowed to settle for a few minutes. The first analysis completed was: a sample of the Coleman supernatant taken using a pipette and exposed to trypan blue stain. That stained sample was then placed on a hemocytometer cell counting slide and viewed under the microscope. This was also done to the HydraSolve #1 aspirate.

The observation made by Dr. Tchkonia, and corroborated by Drs. Giorgadze and Kuo was thus: the HydraSolve # 1 sample was clearly in a cell suspension form. The Coleman sample was in clumps and was not in a cell suspension state. Suction assisted and Ultrasound assisted

samples were not examined in this manner under the microscope as the cell biologists were unanimous in their opinion that it was inconceivable those aspirate samples would be in a state of cell suspension with their thick, chunky and clumpy appearance to the naked eye.

Dr. Tchkonia commented that the lack of blood in the HydraSolve supernatant, in all 4 aspirates, was remarkable and very different from the other non-HydraSolve aspirates. Also, the homogeneous and liquid nature of the supernatant that was devoid of connective tissue chunks was very different from all non-HydraSolve aspirate supernatants (see image 2 and 3 below).

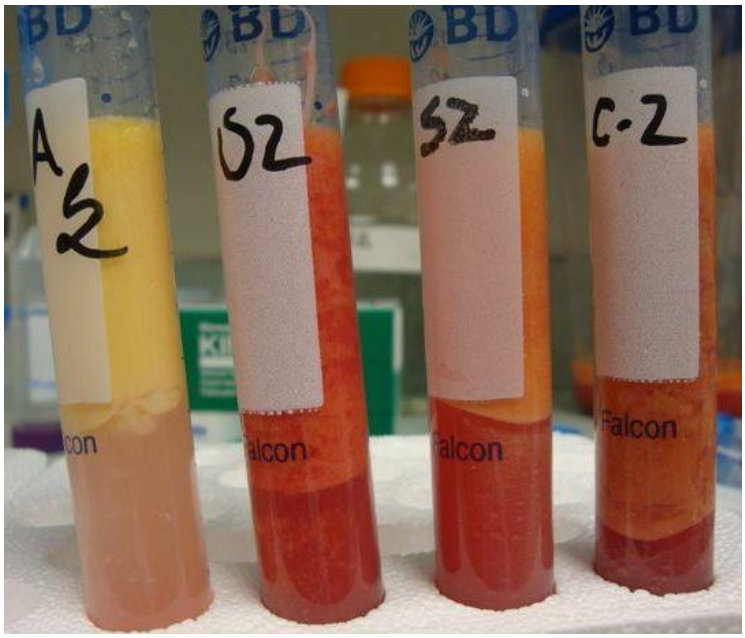


Image 2 **Image 3**

Settled Samples No Centrifuge

C – Coleman, U – Ultrasound (UAL), S – Suction Assisted (SAL), A – HydraSolve

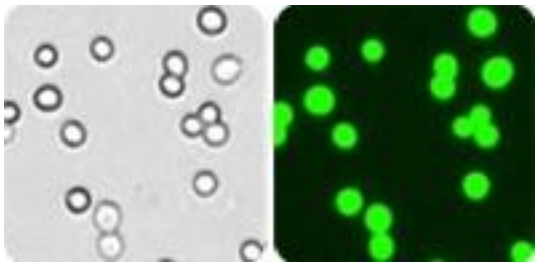
Next, a sample from the supernatant of the first waste container (Coleman) was taken using a pipette and placed in a test tube and labeled. Then a smaller sample was taken using a pipette from the test tube and placed in a 2 ml centrifuge tube. (Epindorf centrifuge.) The sample was spun at 800 rpm for 5 minutes. Then a collagenase digestion was performed on that post-spun sample in a 37 degree C water bath, using 1 mg/ml of collagenase (Worthington type 1) for 45 minutes. Post digestion, the sample was spun again in the centrifuge. Then a sample was taken using a pipette from the supernatant in the centrifuge tube and exposed to two fluorescent dyes for approximately 10 minutes by Dr. Lai. After, a small sample from that post fluorescent dye stained sample was placed onto the Vision Cell Analyzer slide, the slide was placed into the

cell counter and it was read. The identical process and procedure was done to all 7 aspirate samples before being read in the Vision Cell Analyzer.

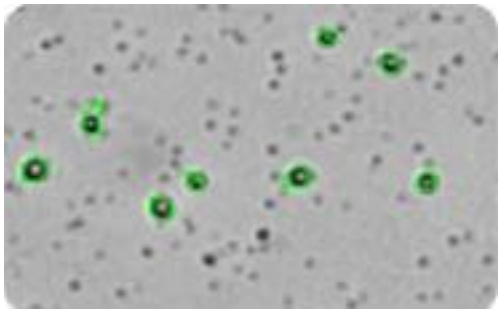
The Vision Cell Analyzer distinguishes adipocytes from lipid droplets; the fluorescent dyes stain only cells and not lipid droplets. (When reading the slides manually through a microscope it is very difficult to distinguish a lipid droplet from an adipocyte.) The first dye stains all cells present, alive and dead cells. The second dye stains only dead cells. The software does a subtraction and provides the % of live cells present. Four separate fields are read and averaged. Dr. Tchkonia had confidence in the science and accuracy of the Vision Cell Analyzer.

The cell counter counts all cells present. Looking at the images on the laptop screen which showed the field of cells being read, one field at a time, Dr. Tchkonia commented that in all fields "it is clear that the majority of cells being read are adipocytes; from what we know of adipose tissue cellular biology, the other cells present are progenitor cells, pre-adipocytes, endothelial cells and macrophages…".

Nexcelom Automated Counter



Brightfield and Fluorescence



Cell Declustering for Counting

Test Results

All samples prepared identically (all post centrifugation; all post collagenase digestion)

Sample	Liposuction Modality	Vacuum Setting	Power Setting	Cannula	Anatomical Location	Viable Cell %
C	Coleman	Hand Syringe	N/A	3mm Coleman	Posterior Flank	85.5
S	SAL	300mmHg	N/A	3mm 3 aperture	Posterior Flank	82.7
U	UAL (Vaser)	300mmHg	70% continuous For 5-minutes	2-ring 3.7mm probe 3mm 3 aperture cannula	Posterior Flank	72.7
A1	HydraSolve	300mmHg	37°C 600psi	3mm 2 aperture	Posterior Flank	98.0
A2	HydraSolve	300mmHg	37°C 600psi	3mm 2 aperture	Abdomen	94.4
A3	HydraSolve	300mmHg	45°C 1100psi	3mm 2 aperture	Abdomen	99.2
A4	HydraSolve	660mmHg	53°C 1300psi	3mm 2 aperture	Abdomen	94.7

CONCLUSION:

Four fat harvesting modalities were evaluated for cell viability using the Vision Cell Analyzer from Nexcelom, Inc. of Lawrence, Massachusetts: Coleman Syringe (CS), Suction Assisted Lipoplasty (SAL) , Vaser-Ultrasonic Assisted Lipoplasty (V-UAL) and HydraSolve Lipoplasty System. The testing was performed under expert guidance, directed by a world authority on adipose tissue cell biology. A total of four PhDs in cell biology were present; all four were actively involved in the process and procedure. Tissue sample preparation of all four fat harvesting modalities was identical, using standard centrifugation and collagenase protocols.

The HydraSolve Lipoplasty System had the best cell viability determination. The four HydraSolve samples ranged from 94.4 % to 99.2 % cell viability, with an average of 96.6 %. The

HydraSolve Lipoplasty System produced excellent cell viability at all machine settings, even at the highest setting. The Coleman modality came in second, SAL third and Vaser-UAL fourth.

The HydraSolve Lipoplasty waste container supernatant was significantly different than the other three supernatants: 1. The HydraSolve supernatant was light yellow in color appearing to be devoid of blood, versus all of the other supernatants that were orange-red in color and clearly contained blood. 2. The HydraSolve supernatant was a homogeneous appearing liquid, devoid of chunks of connective tissue and clumps of fat tissue. Both the SAL and V-UAL supernatants were not homogeneous liquids and did contain obvious chunks of connective tissue and clumps of fat. The Coleman supernatant was not a homogeneous liquid and contained obvious clumps of fat. 3. Microscopically, the HydraSolve supernatant was in a state of cell suspension prior to collagenase digestion, while the Coleman supernatant was not (Drs Tchkonia, Giorgadze and Kuo were unanimous that it was inconceivable that the SAL and V-UAL aspirates would be in a state of cell suspension, based on their obvious chunky and clumpy appearance, so they did not look at those fat tissues under the microscope).

The HydraSolve Lipoplasty System appears to be an ideal fat harvesting modality. It has outstanding cell viability capability, the fat aspirate supernatant appears to be devoid of blood and therefore probably does not require significant cleaning or processing, and the fat aspirate supernatant is in a state of cell suspension in the waste container which may be ideal for injection into the same patient.