Role of the TGF-β pathway in dedifferentiation of human mature adipocytes

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ABSTRACT

Dedifferentiation of adipocytes contributes to the generation of a proliferative cell population that could be useful in cellular therapy or tissue engineering. Adipocytes can dedifferentiate into precursor cells to acquire a fibroblast-like phenotype using ceiling culture, in which the buoyancy of fat cells is exploited to allow them to adhere to the inner surface of a container. Ceiling culture is usually performed in flasks, which limits the ability to test various culture conditions. Using a new 6-well plate ceiling culture approach, we examined the relevance of TGF-β signaling during dedifferentiation. Adipose tissue samples from patients undergoing bariatric surgery were digested with collagenase and cell suspensions were used for ceiling cultures. Using the 6-well plate approach, cells were treated with SB431542 (an inhibitor of TGF-β receptor ALK5) or human TGF-β1 during dedifferentiation. Gene expression was measured in these cultures and in whole adipose tissue, the stromal-vascular fraction (SVF), mature adipocytes and dedifferentiated fat (DFAT) cells. TGF-β1 and collagen type I alpha 1 (COL1A1) gene expression was significantly higher in DFAT cells compared to whole adipose tissue samples and SVF cells. TGF-β1, COL1A1 and COL6A3 gene expression was significantly higher at day 12 of dedifferentiation compared to day 0. In the 6-well plate model, treatment with recombinant TGF-β1 or SB431542 respectively stimulated and inhibited the TGF-β pathway as shown by increased TGF-β1, TGF-β2, COL1A1 and COL6A3 gene expression and decreased expression of TGF-β1, COL1A1, COL1A2 and COL6A3, respectively. Treatment of DFAT cells with recombinant TGF-β1 increased the phosphorylation level of SMAD 2 and SMAD 3. Thus, a new 6-well plate model for ceiling culture allowed us to demonstrate a role for TGF-β in modulating collagen gene expression during dedifferentiation of mature adipocytes.

beta, collagens

Abbreviations: Subcutaneous (SC), collagen type I alpha 1 (COL1A1), collagen type I alpha 2 (COL1A2), collagen type 6 alpha 3 (COL6A3), dedifferentiated (DFAT), extracellular matrix (ECM), Krebs-Ringer-Henseleit (KRH), omental (OM), stromal-vascular fraction (SVF), transforming growth factor beta (TGF-β)

1. INTRODUCTION

Adipose tissue contains connective tissue matrix, preadipocytes, immune cells and mature adipocytes [1]. Mature adipocytes are specialized in lipid storage and are generated from the differentiation of mesenchymal stem cells committed to preadipocytes [2]. Matsumoto et al. demonstrated that adipocytes can dedifferentiate into precursor cells to acquire a fibroblast-like phenotype using ceiling culture [3]. This method is based on the buoyancy of adipocytes, which allows them to adhere to the top inner surface of a reversed culture flask that is completely filled with medium [4]. In 2000, Zhang et al. proposed another culture technique in which adipocytes adhere to the underside of a floating piece of glass [5]. More recently, Jumabay et al. obtained dedifferentiated (DFAT) cells from adipocytes using a method that did not require attachment of the cells to a plastic surface as opposed to ceiling culture. In this experimental model, the isolated adipocytes are incubated in the culture medium for 24h and then transferred to another dish containing a filter. After 5 days, the filter is removed and the DFAT cells sink through the filter to the bottom of the dish [6]. We have recently described a modified version of the ceiling culture approach in 6-well plates, allowing us to decrease the number of cells used and test a larger number of culture conditions [7].

TGF-β1 has often been described as an important regulator of adipocyte physiology because of its role in inhibiting adipogenesis [8-10]. This growth factor also plays a major role in adipose tissue remodelling through induction of ECM protein-coding genes such as collagens [11-13]. We have recently demonstrated that genes coding for proteins of the extracellular matrix including COL1A1, COL1A2, COL6A3 were significantly up regulated during the dedifferentiation process. In that analysis, we did not observe significant changes in gene expression of other types of collagen including COL4A3, COL5A1, COL5A2, COL6A1,

COL6A2, COL8A2, COL20A1 [14]. Moreover, we have previously shown that gene expression of matrix-metalloproteinase 1 (MMP1), fibroblast activated-protein (FAP), dipeptidyl peptidase IV (DPP4) and transforming growth factor β1 (TGF-β1) were strongly induced during dedifferentiation. Finally, recent data support a role for the TGF-β pathway in the dedifferentiation of human pancreatic islet β-cells *in vivo* [15].

Although many groups have used the ceiling culture approach, many aspects of this unique cellular process remain to be characterized. Our previous reports [7, 16] suggest that the TGF-β pathway may be involved in the dedifferentiation process and contribute to the generation of a proliferative cell population that could be useful in cellular therapy or tissue engineering. From the technical standpoint, previous culture systems limited our ability to test various incubation conditions during the process. To the best of our knowledge, no study has ever attempted to modulate the molecular, metabolic or secretory attributes of the cells during dedifferentiation. Our objective was to implement a new 6-well plate culture system and modulate the dedifferentiation process by targeting the TGF-β pathway and its effects on the expression of collagens. We hypothesized that the TGF-β pathway is a significant modulator of COL1A1, COL1A2 and COL6A3 gene expression during the dedifferentiation of mature adipocytes.

2. METHODS

2.1 Tissue sampling

We have complied with all mandatory laboratory health and safety procedures in the course of the experimental work presented in this paper. The project was approved by the Research Ethics Committee of the *Institut Universitaire de Cardiologie et de Pneumologie de Québec* (IUCPQ). Written informed consent was obtained from tissue donors prior to sampling through the management framework of the IUCPQ Obesity Tissue Bank. Adipose tissue samples were obtained from men and women undergoing bariatric surgery. Portions of adipose tissues were digested with collagenase type I in Krebs-Ringer-Henseleit (KRH) buffer for up to 45 minutes at 37°C according to a modified version of the Rodbell method [17]. Adipocyte suspensions were filtered through nylon mesh and washed 3 times with KRH buffer to obtain a purified population of mature adipocytes. The residual KRH buffer of adipocyte isolation, which contained the stromal-vascular fraction, was centrifuged 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. The stromal-vascular cell fraction was then filtered through 140 μm nylon mesh to remove endothelial/mesothelial cells, placed in culture plates and cultured at 37°C under a 5% CO_2 atmosphere. Medium was changed every 2-3 days. Isolated mature adipocytes were used for ceiling culture whereas the stromal vascular fraction was seeded in standard culture flasks containing DMEM-F12 culture medium supplemented with 2.5 % foetal bovine serum. In previous studies, we have demonstrated that adipocytes can successfully dedifferentiate independent of their fat depot origin (SC or OM). We also demonstrated that the dedifferentiation process is relatively independent of obesity level, sex or age of the cell donor [14, 16]. Consistent with these results, data from SC and OM samples were combined in our analyses.

2.2 Ceiling culture

When no treatment was used during the dedifferentiation process, isolated mature adipocytes were counted and 500 000 cells were added to a T-25 flask completely filled with DMEM-F12 culture medium supplemented with 20% calf serum, 2.5 µg/mL amphotericin B and 50 µg/mL gentamicin. Flasks were incubated upside down at 37°C, in 5% CO_2 for seven days. Cells floated up and adhered to the top inner ceiling surface of the flask. After 7 days, the medium was removed and the flasks were inverted so that the cells were on the bottom until day 12 in the same medium [7]. The medium was changed every 3 days. For gene expression analysis, cultures from each patient were harvested at days 4 and 7 of the dedifferentiation process as done previously [14]. One flask per depot per patient was reversed at day 7 and maintained in culture for an additional 5 days (corresponding to day 12). Time points were chosen based on our observations that harvesting cells at day 4 provides a population of round cells that has completely adhered to the flask while cells at day 7 begin to be elongated. All ceiling cultures performed in the laboratory were reversed at day 7. Day 12 represents a time point at which the majority of cells have a fibroblast-like morphology. When DFAT cells were needed for western blot experiments, cultures were maintained in standard condition for more than 12 days and subcultured when cells reached confluence. For other experiments, a ceiling culture model in 6-well plates was used [7]. To do so, 8 ml of DMEM/F12- 20% calf serum were added to each well containing a 1⁄2-inch plastic bushing. A glass slide was put on top of the bushing and mature adipocytes were seeded under each coverslip (**Figure 1**). These mature adipocytes floated and then adhered to the top slide. They were examined at specific time points during the dedifferentiation process. This model required a smaller number of cells and allowed us to use various media during the dedifferentiation process [7]. The drug was added to the media and

when appropriate, the glass slides with the adherent cells were flipped in a new well and then

harvested for RNA extraction.

2.3 RNA extraction and real-time quantitative RT-PCR

Total RNA was isolated from SVF cells, whole adipose tissue and DFAT cells from 5 donors

using the RNeasy lipid tissue extraction kit (Cat No./ID: 74804) and digested with Rnase-free

Dnase (Qiagen, Missisauga, ON, Canada) to remove all traces of DNA. RNA extraction was also

performed from dedifferentiation time course experiments at day 0 (freshly isolated adipocytes),

day 4 and day 7 of ceiling culture and at day 12 from 3 donors using the QIAGEN RNeasy

extraction kit (Cat No. 330503) and in treated DFAT cells. To assess RNA quantity and quality,

an Agilent Technologies 2100 bioanalyzer and RNA 6000 Nano LabChip kit (Agilent, Mountain

View, CA, USA) were used. For each sample, cDNA was obtained using the Quantitect reverse

transcriptase kit (Cat No 205311). The following sequences were used for quantitative PCR

(forward/reverse): ATP synthase, H+ transporting, mitochondrial F1 complex, O subunit

(ATP5O): 5'-AACGACTCCTTGGGTATTGCTTAA-3'/5'-

ATTGAAGGTCGCTATGCCACAG-3', Glucose-6-phosphate dehydrogenase (G6PD): 5'

GCAGGGCATTGAGGTTGGGAG-3'/5'-GATGTCCCCTGTCCCACCAACTCTG-3',

Transforming Growth Factor β1 (TGF-β1): 5'-AAG TTG GCA TGG TAGCCC TT-3'/5'-CCC

TGG ACA CCA ACT ATT GC-3', Transforming Growth Factor β2 (TGF-β2): 5'- CTC CAT

TGC TGA GAC GTC AA-3'/5'- ATA GAC ATG CCG CCC TTC TT-3', Transforming Growth

Factor β3 (TGF-β3): 5'- CAC ATT GAA GCG GAA AAC CT-3'/5'- AAA TTC GAC ATG

ATC CAG GG-3', collagen type one alpha one (COL1A1): 5'- CAC ACG TCT CGG TCA TGG

TA3'/5'- AAG AGG AAG GCC AAG TCG AG-3', collagen type one alpha two (COL1A2): 5'-

AGC AGG TCC TTG GAA ACC TT3'/5'- GAA AAG GAG TTG GAC TTG GC-3', collagen

type 6 alpha 3 (COL6A3): AAG TGC CGA TGT TTC CTC AT3'/5'-TAA TTG AAT CGA

GGA GCC CA-3'. Housekeeping gene expression (ATP5O and G6PD) was measured in each

sample. Results are expressed as ΔΔCt relative to housekeeping gene expression. Graph bars

represent mean values of ΔΔCt values and error bars are the standard error means (SEM). Only

G6PD-normalized results are shown but both housekeeping genes yielded similar results.

2.4 TGF-β1 recombinant treatment and TGF-β receptor 1 inhibitor

Mature adipocytes were counted so that 50 000 cells were seeded in 6-well plates for ceiling

culture in 20% serum. At day 4, slides with adherent adipocytes were reversed into a new plate

containing 2 ml of 5% serum in each well. At day 5, cells (n=7) were treated with 5 ng/ml

recombinant human TGF-β1 or vehicle (0.1% bovine serum albumin) for 24 h (Ref Cat 100-21)

or with 1 μM SB431542 (n=8) (Cat. No 1614), an inhibitor of the TGF-β receptor ALK5 or with

vehicle (dimethylsulfoxyde, DMSO 20mg/ml) for 24h. The cells were then harvested in phenol

buffer (Cat No./ID: 79306) for RNA extraction. Three replicates were cultivated for each

condition and pooled together at day 6 into phenol buffer for RNA extraction and RT-PCR

quantification.

2.5 Western Blotting and antibodies

Proteins were extracted from the organic phase of the RNA phenol-chloroform extraction. First,

100% ethanol was added to the organic phase and incubated for 5 minutes. After centrifugation

(4 500 rpm, 2 minutes, 4°C), the supernatant was incubated 10 minutes with 1.5 ml isopropanol.

The pellet was washed 3 times with 1.5ml ethanol- 0.3M guanidine with 20-minute incubations

and one additional wash without guanidine. The pellet was then incubated at 65°C in Tris pH

7.4–6% SDS until complete dissolution. Sonication was performed as a final disruption step.

Protein samples (30 μg) were run on a 10% SDS-PAGE and transferred to nitrocellulose

membrane. We used a human TGF-β1 antibody (RD System, Cat No. AB-246-NA) and the

Smad2/3 Antibody Sampler Kit (Cell Signaling Technology, Cat No. 12747). β-tubulin was used

as a loading control (Cell Signaling Technology, Cat No. 2146). Densitometric analyses were

performed using Image J software (NIH, Bethesda, MD, USA).

2.6 Statistical analyses

Statistical analyses were performed using JMP software (SAS Institute Inc, Cary, NC, USA).

Expression levels of transcripts were expressed as ΔΔCT relative to G6PD expression (mean

value ± SEM). Comparison of gene expression between the SVF fraction, whole adipose tissue,

adipocytes and DFAT cells were tested using ANOVA followed by a Tukey post hoc test.

Differences in mRNA expression and protein expression between control and treated cells were

tested using matched paired t-test analyses.

3. RESULTS

3.1 Gene expression

We first measured gene expression of TGF-β1, TGF-β2, TGF-β3, COL1A1, COL1A2 and COL6A3 in whole adipose tissue, SVF cells and DFAT cells by real-time quantitative RT-PCR. As shown in **Figure 2**, expression of TGF-β1, TGF-β3 and COL1A1 was significantly higher in DFAT cells compared to the SVF ($p=0.02$, $p=0.01$ and $p=0.02$ respectively), whereas trends were observed for a similar pattern with TGF-β2, COL1A2 and COL6A3 ($p=0.10$, $p=0.08$, $p=0.06$ respectively). TGF-β1, COL1A1and COL6A3 gene expression was significantly higher in DFAT cells compared to whole adipose tissue ($p=0.05$, $p=0.01$, $p=0.02$ and $p=0.03$ respectively) and there was a trend for higher expression of COL1A2 in DFAT cells ($p=0.08$).

We then examined the expression of these transcripts during the dedifferentiation process at day 0, corresponding to mature adipocytes and at days 4, 7 and 12. As shown in **Figure 3**, TGF-β1, COL1A1 and COL6A3 gene expression increased significantly from day 0 to day 12 of the process ($p=0.01$, $p=0.02$, $p=0.02$). The increase in COL1A2, TGF-β2 gene expression from day 0 to day 7 was significant ($p=0.05$, $p=0.02$ and $p=0.02$). A trend was observed for higher expression at day 12 compared to day 0 for COL1A2 ($p=0.06$). Protein expression of TGF-β1 was confirmed by Western blotting at days 0, 4, 7 and 12 in the SC and OM depots (data not shown).

3.2 TGF-β1 recombinant treatment

Using the 6-well plate culture system, we tested the effect of serum starvation on the cells. However, when adipocytes were plated in the well without serum, they did not adhere to the upper slides (data not shown). We then used DMEM/F12 supplemented with 5% serum to test the effect of 5 ng/mL recombinant TGF-β1 or vehicle for 24 hours on collagen gene expression in DFAT cells from 7 patients. As shown in **Figure 4**, supplementation with TGF-β significantly increased TGF-β1, TGF-β2, COL1A1 and COL6A3 gene expression compared to 5% serum alone ($p<0.05$ for all). The treatment had no significant effect on TGF-β3 and COL1A2 gene expression.

3.3 TGF-β receptor 1 inhibitor

We used our 6-well plate model to investigate if endogenous inhibition of TGF-β signaling by SB431542, a TGF-β receptor ALK5 inhibitor, would down-regulate collagen transcripts during the dedifferentiation process. At day 5 of the ceiling culture, cells from n=8 donors were treated with 1 μm SB431542 or with vehicle for 24 hours and gene expression was measured by real-time quantitative RT-PCR. **Figure 5** shows that treating cells with the inhibitor significantly decreased expression of TGF-β1, COL1A1, COL1A2 and COL6A3 ($p=0.04$, $p=0.04$, $p=0.04$ and $p=0.03$, respectively). Trends were observed for a decrease in TGF-β2 and TGF-β3 gene expression.

The activation of the serine/threonine kinase pathway by TGF-β ligands leads to phosphorylation of some members of the intracellular signaling transduction cascade [18]. Protein phosphorylation of SMAD2 and SMAD3 as well as SMAD2, SMAD3 and SMAD4 level was measured using Western Blot analysis in the DFAT cells incubated with or without (vehicle

control) 5 ng/mL recombinant TGF-β1. As shown in **Figure 6**, treatment with recombinant TGF-β significantly increased phosphorylation level of SMAD 2 and SMAD 3 ($p=0.001$ and $p=0.03$, respectively). Protein levels of SMAD 2, SMAD 3 and SMAD 4 were not affected by TGF-β treatment.

4. DISCUSSION

The aim of this study was to test a new ceiling culture system and modulate the process of dedifferentiation using various incubation conditions targeting the TGF-β pathway. Adipose tissue expresses various types of collagen other than COL1A1, COLA2 and COL6A3 [19] and the presence of the three subunits of type VI collagen is required for the stable synthesis of collagen VI [20]. However, following results of our previous study demonstrating up-regulation of COL1A1, COL1A2 and COL6A3 gene expression during dedifferentiation [21], we chose to examine the effects of TGF-β on these transcripts specifically. We first demonstrated that TGF-β1, COL1A1 and COL6A3 gene expression was significantly higher in DFAT cells compared to whole adipose tissue. We also observed a general increase in TGF-β1, COL1A1, COL1A2 and COL6A3 gene expression over time during dedifferentiation. Using the 6-well plate model, we found that incubation of cells with TGF-β (5 ng/mL) during dedifferentiation significantly increased TGF-β1, TGF-β2, COL1A1 and COL6A3 gene expression compared to 5% serum alone ($p<0.05$ for all). Furthermore, when treated with the TGF-β receptor ALK5 inhibitor we observed a significant decrease in TGF-β1, COL1A1, COL1A2 and COL6A3 gene expression during the process, showing that our treatment was effective. Finally, recombinant TGF-β significantly increased the phosphorylation levels of SMAD 2 and SMAD 3 in DFAT cells.

The 6-well plate model of ceiling culture allowed us to treat cells during the dedifferentiation process by targeting and modulating the TGF-β pathway. The ceiling culture method was first described by Sugihara et al. in 1986 to study the biology of adipocytes [4]. Ceiling culture has since become the standard strategy to dedifferentiate mature adipocytes. This culture system allows the cells to be maintained in culture for long periods of time and to efficiently proliferate.

It has proven useful, but the large number of cells required in flask cultures makes it impossible to study the metabolic, molecular and secretory attributes of the cells under various incubation conditions. Here, we show that our 6-well plate model could be helpful to understand the physiological process of dedifferentiation and to identify the triggering factors. Some authors have put forward the hypothesis that dedifferentiation is caused by limited gas exchange, high-serum concentrations and cell-plastic contact. However, the 6-well plate model that we have developed allows for gas exchanges, which rules out a predominant effect of hypoxia. On the other hand, the high serum conditions may be an important aspect of the culture because it may contain high concentrations of growth factors. Here, we tested lower serum concentrations (5%) and still observed cell adherence and dedifferentiation. However, when the cells were cultured in serum-free medium, they could not adhere to the surface. The 6-well plate model represents a relevant approach to examine the impact of various culture environments on the cells.

The response of the cells to the treatments with agonists and antagonists of the TGF-β pathway using the 6-well plate model supports a role for TGF-β in modulating expression of extracellular matrix components during dedifferentiation. TGF-β is a well-known potent inducer of ECM protein-coding genes such as fibronectin and collagens [22]. This multi-functional cytokine has been described as a key factor in matrix remodeling in various physiological and pathological processes [23-27]. However, to the best of our knowledge, we are the first to describe a role for this pathway in human adipocyte dedifferentiation. Due to the limitations in the amount of material, we could not prove a causal impact TGF-β/SMAD signaling during dedifferentiation. However, concomitant with the high expression levels of TGF-β and collagens in DFAT cells, we show that TGF-β signaling is effective at the end of the process. The increase in SMAD 2/3

phosphorylation following treatment with recombinant TGF-β indeed proves that the pathway remains active once the cell are dedifferentiated. We used high concentrations of a soluble and active TGF-β. Thus, we omitted the essential activation steps of this pathway. Furthermore, active TGF-β is cleared from the extracellular space if it does not associate rapidly with surface signaling receptors. However, our data demonstrate that the 6-well plate model allows for detailed characterization of the cells regarding a well-known pathway, and suggest that it could be used to examine other cell programs during dedifferentiation.

Dedifferentiation contributes to the generation of a proliferative cell population that could be useful in cellular therapy or tissue engineering. Modulating the secretory, molecular or metabolic characteristics of the cells could potentially increase the efficiency of the process. The extent of dedifferentiation is difficult to measure and we do not have a recognized marker for this process yet. We have previously used cell size of the remaining mature cells, but we had reported that qPCR was, to date, a more sensitive method to detect short-term changes in the process [16]. Cell size has little sensitivity for short-term incubations and effects that may not dramatically alter the process. Accordingly, we observed changes in collagen gene expression with SB431542, but we were unable to detect qualitative or quantitative changes in the visual aspects of the cultures. More studies are needed to address whether the 6-well plate model can be used to modulate the extent of dedifferentiation.

Some limitations need to be acknowledged. As mentioned, we were unable to address whether TGF-β can modulate the extent of dedifferentiation. Because TGF-β1 gene expression was very low at the beginning of the process, it would be surprising that it would induce dedifferentiation,

even if a previous study has shown that TGF-β1 is crucial for the dedifferentiation of cancer cells to cancer stem cells in a context of osteosarcoma [5]. It was also demonstrated that TGF-β1 contributes to the loss of the myofibroblast phenotype [28]. As mentioned, TGF-β1 inhibits adipogenesis [8, 10] and we cannot rule out that the treatment with recombinant TGF-β1 increases the proliferative rate of DFAT cells. The observed increase in TGF-β signaling and collagen gene expression could reflect cell composition of the culture. As the dedifferentiation process takes place, an increasing number of fibroblasts could contribute to the increase in TGF-β secretion and signaling in DFAT cells. The changes observed in collagen gene expression following the treatment with recombinant TGF-β1 or with TGF-β signaling inhibitor may indirectly suggest a modulation of fibroblast proliferation.

In conclusion, the 6-well plate culture system will help understand and modulate the dedifferentiation process longitudinally instead of focusing exclusively on the resulting DFAT cells. To the best of our knowledge this study is also the first to show a role for TGF-β and the collagens during human mature adipocyte dedifferentiation *in vitro*. Our findings could potentially contribute to a more extensive characterization of the dedifferentiation process with significant interest for tissue engineering and cell-based therapy.

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AUTHOR CONTRIBUTIONS

The author contributions are: Julie Anne Côté: data acquisition, analysis and interpretation of data, manuscript writing, revision of the manuscript, final approval; Julie Lessard: data acquisition, analysis and interpretation of data, revision of the manuscript, approval; Mélissa Pelletier: data acquisition, analysis and interpretation of data, revision of the manuscript, final approval; Simon Marceau: clinical aspects, sample acquisition, review of the manuscript, approval; Odette Lescelleur: clinical aspects, sample acquisition, review of the manuscript, approval; Julie Fradette: interpretation of data, revision of the manuscript final approval, study supervision; André Tchernof: study funding, design and conduction of the study, data collection and analysis, interpretation of data, manuscript writing, revision of the manuscript final approval, study supervision.

COMPETING INTERESTS

André Tchernof receives research funding from Johnson & Johnson Medical Companies for studies unrelated to this manuscript.

FIGURE HEADINGS

Figure 1: A) Picture of the 6-well plate ceiling culture model. Panels 1, 2 and 3: 8 ml of DMEM/F12- 20% calf serum is added to each well containing a 1⁄2-inch plastic bushing; panels 4 and 5: a glass slide is put on top of the bushing; panel 6: mature adipocytes are seeded under each coverslip. **B)** Cells float and adhere to the slides. They can be studied at specific time points.

Figure 2: Expression levels of TGF-β1, TGF-β2, TGF-β3, COL1A1, COL1A2 and COL6A3 in whole adipose tissue, SVF, and DFAT cells (day 12) cells (n=3 donors). Values are mean ± SEM.

Figure 3: Expression levels of TGF-β1, TGF-β2, TGF-β3, COL1A1, COL1A2 and COL6A3 at various time points during the dedifferentiation process (n=3 donors). Values are mean ± SEM.

Figure 4: Expression levels of TGF-β1, TGF-β2, TGF-β3, COL1A1, COL1A2 and COL6A3 in dedifferentiating adipocytes incubated in media containing 5 % serum or 5% serum supplemented with TGF-β1 (5 ng/mL) (n=7 samples). Values are mean ± SEM.

Figure 5: Collagen and TGF-β gene expression after treatment with SB431542 at day 4 of the dedifferentiation process (n=8 samples). Values are mean ± SEM.

Figure 6: SMAD protein levels and phosphorylation measured in DFAT cells incubated with or without 5 ng/mL recombinant TGF-β1 (P- SMAD 2 and SMAD 2, n=9; P-SMAD 3, SMAD 3 and SMAD 2, n=7 samples). Values are mean ± SEM. Protein expression relative to β-tubulin. Bands from a representative blot are shown.

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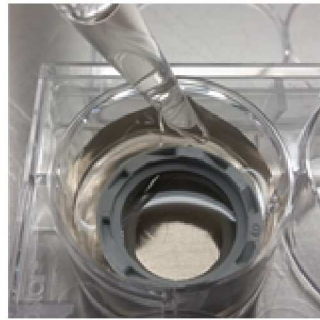
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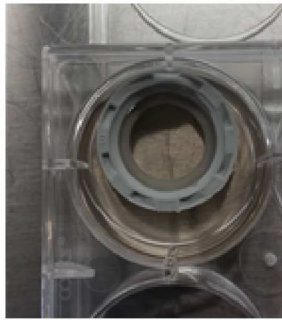
A)

B)
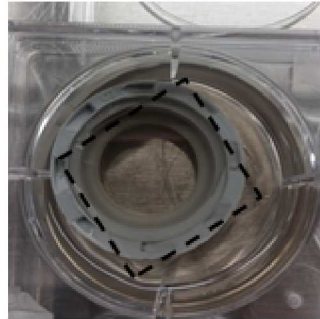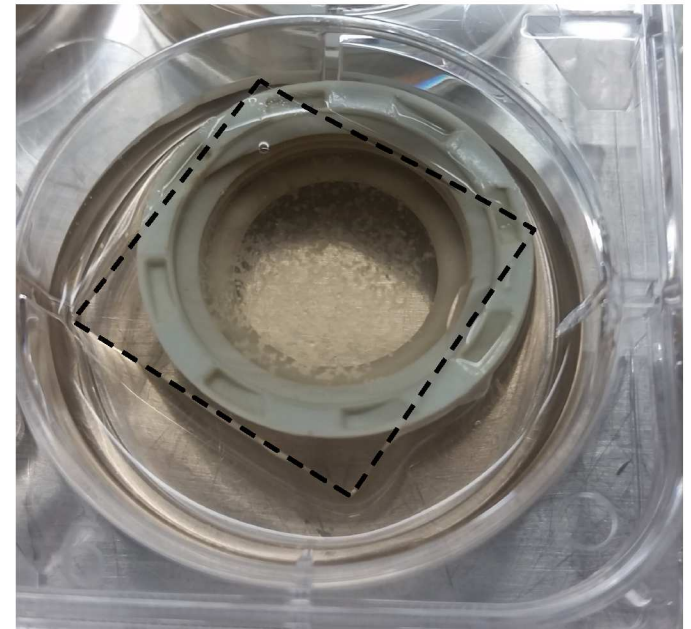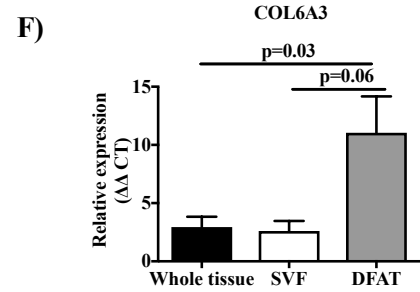
A)

TGF-β1

B)

TGF-β2

C)

TGF-β3

D)

COL1A1

E)

COL1A2

F)

COL6A3

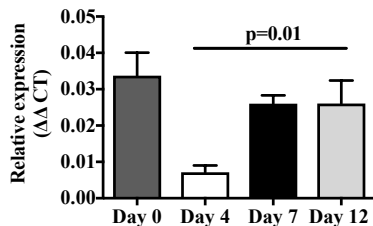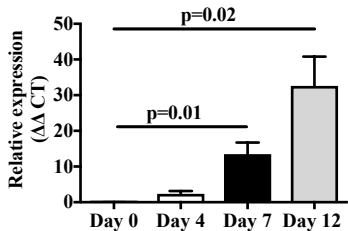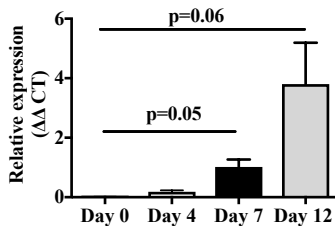A) TGF-β1

B) FEBS Open Bio — TGF-β2

C) TGF-β3

D) COL1A1

E) COL1A2

F) COL6A3

A) TGF-β1

0.007

B) TGF-β2

0.004

C) TGF-β3

NS

D) COL1A1

0.001

E) COL1A2

NS

F) COL6A3

0.009

A) TGF-β1, p=0.04, Expression relative (ΔΔ CT), CTRL vs SB431542

B) TGF-β2, 0.08, Expression relative (ΔΔ CT), CTRL vs SB431542

C) TGF-β3, p=0.09, Expression relative (ΔΔ CT), CTRL vs SB431542

D) COL1A1, 0.04, Expression relative (ΔΔ CT), CTRL vs SB431542

E) COL1A2, 0.04, Expression relative (ΔΔ CT), CTRL vs SB431542

F) COL6A3, 0.03, Expression relative (ΔΔ CT), CTRL vs SB431542

A) SMAD 2

B) P-SMAD 2

C) SMAD 3

D) P-SMAD 3

E) SMAD 4