Evaluation of MicroAire Tissue Collection Method on Adipose Tissue and ADRCs

Confidential Protocol and Report

July 2011

cytori

1.0 Summary

Conventional syringe lipoaspiration, though considered to be the most gentle and best method for acquiring healthy adipose tissue for fat grafting is time consuming and technically more difficult than most other common lipoaspiration methodologies. Utilization of power assisted lipoaspiration with instrumentation such as that provided in the MicroAire systems would provide a faster and easier to use technique for harvesting fat and obtaining tissue for adipose-derived regenerative cell extraction.

Based upon the analyses of fat graft and ADRC generation presented here, use of MicroAire power assisted lipoaspiration appears to disrupt adipose tissue more than syringe based tissue harvest. This may be advantageous when trying to acquire ADRCs in patients with low vascular density. Although data evaluating fat graft quality before and after Puregraft™ processing indicates that the mature adipocyte component of fat graft is damaged more by PAL harvest than by the syringe technique, Puregraft is able to improve fat graft regardless of the harvest method used in this study to yield comparable adipose graft.

2.0 Purpose

The purpose of this study is to assess how the MicroAire lipoaspiration system (MicroAire Surgical Instruments, LLC) affects the biologic properties of the aspirated adipose tissue, and on the yield, viability, and mixture of cell types found within the ADRCs (adipose-derived regenerative cells) obtained from the adipose tissue.

3.0 Definitions

3.1 **CD34:** The CD34 protein is a cluster of differentiation molecule present on certain cells within the human body and is a member of a family of single-pass transmembrane sialomucin proteins that show expression on early hematopoietic and vascular-associated tissue. It is a cell surface glycoprotein and functions as a cell-cell adhesion factor. It may also mediate the attachment of stem cells to bone marrow extracellular matrix or directly to stromal cells.

3.2 **CD45:** Protein tyrosine phosphatase, receptor type, C (PTPRC) is a member of the protein tyrosine phosphatase (PTP) family. PTPs are known to be signaling molecules that regulate a variety of cellular processes including cell growth, differentiation, mitotic cycle, and oncogenic transformation. It is specifically expressed in hematopoietic cells except erythrocytes and plasma cells. This PTP has been shown to be an essential regulator of T- and B-cell antigen receptor signaling. It functions through either direct interaction with components of the

antigen receptor complexes, or by activating various Src family kinases required for the antigen receptor signaling.

3.3 **CD31:** CD31, also known as platelet endothelial cell adhesion molecule 1 (PECAM1), is a type I integral membrane glycoprotein and a member of the immunoglobulin superfamily of cell surface receptors. It is constitutively expressed on the surface of endothelial cells, and concentrated at the junction between them. It is also weakly expressed on many peripheral lymphoid cells and platelets.

3.4 **CD68:** CD68 (Cluster of Differentiation 68) is a glycoprotein which binds to low density lipoprotein. It is expressed on monocytes/macrophages and giant cells.

3.5 **Celase®:** A Cytori Therapeutics proprietary enzyme used for tissue dissociation.

3.6 **Celution® 800/CRS Tissue Processor:** A semi-automated system that can be used to wash and enzymatically digest adipose tissue to release, concentrate and wash a regenerative cell fraction.

3.7 **CFU-F assay:** A culturing method used to determine the frequency of progenitor cells in a population of nucleated cells.

3.8 **Flow Cytometry:** A technique for identifying and sorting cells and their components (as DNA) by staining with a fluorescent dye and detecting the fluorescence usually by laser beam illumination.

3.9 **Process Solution:** Lactated Ringer's solution—the solution used to wash the tissue, dilute the Celase reagent, and wash the ADRC pellet and autologous graft.

4.0 Experimental Procedures

4.1 Patient and Surgical Site Selection

4.1.1 The following parameters were used to select patient population and qualify acceptable procedures for the study inclusion.

4.1.2 Patient Selection

- 4.1.2.1 Male or female
- 4.1.2.2 Age 20-60
- 4.1.2.3 Good health; ASA Class I
- 4.1.2.4 BMI < 30

4.1.2.5 No history of bleeding disorders, diabetes, HIV or lipoatrophy disorders (lupus, scleroderma, etc.)

4.1.2.6 Non-smoker preferable

4.1.3 Site Selection

4.1.3.1 Surgical site chosen based on patient requirements

4.1.3.2 Preferred areas are: abdomen, flanks, inner and outer thighs

4.1.3.3 Exclude back, chest, arms, calf, superficial sculpting

4.2 Surgical Parameters—to be collected at time of procedure

4.2.1 Anesthesia or other analgesic use

4.2.2 Infiltration method:

4.2.2.1 Infiltration Fluid

4.2.2.1.1 Tumescent/wetting solution formula for each test case

4.2.2.1.2 For example: 1 ampoule epinephrine per 1L bag, saline or lactated Ringers.

4.2.2.2 Infiltration temperature: 35-40°C. Approximate temperature

4.2.2.3 Infiltration volume/ratio -- example: 1.5 to 2 IN for every 1 OUT

4.2.2.4 Record

4.2.2.4.1 Total infiltration volume (per body area)

4.2.2.4.2 Start time for infiltration (per body area)

4.2.2.4.3 Infiltration cannula diameter/type—for example, 14 gauge/multi hole pattern

4.2.2.4.4 vacuum level used –for example 50%, or (15 in/Hg)

4.2.2.4.5 Cannula diameter: 3.0 mm, 4 mm

4.2.2.4.6 MicroAire Cannula Style/type

4.3 Experimental data was collected from 5 donor tissues obtained the same day as experiment initiation as described below.

4.4 **Tissue Collection and Distribution**

4.4.1 Tissue was collected from 5 donors using both MicroAire assisted lipoplasty and by 60 CC syringe aspiration. At least 500 mL of adipose tissue and ideally 600 mL was collected from each donor.

4.4.2 Approximately 10 mL of the collected adipose tissue was used for adipose tissue quality assessment and 5 mL of the samples was used for histological analysis.

4.4.3 A minimum of 50 mL and preferably 100 mL was set aside and washed using the Puregraft™ system and then analyzed for hydration, lipid volume and intact graft volume.

4.4.4 A minimum of 110 mL (maximum 250 mL) from each collection method was used for Celution® 800/CRS system processing to evaluate the adipose-derived regenerative cell populations

4.5 **Celution® 800/CRS Processing**

6.6.1 Lipoaspirate was processed using the Celution® 800/CRS processors to generate ADRCs concurrently. After recovery from the device the resuspended ADRC output was transferred into two pre-labeled 15 mL tubes for further analysis.

4.6 **Puregraft™ Analysis**

4.6.1 30 mL of adipose was separated into three separate 15 mL conical centrifuge tubes. Three 10 mL aliquots each of adipose from either MicroAire or syringe samples was placed into the centrifuge and spun for 5 min at 1200 r.p.m. The volumes of aqueous phase, tissue, and free lipid was measured and recorded.

4.6.2 Lipoaspirate was processed for graft preparation using the Puregraft™ system (minimum of 50 mL and max of 100 mL). Tissue was washed twice, according to the Product Information for Use insert, and then retrieved from the system using a Toomey syringe.

4.6.3 Three 10 mL aliquots of washed adipose was placed into the centrifuge and spun for 5 min at 1200 r.p.m. Volumes of aqueous phase, tissue, and free lipid was measured and recorded.

4.7 **Cell Yield and Viability Analysis of ADRCs after Celution® processing**

4.7.1 Cell samples were mixed thoroughly before aliquotting for cell counting.

4.7.2 Three (3) cell counts were performed per sample, and the resulting viable cell concentrations were averaged.

4.7.3 Cell number and viability were determined according to our internal work instruction LWI9316 —Cell Quantification by NucleoCounter™."

4.7.4 Any cell count in a triplicate sample set that was measured to be more than 25% (higher or lower) of the mean values for the other two was repeated to ensure technical sampling error was not the source of this measurement disparity.

4.7.5 Once the cell count was determined by the NucleoCounter™, aliquots of the requisite volume of the ADRCs was aseptically removed and used for the CFU-F assay. The rest of the ADRCs were used for flow cytometric characterization of cell surface marker proteins: CD31, CD34, and CD45.

4.8 CFU-F Assay

4.8.1 ADRCs were resuspended to create a 5000 cell per well suspension and seeded into 6 well plates according to work instruction our internal work instruction LWI9608 —Colony forming unit-fibroblast (CFU-F) Assay.

4.8.2 Tissue culture media was changed every three or four days for the duration of the assay (typically only once since most assays ended with 7-8 days of plating).

4.8.2.1 After up to 10 days of culture the percentage of adherent nucleated cells that grew into colonies was determined (% CFU-F frequency).

4.9 Loose Cell Cytological Analysis for CD31, CD34, CD45, and CD68 expressing Cells

4.9.1 Qualitative evaluation of cells loosely retained within lipoaspirate was performed by centrifuging aspirate from each sample for 5 minutes at 1400 r.p.m. The cell pellet was resuspended to a concentration of 70,000 cells/mL and then 100 µl was spun onto standard cytospin histology slides (800 r.p.m. for 5 minutes) using standard cytopreparation methods and then immune-stained for either cell surface antigens: CD31, CD34, CD45 or CD68.

4.9.2 Digital images of each cell surface marker staining result were captured and qualitatively assessed for presence or absence of CD (31/34/45/68) positive cells.

4.10 Flow Analysis of Celution® 800/CRS processed ADRCs

ADRC output from tissue obtained using the two different collection methods was quantitatively assessed for content of CD31, CD34, and

CD45 cell surface marker protein positive cells using flow cytometry. Forward and side scatter data was used to specify analysis of single nucleated cells while eliminating analysis of contaminating red blood cells and multi-cellular clusters.

4.11 **Lipolysis Assay**

Samples were prepared from approximately 10 mL of adipose tissue collected by each harvesting method. The samples were assessed for responsiveness to agonist –induced glycerol release according to our internal work instruction, LWI9302 —Lipolysis Assay". Relative response to agonist induced lipolysis is directly correlated to mature adipocyte viability. Results were expressed as relative adipose viability determined from this correlation curve.

4.12 **Histological Sample Evaluation**

4.12.1 5 mL of adipose tissue was fixed with neutral buffered formalin, dehydrated, and then paraffin embedded. 5 µm thick paraffin sections were deparaffinized, and stained using hematoxylin and eosin. H & E stained sections were observed and photographed microscopically and qualitatively scored for intact adipocytes.

5.0 **Protocol Acceptance Criteria**

5.1 Feasibility Criteria

5.1.1 Donor tissue was acceptable for use if there is greater than 310 mL of tissue collected.

5.1.2 Histological assessments were considered acceptable for statistical analysis of greater than 1% positive events / 500 nucleated cells could be detected. Data below this was qualitatively assessed and reported as —below the sensitivity of the assay".

5.2 End Assay Results

5.2.1 The viable cell yield post Celution® processing must be >80,000 ADRCs/mL of adipose tissue processed in the syringe acquired tissue.

5.2.2 Viability of ADRCs from control syringe tissue was >70%.

5.2.3 CFU-F assay data is acceptable for any sample if at least an average of 5 colonies / well is obtained from ADRCs.

5.2.4 CD31/34/45 populations are present and CD34 and CD45 are discrete populations. Flow cytometry results can be confirmed through cytospin assay.

6.0 Data Analysis

This was a double armed study, thus the data obtained from tissue harvested using MicroAire was compared with the syringe control with minimal limits described in the Acceptance Criteria section of this protocol. Standard statistical analysis was performed where applicable. Mean and standard deviation were calculated and paired t-test analysis was performed to compare results of MicroAire and syringe acquired samples.

7.0 Rationale

The rationale for performing this study is to obtain data which address questions regarding the feasibility of adipose tissue collection using MicroAire liposuction for physicians desiring to use the lipoaspirate either as graft tissue in an autologous fat grafting procedure or as a means of harvesting tissue to obtain stem and regenerative cells for a variety of potential applications.

7.1 Rationale for Evaluation Methods

7.1.1 Free Lipid Volume: Determination of free lipid volume recovered/unit volume of tissue will be measured to assess grossly the relative loss of adipose due to the tissue collection process.

7.1.2 Lipolysis assay: The primary function of adipose tissue is to act as an energy storage depot. This function is mediated in part by hormonally regulated accumulation and release of triglycerides. The lipolysis assay measures this function which directly reflects the health (quality) of the tissue.

7.1.3 Analysis of ADRCs released from adipose tissue. The NucleoCounter™ assay is verified as acceptable for use to determine and compare the total number and viability of ADRCs present in the output generated from tissue collected by each method.

7.1.4 CFU-F Assay: This assay is used to indirectly evaluate the adherent cell population of ADRCs known to contain the putative adipose-derived stem cells. Stem cells are a potential mediator of the therapeutic mechanism of ADRCs. Failure to detect these cells in ADRC output suggests a significant difference in cellular composition.

7.1.5 Cryopreservation of Adipose Tissue: the growing desire to store lipoaspirate for either cosmetic or therapeutic applications is driving the

need to ensure the viability of the tissue and cells following cryopreservation.

7.1.6 Flow Cytometry and Cytological Analysis

7.1.6.1 Examination of cell surface proteins associated with major subpopulations of non-adipocytes found in adipose will enable evaluation of whether MicroAire method affects the relative identity of the ADRCs subsequently obtained by Celution® 800/CRS device processing.

7.1.6.2 H & E analysis of paraffin embedded adipose tissue will enable determination on MicroAire effect on gross cytological morphology of adipose tissue collected by aspiration.

8.0 Results

8.1 Patient Demographics and Clinical Parameters

8.1.1 Adipose tissue was obtained using both syringe aspiration and power assisted lipoaspiration (MicroAire, 4 mm cannula, two hole) methods from 6 different female donor (Table 1).

Table 1. Patient Demographics and Tissue Sample Information

Run ID #	Donor/Cytori ID#	Gender	Age	Harvest Site/Method	MicroAire	Syringe	Experiment Date	Doctor
MA-1-2647	2647	F	52	Thighs, hips, Abs	~500mL	~600mL	4/19/2011	Mills
MA-2-2668	2668	F	33	inner, outer thighs, flanks, arms	~625mL	~350mL	5/4/2011	Cohen
MA-3-2702	2702	F	44	hips,flanks	~1000mL	~250mL	6/2/2011	Gold
MA-4-2706	2706	F	48	Abs, flanks, axilla	500mL	500mL	6/3/2011	Cohen
MA-5-2722	2722	F	46	arms, back, thighs	750mL	500mL	6/14/2011	Gold
MA-6-2731	2731	F	52	abdomen, flanks	600mL	500mL	6/16/2011	Cohen

8.2 Gross Morphology/Tissue Appearance

8.2.1 Tissue from individual syringes was pooled prior to analysis. PAL samples were either photographed in their original collection canister or else transferred to sterile beakers or bottles prior to photographing.

8.2.2 Gross tissue appearance between syringe and PAL acquired samples was comparable except for samples MA-3-2702 and MA-5-2722 in which the syringe acquired tissue appeared bloodier than the PAL acquired tissue (Figure 1).

Figure 1, Gross Morphology Assessment

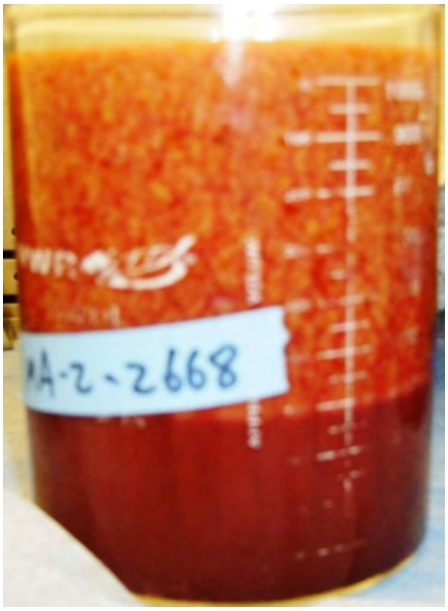
SYRINGE CONTROL **MICROAIRE (PAL)**

A.

Figure 1, Gross Morphology Assessment cont.

SYRINGE CONTROL **MICROAIRE (PAL)**

B.



C.

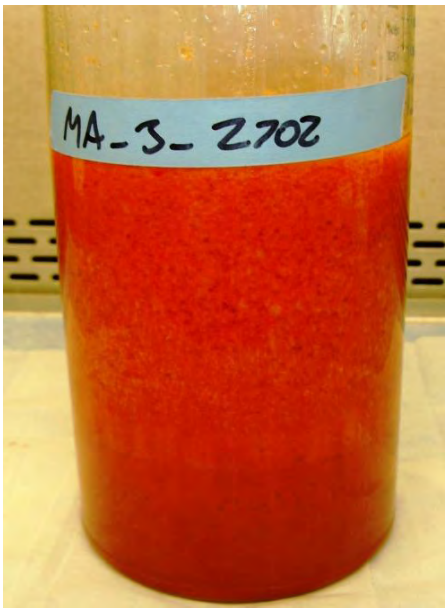


Figure 1, Gross Morphology Assessment cont.

SYRINGE CONTROL **MICROAIRE (PAL)**

D.

E.

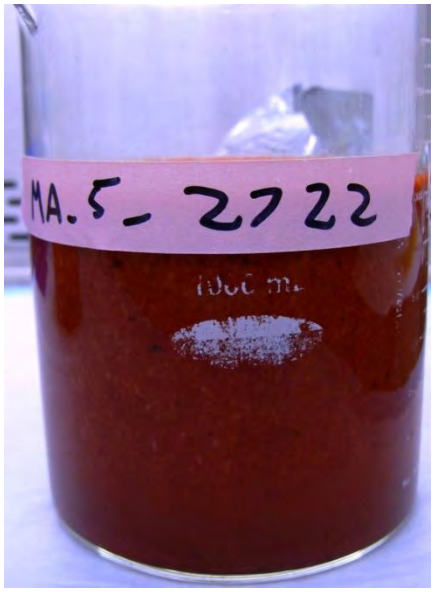
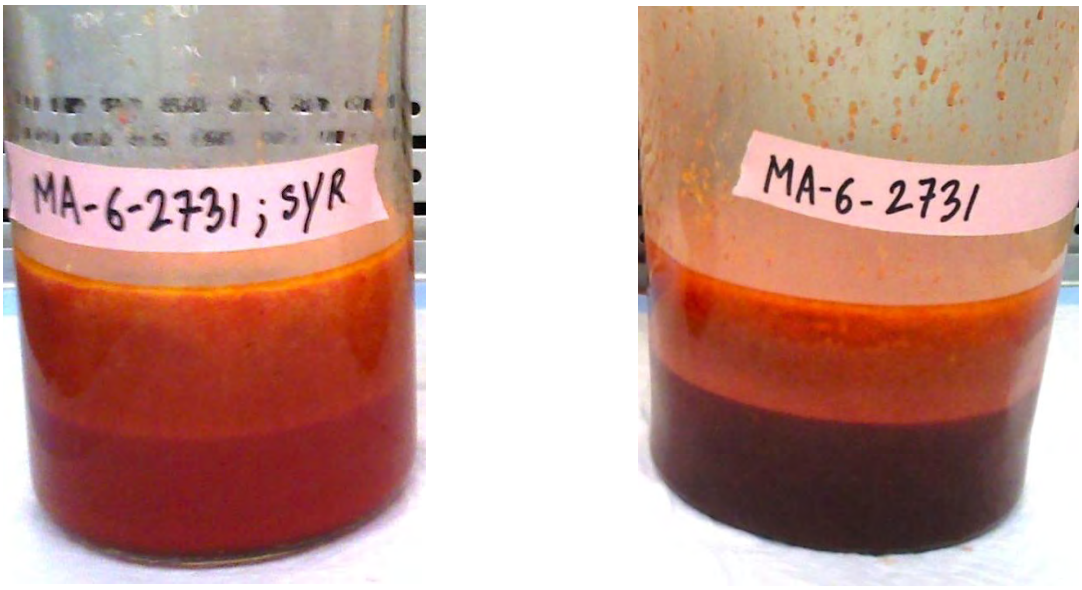


Figure 1, Gross Morphology Assessment cont.

F.

SYRINGE CONTROL **MICROAIRE (PAL)**



8.3 Adipose Graft Analysis

8.3.1 Aqueous content of graft

8.3.1.1 The mean initial aqueous fluid content of tissue collected by PAL was 27 ± 7.0% of total sample volume and for tissue collected by syringe was 26.9 ± 10.2% of total sample volume (Figure 2).

8.3.1.2 The mean initial aqueous fluid content of tissue collected by PAL after Puregraft processing was 13.1 ± 9.9% of total sample volume and for tissue collected by syringe was 11.9 ± 10.9% of total sample volume (Figure 2).

8.3.1.3 No significant difference in aqueous fluid content was observed between syringe and PAL acquired lipoaspirates either before or after processing the tissue with the Puregraft system (P =0.988 and P = 0.626, by paired t test analysis, respectively).

8.3.1.4 A statistically significant reduction in aqueous content of tissue recovered after Puregraft processing was observed in PAL acquired samples compared to tissue prior to processing (P< 0.003). A trend toward significant reduction was also observed in

syringe acquired samples (P =0.053 for syringe by two-sided Paired t test analysis).

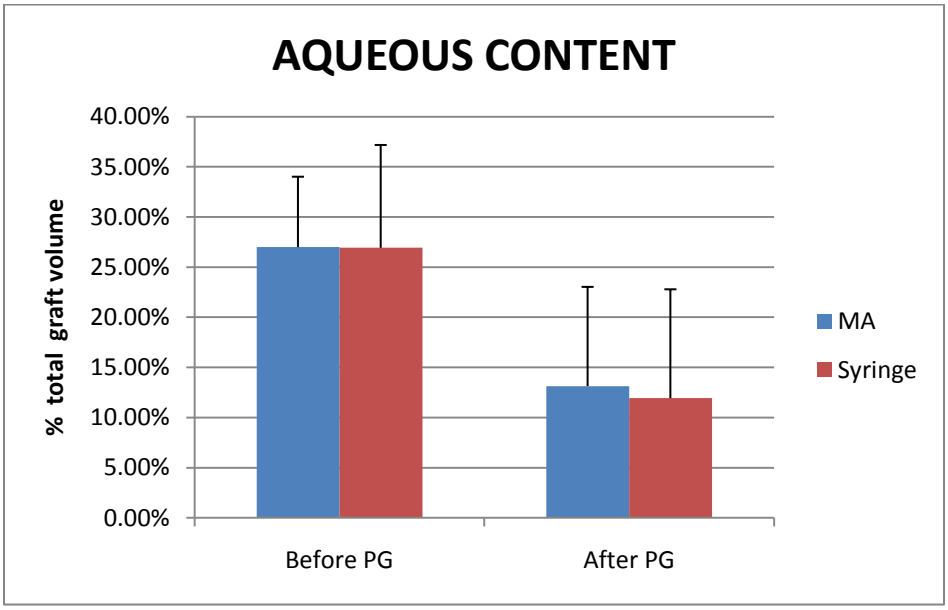


Figure 2. Comparison of aqueous fluid content in fat graft acquired by syringe and MicroAire PAL. N=6 donors.

8.3.2 Lipid content of graft

8.3.2.1 The mean initial free lipid content of tissue collected by PAL was 8.8 ± 3.7% of total sample volume and for tissue collected by syringe was 9.9 ± 5.0% of total sample volume (Figure 3).

8.3.2.2 The mean initial free lipid content of tissue collected by PAL after Puregraft processing was 0.97 ± 0.83% of total sample volume and for tissue collected by syringe was 1.9 ± 1.5% of total sample volume (Figure 3).

8.3.2.3 No significant difference in free lipid content was observed between syringe and PAL acquired lipoaspirates before or after processing the tissue with the Puregraft system (P =0.514 and P = 0.054, by paired t test analysis, respectively).

8.3.2.4 A statistically significant reduction of free lipid in tissue recovered after Puregraft processing was observed in samples compared to tissue prior to processing (P< 0.003 and P <0.004 by two-sided Paired t test analysis, for PAL and syringe tissues, respectively).

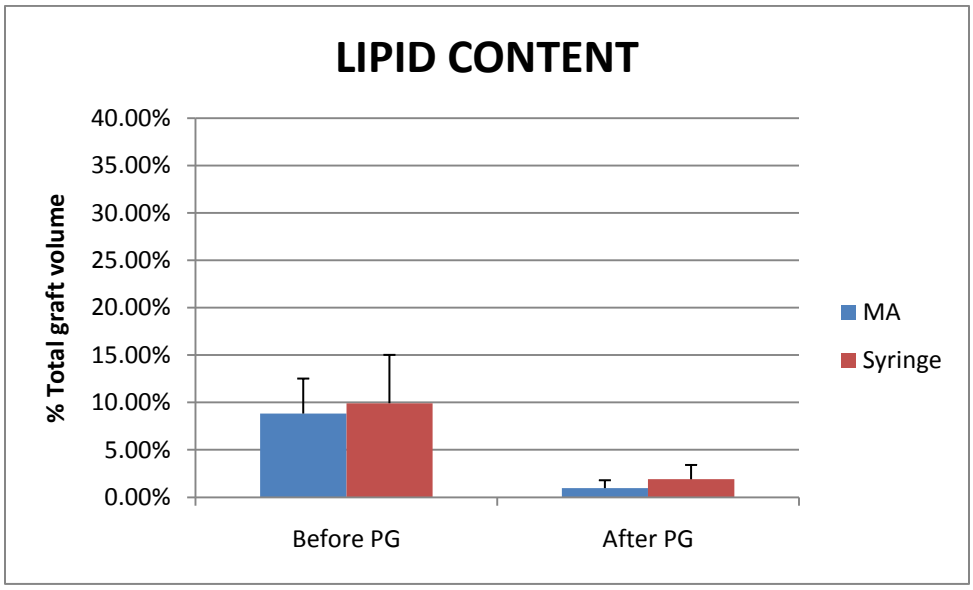


Figure 3. Comparison of free lipid content in fat graft acquired by syringe and MicroAire PAL. N=6 donors.

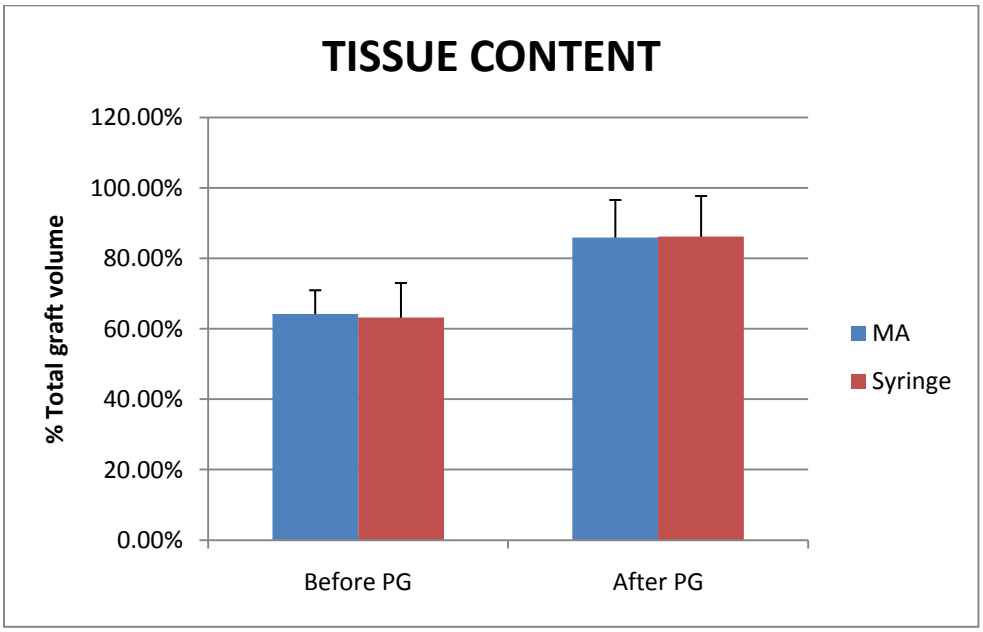


Figure 4. Comparison of tissue content in fat graft acquired by syringe and MicroAire PAL. N=6 donors.

8.3.3 Tissue Content of graft

8.3.3.1 The mean tissue content of graft collected by PAL was 64 ± 6.8% of total sample volume and for graft collected by syringe was 63 ± 9.9% of total sample volume (Figure 4).

8.3.3.2 The mean initial tissue content of graft collected by PAL after Puregraft processing increased to 85.9 ± 10.7% of total sample volume and for tissue collected by syringe was 86.1± 11.6% of total sample volume (Figure 4).

8.3.3.3 No significant difference in tissue content of graft was observed between syringe and PAL acquired samples before or after processing with the Puregraft system (P =0.828 and P = 0.905, by paired t test analysis, respectively).

8.3.3.4 A statistically significant increase in relative tissue content recovered after Puregraft processing was observed in samples compared to that prior to processing (P< 0.0002 and P <0.01 by two-sided Paired t test analysis, for PAL and syringe tissues, respectively).

8.3.4 Histology of Lipoaspirate

8.3.4.1 Representative hematoxylin and eosin stained images of PAL acquired and syringe acquired samples are shown Figure 5 and Appendix F. Regions of intact and disrupted adipose were observed in samples obtained by both methods. There were no obvious differences in

SYRINGE CONTROL **MICROAIRE (PAL)**

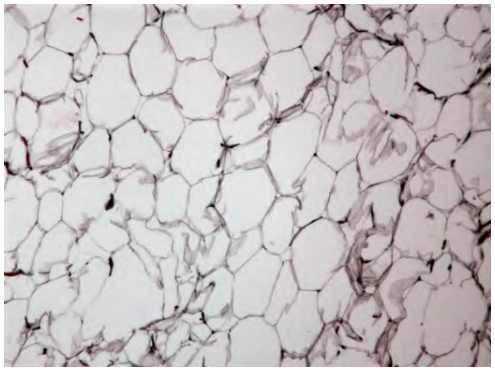
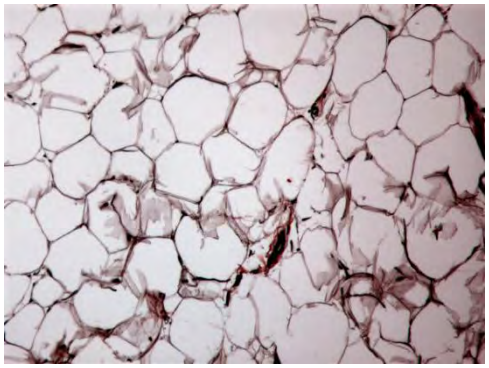
A.  B. 

Figure 5. H & E stained microsections of lipoaspirate acquired by either PAL or syringe. Images were acquired using a 10x objective and captured using a digital camera and SPOT image capture and analysis software.

8.3.5 Lipolysis Assay

8.3.5.1 Relative adipocyte viability was significantly less in samples obtained using PAL compared to syringe acquired tissue (P=0.02 by paired t test analysis) suggesting that the more aggressive mechanical perturbation of the tissue during PAL harvest may result in a lower quality graft (Figure 6).

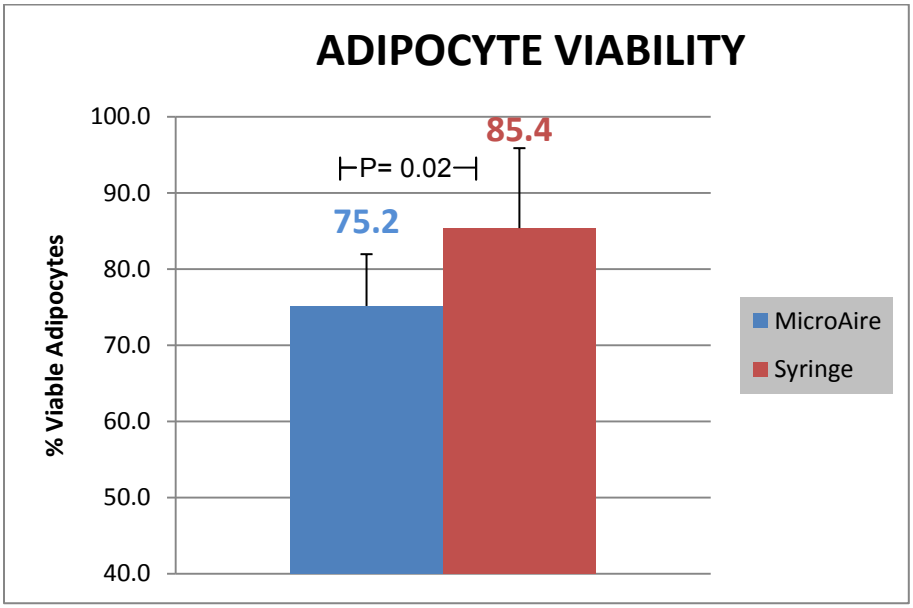


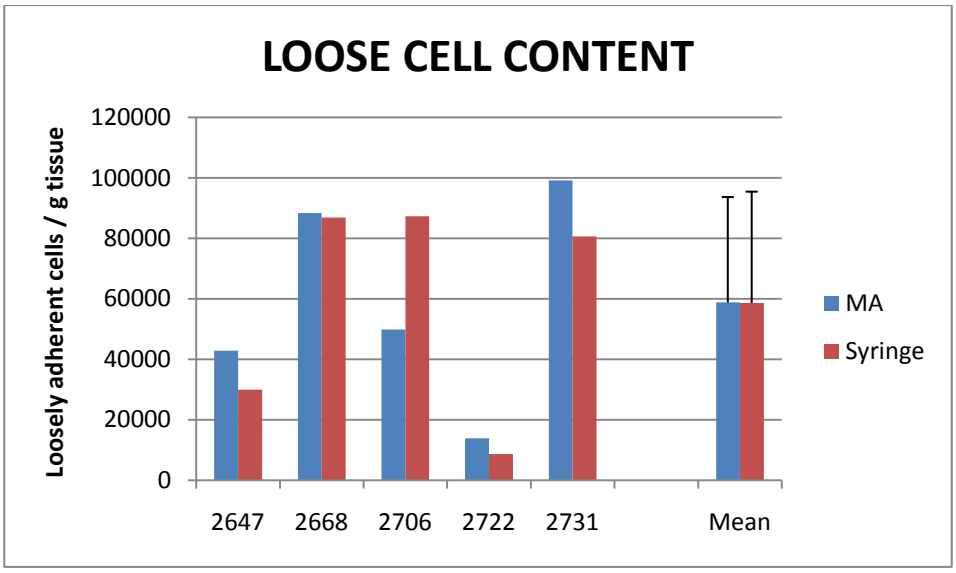
Figure 6. Lipolysis Assay determination of mature adipocyte viability in fat graft tissue acquired by MicroAire PAL or syringe aspiration. N=5* donors.

8.3.6 Loosely Adherent Cell Analysis

8.3.6.1 The amount of cells and disrupted microvasculature retained but not completely integrated within graft tissue is a measure of tissue disruption resultant from tissue harvest. The mean number of loosely adherent cells in PAL tissue was $5.88 \times 10^4 \pm 3.49 \times 10^4$ cells per gram of tissue whereas the number of these cells in syringe acquired tissue was $5.86 \times 10^4 \pm 3.68 \times 10^4$ cells per gram of tissue. While there was no statistically significant difference in mean number of cells (P=0.988), the recovered number of loose cells in PAL acquired tissue was higher than that of syringe acquired tissue in four of five samples (Figure 7A).

8.3.6.2 Viability of loosely adherent cells in graft obtained by PAL (79.7 ± 14.4%) or by syringe (70.8 ± 11.3%) was not statistically different (P = 0.117 by paired t test analysis). See figure 7B.

A.



B.

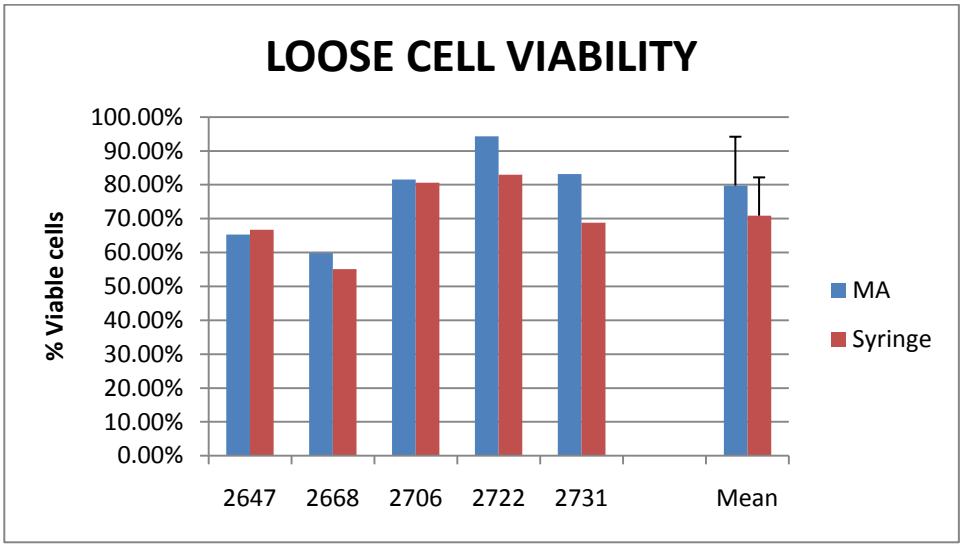


Figure 7. Cell concentration (A) and relative cell viability (B) in loosely adherent cells from PAL and syringe acquired tissues. N=6 donors.

8.3.7 Immunocharacterization of loosely adherent cell content

8.3.7.1 Cytospin preparations of cell pellets obtained during loose cell analysis were immunostained to qualitatively evaluate the presence of different cell subpopulations found within the stromal vascular fraction of adipose obtained using PAL and syringe. Representative images of results are shown in Figure 8 and from each donor in Appendix E.

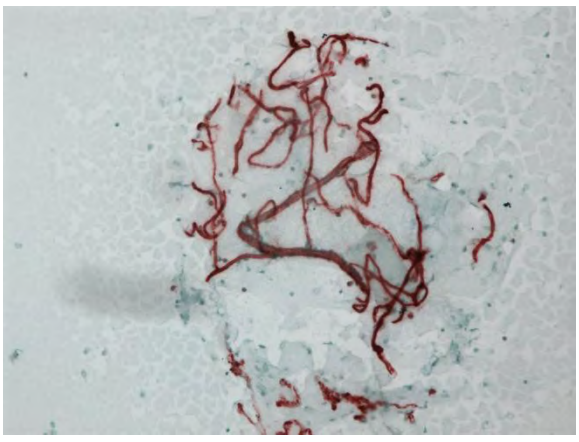
8.3.7.2 CD31 and CD34 stain predominantly microvasculature, although a small number of individual CD34 positive cells are observed independent of the vasculature. Few if any individual cells are stained with CD31.

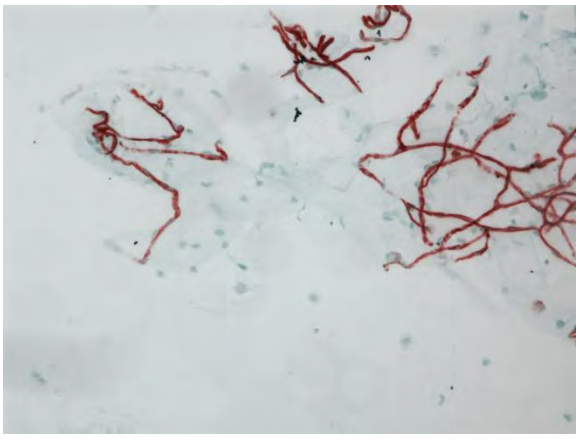
8.3.7.3 CD45 expression is indicative of white blood cells and these cells are seen to be qualitatively similar in abundance in these cell preparations.

8.3.7.4 CD68 stains principally tissue monocyte/macrophages (which are a subset of the CD45 positive cell population. These cells are more or less abundant depending on the patient's BMI. Within the BMI range tested in this study, these cells constitute less than 5% of the total CD45 cell population.

SYRINGE CONTROL **MICROAIRE (PAL)**

CD31





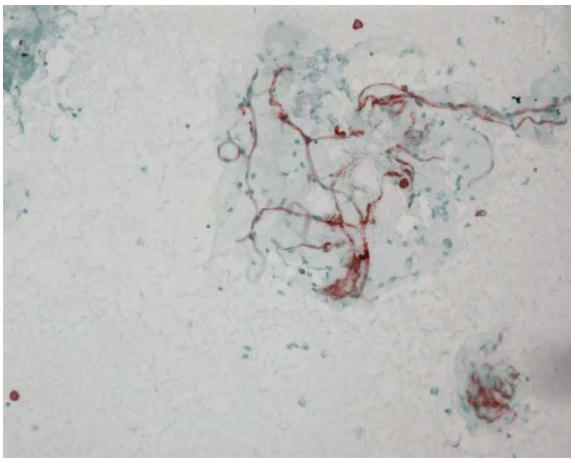
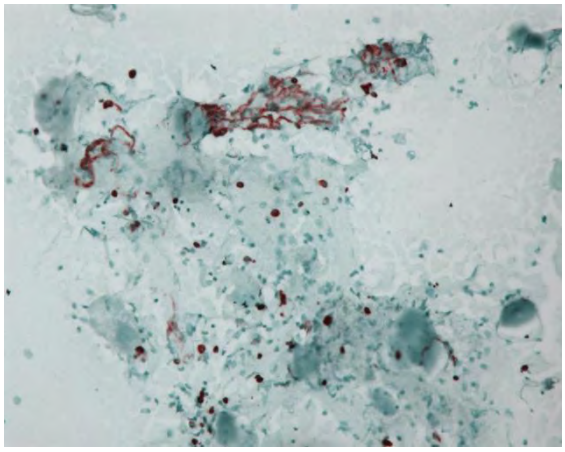
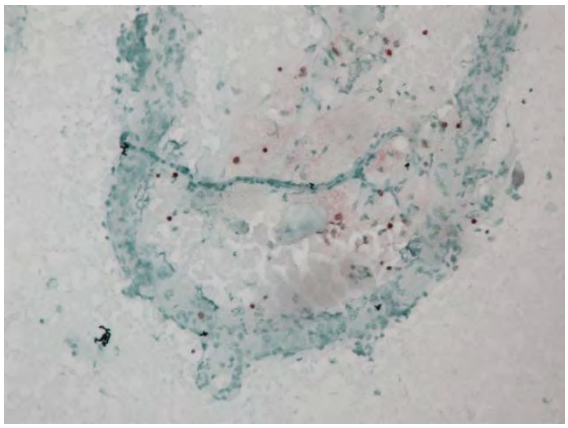
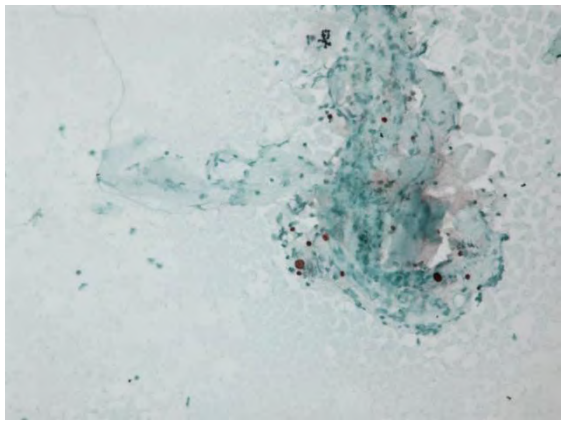
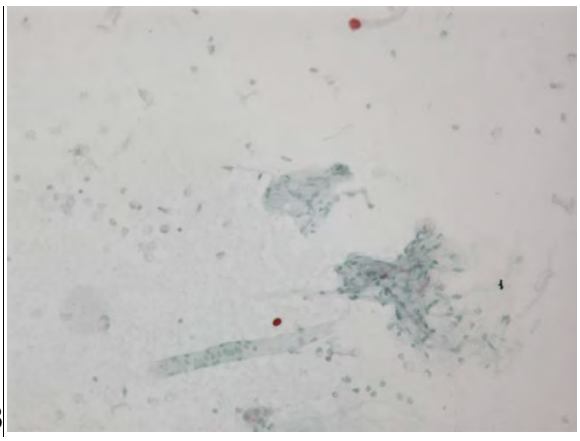
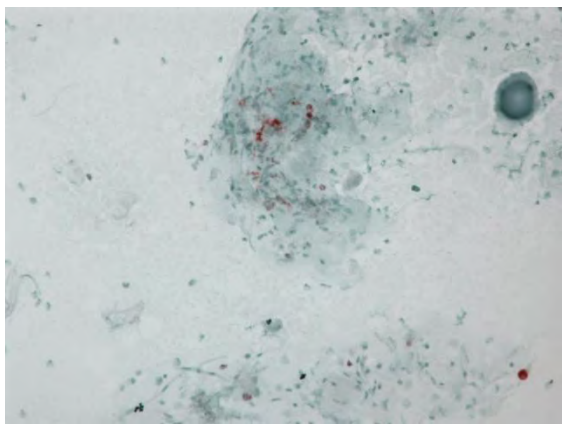
	SYRINGE CONTROL	MICROAIRE (PAL)
CD34		
CD45		
CD68		

Figure 8. Representative images of different stromal vascular subpopulations in loosely adherent cells obtained from PAL and syringe harvested adipose. Images captured using 10x objective lens.

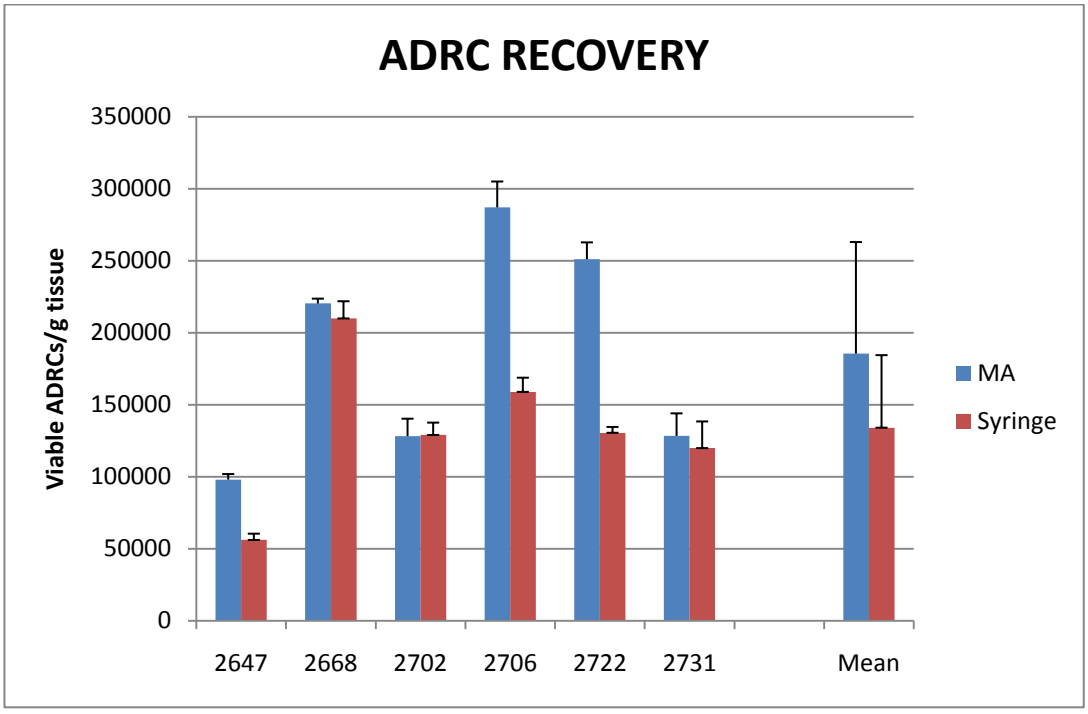
8.4 ADRC Characterization

8.4.1 Cell Recovery and Viability

8.4.1.1 The mean number of adipose derived regenerative cells (ADRCs) from PAL tissue was $1.85 \times 10^5 \pm 0.77 \times 10^5$ cells per gram of tissue whereas the number of these cells in syringe acquired tissue was $1.34 \times 10^5 \pm 0.5 \times 10^5$ cells per gram of tissue. While there was no statistically significant difference in mean number of cells (P=0.08), the recovered number of ADRCs cells in PAL acquired tissue was higher than that of syringe acquired tissue in four of five samples (Figure 9A).

8.4.1.2 Viability of ADRCs obtained by PAL (81.2 ± 6.9%) or by syringe (85.7 ± 2.8%) was not statistically different (P = 0.173 by paired t test analysis). See figure 9B.

A.



B.

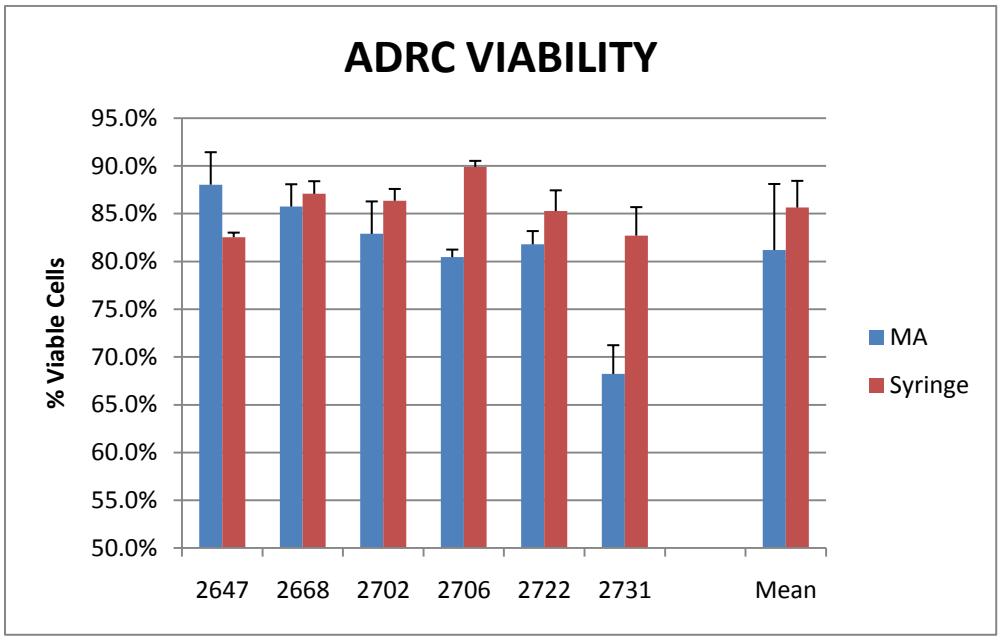


Figure 9. A. Recovered ADRCs from PAL and Syringe acquired tissue. B. Relative cell viability of ADRCs isolated from PAL and syringe acquired tissue. N=6 donors.

8.4.2 CFU-F

8.4.2.1 The mean concentration of adherent colony forming cells, an indirect indicator of adipose-derived stem cells, in PAL tissue was 0.82 ± 0.26% whereas the concentration of these cells in syringe acquired tissue was 0.76 ± 0.2%. There was no statistically significant difference in CFU-F capacity (P=0.386, paired t test) (Figure 10).

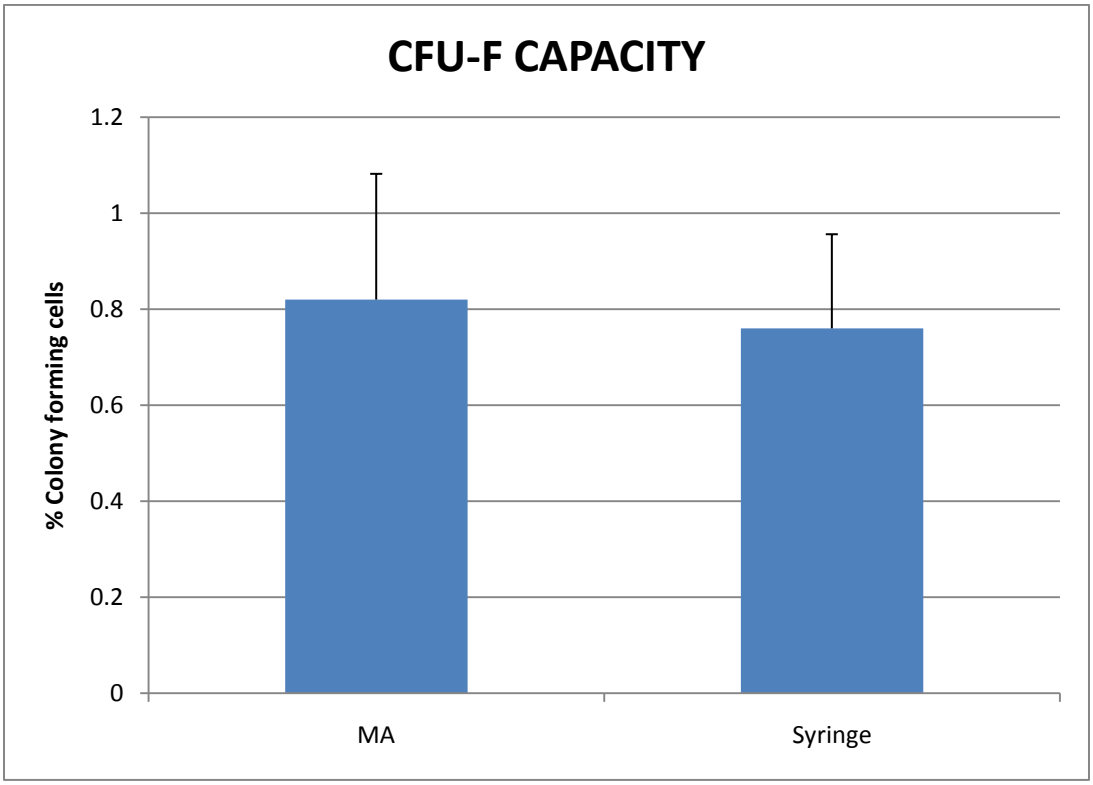


Figure 10. Colony forming unit-fibroblast (CFU-F) capacity of ADRCs isolated from MicroAire power assisted lipoaspiration (MA) and by syringe aspiration. N = 6 donors.

8.4.3 Flow Cytometry

8.4.3.1 No differences in relative subpopulation content was seen between tissue acquired using MicroAire PAL and syringe indicating that no bias in cell recovery of a particular ADRC cell subpopulation occurs when power assisted lipoaspiration is used (Table 2).

Table 2. Cell surface marker protein expression in ADRCs isolated from PAL acquired and syringe acquired tissue.

Harvest Technique	CD45+/CD31-/CD34- Mean ± SD % Total Cells	CD34+/CD31-/CD45- Mean ± SD % Total Cells	CD34+/CD31+/CD45- Mean ± SD % Total Cells
MA	**37.3 ± 4.2**	**34.7 ± 5.1**	**12.8 ± 5.3**
Syringe	**36.8 ± 8.5**	**31.5 ± 10.8**	**13.4 ± 4.99**

9.0 Deviations/Modifications to Protocol

9.1 Cryopreservation was attempted with only one of the donor samples due to lack of tissue volume in the other five; however, since quality of lipoaspirate appears so similar between PAL and syringe it is reasonable to assume that they may be similar in regards to cryopreservation as well.

9.2 The syringe sample from Donor 3 was low volume and histology was not performed on this sample due to lack of available tissue.

9.3 CFU-F results for donors 4 and 5 were estimated because progenitor number in these experiments were higher than the number than within the expected range and colony density in assay wells was too high to ascertain the actual number of colony originator cells.

10.0 Conclusions

10.1 Based upon loose cell and ADRC generation data in combination with lipolysis assay data use of MicroAire power assisted lipoaspiration appears to disrupt adipose tissue more than syringe tissue harvest. This may be advantageous when trying to acquire ADRCs in patients with low vascular density; however, syringe aspiration is preferable to PAL when acquiring tissue that will be used for fat grafting. Graft composition data before and after Puregraft processing indicates that Puregraft is able to process graft regardless of harvest method used in this study to yield comparable adipose graft.

11.0 Appendices

11.1 Appendix A Adipose Tissue Information Worksheet
11.2 Appendix B ADRC Isolation and Cell Count Record
11.3 Appendix C CFU-F Assay Data Sheet
11.4 Appendix D Puregraft Assay Data Sheet
11.5 Appendix E Representative Immunostaining of Loosely Adherent Cells
11.6 Appendix F Representative Histology images of Adipose from PAL and Syringe

12.0 Revision History

Rev #	Date	Reason for Revision	Change Order No.
A		Initial Release	NA

Appendix A - Adipose Tissue Information Worksheet

(Complete for each sample from each donor)

Date of collection: 4/19/11 Sample ID#: 2647

Surgeon: Dr. Mills

Donor gender: male / female Donor Age: 52

Height: 5'4" Weight: 175 BMI: _____

1. Harvest Site(s): Thighs, hips, abs
2. Time of tissue arrival at lab for analysis 2:30pm
3. Total volume of human lipoaspirate received ~600 mL
4. Free Lipid Volume ~20 mL
5. Volume of lipoaspirate tissue set aside for Puregraft™ analysis 130 mL
6. Volume of lipoaspirate tissue set aside for lipolysis assay 10 mL
7. Volume of lipoaspirate tissue set aside for histology evaluation 5 mL
8. Volume of lipoaspirate tissue for Celution® 800/CRS processing 120 mL

Notes: Bloodiness of the sample minimal bloodiness

Information recorded by (Signature/Date): 4/19/11

Reviewed by (Signature/Date) 7/21/11

Appendix A - Adipose Tissue Information Worksheet

(Complete for each sample from each donor)

Date of collection: 4/19/11 Sample ID#: 2647

Surgeon: Dr. Mills

Donor gender: male / female Donor Age: 52

Height: 5'4" Weight: 175 BMI: _____

1. Harvest Site(s): Thighs, hips, abs
2. Time of tissue arrival at lab for analysis 2:30 pm
3. Total volume of human lipoaspirate received ~500 mL
4. Free Lipid Volume ~20 mL
5. Volume of lipoaspirate tissue set aside for Puregraft™ analysis 130 mL
6. Volume of lipoaspirate tissue set aside for lipolysis assay 10 mL
7. Volume of lipoaspirate tissue set aside for histology evaluation 5 mL
8. Volume of lipoaspirate tissue for Celution® 800/CRS processing 120 mL

Notes: Bloodiness of the sample minimal bloodiness

Information recorded by (Signature/Date): [signature] 4/19/11

Reviewed by (Signature/Date) Ken C. Hunt 7/21/11

Appendix A - Adipose Tissue Information Worksheet

(Complete for each sample from each donor)

Date of collection: 5/4/11 Sample ID#: 2668

Surgeon: Cohen

Donor gender: male / (female) Donor Age: 33

Height: 64 in. Weight: 137 lbs. BMI: _____

1. Harvest Site(s): Inner/outer thighs, flanks, arms
2. Time of tissue arrival at lab for analysis 14:00
3. Total volume of human lipoaspirate received ~350 mL
4. Free Lipid Volume ~10 ml mL
5. Volume of lipoaspirate tissue set aside for Puregraft™ analysis ~150 mL
6. Volume of lipoaspirate tissue set aside for lipolysis assay 10 mL
7. Volume of lipoaspirate tissue set aside for histology evaluation 10 mL
8. Volume of lipoaspirate tissue for Celution® 800/CRS processing 120 mL

Notes: Bloodiness of the sample minimal bloodiness

Information recorded by (Signature/Date): [signature] 5/4/11

Reviewed by (Signature/Date) [signature] 7/21/11

Appendix A - Adipose Tissue Information Worksheet

(Complete for each sample from each donor)

Date of collection: 5/4/2011 Sample ID#: Z668

Surgeon: Cohen

Donor gender: male / (female) Donor Age: 33

Height: 64in. Weight: 137 lbs. BMI: _____

1. Harvest Site(s): Inner/outer thighs, flanks, arms
2. Time of tissue arrival at lab for analysis 14:00
3. Total volume of human lipoaspirate received ~625 mL
4. Free Lipid Volume ~25 ml mL
5. Volume of lipoaspirate tissue set aside for Puregraft™ analysis ~150 mL
6. Volume of lipoaspirate tissue set aside for lipolysis assay 10 mL
7. Volume of lipoaspirate tissue set aside for histology evaluation 10 mL
8. Volume of lipoaspirate tissue for Celution® 800/CRS processing 120 mL

Notes: Bloodiness of the sample moderate bloodiness

Information recorded by (Signature/Date): 5/4/11

Reviewed by (Signature/Date) Kevin C. Hart 7/21/11

Appendix A - Adipose Tissue Information Worksheet

(Complete for each sample from each donor)

Date of collection: 6/2/11 Sample ID#: 2702

Surgeon: Gold

Donor gender: male / female Donor Age: 44

Height: 5'7" Weight: 168 BMI: 26.3

1. Harvest Site(s): hips, flank area
2. Time of tissue arrival at lab for analysis 12pm
3. Total volume of human lipoaspirate received ~250 mL
4. Free Lipid Volume ~20 mL
5. Volume of lipoaspirate tissue set aside for Puregraft™ analysis ~50 mL
6. Volume of lipoaspirate tissue set aside for lipolysis assay 10 mL
7. Volume of lipoaspirate tissue set aside for histology evaluation N/A mL
8. Volume of lipoaspirate tissue for Celution® 800/CRS processing 100 mL

Notes: Bloodiness of the sample minimal bloodiness

Information recorded by (Signature/Date): 6/2/11

Reviewed by (Signature/Date) 7/21/11

Appendix A - Adipose Tissue Information Worksheet

(Complete for each sample from each donor)

Date of collection: 6/2/11 Sample ID#: 2702

Surgeon: Gold

Donor gender: male / ~~female~~ Donor Age: 44

Height: 5'7" Weight: 168 BMI: _____

1. Harvest Site(s): hips, flank dried
2. Time of tissue arrival at lab for analysis 12 pm
3. Total volume of human lipoaspirate received ~~250~~ AM 6/2/11 ~1000 mL
4. Free Lipid Volume ~~25~~ AM 6/2/11 ~100 mL
5. Volume of lipoaspirate tissue set aside for Puregraft™ analysis ~300 mL
6. Volume of lipoaspirate tissue set aside for lipolysis assay 10 mL
7. Volume of lipoaspirate tissue set aside for histology evaluation 10 mL
8. Volume of lipoaspirate tissue for Celution® 800/CRS processing 100 mL

Notes: Bloodiness of the sample moderate bloodiness

Information recorded by (Signature/Date): [signature] 6/2/11

Reviewed by (Signature/Date) K. C Fleck 7/21/11

Appendix A - Adipose Tissue Information Worksheet

(Complete for each sample from each donor)

Date of collection: 6/3/11 Sample ID#: 2706 (Syringe)

Surgeon: Cohen

Donor gender: male / (female) Donor Age: 48

Height: 5'5" Weight: 160 BMI: 27

1. Harvest Site(s): Flanks, Abdominals, Axilla
2. Time of tissue arrival at lab for analysis 12:45pm
3. Total volume of human lipoaspirate received 500 mL
4. Free Lipid Volume 20 mL
5. Volume of lipoaspirate tissue set aside for Puregraft™ analysis 100 mL
6. Volume of lipoaspirate tissue set aside for lipolysis assay 10 mL
7. Volume of lipoaspirate tissue set aside for histology evaluation 10 mL
8. Volume of lipoaspirate tissue for Celution® 800/CRS processing 120 mL

Notes: Bloodiness of the sample moderate bloodiness

Information recorded by (Signature/Date): [signature] 6/3/11

Reviewed by (Signature/Date) Kevin e Hucke 7/21/11

Appendix A - Adipose Tissue Information Worksheet

(Complete for each sample from each donor)

Date of collection: 6/3/11 Sample ID#: 2706

Surgeon: Cohen

Donor gender: male / (female) Donor Age: 48

Height: 5'5" Weight: 160 BMI: 27

1. Harvest Site(s): Flanks, Abdominals, Axilla
2. Time of tissue arrival at lab for analysis 12:45
3. Total volume of human lipoaspirate received 500 mL
4. Free Lipid Volume 20 mL
5. Volume of lipoaspirate tissue set aside for Puregraft™ analysis 100 mL
6. Volume of lipoaspirate tissue set aside for lipolysis assay 10 mL
7. Volume of lipoaspirate tissue set aside for histology evaluation 10 mL
8. Volume of lipoaspirate tissue for Celution® 800/CRS processing 120 mL

Notes: Bloodiness of the sample moderate bloodiness

Information recorded by (Signature/Date): 6/3/11

Reviewed by (Signature/Date) 7/22/11

Appendix A - Adipose Tissue Information Worksheet

(Complete for each sample from each donor)

Date of collection: 6/14/11 Sample ID#: 2722

Surgeon: Gold

Donor gender: male / ~~female~~ Donor Age: 46

Height: _____ Weight: _____ BMI: _____

1. Harvest Site(s): Arms, back, thighs
2. Time of tissue arrival at lab for analysis 1:45 pm
3. Total volume of human lipoaspirate received 500 mL
4. Free Lipid Volume ~ 30 mL
5. Volume of lipoaspirate tissue set aside for Puregraft™ analysis 105 mL
6. Volume of lipoaspirate tissue set aside for lipolysis assay 10 mL
7. Volume of lipoaspirate tissue set aside for histology evaluation 10 mL
8. Volume of lipoaspirate tissue for Celution® 800/CRS processing 120 mL

Notes: Bloodiness of the sample mild-moderate bloodiness

Information recorded by (Signature/Date): 6/14/11

Reviewed by (Signature/Date) 7/15/11

Appendix A - Adipose Tissue Information Worksheet

(Complete for each sample from each donor)

Date of collection: 6/14/11 Sample ID#: 2722

Surgeon: Gold

Donor gender: male / ~~female~~ Donor Age: 46

Height: / Weight: / BMI: /

1. Harvest Site(s): Arms, back, thighs
2. Time of tissue arrival at lab for analysis 1:45 pm
3. Total volume of human lipoaspirate received 750 mL
4. Free Lipid Volume 15 mL
5. Volume of lipoaspirate tissue set aside for Puregraft™ analysis 200 mL
6. Volume of lipoaspirate tissue set aside for lipolysis assay 10 mL
7. Volume of lipoaspirate tissue set aside for histology evaluation 10 mL
8. Volume of lipoaspirate tissue for Celution® 800/CRS processing 120 mL

Notes: Bloodiness of the sample Extremely bloody; difficult to distinguish tissue, did not separate

Information recorded by (Signature/Date): [signature] 6/14/11

Reviewed by (Signature/Date) Ken C Hauck 6/14/11

Appendix A - Adipose Tissue Information Worksheet

(Complete for each sample from each donor)

Date of collection: 6/16/11 Sample ID#: MA-2731-SYR

Surgeon: Cohen

Donor gender: male / female Donor Age: 52

Height: 5'6 Weight: 138 BMI: 23

1. Harvest Site(s): Flanks, abdomen
2. Time of tissue arrival at lab for analysis ~5:30pm
3. Total volume of human lipoaspirate received ~500 mL
4. Free Lipid Volume ~50 mL
5. Volume of lipoaspirate tissue set aside for Puregraft™ analysis 135 mL
6. Volume of lipoaspirate tissue set aside for lipolysis assay 10 mL
7. Volume of lipoaspirate tissue set aside for histology evaluation 10 mL
8. Volume of lipoaspirate tissue for Celution® 800/CRS processing 120 mL

Notes: Bloodiness of the sample minimal bloodiness

Information recorded by (Signature/Date): 6/16/11

Reviewed by (Signature/Date) 7/15/11

Appendix A - Adipose Tissue Information Worksheet

(Complete for each sample from each donor)

Date of collection: 6/16/11 Sample ID#: MA-6-2731

Surgeon: Cohen

Donor gender: male / female Donor Age: 52

Height: 5'6 Weight: 138 BMI: 23

1. Harvest Site(s): Flanks, Abdomen
2. Time of tissue arrival at lab for analysis ~5:30pm
3. Total volume of human lipoaspirate received ~600 mL
4. Free Lipid Volume ~100 mL
5. Volume of lipoaspirate tissue set aside for Puregraft™ analysis 135 mL
6. Volume of lipoaspirate tissue set aside for lipolysis assay 10 mL
7. Volume of lipoaspirate tissue set aside for histology evaluation 10 mL
8. Volume of lipoaspirate tissue for Celution® 800/CRS processing 120 mL

Notes: Bloodiness of the sample moderate bloodiness

Information recorded by (Signature/Date): 6/16/11

Reviewed by (Signature/Date) K. C Heck 7/15/11

Appendix B - ADRC Isolation and Cell Count Record

(Complete for each donor)

Human lipoaspirate information:

Cytori ID No MA-1-26479R Date of Lipoaspirate Harvest 4/19/11

Amount of human lipoaspirate processed: 120 mL

Amount of Celase™ used: 2.1 mL

Materials Information:

Material	Lot #	Exp Date
Celution® 805 Consumable Sets	7101819	2011-08
Celase™ Reagent	14792124	2010-04
Nucleocounter cassettes	0111-05	2012-04
Other		
Equipment		
Celution® 800/CRS Device S/N	**Syr:** 1877	**MA:**
Celution® 800/CRS Device Software version	4.1/E1	
NucleoCounter Asset No.	B460	

NucleoCounter® Cell Counting Data:

	Cell Count Post Isolation		
Dead Cells/mL * DF	$2.6e^5$	$2.9e^5$	$2.7e^5$
Total Cells/mL * DF	$1.5e^6$	$1.7e^6$	$1.5e^6$
Viable Cells/mL	$1.2e^6$	$1.4e^6$	$1.3e^6$
Volume	5.2mL		
Total Viable Cells	$6.76e^6$		
Viability	82.3%	83%	82.4%

DF – Dilution Factor

Performed By: AMMAR ASMAR Date: 4/19/11

Verified By: Kevin C Hicok Date: 7/21/11

Appendix B - ADRC Isolation and Cell Count Record

(Complete for each donor)

--continued--

Final Volume Cells Resuspended: 5.2 mL

Final Cell Concentration: 1.3e6 ADRCs/mL

sample: 1 mL for CFU/F assay; 3.3 mL for FACS

NucleoCounter® Cell Counting Data for Loose Cell Analysis:

MA-1-2647-Syr	Cell Count Post Isolation		
Dead Cells/mL * DF	1.59e5	1.7e5	1.57e5
Total Cells/mL * DF	4.86e5	4.89e5	4.86e5
Viable Cells/mL	3.27e5	3.18e5	3.3e5
Volume	2.3mL		
Total Viable Cells			
Viability	67.3%	65.2%	67.7%

avg = 3.25e5 viable cells/mL

DF – Dilution Factor

Performed By: NJ Date: 4/19/11

Verified By: Kori C Hand Date: 7/21/11

Appendix B - ADRC Isolation and Cell Count Record

(Complete for each donor)

Human lipoaspirate information:

Cytori ID No MA-1-2647 Date of Lipoaspirate Harvest 4/19/11

Amount of human lipoaspirate processed: 120 mL

Amount of Celase™ used: 2.1 mL

Materials Information:

Material	Lot #	Exp Date
Celution® 805 Consumable Sets	7101819	2011-08
Celase™ Reagent	14792124	2010-04
Nucleocounter cassettes	0111-05	2012-04
Other		
Equipment		
Celution® 800/CRS Device S/N	**Syr:**	**MA:** 1877
Celution® 800/CRS Device Software version		4.1/E1
NucleoCounter Asset No.	B460	

NucleoCounter® Cell Counting Data:

	Cell Count Post Isolation		
Dead Cells/mL * DF	$3.4e^5$	$3.3e^5$	$2.5e^5$
Total Cells/mL * DF	$2.6e^6$	$2.5e^6$	$2.6e^6$
Viable Cells/mL	$2.2e^6$	$2.2e^6$	$2.3e^6$
Volume	5.2mL		
Total Viable Cells	$1.16e^7$		
Viability	86.7%	86.9%	90.3%

DF – Dilution Factor

Performed By: AMMAR ASMAR Date: 4/19/11

Verified By: K. C Hicok Date: 7/21/11

Appendix B - ADRC Isolation and Cell Count Record

(Complete for each donor)

--continued--

Final Volume Cells Resuspended: 5.2 mL

Final Cell Concentration: 2.23e6 ADRCs/mL

sample: 1 mL for CFU/F assay; 3.6 mL for FACS

NucleoCounter® Cell Counting Data for Loose Cell Analysis:

MA-1-2647	Cell Count Post Isolation		
Dead Cells/mL * DF	2.47e5	2.31e5	2.28e5
Total Cells/mL * DF	6.78e5	7.32e5	6.33e5
Viable Cells/mL	4.32e5	5.01e5	4.05e5
Volume	2.4 mL		
Total Viable Cells			
Viability	63.6%	68.4%	64.0%

average: 4.46e5 viable cells/mL

DF – Dilution Factor

Performed By: NS Date: 4/19/11

Verified By: Ken C Hicok Date: 7/21/11

Appendix B - ADRC Isolation and Cell Count Record

(Complete for each donor)

Human lipoaspirate information:

Cytori ID No MA-2668 SVF Date of Lipoaspirate Harvest 5/4/11

Amount of human lipoaspirate processed: 120 mL

Amount of Celase™ used: 2.1 mL

Materials Information:

Material	Lot #	Exp Date
Celution® 805 Consumable Sets	101860	2011-08
Celase™ Reagent	14792124	2010-04
Nucleocounter cassettes	0111-05	2012-04
Other		
Equipment		
Celution® 800/CRS Device S/N	**Syr:** 1877	**MA:** —
Celution® 800/CRS Device Software version	4.1/E1	
NucleoCounter Asset No.	B460	

NucleoCounter® Cell Counting Data:

	Cell Count Post Isolation		
Dead Cells/mL * DF	$7.3e^5$	$6.6e^5$	$7.6e^5$
Total Cells/mL * DF	$5.31e^6$	$5.79e^6$	$5.58e^6$
Viable Cells/mL	$4.59e^6$	$5.13e^6$	$4.83e^6$
Volume	5.2mL		
Total Viable Cells	2.52e7		
Viability	86.3%	88.6%	86.4%

DF – Dilution Factor

Performed By: AMMAR ASMAR Date: 5/4/11

Verified By: Kimi C Hicok Date: 7/21/11

Appendix B - ADRC Isolation and Cell Count Record

(Complete for each donor)

--continued--

Final Volume Cells Resuspended: 5.2 mL

Final Cell Concentration: 4.85e6 ADRCs/mL

sample: 1 mL for CFU/F assay; 2.06 mL for FACS

NucleoCounter® Cell Counting Data for Loose Cell Analysis:

MA-2-2668	**Cell Count Post Isolation**		
Dead Cells/mL * DF	5.47e5	6.74e5	5.87e5
Total Cells/mL * DF	1.37e6	1.47e6	1.20e6
Viable Cells/mL	8.19e5	7.92e5	6.01e5
Volume	3.5 mL		
Total Viable Cells			
Viability	59.9%	54.1%	51.0%

DF – Dilution Factor

average: 7.4e5 viable cells/mL

Performed By: NP Date: 5/4/11

Verified By: Kevin C Hicok Date: 7/21/11

Appendix B - ADRC Isolation and Cell Count Record

(Complete for each donor)

Human lipoaspirate information:

Cytori ID No MA-2-2668 Date of Lipoaspirate Harvest 5/4/11

Amount of human lipoaspirate processed: 120 mL

Amount of Celase™ used: 2.1 mL

Materials Information:

Material	Lot #	Exp Date
Celution® 805 Consumable Sets	101860	2011-08
Celase™ Reagent	14792124	2010-04
Nucleocounter cassettes	0111-05	2012-04
Other		
Equipment		
Celution® 800/CRS Device S/N	**Syr:** —	**MA:** 1842
Celution® 800/CRS Device Software version		4.1/E1
NucleoCounter Asset No.	B460	

NucleoCounter® Cell Counting Data:

	Cell Count Post Isolation		
Dead Cells/mL * DF	$6.8e^5$	$9.7e^5$	$8.9e^5$
Total Cells/mL * DF	$5.85e^6$	$6.03e^6$	$5.91e^6$
Viable Cells/mL	$5.16e^6$	$5.07e^6$	$5.01e^6$
Volume	5.2mL		
Total Viable Cells	$2.64e^7$		
Viability	88.4%	83.9%	84.9%

DF – Dilution Factor

Performed By: AMMAR ASMAR Date: 5/4/11

Verified By: K. C Hicok Date: 7/21/11

Appendix B - ADRC Isolation and Cell Count Record

(Complete for each donor)

--continued--

Final Volume Cells Resuspended: 5.2 mL

Final Cell Concentration: 5.08e6 ADRCs/mL

sample: 1 mL for CFU/F assay; 1.97 mL for FACS

NucleoCounter® Cell Counting Data for Loose Cell Analysis:

MA-2-2668-SVF	Cell Count Post Isolation		
Dead Cells/mL * DF	6.48e5	6.74e5	6.55e5
Total Cells/mL * DF	1.69e6	1.64e6	1.59e6
Viable Cells/mL	1.04e6	9.60e5	9.39e5
Volume	2.7 mL		
Total Viable Cells			
Viability	61.6%	58.8%	58.7%

average: 9.80e5 viable cells/mL

DF – Dilution Factor

Performed By: KJ Date: 5/4/11

Verified By: Ken C Hicok Date: 7/21/11

Appendix B - ADRC Isolation and Cell Count Record

(Complete for each donor)

Human lipoaspirate information:

Cytori ID No MA3-[illegible]RDate of Lipoaspirate Harvest 6/2/11

Amount of human lipoaspirate processed: 100 mL

Amount of Celase™ used: 1.8 mL

Materials Information:

Material	Lot #	Exp Date
Celution® 805 Consumable Sets	7101826	2011-08
Celase™ Reagent	14792124	2010-04
Nucleocounter cassettes	0111-05	2012-04
Other		
Equipment		
Celution® 800/CRS Device S/N	**Syr:** 1877	**MA:** ——
Celution® 800/CRS Device Software version	4.1/E1	
NucleoCounter Asset No.	B460	

NucleoCounter® Cell Counting Data:

	Cell Count Post Isolation		
Dead Cells/mL * DF	4.5e5	3.3e5	4.0e5
Total Cells/mL * DF	3.12e6	2.7e6	2.8e6
Viable Cells/mL	2.7e6	2.4e6	2.4e6
Volume	5.2mL		
Total Viable Cells	1.3e7		
Viability	85.6%	87.9%	85.8%

DF – Dilution Factor

Performed By: AMMAR ASNAR Date: 6/2/11

Verified By: Kevin C Hicok Date: 7/21/11

Appendix B - ADRC Isolation and Cell Count Record

(Complete for each donor)

--continued--

Final Volume Cells Resuspended: 5.2 mL

Final Cell Concentration: 2.5e6 ADRCs/mL

sample: 1 mL for CFU/F assay; 3.3 mL for FACS

AA 6/2/11

NucleoCounter® Cell Counting Data for Loose Cell Analysis:

	Cell Count Post Isolation		
Dead Cells/mL * DF			
Total Cells/mL * DF			
Viable Cells/mL			
Volume AA 6/2/11	~~5.2mL~~		
Total Viable Cells			
Viability			

N/A insufficient volume of tissue

DF – Dilution Factor

Performed By: AMMAR ASMAR Date: 6/2/11

Verified By: K. C Head Date: 7/21/11

Appendix B - ADRC Isolation and Cell Count Record

(Complete for each donor)

Human lipoaspirate information:

Cytori ID No MA-3-2702 Date of Lipoaspirate Harvest 6/2/11

Amount of human lipoaspirate processed: 100 mL

Amount of Celase™ used: 1.8 mL

Materials Information:

Material	Lot #	Exp Date
Celution® 805 Consumable Sets	7101826	2011-08
Celase™ Reagent	14792124	2010-04
Nucleocounter cassettes	0111-05	2012-04
Other		
Equipment		
Celution® 800/CRS Device S/N	**Syr:** —	**MA:** 1842
Celution® 800/CRS Device Software version		4.1/E1
NucleoCounter Asset No.	B460	

NucleoCounter® Cell Counting Data:

	Cell Count Post Isolation		
Dead Cells/mL * DF	$6.1e^5$	$4.8e^5$	$4.3e^5$
Total Cells/mL * DF	$2.9e^6$	$3.4e^6$	$2.8e^6$
Viable Cells/mL	$2.3e^6$	$2.7e^6$	$2.4e^6$
Volume	5.2mL		
Total Viable Cells	1.28e7		
Viability	78.6%	85%	84.6%

DF – Dilution Factor

Performed By: AMMAR ASMAR Date: 6/2/11

Verified By: K. C Hicks Date: 7/21/11

Appendix B - ADRC Isolation and Cell Count Record

(Complete for each donor)

--continued--

Final Volume Cells Resuspended: 5.2 mL

Final Cell Concentration: 2.47e6 ADRCs/mL

sample: 1 mL for CFU/F assay; 3.5 mL for FACS

NucleoCounter® Cell Counting Data for Loose Cell Analysis:

	Cell Count Post Isolation		
Dead Cells/mL * DF	3.94e5	3.56e5	4.12e5
Total Cells/mL * DF	1.01e6	1.49e6	1.56e6
Viable Cells/mL	6.18e5	1.13e6	1.15e6
Volume	2.6 mL		
Total Viable Cells			
Viability	61.0%	76.0%	73.5%

average: 9.66e5 viable cells/mL

DF – Dilution Factor

Performed By: NS Date: 6/2/11

Verified By: Kim CHeek Date: 7/21/11

Appendix B - ADRC Isolation and Cell Count Record

(Complete for each donor)

Human lipoaspirate information:

Cytori ID No 2706 Date of Lipoaspirate Harvest 6/3/11

Amount of human lipoaspirate processed: 120 mL

Amount of Celase™ used: 2.1 mL

Materials Information:

Material	Lot #	Exp Date
Celution® 805 Consumable Sets	7101826	2011-08
Celase™ Reagent	14792124	2010-04
Nucleocounter cassettes	0111-05	2012-04
Other		
Equipment		
Celution® 800/CRS Device S/N	**Syr:** 1877	**MA:** —
Celution® 800/CRS Device Software version	4.1/E1	
NucleoCounter Asset No.	B460	

NucleoCounter® Cell Counting Data:

	Cell Count Post Isolation		
Dead Cells/mL * DF	3.6e5	4.6e5	4.2e5
Total Cells/mL * DF	3.84e6	4.38e6	4.02e6
Viable Cells/mL	3.48e6	3.93e6	3.6e6
Volume	← 5.2 →		
Total Viable Cells	1.8|e7	2.04e7	1.87e7
Viability	90.6	89.5	89.6

DF – Dilution Factor

Performed By: _____ Date: 6/3/11

Verified By: _____ Date: 7/21/11

Appendix B - ADRC Isolation and Cell Count Record

(Complete for each donor)

--continued--

Final Volume Cells Resuspended: 5.2 mL

Final Cell Concentration: 3.67 x 10^6 ADRCs/mL

sample: 1 mL for CFU/F assay; 2.7 mL for FACS

NucleoCounter® Cell Counting Data for Loose Cell Analysis:

MA-4-2706-SVF	Cell Count Post Isolation		
Dead Cells/mL * DF	2.47e5	2.62e5	2.41e5
Total Cells/mL * DF	1.25e6	1.46e6	1.18e6
Viable Cells/mL	1.01e6	1.20e6	9.39e5
Volume	1	2.5	1
Total Viable Cells	2.5e6	3.0e6	2.35e6
Viability	80.3%	82.1%	79.6%

average: 1.05e6 viable cells/mL

DF – Dilution Factor

Performed By: NJ Date: 6/3/11

Verified By: Kevin C Heath Date: 7/21/11

Appendix B - ADRC Isolation and Cell Count Record

(Complete for each donor)

Human lipoaspirate information:

Cytori ID No 2706 Date of Lipoaspirate Harvest 6/3/11

Amount of human lipoaspirate processed: 120 mL

Amount of Celase™ used: 2.1 mL

Materials Information:

Material	Lot #	Exp Date
Celution® 805 Consumable Sets	7101826	2011-08
Celase™ Reagent	147-92124	2010-04
Nucleocounter cassettes	0111-05	2012-04
Other		
Equipment		
Celution® 800/CRS Device S/N	**Syr:** —	**MA:** 1842
Celution® 800/CRS Device Software version		4.1/E1
NucleoCounter Asset No.	B460	

NucleoCounter® Cell Counting Data:

	Cell Count Post Isolation		
Dead Cells/mL * DF	1.63e6	1.6e6	1.59e6
Total Cells/mL * DF	8.67e6	8.22e6	7.8e6
Viable Cells/mL	7.05e6	6.63e6	6.21e6
Volume	5.2		
Total Viable Cells	3.67e7	3.45e7	3.23e7
Viability	81.2	80.5	79.6

DF – Dilution Factor

Performed By: _____ Date: 6/3/11

Verified By: K C Hart Date: 7/22/11

Appendix B - ADRC Isolation and Cell Count Record

(Complete for each donor)

--continued--

Final Volume Cells Resuspended: 5.2 mL

Final Cell Concentration: 6.63 x 10^6 ADRCs/mL

sample: 1 mL for CFU/F assay; 1.5 mL for FACS

NucleoCounter® Cell Counting Data for Loose Cell Analysis:

MA-4-2706	Cell Count Post Isolation		
Dead Cells/mL * DF	1.33e5	1.37e5	1.33e5
Total Cells/mL * DF	7.92e5	7.32e5	6.72e5
Viable Cells/mL	6.6e5	5.94e5	5.40e5
Volume	← 2.5 →		
Total Viable Cells	1.625e6	1.49e6	1.35e6
Viability	83.2%	81.3%	80.2%

average: 5.98 e5 viable cells/mL

DF – Dilution Factor

Performed By: NJ Date: 6/3/11

Verified By: Kevin C Hruska Date: 7/22/11

Appendix B - ADRC Isolation and Cell Count Record

(Complete for each donor)

Human lipoaspirate information:

Cytori ID No 2722 Date of Lipoaspirate Harvest 6/14/11

Amount of human lipoaspirate processed: 120 mL

Amount of Celase™ used: 2.1 mL

Materials Information:

Material	Lot #	Exp Date
Celution® 805 Consumable Sets	7101860	2011-08
Celase™ Reagent	14826923	2010-05
Nucleocounter cassettes	0611-02	2012-08
Other		
Equipment		
Celution® 800/CRS Device S/N	**Syr:** 1844	**MA:** —
Celution® 800/CRS Device Software version	4.1/E1	
NucleoCounter Asset No.	B460	

NucleoCounter® Cell Counting Data:

	Cell Count Post Isolation		
Dead Cells/mL * DF	$5.8e^5$	$5.7e^5$	$4.4e^5$
Total Cells/mL * DF	$3.66e^6$	$3.54e^6$	$3.60e^6$
Viable Cells/mL	$3.09e^6$	$3.0e^6$	$3.15e^6$
Volume	5.1	5.1	5.1
Total Viable Cells			
Viability	84.2	83.9	87.8

DF – Dilution Factor

Performed By: _____ Date: 6/14/11

Verified By: K. C Hicar Date: 7/15/11

Appendix B - ADRC Isolation and Cell Count Record

(Complete for each donor)

--continued--

Final Volume Cells Resuspended: 5.1 mL

Final Cell Concentration: 3.08×10^6 ADRCs/mL

sample: 1 mL for CFU/F assay; 3.25 mL for FACS

NucleoCounter® Cell Counting Data for Loose Cell Analysis:

MA-S-2722-SVF	Cell Count Post Isolation		
Dead Cells/mL * DF	1.3e4	2.2e4	1.7e4
Total Cells/mL * DF	1.1e5	1.2e5	8.1e4
Viable Cells/mL	9.6e4	1.0e5	6.3e4
Volume	1	3.0	1
Total Viable Cells	3.88e5	3.0e5	1.89e5
Viability	86.0%	82.1%	77.0%

concentration: 8.63 e4 viable cells/mL

DF – Dilution Factor

Performed By: NJ Date: 6/14/11

Verified By: K. C Heck Date: 7/15/11

Appendix B - ADRC Isolation and Cell Count Record

(Complete for each donor)

Human lipoaspirate information:

Cytori ID No 2722 Date of Lipoaspirate Harvest 6/14/11

Amount of human lipoaspirate processed: 120 mL

Amount of Celase™ used: 2.1 mL

Materials Information:

Material	Lot #	Exp Date
Celution® 805 Consumable Sets	7101860	2011-08
Celase™ Reagent	14826923	2010-05
Nucleocounter cassettes	0511-02	2012-08
Other		
Equipment		
Celution® 800/CRS Device S/N	**Syr:** —	**MA:** 1877
Celution® 800/CRS Device Software version		4.1/E1
NucleoCounter Asset No.	B4160	

NucleoCounter® Cell Counting Data:

	Cell Count Post Isolation		
Dead Cells/mL * DF	$1.23e^6$	$1.39e^6$	$1.32e^6$
Total Cells/mL * DF	$7.14e^6$	$7.02e^6$	$7.50e^6$
Viable Cells/mL	$5.91e^6$	$5.64e^6$	$6.18e^6$
Volume	5.1	5.1	5.1
Total Viable Cells	$3.01e^7$	$2.88e^7$	$3.15e^7$
Viability	82.8	80.2	82.4

DF – Dilution Factor

Performed By: _____ Date: 6/14/11

Verified By: Kevin C Hicok Date: 6/14/11

Appendix B - ADRC Isolation and Cell Count Record

(Complete for each donor)

--continued--

Final Volume Cells Resuspended: 5.1 mL

Final Cell Concentration: 5.91×10^6 ADRCs/mL

sample: 1 mL for CFU/F assay; 1.7 mL for FACS

NucleoCounter® Cell Counting Data for Loose Cell Analysis:

MA-5-2722	Cell Count Post Isolation		
Dead Cells/mL * DF	9x10e3	7 e3	9 e 3
Total Cells/mL * DF	1.9e5	1.5e5	1.5e5
Viable Cells/mL	1.3e5	1.4e5	1.4e5
Volume	1	3.0	1
Total Viable Cells	3.9e5	4.2e5	4.2e5
Viability	93.3%	95.2%	74.0%

average: 1.37e5 viable cells/mL

DF – Dilution Factor

Performed By: NJ Date: 6/14/11

Verified By: Kevin C Hauch Date: 7/15/11

Appendix B - ADRC Isolation and Cell Count Record

(Complete for each donor)

Human lipoaspirate information:

Cytori ID No MA-6-2731-SVF Date of Lipoaspirate Harvest 6/16/11

Amount of human lipoaspirate processed: 120 mL

Amount of Celase™ used: 2.1 mL

Materials Information:

Material	Lot #	Exp Date
Celution® 805 Consumable Sets	7101860	2011-08
Celase™ Reagent	14826923	2010-05
Nucleocounter cassettes	0511-02	2012-08
Other		
Equipment		
Celution® 800/CRS Device S/N	Syr: 1844	MA: —
Celution® 800/CRS Device Software version	4.1/E1 → same	
NucleoCounter Asset No.	8460	

NucleoCounter® Cell Counting Data:

	Cell Count Post Isolation		
Dead Cells/mL * DF	$5.4e^5$	$6.1e^5$	$5.9e^5$
Total Cells/mL * DF	$3.84e^6$	$3.06e^6$	$3.3e^6$
Viable Cells/mL	$3.3e^6$	$2.5e^6$	$3.1e^6$
Volume	1	5.1	1
Total Viable Cells	1.68e7	1.28e7	1.58e7
Viability	85.9	80.1	82.3

KCH 7/15/11 math error

DF – Dilution Factor

Performed By: _____ Date: 6/16/11

Verified By: C Huck Date: 7/15/11

Appendix B - ADRC Isolation and Cell Count Record

(Complete for each donor)

--continued--

Final Volume Cells Resuspended: 5.1 mL

Final Cell Concentration: 1.51 x 10^6 ADRCs/mL

sample: 1 mL for CFU/F assay; 4 mL for FACS

NucleoCounter® Cell Counting Data for Loose Cell Analysis:

	Cell Count Post Isolation		
Dead Cells/mL * DF	3.9e5	4.3e5	4.8e5
Total Cells/mL * DF	1.5e6	1.4e6	1.3e6
Viable Cells/mL	1.1e6	1.0e6	8.4e5
Volume	← 2.5 →		
Total Viable Cells	2.75e6	2.5e6	2.1e6
Viability	74.0	70.1	43.6

DF – Dilution Factor

Performed By: _____ Date: 6/16/11

Verified By: K C Hauck Date: 7/15/11

Appendix B - ADRC Isolation and Cell Count Record

(Complete for each donor)

Human lipoaspirate information:

Cytori ID No MA-2731 Date of Lipoaspirate Harvest 6/16/11

Amount of human lipoaspirate processed: 120 mL

Amount of Celase™ used: 2.1 mL

Materials Information:

Material	Lot #	Exp Date
Celution® 805 Consumable Sets	7101860	2011-08
Celase™ Reagent	14826923	2010-05
Nucleocounter cassettes	0511-02	2012-08
Other		
Equipment		
Celution® 800/CRS Device S/N	**Syr:** —	**MA:** 1877
Celution® 800/CRS Device Software version	—	4.1/E1
NucleoCounter Asset No.	B460	

NucleoCounter® Cell Counting Data:

	Cell Count Post Isolation		
Dead Cells/mL * DF	$1.26e^6$	$1.46e^6$	$1.48e^6$
Total Cells/mL * DF	$4.17e^6$	$4.89e^6$	$4.20e^6$
Viable Cells/mL	$2.9e^6$	$3.42e^6$	$2.70e^6$
Volume	⊢ 5.1 ⊣		
Total Viable Cells	$1.48e^7$	$1.74e^7$	$1.38e^7$
Viability	69.8	70.1	64.8

DF – Dilution Factor

Performed By: _____ Date: 6/16/11

Verified By: K C Hock Date: 7/15/11

Appendix B - ADRC Isolation and Cell Count Record

(Complete for each donor)

--continued--

Final Volume Cells Resuspended: 5.1 mL

Final Cell Concentration: 1.53×10^7 ADRCs/mL

sample: 1 mL for CFU/F assay; _____ mL for FACS

NucleoCounter® Cell Counting Data for Loose Cell Analysis:

	Cell Count Post Isolation		
Dead Cells/mL * DF	$1.7e^5$	$2.6e^5$	$3.0e^5$
Total Cells/mL * DF	$1.3e^6$	$1.5e^6$	$1.5e^6$
Viable Cells/mL	$1.1e^6$	$1.2e^6$	$1.2e^6$
Volume (at 6/22/11)	1	2.5	1
Total Viable Cells	~~$3.0e^6$~~ $2.75e^6$	$3.0e^6$	$3.0e^6$
Viability	87.1	82.7	80.0

DF – Dilution Factor

Performed By: _____ Date: 6/16/11

Verified By: K. C Hunt Date: 7/15/11

Appendix C - CFU-F Assay Data Sheet
(Complete for each sample from each donor)

1. Sample ID #: MA-1-2647 SVR

2. Cell plating date: 4/19/11 and Assay End Date: 4/25/11

3. Total Number of Incubation Days: 6

4. Viability of the original sample: 82.54 %

5,000 cells/ well

34	35	35
30	27	33

Average CFU-F in 5,000 cells per well plating density = 33

CFU-F frequency (%) = 0.66

CFU-F assay performed by (signature & date): [signature] 4/26/11

Verified by (signature & date): Karen CHeek 7/21/11

Appendix C - CFU-F Assay Data Sheet
(Complete for each sample from each donor)

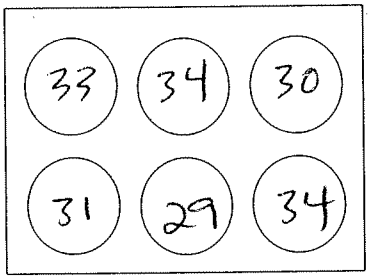
1. Sample ID #: MA-1-2647

2. Cell plating date: 4/19/11 and Assay End Date: 4/25/11

3. Total Number of Incubation Days: 6

4. Viability of the original sample: 88.04 %

5,000 cells/ well



Average CFU-F in 5,000 cells per well plating density = 32

CFU-F frequency (%) = 0.64

CFU-F assay performed by (signature & date): 4/26/11

Verified by (signature & date): Kevin C Hacker 7/21/11

Appendix C - CFU-F Assay Data Sheet
(Complete for each sample from each donor)

1. Sample ID #: MA-2-2668 SVF

2. Cell plating date: 5/4/11 and Assay End Date: 5/9/11

3. Total Number of Incubation Days: 5

4. Viability of the original sample: 87.08%

5,000 cells/ well

19	18	22
22	22	19

Average CFU-F in 5,000 cells per well plating density = 20.5

CFU-F frequency (%) = 0.41

CFU-F assay performed by (signature & date): [signature] 5/10/11

Verified by (signature & date): [signature] 7/21/11

Appendix C - CFU-F Assay Data Sheet
(Complete for each sample from each donor)

1. Sample ID #: MA-2-2668

2. Cell plating date: 5/4/11 and Assay End Date: 5/9/11

3. Total Number of Incubation Days: 5

4. Viability of the original sample: 85.74%

5,000 cells/ well

41	43	38
39	31	34

Average CFU-F in 5,000 cells per well plating density = 38

CFU-F frequency (%) = 0.76

CFU-F assay performed by (signature & date): [signature] 5/10/11

Verified by (signature & date): [signature] 7/21/11

Appendix C - CFU-F Assay Data Sheet
(Complete for each sample from each donor)

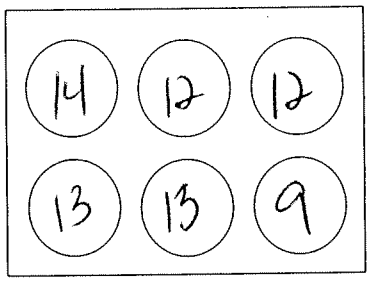
1. Sample ID #: MA-3-2702 SVF

2. Cell plating date: 6/2/11 and Assay End Date: 6/6/11

3. Total Number of Incubation Days: 4

4. Viability of the original sample: 86.36%

5,000 cells/ well



Average CFU-F in 5,000 cells per well plating density = 12.5

CFU-F frequency (%) = 0.25

CFU-F assay performed by (signature & date): 6/6/11

Verified by (signature & date): Kevin C Hicok 7/21/11

Appendix C - CFU-F Assay Data Sheet
(Complete for each sample from each donor)

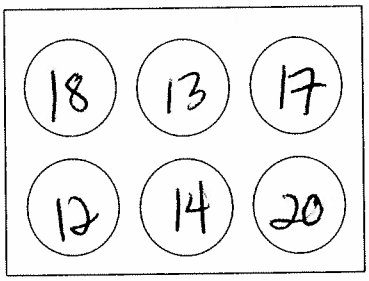
1. Sample ID #: MA-3-2702

2. Cell plating date: 6/2/11 and Assay End Date: 6/6/11

3. Total Number of Incubation Days: 4

4. Viability of the original sample: 82.89%

5,000 cells/ well



Average CFU-F in 5,000 cells per well plating density = 15.5

CFU-F frequency (%) = 0.31

CFU-F assay performed by (signature & date): 6/6/11

Verified by (signature & date): Keri C Hecht 7/21/11

Appendix C - CFU-F Assay Data Sheet
(Complete for each sample from each donor)

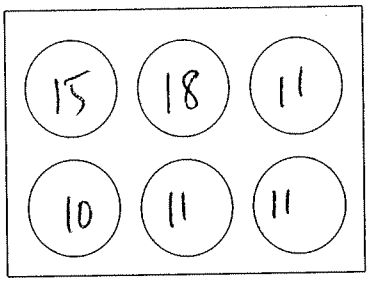
1. Sample ID #: MA-4-2706 SYR

2. Cell plating date: 6/3/11 and Assay End Date: 6/8/11

3. Total Number of Incubation Days: 5

4. Viability of the original sample: 80.7%

5,000 cells/ well



Average CFU-F in 5,000 cells per well plating density = 12

CFU-F frequency (%) = 0.24

CFU-F assay performed by (signature & date): 6/3/11

Verified by (signature & date): 7/21/11

Appendix C - CFU-F Assay Data Sheet
(Complete for each sample from each donor)

1. Sample ID #: MA-4-2706

2. Cell plating date: 6/3/11 and Assay End Date: 6/8/11

3. Total Number of Incubation Days: 5

4. Viability of the original sample: 81.6%

5,000 cells/ well

14	10	10
10	12	8

Average CFU-F in 5,000 cells per well plating density = 10.5

CFU-F frequency (%) = 0.21

CFU-F assay performed by (signature & date): 6/3/11

Verified by (signature & date): 7/22/11

Appendix C - CFU-F Assay Data Sheet
(Complete for each sample from each donor)

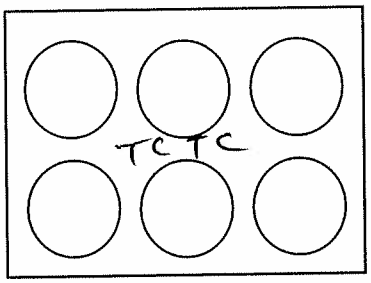
1. Sample ID #: 2722

2. Cell plating date: 6/14/11 and Assay End Date: 6/20/11

3. Total Number of Incubation Days: 6

4. Viability of the original sample: 85.3%

5,000 cells/ well



Average CFU-F in 5,000 cells per well plating density = 75 + KCH 7/15/11

CFU-F frequency (%) = 71.5 KCH 7/15/11

CFU-F assay performed by (signature & date): 6/20/11

Verified by (signature & date): Ken C Hicok 7/15/11

Appendix C - CFU-F Assay Data Sheet
(Complete for each sample from each donor)

1. Sample ID #: 2722

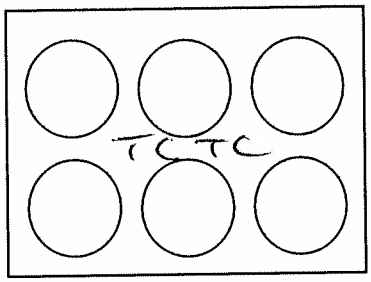
2. Cell plating date: 6/14/11 and Assay End Date: 6/20/11

3. Total Number of Incubation Days: 6

4. Viability of the original sample: 81.8% 5.91 x 10^6 c/ml (viable cell conc.)

5,000 cells/ well

Too confluent to count



Average CFU-F in 5,000 cells per well plating density = 75 +

CFU-F frequency (%) = > 1.5%

CFU-F assay performed by (signature & date): 6/14/11

Verified by (signature & date): Ken C Hsieh 7/15/11

Appendix C - CFU-F Assay Data Sheet
(Complete for each sample from each donor)

1. Sample ID #: MA-6-2731-SVR

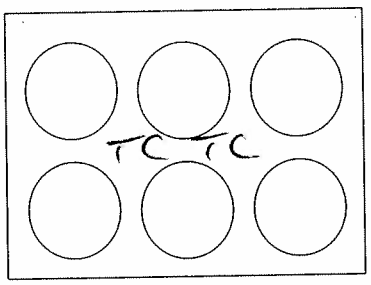
2. Cell plating date: 6/16/11 and Assay End Date: 6/22/11

3. Total Number of Incubation Days: _____

4. Viability of the original sample: ~~89.2%~~ 82.8% gf 6/27/11

5,000 cells/ well

Too confluent to count



Average CFU-F in 5,000 cells per well plating density = 75+

CFU-F frequency (%) = >1.5%

CFU-F assay performed by (signature & date): 6/16/11

Verified by (signature & date): Kevin Heck 7/15/11

Appendix C - CFU-F Assay Data Sheet
(Complete for each sample from each donor)

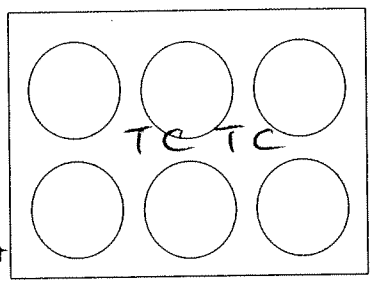
1. Sample ID #: MA-6-2731

2. Cell plating date: 6/16/11 and Assay End Date: 6/23/11

3. Total Number of Incubation Days: 7

4. Viability of the original sample: 83.0%

5,000 cells/ well



TCTC = Too confluent to count

Too many colonies to count

Average CFU-F in 5,000 cells per well plating density = ~75+

CFU-F frequency (%) = > 1.5%

CFU-F assay performed by (signature & date): 6/16/11

Verified by (signature & date): Ken C Hicok 7/15/11

Appendix D - Puregraft Assay Data Sheet
(Complete for each sample from each donor)
Measure before PG, only if have enough tissue.

	Before PG	After PG
Volume added to Puregraft (mL)	NA	pre & post PG
Volume recovered from Puregraft for analysis	NA	10mL x 3reps
Aqueous Vol. Rep 1	1.75 mL	0.125mL
Aqueous Vol. Rep 2	1.8mL	0.2mL
Aqueous Vol. Rep 3	1.8 mL	0.2mL
Graft Vol. Rep 1	8 mL	9.875mL
Graft Vol. Rep 2	7.75mL	9.8mL
Graft Vol. Rep 3	7.9mL	9.8mL
Lipid Vol. Rep 1	0.25mL	0mL
Lipid Vol. Rep 2	0.45mL	0mL
Lipid Vol. Rep 3	0.3mL	0mL

Performed By: AMMAR ASMAR Date: 4/19/11

Verified By: K. C Hicks Date: 7/21/11

Appendix D - Puregraft Assay Data Sheet
(Complete for each sample from each donor)
Measure before PG, only if have enough tissue.

	Before PG	After PG
Volume added to Puregraft (mL)	NA	pre & post PG 10mL x 3 reps
Volume recovered from Puregraft for analysis	NA	
Aqueous Vol. Rep 1	1.5mL	0.2mL
Aqueous Vol. Rep 2	1.75mL	0.25mL
Aqueous Vol. Rep 3	2mL	0.25mL
Graft Vol. Rep 1	7.75mL	9.8mL
Graft Vol. Rep 2	7.25mL	9.75mL
Graft Vol. Rep 3	7mL	9.75mL
Lipid Vol. Rep 1	0.75mL	0 mL
Lipid Vol. Rep 2	1mL	0mL
Lipid Vol. Rep 3	1mL	0mL

Performed By: AMMAR ASMAR Date: 4/19/11

Verified By: Kevin C Hicks Date: 7/21/11

Appendix D - Puregraft Assay Data Sheet
(Complete for each sample from each donor)
Measure before PG, only if have enough tissue.

	Before PG	After PG
Volume added to Puregraft (mL)	NA	
Volume recovered from Puregraft for analysis	NA	pre & post PG 10mL x 3 reps 5/4/11 AA
Aqueous Vol. Rep 1	2.25mL	~~0.4mL~~ 0.2mL
Aqueous Vol. Rep 2	2 mL	0.3mL
Aqueous Vol. Rep 3	2 mL	0.4mL
Graft Vol. Rep 1	6.5mL	9.4mL
Graft Vol. Rep 2	6.4mL	9.5mL
Graft Vol. Rep 3	6.5mL	9.35mL
Lipid Vol. Rep 1	1.25mL	0.4mL
Lipid Vol. Rep 2	1.6mL	0.2mL
Lipid Vol. Rep 3	1.5mL	0.25mL

Performed By: AMMAR ASMAR Date: 5/4/11

Verified By: Kevin C Hicok Date: 7/21/11

Appendix D - Puregraft Assay Data Sheet
(Complete for each sample from each donor)
Measure before PG, only if have enough tissue.

	Before PG	After PG
Volume added to Puregraft (mL)	NA	
Volume recovered from Puregraft for analysis	NA	pre & post PG 10mL x 3 reps
Aqueous Vol. Rep 1	2.25mL	0.9mL
Aqueous Vol. Rep 2	2.4mL	0.75mL
Aqueous Vol. Rep 3	2.3mL	0.6mL
Graft Vol. Rep 1	6.5mL	9mL
Graft Vol. Rep 2	6.4mL	9.25mL
Graft Vol. Rep 3	6.5mL	9.3mL
Lipid Vol. Rep 1	1.25mL	0.1mL
Lipid Vol. Rep 2	1.2mL	0mL
Lipid Vol. Rep 3	1.2mL	0.1mL

Performed By: AMMAR ASMAR Date: 5/4/11

Verified By: Ken Hicock Date: 7/21/11

Appendix D - Puregraft Assay Data Sheet
(Complete for each sample from each donor)
Measure before PG, only if have enough tissue.

	Before PG	After PG
Volume added to Puregraft (mL)	NA	
Volume recovered from Puregraft for analysis	NA	30mL
Aqueous Vol. Rep 1	2.6 mL	1.0mL
Aqueous Vol. Rep 2	2.5 mL	1.25mL
Aqueous Vol. Rep 3	2.6 mL	1.2mL
Graft Vol. Rep 1	6.4 mL	8.75mL
Graft Vol. Rep 2	6.0 mL	8.3mL
Graft Vol. Rep 3	6.0 mL	8.5mL
Lipid Vol. Rep 1	1.1 mL	0.25 mL
Lipid Vol. Rep 2	1.4 mL	0.45 mL
Lipid Vol. Rep 3	1.4 mL	0.3mL

Performed By: _____ Date: 6/2/11

Verified By: _____ Date: 7/21/11

Appendix D - Puregraft Assay Data Sheet
(Complete for each sample from each donor)
Measure before PG, only if have enough tissue.

	Before PG	After PG
Volume added to Puregraft (mL)	NA	
Volume recovered from Puregraft for analysis	NA	30mL
Aqueous Vol. Rep 1	2.5mL	2.2mL
Aqueous Vol. Rep 2	2.75mL	2.25mL
Aqueous Vol. Rep 3	2.25mL	20mL
Graft Vol. Rep 1	6.5mL	7.65mL
Graft Vol. Rep 2	6.5mL	7.65mL
Graft Vol. Rep 3	5.65mL	7.8mL
Lipid Vol. Rep 1	1.0mL	0.15mL
Lipid Vol. Rep 2	0.75mL	0.1mL
Lipid Vol. Rep 3	2.1mL	0.2mL

Performed By: AMMAR ASMAR Date: 6/2/11

Verified By: Kevin C Hazel Date: 7/21/11

Appendix D - Puregraft Assay Data Sheet
(Complete for each sample from each donor)
Measure before PG, only if have enough tissue.

	Before PG	After PG
Volume added to Puregraft (mL)	NA	
Volume recovered from Puregraft for analysis	NA	
Aqueous Vol. Rep 1	2.5	3.4
Aqueous Vol. Rep 2	2.9	3.0
Aqueous Vol. Rep 3	2.5	2.9
Graft Vol. Rep 1	6.5	6.6
Graft Vol. Rep 2	6.6	6.9
Graft Vol. Rep 3	6.5	7.0
Lipid Vol. Rep 1	1.0	0.2
Lipid Vol. Rep 2	0.5	0.3
Lipid Vol. Rep 3	1.0	0.2

Performed By: _____ Date: 6/3/11

Verified By: _____ Date: 7/21/11

Appendix D - Puregraft Assay Data Sheet
(Complete for each sample from each donor)
Measure before PG, only if have enough tissue.

	Before PG	After PG
Volume added to Puregraft (mL)	NA	
Volume recovered from Puregraft for analysis	NA	
Aqueous Vol. Rep 1	3.4	2.1
Aqueous Vol. Rep 2	4.6	2.5
Aqueous Vol. Rep 3	3.6	2.9
Graft Vol. Rep 1	6.6	7.8
Graft Vol. Rep 2	5.4	7.5
Graft Vol. Rep 3	2nd 6/13/11 ~~6.4~~ 6.5	7.1
Lipid Vol. Rep 1	0.5	0.1
Lipid Vol. Rep 2	0.5	0.1
Lipid Vol. Rep 3	0.8	0.2

Performed By: _____ Date: 6/3/11

Verified By: _____ Date: 7/22/11

Appendix D - Puregraft Assay Data Sheet
(Complete for each sample from each donor)
Measure before PG, only if have enough tissue.

	Before PG	After PG
Volume added to Puregraft (mL)	NA	105
Volume recovered from Puregraft for analysis	NA	62
Aqueous Vol. Rep 1	2.5	1.8
Aqueous Vol. Rep 2	2.0	1.6
Aqueous Vol. Rep 3	2.8	2.1
Graft Vol. Rep 1	5.3	8.2
Graft Vol. Rep 2	7.1	8.0
Graft Vol. Rep 3	6.2	8.0
Lipid Vol. Rep 1	1.0	0.2
Lipid Vol. Rep 2	1.9	0.5
Lipid Vol. Rep 3	1.9	0.2

Performed By: _____ Date: 6/14/11

Verified By: Kevin C Heck Date: 7/15/11

Appendix D - Puregraft Assay Data Sheet
(Complete for each sample from each donor)
Measure before PG, only if have enough tissue.

	Before PG	After PG
Volume added to Puregraft (mL)	NA	200
Volume recovered from Puregraft for analysis	NA	105
Aqueous Vol. Rep 1	3.0	2.0
Aqueous Vol. Rep 2	4.5	2.0
Aqueous Vol. Rep 3	2.9	1.9
Graft Vol. Rep 1	6.0	7.9
Graft Vol. Rep 2	5.0	7.8
Graft Vol. Rep 3	6.1	8.0
Lipid Vol. Rep 1	0.9	0.2
Lipid Vol. Rep 2	1.1	0.3
Lipid Vol. Rep 3	1.0	0.2

Performed By: _____ Date: 6/14/11

Verified By: K C Hruska Date: 7/15/11

Appendix D - Puregraft Assay Data Sheet
(Complete for each sample from each donor)
Measure before PG, only if have enough tissue.

	Before PG	After PG
Volume added to Puregraft (mL)	NA	135
Volume recovered from Puregraft for analysis	NA	gf 6/16/11 ~~57~~ 56
Aqueous Vol. Rep 1	5.0	0.8
Aqueous Vol. Rep 2	4.6	0.7
Aqueous Vol. Rep 3	4.4	0.6
Graft Vol. Rep 1	4.6	9.4
Graft Vol. Rep 2	4.7	9.3
Graft Vol. Rep 3	5.1	9.5 ~~/~~ gf 6-27-11
Lipid Vol. Rep 1	0.4	<0.1 0.00
Lipid Vol. Rep 2	0.6	<0.1 0.00
Lipid Vol. Rep 3	0.5	<0.1 0.00

Performed By: _____ Date: 6/16/11

Verified By: Kevin C Hick Date: 7/15/11

MA-6-2731

Appendix D - Puregraft Assay Data Sheet
(Complete for each sample from each donor)
Measure before PG, only if have enough tissue.

	Before PG	After PG
Volume added to Puregraft (mL)	NA	185
Volume recovered from Puregraft for analysis	NA	70
Aqueous Vol. Rep 1	2.5	0.4
Aqueous Vol. Rep 2	2.7	0.3
Aqueous Vol. Rep 3	2.5	0.3
Graft Vol. Rep 1	7.6	9.9
Graft Vol. Rep 2	6.8	9.7
Graft Vol. Rep 3	7.1	9.8
Lipid Vol. Rep 1	0.3	~~<0.1~~ gf 6-27-11 0.00
Lipid Vol. Rep 2	0.4	<0.1 0.00
Lipid Vol. Rep 3	0.3	~~<0.1~~ 0.00

Performed By: _____ Date: 6/16/11

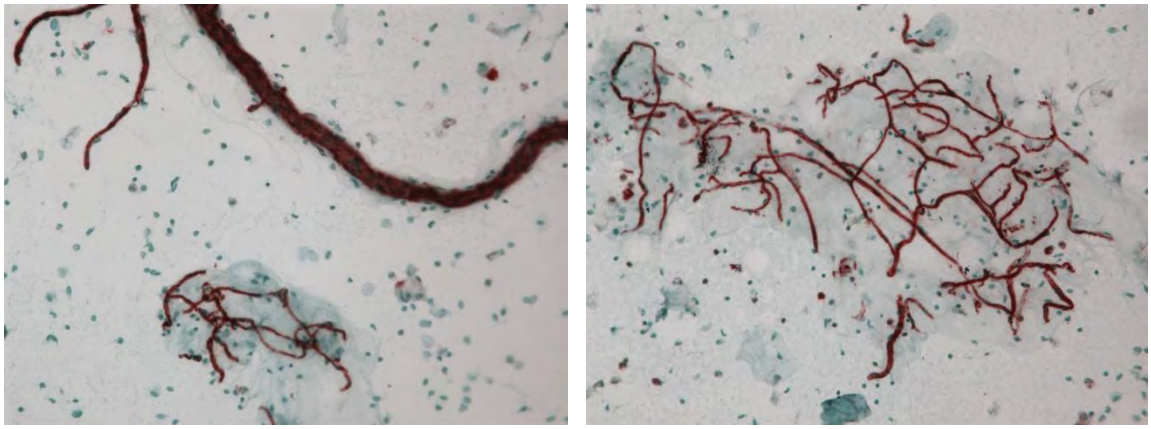
Verified By: _____ Date: 7/15/11

Appendix E. Representative Immunostaining of Loosely Adherent Cells

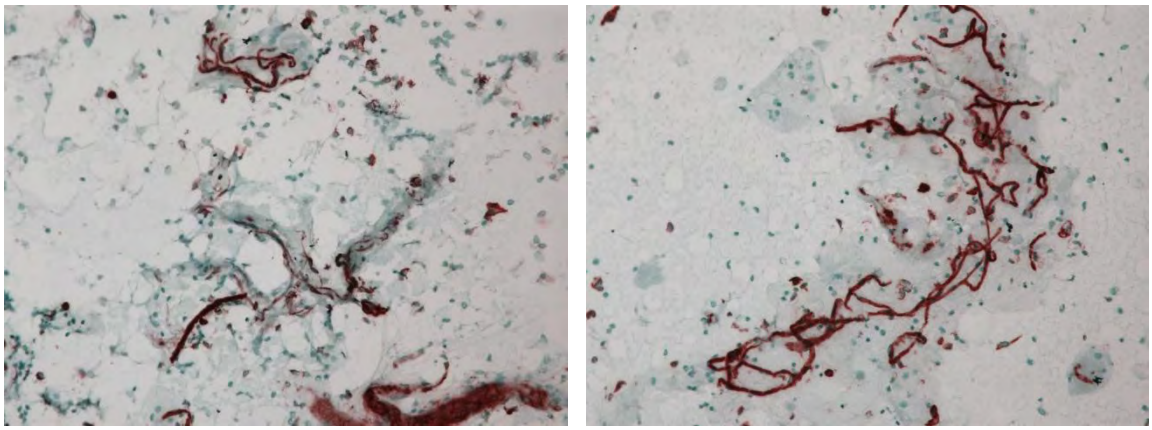
SYRINGE CONTROL **MICROAIRE (PAL)**

Donor 1

CD31



CD34

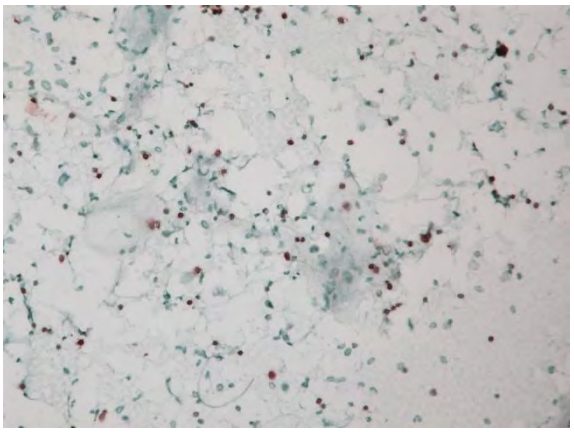


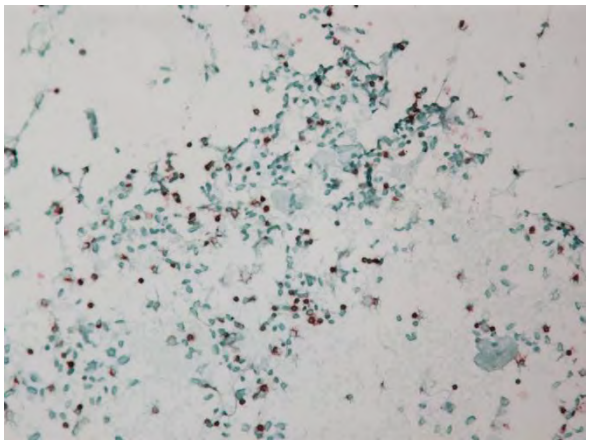
Appendix E. Representative Immunostaining of Loosely Adherent Cells (cont.)

SYRINGE CONTROL **MICROAIRE (PAL)**

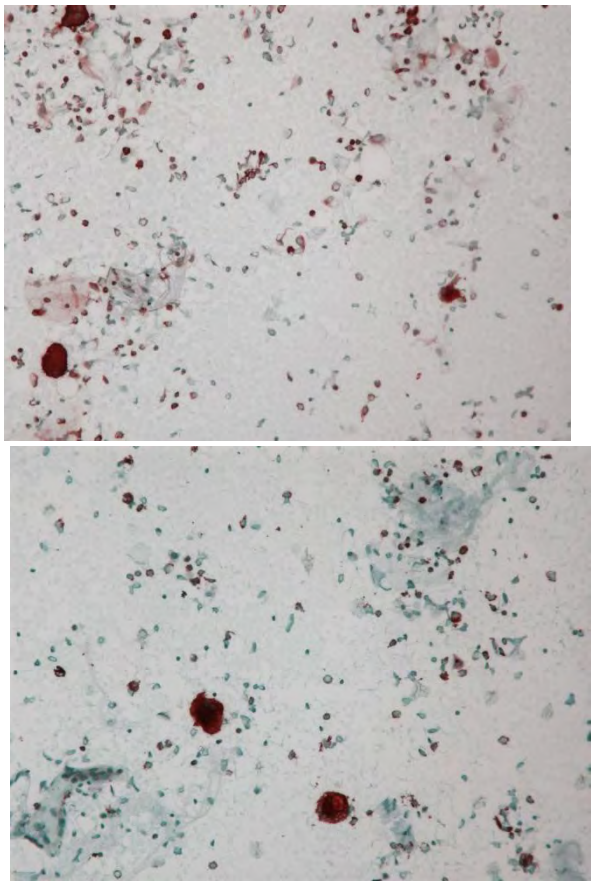
Donor1

CD45





CD68

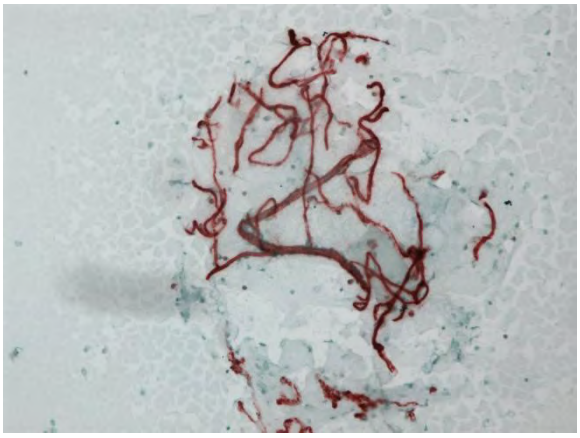


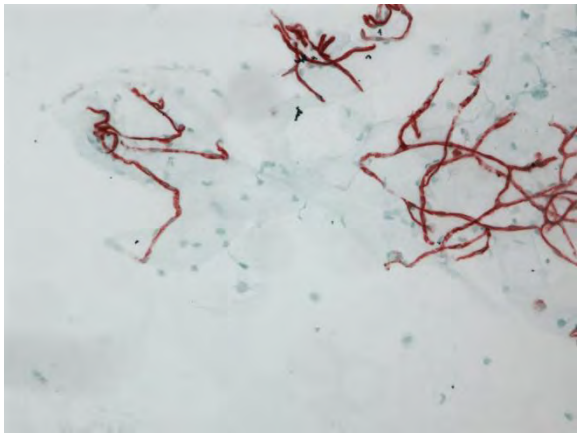
Appendix E. Representative Immunostaining of Loosely Adherent Cells (cont.)

Donor 2

SYRINGE CONTROL **MICROAIRE (PAL)**

CD31





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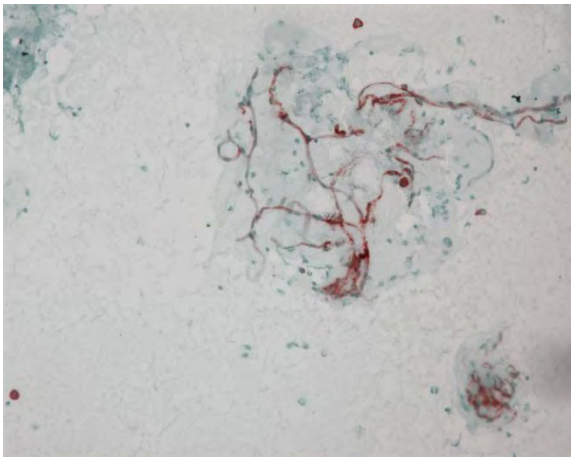
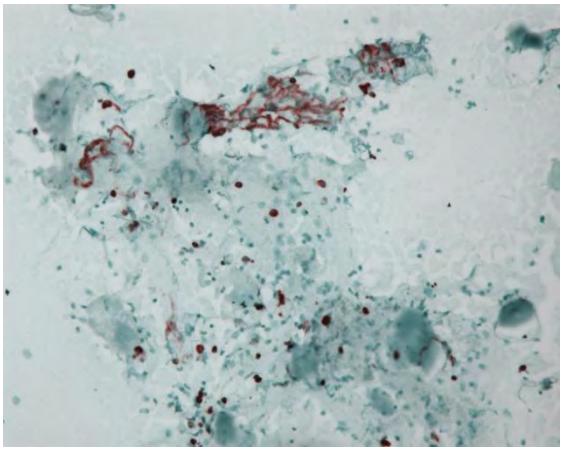
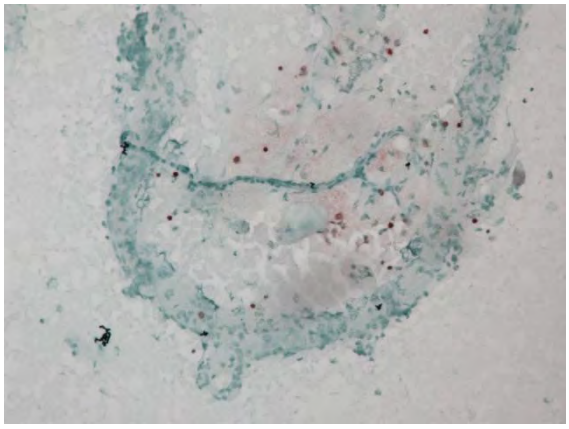
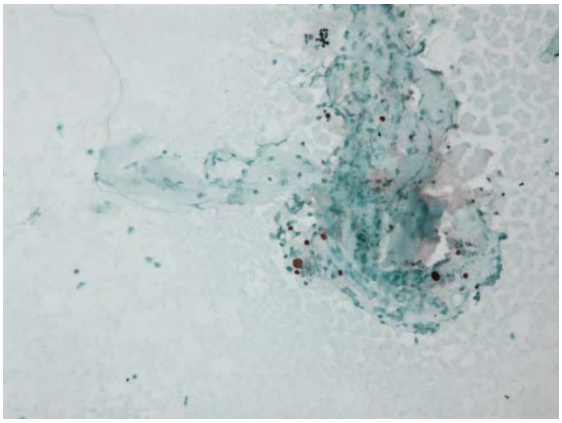
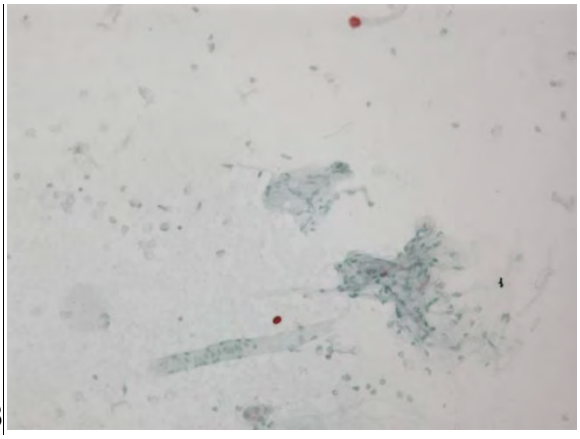
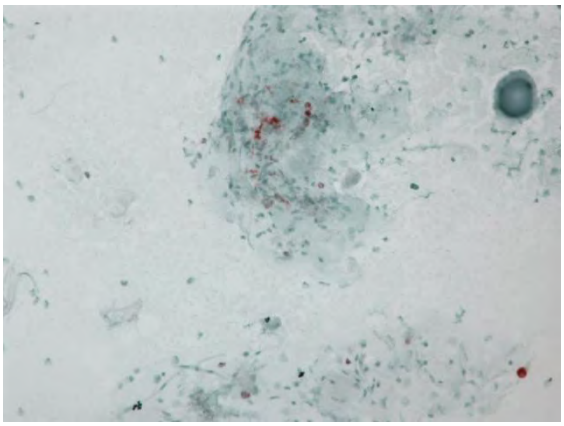
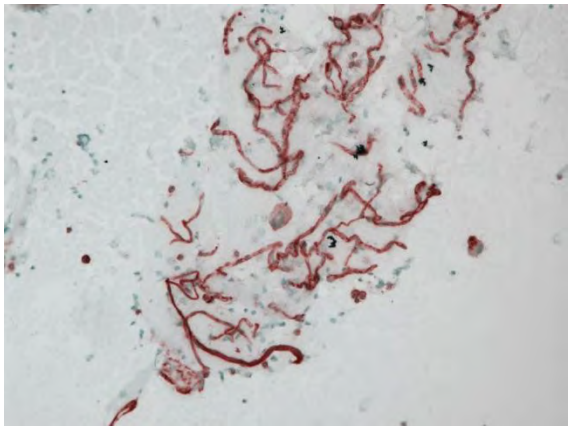
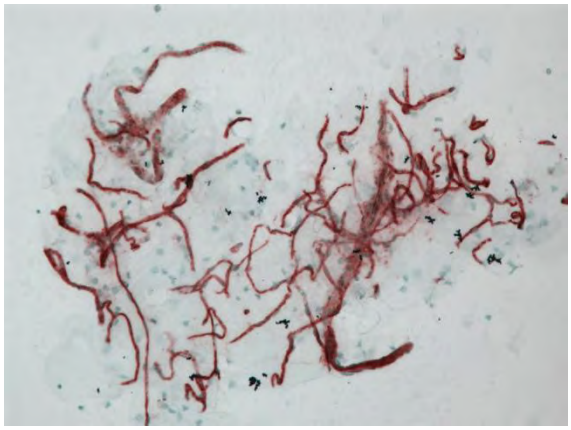




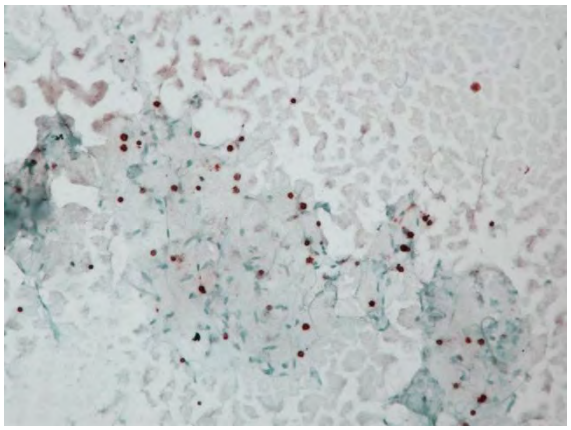
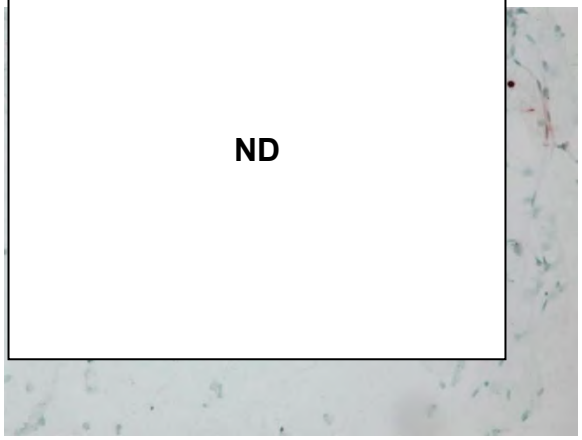
Appendix E. Representative Immunostaining of Loosely Adherent Cells (cont.)

	SYRINGE CONTROL	**MICROAIRE (PAL)**
Donor 2		
CD45		
CD68		

Appendix E. Representative Immunostaining of Loosely Adherent Cells (cont.)

	SYRINGE CONTROL	MICROAIRE (PAL)
Donor 3		
CD31	ND	
CD34	ND	

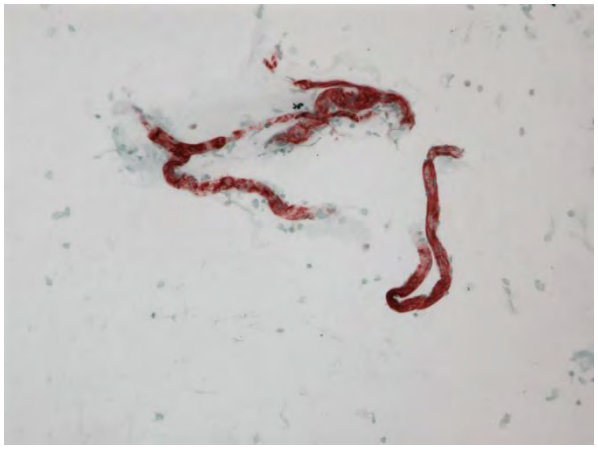
Appendix E. Representative Immunostaining of Loosely Adherent Cells (cont.)

	SYRINGE CONTROL	**MICROAIRE (PAL)**
Donor 3		
CD45	**ND**	
CD68	**ND**	

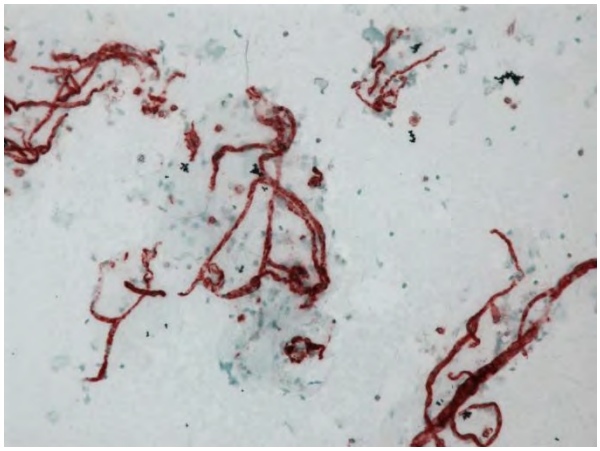
Appendix E. Representative Immunostaining of Loosely Adherent Cells (cont.)

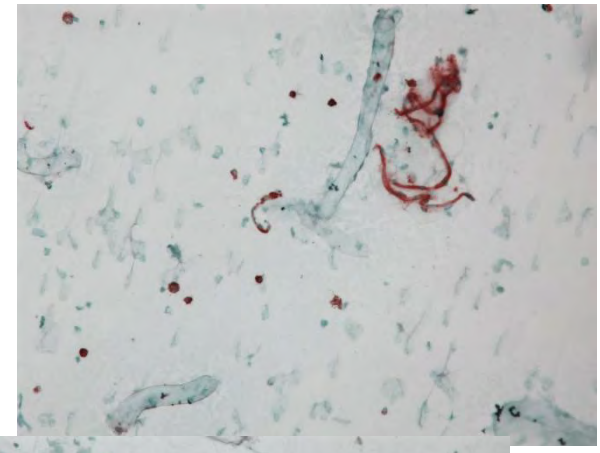
SYRINGE CONTROL **MICROAIRE (PAL)**

Donor 4



CD31





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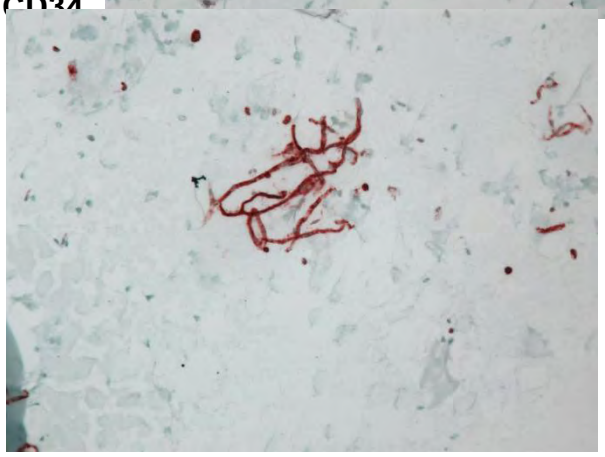
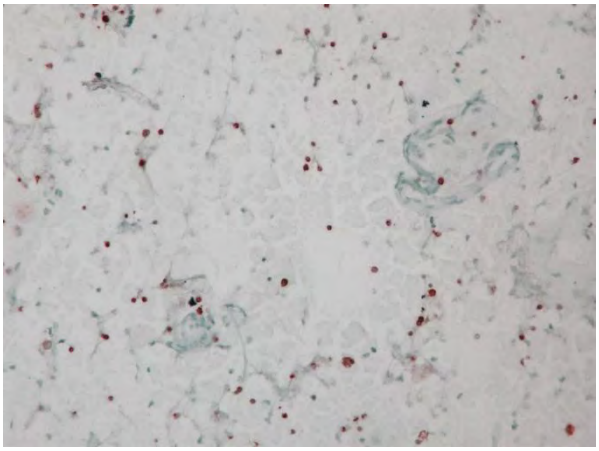


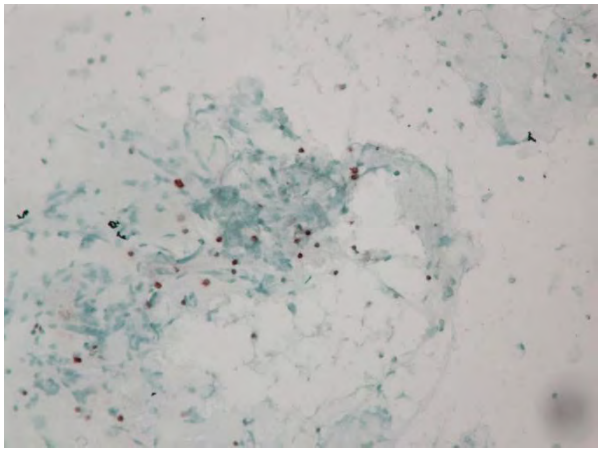
Appendix E. Representative Immunostaining of Loosely Adherent Cells (cont.)

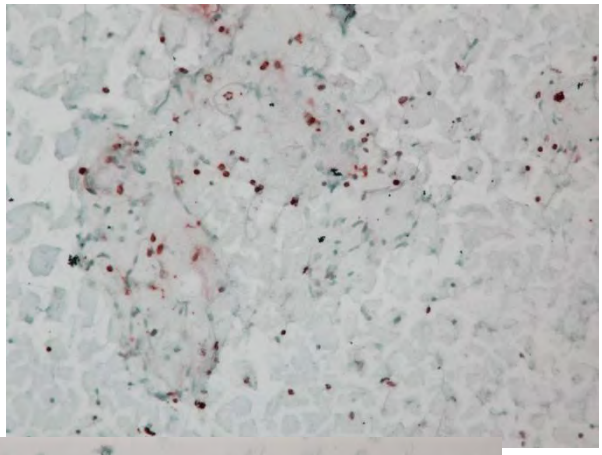
SYRINGE CONTROL **MICROAIRE (PAL)**

Donor 4



CD45





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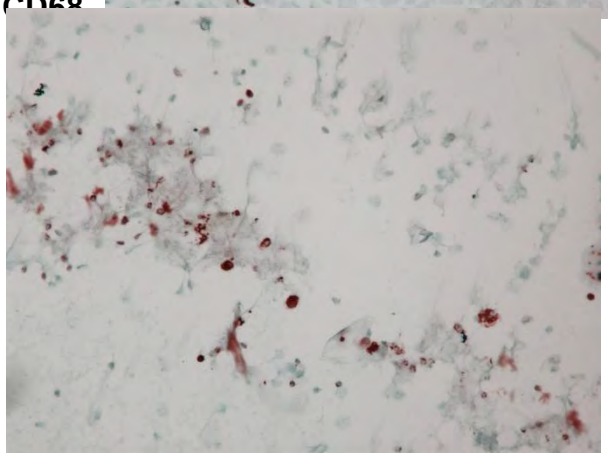
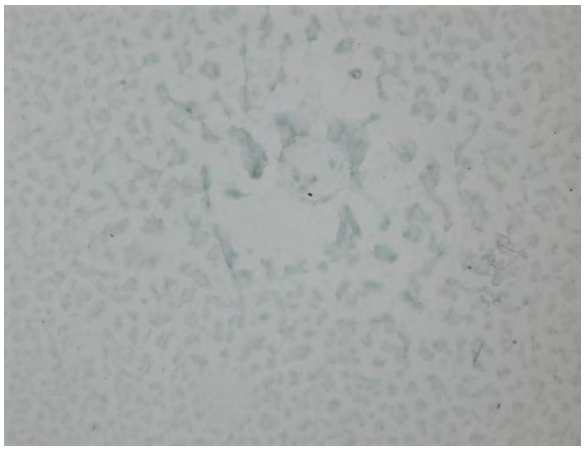


Appendix E. Representative Immunostaining of Loosely Adherent Cells (cont.)

SYRINGE CONTROL **MICROAIRE (PAL)**

Donor 5



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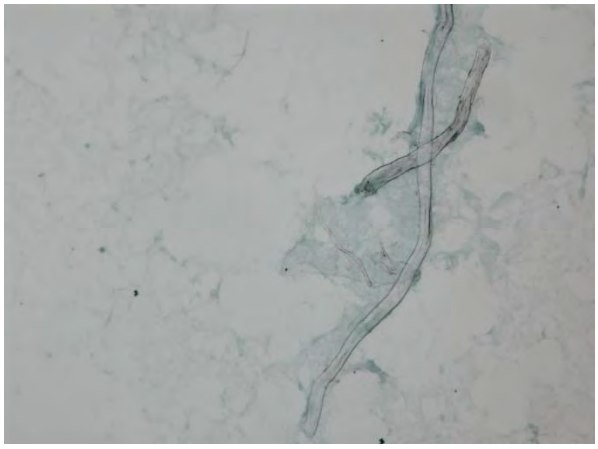
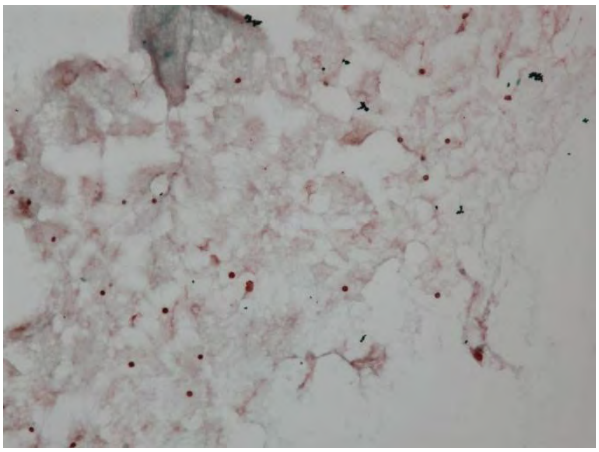


CD34

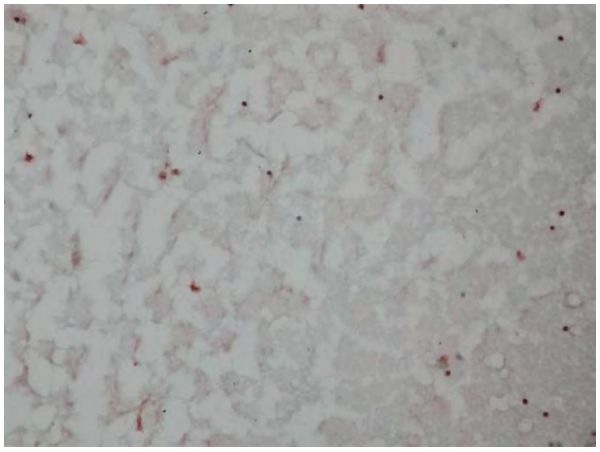
Appendix E. Representative Immunostaining of Loosely Adherent Cells (cont.)

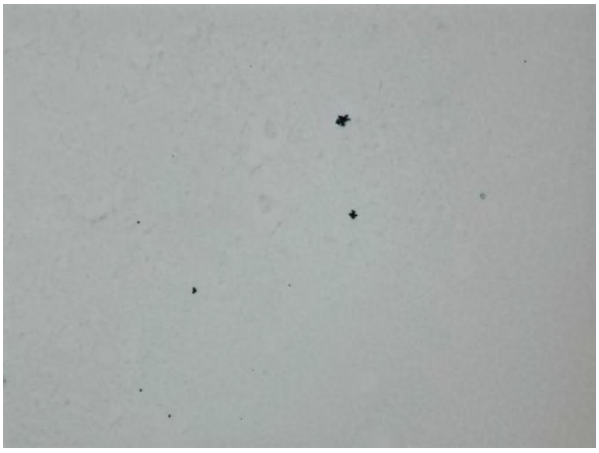
SYRINGE CONTROL **MICROAIRE (PAL)**

Donor 5

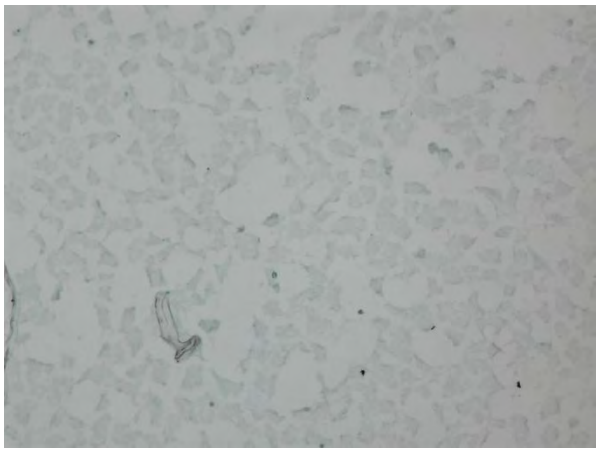


CD45

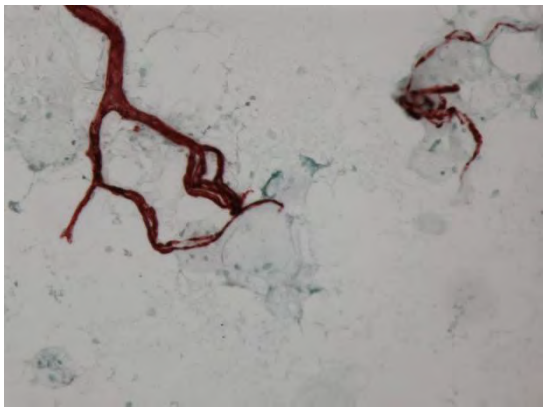
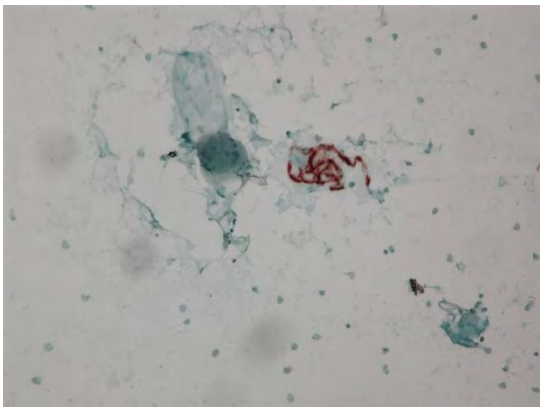
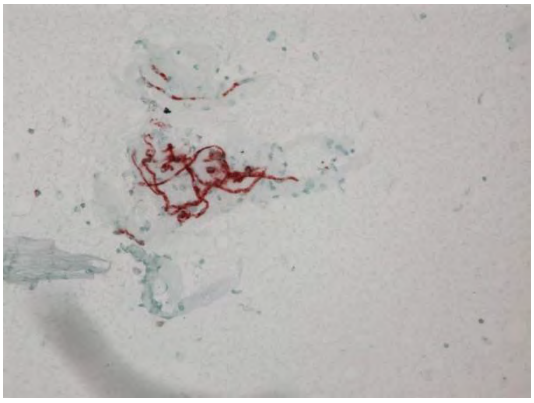
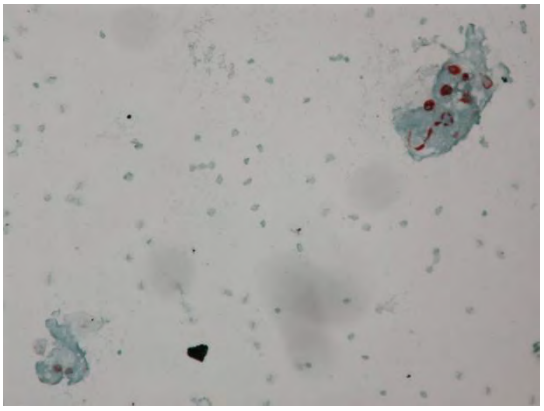




CD68



Appendix E. Representative Immunostaining of Loosely Adherent Cells (cont.)

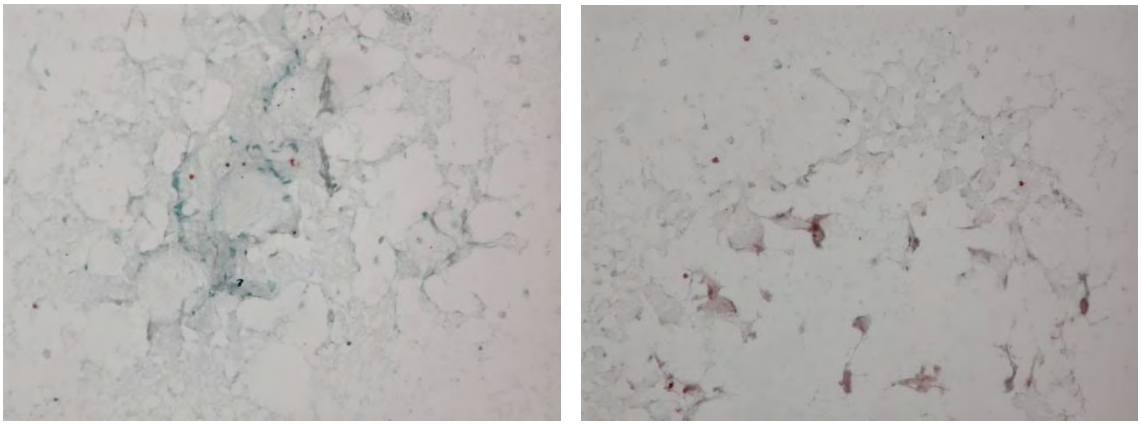
	SYRINGE CONTROL	**MICROAIRE (PAL)**
Donor 6		
CD31		
CD34		

Appendix E. Representative Immunostaining of Loosely Adherent Cells (cont.)

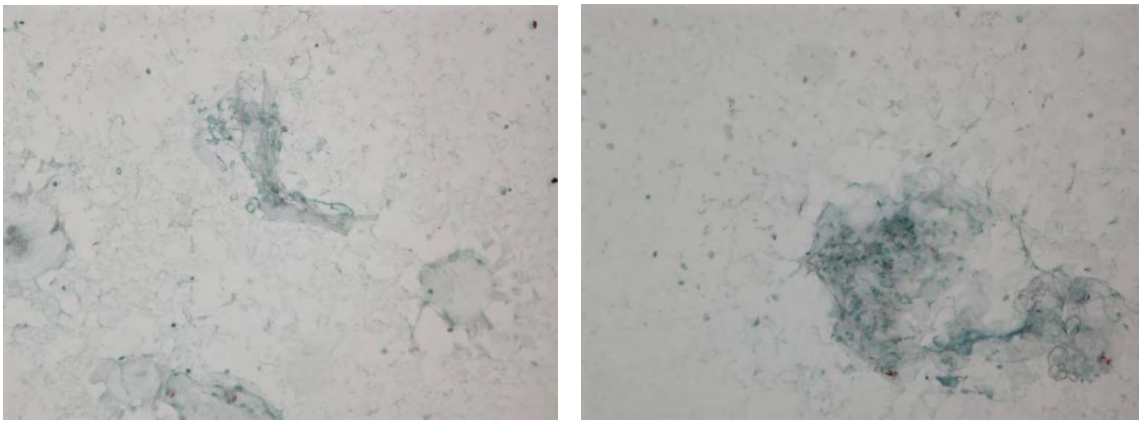
SYRINGE CONTROL **MICROAIRE (PAL)**

Donor 6

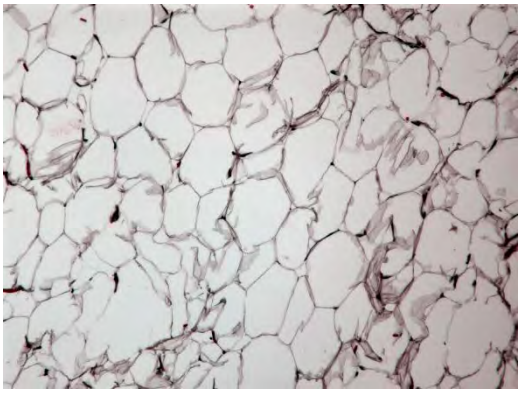
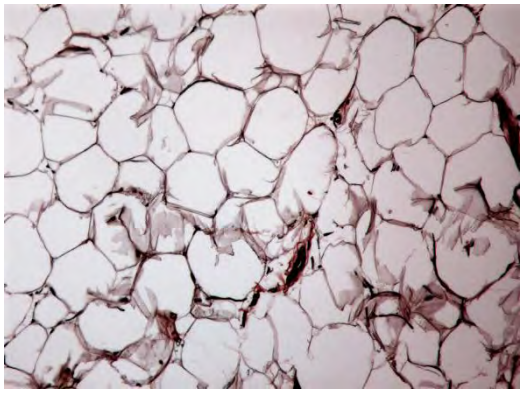
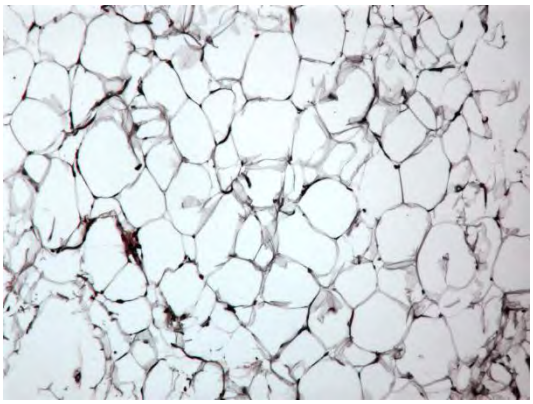
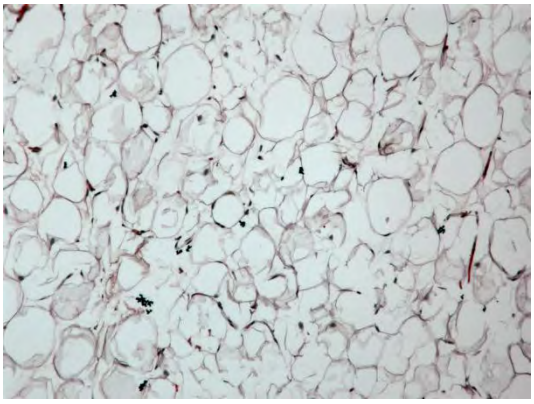
CD45



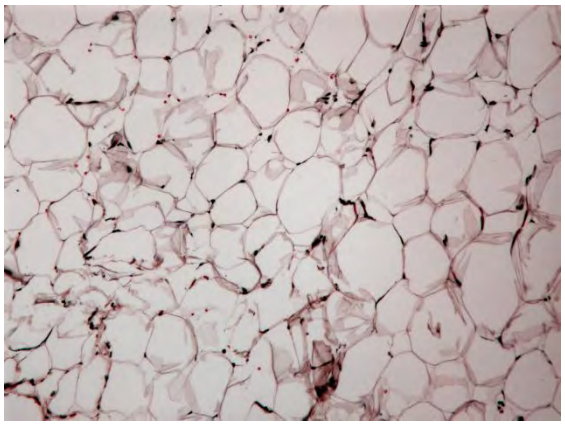
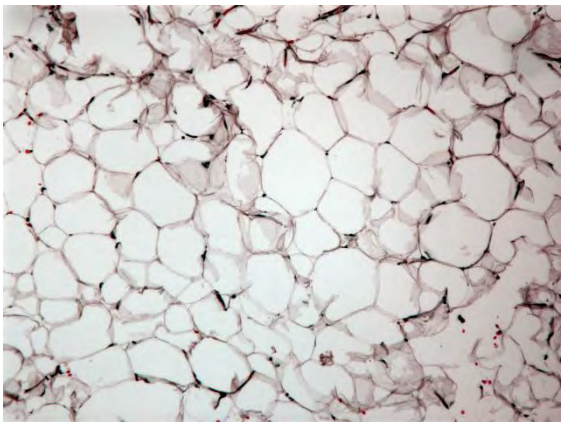
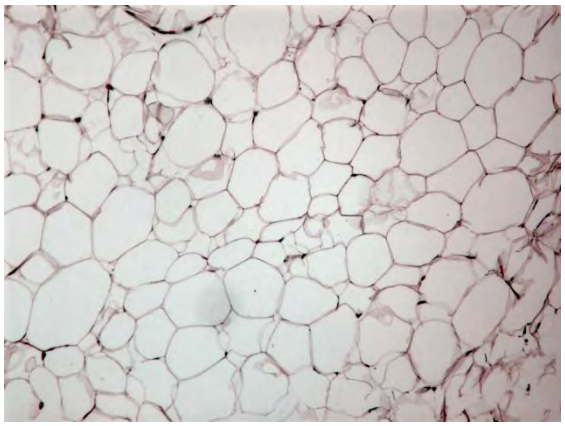
CD68



Appendix F. Representative Histology Images of Adipose from PAL and Syringe

SYRINGE CONTROL	MICROAIRE (PAL)
Donor 1	
	
Donor 2	
	

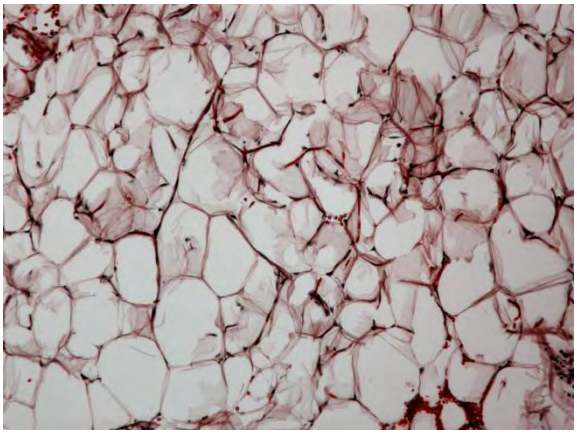
Appendix F. Representative Histology Images of Adipose from PAL and Syringe Cont.

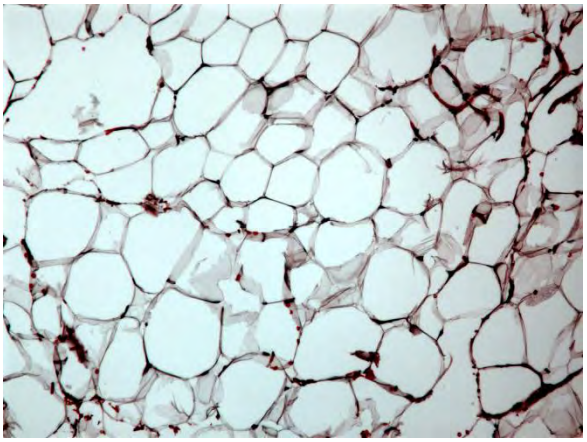
SYRINGE CONTROL	MICROAIRE (PAL)
Donor 3	
Not Captured	
Donor 4	
	

Appendix F. Representative Histology Images of Adipose from PAL and Syringe Cont.

SYRINGE CONTROL **MICROAIRE (PAL)**

Donor 5





Donor 6