Characterization and visualization of the liposecretion process taking place during ceiling culture of human mature adipocytes

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ABSTRACT

Objective: To investigate and further characterize the process of mature adipocyte dedifferentiation. Our hypothesis was that dedifferentiation does not involve mitosis but rather a phenomenon of liposecretion. **Methods:** Mature adipocytes were isolated by collagenase digestion of human adipose tissue samples. Ceiling cultures were established using our six-well plate model. Cells were treated with cytosine β-D-arabinofuranoside (AraC) or Vincristine (VCR), two agents blocking cell division, and were compared to vehicle. Liposecretion events were visualized by time-lapse microscopy, with and without AraC in adipocytes transducted with a baculovirus. Microscopic analyses were performed after labeling phoshorylated histone 3 and cyclin B1 in ceiling cultures. **Results:** Treatment with AraC almost entirely prevented the formation of fibroblasts up to 12 days of ceiling culture. Similar results were obtained with VCR. The antimitotic effectiveness of the treatment was confirmed in fibroblast cultures from the adipose tissue stromal-vascular fraction by proliferation assays and colony forming unit experiments. Using time-lapse microscopy, we visualized liposecretion events in which a large lipid droplet was rapidly secreted from isolated mature adipocytes. The same phenomenon was observed with AraC. This was observed in conjunction with histone 3 phosphorylation and cyclin B1 segregation to the nucleus. **Conclusion:** Our results support the notion that dedifferentiation involves rapid secretion of the lipid droplet by the adipocytes with concomitant generation of fibroblast-like cells that subsequently proliferate to generate the dedifferentiated adipocyte population during ceiling culture. The presence of mitotic markers suggests that this process involves cell cycle progression, although cell division does not occur.

INTRODUCTION

Sugihara et al. first introduced the ceiling culture model in 1986 to study the biology of mature adipocytes. Using this culture system, they observed important morphological changes as unilocular fat cells changed into fibroblast-like cells over the course of a few days, a process they have named dedifferentiation (Sugihara et al., 1986). Since the introduction of ceiling culture, several groups have used this model to generate dedifferentiated fat cells (Poloni et al., 2012; Wei et al., 2013; Yagi et al., 2004; Zhang et al., 2000). In most of these studies, dedifferentiation is described as the conversion of lipid-filled mature adipocytes to fibroblast-like cells, suggesting that the phenotype change occurs through a progressive loss of lipids, as seen during lipolysis, although the exact mechanism remains unclear.

In 2016, Maurizi et al., using time-lapse and electron microscopy experiments, suggested that mature adipocytes quickly release their lipid vacuole by a phenomenon termed liposecretion (Maurizi et al., 2017). According to that study, post-liposecretion fibroblast-like cells are characterized by a central elongated nucleus and have well-developed organelles. Furthermore, transcriptome data showed that expression of genes involved in lipid transport were up-regulated in mature adipocytes undergoing dedifferentiation. The authors also observed liposecretion in explants of human subcutaneous adipose tissue (Maurizi et al., 2017). More recently, they investigated the molecular mechanisms potentially involved in the liposecretion phenomenon. Their results showed that the process is regulated by several autocrine and external signaling molecules (Maurizi et al., 2018). The objective of the present study was to further characterize and visualize the process of liposecretion in human mature adipocytes undergoing ceiling culture. Considering the uncertainty as to whether cell division occurs (Jumabay et al., 2014; Maurizi et

al., 2017), we tested the hypothesis that dedifferentiation of mature adipocytes does not involve cell division, but rather a phenomenon of liposecretion.

METHODS

2.1 Tissue sampling

Tissue specimens were obtained from the Biobank of the *Institut universitaire de cardiologie et de pneumologie de Québec - Université Laval* according to institutionally-approved management modalities. All participants provided written, informed consent. The study was performed with adipose tissue samples from 26 participants (6 men, 20 women) scheduled for bariatric surgery. According to a modified version of the Rodbell method, adipose tissue was digested for 45 min with collagenase type I in Krebs-Ringer-Henseleit (KRH) buffer at 37°C (Rodbell, 1964). Adipocyte suspensions were filtered through nylon mesh and washed 3 times with KRH buffer. The residual KRH buffer, which contained the stromal-vascular fraction, was centrifuged (1,500 rpm, 5 min) and the pellet was washed in DMEM-F12 culture medium supplemented with 10% calf serum, 2.5 µg/mL amphotericin B, and 50 µg/mL gentamicin. To remove endothelial/mesothelial cells, the stromal-vascular cells were then filtered through 140 µm nylon mesh and then placed in culture plates and cultured at 37°C under a 5% CO_2 atmosphere. Medium was changed every 2-3 days. The isolated mature adipocytes were used for ceiling culture.

2.2 Ceiling culture

To dedifferentiate mature adipocytes, we used a ceiling culture model in six-well plates that was developed in our laboratory (**Figure 1**). In this model (Côté et al., 2017b), a glass slide is placed

at the bottom of each well and eight ml of DMEM/F12- 20% calf serum is added to each well containing a 1⁄2" plastic bushing. Then, a second glass slide is put on top of the bushing and 50,000 mature adipocytes are seeded under each coverslip. The cells then float and adhere to the slides and undergo dedifferentiation (Lessard et al., 2015). The use of smaller number of cells compared to the original ceiling culture method which was performed in reversed flasks allows testing of various time points and culture conditions. To examine the cells during the early events of dedifferentiation, cell adherence was improved by pre-incubating the top glass slides in a 20 mg/L poly-L-lysine solution for at least 24 hours.

2.3 Cell-cycle-arrest experiments

Cytosine β-D-arabinofuranoside (Cytarabine, AraC) was used as a selective inhibitor of DNA synthesis, causing cell cycle arrest in the G_2 phase (Azuma et al., 2001). Mature adipocytes in ceiling culture were seeded at a constant density of 50,000 cells in each condition with 5µM or 10 µM of AraC from day 0 to day 4, 7 or 12. Cells were also incubated with 1-5 µM of Vincristine (22-Oxovincaleukoblastine, VCR), a mitosis inhibitor blocking cells in the M phase through interaction with tubulin (Prakash and Timasheff, 1991). After treatment, the glass slides with adherent cells were transferred into a new culture plate. Cells were fixed in 10% formalin for at least 1 hour and washed three times with PBS. Images of the cells were acquired using the Olympus motorized inverted research microscope IW81 (Olympus Corporation, Tokyo, Japan). Images of control and treated cells were taken at x4 magnification using phase contrast microscopy on days 4, 7 and 12 of the dedifferentiation process. The surface of fibroblastic cells was measured by image analysis with the ImageJ software. Fibroblasts were counterstained in three images chosen randomly for each condition (control and AraC) from three patients. The surface occupied by fibroblasts was expressed as a percentage of the total field surface. The anti-

proliferative activity of AraC and VCR was tested on primary stromal cells in standard culture conditions to rule out cytotoxic effects. Cytotoxicity of the cell cycle arrest agents was also tested with the ToxyLight[TM] nondestructive bioassay kit (Lonza, Walkersville, Inc). Luminescence was measured in the culture medium after 24 hour treatments (AraC 5µM and VCR 2µM) with the Gen 5 plate reader (Biotek). Each condition was tested in duplicate from three patients. Data were normalized on the amount of proteins measured by Pierce[TM] BCA protein Assay Kit (Thermo Scientific). Similarly, the efficiency of the inhibitors was tested using colony forming unit (CFU) experiments and proliferation assays using the stroma-vascular fraction of adipose tissue. For the CFU, cells were seeded at 100 cells/ cm^2 in a 6-well plate and cultivated for 14 days. Cells were then fixed and stained with crystal violet 1%. Pictures of each condition were captured using the Zeiss Axio Observer Z1 microscope (Carl Zeiss, Oberkochen, Germany) and colony number was measured using Zen lite software. A colony was defined by a diameter of 2 mm consisting of at least 40 cells. Results are expressed as the total number of colonies in each condition (in duplicate). For proliferation assays, cells were seeded at 25% density in a 96-well plate. To allow cell adhesion, baseline was measured after 24 hours. Following treatment, cells were cultivated for 7 days. Measures were taken using the WST-1 Cell Proliferation Assay Kit (Abcam, Cambridge, United Kingdom). Results are expressed in fold over control.

2.4 Time-lapse microscopy experiments

Ceiling cultures of mature adipocytes with or without AraC were used to perform time-lapse microscopy experiments. Live images of the mature cells incubated at 37 °C in a humidified atmosphere containing 5% CO_2 were captured using the Zeiss Axio Observer Z1 microscope (Carl Zeiss, Oberkochen, Germany). Images were taken every 30 minutes in different Z positions with a 10x LD-A-Plan objective in phase contrast. We performed two independent experiments,

the first one starting at day 0 of the process (day 0 refers to mature adipocytes) up to day 8 and the second experiment starting at day 4 of the process up to day 11. Time-lapse image analysis was performed with ZEN 2 Blue Edition Digital Imaging Software.

2.5 Transduction experiments

To visualize the nuclei in time-lapse experiments, cells were treated with the CellLight® Histone 2B-GFP reagent (Molecular Probes, Life Technologies) using BacMam 2.0 technology. Prior to plating, they were incubated in a microtube with the BacMam 2.0 reagent at 37°C for 15 minutes. Afterward they were plated and incubated overnight (about 16 hours) in the culture condition described previously. The cells were visualized using the Zeiss Axio Observer Z1 microscope (Carl Zeiss, Oberkochen, Germany). Time-lapse images were taken with an Axiocam 506 every 30 minutes for approximately 5 days in different Z positions with a 10x LD-A-Plan objective for TL Phase and GFP.

2.6 Immunofluorescence experiments

For immunofluorescence experiments, cells were fixed with 10% formalin for at least 1 hour. Cells were permeabilized with 0.1% triton X-100 and blocked with 0.3% bovine serum albumin (BSA) in PBS 1X. Due to a high level of auto-fluorescence, mature adipocytes were treated twice, for 15 min, with a quenching solution of ammonium chloride (50 mM) after fixation. Cells were washed twice in PBS and incubated with various primary antibodies overnight at 4°C. Two different markers of cell mitosis were labeled. The first immunostaining experiment was performed with the HSC Mitotic index kit/Phospho-Histone 3 rabbit polyclonal antibody (1:1000, Molecular Probes, Invitrogen) on cells treated with 0.1 µM of Nocodazole. Another experiment was performed with the Cyclin B1 Rabbit polyclonal antibody (1:100, ThermoFisher). For all the

immunostaining experiments, cells were incubated for 1 hour with AlexaFluor 488 goat anti-rabbit IgG (H+L) as a secondary antibody (1:1000, Molecular Probes, Invitrogen). Nuclei were stained with 4',6-diamino-2-phenylindole (DAPI) and incubated with the second antibody for 1 hour (1:1000, Molecular Probes, Invitrogen). A negative control was generated for each experiment using the same conditions, but without the primary antibody. Coverslips were placed on a microscope slide with 0.12 micron secure-seal spacer with glycine mounting medium and sealed with nail polish. Digital images were captured using Zeiss Axio observer Z1 inverted microscope (Carl Zeiss, Oberkochen, Germany) with an Axiocam 506 using a Plan-Apochromat 20x objective for imaging of transmitted light differential interference contrast (TL DIC), DAPI and AlexaFluor 488. Confocal images were taken on a LSM800 confocal system (Zeiss) to visualize DAPI and AlexaFluor 488 staining, as well as transmitted light images using the ESID (electronically switchable illumination and detection module) detector. Images were analyzed using the Zen lite 2.3 Digital Imaging software.

2.7 Statistical analyses

Repeated measures ANOVA was performed with the JMP statistical software (SAS Institute, Cary, NC) to determine the effect of culture condition (Control vs. AraC vs. VCR), culture time (days 4, 7 and 12) and the culture condition * time interaction.

RESULTS

We first examined whether cell division was necessary for the dedifferentiation process to take place in ceiling culture. We incubated mature adipocytes with 5 µM AraC, or vehicle and found that over 12 days of culture, the generation of fibroblast-like cells was almost completely prevented with the treatment (**Figure 2A**). In control conditions, the surface occupied by

fibroblast-like cells increased exponentially over the 12 days of culture, but this was completely blunted in the presence of 5 µM AraC (**Figure 2B**). The condition * time interaction was highly significant (**Figure 2C**). Similar results were also obtained using 10 µM AraC (data not shown). VCR (1 µM) was also tested and prevented the generation of fibroblast-like cells over 11 days of culture compared to vehicle conditions (**Figure 3**). The effects of AraC and VCR on cell division were also tested in fibroblast monolayer cultures established from the stromal-vascular fraction of adipose tissue. As shown in **Figure 4A**, the same doses of both agents did not decrease the number of living cells over time but effectively prevented cell division in this population. Bioluminescence analysis of cytolysis through release of adenylate kinase from damaged cells was not increased by the treatments relative to controls in three duplicate cultures (not shown). In **Figure 4B,** the left panel shows results of the proliferation assays. As illustrated, AraC and VCR successfully inhibited cell proliferation over 7 days. The right panel of the same figure shows CFU results. Data show colony formation in the control condition, whereas no colonies were observed in the treated conditions. **Figure 4C** shows a representative colony forming unit experiment. In the absence of AraC and VCR the presence of multiple colonies can be seen. This is not observed in the treated conditions, where only a few isolated cells were found.

Markers of cell division were examined in mature adipocytes in ceiling culture. Cells were labeled with an antibody recognizing histone 3 phosphorylation on serine 10 (Phospho-H3). To increase the number of cells arrested in the G_2/M phase, cells were incubated in medium containing 0.1 µM Nocodazole. **Figure 5A** shows positive Phospho-H3 staining co-localizing with the cell nucleus in a mature cell undergoing ceiling culture. This image is representative of several others showing positive Phospho-H3 staining in cells that had a circular/spherical shape. **Figure 5B** shows Phospho-H3 labeling co-localizing with the cell nucleus in an adipocyte with

the elongated shape typically observed in ceiling culture. In **Figure 5C**, positive Phospho-H3 labeling co-localizing to the nucleus in a mature adipocyte was observed. Mature adipocytes in ceiling culture were also labeled for Cyclin B1. As shown in **Figure 6A** labeling of Cyclin B1 in mature adipocytes undergoing ceiling culture revealed slight immunoreactivity in the cytoplasm, but most of the labeling co-localized in the nucleus in a mature cell with elongated shape. **Figure 6B** shows confocal images of Cyclin B1 labeling in a mature adipocyte colocalized with DAPI staining in the nucleus.

With labeling experiments apparently pointing toward a cell division process, time-lapse microscopy experiments were performed in dedifferentiating mature adipocytes. As illustrated in the still pictures from time-lapse microscopy movie files in **Figure 7**, one event showed morphological changes that take place during ceiling culture. **Figure 7A** shows the elongation of round mature adipocytes, a shape typically found in the ceiling culture model (Lessard et al., 2015). Another event involved minimal elongation of the mature cell throughout the process as shown in **Figure 7B**. Movie files are available in the **Supplemental material**.

Time-lapse microscopy experiments were performed with mature adipocytes that were transfected with a baculovirus targeting the histones 2B in the nuclei. The sequential images derived from the baculovirus transduction time-lapse microscopy experiments are shown in **Figure 8**. **Figure 8A and 8B** show dedifferentiation events occurring between 66 and 96 hours and between 74 and 101 hours of culture respectively. We clearly observed secretion of a large lipid droplet from single-nucleus adipocytes in the absence of any cell division event. This was the case for all of our experiments (not shown). An elongated nucleus was observed in some (**Figure 8A**) but not all (**Figure 8B**) experiments. Movie files are available in **Supplemental**

material. As shown in **Figure 8C,** a mature adipocyte becomes a fibroblast-like cell retaining the nucleus after 110 hours in ceiling culture. The secreted lipid droplet did not stain for histone 2B and therefore, did not contain a nucleus, which ruled out cell division.

The dedifferentiation of mature adipocytes in ceiling culture in the presence of AraC can be observed in **Figure 9**. Secretion of the lipid droplet is visible from single-nucleus adipocytes. As in Figure 8, no cell division is observed. The liposecretion process still occurs in the presence of the inhibitor. The movie file is available in **Supplemental material**

DISCUSSION

The aim of the present study was to provide novel information on the cellular process taking place in mature adipocytes undergoing ceiling culture. Due to reports either suggesting a phenomenon of liposecretion (Maurizi et al., 2017) or asymmetrical cell division (Jumabay et al., 2014), we tested the hypothesis that dedifferentiation of mature adipocytes does not involve cell division, but rather a phenomenon of liposecretion. As expected, we found that fibroblast division is essential to obtain a confluent culture of dedifferentiated cells in ceiling cultures. Regarding mature adipocytes, the presence of mitosis markers including labeling of phosphorylated histone 3 and segregation of cyclin B1 to the nucleus initially pointed toward a cell division event. However, time-lapse microscopy experiments with fluorescent labeling of histone 2B allowed ruling out cell division of mature adipocytes during dedifferentiation, and provided support to the liposecretion phenomenon initially described by Maurizi et al. (Maurizi et al., 2017).

Our experiments show clear labeling of proteins associated with mitosis during ceiling culture,

which was initially consistent with a cell division process taking place in human mature adipocytes. Consistent with these findings, we have previously shown that the expression of many genes coding for proteins implicated in the cell cycle increased in both the early (e.g. CCNG1, cyclin G1) and the late (e.g. CCNB1, cyclin B1) stages of dedifferentiation (Côté et al., 2017a). The same study indicated downregulation of CEBPA and other adipocyte-functioning genes (e.g. ADIPOQ), occurring simultaneously to an activation of genes related to cell cycle and stem cell renewal (WNT5A, FGF5, FGF7, MAP2K4) (Côté et al., 2017a). Recently, a possible role of the WNT5A in adipocyte dedifferentiation has been put forward (Zoico et al., 2016). By using a co-culture model of A375 melanoma cells and 3T3-L1 adipocytes, it has been found that after a few days of co-culture, adipocytes were transformed into fibroblastic-like cells. Co-culture increased melanoma migration, while the addition of an inhibitor of the WNT5A pathway to the culture model, reduced cell migration (Zoico et al., 2016). As the FGF and WNT pathways have been proposed to interact with each other (Ornitz and Itoh, 2015), they are possibly involved in adipocyte reprogramming and dedifferentiation.

Despite of positive cell cycle markers, our other results suggest that dedifferentiation does not involve cell division. Phosphorylation of serine 10 on histone 3 is involved in chromatin condensation and peaks during mitosis (Goto et al., 1999; McIntosh, 2016). Its presence is first evident in the late G2 phase of the cycle and is complete during prophase (Rhind and Russell, 2012). Cyclin B1 accumulates in the cytoplasm during the G2 phase and migrates in the nucleus for degradation as an early event of mitosis (Pines and Hunter, 1991). Thus, cell cycle changes appear to advance at least up to the G2/M transition or early mitotic phase. The G2/M transition is the habitual consequence of cell cycle initiation at the G1/S transition, and examples of cells arresting in the G2 phase are rare (Rhind and Russell, 2012). The presence of phospho-H3 and

nuclear cyclin B1 labeling in our cells suggests that mature adipocytes in ceiling cultures may represent one such example. The precise kinetics of the cell cycle changes relative to the release of the lipid droplet could not be directly quantified in the present study. Additional work is needed to reconcile the presence of mitosis markers and the absence of cell division in mature adipocytes undergoing ceiling culture.

Because mature adipocytes convert to fibroblast-like cells following rapid secretion of their lipid droplet, a progressive transformation of mature adipocytes into a different cell phenotype with gradual lipid degradation and release through lipolysis can also be ruled out. Regarding the fate of the secreted lipid droplet, it is unlikely that it has the machinery to undergo subsequent cell division. We hypothesize that the droplets remain in the medium and eventually aggregate at the time of culture reversal, consistent with the frequently observed lipid layer floating on the medium at the end of the culture process.

Our experiments provide little information on the frequency of the liposecretion phenomenon and whether it takes place *in vivo*. Previous observation of isolated lipid droplets in human tissue explants by electron microscopy indirectly suggested that liposecretion may, indeed, occur in whole tissue samples (Maurizi et al., 2017). Further studies are needed to confirm this notion. Regarding the occurrence rates in ceiling culture, our observations of large microscope fields in real-time suggest that liposecretion is not a highly frequent phenomenon. Only a few events can be observed in each experiment and the timing of the liposecretion phenomenon is slightly variable between experiments (see **Figure 7** vs. **Figure 8A** vs. **Figure 9**). To date, the reasons explaining such heterogeneity are unknown and future research is needed before attempting to address this issue. Despite liposecretion rarity, confluence of the fibroblast-like cells is usually

reached after 12-15 days of ceiling cultures (Lessard et al., 2015). This implies that proliferation of a few cells is required. Consistent with this observation, our cell cycle arrest experiments demonstrated that confluence cannot be reached in conditions where cell proliferation is inhibited. This was not due to a cytotoxic effect. Hence, we propose that proliferation of a small number of fibroblast-like cells resulting from the liposecretion process is an absolute requirement to generate a sizeable population for tissue engineering purposes. Moreover, time-lapse experiments of cells treated with AraC were performed. We found that the liposecretion process still occurs in the presence of this inhibitor blocking cells in the G_2 phase. This suggests that progression to the mitotic phase is not required for the phenomenon of liposecretion. This is supported by the fact that morphological nucleus changes reflecting segregation were not observed.

In conclusion, despite the presence of mitosis markers and even nucleus elongation in some experiments, we report that the process of mature adipocyte dedifferentiation does not involve cell division. It rather involves liposecretion of a large lipid droplet by the adipocytes with concomitant generation of fibroblast-like cells that subsequently proliferate to generate the dedifferentiated adipocyte population.

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FIGURE HEADINGS

Figure 1: Representation of the ceiling culture approach in six-well plates. Only one well is represented.

Figure 2: **(A)** Mature adipocytes isolated by collagenase digestion and cultivated in ceiling culture with control vehicle or 5µM cytosine β-D-arabinofuranoside (AraC). Cells were incubated with vehicle or AraC from day 0 to day 4, 7 or 12 as indicated. Cells were fixed in 10% formalin at least 1 hour on specific days (4, 7 or 12 as indicated) and washed with PBS. All images were obtained in phase contrast, inverted microcopy at 4x magnification. **(B)** Representative image analysis for the quantification of the field surface occupied by fibroblasts. **(C)** Quantification of the field surface occupied by fibroblasts measured from 3 randomly selected images from 3 independent cultures treated with 5µM AraC or vehicle. Data are express as mean ± SEM.

Figure 3: Mature adipocytes isolated by collagenase digestion and cultivated in ceiling culture with control vehicle (CTL) or with 1 µM Vincristine (VCR) from day 0 to day 4, 7 or 11 as indicated. Cells were fixed in 10% formalin for at least 1 hour on day 4, 7 or 11 as indicated and washed with PBS. All images were obtained in phase contrast, inverted microscopy at 4x magnification.

Figure 4: **(A)** Fibroblasts from the stromal-vascular fraction of adipose tissue. Control vehicle, 5 µM AraC treatment and 2 µM VCR treatment. All images were obtained by phase contrast, inverted microscopy at 4x magnification. Images are representative of n=3 experiments.

(B) The left panel shows results of the proliferation assay. Data are expressed by mean ± SD

(n=4) of fold over control. *P<0.01. The right panel shows results of the colony forming unit experiments. Results are expressed by mean number of colonies ± SD (n=3). Non detectable (n.d.) colonies were observed in the AraC- and VCR-treated conditions.

(C) Representative images of the CFU experiments (n=3).

Figure 5: Fluorescence microscopy labeling of phosphorylated histone 3 (Phospho-H3) in mature adipocytes undergoing ceiling culture and treated with 0.1 µM of Nocodazole. Images show transmitted light differential interference contrast images (TL DIC), DAPI staining (blue fluorescence) and Phospho-H3 staining (green fluorescence) in mature adipocytes undergoing ceiling cultures for **(A)** 6 days; **(B)** 7 days; and **(C)** 10 days. Images obtained at 20x magnification. **(D)** Negative control

Figure 6: Fluorescence microscopy **(A)** labeling of Cyclin B1 in mature adipocytes undergoing ceiling culture. Images show transmitted light differential interference contrast images (TL DIC), DAPI staining (blue fluorescence) and Cyclin B1 staining (green fluorescence) in mature adipocytes undergoing ceiling cultures for 6 days. Confocal microscopy **(B)** negative control **(C)** of Cyclin B1 co-localized with the nucleus of a mature adipocyte undergoing ceiling culture for 7 days. Images show ESID, DAPI (blue fluorescence) and Cyclin B1 (green fluorescence). Both images were obtained at 20x magnification.

Figure 7: Sequential images derived from two time-lapse microscopy experiments. White asterisks show the liposecretion event **(A)** In experiment 1, morphological changes are shown from 120h to 126h of ceiling culture. **(B)** In experiment 2, morphological changes are shown from 48h to 70h of ceiling culture. Movie files are available in **Supplemental material**. Experiments visualized at 10x magnification.
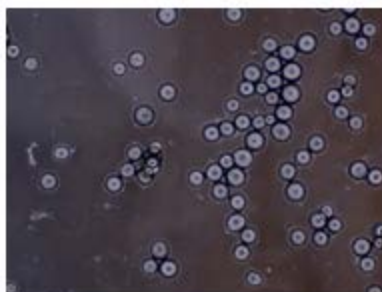
Figure 8: Sequential images derived from two baculovirus transduction time-lapse microscopy experiments. Adipocyte nucleus is visualized by green fluorescence. The event of liposecretion is indicated by the white asterisk. **(A)** Liposecretion event shown from 66h to 96h of ceiling culture post transduction. **(B)** Liposecretion event shown from 74h to 101h of ceiling culture post transduction. Image **(C)** shows fibroblast-like cell derived from a mature adipocyte after 110h of ceiling culture. Movie files are available in **Supplemental material**. Experiments were visualized at 10x magnification.

Figure 9: Sequential images derived from a baculovirus transduction time-lapse microscopy experiment. Adipocyte nucleus is visualized by green fluorescence. The event of liposecretion is indicated by the white asterisk. **(A)** Liposecretion event shown form 73.5h to 133.5h of ceiling culture with AraC 5µM post-transduction. The movie file is available in **Supplemental material.** Cells were visualized at 10x magnification.

Top coverslip treated with poly-L-Lysine

Plastic bushing

Bottom glass slide

6-Well filled with DMEM-20% Calf Serum

Top coverslip is reversed

Days 4,7,12

Fixation with 10% formalin (if needed)

Immunostaining

Observations
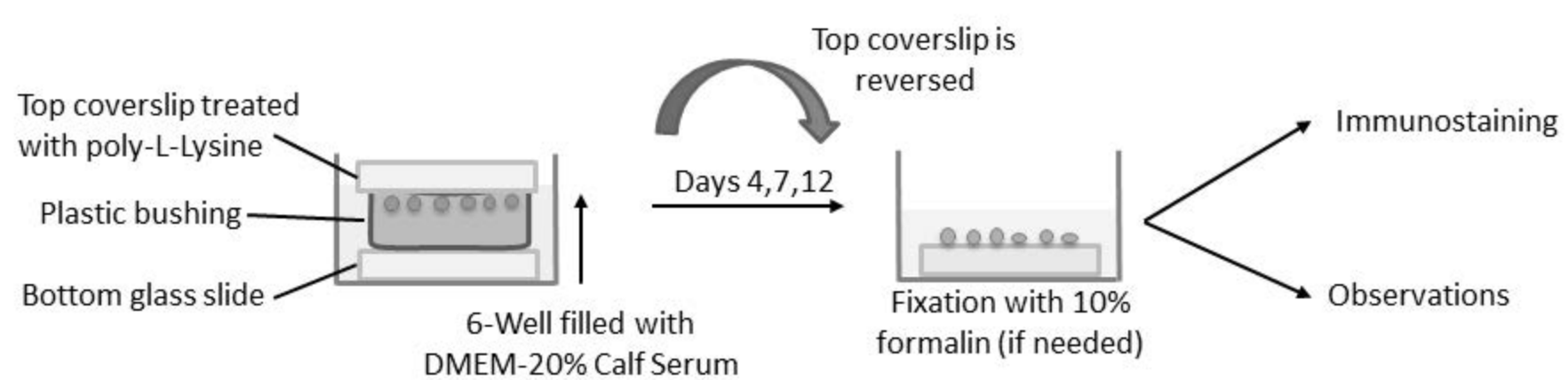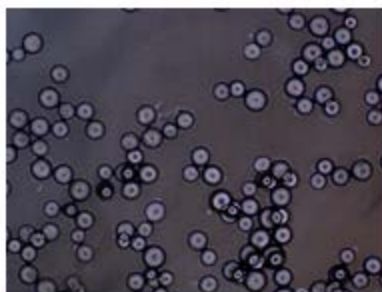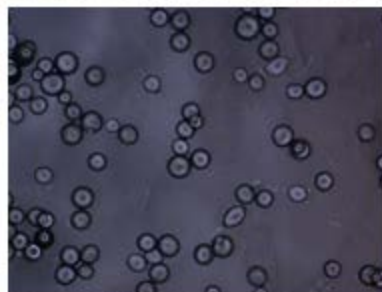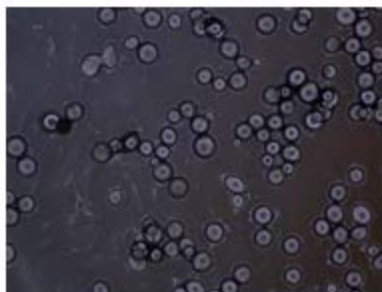
	Control	AraC
Day 4		
Day 7		
Day 12		
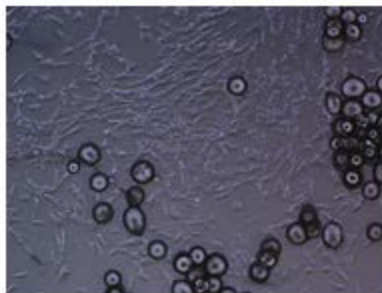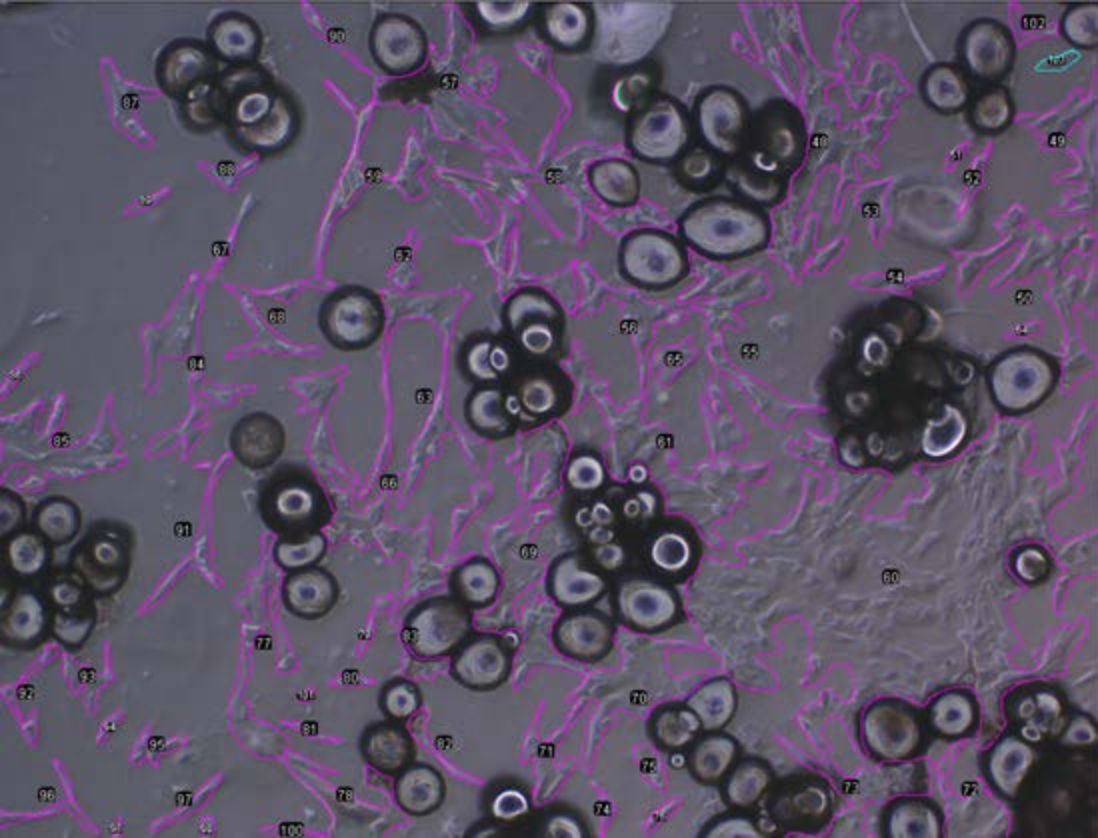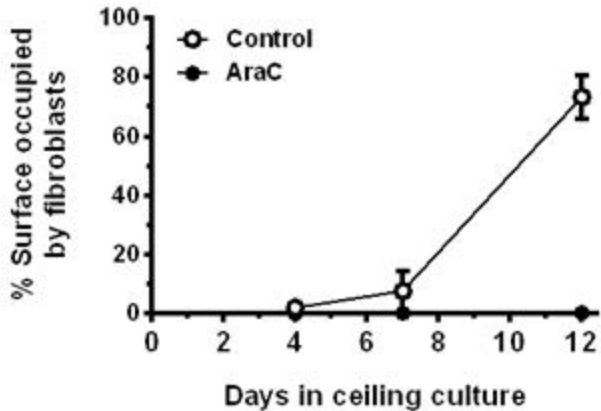
Repeated Measures ANOVA
Control vs. AraC: p<0.0001
Time: p<0.0001
Time*Conditon: p<0.0001
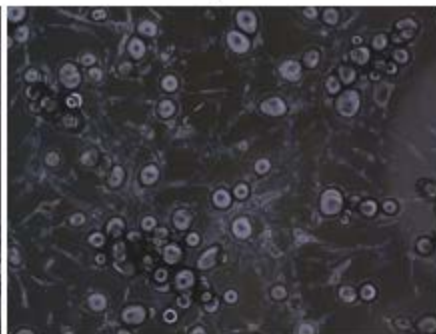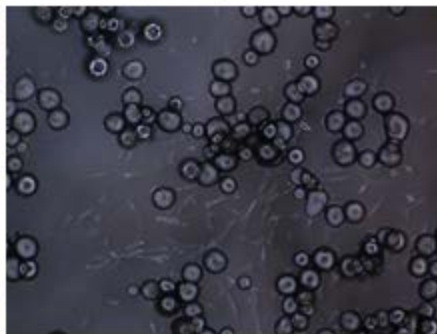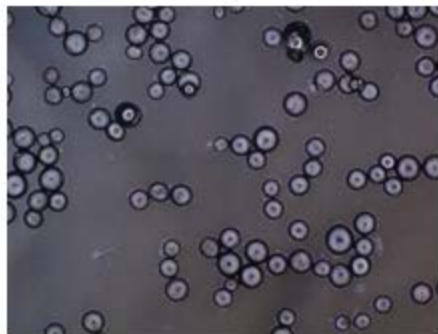
	Day 4	Day 7	Day 11
CTL			
VCR			
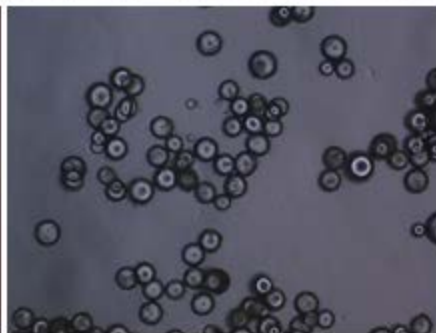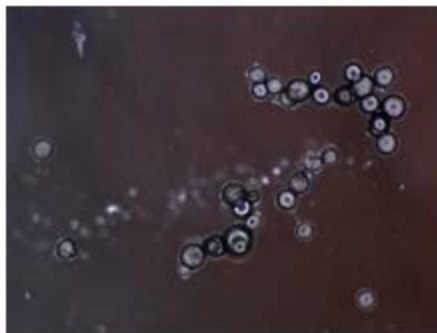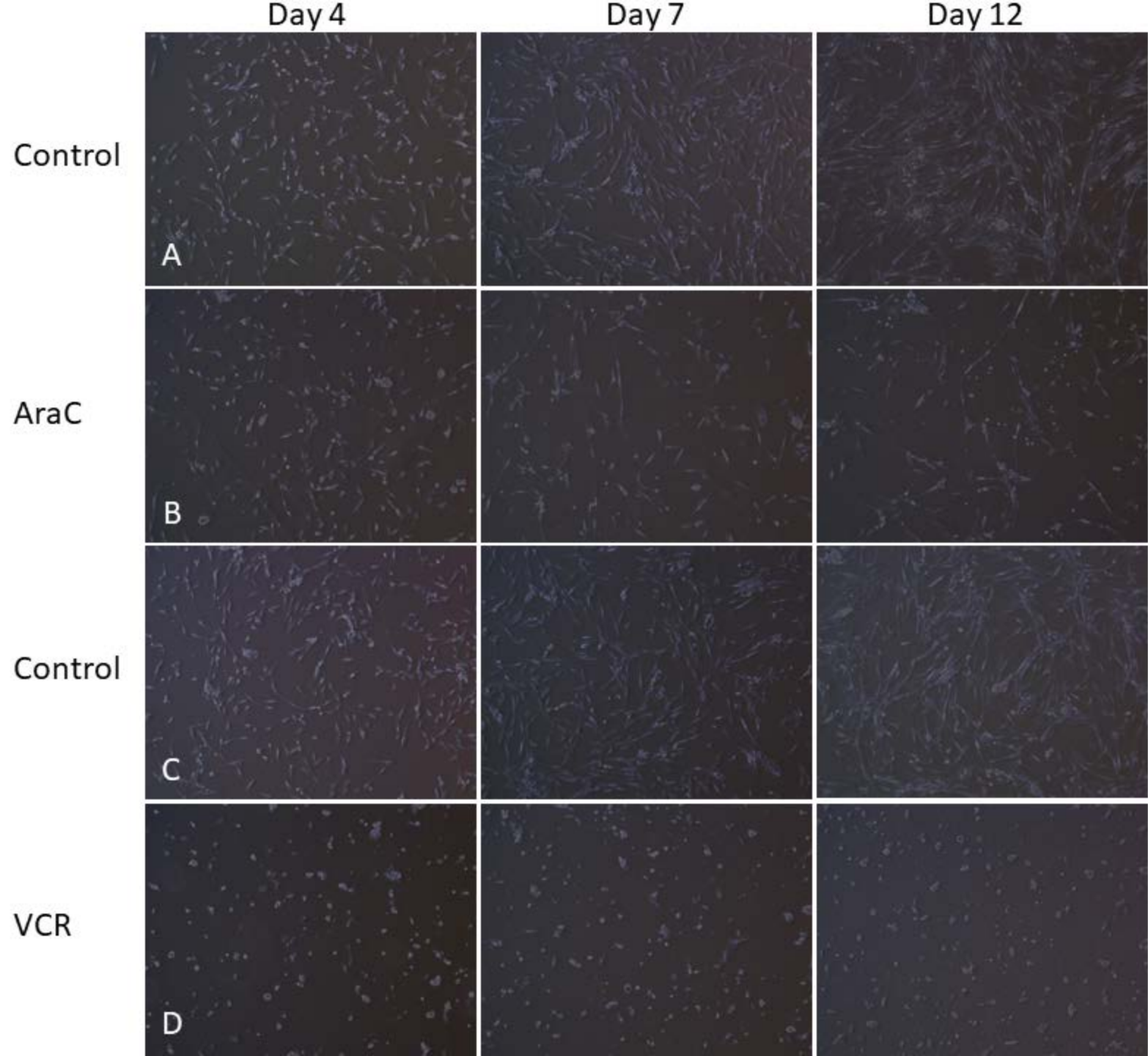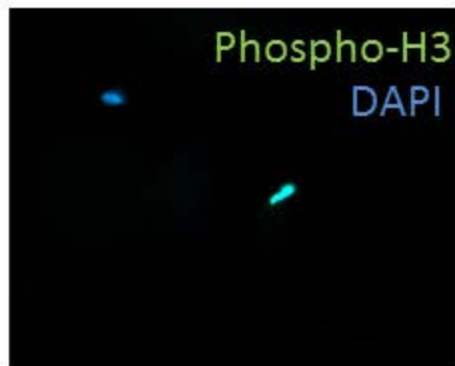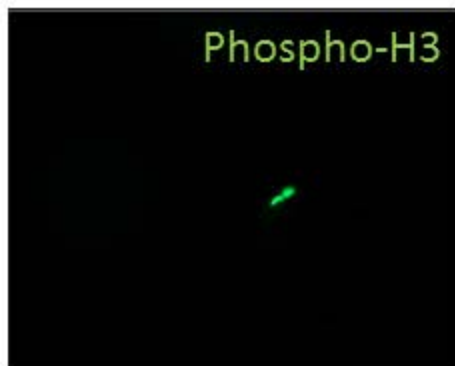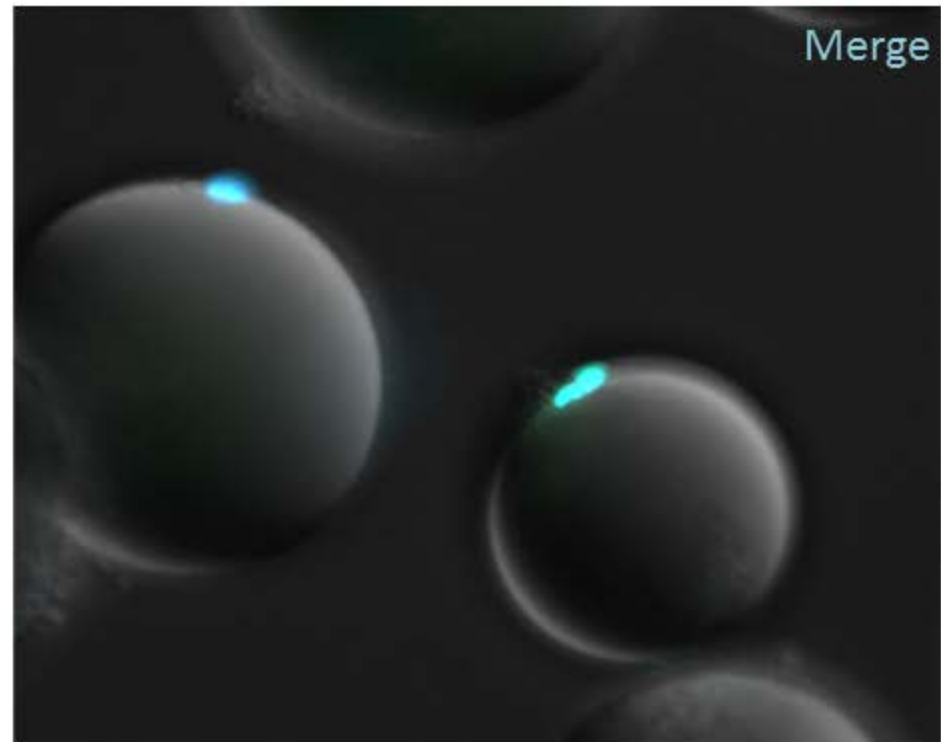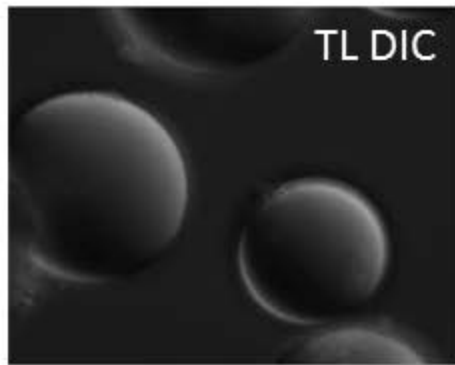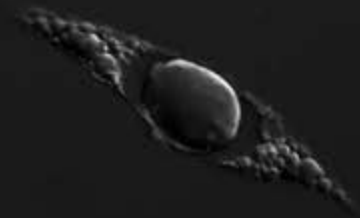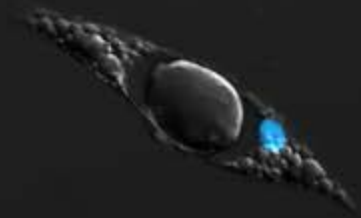
TL DIC | DAPI | Phospho-H3 | Merge
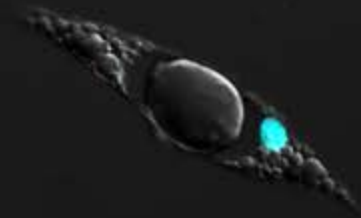
TL DIC	DAPI	Merge
Phospho-H3	Phospho-H3 DAPI	

TL DIC

DAPI

Cyclin B1

DAPI
Cyclin B1

Merge

4th d (96h)

110h → 116h

117h → 122h

123h → 128h

129h → 134h

Lipid droplet

Fibroblast

Nucleus